

Sections:

BIOLOGY

ENVIRONMENTAL ENGINEERING

**ON THE FRESHWATER TRICLADID FLATWORMS (PLATYHELMINTHES,
TRICLADIDA) IN THE URBAN AREAS OF CRAIOVA (ROMANIA) –
PRELIMINARY DATA**

Babalean Anda Felicia^{1*}

¹University of Craiova, Faculty of Horticulture, e-mail: anda_babalean@yahoo.com

Keywords: *Polycelis tenuis*, *Dendrocoelum lacteum*, *Girardia tigrina*

ABSTRACT

The paper presents preliminary data on the freshwater tricladid flatworms sampled in some springs and running waters of three urban areas in Craiova municipality: The Botanical Garden, The Romanescu Park and Balta Craioviței area. The faunistic account comprises: Polycelis tenuis Ijima, 1884; Dendrocoelum lacteum (Müller, 1774) and Girardia tigrina (Girard, 1850) (an asexual population of presumable Girardia tigrina). The gross anatomy of the copulatory complex is presented and briefly discussed for P. tenuis and D. lacteum in relation with the literature. P. tenuis is for the second time reported in Romania. Short notes on the above mentioned species biology are given.

INTRODUCTION

Craiova is a town located in Oltenia Plain, SW Romania. The literature on the history of Craiova since the medieval period (Ciobotea et al., 1999) mentions numerous springs, watercourses, lakes and marshlands on the present territory of the city: Valea Vlăiciei Brook, Stan Jianu Brook and the spring of Hagi Stan Jianu, Valea Orbeților Brook, Valea Episcopiei Brook, Valea Tabacilor Brook, Șerca Brook, Belcineanu Pond, Valea Fetii Brook and pool, Bibescu Pond with many springs, Valea Hanului Doctorului Brook, Craiovița and Geanoglu pools.

During the development of the city, there have been elaborated several plans of urban systematization and most watercourses have disappeared as they were integrated in the sewerage system and introduced into the underground. Lakes and marshlands were drained, sanitized, or arranged in parks and recreational areas. Consequently, from the multitude of wetlands, in the urban part of Craiova there were preserved: Jianu Brook and Jianu Spring in the Botanical Garden, Bibescu Pond and Valea Fetii Brook in Romanescu Park and Balta Craioviței Lake.

This paper is a contribution on the Tricladida flatworms in the above mentioned areas for which the author does not know any data to have been reported so far.

MATERIAL AND METHODS

The worms were removed from the underside of the submerged stones with a paint brush, fixed in Beauchamp 12 + 24 hours and preserved in ethanol 75⁰. Selected specimens underwent paraffin inclusion, sagittal and frontal (horizontal) sections at 5 microns and Haematoxylin-Eosin staining.

The collecting sites and moments:

Romanescu Park: Bridge no.1: 2.04.2017 – *D. lacteum* – 1 ind., 5.01.2018 – *D. lacteum* – 6 ind.; Bridge no. 3: 2.04.2017 – *G. tigrina* – 20 ind., 5.01.2018 – *G. tigrina* – 23 ind.; High Bridge: 2.04.2017: *D. lacteum* – 2 ind., *P. tenuis* – 2 ind. *G. tigrina* – 2 ind.; Spring: 2.04.2017 – *D. lacteum* – 1 mature + 3 youngs, *P. tenuis* – 18 ind.; Concrete canal: 2.04.2017 – *D. lacteum* – 2 ind., *P. tenuis* – 2 ind., *G. tigrina* – 4 ind., 16.04.2017 – *D. lacteum* – 2 ind., *G. tigrina* – 3 ind.

Botanical Garden: Lake no. 1: 16.02.2018 – *D. lacteum* – 1 ind. (deteriorated during fixation); Rivulet no.1: 15.04.2016 – *D. lacteum* – 1 ind., *P. tenuis* – 10 ind., 23.03.2017 – *D. lacteum* – 2 ind., *P. tenuis* – 13 ind.; Rivulet no.2: 23.03.2017 – *D. lacteum* – 8 ind., *P. tenuis* – 3 ind.; Jianu Fountain Spring: 23.03.2017 – *P. tenuis* – 14 ind.

Balta Craiovitiei Lake: Zeus spring: 10.04.2017 – *P. tenuis* – 43 ind.

Abbreviations for all figures: ad – adenodactyl; adb – adenodactyl bulb; adpp – adenodactyl papilla; adp – adenodactyl pore; bc – bursal canal; bh – bursal horns; cb – copulatory bursa; cm – circular musculature; com – common atrium; cod – common oviduct; eg – eosinophilic glands; ejd – ejaculatory duct; f – flagellum; fd – folds of the common atrium wall; g – gonopore; ma – male atrium; p – penis; pb – penis bulb; pp – penis papilla; sv – seminal vesicle; spv – spermiducal vesicles; spd – sperm ducts.

The histological slides are deposited in author personal collection and may be lend on request.

RESULTS AND DISCUSSIONS

The systematic account according to Sluys et al. 2009 includes:

Subord. Continenticola Carranza et al., 1998

Superfam. Planarioidea Stimpson, 1857

Fam. Planariidae Stimpson, 1857

1. *Polycelis tenuis* Ijima, 1884

Material examined – serial frontal and sagittal sections on 9 specimens:

No. 5 – serial frontal sections on 9 slides, Botanical Garden, 15.04.2016

No. 6 – serial sagittal sections on 35 slides, Botanical Garden, 15.04.2016

No. 7 – serial sagittal sections on 31 slides, Botanical Garden, 15.04.2016

No. 12 – serial frontal sections on 5 slides, Botanical Garden, 15.04.2016

No. 9 – serial frontal sections on 4 slides, Botanical Garden, 23.03.2017

No. 10 – serial sagittal sections on 34 slides, Botanical Garden, 23.03.2017

No. 11 – serial sagittal sections on 19 slides, Botanical Garden, 23.03.2017

No. 13 – serial frontal section on 7 slides, Balta Craioviței-Zeus spring, 10.04.2017

Pt1 – serial frontal sections on 2 slides, the Romanescu Park (spring), 2.04.2017

The reconstruction of the copulatory apparatus was done using the slides of the specimen no. 10 – Fig. 5.

External morphology

The general aspect of living adult specimens is presented in Fig. 1 a, b. The mature specimens in fully stretched state are up to 11 mm long and 2 mm wide. The colour of the body is dark brown to black on the dorsal side and brownish on the ventral side in the living worms. The ocular bands include the mid-dorsal part of the head, measure 1/3 of the worm length in fixed specimens and consist of 30 – 35 eyes on each band. The pharynx represents ¼ of the body length and it is located in the posterior half of the body.

In all specimens, part of the copulatory complex (the large common atrium) is visible in living animals as a round white area situated in the middle of the posterior third part of the body – Fig. 1 a, b. The pharynx, the copulatory complex and part of the sperm ducts are visible on the ventral side of the fixed worms – Fig. 1c.

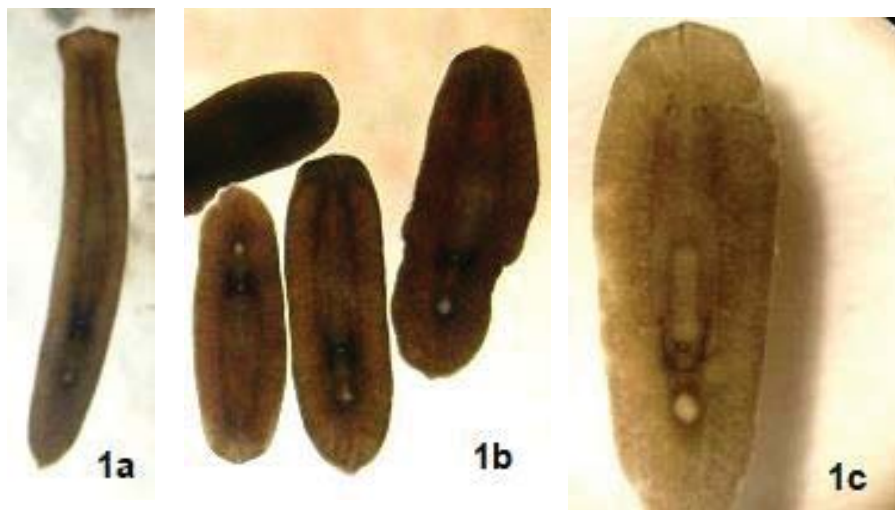


Fig. 1 *Polycelis tenuis* – external morphology

a – specimen from Zeus spring, 10.04.2017, swimming; b – living specimens from Zeus spring, 10.04.2017, in rest state on the water surface ; c – fixed specimen from the Botanical Garden, 15.04.2016

The copulatory complex – Fig. 2, 3, 4

The sperm ducts present spermiducal vesicles on both sides of the pharynx; they enter the penis bulb separately – Fig. 2a, 4a. The general aspect of the penis is of an elongate cone. The slides of all sagittal and frontal sections show the penis consisting of a well developed muscular, almost spherical bulb and a conic-cylindrical papilla. In most cases the bulb lacks a seminal vesicle, revealing only 2 ducts dichotomically branched surrounded by musculature – Fig. 4a, corresponding to the entrance of the sperm ducts. In few specimens the penis bulb houses a small seminal vesicle – Fig. 2a, 2b. The penis papilla reveals a compartmented/folded ejaculatory duct – Fig. 2a, 2b. The common atrium appears as a large space delimited by a folded wall; the folders take the aspect of adenodactyls– Fig. 2a, 3a.

The copulatory bursa is horn shaped. The bursal canal is large and opens into the common atrium. The two oviducts – Fig. 3b, 4b join into a common oviduct which runs between the bursal canal and the male atrium to open into the common atrium. The oviduct is surrounded by branched eosinophilic glands – Fig. 4b.

Habitat

Polycelis tenuis was found on submerged stones and sand in springs (Fântâna Jianu, Romanescu Park), concrete canals (Romanescu Park), and among vegetation in the spring of Balta Craioviței-Zeus. Water temperature – 13-14° C in April in Romanescu Park; 18° C in Zeus spring.

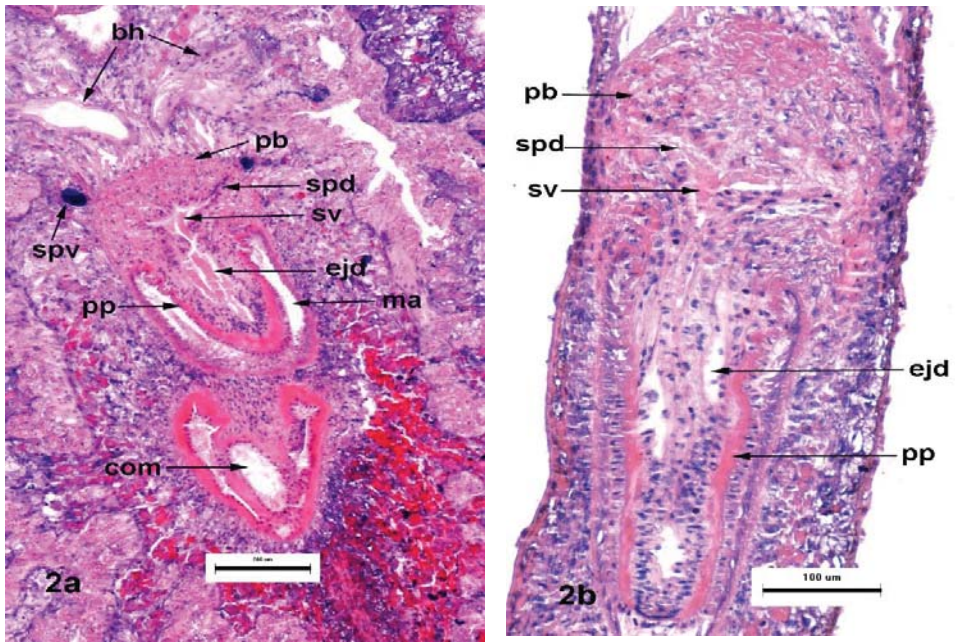


Fig. 2 *Polycelis tenuis* – aspects of the copulatory complex in different specimens:
 a – Botanical Garden, 23.03.2017, frontal section (slide 9.3.4), bar = 200μm; b –
 Botanical Garden, 15.04.2016, sagittal section (slide 6.25.3), bar = 100μm

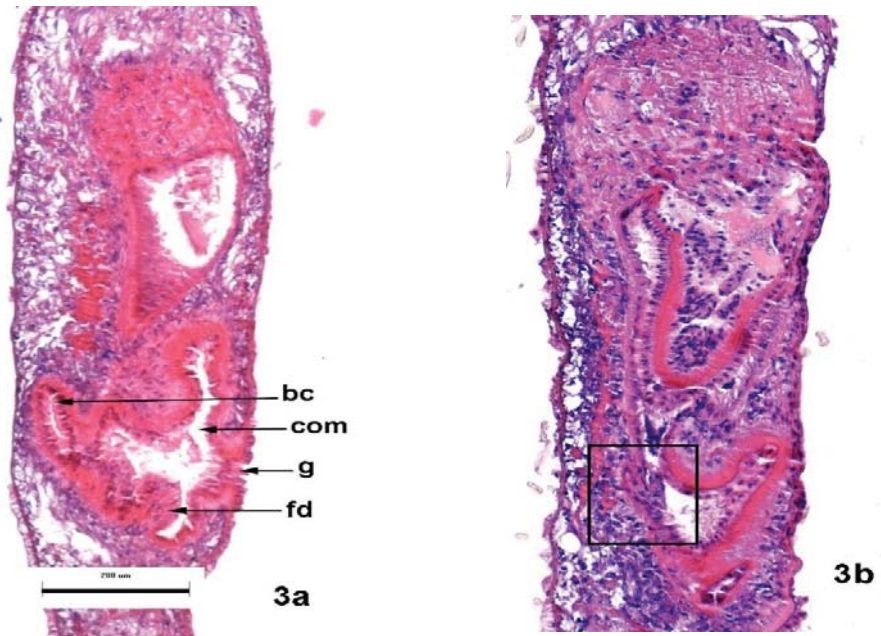


Fig. 3 *Polycelis tenuis* – aspects of the copulatory complex in different specimens:

a – Botanical Garden, 23.03.2017, sagittal section (slide 10.30.2), bar = 200µm; b – Botanical Garden, 15.04.2016, sagittal section (slide 7.20.3) – the entrance of the common oviduct into the common atrium – in square

Discussions

The general aspect of the penis and the presence/lack of adenodacyls in *P. tenuis* is analysed in the English literature (Ball & Reynoldson 1981, Reynoldson & Young 2000). The folded, irregular ejaculatory duct is part of the original description in *P. tenuis* (Ijima, 1884, Fig. 2 – Bd.XL). This feature was also reported by Năstăsescu (1976). The lack of the seminal vesicles in most specimens might be associated with the physiological state. Instead, the dichotomy of the two vasa deferentia within the penis bulb is a mystery and lead to the idea of more than two entries (sperm ducts) into the penis bulb. The horn shaped bursa present in *P. tenuis* and *P. nigra* is discussed elsewhere (Taylor & Reynoldson 1962).

Polycelis tenuis was first recorded in the E and SE of Romania - Bucharest and Dobrogea by Năstăsescu (1976). In Oltenia Plain it is the second record of this species in Romania, thus extending to west the species geographical range.

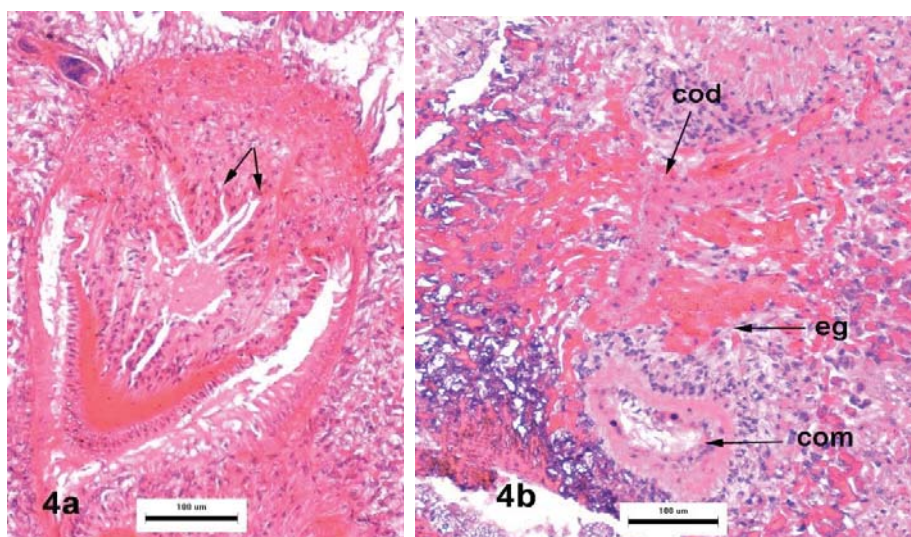


Fig. 4 *Polycelis tenuis* – aspects of the copulatory complex in different specimens: a - Botanical Garden, 15.04.2016, frontal section (slide 5.4.2) – the dichotomy of the sperm-ducts pointed by arrows, bar = 100µm; b – Balta Craioviței-Zeus, 10.04.2017, frontal section (slide 13.5.2), bar = 100µm

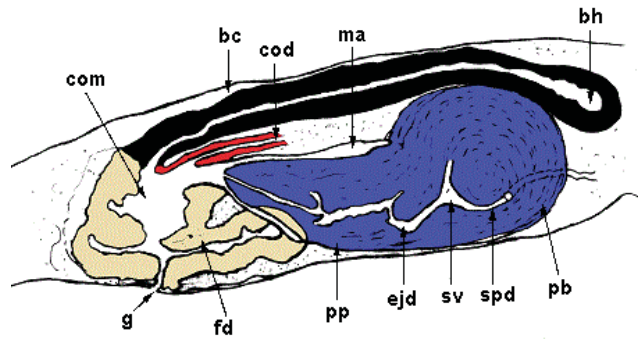


Fig. 5 *Polycelis tenuis* – the sagittal reconstruction of the copulatory apparatus (scale bar not available)
Fam. Dendrocoelidae Hallez, 1892

2. *Dendrocoelum lacteum* (Müller, 1774)

Material examined – serial frontal and sagittal sections on 3 specimens:

Dd1 – serial sagittal sections on 45 slides, Romanescu Park, 5.01.2018

Dd2 – serial sagittal sections on 35 slides, Botanical Garden, 23.03.2017

Dd3 – serial frontal sections on 11 slides, Romanescu Park, 5.01.2018

The copulatory apparatus was reconstructed using the sagittal serial sections on Dd1 specimen – Fig. 8.

External morphology

The general aspect of mature living specimens is presented in Fig. 6. The size of the living worms ranges between 18 mm/3mm in specimens from the Romanescu Park and 25 mm/3.5 mm in specimens from the Botanical Garden. The colour of the living worms varies from gray in the sample of the Romanescu Park to white and light pale-pink in the sample of the Botanical Garden.

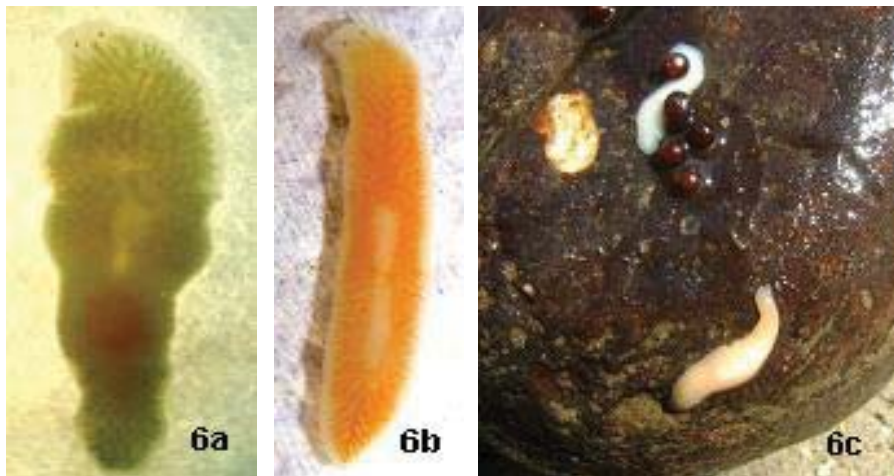


Fig. 6 *Dendrocoelum lacteum* – the general aspect of living specimens: a – individual with a cocoon inside the body, Romanescu Park, 2.04.2017; b,c – Botanical Garden, 23.03.2017

The copulatory complex – Fig. 7

The sperm ducts enter the penis bulb separately and run separately on the lateral sides of the bulb. The penis is a short cone. The bulb consists of interwoven layers of musculature. The papilla is delimited by an internal layer of longitudinal muscles and an external layer of circular muscles which do not extend onto the flagellum. In frontal section (Dd3.6.3) the penis reveals a large cavity corresponding to the seminal vesicle and a short ejaculatory duct. The penis cavity is lined with a tall vacuolated epithelium which extends outside the penis in an everted flagellum. The adenodactyl is oval-elongated, almost of the same size of the penis, placed on the left side of the penis. It consists of a muscular bulb and a hollow papilla with a pore. The adenodactyl penetrates the common atrium.

The copulatory bursa is an ovoid sac lined with a low epithelium. The bursal canal opens into the common atrium. The oviducts join into a common oviduct which opens posterior into the common atrium.

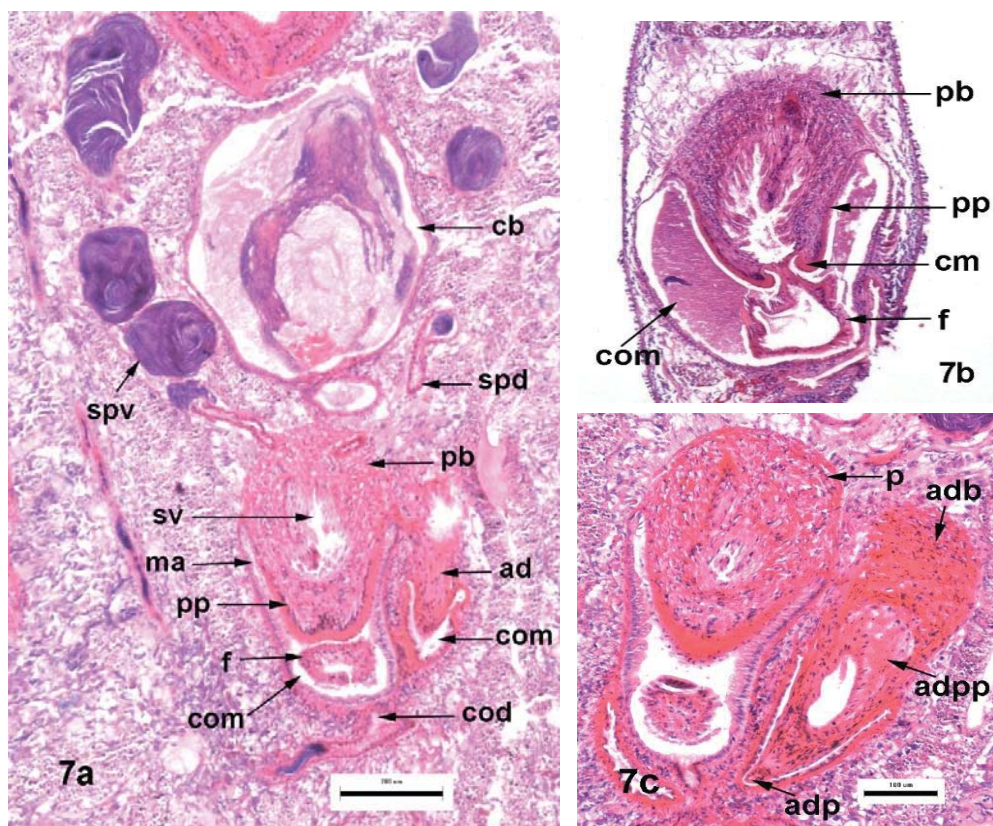


Fig. 7 *Dendrocoelum lacteum* (Dd1 – Romanescu Park, 5.01. 2018) – the copulatory apparatus in sagittal and frontal sections:
 a – frontal section (slide Dd3.5.2), bar = 200µm; b – sagittal section (slide Dd1.25.2); c – frontal section (slide Dd3. 4.2) – the adenodactyl, bar = 100µm

Habitat

Dendrocoelum lacteum was found in small and slow running waters, under rocks situated near the banks, nearly without flow. Water temperature – 4.5° C in January.

Discussions

The taxonomic importance of some morphological characters was analysed and discussed in the genus *Dendrocoelum*: the adenodactyl structure and especially its musculature (Harrath et al., 2012), the penial flagellum and the bursal canal sphincter (Stocchino et al., 2013).

With respect to the aspect of the adenodactyl cavity, the examined specimens differ from *Dendrocoelum lacteum* described and figured elsewhere (Kenk, 1978). In the analysed specimens, the adenodactyl with a strong bulb and a large cavity (hollow) within the papilla differs from that of *Dendrocoelum lacteum* figured by Kenk (1978, fig. 50) in which the papilla of the adenodactyl houses a duct. However, Ball and Reynoldson (1981) state a hollow adenodactyl in *Dendrocoelum lacteum*. Adenodactyl with a large cavity (hollow) and not a duct within the papilla is present in some *Dendrocoelum* s.l. species: *Dendrocoelum lacustre* (Stankovič, 1932-1938; *Dendrocoelum ochridense* (Stankovič & Komárek, 1927 (Kenk, 1978, fig. 41, 44); *Dendrocoelum* (*Dendrocoelides*) *debeauchampianum* Codreanu & Balcesco, 1967; *Dendrocoelum* (*D.*) *atriostrictum* Codreanu & Balcesco, 1967; *Dendrocoelum* (*D.*) *banaticum* Codreanu & Balcesco, 1967 (Codreanu & Balcesco, 1967, fig. 1, 2, 3); *Dendrocoelum* (*D.*) *vallanti* de Beauchamp, 1954; *Dendrocoelum* (*D.*) *collini* (de Beauchamp, 1919); *Dendrocoelum* (*D.*) *polymorphum* Codreanu & Balcesco, 1967; *Dendrocoelum* (*D.*) *racovitzai* de Beauchamp, 1949; *Dendrocoelum* (*Neodendrocoelum*) *plesiophthalmus* de Beauchamp, 1937 (Gourbault, 1972, fig. 8.3, 10.1, 11.2, 12.1, 15.2); *Dendrocoelum amplum* Harrath & Sluys, 2012 (Harrath et al., 2012); *Palaeodendrocoelum romanodanubialis* Codreanu, 1950 (Codreanu, 1950, fig. 3). Most probably, the cavity of the adenodactyl – duct/hollow – is correlated with the physiological state as the same type of cavity is present in different morphological forms of *Dendrocoelum*, for instance in *Dendrocoelum* with flagellum and in *Dendrocoelides* without flagellum. By its histological structure, the flagellum of the specimen Dd1 satisfies the condition of the true flagellum discussed by Stocchino et al. (2013).

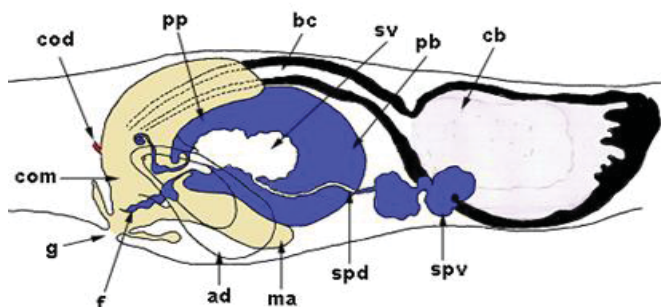


Fig. 8 *Dendrocoelum lacteum* – the sagittal reconstruction of the copulatory apparatus (scale bar not available)

Superfam. Geoplanoidea Stimpson, 1857
Fam. Dugesiidae Ball, 1974

3. *Girardia tigrina* (Girard, 1850)

Only asexual specimens were collected in April and January from Romanescu Park. The general aspect of living and fixed specimens is presented in Fig. 9. The body is brown-olive with white-yellow and dark-brown spots. The lateral sides of the body are white transparent with brown spots. The pharynx is pigmented. The head is sharp triangular, with triangular auricles. The body of the living worms is up to 11 mm long and 1.5 mm wide for the worms sampled in January and up to 14-15 mm long for the worms sampled in April.

Some specimens sampled in April underwent asexual reproduction by fission, completely developed in nearly one hour. The resulted head and tail showed their own slow movement, the tail being faster than the head. The body of one individual shows clear marks of fission in two points – Fig. 9d. No specimen collected in January showed any sign of asexual reproduction.

Habitat – low polluted waters, temperature – 4.5° C in January, 13-14° C in April.

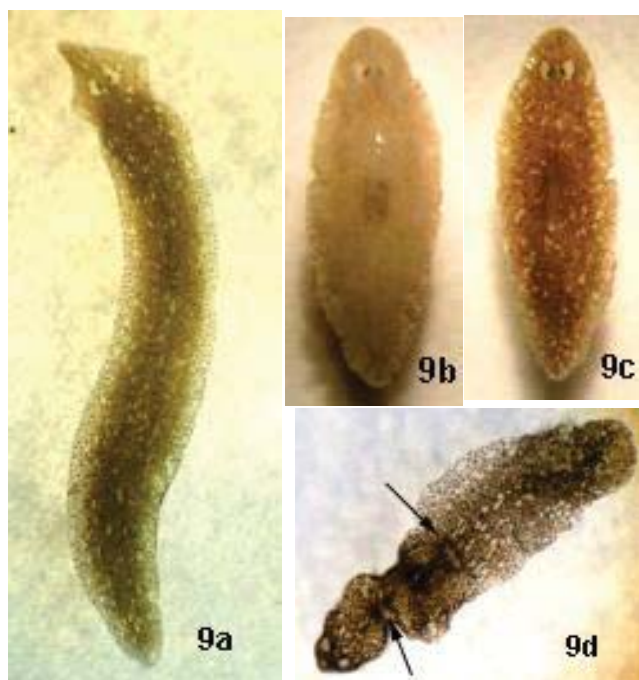


Fig. 9 *Girardia tigrina* – Romanescu Park

a – living worm, 5.01.2018; b, c – fixed worm, 5.01.2018 ventral and dorsal view; d – living worm in fission, 2.04.2017

Discussions

Because only asexual individuals were sampled, the assignment to *Girardia tigrina* is presumable. The asexual population assigned to *G. tigrina* resembles the general description of *Girardia tigrina* found elsewhere with respect to the external morphology (Ball & Reynoldson 1981, Kawakatsu et al. 2012, Reynoldson & Young 2000, Sluys et al. 2005 and included references). For the specimens from Craiova there are to be noticed the lateral white, transparent, brown-spotted marginal strips.

CONCLUSIONS

The preliminary data of this paper need more detailed and comprehensive studies to confirm the species identity and to establish their phylogenetic affinities on molecular and histological grounds.

The paleo-geographical evolution and the features of the hydrographic and hydrogeological basins of Oltenia Plain and Craiova area (Pleniceanu 1999, Savin 2000) outline the phylo-geographic perspectives on the knowledge of the freshwater Tricladida in this region.

ACKNOWLEDGMENT

Author is deeply grateful to: Dr. M. Niculescu for access to the histological facilities, Mrs. C. Micu for preparing the histological slides, Dr. G. Mogoşanu and Prof. M. Mogoanta at the Faculty of Medicine in Craiova for the Nikon photos, Dr. A. Vlăduţ for the help with the English translation of a draft of the manuscript, Prof. Dr. M. Năstăsescu and Prof. Dr. R. Sluys for general discussions and suggestions on the manuscript. The responsibility for the paper remains to author.

REFERENCES

- Ball I. R., Reynoldson T. B., 1981, British Planarians, Platyhelminthes: Tricladida, Keys and notes for the identification of the species, Cambridge University Press, pp. 141.
- Ciobotea D., Bădescu I., Zarzără I., Pleniceanu V., Avram C., Lucaks S., 1999, Grădinile și Parcurile Craiovei. Pagini de Istorie și Civilizație, Vol. VI, 35-69.
- Codreanu R., 1950, O nouă tricladă epigee relictă din Defileul Dunării: *Palaeodendrocoelum danubialis* n.g., n. sp. Analele Academiei R.P.R., III (16), 599-622.
- Codreanu R., Balcesco D., 1967, Sur le rapports entre les sous-genres *Paradendrocoelum* Kenk 1930 et *Dendrocoelides* de Beauchamp 1919 d'après les espèces obscuricoles du Banat et de l'Olténie. Revue Roumaine de Biologie, Zoologie, 12 (5), 337-349.
- Gourbault N., 1972, Recherches sur les Triclaides paludicoles hypogés, Mémoires du Muséum National d'histoire Naturelle, nouvelle série, série A, Zoologie, LXXIII, pp. 249 + 3 planches.
- Harrath A. H., Sluys R., Ghlala A., Alwasel S., 2012, The first subterranean freshwater planarians from north Africa, with an analysis of adenodactyl structure in the genus *Dendrocoelum* (Platyhelminthes, Tricladida, Dendrocoelidae). Journal of Cave and Karst Studies, 74 (1), 48-57, DOI: 10.4311/2011LSC0215
- Ijima I., 1884, Untersuchungen über Bau und die Entwicklungsschichte der Süsswasser Dendrocoelen. Zeitschrift für wissenschaftliche Zoologie, Bd. XL.
- Kenk R., 1978, The planarians (Turbellaria: Tricladida Paludicola) of Lake Ohrid in Macedonia. Smithsonian Contribution to Zoology, 280, 1-56.
- Năstăsescu M., 1976, Despre trei specii ale genului *Polycelis* Ehrenberg 1831 (Tricladida – Paludicola) prezente în apele României. Studii și Cercetări de Biologie, seria Biologie Animală, 28 (1), 3-8.
- Pleniceanu V., 1999, Apele din Câmpia Olteniei, Edit. Universitaria, Craiova, pp. 354.
- Reynoldson T. B., Young J. O., 2000, A key to the freshwater Triclaids of Britain and Ireland with notes on their ecology, Freshwater Biological Association, No. 5, pp. 72.
- Savin C., 2000, Apele subterane din zona Craiova, Edit. Tipored, pp.313.
- Sluys R., Kawakatsu M., Ponce De Leon R., 2005, Morphological stasis in an old and widespread group of species: Contribution to the taxonomy and biogeography of the genus *Girardia* (Platyhelminthes, Tricladida, Paludicola). Studies on Neotropical Fauna and Environment, 40 (2), 155-180.

Sluys R., Kawakatsu M., Riutort M., Baguña J., 2009, A new higher classification of planarian flatworms (Platyhelminthes, Tricladida). *Journal of Natural History*, 43 (29-30), 1763-1777, DOI: 10.1080/00222930902741669

Stocchino A. G., Sluys R., Marcia P., Manconi R., 2013, Subterranean aquatic planarians of Sardinia, with a discussion on the penial flagellum and the bursal canal sphincter in the genus *Dendrocoelum* (Platyhelminthes, Tricladida, Dendrocoelidae). *Journal of Cave and Karst Studies*, 75 (2), 93-112. DOI: 10.4311/2011LSC0252.

Taylor M. C., Reynoldson, T. B., 1962, The Population Biology of Lake-Dwelling *Polycelis* Species with Special Reference to *P. nigra* (Müll.) (Turbellaria, Tricladida). *Journal of Animal Ecology*, 31(2), 273-291.

Web references:

Kawakatsu M., Nishino M., Ogata K., Kuranishi N. B., Kobayashi N., Ohtaka A., 2012, *Two North American Freshwater Planarian Species Now Naturalized in Japan: Girardia tigrina (Girard, 1850) and Girardia dorotocephala (Woodworth, 1897) ---In Connection with the Field Survey of Benthic Invertebrates---*, Kawakatsu's Web Library on Planarians: December 15, 2012.

**RESEARCHES ON THE INFLUENCE OF CLIMATE CHANGE
ON THE QUALITY OF WINE-PRODUCTION PRODUCTION IN THE
SÂMBREȘTI VINEYARD**

Băducă Cîmpeanu C.¹, Marinică Ion², Mete L.³

¹University of Craiova, Faculty of Horticulture

²University of Craiova, Faculty of Science, Craiova

³University of Craiova, Faculty of Horticulture, Master Vine-Wine Expertise
Correspondence author. E-mail: cbaduca@gmail.com, metelaurentiu@yahoo.com

Keywords: *grapevine, climatic changes, vineyard, ripening*

ABSTRACT

The Sâmburești vineyard is one of the most famous vineyards in Romania for the production of high quality wines, especially tomatoes. The prestige of Sâmburești wines is based on their quality due, first of all, to the grapes harvested at the optimum maturity, depending on the characteristics of the wine to be obtained. Grape breeding is a phenomenon closely related to the climatic conditions specific to the wine year. The climatic conditions specific to the Sâmburești vineyard are very favorable for obtaining high quality. In recent years, the phenomenon of climate change, also known as global warming, has a strong impact on viticulture and winemaking all over the world. In order to see to what extent climate change influences the quality of wine-growing, we conducted a study of the 5 main varieties cultivated in the Sâmburești vineyard (3 varieties for red wines and two varieties for white wines), which covered the wine years 2015, 2016 and 2017. The results show that under the influence of climate change, grape ripening is much earlier, which leads to the accumulation of higher amounts of sugar in grapes to technological maturity.

INTRODUCTION

Seasonal fluctuations in yield, grape composition and wine attributes, largely driven by variable climatic conditions, are major challenges for the wine industry aiming to meet consumer expectations for consistent supply, wine style and product quality (Cichi Daniela Doloris, 2006; Clingeleffer P.R., 2010). It is projected to have a significant effect on European viticultural geography. Detrimental impacts on winegrowing are predicted in southern Europe, mainly due to increased dryness and cumulative thermal effects during the growing season (Malheiro A. e.a., 2010).

The effect of temperature on berry composition is biologically interesting and has practical implications for wine attributes (Bonada M. and Sadras V.O., 2015). Vine phenology and grape ripening are highly dependent on water uptake conditions. Mild water deficit stress enhances grape quality for the production of red wines (Maracineanu L.C., 2011). Vine water status can accurately be assessed by means of stem water potential or carbon isotope discrimination measured on grape sugars. Quality losses through severe water stress can be avoided through the use of drought-adapted plant material, appropriate canopy management, yield reduction or the implementation of deficit irrigation (Van Leeuwen C. e.a., 2009). Temperature and solar radiation

influence *Vitis vinifera* L. berry ripening. Both environmental conditions fluctuate cyclically on a daily period basis and the strength of this fluctuation affects grape ripening too. Additionally, a molecular circadian clock regulates daily cyclic expression in a large proportion of the plant transcriptome modulating multiple developmental processes in diverse plant organs and developmental phases. Circadian cycling of fruit transcriptomes has not been characterized in detail despite their putative relevance in the final composition of the fruit (Carbonell-Bejerano P. e.a., 2014).

The terroir and typic concepts are complex and have several dimensions little known outside the wine branch. Controlled by manufacturers and market executives, these concepts could be relevant communication vectors if consumers understand them too (Jourjon F. e.a., 2013, Muntean Camelia e.a., 2016).

The physiology of grapevine has already suffered significant impacts of global climate change over the last decades. Harvesting takes place earlier, although grape growers tend to wait longer for maturity. The content of the grains in sugar (and alcohol in wine) tends to increase, while phenolic and aromatic maturity is not always attained. Acidity tends to decrease with potential effects on wine aging capacity. Water supply is becoming shorter in many regions (Delrot S. e.a., 2010).

MATERIAL AND METHODS

We conducted a study in the vineyard of Sâmburești, where we followed the way the influence of climate change on the quality of the wine-growing production is felt. In order to accomplish the proposed goal, the research followed two directions: analysis of climatic data and oenological data analysis.

With regard to climatic data, we first performed an analysis of the multi-annual values for temperature, precipitation and insolation, the main climatic indicators for assessing climate favorability for viticulture. The benchmark ranges for a period of 40 years (1975-2014). The study of the multi-annual climate data aimed to characterize the specific climate of the Sâmburești vineyard by calculating the multi-annual average values for each month for temperature, precipitation and insolation. We have also watched to what extent, from these data, the phenomenon of climate change is confirmed or not.

With regard to oenological data we analyzed the productivity and quality of grapes at full maturity for a number of 5 varieties: 2 white varieties (Chardonnay and Sauvignon) and 3 red varieties (Merlot, Cabernet Sauvignon, Black Feteasca). All of these varieties are part of the new assortment of the Sâmburești vineyard, they are found in the young plantations, established in 2008-2010, through the program of conversion and restructuring in viticulture. Driving systems are different: Guyot for white varieties; the hop planted for red varieties, the planting distances are the same for all varieties: 2.2 m between rows, 1 m between plants per row, resulting in a density of 4545 vines/ha.

For all varieties, we followed the date when the full yield (corresponding to the maximum grain weights) of the production/hub (by multiplying by 4545 we obtained production per hectare), the sugar content and the total acidity of the grapes, ie the parameters determined at tracking the baking process.

RESULTS AND DISCUSSIONS

The analysis of the multiannual climatic synthesis data for the Sâmburești vineyard, presented in Table 1 shows that this vineyard enjoys particularly favorable climatic conditions for a quality viticulture. Along with soils and relief, the climate represents the natural environment of vine cultivation in the vineyard of Sâmburești, particularly favorable for obtaining high quality wines, both white and especially red.

The analysis of temperature data shows that one month in the year (January) has a negative multiannual average for a 40-year period. The other winter months (December and February) have positive multiannual average values, although over the course of these months they have recorded negative average temperatures. Also, negative monthly average temperatures were recorded outside the winter months in March and November, but they were rare and accidental. For the vegetative growing season, the values are very good.

For precipitations and the duration of sunshine, values are also favorable to vines, but with absolute values that vary within very wide limits, indicating that each viticultural year is different and which significantly influences wine-growing and its quality.

Table 1
Parameters of multiannual climatic indicators in the Sâmburești vineyard (1975 – 2014)

Temperature, °C				Precipitations, mm			Insolation, ore		
T ^o medie	Min.	Average	Max.	Min.	Average	Max.	Min.	Average	Max.
January	- 5,2	- 0,5	5,8	1,1	51,1	76,5			
February	- 5,7	0,8	7,4	2,5	33	84,3			
March	- 0,2	5,8	10,4	0,8	34,5	86,0			
April	7,1	11,4	14,3	2,4	45	151,4	138,1	193,0	292,8
May	13,5	16,7	20,5	18,4	78,0	281,8	146,9	250,9	350,5
June	17,6	20,2	23,1	8,4	74,7	165,2	212,3	281,6	364,0
July	19,5	22,4	26,9	7,4	71,6	211,4	257,1	313,4	399,3
August	17,4	21,9	25,3	5,6	59,3	191,8	211,5	290,6	399,3
September	13,6	17,4	21,9	2,6	46,9	191,8	135,4	222,2	282,6
October	8,8	11,6	14,7	0,2	47,6	140,2			
November	- 0,1	5,4	10,5	0,2	41,4	123,7			
December	- 3,1	0,8	5,2	0,2	43,2	139,9			
Annual	9,7	11,5	12,5	323,2	611,0	1156,8	1101,3	1541,8	2088,5

The average annual average temperature in the Sâmburești vineyard is 11.5°C. The coldest year was 1976 with an average temperature of 9.7°C, and the warmest year was 2007 with an average temperature of 12.5°C. Thus, the amplitude of annual averages was 2.8 ° C. The frequency of very cold years (mean temperature ≤10.0°C) is 6.0 % and very hot years (years with mean ≥12.0°C) was 16.0 %, which shows a higher high share of the very warm years compared to the very cold. The annual average temperature variation chart has an increasing linear trend, confirming the trend of global warming.

In the vineyards of Sâmburești, the spring is warm and warm, the summers are warm, with many tropical and hectic days, the autumns are long and warm and the winters are mild. In some time periods, "warm windows" are recorded, and agro-meteorological frost has a low frequency (8 % in December and 10 % in January). Under these conditions, the thermal regime is particularly favorable for the cultivation of all varieties of vine, especially those for red wines.

An overview of recording data from 1975 to 2014 for the duration of the Sunshine shows that the average monthly sunshine duration was 258.6 hours, the minimum duration was 135.4 hours recorded in the rainy month of September 1998 (94.5 L/m²), the maximum duration was 399.3 hours recorded in an excessively drought and heat month July 2007 (7.4 L/m²). The overall monthly sunshine graphs have a strongly increasing linear trend (excluding September), which is directly related to the global warming climate process, which has increased the number of warm days as well as those with variable or clear sky. The analysis of the monthly regime shows that it is particularly favorable to the vineyard culture with a peak in the warmest month - July.

If we refer to the multiannual average temperature, which is 11,5°C for the reference range (1975-2014), it is noted that starting with 2007, with one exception (2011), all years had a mean temperature above the multiannual average, an aspect that must be borne in mind by the global warming phenomenon. This is confirmed by the average of the last 10 years, when a much higher average (11,75°C) than the average of the first 10 years of the reference range (10,87°C) results. So, at the Sâmburești vineyard level, the global warming phenomenon is as real as it can be demonstrated by the average annual temperatures.

This trend accelerated after 2014, 2015 being the first year in which the average global temperature exceeded by 1°C, the average of the last century. In 2016, the exceedance was even higher than in 2015, and 2017 is in the first three years the warmest on our planet, with extreme weather phenomena, according to the WMO provisional climate statement and the NASA Goddard Institute for Space Studies (GISS) report in New York. The report also highlights the impact on human safety, well-being and the environment.

The data in Table 2 highlights how the influence of climate change on wine-growing, the impact on grape production and quality parameters is manifested.

The study conducted on the 5 basic varieties of the Sâmburești vineyard range shows that all varieties reach maturity in 2016 more quickly than in 2015 with 2-4 days and in 2017 as compared to 2016, which means that in 2017 the full maturity of was reached 5-8 days earlier than in 2015 at all varieties studied. Thus, depending on the variety, the grapes have reached full maturity as follows; between September 8 and 18, 2015, between September 5 and September 15, 2016, and between September 3 and September 11, 2017. The main consequence of the early grapes' arrival at full maturity is that of the main parameters of grape composition - the contents in sugar and total acidity.

The full maturity moment is much more relevant for the study of the maturing dynamics compared to the time of harvest, as the latter varies according to the technological objectives to be pursued, and to what extent the grapes must be copts at the time of harvesting, according to the type of wine to be obtained.

The analysis of the data in Table 2 shows that in 2015 the grapes presented at full maturity between 206 and 212 g/L, in 2016 between 202 and 209 g/L, and in 2017 between 200 and 207 g/L. At the same time, total grape acidity values were between 4.05 and 4.06 g/L of H₂SO₄ in 2015, between 4.21 and 4.64 g/L of H₂SO₄ in 2016 and between 4.48 and 4,75 g/L H₂SO₄ in 2017.

Another very important element to be taken into account when assessing vineyard production is the yield or the level of production. If, in 2015, grape yields were between 2.04 and 2.18 kg/vine (9.27 to 9.90 t/ha), in 2016, at the time of reaching full maturity earlier, grape yields were between 2.08 and 2.28 kg/vine (9.45 and 10.18 t/ha), while in 2017 they were between 2.1 and 2.28 kg/vine (9.54 and 10.36 t/ha).

Table 2

Productivity and quality of grapes at full maturity

Year		Chard.	Sv. Bl.	Merlot	Cabernet S.	Fetească N.
2015	Date F.M.	8.IX	10.IX	14.IX	18.IX	18.IX
	Sugars, g/L	212	206	206	212	208
	A.T., g/L H ₂ SO ₄	4,05	4,12	4,32	4,40	4,46
	Kg/vine	2,12	2,04	2,18	2,04	2,05
2016	Date F.M.	5.IX	8.IX	10.IX	14.IX	15.IX
	Sugars, g/L	209	202	204	209	205
	A.T., g/L H ₂ SO ₄	4,21	4,31	4,44	4,52	4,64
	Kg/vine	2,16	2,08	2,24	2,08	2,18
	Date F.M.	3.IX	5.IX	7.IX	10.IX	11.IX

2017	Sugars, g/L	204	200	202	207	201
	A.T., g/L H ₂ SO ₄	4,48	4,56	4,60	4,70	4,75
	Kg/vine	2,21	2,15	2,28	2,10	2,21

Chard - Chardonnay, Sv. Bl. - Sauvignon Blanc, F.M. - Full maturity

The results show that climate change significantly impresses on vineyard production in the vineyard of Sâmburești. In the wine industry the quality of grape raw material is a major factor in the quality of wines. The sugar and acidity content of the grapes at the time of full maturity is very good, especially since it is going to improve over the overmature, which creates particularly favorable premises for obtaining high-class, competitive domestic and export wines. In this respect, the global warming phenomenon has helped the quality of wine-growing.

CONCLUSIONS

The phenomenon of climate change is manifested in the vineyard of Sâmburești, with all the elements that define it, of which in this study we highlighted the significant increase of the average annual temperatures in the last decade. To characterize the climate of the vineyard we analyzed the climatic data for a period of 40 years (1975-2014). The data recorded during this study (2015-2017) show that this phenomenon was particularly noticeable in the last 3 years. The phenomenon of climate change, also known as global warming, has triggered a hurry in the baking process, which made grapes reach technological maturity sooner, even if it meant lower sugar contents, but they knew increases important to the technological maturity, respectively the harvest time. The limitation of grape production and the very good values of the main chemical constituents of grapes (sugars and acidity) have a predominant influence on the quality of the wine. From this point of view, it can be said that in the vineyard of Sâmburești during the years 2015-2017, have had a beneficial influence on wine-growing. In the future, it is to be expected that in the short and medium term, climate change will continue to be beneficial for wine-growing but in the medium to longer term, less favorable side-effects will start to appear.

REFERENCES

- Bonada M., Sadras V.O., 2015. Review: critical appraisal of methods to investigate the effect of temperature on grapevine berry composition. *Australian Journal of Grape and Wine Research*, vol. 21, nr. 1, pag. 1–17.
- Carbonell-Bejerano P., Rodríguez Virginia, Royo Carolina, Hernáiz Silvia, Morogonzález L.C., Torres-Viñals M., Martínez-Zapater J.M., 2014. Circadian oscillatory transcriptional programs in grapevine ripening fruits. *BMC Plant Biology*, vol. 14, nr. 78, pag. 1-15.
- Cichi Daniela Doloris, 2006. Modificările termice din ecosistemul viticol. (Cauze, efecte asupra viței de vie, studii). Editura Universitaria Craiova.
- Clingeffer P.R., 2010. Plant management research: status and what it can offer to address challenges and limitations. *Australian Journal of Grape and Wine Research*, vol. 16, Nr. Special, pag. 25–32.
- Delrot S., Medrano H., Or E., Bavaresco L., Grando S., 2010. *Methodologies and Results in Grapevine Research*; Springer: New York, USA, pag. 5–6.
- Jourjon F., Wilson D., Symoneaux R., 2013. Le terroir: mythe ou réalité pour les consommateurs de vin? *Revue Suisse Viticulture, Arboriculture, Horticulture*, vol. 45, nr. 2, pag. 122–125
- Malheiro A., Santos J., Fraga H., Pinto J., 2010. Climate change scenarios applied to viticultural zoning in Europe. *Climate Research*, vol. 43, no. 3, pp. 163–177.

Maracineanu L.C., 2011. Aplicații ale ecologiei în viticultură. Editura Universitaria, Craiova.

Muntean Camelia, Stoica Felicia, Băducă Constantin, 2016. Establishing the quality indicators of the red wines in Drăgășani vineyard. Analele Universității din Craiova, seria Biologie, Horticultură, Tehnologia Prelucrării Produselor Agricole, Ingineria Mediului, vol. XXI (LVII), pag. 331-336.

Van Leeuwen C., Trégoat O., Choné O., Bois B., Pernet D., Gaudillère J.P., 2009. Vine water status is a key factor in grape ripening and vintage quality for red Bordeaux wine. How can it be assessed for vineyard management purposes? Journal International of Sciences Vigne Vin, vol. 43, nr. 3, pag. 121-134.

**CALAMINTHA OFFICINALIS MOENCH (LAMIACEAE) – HISTO-
ANATOMICAL AND PRELIMINARY CHROMATOGRAPHIC RESEARCHES**

Bejenaru Cornelia¹, Bejenaru Ludovic Everard², Biță Andrei²,
Mogoșanu George Dan^{2*}, Scorei Iulia Daria³, Blendea Antonia¹

¹Department of Vegetal & Animal Biology, Faculty of Pharmacy, University of Medicine and
Pharmacy of Craiova

²Department of Pharmacognosy & Phytotherapy, Faculty of Pharmacy, University of Medicine and
Pharmacy of Craiova

³BioBoron Research Institute, S.C. Natural Research S.R.L., Craiova

* Correspondence author. E-mail: mogosanu2006@yahoo.com

Keywords: *Calamintha officinalis Moench, Lamiaceae, histo-anatomy, polyphenols, thin-layer chromatography.*

ABSTRACT

The paper contains the histo-anatomical researches on the root, aboveground stem and leaf of Calamintha officinalis Moench species, as well as preliminary chromatographic analysis of the polyphenols content of Calaminthae officinalis herba. In the thin-layer chromatogram, chlorogenic acid was identified from the six-polyphenol components specific bands.

INTRODUCTION

Calamintha officinalis Moench, Calamint, Mountain Mint, *Lamiaceae* family, is an herbaceous, perennial, Eurasian species, 50–60 cm height, which blooms in July–September, spontaneous on well-drained dry grassy banks, calcareous soils, in the forests glades and meadows (Ciocârlan 2000, Singh et al. 2012a & 2012b).

The medicinal product *Calaminthae officinalis herba* contains many active principles, as follows: essential oil (β -bisabolene, germacrene D, piperitenone, *cis*-piperitone oxide), flavonoids (eriocitrin, eriodyctiol, acacetin), triterpenes, catechic tannin, phenolic acids (chlorogenic acid, *p*-coumaric acid), mineral salts (Karousou et al. 2012, Monforte et al. 2012, Singh et al. 2012a & 2012b).

The extractive preparations obtained from the aerial parts of *C. officinalis* exhibit numerous benefits, due to some useful pharmacological actions, such as: aromatic, antimicrobial and preservative, diaphoretic, expectorant, stomachic, anti-spasmodic, anti-inflammatory and anti-ulcer against alcohol-induced gastric mucosa injury in rats, bitter tonic, cicatrizing, hypoglycemic, antioxidant and antiproliferative *in vitro* on MCF-7 breast cancer cell line (Lemhadri et al. 2004, Moattar et al. 2015, Monforte et al. 2012, Singh et al. 2012a & 2012b).

Information about *C. officinalis* histo-anatomy is found in some specialty works (Singh et al. 2012a, Toma & Rugină 1998). The aim of our paper was the histo-anatomical study on the roots, aboveground stems and leaves of *C. officinalis* and the preliminary chromatographic investigation of polyphenols from the aerial parts (*Calaminthae officinalis herba*).

MATERIAL AND METHODS

Histo-anatomical analysis

The vegetal material was harvested in July 2016, from *C. officinalis* plants in blossom, spontaneous in the surroundings of Bozovici commune, Caraș-Severin County (southwestern Romania).

The roots, aboveground stems and leaves were fixed and stored in 70 % ethanol. Using botanical razor, the cross-sections and longitudinal-radial sections were performed.

After pre-washing with distilled water, the sections were clarified using Javel water (10 % sodium hypochlorite solution); the clarifying solution was subsequently removed by washing with distilled water.

A Congo red–chrysoidine mixture (Genevese reagent) was used for the staining of sections. Various colors were obtained starting from the composition of cell membranes: pink to red for cellulose and mucilages, pale red for cytoplasm, yellow for suberin and brown for lignin (Andrei & Paraschivoiu 2003).

Stained and mounted sections were analyzed on a Krüss binocular photon microscope (×4, ×10, ×20, and ×40 objectives).

Nikon Eclipse 55i binocular microscope coupled with a Nikon DS-Fi1 high definition charge-coupled device (CCD) video camera and Image-Pro Plus ver. 6.0 software package (Media Cybernetics) were utilized for shooting and for image acquisition and processing, respectively.

Considering the works of some classical authors (Toma & Rugină 1998), the histo-anatomical review of sections was validated accordingly.

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols was performed on the aerial parts of *C. officinalis* (*Calaminthae officinalis herba*), using TLC CAMAG system (Muttentz, Switzerland) (Altemini et al. 2015, Bojić et al. 2013, Gîrd et al. 2014, Jug et al. 2018):

- stationary phase: TLC silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany) 10×10 cm precoated glass plates, pre-washed with chloroform–methanol (1:1, v/v) and activated by oven-drying (110°C, 30 minutes);
- mobile phase: ethyl acetate–formic acid–methanol–water (15:1:0.1:1, in volumes);
- 10 mL of mobile phase were added in the developing twin-chamber and then oversaturated for 20 minutes;
- sample: 20 % methanolic extract of *Ononidis arvensis herba*;
- standards (Merck): 0.05 % methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin;
- migration distance: 62 mm (sample application line – 8 mm, solvent front – 70 mm);
- sample (8 µL, 10 µL) and standards (2 µL) application: CAMAG Linomat 5 semiautomatic system – spray gas nitrogen, syringe volume 100 µL, dosage speed 150 nL/s, predosage volume 0.2 µL, bands length of 8 mm;
- plate drying: 5 minutes, at 25°C (cold air dryer);
- photographing the chromatographic plate: UV light (λ 254 nm);
- detection: CAMAG TLC Scanner 3 photodensitometer, for densitogram and *in situ* UV light (λ 280 nm) spectra, without derivatization, deuterium–wolfram lamp, scanning speed 40 mm/s, data resolution 200 µm/step, measurement mode absorption;
- winCATS ver. 1.4.3 software package.

RESULTS AND DISCUSSIONS

Histo-anatomical analysis

Root

The cross-section in the lower third of the root highlighted round shape and secondary structure due to the libero-ligneous cambium. The following histological sequence was evidenced from the outside towards the inside of root: Rhizodermis is exfoliated. The first discernible layer is exodermis, consisting of large cells with suberin-impregnated walls. From place to place, passage cells are observed. The cortical parenchyma is made up of large oval cells, with thin, cellulosic walls that delineate small intercellular spaces. The last layer of the bark is endodermis, made up of a single layer of heterodiametric cells. At this level, Casparian strips and passage cells are observed. The conducting tissues are arranged on two concentric rings; predominates the secondary tissues, generated by the secondary meristem (libero-ligneous cambium). The phloem tissue forms a thin, external ring, consisting of sieve tubes, phloem parenchyma and annex cells. Occupying the central area of the root, the xylem tissue is made up of few metaxylem vessels of different calibers, disordered placed in the libriform tissue mass, pushing to the center small diameter protoxylem vessels, accompanied by some xylem parenchyma. The medullary rays are multicellular, uniseriate, cellulosic, at the level of the phloem tissue, and multi-cellular, uniseriate, lignified and sclerified, at the level of the xylem tissue ring. The medullary parenchyma is missing (Figure 1).

Aboveground stem

In the upper third, the aboveground stem exhibited four-edged shape and secondary structure due to the libero-ligneous cambium. On cross-section, from the outside to the inside of aboveground stem, the following histological sequence has been observed: The epidermis has approximately isodiametric cells, having thickened outer wall covered by a thin cuticle with toothed relief. The epidermal cells are slightly tangential elongated, with thin radial walls and thick tangential external and internal walls.

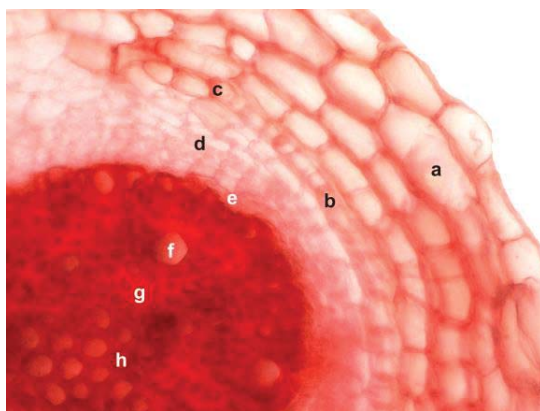


Figure 1. Cross-section through *C. officinalis* root: (a) cortical parenchyma; (b) endodermis; (c) Casparian strips; (d) phloem fascicle; (e) libero-ligneous cambium; (f) metaxylem; (g) libriform tissue; (h) protoxylem (Congo red–chrysoidine staining, $\times 400$).

From place to place, there are stomata, rare multi-cellular, uniseriate and elongated tector trichomes, but also short glandular trichomes with a unicellular secretory gland. The bark is organized into 5–7 layers of angular collenchyma, at the ribs' level,

and 2–3 layers of chlorenchyma, between the ribs. The inner area of the bark is parenchymatous. Inside the bark, there is a single layer of primary-type endodermis, made up of large cells, impregnated with suberin. The conducting tissues are organized into four big libero-ligneous fascicles of collateral open type, at the ribs' level, and four small libero-ligneous fascicles, resulting from the activity of libero-ligneous cambium. The small libero-ligneous fascicles are made up of phloem and xylem tissues only of secondary origin. The phloem tissue is made up of sieve tubes, some phloem parenchyma and annex cells. The medullary rays are multicellular, multiseriate, cellulosic. The periphloemic appearance of some discontinuous packages of sclerenchyma fibers is observed. The xylem tissue is made up of highly lignified and well-represented libriform tissue, near the intra-fascicular cambium, and of metaxylem with different calibers, disposed in radial strings towards the inner side of the large conducting fascicles. On the longitudinal-radial sections, the xylem vessels exhibit reticulate and helical thickenings. The primary xylem tissue is poorly represented, consisting of few primary xylem vessels and xylem parenchyma. Between the conducting fascicles of the xylem area, the medullary rays are wide and strongly lignified. The medullary parenchyma is well developed, of meatus type (Figure 2).

Leaf

Leaf's limb

In cross-section, the following histological sequence was evidenced from the outside towards the inside of leaf's limb: The upper epidermis consists of a single layer of large, flattened cells, having thickened tangential external and internal walls and thin radial walls.

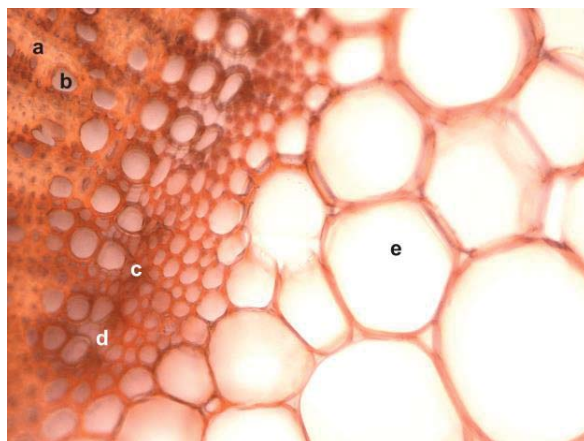


Figure 2. Cross-section through *C. officinalis* aboveground stem: (a) libriform tissue; (b) metaxylem; (c) protoxylem; (d) xylem parenchyma; (e) medullary parenchyma (Congo red–chrysoidine staining, ×200).

The outer walls are bulged and covered by a thin cuticle with toothed relief. Glandular trichomes with multicellular, seriate, unbranched pedicle, made up of 2–3 cells and a unicellular gland, are found in patches. The mesophyll consists of a single layer of palisade parenchyma, with large, elongated and chloroplast-rich cells, but also of 3–4 layers of lacunose parenchyma, having small cells with disordered layout and aeriferous spaces. Numerous small libero-ligneous conducting fascicles are found into the mesophyll. The mesophyll has bifacial type with dorsiventral structure. The lower epidermis consists of a single layer of small, tangential elongated cells, with thin radial

walls and slightly thickened tangential external and internal walls. The cuticle has a toothed relief. At this level, there are numerous diacytic stomata and glandular trichomes, with unicellular, short pedicle and multicellular gland, placed into the epidermal excavations. The median rib is slightly protruding and rounded to the abaxial face. In the central area, into the leaf's parenchyma, a single libero-ligneous conducting fascicle is found. Into the libero-ligneous fascicle, the xylem vessels have a seriate layout and the medullary rays are uniseriate, cellulose. The leaf's limb has bifacial, dorsiventral, hypostomatic structure (Figure 3).

Petiole

On the cross-section, the petiole highlighted semi-elliptical shape, with two laterally adaxial wings. From the outside towards the inside, the following tissues sequence was evidenced: The epidermis is made up of a single layer of large, isodiametric cells, with thickened tangential external and internal walls and thin radial walls.

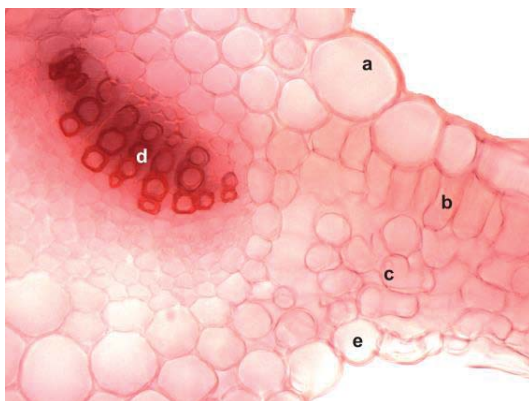


Figure 3. Cross-section through *C. officinalis* leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) libero-ligneous fascicle; (e) lower epidermis (Congo red–chrysoidine staining, ×400).

The outer walls are bulged and covered by a thin cuticle with toothed relief. From place to place are found diacytic stomata, rare multicellular, uniseriate, elongated tector trichomes, but also short glandular trichomes with a unicellular secretory gland. To the abaxial side and at the wings level, there are two layers of angular collenchyma. Into the fundamental parenchyma of meatus type, a large, centrally disposed libero-ligneous fascicle, with the primary xylem vessels arranged in radial strings, and two small conducting fascicles, at the wings level, are found (Figures 4 and 5).



Figure 4. Cross-section through *C. officinalis* petiole: overview (Congo red–chrysoidine staining, $\times 40$).

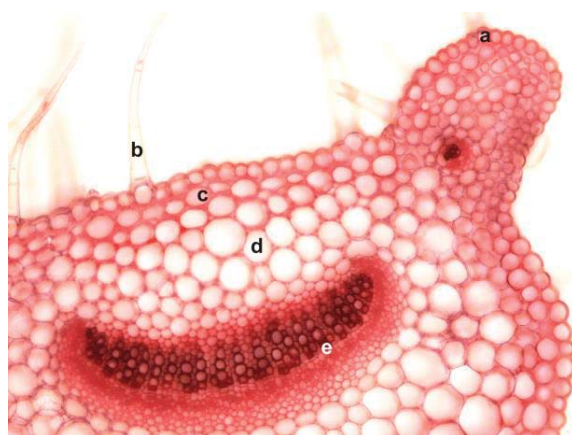


Figure 5. Cross-section through *C. officinalis* petiole: (a) epidermis; (b) tector trichome; (c) angular collenchyma; (d) fundamental parenchyma; (e) libero-ligneous conducting fascicle (Congo red–chrysoidine staining, $\times 100$).

TLC analysis

The experimental data about preliminary TLC analysis of polyphenols from *Calaminthae officinalis herba* are highlighted in Figures 6–8. A concentration of 153.9 $\mu\text{g}/\text{mL}$ chlorogenic acid (R_f 0.32) was quantified in 20 % methanolic extract, corresponding to 76.95 mg/100 g of dried vegetal product.

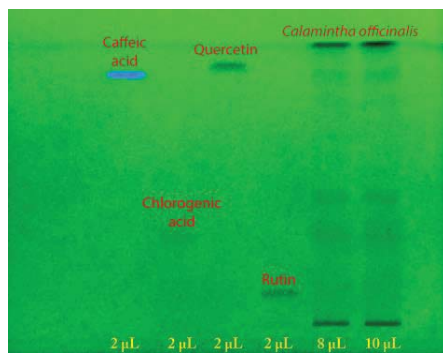


Figure 6. TLC chromatogram of polyphenols from *Calaminthae officinalis herba* 20 % methanolic extract (UV 254 nm, without derivatization). From left to right: first four bands – standards (2 µL); last two bands – sample (8 µL and 10 µL).

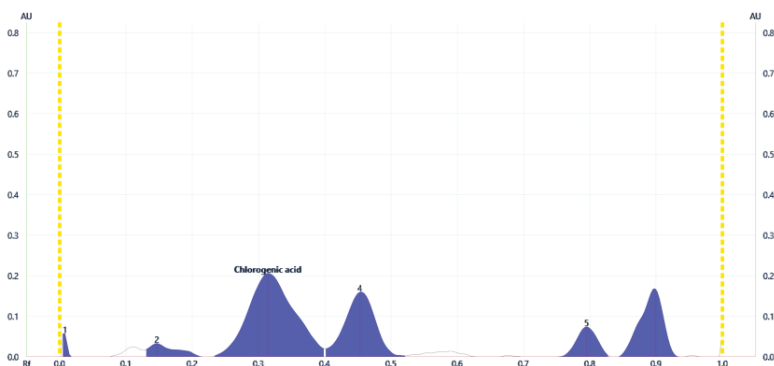


Figure 7. Densitogram of polyphenols (UV 280 nm, without derivatization) separated from *Calaminthae officinalis herba* 20 % methanolic extract. Chlorogenic acid was identified at R_f 0.32.

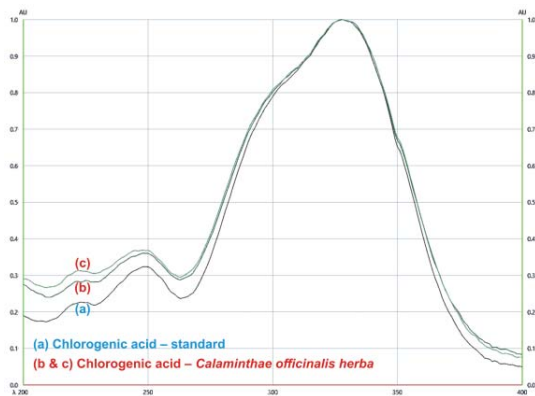


Figure 8. *In situ* UV spectra (UV 280 nm) of chlorogenic acid standard and compound separated from the analyzed sample.

CONCLUSIONS

The study highlighted the histo-anatomical specific features of the roots, aboveground stems and leaves of *Calamintha officinalis* and the preliminary TLC investigations on the polyphenols from *Calaminthae officinalis herba*. In the lower third, the root has round shape and secondary structure. In the upper third, the aboveground stem showed four-edged shape and secondary structure. The leaf's limb has bifacial, dorsiventral, hypostomatic structure and the petiole semi-elliptical shape, with two laterally adaxial wings. Chlorogenic acid was quantified in the 20 % methanolic extract (153.9 µg/mL).

REFERENCES

- Altemini A., Watson D. G., Kinsel M., Lightfoot D. A., 2015, Simultaneous extraction, optimization, and analysis of flavonoids and polyphenols from peach and pumpkin extracts using a TLC-densitometric method. *Chem. Cent. J.*, 9:39.
- Andrei M., Paraschivoiu Roxana Maria, 2003, *Microtehnică botanică*, Edit. Niculescu, Bucureşti, 2003, 222 pp.
- Bojić M., Simon Haas V., Sarić D., Maleš Z., 2013, Determination of flavonoids, phenolic acids, and xanthines in mate tea (*Ilex paraguariensis* St.-Hil.). *J. Anal. Methods Chem.*, 2013:658596.
- Ciocârlan V., 2000, *Flora ilustrată a României. Pteridophyta et Spermatophyta*, ediția a 2-a revizuită și adăugită, Edit. Ceres, Bucureşti, 1138 pp.
- Gîrd C. E., Nencu I., Costea T., Duțu L. E., Popescu M. L., Ciupitu N., 2014, Quantitative analysis of phenolic compounds from *Salvia officinalis* L. leaves. *Farmacia*, 62(4):649–657.
- Jug U., Glavnik V., Kranjc E., Vovk I., 2018, High-performance thin-layer chromatography and high-performance thin-layer chromatography–mass spectrometry methods for the analysis of phenolic acids. *J. Planar Chromatogr.*, 31(1):13–22.
- Karousou R., Hanlidou E., Lazari D., 2012, Essential-oil diversity of three *Calamintha* species from Greece. *Chem. Biodivers.*, 9(7):1364–1372.
- Lemhadri A., Zeggwagh N. A., Maghrani M., Jouad H., Michel J. B., Eddouks M., 2004, Hypoglycaemic effect of *Calamintha officinalis* Moench. in normal and streptozotocin-induced diabetic rats. *J. Pharm. Pharmacol.*, 56(6):795–799.
- Moattar F. S., Sariri R., Giahi M., Yaghmaee P., Ghafoori H., Jamalzadeh L., 2015, Antioxidant and anti-proliferative activity of *Calamintha officinalis* extract on breast cancer cell line MCF-7. *J. Biol. Sci.*, 15(4):194–198.
- Monforte M. T., Lanuzza F., Pergolizzi S., Mondello F., Tzakou O., Galati E. M., 2012, Protective effect of *Calamintha officinalis* Moench leaves against alcohol-induced gastric mucosa injury in rats. Macroscopic, histologic and phytochemical analysis. *Phytother. Res.*, 26(6):839–844.
- Singh P. P., Jha S., Irchhaiya R., 2012a, Pharmacognostical and physico-chemical investigation of the leaf of *Calamintha officinalis* Moench. *Asian Pac. J. Trop. Biomed.*, 2(3):S1362–S1366.
- Singh P. P., Jha S., Irchhaiya R., Fatima A., Agarwal P., 2012b, A review on phytochemical and pharmacological potential of *Calamintha officinalis* Moench. *Int. J. Pharm. Sci. Res.*, 3(4):1001–1004.
- Toma C., Rugină Rodica, 1998, *Anatomia plantelor medicinale*. Atlas, Edit. Academiei Române, Bucureşti, 320 pp.

**ONONIS ARVENSIS L. (FABACEAE) – HISTO-ANATOMICAL
AND PRELIMINARY CHROMATOGRAPHIC RESEARCHES**

Biță Andrei¹, Bejenaru Cornelia², Blendea Antonia², Scorei Iulia Daria³,
Bejenaru Ludovic Everard^{1*}, Mogoșanu George Dan¹

¹Department of Pharmacognosy & Phytotherapy, Faculty of Pharmacy, University of Medicine and
Pharmacy of Craiova

²Department of Vegetal & Animal Biology, Faculty of Pharmacy, University of Medicine and
Pharmacy of Craiova

³BioBoron Research Institute, S.C. Natural Research S.R.L., Craiova

* Correspondence author. E-mail: ludovic_bejenaru@yahoo.com

Keywords: *Ononis arvensis* L., *histo-anatomy*, *polyphenols*, *thin-layer chromatography*.

ABSTRACT

For Ononis arvensis L. species, the paper presents the histo-anatomical researches on the aboveground stem and leaf, but also the preliminary chromatographic analysis of polyphenols content from the flowering aerial part. Of the nine specific bands for polyphenolic components from the thin-layer chromatogram, rutin was identified.

INTRODUCTION

Ononis arvensis L. sin. *O. hircina* Jacq., Field Restharrow, *Fabaceae* family, is an herbaceous, perennial, Eurasian continental species, 30–70 cm height, which blooms in June–July, being common in oak and beech forests, in the meadows and riversides. Unlike *O. spinosa* L., *O. arvensis* has no thorns and is a common species in Transylvania, northern Moldavia and Oltenia regions (Ciocârlan 2000).

Ononis species contain some useful active principles, such as: tetracyclic triterpene saponins (α - and β -onocerin), triterpene alcohols, isoflavones, lignans, coumarins, sterols (β -sitosterol), glycosylated phenyl benzyl ketones (onospin), essential oil, mineral salts (Langer et al. 1995, Mezrag et al. 2017, Sichinava et al. 2014, Tumova et al. 2011).

Ononidis radix and *Ononidis herba* medicinal products and their extractive preparations exhibit important pharmacological actions: diuretic, depurative, anti-lithiatic, antimicrobial, anti-inflammatory, cicatrizing, antioxidant, cytotoxic (Baldemir & Coşkun 2016, Dénes et al. 2017, Mezrag et al. 2017). The flowering aerial parts from *O. arvensis* species are recommended in Turkish ethnopharmacology for the treatment of urinary infections and skin ailments (Baldemir & Coşkun 2016).

The specialty papers contain some data about *O. arvensis* histo-anatomy (Dénes et al. 2017, Langer et al. 1995, Sichinava et al. 2014). The aim of our paper was the histo-anatomical analysis of the aboveground stem and leaf of *O. arvensis* species and the preliminary chromatographic investigation of polyphenols content from the flowering aerial part (*Ononidis arvensis herba*).

MATERIAL AND METHODS

Histo-anatomical analysis

Starting from *Ononis arvensis* plants in blossom, the vegetal material was collected in July 2017, from the surroundings of Stoenești commune, Vâlcea County (southwestern Romania).

The aboveground stems and leaves were fixed and stored in 70 % ethanol. The cross-sections and longitudinal-radial sections were obtained using botanical razor.

After prewashing with distilled water, the sections were clarified using 10 % sodium hypochlorite solution (Javel water). Then, the clarifying solution was removed by washing with distilled water.

For the staining of sections, Genevese reagent (a Congo red–chrysoidine mixture) was used. Taking into account the chemical composition of cell membranes, various colors were obtained: pink to red for cellulose and mucilages, pale red for cytoplasm, yellow for suberin and brown for lignin (Andrei & Paraschivoiu 2003).

To a Krüss binocular photon microscope, stained and mounted sections were analyzed on $\times 4$, $\times 10$, $\times 20$, and $\times 40$ objectives.

Nikon Eclipse 55i binocular microscope coupled with a Nikon DS-Fi1 high definition video camera was used to take photos.

Image-Pro Plus ver. 6.0 software package (Media Cybernetics) was applied for image acquisition and processing.

The histo-anatomical analysis of sections was achieved starting from some classical works (Toma & Rugină 1998).

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols was made for the aerial parts of *O. arvensis* species (*Ononidis arvensis herba*), by applying TLC CAMAG (Muttentz, Switzerland) system (Altemini et al. 2015, Bojić et al. 2013, Gîrd et al. 2014, Jug et al. 2018):

- stationary phase: TLC silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany) 10×10 cm precoated glass plates, prewashed with chloroform–methanol (1:1, v/v) and activated by oven-drying (110°C, 30 minutes);

- mobile phase: ethyl acetate–formic acid–methanol–water (15:1:0.1:1, in volumes), 10 mL added in the developing twin-chamber and then oversaturated for 20 minutes;

- sample: 20 % methanolic extract of *Ononidis arvensis herba*;

- standards (Merck): 0.05 % methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin;

- migration distance: 62 mm (sample application line – 8 mm, solvent front – 70 mm);

- sample (8 μ L, 10 μ L) and standards (2 μ L) application: CAMAG Linomat 5 semiautomatic system – spray gas nitrogen, syringe volume 100 μ L, dosage speed 150 nL/s, predosage volume 0.2 μ L, bands length of 8 mm;

- plate drying: 5 minutes, at 25°C (cold air dryer);

- photographing the chromatographic plate: UV light (λ 254 nm);

- detection: CAMAG TLC Scanner 3 photodensitometer, for densitogram and *in situ* UV light (λ 280 nm) spectra, without derivatization, deuterium–wolfram lamp, scanning speed 40 mm/s, data resolution 200 μ m/step, measurement mode absorption;

- winCATS ver. 1.4.3 software package.

RESULTS AND DISCUSSIONS

Histo-anatomical analysis

Aboveground stem

In the lower third, the cross-section through the aboveground stem has a round contour and secondary structure due to the libero-ligneous cambium. On the cross-section, from the outside to the inside of the aboveground stem, the following histological sequence has been highlighted: The epidermis is made up of large cells, having thickened outer wall covered by a thick cuticle with toothed relief. The epidermal cells are slightly tangential elongated, with thin radial walls and thick tangential external and internal walls. Stomata, cudgel-shaped glandular trichomes and multicellular, long tector trichomes are found in patches. The cortex is organized into 2–3 layers of chlorenchyma to the outside and of a meatus-type cortical parenchyma to the inside. The conducting tissues are organized into numerous libero-ligneous conducting fascicles of collateral-open type. The phloem tissue is made up of sieve tubes, phloem parenchyma and of annex cells. There is a sclerenchyma calotte on the phloem pole of each conducting fascicle. At the level of phloem tissue, the medullary rays are multicellular, multiseriate, cellulosic; near the sclerenchyma calottes, the medullary rays continue with a funnel-like dilatation parenchyma consisting of large, flattened cells. The circular-sinuuous libero-ligneous cambium is found between the xylem and the phloem tissues. The xylem tissue is made up of metaxylem vessels of different sizes, arranged on radial strings in the libriform tissue. The metaxylem shows reticulate and helical thickenings, exhibited on the longitudinal-radial sections. Few protoxylem vessels accompanied by xylem parenchyma are found in the inner area of the xylem tissue. The medullary rays are multicellular, multiseriate (rarely uniseriated) and also lignified. The well-developed medullary parenchyma is of meatus type. There is a medullary gap in the central area (Figures 1–6).



Figure 1. Cross-section through *O. arvensis* aboveground stem: overview (Congo red–chrysoidine staining, $\times 40$).

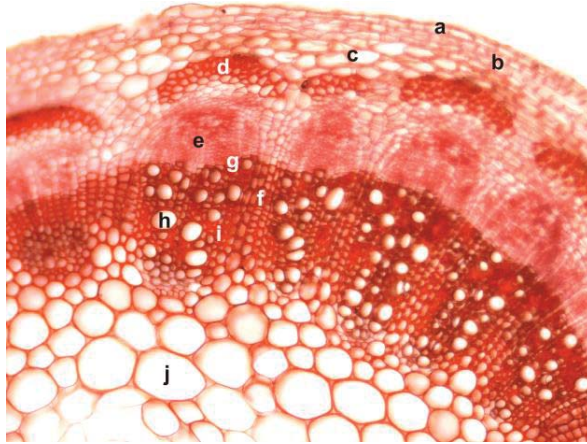


Figure 2. Cross-section through *O. arvensis* aboveground stem: (a) epidermis; (b) chlorenchyma; (c) cortical parenchyma; (d) sclerenchyma calotte; (e) phloem tissue; (f) medullary ray; (g) libero-ligneous cambium; (h) metaxylem; (i) libriform tissue; (j) medullary parenchyma (Congo red–chrysoidine staining, $\times 100$).

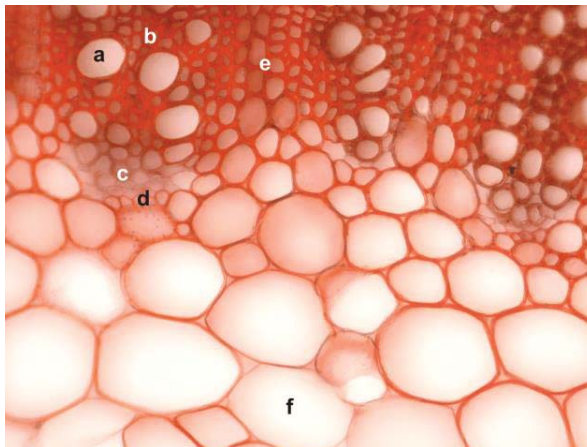


Figure 3. Cross-section through *O. arvensis* aboveground stem: (a) metaxylem; (b) libriform tissue; (c) protoxylem; (d) xylem parenchyma; (e) lignified medullary ray; (f) medullary parenchyma (Congo red–chrysoidine staining, $\times 200$).

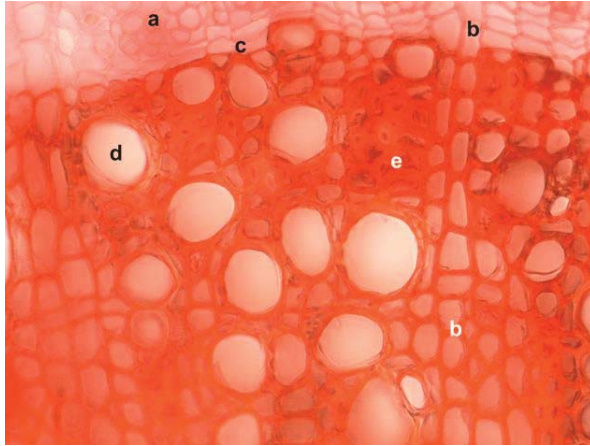


Figure 4. Cross-section through *O. arvensis* aboveground stem: (a) phloem tissue; (b) medullary ray; (c) libero-ligneous cambium; (d) metaxylem; (e) libriform tissue (Congo red-chrysoidine staining, $\times 400$).

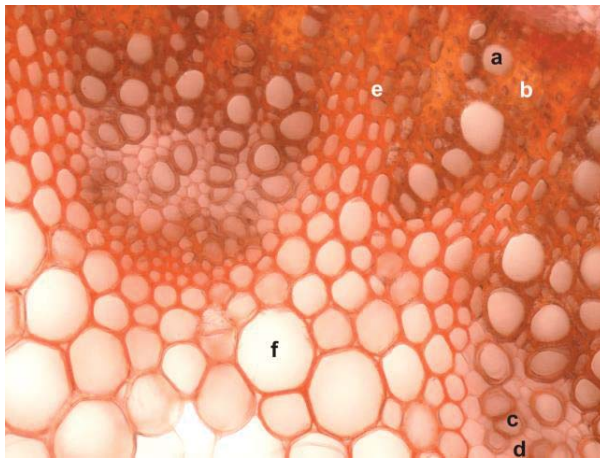


Figure 5. Cross-section through *O. arvensis* aboveground stem: (a) metaxylem; (b) libriform tissue; (c) protoxylem; (d) xylem parenchyma; (e) medullary ray; (f) medullary parenchyma (Congo red-chrysoidine staining, $\times 200$).

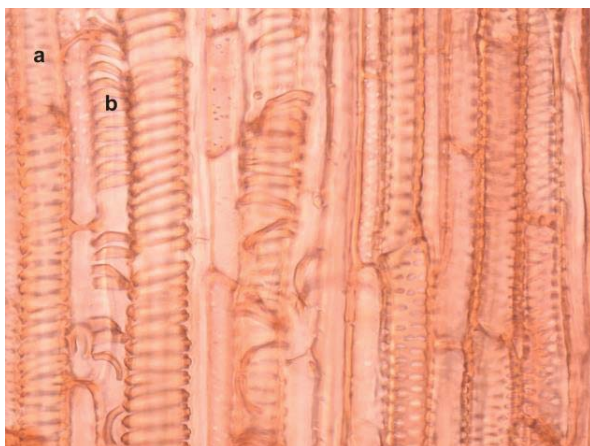


Figure 6. Longitudinal-radial section through *O. arvensis* aboveground stem: (a) reticulate xylem vessel; (b) helical xylem vessel (Congo red–chrysoidine staining, $\times 400$).

Leaf

From the outside towards the inside of leaf's limb, the following histological sequence is evidenced in cross-section: The upper epidermis is made up of a single layer of large, flattened cells, with thickened tangential external and internal walls and thin radial walls. The external walls are bulged and covered by a thick cuticle with toothed relief. From place to place are found stomata and multicellular, long, cudgel-shaped glandular trichomes. The mesophyll consists of two layers of palisade parenchyma made up of large, elongated and chloroplast-rich cells, but also of 3–4 layers of lacunose parenchyma, composed of small cells with disordered layout, leaving aeriferous spaces between them. Into the mesophyll, there are many small libero-ligneous conducting fascicles, surrounded by assimilatory sheaths. The species presents C₄ photosynthesis. The mesophyll has bifacial type and dorsiventral structure. The lower epidermis is made up of a single layer of small, tangential elongated cells, with thin radial walls and thickened tangential external and internal walls. The cuticle has a toothed relief. Stomata and multicellular, long, cudgel-shaped glandular trichomes were found at this level. In cross-section, on the abaxial face, the median rib is protruding and is rounded like a trough. Under the upper epidermis, there are two layers of palisade parenchyma. In the central area, there is only one libero-ligneous conducting fascicle that is placed in the leaf's parenchyma. Into the libero-ligneous fascicle, the xylem vessels have a seriate layout and the medullary rays are multicellular, uniseriate, cellulosic. The libero-ligneous fascicle is surrounded by assimilatory sheath and periphloemic protected by a sclerenchyma calotte. The leaf's limb has bifacial, dorsiventral, amphistomatic structure (Figures 7 and 8).



Figure 7. Cross-section through *O. arvensis* leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) libero-ligneous fascicle; (e) assimilatory sheath; (f) lower epidermis; (g) stomate (Congo red–chrysoidine staining, $\times 200$).

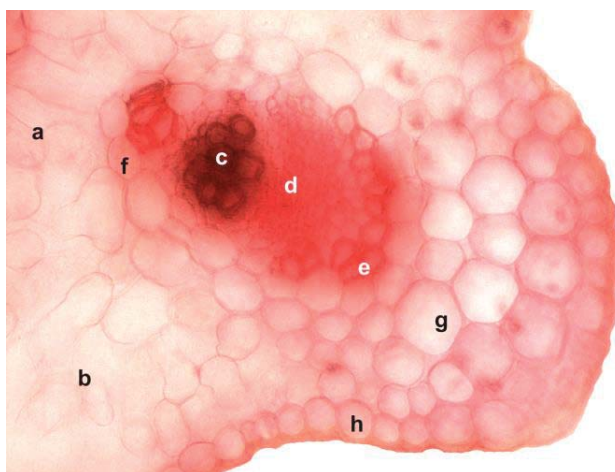


Figure 8. Cross-section through *O. arvensis* leaf's limb: (a) palisade parenchyma; (b) lacunose parenchyma; (c) metaxylem; (d) phloem tissue; (e) sclerenchyma calotte; (f) assimilatory sheath; (g) leaf's parenchyma; (h) lower epidermis (Congo red–chrysoidine staining, $\times 400$).

TLC analysis

Figures 9–11 exhibited the experimental data concerning preliminary TLC analysis of polyphenols from *Ononidis arvensis herba*. An amount of $130.8 \mu\text{g/mL}$ rutin (R_f 0.13) was determined in the 20 % methanolic extract, corresponding to 64.5 mg/100 g of dried vegetal product.

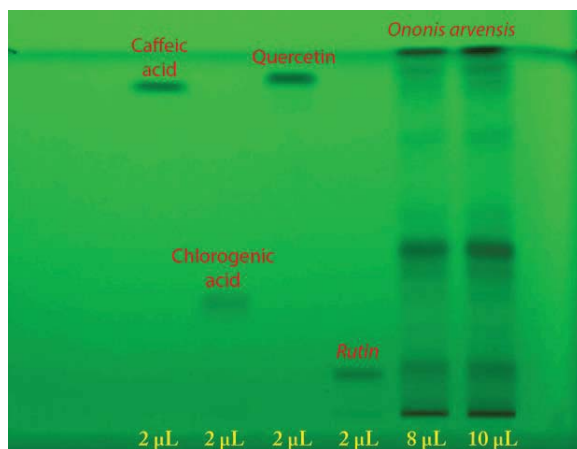


Figure 9. TLC chromatogram of polyphenols from *Ononis arvensis herba* 20 % methanolic extract (UV 254 nm, without derivatization). From left to right: first four bands – standards (2 μ L); last two bands – sample (8 μ L and 10 μ L).

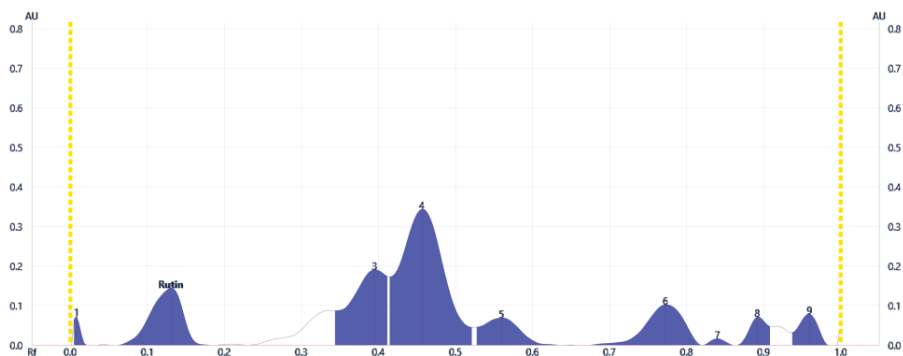


Figure 10. Densitogram of polyphenols (UV 280 nm, without derivatization) separated from *Ononis arvensis herba* 20 % methanolic extract. Rutin was identified at R_f 0.13.

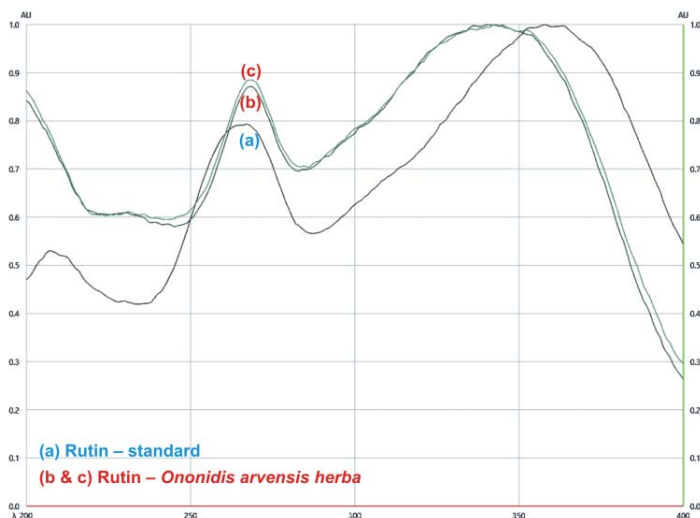


Figure 11. *In situ* UV spectra (UV 280 nm) of rutin standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical researches on the aboveground stem and leaf of *Ononis arvensis* species and the preliminary TLC analyses of the polyphenols from *Ononidis arvensis herba* were accomplished. In the lower third, the aboveground stem has circular contour and secondary structure (libero-ligneous cambium). The leaf's limb has bifacial, dorsiventral, amphistomatic structure. Rutin was identified and quantified (64.5 mg/100 g of dried vegetal product) among the nine specific bands for polyphenolic components from the thin-layer chromatogram.

REFERENCES

- Altemini A., Watson D. G., Kinsel M., Lightfoot D. A., 2015, Simultaneous extraction, optimization, and analysis of flavonoids and polyphenols from peach and pumpkin extracts using a TLC-densitometric method. *Chem. Cent. J.*, 9:39.
- Andrei M., Paraschivoiu Roxana Maria, 2003, *Microtehnica botanică*, Edit. Niculescu, București, 2003, 222 pp.
- Baldemir A., Coşkun M., 2016, Comparative morphological studies on three endemic *Ononis* L. (*Leguminosae*) species growing in Turkey. *Biol. Divers. Conserv.*, 9(1):82–91.
- Bojić M., Simon Haas V., Sarić D., Maleš Z., 2013, Determination of flavonoids, phenolic acids, and xanthenes in mate tea (*Ilex paraguariensis* St.-Hil.). *J. Anal. Methods Chem.*, 2013:658596.
- Ciocârlan V., 2000, *Flora ilustrată a României. Pteridophyta et Spermatophyta*, ediția a 2-a revizuită și adăugită, Edit. Ceres, București, 1138 pp.
- Dénes T., Bartha S. G., Kerényi M., Varga E., Balázs V. L., Csepregi R., Papp N., 2017, Histological and antimicrobial study of *Ononis arvensis* L. *Acta Biol. Hung.*, 68(3):321–333.
- Gîrd C. E., Nencu I., Costea T., Dușu L. E., Popescu M. L., Ciupitu N., 2014, Quantitative analysis of phenolic compounds from *Salvia officinalis* L. leaves. *Farmacia*, 62(4):649–657.

Jug U., Glavnik V., Kranjc E., Vovk I., 2018, High-performance thin-layer chromatography and high-performance thin-layer chromatography–mass spectrometry methods for the analysis of phenolic acids. *J. Planar Chromatogr.*, 31(1):13–22.

Langer R., Engler S., Kubelka W., 1995, Comparative root anatomy of some perennial taxa of the genus *Ononis* L. *Pharmazie*, 50:627–629.

Mezrag A., Malafronte N., Bouheroum M., Travaglino C., Russo D., Milella L., Severino L., De Tommasi N., Braca A., Dal Piaz F., 2017, Phytochemical and antioxidant activity studies on *Ononis angustissima* L. aerial parts: isolation of two new flavonoids. *Nat. Prod. Res.*, 31(5):507–514.

Sichinava M. B., Mchelidze K. Z., Churadze M. V., Alaniia M. D., Aneli D. N., 2014, Chemical composition and microstructural peculiarities of overground and underground vegetative organs of field restharrow (*Ononis arvensis* L.). *Georgian Med. News*, 231:88–94.

Toma C., Rugină Rodica, 1998, Anatomia plantelor medicinale. Atlas, Edit. Academiei Române, București, 320 pp.

Tumova L., Tuma J., Dolezal M., 2011, Pyrazinecarboxamides as potential elicitors of flavonolignan and flavonoid production in *Silybum marianum* and *Ononis arvensis* cultures *in vitro*. *Molecules*, 16(11):9142–9152.

SUCCESSFUL AGRICULTURAL WATER MANAGEMENT FOR WATER POOR ENVIRONMENTS LIKE KONYA BASIN, TURKEY

Bilal Acar, Ahmet Melih Yılmaz, Mehmet Akif Kalender

Department of Farm Buildings and Irrigation, Faculty of Agriculture, University of Selçuk, Konya-Turkey. *Corresponding E-mail:* biacar@selcuk.edu.tr

Keywords: *Agriculture, Irrigation, water scarcity, crop pattern, environment.*

ABSTRACT

How to manage limited water supplies efficiently in water shortage environments is an urgent question to be answered. In examine our previous studies, following practical strategies are recommended for those regions: crop pattern should be designated in regard to the current water resources e.g. increasing cultivated lands of low water consuming crops; deficit irrigation is also good choice for water productivity even for energy efficiency. About 20-25 % deficit irrigation by drip system had no significant yield reduction by comparison to the full irrigation treatment for some field crops such as sugar beet, sunflower and corn plant; developing new crop cultivars using less water; and possibilities of bringing water from the neighbor river basins. In this paper, some applicable agricultural water management strategies were examined with detail for the water scant regions such as Konya, Turkey.

INTRODUCTION

Sustainable use of water resources is one of the most important key issues in agro-production in water shortage environments. Achievement of maximum yield from unit water is actual interest for farmers especially in those regions.

It is great job for water planners or managers to improve the water use efficiency in agriculture for coming 50 years if agro-production and climate trend continue with this present status. In those regions, water scarcity is very serious problem and there is a great challenge between the countries for reaching the fresh water resources. In general, withdrawals of water are about 70 %, and 20 % and 10 % in the sectors of irrigation, industry and municipality, respectively (Molden 2007).

As mentioned above agriculture is the single maximum user of fresh water resources as about 70 % worldwide even 75 % or more somewhere else (Anonymous 2003, Cihan § Acar 2016) and for minimizing the water use in such sector, one of the most important practical solution is correct irrigation program especially regions having the poor water supplies (Yavuz et al. 2015a, 2016a).

Morison et al. (2008) also reported that about 80-90 % of the total fresh water has used in agricultural activities and most of them in irrigation event. The water use therefore is unsustainable in many parts of the world and water resources, highly affected by climate change, are under great pressure. The notable efforts, therefore, have to be performed for maximum crop yield per unit water.

Well management of irrigation water supplies is perhaps one the most important issues for maximizing the yield and quality in agriculture. In order to increase agro-production, beside cultivation of farmlands with modern agricultural equipment's,

application of plant nutrients, and so on, maybe the most important is irrigation among the all those inputs. By irrigation, not only crop water requirement is met, but it also facilitate the crops for other activities such as easy uptaking the plant nutrient elements. In result, efficient management of irrigation water helps the water savings or minimizing the energy cost of irrigation. Energy cost is very high within agro-production inputs in Turkey (Bah § Acar 2017).

EFFICIENT WATER USE STRATEGIES IN AGRICULTURE FOR KONYA, TURKEY

Konya plain, one of the main agro-production centers of Turkey, is known as semi-arid climate having huge arable lands but limited fresh water supplies. In accordance of long-term meteorological data, annual precipitation is around 323 mm in many parts and only 90-100 mm of that has recorded in crop vegetation period. Irrigation is compulsory for better and qualified yield due to the low amount with none uniform rainfall distribution through the year. The available surface and groundwater potentials of Konya plain are about 2.94 billion m³, 66 %, and 1.51 billion m³, 34 %, respectively with a total of 4.45 billion m³ (Anonymous 2015).

In general, groundwater resources have been used during the intense irrigation season between early June and September. In accordance of estimation, more than safely available water amount has used in irrigation and this is an indicator of none sustainable usage of groundwater resources (Anonymous 2015). In that case there is a gradual depletion in groundwater level year by year; about 14-15 m from 1974 to 2007 and 10-11 m from 1997 to 2007 (WWF 2008). Perhaps the main cause of such water level reduction is increasing the cultivated lands with favor of high water consuming crops such as corn without care of current water resources. Yavuz et al. (2016b) stated that ground water resources have to be used in irrigation of both field crops and vegetable plants since surface water resources are limited in most irrigation seasons. Therefore, many deep wells are available in Konya basin of Turkey. One of the most important production costs is energy and is used for pumping water from the groundwater reservoir to the irrigation lands. They suggested using deficit irrigation to reduce applied water to the crops. This results also reduction in electricity cost of irrigation. As known that energy productivity is necessarily prerequisites in all sectors including agriculture since energy cost is greater in Turkey than many of the other countries.

Total land potential of our basin, Konya Closed Basin, having or not projects is about 542 000 ha. In that amount, 370 000 ha of that is situated at Konya plain. All high water using summer crops and low water usage winter cereals have the shares of 60 % and 40 % land use, respectively. In examine the whole crop pattern together, net average seasonal water usage of crops is around 500 mm. Sprinkler is common irrigation technique and irrigation efficiency is satisfactory about 73 % in basin (Topak et al. 2008). Since, irrigation water directly has been taken from the deep wells within the irrigation areas so almost none water losses have occurred in water distribution networks. The other important issue that famers have very great experiences about all irrigation methods including sprinkler irrigation system management and that has resulted high water application efficiency. In accordance of our previous estimation, our safely available water is about 2.31 billion m³ meeting irrigation of almost 342 000 ha farmlands. It clear shows that about 200 000 ha land is abundant and has been also irrigated by using the over water withdrawals from the groundwater supplies. In that case, every year about 1.40 billion m³ water has used more from groundwater resources. On the other word, in accordance of our projections, those resources are not sustainable with this present usage. Yavuz et al. (2015b) also stressed that fresh water use in agriculture is

as about 75 %, more than the world average, so water saving strategies should be started firstly in irrigation.

Topak & Acar (2010) suggested that application of new irrigation techniques such as sprinkler and drip system by well management and finding the new irrigation water resources or making the some water development works are needed urgently for minimizing the stress on over water extraction from groundwater reservoir.

Water requirement of crop should be met on time to obtain maximum benefit. Correct irrigation program is needed to overcome that. Irrigation program or schedule is defined as when and how much water will be applied in one irrigation process.

Efficient management of limited water resources especially in water shortage regions is an urgent question to be answered. In that standing point, we need to use correct agronomy resulting using crops less water in both irrigated and none irrigated / rainfed farming system. Development of new crop cultivars require less amount of water is vital important for sustainable water resources (Parry et al. 2005).

The one of the most important practical alternatives in arid or semi-arid lands is application of deficit irrigation. It can be defined as the water application for crops lower than the full crop water requirements. By this way, some of the irrigation water is saved and such amount is diverted for the irrigation of extra-cropped lands (Fereris & Soriano 2007). The main target of deficit irrigation strategy is to improve the water productivity in agriculture or increase the income of farmers. More lands could be irrigated by same amount of water and crop production as well as the outputs will be improved by this way.

Our previous studies in region clearly showed that correct management of deficit irrigation by drip system has resulted significant water savings with little yield reduction. By this way, comparison to the full irrigation, up to 25 % deficit irrigation has not resulted remarkable yield reduction for some field crops such as sugar beet, corn, sunflower and dry bean (Acar et al. 2014).

On the other hand, a study by Fereres et al. (2003) showed that in regard to the water saving, deficit irrigation technique has resulted more successful in trees or vines by comparison to the field crops. The reason behind that a given deficiency in the applied water has caused higher decrease in transpiration than field crops so that better water saving is available. By the standpoint of water use efficiency, deficit irrigation is very beneficial technique firstly for trees.

The other applicable solution is government support or subsidizes the farmers who are processing the rainfed agriculture. Development or selections of new crop cultivars having very resistance to the dry conditions are very important for efficient water use in agriculture. Increasing the cropped lands with rainfed system has resulted not over water extractions from the groundwater supplies. Cereals, chickpea, lentil even squash are very alternative crops for preventing over water use from water resources.

CONCLUSIONS

Agriculture is the maximum fresh water user sector in Konya province of Turkey. One of the most important factors affecting the sustainable use of water resources in region is increase-irrigated land of high water consuming crops such as corn without control. Thus, current crop pattern is the main reason of the over water extraction from the groundwater resources. The area having the low water consuming crops such as cereals should be improved for sustainable water resources. In addition, modern irrigation technologies should be used more under well management. The other alternative is deficit irrigation by drip system and by this way up to 25 % water saving can be accomplished without significant yield reduction. For sustainable irrigation, there should be good cooperation between water management organizations and farmers. A proper training for water managers and farmers is very important role to play for efficient

water use in agriculture especially for water scant environments. We never forget that water is the backbone of the agriculture and sustainable use of water supplies is necessarily prerequisites for regions having water poor environments.

REFERENCES

Acar B., Topak R., Yavuz D., Kalender M.A., 2014, Is drip irrigation technique sustainable solution in agriculture for semi-arid regions? A case study of Middle Anatolian Region, Turkey. *International Journal of Agriculture and Economic Development*, 2 (2): 1-8.

Anonymous., 2003, Global Water Resources and Water for Agricultural Use in Japan. The Planning of Subcommittee, The Committee of Agricultural and Rural Development, The Council of Food, Agriculture and Rural Areas Policy (in MAFF), 20 ps.

Anonymous., 2015, DSİ. IV. Bölge Müdürlüğü Verileri. <http://www2.dsi.gov.tr/bolge/dsi4/>. Access date: 2 February (In Turkish).

Bah A., Acar B., 2017, Analysis of water use in irrigation for Konya-Çumra province of Turkey. *World Journal of Innovative Research*, 2 (1): 14-17.

Cihan I., Acar B., 2016, Performance of ova water user association in Konya-Turkey. *World Journal of Innovative Research*, 1 (2): 25-28.

Fereres E., Goldhamer D.A., Parsons L.R., 2003, Irrigation water management of horticultural crops. *Horticultural reviews*. Compiled for the American Society of Horticultural Science's 100 th Anniversary *HortScience*, 38: 1036-1042.

Fereres E., Soriano M.A., 2007, Deficit irrigation for reducing agricultural water use. *Journal of Experimental Botany*, 58 (2): 147-159.

Morison J.I.L., Baker N.R., Mullineaux P.M., Davies W.J., 2008, Improving water use in crop production. *Philosophical Transactions of the Royal Society B*, 363: 639-658.

Molden D., 2007, Summary, water for food-water for life. A Comprehensive assessment of water management in Agriculture, 40 ps.

Parry M.A., Flexa J., Medrano H., 2005, Prospects for crop production under drought: research priorities and future directions. *Am. Appl. Biol.*, 147: 211-226.

WWF., 2008, Türkiye' deki Ramsar alanları değerlendirme raporu. Doğal Hayatı Koruma Vakfı, 129 s., İstanbul (In Turkish).

Topak R., Suheri S., Acar B., 2008, Climate-Agricultural Drought-Irrigation with Environmental Concern in Konya Closed Basin, 11-12 September 2008, proceedings, Konya: 67-76 (In Turkish).

Topak R., Acar B., 2010, Sustainable irrigation and importance of technological irrigation systems for Konya Basin. *TABAD, Tarım Bilimleri Araştırma Dergisi*, 3 (2): 65-70.

Yavuz D., Yavuz N., Seymen M., Turkmen M., 2015a, Evapotranspiration, crop coefficient and seed yield of drip irrigated pumpkin under semi-arid conditions. *Scientia Horticulturae*, 197: 33-40.

Yavuz D., Seymen M., Yavuz N., Turkmen M., 2015b, Effects of irrigation interval and quantity on the yield and quality of confectionary pumpkin grown under field conditions. *Agricultural Water Management*, 159: 290-298.

Yavuz D., Yavuz N., Suheri S., 2016a, Design and management of a drip irrigation system for an optimum potato yield. *Journal of Agricultural Science and Technology*, 18: 817-830.

Yavuz D., Yavuz N., Suheri S., 2016b, Energy and water use for drip-irrigated potato in the Middle Anatolian region of Turkey. *Environmental Progress & Sustainable Energy*, 35: 212-220.

EVALUATION OF THE PHYTOREMEDIATION PROPERTIES OF THE INVASIVE SPECIES *SOLIDAGO* GENUS

Bobuľská Lenka^{1*}, Čekanová Katarína¹, Demková Lenka¹, Oboňa Jozef¹, Sarvaš Ján²

¹University of Prešov, 17. Novembra 1, Prešov, Slovakia

²University of Economics in Bratislava, Research and Educational Centre of Bio Energy, Kapušany, Slovakia

* Correspondence author. E-mail: lenka.bobulska@unipo.sk

Keywords: *bioremediation, heavy metals, invasive plants, Goldenrod*

ABSTRACT

*Regeneration of heavy metal-polluted and heavy metal-degraded sites has remained a global challenge despite the existence of numerous conventional physico-chemical techniques that can be applied. Application of the inexpensive "green" and sustainable technique of phytoremediation is unrivalled. Because invasive plants can survive in harsh conditions and they represent big threat for natural biodiversity, the knowledge about their ecology in polluted sites is still important. This study aimed to investigate the potential phytoremediation ability of invasive species of *Solidago* genus in the polluted area in Slovakia. The elements tested were Cd, Cu, Pb and Cr in the soil samples, as well as in the plant material. Samples to obtain heavy metal concentration were determined using by atomic absorption spectroscopy (AAS). Bioconcentration (BCF) and translocation factors (TF) were also calculated. As expected, in the polluted areas that are located close to the source of pollution, the limit values of the heavy metals exceeded several times. Cr, Cu and especially Pb were the elements removed most efficiently by different parts of the plant material (mainly by the plant leaves and root material). Based on the BCF and TF, *Solidago* genus might be considered as potential accumulator of Pb, Cu and Cr.*

INTRODUCTION

Pollution of soil environment by toxic substances is a very serious problem. Although heavy metals are naturally occurring elements that are found throughout the earth's crust, most environmental contamination and human exposure result from anthropogenic activities such as mining and smelting operations, industrial production and use, and domestic and agricultural use of metals and metal-containing compounds (Tchounwou et al. 2014, Angelovičová et al. 2015). Their bioavailability is influenced by physical factors such as temperature, phase association, adsorption and sequestration. It is also affected by chemical factors that influence speciation at thermodynamic equilibrium, complexation kinetics, lipid solubility and octanol/water partition coefficients. Biological factors such as species characteristics, trophic interactions, and biochemical/physiological adaptation, also play an important role (You et al. 2018, Schweizer et al. 2018). Phytoremediation as a remediation technology that has been receiving attention lately as the results from field trials indicate a cost savings compared to conventional treatments. It uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. The genus *Solidago* is an invasive species in Slovakia. It occurs both in habitats only weakly impacted anthropogenic factors as well as in habitats subjected to intensive transformation by humans. In natural and semi-natural population in Slovakia, this genus is represented by three species, two of them

are considered as invasive species: *Solidago canadensis* (L.) (Canadian goldenrod) and *Solidago gigantea* Aiton (Giant goldenrod) (Pavek 2011). These species were brought to Europe from North America in the 18th century (Guzikowa & Maycock 1986) and the expansion of *Solidago* sp. can be attributed to the fact that it produces large numbers of seeds with the greater germination potential of all *Solidago* sp. (Szymura 2012). Nowińska et al. (2012) and Yang et al. (2008) presented that this invader tolerates high concentration of heavy metals in soil which is demonstrated by the presence at sites near emitters of pollution. It also has been emphasized that the plant had great tolerance of chemical properties of the ground, such as the soil pH and the content of minerals and organic matters (Pużyńska et al. 2012, Bielecka et al. 2017).

The main aim of this study was to investigate the potential phytoremediation ability of invasive species *Solidago* genus in the polluted area in Slovakia.

MATERIAL AND METHODS

Study area

The research was conducted in two environmentally loaded areas of Košice city. The localities are known for its industrial activities, predominantly focused on steel production. The main problems in this region is high accumulation of lead and cadmium in soil and water ecosystems. The control site was also selected in the locality with low anthropogenic impact on environment (Figure 1).

Plant and soil assays

We collected 5 individuals of *Solidago* sp. from 5 places within the locality (total set of 75 plant materials were collected). Soil samples were also collected (5 on each locality, the total of 15 soil samples). Plant material and soil samples were transported into the laboratory and dried at room temperature. After drying, plant material was divided into individual parts: roots, plant stem, leaves and flowers. Soil samples were homogenized and sieved through 2 mm sieve opening and stored in plastic bags until analyzed. Total content of heavy metals (Cd, Pb, Cr and Cu) were determined in accredited laboratory by AAS (Atomic Absorption Spectrometry) method for biological and soil material. Additionally, soil pH/KCl, soil moisture and organic carbon were also determined in soil samples.



Figure 1. Sampling points 1 and 2 indicate polluted site in Košice city, sampling point 3 shows control site

Bioconcentration factor (BCF) and translocation factor (TF)

The bioconcentration factor is defined as a ratio of the monitored contaminant in the biological material in relation to soil/substrate dry material in mg kg⁻¹ (Dryżalowska & Falandysz 2014). Green plants are able to uptake and accumulate heavy metals and metalloids from soil/substrate, which has a significant impact on the health risk resulting from their consumption.

The BCF was calculated as follows:

$$BCF = \frac{\text{Total content of heavy metal in dried biological samples (mg/kg DW)}}{\text{Total content of heavy metal in dried soil samples (mg/kg DW)}}$$

The translocation factor helps to recognize in which part of the plant material are heavy metals predominantly accumulated. TF is defined as a ratio of the heavy metals concentration in overhead part of the plant in relation to the concentration of heavy metals in plant roots (Singh et al. 2010).

The TF was calculated as follows:

$$TF = \frac{\text{Total content of heavy metal in the overhead part of the plant (mg/kg)}}{\text{Total content of heavy metal in plant roots (mg/kg)}}$$

RESULTS AND DISCUSSIONS

For the evaluation of phytoremediation ability of *Solidago* genus, determination of soil pH, humidity and other factors are also crucial. Therefore, the value of the soil reaction is one of the very important soil property. The pH value of the soil affects the solubility of the heavy metals in the soil, hence its use by living organisms. Increased acidity of the soil reduces/increases the solubility of many substances (Angelovičová et al. 2015). Another primary factors are organic carbon and soil moisture and its availability. Table 2 shows the average values of selected physico-chemical properties in soil environment.

Table 1

Average values of selected physical and chemical soil properties

Sampling points	Soil moisture (%)	pH/KCl	Organic carbon (%)
1	16.77	6.9	2.5
2	15.44	6.7	2.2
3 (control site)	19.49	6.3	3.3

In all sampling points, soil reaction can be classified as slightly acidic and neutral environment. According to organic carbon content, the soil is moderate humus (Fejér & Bobuřská 2015) and the biggest portion of organic matter represents control site. Table 2 shows the average values of selected heavy metals in the individual parts of plant material, as well as in the soil system.

Table 2

Average values of heavy metals in the individual parts of plant material and soil system

Sampling points	Parts of plant material	Cd (mg/kg)	Pb (mg/kg)	Cr (mg/kg)	Cu (mg/kg)
1	Roots	0.56	22.09	18.82	31.18
	Plant stem	0.33	9.26	12.48	22.87
	Leaves	0.30	701.50	26.20	46.92
	Flowers	0.18	135.24	34.00	199.56
	Soil	5.71	278.82	387.34	516.75
2	Roots	0.63	34.95	440.11	48.92
	Plant stem	0.34	17.56	21.38	29.27
	Leaves	0.24	155.00	17.91	22.30
	Flowers	0.21	45.49	29.39	112.45
	Soil	3.33	167.74	161.83	262.34
3 (control site)	Roots	0.51	6.91	38.43	75.10
	Plant stem	0.33	1.10	43.75	67.51
	Leaves	0.25	32.71	14.48	17.84
	Flowers	0.23	5.80	25.71	90.63
	Soil	0.97	38.48	52.52	59.47
Limit values (for soils)		1.00	70.00	60.00	50.00

Because of heavy metal accumulation by roots, the concentration of metals is different in each part of the plant. The concentration of Cd in all parts of *Solidago* sp. stagnates despite the varying concentrations in the soil at each site. The greatest accumulation of Cd is in the root and the lowest in bloom. The highest Pb accumulation was in leaves, where sampling site which was most polluted exceeded 2.5 times the Pb content in the soil. At other sites, the metal content in the leaves almost bounded the Pb content in the soil. Additionally, the high concentration of Pb was also measured in the flowers. It seems that with the increasing concentration of Pb in the soil, the Pb content in the flowers is also directly increased and the accumulated metal in the flowers is able to contain about a sixth of the metal compared to the soil. In the above-ground areas, the accumulated Cr content at each site was low compared to the amount of metal in the soil. Content of Cu was generally highest in flowers. At the control site, the Cu content in the root, stalk and flowers exceeded the soil content of determined metal.

Table 3 represents the sum of the bioconcentration factor (BCF) and translocation factor (TF) in the above-ground part of the plant material.

Table 3

Sum of the BCF and TF in the above ground part of the plant material

Sampling points	Sum of the factors	Cd (mg/kg)	Pb (mg/kg)	Cr (mg/kg)	Cu (mg/kg)
1	BCF	0.06	0.27	0.18	0.43
	TF (Plant stem)	0.59	0.42	0.66	0.73
	TF (Leaves)	0.54	1.76	1.39	1.50
	TF (Flowers)	0.32	6.12	1.81	6.40
2	BCF	0.14	3.03	0.19	0.52
	TF (Plant stem)	0.65	0.16	1.14	0.90

	TF (Leaves)	0.49	4.73	0.38	0.24
	TF (Flowers)	0.45	0.84	0.67	1.20
3 (control site)	BCF	0.85	1.03	1.60	2.96
	TF (Plant stem)	0.65	0.16	1.14	0.90
	TF (Leaves)	0.49	4.73	0.38	0.24
	TF (Flowers)	0.45	0.84	0.67	1.20

More precise determination of the accumulation of heavy metals provides BCF. The Cd value is in all locations $BCF < 1$, representing a minimum accumulation of this metal. According to BCF in details, Pb is preferentially accumulated in above-ground parts of plant material, mainly in leaves. Cr is preferably accumulated by root system. TF values that represents the ratio of the heavy metal content in the above-ground portions and the root content, the highest values shows Pb. According to Baker (1981), *Solidago* sp. can be considered to a good accumulator of Pb and Cu.

According to one-way ANOVA (Table 4), Pb and Cr showed the significantly highest correlation among the localities. The highest accumulation ability of Pb was confirmed in leaves, for Cr it is in roots and Cu in flowers.

Table 4

One-way ANOVA for the metals in the plant material among the localities and parts in plants

Heavy metals	factor	Df	F value/p value
Cd	Locality	2	0.102
Pb		2	6.988**
Cr		2	5.544**
Cu		2	0.615
Cd	Plant parts	3	204.000***
Pb		3	10.140***
Cr		3	7.328***
Cu		3	15.450***

Invasive plant species are often characterized as very resistant to changes of pH, climate and other environmental conditions (Bielecka et al. 2017). Work by Yang et al. (2005) has shown that many species of *Solidago* have developed various mechanisms for the accumulation of metals (Cu, As, Zn, etc.) and resistance to the metal stress. The largest plant intake among the analyzed metals reached Pb, which was also proven in studies of Yang et al. (2007) where *Solidago canadensis* was studied and showed the interaction with this metal in highly contaminates site. The amount of Pb from each site did not exceed $TF > 1$, which confirms that Pb is predominantly accumulates in the above part of plant material compared to the roots system. Cr, unlike other metals, is found in various plant parts, but with respect to BCF, this metal shows lower portion in the samples compared to Pb and Cu. The plant organ of Cd accumulation is preferably the root system according to TF. Metals from the highest content to the lowest have the following sequence: $Pb > Cu > Cr > Cd$. The results of the statistical comparison between the values of metals in the selected sites can confirm that *Solidago* sp. is able to accumulate some heavy metals. Hinman (2005) created a list of plants that were studied for phytoremediation, including *Solidago canadensis*. Invasive plants have a strong prerequisite to become hyperaccumulators, which was also shown in the work of Dissanayake et al. (2002) who studied the potential accumulation effect in contaminated soil for two invasive plants: *Lantana camara* L and *Wedelia trilobata* L. with a positive result.

CONCLUSIONS

In the industrial areas, pollution of soil environment became very serious problem. Polluted soils are no longer suitable for agricultural production because they lose common biochemical properties which may cause the reduction of soil fertility and decline soil quality and health. Phytoextraction is a method in which the content of pollutants is accumulated in plants, preferably in above ground parts. Accumulated metal can be removed without big problems by harvesting plants without any major interference with the environment. Such environmentally friendly methods can be the most sensible way of removing pollutants compared to the technologies that cause secondary air or groundwater pollution and are usually extremely expensive. Goldenrod genus (*Solidago* sp.), a very widespread plant in Slovakia, for its invasive nature and relatively large biomass was a promising candidate for its use of a hyperaccumulator of heavy metals in soil ecosystem. The invasive *Solidago* sp. is considered as Pb and Cu accumulator and the possible accumulator of Cr. For better and more accurate determination, we recommend the analysis of a particular species. Considering, that the plant species are invasive, they cannot be directly used and thus directly control the rate and capacity of the accumulation, as well as the application and regulation of the metal in the plants.

ACKNOWLEDGEMENT

The work was supported by the Agency of Ministry of Education, Science and Sport of the Slovak Republic, the grant VEGA n. 1/0326/18 and VEGA n. 2/0013/16.

REFERENCES

- Angelovičová L., Bobuřská L., Fazekašová D., 2015. Toxicity of heavy metals to soil biological and chemical properties in conditions of environmentally polluted area Middle Spiš (Slovakia). *Carpathian Journal of Earth and Environmental Sciences*, 10(1): 193-201.
- Baker A.J.M., 1981. Accumulators and excluders – strategies in the response of plant to plant heavy metals. *Journal of Plant Nutrition*, 3(1-4): 643-654.
- Bielecka A., Królak E., Biardzka E., 2017. Habitat conditions of Canadian goldenrod in a selected region of eastern Poland. *Journal of Ecological Engineering*, 18(4): 76-81.
- Dissanayake U.S., Tennakoon K.U., Priyantha N., 2002. Potential of two invasive plant species, *Lantana camara* L and *Wedelia trilobata* L., for selective heavy metal uptake. *Ceylon Journal of Science (Biological Science)*, 29: 1-11.
- Dryżalowska A., Falandysz J., 2014. Bioconcentration of mercury by mushroom *Xerocomus chrysenteron* from the spatially distinct location: levels, possible intake and safety. *Ecotoxicology and Environmental Safety*, 107: 97-102.
- Fejér J., Bobuřská L., 2015. *Soil Science (in Slovak)*. Prešov: FHPV, Prešovská Univerzita v Prešove, 121 p.
- Guzikowa M., Maycock P.F., 1986. The invasion and expansion of three North American species of goldenrod (*Solidago canadensis* L. sensu lato, *S. gigantea* Ait. and *S. graminifolia* (L.) Salisb.) in Poland. *Acta Societatis Botanicorum Poloniae*, 55:367-384.
- Hinman C., 2005. Sampling of plant species studied for phytoremediation. Low impact development. Technical guides manual for puget sound. Washington: Washington State University, 256 p.
- Nowińska K., Kokowska-Pawlowska M., Patrzalek A., 2012. Metals in *Calamagrostis epigejos* and *Solidago* sp. from reclaimed post-industrial wetlands. *Ecologia terenów Wiejskich*, Nr 3/III, Polska Akademia Nauk, Oddział w Krakowie, pp. 91-100.

Pavek P.L.S., 2011. Plant guide for Canada goldenrod (*Solidago canadensis*). USDA Natural Resources Conservation Service: Pullmann, WA.

Pużyńska K., Stokłosa A., Stupnicka-Rozdynkiewicz E., 2012. The impact of ecological conditions on *Solidago* sp. occurrence. *Zeszyty Naukowe Uniwersytetu Przyrodniczego we Wrocławiu, Rolnictwo C*, 584: 89-98.

Schweizer S.A., Seitz B., van der Heijden M.G.A., Schulin R., Tandy S., 2018. Impact of organic and conventional farming systems on wheat grain uptake and soil bioavailability of zinc and cadmium. *Science of the Total Environment*, 639: 608-616.

Singh R., Singh D.P., Kumar N., Bhargava S.K., Barman S.C., 2010. Accumulation and translocation of heavy metals in soil and plants from fly ash contaminated area. *Journal of Environmental Biology*, 31(4): 421-430.

Szymura M., 2012. Evaluation of ability for generative and vegetative reproduction of goldenrods occurred in Poland. *Zeszyty Naukowe Uniwersytetu Przyrodniczego we Wrocławiu, Rolnictwo CI*, 585: 103-112.

Tchounwou P.B., Yedjou C.G., Patlolla A.K., Sutton D.J., 2014. Heavy metals toxicity and the environment. Lush A. (Ed.). *Molecular, Clinical and Environmental Toxicology*, Volume 3: Environmental Toxicology. Springer: Berlin, pp. 133 – 164.

Yang R.Y., Tang J.J., Yang Y.S., Chen X., 2007. Invasive and non-invasive plant differ in response to soil heavy metal lead contamination. *Botanical Studies*, 48(4): 453-458.

Yang R.Y., Yu G.D., Tang J.J., Chen X., 2008. Effects of metal lead on growth and mycorrhizae of an invasive plant species (*Solidago canadensis* L.). *Journal of Environmental Science*, 20: 739-744.

Yang X.E., Jin X.F., Feng Y., Islam E., 2005. Molecular mechanisms and genetic basis of heavy metals tolerance/accumulation in plants. *Journal of Integrative Plant Biology*, 47(9): 1025-1035.

You T., Liu D., Chen J., Yang Z., Dou R., Gao X., Wang L., 2018. Effects of metal oxide nanoparticles on soil enzyme activities and bacterial communities in two different soil types. *Journal of Soils and Sediments*, 18(1): 211-221.

**RESEARCH ON THE PHYSIOLOGY OF SOME WOODY SPECIES
IN THE COMANESTI HILLS FROM MEHEDINTI COUNTY, ROMANIA**

Buse-Dragomir Luminita¹, Nicolae Ion²

1. University of Craiova, e-mail: luminita25dragomir@yahoo.com

2. University of Craiova, e-mail: ionnicolaebio@yahoo.com

Key words: forest, photosynthesis, transpiration, respiration, chlorophyll

ABSTRACT

The forest from the hills of Comanesti is characterized by the presence of a large number of wood species. They are in a continuous competition for water and mineral sources, but also for light. The photosynthetic productivity is influenced by the density of trees and shrubs, the amount of light reaching the leaves, the amount of water in the environment.

Transpiration records a noticeable diurnal variation, but also a seasonal variation in all species that were studied.

The content of chlorophyll pigments shows significant variations due to the amount of light and nutrients in the soil, and the compensation point of light varies widely, plants exhibiting ombrophilous or heliophilous particularities depending on it.

INTRODUCTION

Comănești Hills are located in Mehedinți County, west of the Motru River, and belong to the commune Bala, the village of Comănești. These appear as a set of prolonged ridges, separated by wide valleys, generally with a more evolved, relatively lower relief, which carries the traces of a less violent denudation than that from the east of Jiu river (Sîrbu Anca, 2007).

The altitude varies between 150-402 m, the coordinates being 44 °58 latitude and 22 °54 longitude ([http://google. maps](http://google.maps)).

The area has been proposed for protection and conservation and is now fully owned by the state, being used as a forest found. The site hosts two threatened species at European level and 36 vascular taxa included in the Red List of Romania (Sîrbu Anca et al., 2007). The skeletal substrate represented by erodisols and regosols, which is slightly brittle, causes, during the torrential rains that the respective hills are subjected to, the erosion process.

The research carried out in 2018 in Comănești Forest was aimed at establishing the eco-physiological particularities of some wood species, a particular emphasis being placed on the influence of light and water factors, factors that play a primordial role in forest ecosystems.

More recent information on the flora and vegetation in the research area comes from Costache (2011). After Ciocârlan (2009), the territory under investigation falls within the Central European Region, Danubian-getic province, Getic Plateau District

The physical-geographic, pedo-climatic and phyto-geographical aspects justify the great floristic diversity and complexity of the vegetal groups existing in this part of

the country, as evidenced by Roman (1974), in the southern part of the Mehedinti Plateau, as well as in the Upper Basin of Motru, (Maloş 1977, quoted by Costache, 2011) of course with some peculiarities.

The Comăneşti Hills are crossed from east to west by the Pistriţa brook, a tributary of the Motru River. On the northern side, another tributary flows from it, the stream of Călugăriţa.

It was considered important to study the eco-physiology of some ligneous species, because the plants from these forest ecosystems, living in communities, find themselves in special living conditions than those in the isolation state. By coexisting in large numbers on limited surfaces, mutual relationships are established between them, where competition for light, water and mineral salts plays a decisive role.

When the association is heterogeneous, the phenomenon of competition appears, the more endowed individuals monopolize a larger share of water or nutrient reserves, all these factors influence the course of the physiological processes.

The vegetation period is also decisive for the CO₂ balance and for the annual yield of the production. If the vegetation period is long enough, even with a rather modest assimilation intensity, an appreciable gain of biomass is achieved. If CO₂ assimilation is possible only for a relatively short period of time, even if the plants have a high photosynthetic capacity, the yield of production remains very low. The most useful contribution that physiologists can make to forestry is to determine which physiological processes are inhibited by particular stresses and suggest to tree breeders what characteristics will minimize the inhibitory effects of these stresses (Kramer PJ, 1986)

Abiotic and biotic stresses elicit changes in normal physiology of trees. Plant growth regulators (PGR) are involved in the stress response and appear to have two roles: 1) to minimize the impact of the stress on the tree and; 2) to trigger stress resistance mechanisms. In the latter case the PGR-induced changes appear to enhance resistance to subsequent stress. This cross-adaptation to stress is important in trees (<https://link.springer.com/article/>).

Leaf physiological parameters were mainly influenced by the light gradient, whereas crown morphological and whole-tree parameters were mainly influenced by tree size. When tree is small, a greater proportion of whole-tree biomass was allocated to roots. However, physiological differences between the species decreased with decreasing light and most morphological differences tended to disappear with increasing tree size, suggesting that many species differences in shade-tolerance are expressed mainly during the seedling stage (Delagrange et al, 2004).

Evergreen species are thought to have leaf traits supporting lower photosynthesis and transpiration rates, in order to conserve water during dry periods. Evergreen species had similar assimilation rates but lower photosynthetic water-use efficiency (PWUE) than deciduous species, possibly to extend their leaf life spans by protecting their photosynthetic machinery from overheating through evaporative cooling. Species of humid and semi-arid environments did not differ with respect to assimilation rate or PWUE, but semi-arid species did have smaller leaf sizes and greater leaf potassium and phosphorus concentrations. These traits may enable semi-arid species to maximize growth during episodes of favorable moisture availability (Kyle W, 2013).

Wendy S. et al (2012) find that chlorophyll content index was highly correlated with foliar N concentration, which may be useful in detecting nutrient deficiencies, although the relationship was species dependent.

MATERIALS AND METHODS

Research has been done on the species: *Cornus sanguinea*, *Acer tataricum*, *Carpinus betulus*, *Carpinus orientalis*.

On the biological material which was the subject of this research were determined: the intensity of leaf transpiration, the intensity of photosynthesis, the respiration intensity, the light compensation point, the content of assimilating pigments.

Transpiration, photosynthesis and leaf respiration were determined using the LCi portable apparatus, measuring in parallel the photosynthetic radiation (PAR) and the temperature in the assimilation chamber. The advantage of using this apparatus is that it is possible to do a lot of determinations without the detachment and damage of the plant material.

The light compensation point (the amount of light intensity at which photosynthesis is equal to respiration) was calculated by the determination of photosynthesis at different light intensities. The values obtained were compared to the intensity of respiration in the dark.

The chlorophyll pigment content of the leaves was determined with the Minolta portable chlorophyll-meter, the data being expressed in SPAD units.

The intensity of net assimilation was determined in July 2018 by the method of rings. Leaf halves were detached, dried at 105 °C, and after 48 hours the halves remaining on the plants were detached, the difference after drying and weighing at the analytical balance representing the amount of biomass accumulated in that range of time.

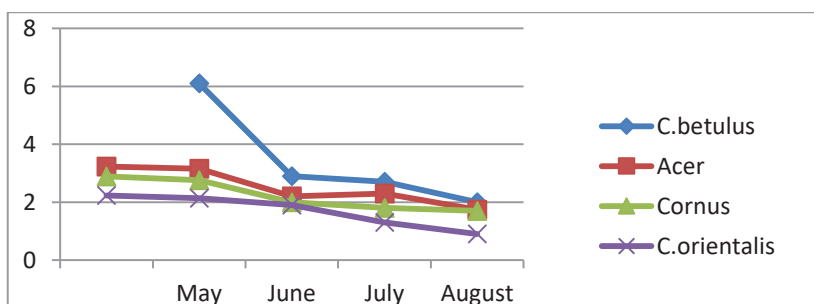
RESULTS AND DISCUSSIONS

Intensity of transpiration

The determinations made in May - August 2018 showed significant variations in the intensity of transpiration. The highest values were recorded in spring for all plants under study, values due to both the higher amount of water in the soil and the fact that the younger leaves have a thinner cuticle and can lose a lot of water at this level.

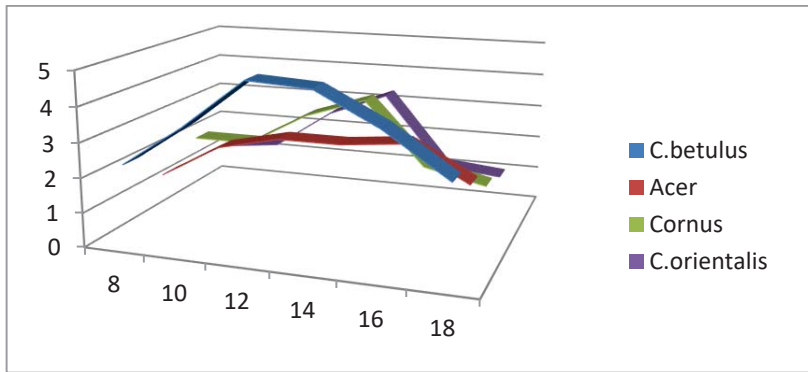
In the summer months, low sweat intensity can not be correlated with air temperature but with low water absorption due to low soil moisture.

The most intense transpiration was observed at *Carpinus betulus*, the lowest values being recorded at *Carpinus orientalis* (graphic1).



Graphic 1. Seasonal variation of transpiration ($\text{mmol H}_2\text{O} / \text{m}^2 / \text{s}$)

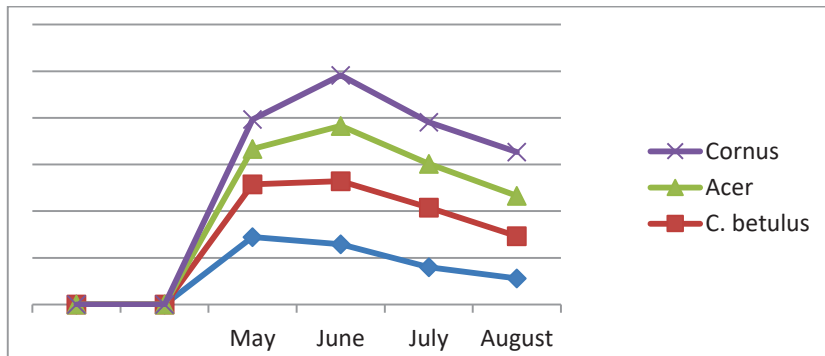
The diurnal variation of transpiration was determined at different times of the day, from the data shown in graph 2 being observed a peak of the process at around 15⁰⁰ o'clock and a minimum in the early morning hours. The largest diurnal fluctuations of transpiration are found in *Carpinus betulus* (graphic 2).



Graphic 2. Diurnal variation of transpiration intensity (mmolH₂O / m² / s)

Intensity of photosynthesis

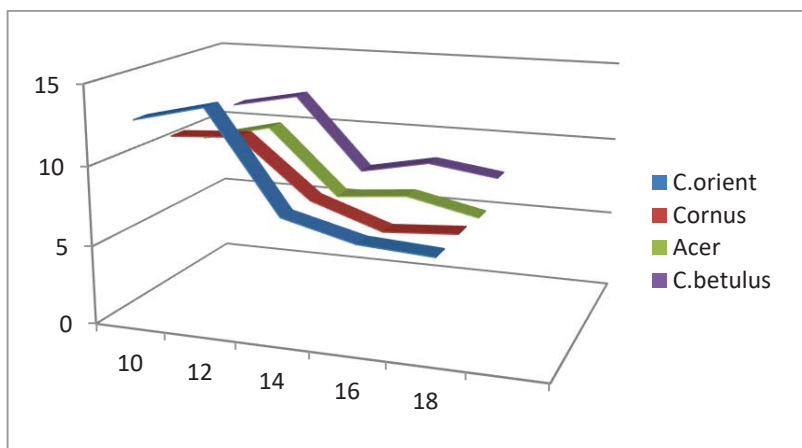
Due to the different stages of development and climatic conditions, in 2018, there was a seasonal variation of photosynthesis, with a maximum in June and minimum values in August (graphic 3). The highest values of photosynthesis intensity were recorded in *Cornus sanguinea*.



Graphic 3. Seasonal variation in the intensity of photosynthesis (µmolCO₂/m²/s)

The diurnal variation of photosynthesis was determined in June 2018.

Leaf determinations at an active photosynthetic radiation of 30-90 µmol / m² / s and at a temperature of 25-31 ° C revealed that the intensity of the photosynthesis process varied between 6.9 and 12.73 µmol / m² / s (graphic 4). The greatest variations of this process were observed in *Cornus sanguinea*.



Graphic 4. Diurnal variation in the intensity of photosynthesis ($\mu\text{molCO}_2/\text{m}^2/\text{s}$)

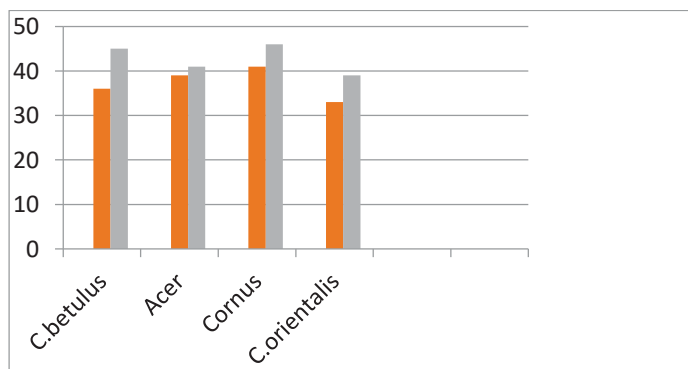
Content in chlorophyll pigments

Research on assimilating pigments has shown their great variability, quantitative and even qualitative, due to their sensitivity to internal and external factors.

The study of chlorophyll pigments in different populations and under different ecological conditions could provide some information on their role in ecosystems. Knowing the correlation between the content of chlorophyll pigments and the photosynthesis process, the in-depth investigation of this physiological index could allow the characterization or differentiation of some locations.

Forest ecosystems, through the complex structure of the vegetal component built both vertically and horizontally, determine a great variability of the foliar exposure conditions for the reception of sunlight. The assimilating pigments, as the main solar energy receptors, react by adapting to these differences, manifested by changes in the total content and changes in the ratio of chlorophylls a and b.

For the study of chlorophyll pigments, determinations were made in the months of May and July 2018. Graphical data shows that all species had a higher content in July (graphic 5).

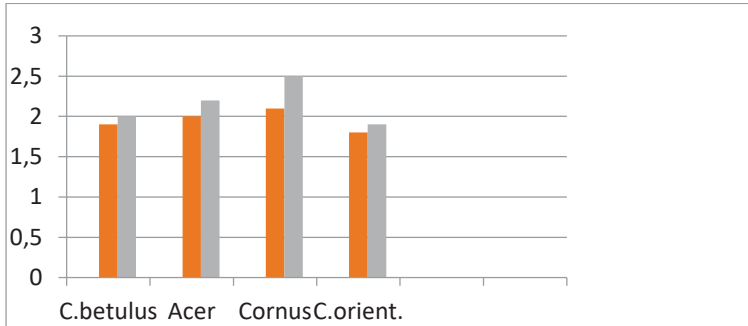


Graphic 5. chlorophyll content of leaves (SPAD units)

Intensity of net assimilation

From the data presented in graphic 6, it appears that there were significant differences in the two months of determinations, with higher values being recorded in July.

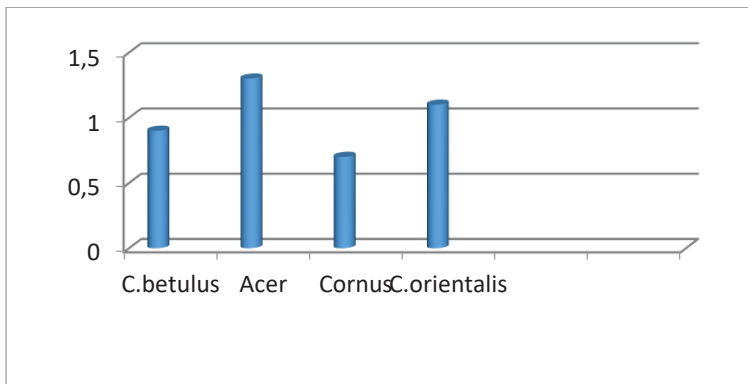
Among the species that were studied, the most intense assimilation process was observed at *Cornus sanguinea* (2.5 g dry substance / day / m² leaf).



Graphic 6. Intensity of net assimilation (g dry substance/ day / m² leaf)

Intensity of leaf respiration

The intensity of leaf respiration was determined with the Lci analyzer, by covering the assimilation chamber with foil paper, to prevent light penetration and the photosynthesis process. It was necessary to know this parameter in order to determine the value of the light compensation point for each of the studied species.



Graphic 7. Leaf respiration intensity (µmolCO₂ / m² / s)

The determinations were carried out in May at 26 °C. From graphic 7, it appears that the leaves of *Cornus sanguinea* have the least intense respiration, the most intense process being measured in the leaves of *Acer tataricum*.

The light compensation point

In the Comanesti forest, after the appearance of the leaves of the tall trees, the shading of the low vegetation is very pronounced.

Knowing that young trees, shrubs and grasses can not accumulate biomass if they do not get enough light, it was important to know the minimum growth limit, which

must be above the light compensation point. Knowledge of PC value is of great importance because under this value plants no longer produce organic substances, and the body lives out of its reserves. (Atanasiu, L., Polescu, L., 1988).

To find out the compensation point, we compared the intensity of photosynthesis determined at different times of the day, so at different intensity of light, with the value of respiration intensity in the dark. Data obtained reveals that among the studied species, *Cornus sanguinea*, *Acer tataricum* have the lowest values of the compensation point, behaving as ombrophyllous plants (Table 1).

Table 1

Compensation point of light (lx)	
Plant	Compensation point (lx)
<i>Carpinus betulus</i>	320
<i>Acer tataricum</i>	310
<i>Cornus sanguinea</i>	200
<i>Carpinus orientalis</i>	600

The highest value of the compensation point was recorded at *Carpinus orientalis*, that has a heliophyllous character, as it is also present in the specialized literature (Nețoiu C et al., 2008).

CONCLUSIONS

The determinations made in May-August 2018 revealed significant variations in the intensity of leaf transpiration. The highest values were recorded in spring for all plants under study, values due to both the higher amount of water in the soil and the fact that the younger leaves have a thinner cuticle and can lose a lot of water at this level.

The most intense transpiration was recorded at *Carpinus betulus*, the lowest values being measured at *Carpinus orientalis*,

The most intense photosynthesis was recorded in *Cornus sanguinea*, and the low assimilation being found at *Carpinus orientalis*.

Research on assimilated pigments showed their large quantitative variability, due to their dependence on internal and external factors.

Cornus sanguinea and *Acer tataricum* have the lowest values of the compensation point, acting as ombrophyllous plants. The highest value of the compensation point was recorded in *Carpinus orientalis*, which can be included in the heliophyllous species group.

REFERENCES

- Atanasiu, L., Polescu, L. 1988. *Fotosinteza, sau cum transformă plantele lumina soarelui*, Ed. Albatros, București,
- Ciocârlan V. 2009. *Flora ilustrată a României*, Edit. Ceres București
- Costache I., 2011. *Flora și vegetația bazinului hidrografic inferior al Jiului*, Ed. Universitaria, Craiova
- Delagrange S., Messier C., Martin J. Lechowicz Pierre Dizengremel , 2004, *Physiological, morphological and allocational plasticity in understory deciduous trees: importance of plant size and light availability* , *Tree Physiology*, Volume 24, Issue 7, 1 P 775–784,
- Kyle W. Tomlinson, Lourens Poorter, Frank J. Sterck, Fabian Borghetti, David Ward, Steven de Bie and Frank van Langevelde, 2013, *Leaf adaptations of evergreen and deciduous trees of semi-arid and humid savannas on three continents*, *Journal of Ecology* 101, p 430–440

Kramer J. P., 1986, The rol of physiology in forestry, *Tree Physiology*, vol.2, Issue 1-2-3-1, p1-16

Nețoiu C., Vișoiu D., Bădele O., 2008, *Dendrologie*, Ed. Eurobit, Timișoara

Roman N., 1974, *Flora si vegetatia din sudul Podisului Mehedinti*, Ed Academiei

Sîrbu Anca et al., 2007. *Arii speciale pentru protecția și conservarea plantelor în România*, Ed. Victor B Victor, București. 396 pp.

Wendy S, Klooster Bert M., Cregg R., Thomas Fernandez, Pascal Nzokou. 2012. *Growth and physiology of deciduous shade trees in response to controlled-release fertilizer*, Elsevier

<http://google.maps>

<https://link.springer.com/article/10.1007/BF00043955>

ECOTECHNOLOGICAL CONCEPTS FOR LOGGING

Chisăliță Ion^{1,2*}, Ciontu Cătălin-Ionel¹, Cântar Ilie-Cosmin¹

1 – National Institute for Research and Development in Forestry „Marin Drăcea”

2 – University of Agricultural Science and Veterinary Medicine of Banat „King Michael I of Romania” from Timisoara, Faculty of Horticulture and Forestry

*chisalita_ion@yahoo.com

Keywords: *ecotechnological, logging, forest, skidder, cableway*

ABSTRACT

The paper aims to present some of the ecotechnological concepts in logging, applicable in our country. The first part of the paper defines the new technologies of logging in the current concept, as well as the modern ecoproductive machinery intended to be used with the new technologies.

The work continues with the presentation of the material and the research methods used. Thus, books, magazines, brochures and international databases were used. The research methods used were: bibliographic documentation and practical experience, as well as analysis and synthesis for the elaboration of the results and conclusions of the study.

In the results and discussions are presented the basic principles regarding the ecotechnology in the logging and respectively the ecotechnological trends together with the characteristics of the current systems and their swot analysis.

The paper ends with the conclusions that bring together the most important ideas regarding the ecotechnological concepts in the logging as well as the most pertinent strengths and weaknesses of the swot analysis realized.

INTRODUCTION

New logging technologies in the present context represent the mode of succession of operations in the structure of the process specific to this activity, carried out with a system of machines that needs to be performing from the point of view of forest ecosystem protection and achieving the maximum economic efficiency.

Protecting the forest ecosystem means minimal damages for trees, seeds and soil on the logging process, so that the impact always falls below the threshold of supportability.

Some modern competitive machinery regarding ecoproductivity criteria such as funiculars and forwarders can work very well individually, but can produce spectacular productivity when used in an integrated way (Dima 2013). By integrated mode of operation is meant the optimal combination of machines, for their exploitation under the specific conditions of the field, of the quantity and the dimensional quality of the timber extracted from the forest.

Modern low-impact technologies can not be conceived without the large-scale introduction of funiculars, all the more because of the lack of accessibility in Romania and without the prospect of having an optimal road network in the near future.

MATERIAL AND METHOD

The materials used to achieve the proposed aim and objectives in this paper, are books, magazines, brochures, international databases and specialized papers addressing the issue of the ecotechnological concepts of logging.

Below are presented research methods used (bibliographic documentation, practical experience, analysis and synthesis).

The bibliographic documentation, ie the collection from all possible sources for the achievement of the objectives of this paper, was made using sources from the Romanian and foreign literature, regarding the low impact exploitation logging activity. In this way, documentation has been carried out on how forest exploitation technologies with a low impact on the forest ecosystem are designed and used in similar forests.

The Code of Best Forest Practice Ireland 2000 has also been an important bibliographic source through its objectives to promote forest practices in the forestry technology process to improve the use standards and reduction of environmental impact. This is a document that has been reviewed and adjusted by numerous experts from FAO member countries, universities, research institutes, non-governmental organizations, and the private sector (F.A.O Forest Harvestin Bulletin 2003).

The practical experience used for conducting this research was accumulated in the forestry field for a long time in the centralized economy before 1989 and on the market economy after 1989 and to the present with a long transition period between the two phases.

The experience in the wood exploitation and processing activity is also based on the activity carried out in the area of the private commercial companies, for a period of approx. 10 years after 2001 and where it was possible to observe the evolution of this field in the Romanian market economy (Chisăliță et al 2015).

The analysis and synthesis, used for the processing of data collected from the specialized bibliography, were used to formulate the conclusions of this study.

RESULTS AND DISCUSSIONS

Basic principles for ecotechnologies in logging

Concerning the basic principles on ecotechnology in logging, the ecotechnological methods of logging are characterized by (Horodnic 2014):

- rationalizing the use of tractors;
- extension of cable installations as basic equipment;
- developing optimal transport networks to reduce collection distances;
- the use of tandem trolleys with performing machines that collect the wood in the young crop;
- application of the methods of exploitation in short assortments („Shortwood Harvesting System”) and technology („Forwarding”).

Logging technology with reduced impact can be defined as a way of organizing the activity based on careful planning and strict verification of the implementation of those woodworking operations correlated with a modern machine system that avoids the negative effects that can occur in the case of conventional logging systems (Horodnic 2014).

Regardless of what kind logging equipment with low-impact we are talking about, the common features of ecotechnologies are:

- establishing an optimal pace of interventions in crop in order to ensure a sufficiently long period for regeneration and a rate of increase in the volume and quality of the remaining trees (frequent interventions increase the risk of damage);
- minimizing the damage to the crop remaining or its development environment;

- minimizing the width of the collection paths and the surface on which they are carried out;
- carrying out the activity in favorable conditions by the technological adaptation to the state of the land and the weather;
- the design of the works must include alternative variants for different working conditions on the same exploited surface;
- reducing the pressure of the ground by using low-pressure tires and / or large contact surface;
- appropriate training of workers and coordinators of logging activities; in many cases, forest workers are poorly trained and underpaid, resulting in negative environmental impacts and economic losses;
- providing protection equipment and machines with improved ergonomic features; additional indirect costs in the event of work-related accidents may be up to 6 times higher than direct operating costs;
- proper realization and maintenance of forest roads by: ensuring a profile adapted to the area, permanent maintenance of the wear layer and drainage system of precipitation water, avoiding collecting by pulling or semi-pulling on forestry roads;
- directed tree felling, also taking into account the way of collection; In the case of gathered with the cable by pulling (independent winches or mounted on the chassis of the forestry tractor), it is preferable to target the oblique fall in relation to the approach path;
- correct placement of storage surfaces (primary platforms); it is preferable to use the edge of the road over a longer length rather than create a single extended surface within the crop;
- post-exploitation assessment is particularly important for both the operating company and the forest manager as measures for improving the logging can be established;
- rehabilitation of the degraded land following the collection of wood and repairing the damages on the affected area due to the negative impact of some exploitation operations is mandatory;
- collection with low impact results in higher costs (about 20 %) due to design and organization activity that involves time and effort in addition to the conventional system.

The tandem use of machinery and the achievement of an integrated work on the basis of ecoproductive technologies means respecting principles and achieving higher economic and ecological outcomes. Cableways, forwarders, skidders can work very well individually, but they can generate spectacular productivity with a proportional reduction in impact on the soil if used in an integrated fashion. By integrated working method is meant the optimal combination of the equipment for the exploitation under specific conditions of land and the wood extract from the forest (Dima 2013).

This beneficial concept both economically and ecologically is based on the following:

- large-scale introduction of cableways, especially as ecological machines. Modern cable installations with powerful drive groups and trolleys that can carry up to 4 tons of load are a solution that suits the conditions in Romania (Ionașcu et al 1999). These machines can be used for gathering wood in tandem with trolleys or independent winches, but mostly skidding and nearing to a distance of 1000 m where they can achieve an average productivity of 100 cubic meters each day (www.wyssen.com).

- the downstream transport of wood should be made with modern high-capacity skidders, which can also be used to collect wood at the foot of the slopes by skidding and nearing (www.irum.ro) and which according to the experiments can reach a productivity of 1000 cubic meters / month. The alternative to skidder is the 12, 14 or 18

tonne forwarders, the ability to transport the timber suspended in the primary platform (Pulkki 2013).

➤ in the case of production of 2500-3000 mc / month, depending on the nature of the exploited wood, the processor heads can be used either for resinous or hardwoods, so that the wood can be cut and measured in the shortest possible time. Measurement of this wood as it is sorted and cut by processors is done automatically.

➤ wood harvesting (felling, cross cutting and trimming out) will be done with modern mechanical saws (Stihl, Husqvarna, Dolmar, Jonsered, etc.). Where conditions of species and relief permit, harvesters are used which perform verified productivity in Romania of approx. 200 mc / day (Oprea et al 2004).

Improved technological lines, those that are always proposed but not properly implemented, and due to ambiguities in norms and legislation, but also in the company capitalization mechanism, in the present conception are based on the forwarder that is approaching as opposed to classical ones that rely solely on the skidder tractor in the full process of exploitation.

These modern technological lines have the great advantage of productivity and ecological character (ecoproductive technologies, ecotechnologies, low impact technology systems) include the following:

➤ the use of the skidder tractor only when the winch is mounted on the tractor as well as the short-distance skidding and difficult trails inside the parquet;

➤ the proximity of the woodworking by using a forwarder tractor that has a higher productivity than the skidder, does not destroy the collecting routes it runs, consequently reduces operating costs while improving the environmental impact;

➤ the use of cableways, also only inside the surface of the felling area, on trails with slopes unconventional to the tractor, and on the nearest distance that exceeds the surface of the felling area, use the forwarder tractor;

➤ the studies and experiments carried out have revealed the following differences between the technological lines based on the current working technique in the logging in our country, respectively with skidder tractor at the hauling and the improved (proposed) technological lines based on the forward tractor at hauling. Comments were made on the TAF 650 tractors and the John Deere forward tractor (Oprea et al 2004):

➤ labor productivity gains through the introduction of improved technology lines of 20-53 %;

➤ forward tractor productivity up to 2 times greater than that of the skidder tractor for the same close distances as a result of higher travel speeds to forwarder tractors (www.interforst.at);

➤ labor cost reductions by introducing improved technology lines, 28-34 % due to higher productive efficiency and forward tractor over the skidder tractor;

➤ maintenance costs - working mc / wood collected on the forward tractor compared to the skidder tractor, by 15-23 % lower (for distances between 1000 - 2000 m).

Trends in the ecotechnology of wood exploitation

Today in Romania, analyzing the context in which logging takes place, we find that these are generally not conducive to ecoproductive forest exploitation for at least the following reasons:

➤ there is a tendency to reduce the areas that are through cuts and which cause a small volume of exploitation on the surface of the felling area, restricting the use of eco productive machinery;

➤ operators are constantly aiming at reducing operating costs, making as much rebate as possible from the observance of forestry restrictions and in the absence of

clear rules and procedures for exploitation in the Romanian system and easy to apply and monitor.

"Reduced Impact Forest Harvesting" is a working system based on the controlled implementation of specific harvesting and harvesting operations with low levels of damage to soil, water and further development of trees, a system that ensures perpetuation of forest functions and its economic viability after exploitation (F.A.O. Forest Harvesting Bulletin 2003).

In the current context of low-impact logging on the forest ecosystem, the objectives of the wood exploitation act can be concretized as follows:

- permanent placement in the exploitation process on the surface of the felling area, the operations to be collected, removed and near, in the ecological restrictions, together with the technical and economic ones;

- maximizing the value of the exploited timber, the final product of the wood exploitation (primary raw wood sorts or raw wood sorts) by efficient sorting techniques, applied with professionalism;

- minimizing the costs of timber exploitation through appropriately applied techniques and technologies while at the same time providing a modern machine system to be used in an integrated system according to the characteristics and working conditions on the surface of the felling area.

In this context, woodworking engineering must serve and apply "low impact technologies" that apply the principles of industrial ecology to forestry to reduce the impact on the forest ecosystem.

Features of current collection systems

Currently known collection systems are: manual, tractor, cart-load, cableways, helicopter. A swot analysis of collection systems sets out the opportunities and barriers to their application according to the field conditions and the aim of reducing the impact on the forest ecosystem in the harvesting process.

Table nr. 1

SWOT analysis

Collection system	Strong points	Weaknesses
Manual	<ul style="list-style-type: none"> • apply without arrangement of collection paths; • simple equipment to equip workers • low investment; • easy to organize. 	<ul style="list-style-type: none"> • hard work; • low productivity; • risks of occupational diseases.
Tractor	<ul style="list-style-type: none"> • average costs; • relatively simple technology; • generally skilled workforce. 	<ul style="list-style-type: none"> • damage to the relatively large environment; • high collection roads densities, a large area get out of production; • slope limitation.
Cart-load	<ul style="list-style-type: none"> • reduced damage to the soil, the trees and the seed; • low costs and very small investment; • very high mobility compared to the density of forest. 	<ul style="list-style-type: none"> • small collection distances; • small pieces of wood; • limited to the collected operation, at most removed.

Cableways	<ul style="list-style-type: none"> • reduced impact on the forest ecosystem; • relatively average costs compared to achieved productivity. 	<ul style="list-style-type: none"> • difficult assembly and disassembly involving qualified workforce; • Relatively large investments.
Helicopter	<ul style="list-style-type: none"> • high productivity; • low density of forest roads; • reduced environmental impact. 	<ul style="list-style-type: none"> • very expensive; • requires a very precise organization.

Table nr. 2

Opportunities and barriers

Collection system	Opportunities	Barriers
Manual	<ul style="list-style-type: none"> • working distances up to 100 m, optimum <50 m. 	<ul style="list-style-type: none"> • distances greater than 100 m; • limited working time.
Tractor	<ul style="list-style-type: none"> • working distances up to 500 m, optimally up to 200 m; • gradients of less than 60 % on dry land; less than 40 % on wet ground; less than 25 % on snow and less than 10 % on ice. 	<ul style="list-style-type: none"> • distances greater than 500 m; • gradients of more than 60 % on dry land, more than 40 % on wet ground, more than 25 % on snow and more than 10 % on ice.
Cart-load	<ul style="list-style-type: none"> • longitudinal sloping on full 20 % ramp; • longitudinal slope on full slope driving and 40 % downhill racing. 	<ul style="list-style-type: none"> • longitudinal tilt on full ramping 20 % higher; • longitudinal sloping in full slope and ramping more than 40 %; • Working distance greater than 2000 m.
Cableways	<ul style="list-style-type: none"> • working distances less than 2000 m; • gravity cableways at land inclinations above 15°; • Non-gravity cableways at inclinations of less than 15°. 	<ul style="list-style-type: none"> • operating costs higher than the value of the wood in the area to be exploited; • distances less than 300 and above 2500 m; • Load weight of up to 4 tons.
Helicopter	<ul style="list-style-type: none"> • lack of forest roads; • the value lean of the wood mass to be exploited higher than the operating costs. 	<ul style="list-style-type: none"> • operating costs higher than the value of the wood to be exploited

CONCLUSIONS

The tandem use of the equipment and the achievement of an integrated work is all the more justified because the road infrastructure is well below the optimum but also of poor quality and the accessibility of the national forestry fund covers only 65 % of our forests, being even lower in the area of exploitable stands.

Using only skidders in the integrated manner with the funicular, at their high productivity, can lead to the clogging of the primary platform access road in a week, due to the creation of narrow places in the tandem of unpredicted machinery to work in an integrated way. The alternative to skidders in this case is the forwarders.

The use of processor heads leads to the management of the wood in the primary platform and to dischargement in the assortments, knowing the algorithms for transforming the standing wood mass into wood assortments, taking into account the technological consumes and waste highlighting in the exploitation process (Chisăliță 2014).

With regard to improved technology lines, the forwarder can also move on forest roads where, due to their condition, they are impractical for specialized transport vehicles. The displacement of the forwarder tractors in the logging yard, although to be done on more demanding routes, does not burden the operating costs significantly regarding the other advantages outlined above.

Regarding the trends in the ecotechnology of wood exploitation, analyzing the context in which the forest exploitation takes place in Romania, we find that these are generally not favorable for ecoproductive forestry exploitations. From a structural point of view, a low-impact technology system does not differentiate from a conventional one, but what has to be done constantly is to choose those exploitation ways that reduce the consequences of damage to the residual trees, seedling and soil below the threshold of supportability.

Swot analysis of collection systems reveals the fact that cableways and cable installations have the least impact on the forest ecosystem. Their limitations are related to the small distances and small dimensions of the wood collected in the case of the workshop and, in turn, to the large investments and the need for qualified workforce in the case of cable installations.

Harness logging are suitable to be used up to 500 m (optimally up to 200 m) and on land with less than 60 % slope land. Funiculars can be used up to 2000 m for slopes up to 15° for non-gravitational funiculars and over 15° for gravitational funnels.

REFERENCES

Chisăliță, I., 2014, Exploatare forestiere. Îndrumar de proiectarea și organizarea, Editura Eurostampa, Timișoara;

Chisăliță, I., Moatăr, M., Crăciunescu, A., Ștefan, C., Banu, C., Foră, C., Alina, D., Stanciu, S., 2015, Time to change the management of the forest exploitation in Romania - a national priority geoconference on water resources, Forest, marine and ocean ecosystems, VOL II (SGEM 2014), Book Series: International Multidisciplinary Scientific GeoConference-SGEM, pp. 509-519;

Dima, P., 2013, Utilaje forestiere folosite integrat, Meridiane Forestiere, anul 16 , nr.2. pp. 79;

Horodnic, S., 2014, Sisteme tehnologice forestiere cu impact ecologic redus, Editura Universității, Suceava;

Ionașcu, Gh., Iordache, E., Derczeni, R., 1999, Noutăți în construcția și exploatarea instalațiilor cu cablu la colectarea lemnului, în „Pădurea românească în pragul mileniului trei”- Lucrările sesiunii științifice jubiliare consacrate aniversării a 50 de ani de învățământ silvic superior la Brașov, Editura Universității „Transilvania” din Brașov;

Oprea, I., Sbera, I., 2004, Tehologia exploatare lemnului, Editura Tridona, Oltenița;

Pulkki, R., 2013, Machine specifications and operating techniques, Faculty of Forestry, Lakehead University, Canada;

Pulkki, R., 2013, Cutofflength, treelength or full tree harvesting?, Faculty of Forestry, Lakehead University, Canada;

***, 2000, Code of Best Forest Practice Ireland: planning the thinning operation, Forest Service, Department of the Marine and Natural Resources, Dublin, Ireland , pp.124-128;

***, 2003, F.A.O. Forest Harvesting Bulletin, vol.13;

***, S.C.IRUM S.A.Reghin, <http://www.irum.ro>

***, Wyssen Seilbahnen AG, <http://www.wyssen.com>

**FOREST SOILS FROM BANAT. DISTRIBUTION BY PHYTO-CLIMATIC ZONES
AND COUNTY FOREST ADMINISTRATIONS**

Chisăliță I. ^{1,2*}, Ciontu C. I. ¹, Căntar I. C. ¹

1 – National Institute for Research and Development in Forestry „Marin Drăcea”

2 – University of Agricultural Science and Veterinary Medicine of Banat „King Michael I of Romania” from
Timisoara, Faculty of Horticulture and Forestry

*chisalita_ion@yahoo.com

Keywords: *County Forest Administrations, Banat, soils, phyto-climatic areas*

ABSTRACT

Using existing forest inventory database from Timisoara Forest Research Station, an analysis has been made of the distribution of forest soils in Banat, by classes and types of soil, phyto-climatic belt and County Forest Administrations. In the first part of the paper, a presentation of forest distribution in the region were made. The calculations show that the largest areas are occupied by cambisols (281,389.4 ha - 62 %) and luviosoluri (131.992.4 ha - 29 %). Cambisols are spread mostly on the mountain-premonton altitudinal (FM1+FD4) where it occupies 132,365.4 ha (47 %) followed by FD3 with 103,617.9 ha (37 %). These soils generally provide favorable conditions for beech. Luvisols are mostly distributed in FD2 (68,373.6 ha - 52 %) followed by FD3 (31.754,2 ha - 24 %) and FD1 (20.101,0 ha - 15 %). It is worth mentioning that these soils are favorable for Turkey oak, occupying 21 % of the Timiș County Forest Administration surface. Chernisols (6 %) are almost entirely composed from rendzine, mostly located in FD3 (15,040.5 ha - 58%). Knowing the distribution of soils on phyto-climatic levels, correlated with their favorability for certain species can serve both as an overview and as a general orientation for forest management.

INTRODUCTION

Soil, as an integral part of the forest sites, has a very important role in forest life. In a certain climatic context, the productivity of forest vegetation depends on the favorability of soil conditions, the extent to which trees can develop the root system (soil thickness, ecological useful volume), and the nature of the ecological soil complex (Chiriță et al 1977).

Under comparable regional and local climatic conditions, the relationship between water, air, and nutrients can be different from one soil to another and can cause fundamental changes in the stands composition and their vigor of growth when water, air and soil nutrients they are not balanced (Păunescu 1975).

According to the ecosystem concept, the forest ecosystem is an open system (Malița et al 1979) based on the existence of a main process of production, transformation and decomposition of organic matter, whose intermediate product is humus, whose qualitative qualities "depend on the biochemical content of the necromase, the specificity of the decomposing populations, the thermal, ionic and water regime of the soil" (Doniță et al 1990). As such, the types of forest ecosystems are classified within humification groups, being homogeneous from the point of view of biosynthesis, bio-decomposition and water regime of air, ionic and soil acidity (Doniță et al 1990).

Within the ecosystem, the forest sites has a more pronounced autonomy and a higher permanence than biocenosis, resulting in the importance of knowledge of forest soils, components of the forest sites.

Forest soils are heavily influenced by relief, slopes and exposition the thickness of the soil profiles, "especially the humus horizon, the regions of humidity, trophicity, consistency and temperature in soils" (Chiriță et al 1977). But the slope of the forest lands is very important and in another aspect, "the existence of a general predisposition of the Romanian geographic space to the floods" (Giurgiu 1978). As for a particular situation in the Banat forest area in terms of land slope, an analysis has been made in the work.

The zoning of the forests in our country, ie the establishment of territorial zoning units on latitude and altitude was made on the basis of the natural spread of large forest formations indicating special qualitative microclimates that meet on surface stretched under geographic zoning conditions (Doniță et al 1981). Geographic zoning conditions mean relief conditions in which soils and vegetation are formed only under the influence of the local climate and not local climates or special substrate conditions. For Romania the indicators formations are spruce, beech, sessile oak, pedunculate oak, Turkey oak, Hungarian oak, greyish oak and pubescent oak. A single zoning unit has been used that usually has a sub-zone rank or a bioclimatic layer. The name of altitudinal plant layer starts in all cases with the altitude-related character of their area, linked to the zonal forestry formation that inhabits it (Chiriță et al 1977).

In the paper was presented and analyzed the distribution of forest soils on altitudinal plant layer.

MATERIAL AND METHODS

In the first part of the paper there was a concise presentation of Banat forests, using data from the specialized literature and forest management plan, were presented the distribution of the forests in the region, the geographical and ecological conditions, the altitudinal ranges of the plants layers, etc.

In order to achieve the goal, the database of the existing forest inventory at the Timisoara Forest Research Station was used. Data was used up to the year 2000, before massive forest retrocessions when there was a larger volume of centralized data for analysis. Data processing was done on soil types and classes, respectively on bioclimatic zones. The analysis was made both on the whole region and on the forestry directions, given the complex character of the phytogeographical conditions in the region. All the forest units (describing the soil types) of the two forestry directions have been included. In the analysis of the distribution of forests on climatic zones reference was also made to the distribution of the main forest species.

Given the fact that the inclination of the land influences both the formation and the soil characteristics and the hydrological regime under separate conditions in the Banat forest area, an analysis was also made of the distribution of the forest lands by slope categories and forest directions. The categories used are (Târziu 1997): without inclination (< 3G), gentle (3-5 G), moderate (3-15 G), fast (15-25 G), very fast (25-45 G), the steep (> 45 G).

The location of the main profile network, the description of these profiles, as well as the location and description of the control profiles in each forest units was done simultaneously with the collection of data such as: the relief shape, the terrain configuration, the exhibition, the slope, the altitude, the type of the site, litter and type of flora. The main profile network was drawn up on the basis of a preliminary naturalistic study, which, at the time of the forest management planning work, it got indesit, if necessary was (***) Îndrumar pentru amenajarea pădurilor 2000). When analyzing the

distribution of soils, some general appreciations were made regarding their favorability for certain species according to some data from management plan forestry. The processed data concerned a forest area of 373,510.9 ha from the Caraș-Severin County Forest Administration and 79,114.9 ha from the Timișoara County Forest Administration, with a total of 452,625.8 ha for the whole Banat Forest area.

RESULTS AND DISCUSSIONS

The forests occupy in Banat approx. 28.7 % of the total area of the region, most forest areas (over 65 %) being located at the mountains (Bîndiu et al 1995). The county of Timiș, typical of the plain, has only 11,8 hectares of woodland, while Caras-Severin County has extensive hectares of massive forests (about 45 % of the total), with a country record (Bîndiu et al 1995).

In Banat there is an overlap of mountain and northern elements with elements of Mediterranean and Southern origin, mountain elements descending appreciably to the plain and vice versa (Grigore, Schrott, 1973), Banat being a bridge between the Balkans and Central Europe, between the East and the West, there is almost everywhere an interference of very complex geographic and ecological areas (Bîndiu et al 1995). The forests of Banat vegetate in all bioclimatic areas from the subalpine forest layer to the forest steppe.

Layers occupy the following altitudinal intervals (Doniță et al 1981): spruce forests over 1450 m (only in the ecological subregion F₂ Tarcu-Poiana Ruscăi), mixtures of beech with resinous, 1350-1450 m (in subregion F₁ Cerna-Semenic) and 900-1350m (in F₂ Tarcu-Poiana Ruscăi), mountain beech 800-1250m in F₁ and 800-1350m in F₂, hilly beech 500-800 m in both subregions, sessile oak and sessile oak with Turkey oak 300-500 m, Turkey oak-Hungarian oak (150 m) 200-300 m, oaks of meadow 100-200 m (only in the subregion F₂), oaks of meadow and depression 70-200m (only in O1 sub-region, Timis Plain).

The first three floors of vegetation: subalpine forest, mountain spruce and mountain mixes are quite poorly represented (totaling only 5 % of the forests of Banat). Spruces are frequent only in the Tarcu-Godeanu massif and at high altitudes. The most extensive forest areas, on the whole of the region, are located on the montane-premontaneous layer (33 %) and the hilltop layer sessile oak, beech and sessile oak-beech (34 %). Beech are best represented in the mountain area, both vertically and horizontally.

It is worth mentioning that in Banat the beech form a higher limit for the forest vegetation, but the beech is well represented on the hilly layer of sessile oak, beech and sessile oak-beech trees. Majority forms pure arboretums. The sessile oak make the transition to the beech in the upper part or to Turkey oak - Hungarian oak at the bottom. The last 3 layers of vegetation totalizing 6 % of Banat forests. Oak and plains are represented fragmentarily.

Forest soils have formed in different soil inclination conditions. In the Caraș-Severin County Forest Administration, slopes very quickly occupy 67 % of forest area, and slopes quickly occupy 25 %. This requires a great deal of planning and cutting due to the hydrological role of the forest and the recent increase in flood numbers and intensity.

In the Timis County Forest Administration, 33 % of the forests are on very fast slopes and 25 % on fast slopes.

Throughout the surface of the Banat forest were recorded soils classified in the following classes: 1 – Cambisols (281.389,4 ha – 62 %), 2 – Spodosols (3.524,8 ha – 1 %), 3 – Pelisols (9.586,7 ha – 2 %), 4 – Chernisols (25.837,6 ha - 6 %), 5 – Luvisols (131.992,4 ha – 29 %), 6 – Anolisols (294,9 ha), totalizing 452.625,8 ha (100 %).

So cambisols and luvisols occupy the vast majority of the Banat forest land (91 %). Cambisols are predominantly spread on the mountainous-premontaneous layer of beech forests (FM1 + FD4), where they occupy 132,365.4 ha (47 %), followed by FD3 - the hilltop layer sessile oak, beech and sessile oak-beech forests with 103,617.9 ha (37 %). On the FM1 + FD4 layer they are mostly distributed in the Caraş-Severin County Forest Administration (94 %), the eutricambisols occupying 47 %, and the districambisols 53 %. In the Timis County Forest Administration the cambisols are mostly distributed in FD3 (39 %) followed by FM1 + FD4 (35 %) and FD2 (25 %). In these layers, the eutricambisols represent (97 %) and are spread on slopes very fast (mostly) or fast, which ensure a good drainage of precipitation waters, and so a large part of the water does not participate in the processes of pedogenesis.

Cambisols rich in humus and in exchange bases, airy and permeable, slightly acidic, provide superior edaphic conditions for high productivity beech and for oak or oak mixes with beech. But the beech forms in Banat the stretched out forests and the poorer cambisols in the bases of exchange and even more acidic but with a large edafic volume and good ventilation.

Table 1

The distribution of forest soils on bioclimatic areas in Banat forests*

Bioclimatic area	Soil class	Soil types	D.S. Caraş-Severin	%	D.S. Timiş	%	Total Banat (ha)	%
1. Subalpine forest layer (F-sa)	Cambisols (CAM)	Districambisols (DC)	260,5	59	-	-	260,5	59
	Spodosols (SPO)	Prepodzol (EP)	121,3	27	-	-	121,3	27
	Pelisosols (PEL)	Pelisosols (PE)	62,0	14	-	-	62,0	14
	Total Fsa		443,8	100	-	-	443,8	100
2. Mountain layer of spruce (FM ₃)	Chernisols (CER)	Rendzina (RZ)	82,8	-	-	-	82,8	-
	Cambisols (CAM)	Eutricambisols (EC)	4749,2	-	-	-	4749,2	-
		Districambisols (DC)	12739,5	-	-	-	12739,5	-
	Spodosols (SPO)	Total	17488,7	95	-	-	17488,7	95
		Prepodzols (EP)	672,7	-	-	-	672,7	-
		Podzols (PD)	230,3	-	-	-	230,3	-
		Total	903,0	5	-	-	903,0	5
	Pelisosols (PEL)	Pelisosols (PE)	20,8	-	-	-	20,8	-
		Vertosols (VS)	0,6	-	-	-	0,6	-
		Total	21,4	-	-	-	21,4	-
Total FM ₃		18495,9	100	-	-	18495,9	100	
3. Mountain-mixture layer (FM ₂)	Cambisols (CAM)	Eutricambisols (EC)	51,0	-	-	-	51,0	-
		Districambisols (DC)	3656,1	-	-	-	3656,1	-
	Spodosols (SPO)	Total	3707,1	62	-	-	3707,1	62
		Prepodzols (EP)	1543,1	-	-	-	1543,1	-
		Podzols (PD)	495,1	-	-	-	495,1	-
		Total	2038,2	34	-	-	2038,2	34
	Pelisosols (PEL)	Pelisosols (PE)	241,6	-	-	-	241,6	-
		Vertosols (VS)	14,1	-	-	-	14,1	-
	Total FM ₂		255,7	4	-	-	255,7	4
	4. Mountaineering-premontan layer beech (FM ₁ +FD ₄)	Chernisols (CER)	Rendzina (RZ)	6001,0	100	-	-	6001,0
Peluosols (EL)			180,3	-	-	-	180,3	-
Luviosols (LUV)		Planosols (PL)	3802,0	-	-	173,6	2	3975,6
		Total	3982,3	3	-	173,6	-	4155,9

5. The hilltop layer of sessile oak, beech and sessile oak-beech (FD ₃)	Cambisols (CAM)	57404,6	-	9245,8	-	66650,4	-
	Districambosols (DC)	65715,0	-	-	-	65715,0	-
	Total	123119,6	89	9245,8	98	132365,4	90
	Spodosols (SPO)	441,6	-	20,7	-	462,3	-
	Pelisosols (PEL)	2244,6	-	-	-	2244,6	-
	Vertosols (VS)	43,2	-	4,0	-	47,2	-
	Total	2287,8	2	4,0	-	2291,8	2
	Total FM ₁ +FD ₄	137462,1	100	9444,1	100	146906,2	100
	Chernisols (CER)	15040,5	11	-	-	15040,5	10
	Luvosols (LV)	1142,0	-	550,3	-	1692,3	-
	Planosols (PL)	28753,1	-	1308,8	-	30061,9	-
	Total	29895,1	21	1859,1	15	31754,2	20
	Districambosols (DC)	6409,9	-	673,0	-	7082,9	-
	Total	93378,7	65	10239,2	85	103617,9	67
Pelisosols (PEL)	3339,7	*	3,6	-	3343,3	-	
Vertosols (VS)	1044,9	-	2,2	-	1047,1	-	
Total	4384,6	3	5,8	-	4390,4	3	
Total FD ₃	142698,9	100	12104,1	100	154803,0	100	
6. The hilly layer of the quercus (sessile oak, Turkey oak, Hungarian oak, mixtures of these) and hills (FD ₂)	Chernisols (CER)	2993,6	-	-	-	2993,6	3
	Luvosols (LV)	2015,6	5	4802,8	-	6818,4	-
	Planosols (PL)	40566,8	-	20988,4	-	61555,2	-
	Total	420582,4	66	25791,2	79	683736	71
	Districambosols (DC)	16778,8	-	6551,4	-	23330,2	-
	Total	100,5	-	135,3	-	235,8	-
	Planosols (PL)	16879,3	26	6686,7	20	23566,0	24
	Pelisosols (PE)	1392,2	-	12,0	-	1404,2	-
	Vertosols (VS)	331,4	-	180,1	-	511,5	-
	Total	1723,6	3	192,1	1	1915,7	2
	Total FD ₂	64178,9	100	32670,0	100	96848,9	100
	Luvosols (LUV)	283,9	1	451,8	-	735,7	-
	Planosols (PL)	3872,1	-	15493,2	-	19365,3	-
	Total	4156,0	99	15945,0	97	20101,0	97
Cambisols (CAM)	-	-	116,1	1	116,1	1	
7. The hilly layer quercus with oak (sessile oak, Turkey oak,	Cambisols (CAM)	-	-	116,1	1	116,1	1
	Districambosols (DC)	-	-	-	-	-	-
	Total	-	-	-	-	-	-
	Spodosols (SPO)	-	-	-	-	-	-
	Pelisosols (PEL)	-	-	-	-	-	-
	Vertosols (VS)	-	-	-	-	-	-
	Total	-	-	-	-	-	-
	Total FM ₁ +FD ₄	-	-	-	-	-	-
	Chernisols (CER)	-	-	-	-	-	-
	Luvosols (LV)	-	-	-	-	-	-
	Planosols (PL)	-	-	-	-	-	-
	Total	-	-	-	-	-	-
	Districambosols (DC)	-	-	-	-	-	-
	Total	-	-	-	-	-	-
Pelisosols (PEL)	-	-	-	-	-	-	
Total	-	-	-	-	-	-	
Total FD ₂	-	-	-	-	-	-	
Luvosols (LUV)	-	-	-	-	-	-	
Planosols (PL)	-	-	-	-	-	-	
Total	-	-	-	-	-	-	
Cambisols (CAM)	-	-	-	-	-	-	

Hungarian oak, mixtures of these (FD ₁)	Andisols (AND)	Andosols (AN)	54,8	-	19,3	-	74,1	-
	Pelisols (PEL)	Vertosols (VS)	2,9	-	321,2	2	324,1	2
	Total FD ₁		4213,7	100	16401,6	100	20615,3	100
8. Forest field (FC=CF)	Chernisols (CER)	Chernozem (CZ)	-	-	46,8	1	46,8	1
	Luvisols (LUV)	Preluosols (EL)	-	-	3232,7	-	3232,7	-
		Luvosols (LUV)	-	-	3926,2	-	3926,2	-
		Alosols (AL)	-	-	260,5	-	260,5	-
		Planosols (PL)	-	-	188,3	-	188,3	-
	Total	-	-	7607,7	93	7607,7	93	-
	Cambisols (CAM)	Eutricambisols (EC)	-	-	267,7	3	267,7	3
	Andisols (ABD)	Andosols (AN)	-	-	220,8	3	220,8	3
	Pelisols (PEL)	Vertosols (VS)	16,6	-	-	-	16,6	-
	Total CF	-	16,6	-	8143,0	100	8159,6	100
9. Silvosteppe (Ss)	Chernisols (CER)	Chernozem (CZ)	-	-	43,1	12	43,1	12
	Pelisols (PEL)	Peliosols (PE)	-	-	9,1	-	9,1	-
		Vertosols (VS)	-	-	299,9	-	299,9	-
		Total	-	-	309,0	88	309,0	88
	Total Ss	-	-	352,1	100	352,1	100	-
Total			373510,9	-	79114,9	-	452625,8	-
% Banat			83		17		100	

* The soil classes and types are in accordance with the Romanian Soil Taxonomy System (SRTS 2003)

In both County Forest Administrations occupied by cambisols are located on the two mentioned layers (84 % Caraş-Severin County Forest Administration and 74 % Timiş County Forest Administration), mentioning that in the Timiş County Forest Administrations 25 % of the areas are located on the FD2 layer. Luvisols are mostly distributed in FD2 (68,373.6 ha - 52 %) followed by FD3 (31.754,2 ha - 24 %) and FD1 (20.101,0 ha - 15 %). On the County Forests Administrations predominate in FD2 (Caraş-Severin County Forest Administration 42,582.4 ha - 53 % respectively Timiş County Forest Administration 25,791.2 ha - 50 %). Altitude differences are grouped on the upper layers in the Caraş-Severin County Forest Administration (42 %) and in the lower layers in the Timiş County Forest Administration (46 %). In FD2 they are predominantly represented by planosols (90 %) and luvisols (19 %). Planosols occupy 95 % of the forest lands in the Caraş-Severin County Forest Administration and 81 % in the Timiş County Forest Administration. In FD3 majority there are the planosols (95 %), occupying 96 % of the lands of Caraş-Severin County Forest Administration and 70 % in Timiş County Forest Administration, followed by luvisols. It should be noted that these soils provide favorable conditions for the Turkey oak, a species that occupies 21 % of the forestry area of the Timis County Forest Administration.

Chernisols occupy 6 % of the forest area of Banat, being half the majority rendzine (only 89.9 hectares of chernozems in the area of forest plain and forest steppe). They are distributed almost on all vegetation layers (some with very small areas), mainly in FD3 (15,040.5 ha - 58 %) followed by FM1 + FD4 (7.630.8 ha - 30 %) and FD2 (2.993,6 ha 12 %). The rest of the forest areas are occupied by pelisols (2 %), spolisoluri (1 %) and only 284.9 ha of andisols.

CONCLUSIONS

The forests of Banat vegetate in special conditions at the interference of very complex geographic and ecological areas. The forest lands are situated predominantly by sloping very quickly slopes, in the Caraş-Severin County Forest Administration they occupy 67 % and in the Timiş County Forest Administration 33 %, which requires an increased care for the planning and execution of cutting trees.

Banat forest soils belong mostly to the cambisol class 281,389.4 ha (62 %) and luvisolles 131,992.4 ha (29 %). Cambisols are spread on the whole of the region, mostly on the montane-premontan layer of beech (FM1 + FD4) where it occupies 132,365.4 ha (47 %) followed by FD3 the hilltop layer sessile oak, beech and sessile oak-beech with 103,613.9 ha (37 %). The paper presents their distribution by types and County Forest Administrations. Cambisols are generally favorable for beech.

Luvisols are mostly spread in FD2 68,373.6 ha (52 %), both in the Caraş-Severin County Forest Administration 42,582.4 ha (53 %) and in Timiş County Forest Administration 25,791.2 ha (50 %). They are predominantly represented by planosols (95 %) in the Caraş-Severin County Forest Administration and (81 %) in the Timis County Forest Administration. These soils provide favorable conditions for the Turkey oak.

Chernisols occupy 6 % of the forest area of Banat being almost entirely made of rendzine.

The distribution of soils on phytoclimatic levels, correlated with their favorability for certain species, can serve as a general orientation for forest management.

REFERENCES

- Bîndiu, C., et al., 1995, Pădurea seculară. Cercetări ecologice în Banat, Ed. Mirton, Timișoara;
- Chiriță, C., et al., 1977, Stațiuni forestiere, Ed. Academiei R.S.R.;
- Doniță, N., et al., 1981, Zonarea și regionarea ecologică a pădurilor din R.S.R., Redacția de propagandă tehnică agricolă;
- Doniță, N., et al., 1990, Tipuri de ecosisteme forestiere din România, seria a II-a, Redacția de propagandă tehnică agricolă;
- Grigore, S., Schrott, J., 1973, Flora și vegetația Banatului, Tipografia Universității Timișoara;
- Giurgiu, V., 1978, Conservarea pădurilor, Ed. Ceres;
- Malița, M., et al., 1979, Sisteme în științele naturii, Ed. Academiei R.S.R.;
- Păunescu, C., 1975, Solurile forestiere I, Ed. Academiei R.S.R.;
- Tîrziu, D., 1997 – Pedologie și stațiuni forestiere, Ed. Ceres;
- ***, 2003, Sistemul roman de taxonomie a solurilor (SRTS);
- ***, 2000, Îndrumar pentru amenajarea pădurilor.

THE FOREST SOILS FROM ARAD COUNTY

Cântar Ilie-Cosmin^{1*}, Dincă Lucian²

¹„Marin Drăcea”, National Institute for Research and Development in Forestry, Timisoara

²„Marin Drăcea”, National Institute for Research and Development in Forestry, Braşov

* Correspondence author. E-mail: cantar.cosmin@yahoo.com

Key words: *luvisol, eutric cambisol, gleysol, humus*

ABSTRACT

The aim of this paper is to realize a description of forest soils from Arad County. The work material is represented by soil analysis data from forest management plans made in the period 1985-2015 from 22 forest districts, meaning 483 soil profiles and 1332 pedo-genetical horizons. The soils found in Arad are characteristic to the area of low hills (luvisols, preluvisols, eutric cambisols). However, field soils (phaeozems), mountain soils (dystric cambisols) and even azonal soils (fluvisols, gleysols) are present, in the order of their spreading: luvisol (moderately acid, mezobasic in Ao and Bt and oligomezobasic in El, with a high total cationic exchange capacity, well supplied with nitrogen and intensely humiferous), eutric cambisol (moderately acid, mezobasic, with a high total cationic exchange capacity, very well supplied with nitrogen and intensely humifeous), preluvisol (moderately acid, mezobasic, with a high total cationic exchange capacity, very well supplied with nitrogen and intensely humiferous), gleysol (eubazic, with a very high total cationic exchange capacity, very well supplied with nitrogen and moderately humiferous).

INTRODUCTION

Based on data from the National Statistical Institute, the forest area from Arad Forest District is 211.470 ha for the year 2015 (www.insse.ro). The state administration, Romsilva National Forest Institute, manages through its 10 Forest Districts, 102.329 ha (www.rosilva.ro). The difference between the above numbers is represented by private forests which occupy an important percentage in this county.

Forest soils are an essential part of forest ecosystems (Spârchez *et al.*, 2011, Dincă *et al.*, 2006). The purpose of this paper is to realize a description of soils from Arad county's forest area.

MATERIAL AND METHODS

During the forest management activity, amongst other works, soil samples from profiles situated in characteristic areas are also harvested. These samples are then analyzed at the soil laboratory from "Marin Drăcea" National Institute for Research and Development in Forestry. The following parameters are taken into account: pH, humus content, content of carbonates, base exchange capacity (Sb), hydrogen exchange capacity (Sh), total cationic exchange capacity (T), base saturation degree (V), texture, total nitrogen. The methods used in the analysis of soil samples are acknowledged at national and international level (Dinca L. *et al.*, 2012).

The present paper takes into account soil samples harvested in the period 1985-2015 from 22 forest districts belonging to Arad Forest Management District (Amenajamentele Ocoalelor Silvice: Beliu (1995, 2006, 2015); Bârzava (2007); Ceala (1992, 2002, 2012); Chişinău Criş (2002, 2012); Gurahonţ (1994, 2005, 2014); Lipova (2007); Radna (2006, 2015); Săvârşin (1985, 1996, 2006); Sebiş Moneasa (2005, 2014); Valea Mare (2009, 2012)). As a whole, 483 soil samples and 1332 pedo-genetical horizons were analyzed. The repartition of soil types and main chemical properties of soils (pH, base saturation degree, total cationic exchange capacity, humus and nitrogen content) were analyzed.

RESULTS AND DISCUSSION

Types of soils from Arad Forest District

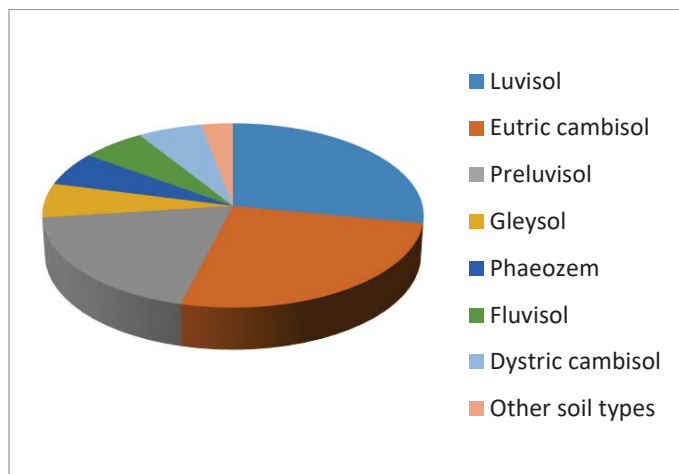


Figure 1. The percentage of forest soils identified in Arad District

The most widespread types of soils are the ones from Luvisols (47 %) and Cambisols (32 %) classes. As types of soils, the most widespread is luvisol (28 %), followed by eutric cambisol (26 %) and preluvisol (19 %). These soils are followed as spreading by a group of 4 soils that occupy each 6 % of the county's forest area: gleysol, phaeozem, dystric cambisol and fluvisol (figure 1). Other types of soil (alosols, soloncheaks, solonetz, rendzic leptosols, entiantrosols) represent 3 % of the total forest soils from this area.

At the level of our country, dystric cambisol occupies the first place as spreading in forest soils (2.292.35 ha, meaning 35 %), the luvisol 2nd place (1.440.052 ha, meaning 22 %), eutric cambisol the 3rd place (with a total area of 869.909 ha, meaning 13 %), and preluvisol the 5 place (335.050 ha, meaning 5 %), (Dincă L. *et al.*, 2014). It can be observed that all 4 mentioned soils are also occupying significant areas in Arad Forest District. Iliuţă *et al.* (2012) have identified on 182.080 ha from Arad Plain the presence of chernozems on 44 % of the area, followed by eutric cambisol (22 %), vertisol (12 %), phaeozem (8 %) and gleysol (5 %). Vlad *et al.*, (2007) have identified the following percentages occupied by soils from the total agricultural acreage in the surveyed area from Arad county (775.409 ha of which 511.520 ha is represented by agricultural fields): 30.53 % chernozems, 25.11 % luvisols, 14.91 %, protisols, 11.82 % pelisols, 6.80 %

cambisols and of total forest acreage in surveyed area 47,79 % are luvisols and 46,68 % are cambisols. Şohrenţ and Rusu (2009), have found in Miniş-Măderat vineyards perimeter from Arad County, 6 types of soils: preluvisol (30 %), eutric cambisol (24 %), vertisol (20 %), fluvisol (10 %), chernozem (10 %) and gleysol (6 %).

Soil solution reaction

In regard with the soil solution reaction, this was calculated on pedogenetic horizons, differentiated for the most widespread types of soils (eutric cambisol, luvisol), but also for the ones at which the values of this parameter re different (large at phaeozem and fluvisol and lower for dystric cambisol) (figure 2). The lowest values of pH are registered for dystric cambisol, this being a strongly acid soil, while the largest pH values are for fluvisol and phaeozem, weakly alkaline soils. All the other soils are moderately acid. In the second horizon, the pH is larger, due to the rocks formed on parental materials that are rich in calcium and pheromagnesium minerals (Târziu *et al.*, 2004). The largest amplitude pH variation is found in fluvisols, soils formed through the accumulation of slime (which are of different types and have as such a variable pH).

Borza *et al.* (2007) have found the following pH distribution for Arad County's soils: highly acid (pH<5)-3.2 %, moderate acid (pH=5.1-5.8)-38.4 %, low acid (pH=5.9-6.7)-38.4 %, neutral (pH=6.8-7.2)-6.6 %, low alkaline (pH=7.3-8.4)-19.3 % and moderate and excessive alkaline (pH>8.5)-2.2 %. The luvisol reaction in the Gurahont Depression is as follows: of the 8.938 ha of arable land, 1.609 (18 %) are acid, 5.810 ha (65 %) are less acid, 536 ha (6 %) have a neuter reaction and 983 ha (11 %) have an alkaline reaction (Balaşcău and Borza, 2011). Acid preluvisols were also identified in other areas of the country (Chisăliţă *et al.*, 2015, in O.S. Făget, D.S. Timiş). Iliuţă and Țărău (2013), found from 755.409 ha, of which 511.520 ha arable terrains that moderate and strong acid covers 150.000 ha, moderate alkaline cover 45.000 ha and strong and excessive alkaline 16.000 ha.

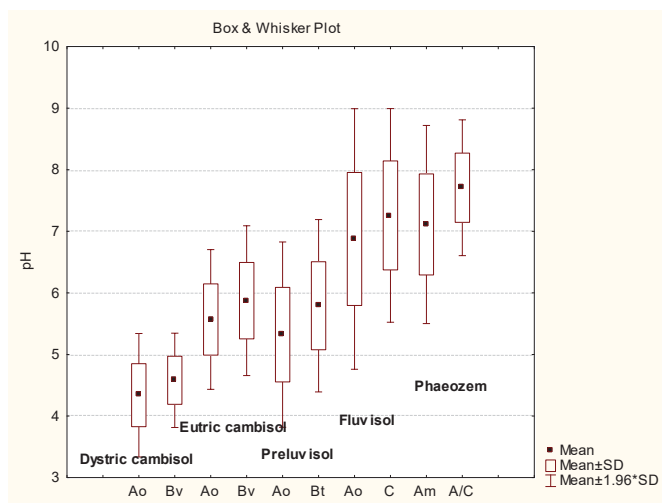


Figure 2. pH variation of genetic horizons for the most widespread forest soils from Arad

Base saturation degree

The values on pedo-genetic horizons were also calculated for the base saturation degree. The most widespread soils from the area (luvisol, eutric cambisol and preluvisol) were analyzed, together with gleysol, a soil with a good representation in this county, in comparison with other areas of the country (figure 3).

It can be observed that the variation amplitude of this parameter is high for all soils. By analyzing the average value of the base saturation degree, it can be observed that it is situated in the interval 55 – 65 % for eutric cambisol and preluvisol, both soils being mezobasic, the luvisol is mezobasic in Ao and Bt and oligomezobasic in EI, while gleysol is eubasic.

Total cationic exchange capacity

In regard with the total cationic exchange capacity, an average value per profile was calculated and rendered as table for each type of soil (Table 1).

All soils have a large cationic exchange capacity (figure 4). The largest value of this parameter is registered for gleysol, while the lowest is for dystric cambisol.

For luvisols from Arad area, Mihuț and Niță (2014), have established that the total cation exchange capacity is very small in A and E horizons (under 10 me/100 g soil), may increase up to double the horizon iluvial becoming middle (20-35 me/100 g soil).

Humus

A very important soil parameter is the humus content (Dincă L., 2015). In regard with it, the average content from the A horizon was determined for each type of identified soil (Table 1). Gleysol and fluvisol are moderately humifer soils, while dystric cambisol, luvisol, eutric cambisol and preluvisol are intensely humiferous (figure 5).

The humus quantities from this district are similar with the average values for forest soils calculated for the entire country (Dincă *et al.*, 2012). Toth *et al.* (2012), have established that for Cacica are, the humus content in soils indicates a good supply, the weighted average being 3.24 %.

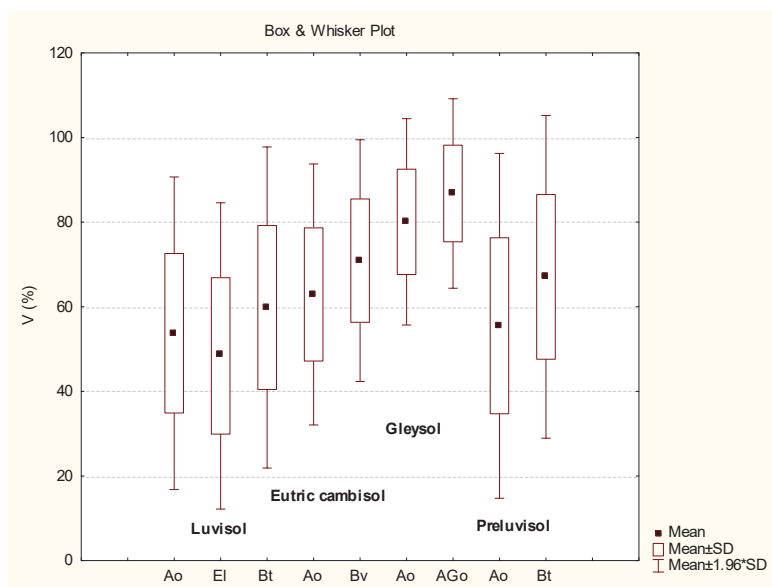


Figure 3. Base saturation degree variation for the most widespread soils from Arad County

Table 1

Average content of humus, nitrogen and total cationic exchange capacity for forest soils from Arad county

Eutric cambisol	Dystric cambisol	Preluvisol	Luvisol	Gleysol	Fluvisol
Total average cationic exchange capacity per soil type (T-me 100 g ⁻¹ sol)					
22.75	21.74	22.50	22.17	26.06	24.65
Average humus content in the A horizon per soil type (H-%)					
5.21	8.05	5.04	6.10	3.86	4.11
Average nitrogen content in the A horizon per soil types (%)					
0.27	0.43	0.25	0.31	0.20	0.24

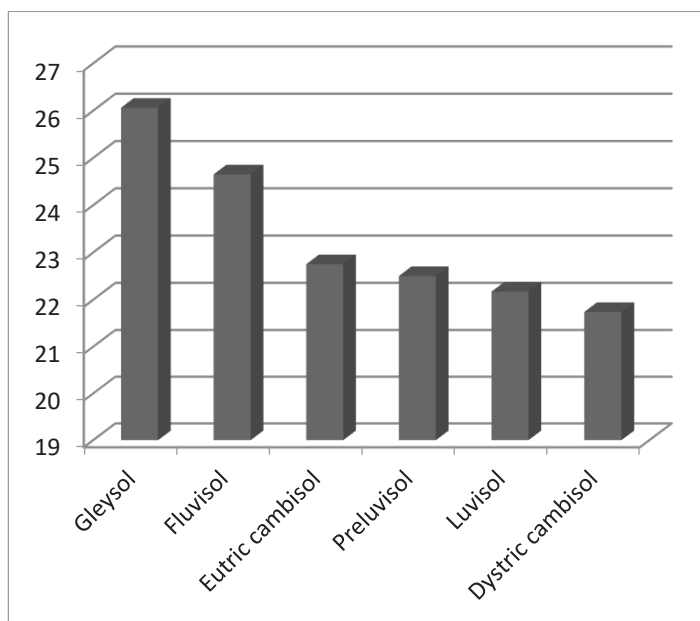


Figure 4. The variation of total cationic exchange capacity for the most widespread forest soils from Arad county

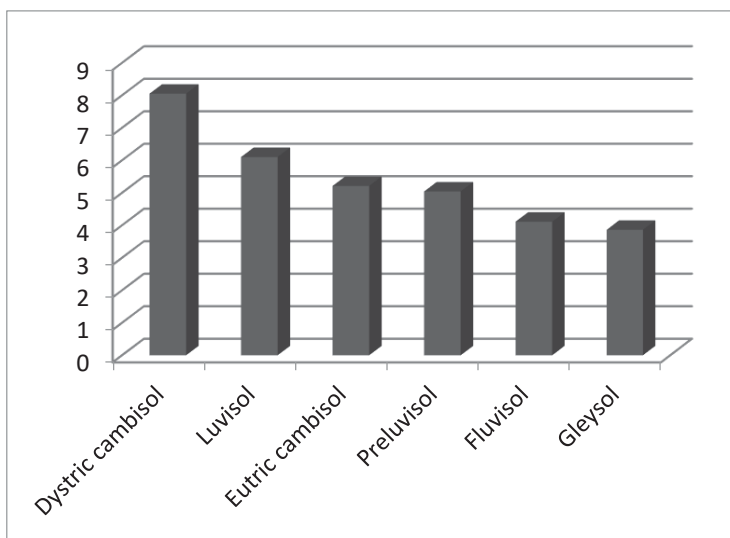


Figure 5. The variation of humus content for the most widespread forest soils from Arad county

Nitrogen

In regard with the nitrogen supply, fluvisols, preluvisols and eutric cambisols are very well supplied soils with this element, while dystric cambisols and luvisols are well supplied with nitrogen.

CONCLUSIONS

In Arad, a similar number of soils (12) with the ones from Maramureş County is found, but larger than the ones from Giurgiu County, which has only 7 types of soils (Crişan et al., 2017). Unlike other counties, gleysols are well represented in Arad.

Luvisol is a moderately acid soil, mezobasic in Ao and Bt and oligomezobasic in E1, with a high total cationic exchange capacity, well supplied with nitrogen and intensely humiferous. Eutric cambisol is a moderately acid soil, mezobasic, with a high total cationic exchange capacity, very well supplied with nitrogen and intensely humiferous. Preluvisol is a moderately acid soil, mezobasic, with a high total cationic exchange capacity, very well supplied with nitrogen and moderately humiferous. Gleysol is a eubasic soil, with a very large total cationic exchange capacity, very well supplied with nitrogen and moderately humiferous. Dystric cambisolul is a strongly acid soil, oligomesobasic, with a high total cationic exchange capacity, well supplied with nitrogen and intensely humiferous. Phaeozem is a weak alkaline soil, with a high total cationic exchange capacity. Fluvisol is a weak alkaline soil, with a high total cationic exchange capacity, very well supplied with nitrogen and moderately humiferous.

REFERENCES

Balaşcău D., Borza I., 2011, *Soil quality in Gurahonţ depression*, JOURNAL of Horticulture, Forestry and Biotechnology, Volume 15(2), 157- 161.

Borza I., Ţărău D., Sala F., Ţărău I., Iordache M., 2007 *Quality state of soils from West of Romania and measures for their fertility restoration*, Research Journal of Agricultural Science, 39 (2), 161-166.

Chisăliță I., Dincă L., Spârchez Gh., Crăciunescu A., Vișoiu D., 2015, *The influence of some stagnoluvisols characteristics on the productivity of Quercus cerris and Qurecus frainetto stand from O.S. Făget, D.S. Timiș*, Research Journal of Agricultural Science, 47 (3), 23-28.

Crișan V.E., Enescu R., Dincă M., 2017, *Descrierea solurilor din cadrul Direcțiilor Silvice Giurgiu și Maramureș*, Revista de Silvicultură și Cinegetică Brașov nr. 39, pag. 85-89.

Dincă L., Dincă M., Băcăințan N., 2006, *Utilizarea programelor de modelare în Pedologia forestieră*, Analele ICAS, Seria I, Vol. 49, Editura Tehnică Silvică, 45-53.

Dincă L., Lucaci D., Iacoban C., Ionescu M., 2012, *Metode de analiză a proprietăților și soluției solurilor*, Editura Tehnică Silvică, 173 p.

Dincă L., Spârchez G., Dincă M., Blujdea V., 2012, *Organic carbon concentrations and stocks in Romanian mineral forest soils*, Annals of Forest Research, 55 (2), 229-241.

Dincă L., Spârchez G., Dincă M., 2014, *Romanian's forest soil GIS map and database and their ecological implications*, Carpathian Journal of Earth and Environmental Sciences, 9 (2), 133-142.

Dincă L., 2015, *Describing an own calculation program for the organic carbon from soils (STOCS) and its main applications*, Research Journal of Agricultural Science, Timisoara, 47 (3), 37-43.

Iliuță A., Țărău D., Bești B., 2012, *Yield capacity limitative factors in Arad Plain soils*, Research Journal of Agricultural Science, 44 (4), 77-81.

Iliuță A., Țărău D., 2013, *Fertility limitative pedological factors approach and soil yield capacity recovery actions in Arad county*, Research Journal of Agricultural Science, 45 (4), 83-89.

Mihuț C., Niță L., 2014, *Research on some degraded soils in Arad and mitigation measures to impose*, Research Journal of Agricultural Science, 46 (3), 3-7.

Spârchez Gh., Târziu D., Dincă L., 2011, *Pedologie*, Editura Lux Libris, Brașov, 293 p.

Șohrenț M., Rusu I., 2009, *The main types of soils in perimeter vineyards Miniș-Măderat, Arad county*, Research Journal Of Agricultural Science, No. 41 (3), 152-155.

Târziu D., Spârchez Gh., Dincă L., 2004, *Pedologie cu elemente de Geologie*, Editura Silvodel, Brașov, 2004, 343 p.

Toth A., Turi Șt., Turi P., Duma-Copcea A., 2012, *Characterization of soils pedo Pecica near the village, Arad*, Research Journal of Agricultural Science, 44 (4), 144-148.

Vlad H., Borza I., Țărău D., Dologa D., Iliuță A., Băc C., Florea D., 2007, *Pedological factors, component of Arad county rural area*, Research Journal of Agricultural Science, 39 (2), 155-160

*** Amenajamentele Ocoalelor Silvice: Beliu (1995, 2006, 2015); Bârzava (2007); Ceala (1992, 2002, 2012); Chișinău Criș (2002, 2012); Gurahonț (1994, 2005, 2014); Lipova (2007); Radna (2006, 2015); Săvârșin (1985, 1996, 2006); Sebiș Moneasa (2005, 2014); Valea Mare (2009, 2012).

www.insse.ro

www.rosilva.ro

**PLANTS CONSERVED IN "ALEXANDRU BELDIE"
HERBARIUM – ALYSSUM GENUS**

Cântar Ilie-Cosmin^{1*}, Vechiu Emilia², Dincă Lucian²

¹ „Marin Drăcea”, National Institute for Research and Development in Forestry, Timisoara

² „Marin Drăcea”, National Institute for Research and Development in Forestry, Braşov

* *Correspondence author. E-mail:** cantar.cosmin@yahoo.com

Key words: *Alyssum, herbarium, species, botanists*

ABSTRACT

The purpose of the present paper is to describe the Alyssum Genus species present in Al. Beldie Herbarium from I.N.C.D.S. Bucharest. A data base was created for the species of this genus, containing data regarding their gathering place and date, the specialists who has collected them and the conservation degree of each exemplar, noted from 1 to 4. The Genus was divided in six sections, with species gathered from different parts of Europe, preponderantly from Romania. All the 29 species present in the Herbarium were gathered between 1833 and 1993 by Romanian and foreign specialists who have kept them in a very good conservation state. Furthermore, different diagrams were realized regarding the number of species encountered and their gathering periods.

INTRODUCTION

Brassicaceae Family contains 321 Genera and approximately 3700 species (Al-Shehbaz 1987). Alyssum Genus belongs to the Brassicales Order and contains 195 species distributed all around Europe, Asia, North Africa and North America (Li et al 2014). The Genus is represented by annual, biennial or perennial herbaceous plants, with oval elongated leaves and yellow or white (pink or purple at some species) hermaphrodite stellar flowers. The stem is sessile or ramified, while the fruit is a silica or silica (Baskin et al 1974). Dudley (1964) has divided the Genus in six sections, namely Meniocus (Desv.) Hook, Psilonema (C. A. Meyer) Hook, Alyssum and Gamosepalum (Hauskn.) Dudley, Tetradenia (Spach) Dudley, and Odontarrhena (C. A. Meyer) Koch. where all species are perennial and hyper accumulating Ni (Broadhurst Catherine et al 2016).

Recent phylogenetic analyses show that the Alyssum Genus is polyphyletic (Al-Shehbaz 1987).

The Alyssum name originates from Greek, where “a” means “no” and “lyssa” relates to madness, anger. As such, the name ascertains to the plant’s reputation in medicinal purposes. It is considered that an infusion created from its leaves and flowers can be administered as antidote against madness or the bites of a rabid dog. The tea can also be used as sedative against anger (Dudley 1966).

MATERIALS AND METHODS

Marin Drăcea National Institute for Forestry Research-Development (INCDS) from Bucharest holds a Herbarium named after Alexandru Beldie, an important Romanian botanist. The Herbarium contains over 40.000 plates of some plant species

kept in their original maps and organized in almost 600 drawers (Vasile Diana et al 2017). The plant collection is registered in INDEX HERBARIORUM and is composed of plants that come from privately donated collections as well as from foreign collections obtained through exchanges. Amongst them we mention the 32 *Arabis* genus species (Dincă et al 2017a), the 33 *Orobanche* genus species (Scărlătescu et al 2017), 9 *Melica* species and 11 *Eragrostis* genus species (Cântar et al 2017), 19 *Androsace* genus species (Dincă Maria et al 2017), 15 *Veronica* genus species (Dincă et al 2017b), 69 *Potentilla* genus species (Crișan et al 2017), or 17 *Amaranthus* genus species (Dincă et al 2018).

The study material was thus composed of 83 plates belonging to the *Alyssum* Genus and present in Al. Beldie Herbarium. All 29 species representative of the *Alyssum* Genus and found within the Herbarium were introduced in a data base where they were grouped based on the species, gathering place and date, specialist who has collected them and their degree of conservation. Table number 1 presents an excerpt of the *Alyssum* Genus data base.

Furthermore, based on the investigations from the specialty literature, some *Alyssum* species present in the Herbarium were also described.

RESULTS AND DISCUSSIONS

The species found in the Herbarium were the following: *Alyssum alyssoides* L., *Alyssum arduini* Frits., *Alyssum argenteum*, *Alyssum borzaeanum*, *Alyssum caliacre*, *Alyssum campestre* L., *Alyssum corsicum* Duby, *Alyssum desertorum*, *Alyssum diffusum*, *Alyssum edentulum* W. et K., *Alyssum eximium* Nyár., *Alyssum halmifolium* L., *Alyssum hirsutum*, *Alyssum hispidum* L & P., *Alyssum leucadaeum* Guss., *Alyssum linifolium* Steph., *Alyssum macrocarpum* D.C., *Alyssum maritimum* Lam., *Alyssum minutum* Schl., *Alyssum murale* Waldst. & Kit., *Alyssum petraeum* Ard., *Alyssum repens* Baumg., *Alyssum rostratum* Stev., *Alyssum rupestre* Ten, *Alyssum saxatile* L., *Alyssum sinuatum*, *Alyssum tortuosum*, *Alyssum wierzbickii* Heuff. and *Alyssum wulfenianum* Bernh.. The most numerous *Alyssum* species present in the Herbarium are: *A. repens* Baumg (17 plates), *A. alyssoides* L. (11 plates), *A. arduini* Frits. and *A. borzaeanum* (6 plates) (Fig. 1).

Table 1

The inventory of *Alyssum* Genus (excerpt from the data base)

Drawer number	Plate number	Herbarium/ Botanic collection/ Institution	Name of species	Gathering date	Gathering place	Collected/ Determined by:	Conservation Degree (1..4)
51	1	Al. Beldie Herbarium, Bucharest	<i>Alyssum alyssoides</i> L.	1947.08.02	Poiana Țapului	Al. Beldie	1
51	36	Dr. C.Baenitz/ Herbarium Europaeum	<i>Alyssum minutum</i> Schl	1993.05.26	Bunardzik, pr. Filipopol	V. Střibrný	1
51	68	Museum Botanicum Universitatis, Cluj / Flora Romaniae exsiccata	<i>Alyssum arduini</i> Fritsch	1936.04.06	Oltenia, distr. Gorj 470 m	E. Pop	1
51	15	Museum Botanicum Universitatis, Cluj / Flora Romaniae exsiccata	<i>Alyssum borzaeanum</i>	1925.05.25	Transsilvania, distr Cluj 410 m alt	E.L. Nyárády	1

51	48	Bucharest's Polytechnics Herbarium, Silviculture Faculty/ Botanic Laboratory	<i>Alyssum repens</i> Baumg.	1943.08.01	Bucegi: Bucşoiu 2400 m	Al. Beldie	1
51	66	Museum Botanicum Universitatis, Cluj / Flora Romaniae exsiccata	<i>Alyssum rostratum</i> Stev.	1922.06.13	Dobrogea, distr. Tulcea 100 m	Al. Borza	1

***Alyssum alyssoides* L.** (Fig. 2) is an annual plant original from Europe and Asia but that was also introduced in the United States, Canada and Argentina (Dudley 1964). It can grow up to 35 cm, with a stem and erect stem, linear-obceolante leaves and obovate leaves that can reach 4 mm in length and are usually milky. The fruits are orbicular, emarginated or truncated, reaching 2,5-3,5 mm. The plant blooms between March-August and can also grow at altitudes of up to 2000 m (Akyol et al 2017). The seeds germinate during summer and early autumn, but only the seedlings that germinate during autumn survive (Baskin et al 1974). The plant is spread in dry, steppe and mountain areas.

***Alyssum borzaeanum* Nyár.** (Fig. 3) is an endemic species spread out along the Black Sea's shore, Ukraine, Turkey, North Greece and Bulgaria. In Bulgaria it can be found on a surface of approximately 100 km² and is inscribed in the Red List as endangered species. The plant is perennial and can reach 10-30 cm in height. The base leaves are covered with stellar, oblanceolate leaves, while the flowers are numerous and yellow. It usually grows in steppe pastures, sometimes even on rocky areas or on shores (<http://www.iucnredlist.org>). In Romania the plant was first mentioned by Nyárády in 1926 (Păunescu Anca 2008).

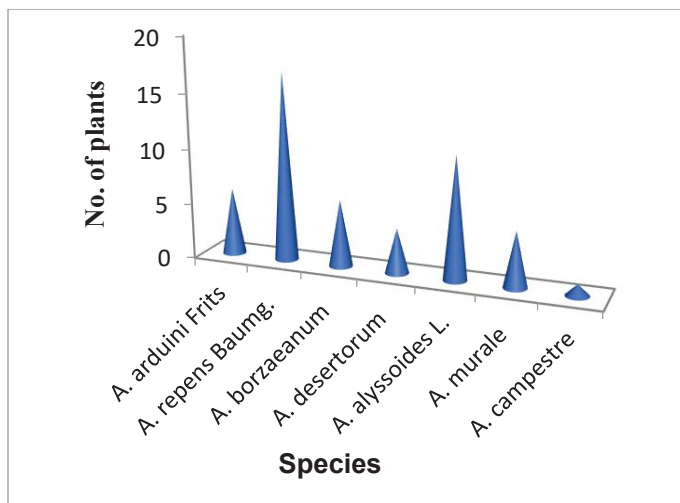


Figure 1. *Alyssum* Genus species present in the herbarium

Alyssum desertorum originates from Central and South-East Europe, as well as from Central and West Asia (Dudley 1964). The plant is annual and can grow up to 25 cm in height. The stem is covered with stellar hairs, while the leaves are oblanceolate, sessile and 0,5 up to 3 cm. The flowers range from pale yellow to white and can grow

up to 2 mm (<http://www.efloras.org/>). The plant blooms from April until June (Dudley 1964). It can be found in perturbed areas, arid or rocky and on slopes with altitudes between 700 and 1200 meters. It usually grows together with *A. simplex* and *A. alyssoides*.

Alyssum repens Baumg. (Fig. 4) preponderantly found in South-East and Central Europe, Turkey and Caucasus. The leaves are oblong-ovate up to lanceolate, while the flowers are yellow-orange in color and with a diameter of 4-5 mm. The plant blooms during summer, from June until September. In Romania the plant can be found in the Carpathians or Apuseni Mountains (Dudley 1966).

Alyssum murale Waldst. & Kit (Fig. 5) is a species with a long blooming period, from June until September. It is a perennial plant, with linear-oblic green leaves, while the flowers are small and yellow. It usually grows on poor, rocky and well drained soils. The plant reproduces easily, through seeds. It is usually found in South-East and Central Europe (Dudley 1966).

Alyssum murale is a species that hyper accumulates Nickel (Ni), and as such used for the phytoremediation of soils rich in heavy metals (Broadhurst Catherine et al 2016).



Figure 2 *Alyssum alyssoides*

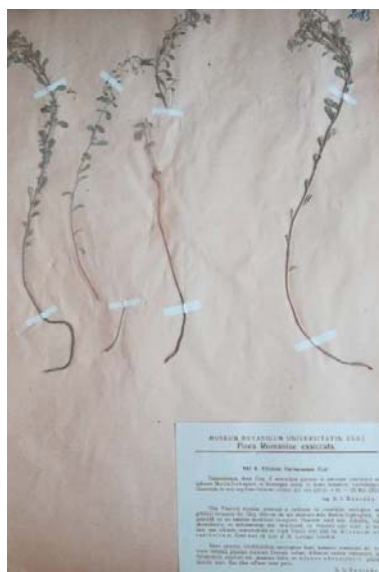


Figure 3 *Alyssum borzaeanum* Nyár.



Figure 4 *Alyssum repens*



Figure 5 *Alyssum murale* Waldst. & Kit.

The plant's gathering year. The *Alyssum* plants present in Al. Beldie Herbarium were collected between 1883 and 1993, namely on a time period of 110 years. The first plant that was collected was *Alyssum edentulum* W.K. in 1883 in Cernei Mountains. The periods in which most plants were gathered were 1920-1929 and 1930-1939, while the least plants were harvested during 1940-1980.

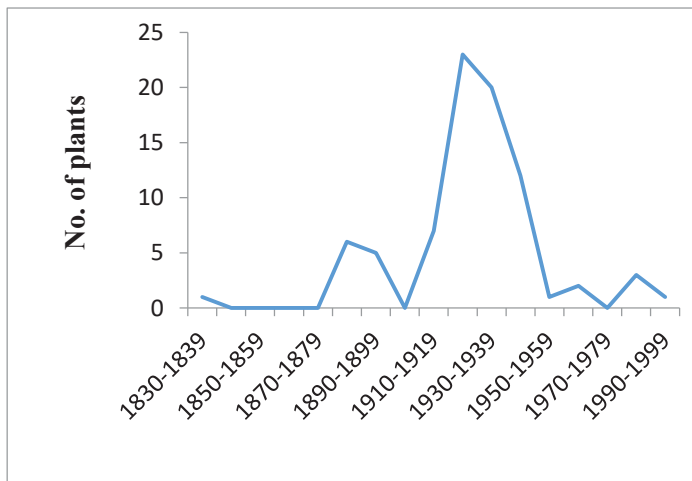


Figure 6. Harvesting periods of *Alyssum* Genus plants

The *Alyssum* plants were mainly gathered from Romania, namely from Banat, Bucegi Mountains, Mureș, Ilfov, Cluj, Buzău, Oltenia, Prahova etc (Fig. 7). Few plants were gathered from outside Romania, from countries such as Italy and France. Most plants were collected from Dobrogea.

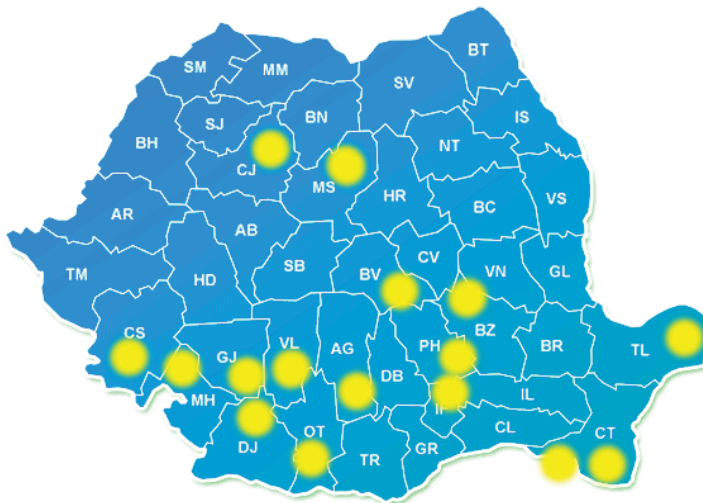


Figure 7. Place of harvest for Alyssum Genus in Romania

The plants were collected by Romanian and foreign specialist. Most plants were determined by Al. Borza and E.L. Nyárády. Amongst the renowned Romanian botanists who have collected plants from this Genus we mention Al. Beldie, C.C. Georgescu, E. Pop, I. Morariu, S. Paşcovschi, M. Ciucă, M. Haret, Şt. Purceleanu and T. Bunea. Amongst the foreign botanists we mention E. Reverchon, G.A. Poscharsky, H. Groves, J. Neuwirth, K. Richter, P. Ascherson etc.

CONCLUSIONS

From amongst the 195 *Alyssum* Genus species that can be found in Europe, Asia, North Africa and North America, 29 species were identified in “Al. Beldie” Herbarium. The plants were collected between 1833-1993, with a focus during 1920-1939. The oldest plant is *Alyssum edentulum* W.K harvested in 1833 from Cernei Mountains.

Most of the species were collected from Romania, especially from Dobrogea. The plants were kept in a very good conservation state. Within the species we can also find species from the Red List, such as *Alyssum borzaeanum* Nyár.

The most numerous species found in the Herbarium are *Alyssum repens* Baumg (17 plates), followed by *Alyssum alyssoides* L. (11 plates). They were gathered by renowned specialists such as Al. Beldie, E.L. Nyárády, S. Paşcovschi, C.C. Georgescu, M. Haret or T. Bunea.

REFERENCES

Akyol, Y., Kocabaş, O., Bozdağ, B., Minareci, E., Özdemir, C., 2017, Vascular anatomy of *Alyssum alyssoides* and *A. desertorum* (Brassicaceae) from Eastern Anatolia, Turkey. *Phytologia Balcanica: International Journal of Balkan Flora and Vegetation*, Vol. 23, No. 1, pp. 3-6.

Al-Shehbaz, I.A., 1987, The genera of Alysseae (Cruciferae;Brassicaceae) in the Southeastern United States. *Journal of the Arnold Arboretum* Vol. 68, No. 2, pp. 185-240.

Baskin, J. M., Baskin, C. C., 1974, Effect of vernalization on flowering of the winter annual *Alyssum alyssoides*. Bulletin of the Torrey Botanical Club. Vol. 101, No. 4, pp. 210-213.

Broadhurst Catherine L., Rufus L.C., 2016, Growth and Metal Accumulation of an *Alyssum murale* Nickel Hyperaccumulator Ecotype Co-cropped with *Alyssum montanum* and Perennial Ryegrass in Serpentine Soil. *Frontiers in Plant Science*, No. 7, pp. 451.

Cântar I.C., Dincă Maria., 2017, Doua genuri de plante din familia Poaceae (*Melica* si *Eragrostis*) existente in Herbarul Al. Beldie al INCDS Bucuresti. *JOURNAL of Horticulture, Forestry and Biotechnology*, Vol. 21, No. 3, pp. 68-76.

Crișan V., Dincă L., Oneț C., Oneț Aurelia, 2017, Collection species from *Potentilla* genus. *Natural Resources and Sustainable Development*, pp. 27-34.

Dincă L., Cântar I.C., Dincă Maria., 2017a, The characteristics of plant species from *Arabis* type present in Al. Beldie Herbarium from I.N.C.D.S. Bucharest. *Annals of West University of Timișoara, ser. Biology*, Vol. 20, No. 2, pp. 115-122.

Dincă L., Enescu Raluca., Oneț Aurelia., 2017b, Plant species from "Al. Beldie" Herbarium - *Veronica* genus - short description. *Natural Resources and Sustainable Development*, Vol. 9, pp. 43-50.

Dincă L., Dincă Maria, Pantea S., Timiș-Gânsac Voichița, Oneț C., 2018, *Amaranthus* plant - between myth and usage. *Natural Resources and Sustainable Development*, Vol. 8, No. 1, pp. 9-16.

Dincă Maria, Dincă L., Vasile Diana, 2017, A short description of *Androsace* genre plants present in Alexandru Beldie Herbarium from I.N.C.D.S. Bucharest. *Currents Trends in Natural Sciences*, Vol. 6, No. 12, pp. 16-24.

Dudley T.R., 1964, Synopsis of the genus *Alyssum*. *Journal of the Arnold Arboretum*. Vol. 45, No. 3, pp 358–373.

Dudley T.R., 1966, Ornamental madworts (*Alyssum*) and the correct name of the goldentuft *Alyssum*. *Arnoldia* Vol. 26, No. 6-7, pp. 33-45.

Li, Y., Kong, Y., Zhang, Z., Yin, Y., Liu, B., Lv, G., Wang, X., 2014, Phylogeny and biogeography of *Alyssum* (*Brassicaceae*) based on nuclear ribosomal ITS DNA sequences. *Journal of genetics*, Vol. 93, No. 2, pp. 313-323.

Păunescu Anca, 2008, Histological investigation of the secondary somatic embryogenesis of *Alyssum borzaeanum* (*Brassicaceae*). *PHYTOLOGIA BALCANICA*, Vol. 14, No. 1, pp. 111 –117.

Scărlătescu V., Vasile Diana, Dincă L., 2017, Plant species from "Al. Beldie" Herbarium - *Orobanche* genre - short description. *ProEnvironment Promediu*, Vol. 10, No. 31, pp. 191-198.

Vasile Diana, Dincă L., Indreica A., Voiculescu I., 2017, Herbarul Alexandru Beldie - o colecție de plante și o importantă bază de date pentru specialiști. *Revista de Silvicultură și Cinegetică*, 39, pp. 114-119

<http://www.efloras.org>

<http://www.iucnredlist.org>

THE FLORISTIC AND VEGETATION STUDY IN THE BANU MARACINE VITICULTURAL CENTER

Cocoloș (Firică) Marcela Alina^{1*}, Giugea Nicolae¹, Mărăcineanu Liviu¹

^{1*} University of Craiova, Craiova Faculty of Horticulture

* Correspondence author. E-mail: alinamfirica@yahoo.com

Keywords: weeds, plantation, vine, viticulture, areal

ABSTRACT

This paper presents an analysis of the seedlings found in the Banu Mărăcine viticultural center, the degree of spreading, as well as some aspects related to their biology and ecology. It was used the method of comparative observation on the evolution of weeds and the changes which appear due to climatic factors.

INTRODUCTION

The floral and vegetation study is a theoretical analysis in order to know the main seedlings of the central viticulture Banu Mărăcine. Demonstration of the connection between plants and living conditions is reflected in the area of spreading of different species and in the way of grouping of different species on different territories. To assess the climatic and edaphic conditions typical of a habitat, we use indicator plants, which can offer rigorous information and with much lower costs compared to chemical analyzes or meteorological measurements. It has been used to identify plantlets and mature plants "Determination of weeds in agricultural crops"

MATERIAL AND METHODS

The research method that led to the realization of this work was to prepare a work plan, the selection of the required bibliography, field trips to identify the species, collecting material for the determination and classification. By studying the weeds in the area we have compiled systematic files with information on the characteristics of plant recognition and their influence on vine culture. We determined the systemic classification based on the sampling of specimens in the mentioned area, assessing the degree of development of the plant according to the pedological factors and the performance of the agricultural works.

To write the paper we used bibliographic material regarding the researched area, botanical, systematic and geobotanic works with zonal character. To this we applied information accumulated by field observations and data taken from the trader and specialists. We considered a number of specialized works with relevant content for the topic, applied to the data from the study of the plants found in different areas of the Banu Mărăcine wine-growing center. In the assessment of the soil condition it was used the geobotanic survey method promoted by Chirilă C. and Micu I., 1971.

RESULTS AND DISCUSSIONS

In the vineyard plantations, the weeds presented are numerous both in diversity and in the relations of abundance and domination that are established between them. According to studies conducted to date, from the point of view of vegetation "the territory falls within the Quercinese forest vegetation area. The whole area has been covered in the past by forests, but by expanding agricultural areas, only isolated forests meet today, especially on the slopes of the valleys." (Popa A., Giugea N., Genoiu T.C., 2015) It should be noted that the primary grass vegetation does not imply a monotonous aspect, according to the above mentioned work, complex variety of species: *Festuca rupicola*, *Achillea mellifolium*, *Hordeum murinum*, *Lolium perenne*, *Cynodon dactylon*, *Trifolium repens* and others.

In the vineyard plantations, the weeds presented are numerous both in diversity and in the relations of abundance and domination that are established among them. It has been found that in well-grown plots of land, the weed impregnation is poor, while in those with poor quality works there is a high degree of weed enrichment. In the vineyards of Banu Mărăcine prevail the annual summer weeds, and from the perennial ones predominate those with rhizomes. Among the species identified by the research of each plot, the more numerous are the following: *Sorghum halepense*, *Convolvulus arvensis*, *Elymus repens*, *Cynodon dactylon*, *Cardaria draba* (Urda vacii), *Polygonum aviculare*, *Rumex crispus* and *Sonchus arvensis*. (Popescu Gh., Costache. I., Răduțoiu D., 2003).

In the weed species encountered in the Banu Maracine vineyard, the following characteristics are presented:

Sorghum halepense - The Poaceae family (Gramineae). Stony-planter plant with salty rhizome, which can reach 1.5 meters. Leaves linear-lanceolate, acuminate. It is multiplied by seeds, a plant producing 1200-6000 seeds. It prefers all types of soil, especially alluvial, thermophilic, found in the continental plain of the plain. (Andrei M., 1997).

Elymus repens - Poaceae Family (Gramineae). The plantula has a linear narrow leaf of 80-100 mm in length and one mm in width with stiff, short bristles. The mature plant has rhizomes in the upper layer of the soil, mostly between 2 and 12 cm deep. The adventives roots reach up to one meter deep underground. The rizomes are renewed annually and their activity lasts for 12-15 months after which they die. In one square meter can be found up to 3.000 grams of rhizomes, the total length of which ranges from 4 to 6 meters, and which have together over 25,000 buds (Chirilă C., 2001). The stem is one meter high, and the lower leaves have hairy patent vagina. The ligula is short and the auricles are narrow, long and surround the stem, especially on the last leaf. The inflorescence is a compound spike. It blooms from June to August. One plant produces about ten thousand kernels.

Convolvulus arvensis (Fig. 1) Convolvulaceae Family. The plant has epigenous cotyledons, long petiolate, with a pointed tip, slightly longer than wide. The hypocotyl is dirty-reddish. The mature plant has a highly developed root system. From this, radicular buds can form many suckers. The stem is volubilous when it has support, or creeping when it has no support. The leaves are hatched or sagitt. The fruit is a capsule. One plant produces 500-600 piriform seeds. It is frequently met and often abundant on all soil types and in all cultures. It is frequently multiplied and vegetative by stolons. (Crăciun I., 1989).



Fig. 1: *Convolvulus arvensis* with *Rumex pulcher* Fig. 2: *Sonchus arvensis*

Sonchus arvensis (Fig. 2) is a perennial, polycarpical weed, with suckers of the Asteraceae family. It is multiplied by radicular shoots and seeds. The first leaves have a wide elliptical tongue, the edge being toothed and the following are narrower. The leaves are runny, glabrous, thorny on the edges (Ciocârlan V, Chirilă C., 1982, Determinatorul buruienilor din culturile agricole, Ed. Ceres, București). The root penetrates into the soil up to 50 cm deep and produce horizontal branches up to a meter long that are kept at a shallow depth (6-15 cm from the surface of the soil). The germination is done in late spring, when the soil is worm. It blooms and capitulates from June - July until September.

Cirsium arvense a species with a high generative multiplication capacity but also with highly active vegetative propagation through suckers, is spread in all vineyards in the country. The stem can often exceed 1.5 meters in height. The fruit is an anchen. It blooms from June to August. (Sîrbu C., 2003)

CONCLUSIONS

It can be concluded that not all plant species meeting in the Banu Mărăcine viticultural center are harmful, as it results from the comparative analysis of the degree of soil enrichment on different surfaces. As it emerges from the study by Șarpe N. 1987, weed species are framed in two sections: weed species common in almost all vineyards in the country and uncommon weed species. In the plantation there is a multiplication of a gradual multiplication of weeds with very high harmful potential, such as *Sorghum halepense* and *Dragon Cardia*. The floral inventory drawn up after the verdict can serve as a basis for further studies showing the dynamics of flora under the influence of edaofclimatic and anthropogenic factors on the researched field.

REFERENCES

- Andrei M., 1997. Morfologia generală a plantelor, Ed. Enciclopedică, București
- Ciocârlan V, Chirilă C., 1982. Determinatorul buruienilor din culturile agricole, Ed. Ceres, București
- Chirilă C., 2001. Biologia buruienilor. Organografie, Corologie, Dinamică, Importanță., Ed. Ceres, București, pag. 303
- Chirilă C. și Micu I., 1971. Contribuții la cunoaștere buruienilor din podgoria Dealu Mare
- Crăciun I., 1989. Dicționar de biologie, Ed. Albatros, București
- Popa A., Giugea N., Genoiu T.C., 2015. Oltenia, mica Românie viticolă, Ed. Aius, Craiova
- Popescu Gh., Costache. I., Răduțoiu D., 2003. Buruienile din plantațiile pomi-viticole și grădinile de legume de la Ferma Tâmburești a SDE Banu-Mărăcine. Aplicații tehnologice „Ghid de practică”. Tipografia Universității din Craiova
- Sîrbu C., 2003. Podgoriile Cotnari, Iași și Huși, Edit. Ion Ionescu de la Brad, Iași.
- Șarpe N., 1987. Combaterea integrată a buruienilor din culturile agricole, Edit. Ceres, București.

THE BIODEGRADATION OF RESIDUE GREEN ROBUSTA AND ARABICA COFFEE IN SOIL DURING INCUBATION, AND THEIR EFFECTS ON SOIL CHEMICAL PROPERTIES

Gougoulias Nikolaos^{1*}, Wogiatzi Eleni¹, Giannoulis Kyriakos¹, Chounta Stamatina¹, Salonikioti Afroditi¹, Chouliara Adamandia²

¹Department of Agronomy Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece

²Informatics' Department, Informatics' Scientist, data exploration, Macedonia University of Thessaloniki, Greece

*Correspondence author. email: ngougoulias@teilar.gr

Keywords: *Green coffee; soil chemical properties; waste*

ABSTRACT

The present work was carried out in vitro (incubation experiment), and 1.25 and 2.5 g of air dried and well milled residue green Arabica coffee or residue green Robusta coffee, were applied to 50 g of soil, respectively. All treatments were incubated for 15 weeks at a constant temperature of 28 °C and after the incubation period, suitable chemical analyses were performed. The results of the experiment showed that the residue Arabica added in soil, are subjected to same degradation, in comparison to the residue Robusta, after a long incubation period. The residue Arabica as compared with of residue Robusta, increased the content of the available forms P, Cu and Zn, while and both materials increased the content of samples in available forms K, Fe and Mn, in comparison to the control (soil). These results, confirm the role of residue green coffee (Arabica or Robusta) as organic soil amendment.

INTRODUCTION

In modern alternative forms of agriculture is mandatory to replace chemicals with natural additives. The addition of various materials in the soil, affects the composition and biological activity of microflora, which determines the biochemical status of soil fertility (Gougoulias et al. 2014). When applying of organic fertilizers in the soil, dominate microbial nutrition (Gougoulias et al. 2013, Riber et al. 2014). Furthermore, by using composts prepared with agricultural wastes improves soil fertility and protect environment (Poulsen et al. 2013).

Literature on coffee waste as a soil amendment is limited, however studies have shown that coffee waste is a valuable organic fertilizer, particularly for sandy soils (Zake et al. 2000; Kasongo et al. 2011).

Green coffee beans contain phenolic compounds, and tocopherols, which exert strong antioxidant effect (Farah and Donangelo, 2006).

The objectives of this research were to study in vitro, the biodegradation in soil of the green Robusta and Arabica coffee solid residue, obtained after the removal of the extract with hot water, and the consequences of that biodegradation on chemical properties of soil.

MATERIAL AND METHODS

Commercial green Robusta and Arabica coffee beans from the local market in Larisa, were dried in a dark place at room temperature, finely ground, shaken at 150 rpm for 30 min using boiling water. The extraction of the green Robusta coffee with boiling water solvent results in the extraction of the polyphenols, where can find applications either in the form of powder or as extracts highly concentrated at polyphenols. The green coffee solid residue, which remained after the removal of the extract, was air dried and was used for the incubation experiment.

Incubation experiment: In this study, 1.25 g and 2.5 g air-dried and milled residue green Robusta coffee or Arabica were applied to 50g of soil respectively, (Table 1) and their effects on the chemical properties of soil, after 15 weeks of incubation at 28 °C were studied. Thus an experimental unit is constituted by 50 g of soil, and a variable amount of residue green Robusta coffee or Arabica. The experimental design was completely randomized with four replications. During the first three weeks of the incubation period, the moisture was maintained at two-thirds of field capacity, but for the next three weeks the soils were left to dry. This process was repeated until the end of the incubation period according to (Wu and Brookes, 2005) they reported that the alternation of drying and rewetting soil samples enhances mineralization of both soil biomass organic matter and non-biomass organic matter. At the end of the incubation period, soil samples were analyzed.

Methods of analyses: Samples were analyzed using the following methods which are referred by (Page et al. 1982).

Organic matter was analyzed by chemical oxidation with 1 mol/l $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol/l $FeSO_4$.

Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively. Available P forms (Olsen P) was extracted with 0.5 mol/l $NaHCO_3$ and measured by spectroscopy. Exchangeable forms of potassium and sodium were extracted with 1 mol/l CH_3COONH_4 and measured by flame photometer.

Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol/l H_2SO_4 and all forms were measured by spectroscopy.

Available forms of Mn, Zn, and Cu were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol/l + $CaCl_2$ 0.01 mol/l + triethanolamine 0.1 mol/l) and measured by atomic absorption.

For the determination of total metals Mn, Cu and Zn, 1 g of material, digestion at 350 °C + 10 ml HNO_3 + 5 ml $HClO_4$. According to the method described by (Varian, 1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus), with the use of flame and air-acetylene mixture.

Statistical analysis: Data analysis was made using the MINITAB (Ryan et al. 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences ($P=0.05$) between treatments were found.

Table 1

Chemical properties of soil samples, residue green Robusta or Arabica coffee used in the experiment

Property	Soil	Residue green Arabica coffee	Residue green Robusta coffee
Texture	Sandy Loam		
pH	7.84 ± 0.3		
* EC, dS/m	0.48 ± 0.06		
Organic matter (%)	0.87 ± 0.06		
CaCO ₃ (%)	8.48 ± 0.5		
N -Total (g/kg)	1.28 ± 0.12	19.98 ± 0.6	20.79 ± 0.9
N-NH ₄ ⁺ (mg/kg)	49.4 ± 9.8		
N-NO ₃ ⁻ (mg/kg)	118.2 ± 15.2		
K-exchangeable (mg/kg)	229.6 ± 7.7		
K-Total (g/kg)	4.75 ± 0.26	0.36 ± 0.02	0.43 ± 0.02
Na-exchangeable (mg/kg)	232.3 ± 9.6		
Na-Total (g/kg)	0.38 ± 0.05	0.172 ± 0.09	0.17 ± 0.02
CEC (cmol/kg)	19.8 ± 1.3		
P -Olsen (mg/kg)	13.5 ± 3.8		
P -Total (g/kg)	0.34 ± 0.06	1.183 ± 0.06	1.51 ± 0.09
Cu -DTPA (mg/kg)	0.81 ± 0.05		
Zn -DTPA (mg/kg)	1.01 ± 0.09		
Mn -DTPA (mg/kg)	2.75 ± 0.19		
Cu -Total (mg/kg)	10.33 ± 1.11	9.27 ± 0.56	3.97 ± 0.22
Zn -Total (mg/kg)	39.45 ± 1.72	5.49 ± 0.28	0.12 ± 0.01
Mn -Total (mg/kg)	532 ± 31.3	2.90 ± 0.16	2.02 ± 0.14
Fe-Total (mg/kg)		62.61 ± 3.13	99.18 ± 5.22
Mg-Total (mg/kg)		1586.9 ± 75.6	1351.5 ± 61.4

*Electrical conductivity, (EC) and soil pH is determined in (1:5) soil/water extract; Data represent average means and SE deviation. (n)=4.

RESULTS AND DISCUSSIONS

The results of the laboratory experiment at the end of the incubation period, they showed a high rate of biodegradation of organic matter from the application of the two rates of residue green Arabica or Robusta coffee compared with control (soil). The biodegradation of organic matter of residue green Arabica and Robusta coffee ranged from 67.9 to 70.6 %, while the control they showed a biodegradation of the organic matter by 22.9 % (Figure 1). The increased biodegradation of soil organic carbon probably was due to increased microbial activity. In similar laboratory studies, it was found that incorporation of coir residue on soil, after his use as substrate, they showed the lower rate of biodegradation of organic matter, about 35 % (Gougoulas et al. 2017).

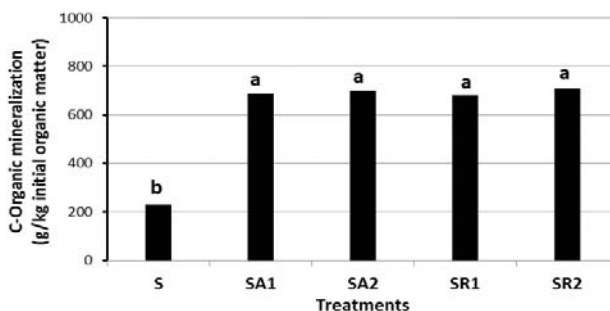


Figure 1. Effect of residue green Arabica and Robusta coffee on soil content in organic matter. (Bar values with the same letter on the top are not significantly different according to Tukey's test ($P > 0.05$)); S, control (soil); SA1, Arabica 1.25 g per 50 g of soil; SA2, Arabica 2.5 g per 50 g of soil; SR1, Robusta 1.25 g per 50 g of soil; SR2, Robusta 2.5 g per 50 g of soil.

The available forms of P were increased by Arabica or Robusta treatments, the greatest increase was observed when residue green Arabica coffee were added (Table 2). Nitrate forms increased by the addition of residue Arabica or Robusta, the greatest increase was observed when green Arabica coffee were added, at the higher dose. Moreover, ammonium content increased by the addition of residue Arabica or Robusta treatments (Table 2). The exchangeable Na content either by the addition of residue green Arabica coffee or by the addition of residue green Robusta coffee, was not showed significantly differences. The exchangeable K content were increased by residue green Arabica coffee or Robusta, the greatest increase was observed when residue at the higher doses were added (Table 2). The addition of residue green Arabica coffee or Robusta increased electrical conductivity where residue at the highest dose was added. Contrary, the addition of residue green Arabica coffee or Robusta reduced the pH of the soil compared to control. The decrease of soil pH at the end of the incubation period, probably is due to the stronger decomposition of soil organic matter, and in the oxidation of the $N-NH_4^+$ to $N-NO_3^-$ (Table 2).

Table 2
Chemical properties of soil mixtures at the end of the incubation period

Treatments	Property						
	Available forms			Exchangeable forms		Extract 1: 5 in H_2O	
	(mg kg^{-1} soil)			(mg kg^{-1} soil)		(dS m^{-1})	
	P-Olsen	N- NH_4^+	N- NO_3^-	Na	K	EC	pH
S	14.1c	25.9b	82.5c	184.2a	282.8c	0.51c	7.71a
SR1	35.1b	110.3a	337.9b	185.0a	430.8b	0.53c	7.00b
SR2	37.4b	130.5a	680.0a	185.3a	657.3a	0.82a	7.10b
SA1	82.83a	134.0a	353.4b	187.4a	429.0b	0.54c	7.31b
SA2	75.07a	138.1a	314.7b	191.2a	631.8a	0.65b	7.23b

S, control (soil); SA1, Arabica 1.25 g per 50 g of soil; SA2, Arabica 2.5 g per 50 g of soil; SR1, Robusta 1.25 g per 50 g of soil; SR2, Robusta 2.5 g per 50 g of soil; Columns with the same letter do not differ significantly according to the Tukey's test ($P=0.05$).

The addition of residue green Arabica coffee increased both the Cu-available and Zn-available forms at the end of the incubation period compared to the control (Table 3). The addition of residue green Arabica coffee or Robusta increased Mn-available forms and Fe-available forms, the greatest increase was observed where the higher doses was added (Table 3).

Table 3

Chemical properties of soil mixtures at the end of the incubation period

Treatments	Property			
	DTPA - (mg kg ⁻¹ soil)			
	Cu	Zn	Mn	Fe
S	1.20b	1.03b	2.09c	0.62c
SR1	1.27b	1.17b	4.97b	0.99b
SR2	1.27b	1.21b	5.97ab	1.58a
SA1	1.67a	1.79a	5.70b	1.02b
SA2	1.67a	1.58a	6.60a	1.31a

S, control (soil); SA1, Arabica 1.25 g per 50 g of soil; SA2, Arabica 2.5 g per 50 g of soil; SR1, Robusta 1.25 g per 50 g of soil; SR2, Robusta 2.5 g per 50 g of soil; Columns with the same letter do not differ significantly according to the Tukey's test (P=0.05).

Total forms of Na and Mn increased by the addition of residue green Arabica coffee or Robusta compared to the control at the end of the incubation period (Table 4). Also, the addition of residue green Arabica coffee or Robusta increased both the total K and total P, the greatest increase was observed where the higher doses were applied. The addition of residue green Arabica coffee or Robusta increased both the Cu-total and Zn-total forms at the end of the incubation period compared to the control. The greatest increase was observed where the residue green Arabica coffee was added (Table 4).

Table 4

Total forms of elements of the soil mixtures at the end of the incubation period

Treatments	Property						
	Total forms - (mg kg ⁻¹ soil)						
	Na	K	P	Cu	Zn	Mn	P-organic
S	389.6b	4760.4c	362.1c	11.20d	41.20d	556.8b	87.40d
SR1	413.7a	5448.1b	482.9b	14.83c	47.67c	628.7a	374.8a
SR2	413.7a	6226.4a	582.9a	14.49c	55.09b	673.5a	149.9b
SA1	413.1a	5752.3b	487.3b	18.10b	59.88ab	646.2a	149.8b
SA2	430.8a	5940.4ab	515.9a	29.41a	64.39a	660.4a	100.0c

S, control (soil); SA1, Arabica 1.25 g per 50 g of soil; SA2, Arabica 2.5 g per 50 g of soil; SR1, Robusta 1.25 g per 50 g of soil; SR2, Robusta 2.5 g per 50 g of soil; Columns with the same letter do not differ significantly according to the Tukey's test (P=0.05).

CONCLUSIONS

The results of the experiment showed that the residue Arabica added in soil, are subjected to same degradation, in comparison with the residue Robusta, after a long incubation period. The residue Arabica compared with of residue Robusta, increased the content of the samples at the available forms P, Cu and Zn. However, exchangeable forms of Na, available forms of K, Fe and Mn was not significantly affected by of residue Robusta and Arabica addition. These results, confirm the role of residue green Arabica coffee or Robusta as organic soil amendment, and very possibly is important components for composting products, while that recycling constitutes a useful practice for environment protection.

REFERENCES

- Farah A., Donangelo C.M., 2006, Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology*, 18(1), 23-36.
- Gougoulas N., Vagelas I., Papachatzis A., Stergiou E., Chouliaras N., Chouliara A., 2013, Chemical and biological properties of a sandy loam soil amended with olive mill waste, solid or liquid form, in vitro. *International journal of Recycling of Organic waste in Agriculture*, 2(1),13.
- Gougoulas N., Vagelas I., Papachatzis A., Kalfountzos D., Giurgiulescu L., Chouliara A., 2014, Mixture of solid with water soluble, olive oil mill waste application, as soil amendment in greenhouse cultivation of vegetables (Case study). *Carpathian Journal of Food Science and Technology*, 6(2), 63-68.
- Gougoulas N., Vagelas I., Giurgiulescu L., Touliou E., Kostoulis V., Chouliara A. (2017). The coir substrate for soilless cultures, reused as soil amendment (study in vitro and in vivo). *Carpathian Journal of Food Science and Technology*, 9(4), 61-70.
- Kasongo R.K., Verdoodt A., Kanyankagote P., Baert G., Ranst E.V., 2011, Coffee waste as an alternative fertilizer with soil improving properties for sandy soils in humid tropical environments. *Soil use and Management*, 27(1), 94-102.
- Page A.L., Miller R.H., Keeney D.R.,1982, *Methods of Soil Analysis. Part 2, Chemical and Microbiological properties.* Agronomy (9), ASSSA, Mad. Wisc. USA.
- Poulsen P.H.B., Magid J., Luxhøi J., de Neergaard A., 2013, Effects of fertilization with urban and agricultural organic wastes in a field trial—waste imprint on soil microbial activity. *Soil Biology and Biochemistry*, 57, 794-802.
- Riber L., Poulsen P.H., Al-Soud W.A., Hansen L.B.S., Bergmark L., Breynd A. and Sørensen S.J., 2014, Exploring the immediate and long-term impact on bacterial communities in soil amended with animal and urban organic waste fertilizers using pyrosequencing and screening for horizontal transfer of antibiotic resistance. *FEMS microbiology ecology*, 90(1): 206-224.
- Ryan B.F., Joiner B.L., Cryer J.D., 2005, *MINITAB Handbook: Updated for release 14*, 5th edition. Brooks/Cole-Thomson Learning Inc., Kentucky, KY.
- Varian M., 1989, *Flama Atomic Absorption Spectroscopy.* Analytical Methods. Varian Australia. Publ. NO: 85-100009-00.
- Wu J. and Brookes P.C., 2005, The proportional Mineralisation of Microbial Biomass and Organic Matter caused by air-drying and rewetting of a grassland soil. *Soil Biology & Biochemistry*, 37: 507-515.
- Zake Y.K., Bwamiki D.P., Nkwiine C., 2000, Soil management requirements for banana production on heavy soils around Lake Victoria in Uganda. *Acta Horticulturae (ISHS)*, 540, 285– 292.

DESCRIBING FOREST SOILS FROM GALATI COUNTY

Deleanu Elena^{1*}, Ionescu Monica¹, Lucaci Dora²

¹ „Marin Dracea” National Institute for Research and Development in Forestry, Bucuresti

² „Marin Dracea” National Institute for Research and Development in Forestry, Braşov

*Correspondence author. e-mail: mdeleanuelena@gmail.com

Keywords: *soil properties, forest soil description, Galaţi County*

ABSTRACT

The aim of this paper is to describe forest soils from Galati County, on the basis of the soil analysis data from forest management plans made in the period 1985-2013, namely 134 soil profiles and 423 pedo-genetic horizons. The data is part of a huge national database created by “Marin Dracea” National Institute for Research and Development in Forestry. The most widespread types of soils are phaeozem (24 %) and fluvisols (20 %), followed by preluvsols (19 %), arenosols (13 %) and chernozem (11 %). Other types of soils identified in this County are: luvisols, regosols, erodosols and vertisols.

INTRODUCTION

For the year 2015 the total surface occupied by forests in Galaţi County is of 37157 ha (www.insse.ro). Galaţi Forest Administration has in its management 4 Forest Districts: Galaţi, Griviţa, Hanul Conachi and Tecuci. As such, this Administration manages a total forest surface of 20552 ha, divided as it follows: resinuous 1 %, common beech 1 %, oak 16 %, diverse hard species 65 % and diverse soft species 18 % (www.romsilva.ro).

Knowing these characteristics is extremely important in order to adopt adequate silvicultural measures (Târziu et al., 2004, Spârchez et al., 2011).

As such, the purpose of the present paper is to describe the soils belonging to the forest fund from this County.

MATERIAL AND METHOD

The present paper intends to monitor the physical-chemical properties of forest soils from Galati, starting with the year 1985 up to present days.

The analysed properties were: pH, carbonates content, humus content, nitrogen content, the basis exchange capacity, hydrogen exchange capacity, total cationic exchange capacity, base saturation degree and texture. All these analysis are centralized in the analysis bulletins and are part of an extended national database realized by INCDS “Marin Dracea”, based on forest management plans. The accredited national and international methodologies were used as methods in the analysis of soil samples (Dincă L. et al., 2012, Edu et al., 2013). For the present paper, soil samples gathered in the period 1985-2013 from 4 forest districts belonging to Galati Forest District were analysed. As a total, 134 soil profiles and 423 pedo-genetical horizons were analysed (Amenajamentele Ocoalelor Galati, Griviţa, Hanul Conachi, Tecuci).

RESULTS AND DISCUSSIONS

Types of soils from Galati County. First and foremost, the existent soil types and their predominance were taken into account. With this purpose in mind, the following diagram was realized in order to emphasize the predominance of each encountered type of soil (Figure 1).

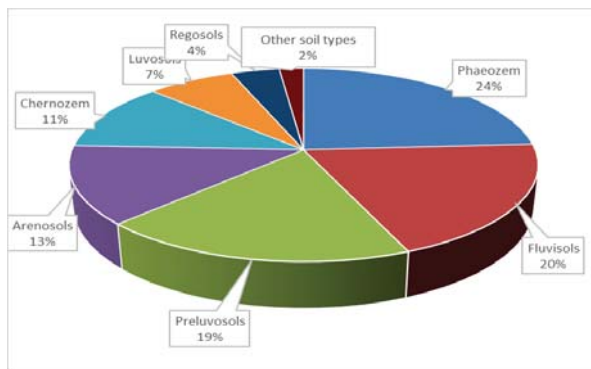


Figure 1. The predominance of forest soils identified in Galati County

As such, the most widespread soil types are the ones from Protisols class that occupy 37 % of the soils from this County, followed by Cernisols class with 35 % and Luvisols class with 26 %. The most widespread types of soils are phaeozem (24 %) and fluvisols (20 %), followed by preluvosols (19 %), arenosols (13 %) and chernozem (11 %). Other types of soils identified in this County are: luvisols, regosols, erodosols and vertisols.

At a national level, luvisols occupies the second place as spreading in forest soils (1.440.052 ha), preluvosols the fifth place (335.050 ha,) and phaeozem the sixth place (235.282 ha) (Dincă L. et al., 2014).

A diagram for the soil reaction was realized for the most widespread soils (phaeozem, fluvisols, preluvosols, chernozem), differentiated on genetic horizons (Fig. 2).

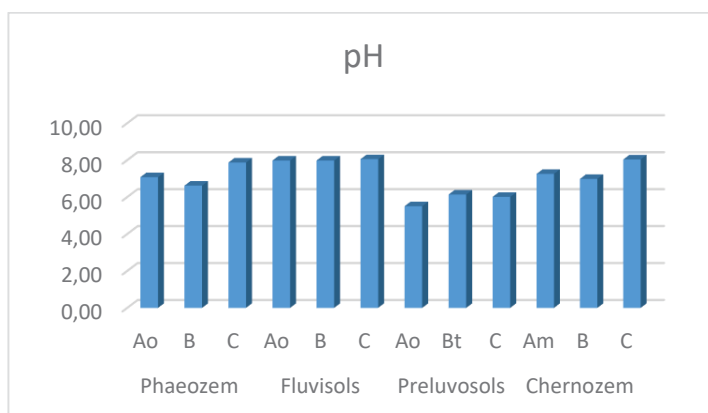


Figure 2. pH variations for the most widespread forest soils from Galati County

The lowest pH average values for the horizons are found for preluvosols (5,51 in the Ao horizon, 6,14 in Bt, 6,01 in C), while the highest values were identified for

fluvisols, recording a value of 8. Phaeozem and chernozem have very close values. By interpreting the average pH values, the reaction is neutral towards alkaline.

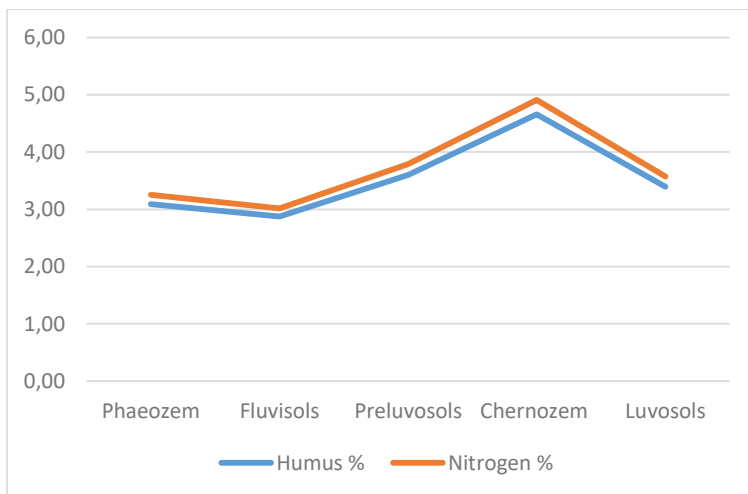


Figure 3. The variation of humus and nitrogen for the most widespread forest soils from Galati County

Average values per profile were calculated in order to obtain the total cationic exchange capacity. The results are then rendered per soil type (Table 1). Chernozem has a very high total cationic exchange capacity, followed by phaeozem, luvosols, preluvosols and fluvisols, whose cationic exchange capacity is also high.

A very important soil parameter is the humus content. As such, for each soil type identified in Galati County, an average value of humus content in the A horizon was calculated (Table 1).

Table 1.

Total cationic exchange capacity and average humus and nitrogen content for forest soils from Galati County

Phaeozem	Fluvisols	Preluvosols	Chernozem	Luvosols
Total average cationic exchange capacity per type of soil (T-me 100 g ⁻¹ soil)				
25,68	17,90	19,72	27,09	19,85
Average humus content in A horizon (H-%)				
3,09	2,87	3,60	4,66	3,39
Average nitrogen content in A horizon (%)				
0,16	0,14	0,19	0,25	0,18

The highest content of humus is registered for chernozem, followed by preluvosols and luvosols, being moderately humiferous soils (Dănescu et al. 1996). The humus values for the first horizon in forest soils from Olt County was of 4,20 % for preluvosols (Deleanu et al., 2017). As a conclusion, the average humus content for soils from Galati County is generally registered within the limits established at a national level in other investigations regarding forest soils: preluvosol = 31,5 g/kg, phaeozem = 30,2 g/kg, luvosol = 27,5 g/kg (Dinca et al. 2012). Similar with humus, nitrogen was only

calculated in the first horizon, as both elements are accumulated through the decomposition of organic matter at the surface and in the first centimetres of the soil's profile (fig. 3). The lowest quantity of nitrogen is accumulated by fluvisols, which is in this case a well-supplied soil with this element, while all the other soils are very well supplied with nitrogen. A graphic was created for the degree of saturation in basis (V), for the most widespread types of soils (Figure 4).

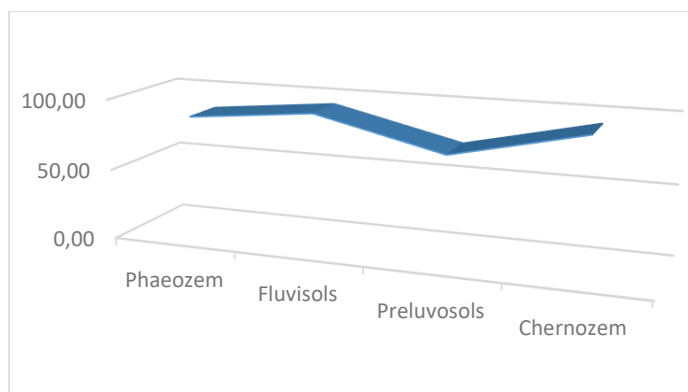


Figure 4. The variation of the base saturation degree for the most widespread forest soils from Galați County

As such, it can be observed that the largest variation amplitude is found in the case of fluvisols and chernozem, while the lowest is recorded for preluvosols. For phaeozem, V registers average values of 85,81 %.

CONCLUSIONS

Forest soils from Galați County belong preponderantly to the Protisols, Cernisols and Luvisol classes. The most widespread types of soils are phaeozem (24 %) and fluvisols (20 %), followed by preluvosols (19 %), arenosols (13 %) and chernozem (11 %). Other types of soils identified in this County are: luvosols, regosols, erodosols and vertisols.

Chernozem has a very high total cationic exchange capacity, followed by phaeozem, luvosol, preluvosol and fluvisol, whose cationic exchange capacity is recorded in the high category.

The highest content of humus is recorded for chernozem, followed by preluvosols and luvosol, situated in the moderately humiferous category.

The lowest quantity of nitrogen is accumulated by fluvisols, which is in this case a well-supplied soil with this element, while all the other soils are very well supplied with nitrogen.

REFERENCES

- Dănescu F., Surdu A., Preda I., Bicu C., Păun R., 1996, Modificările pozitive înregistrate de însușirile fizice și hidrofizice ale solului în UP I Letca – OS Ghimpați, ca urmare a aplicării tehnologiei de refacere a arboretelor ce include afânarea adâncă a solului, *Revista Pădurilor*, 1.
- Deleanu E., Ionescu M., Lucaci D., 2017, Data regarding forest soils from Olt county, *Analele Universității din Craiova, seria Agricultură – Montanologie – Cadastru Vol. XLVII 2017*, p. 284-290.
- Dincă L., Lucaci D., Iacoban C., Ionescu M., 2012, Metode de analiză a proprietăților și soluției solurilor. Editura Tehnică Silvică, p. 173.

Dincă L., Spârchez, Gh., Dincă, M., Blujdea, V., 2012, Organic carbon concentrations and stocks in Romanian mineral forest soils. *Annals of Forest Research*, 55 (2), p. 229-241.

Dincă L., Spârchez G., Dincă M., 2014, Romanian's forest soil GIS map and database and their ecological implications. *Carpathian Journal of Earth and Environmental Sciences*, 9 (2): p. 133-142.

Edu (Deleanu) E.M., Mihalache M., Ionescu M., 2013, Determination of organic carbon in forest soils by comparative analysis of methods: Walkley Black method with the Gogoasa modification versus dry combustion dumars method, *Research Journal of Agricultural Science, Timișoara*, Vol 45, nr 1, p. 13-19.

Spârchez G., Târziu D.R., Dincă L., 2011, *Pedologie*. Editura Lux Libris, Brașov, p. 293.

Târziu D., Spârchez G., Dincă L., 2004, *Pedologie cu elemente de Geologie*. Editura Silvodel, Brașov, p. 343.

***Amenajamentele Ocoalelor Galati (1987, 1994, 2013), Grivița (1996, 2006), Hanul Conachi (1994, 2004), Tecuci (1985, 1996, 2006).

*** www.insse.ro

*** www.romsilva.ro

PLANTAGO GENUS COLLECTION SPECIES PRESENT IN ALEXANDRU BELDIE HERBARIUM

Deleanu Elena^{1*}, Ionescu Monica¹ Dinca Maria²

¹„Marin Drăcea” National Institute for Research and Development in Forestry, Bucuresti

²„Marin Drăcea” National Institute for Research and Development in Forestry, Braşov,

*Correspondence author. e-mail: mdeleanuelena@gmail.com

Keywords: *Plantago*, botanists, plants, leaves.

ABSTRACT

The present paper describes morphologically and ecologically the *Plantago* species present in “Alexandru Beldie” Herbarium from “Marin Drăcea” National Institute for Research and Development in Forestry (INCDS) from Bucharest. The Herbarium contains 231 plates of this genre that belong to 48 species. Some representative species of this genre are then described (*Plantago lanceolata* L., *Plantago media* L., *Plantago major* L., *Plantago maritima* L., *Plantago coronopus* L.). Furthermore, statistics and diagrams concerning the place and year of harvest are also present, together with annotations made by the botanists that have gathered them.

INTRODUCTION

The Alexandru Beldie Herbarium from *Marin Drăcea* National Institute for Research and Development in Forestry (INCDS) located in Bucharest, contains an impressive collection (40 000 plates) of certain plants, especially from mountain areas. As such, some of the mountain plants present in the Herbarium are the 32 *Arabis* genre species (Dincă et al., 2017), 15 *Ornithogalum* species (Enescu R. et al., 2017), 15 *Veronica* species (Dincă et al., 2017), 69 *Potentilla* species (Crişan et al., 2017), 19 *Androsace* species (Dincă et al., 2017), 11 species of *Eragrostis* genus (Cântar C. et al., 2017) or the 112 *Hieracium* species (Dincă et al., 2017).

The plants from the collection are kept in their original folders and are arranged in 600 drawers (Vasile et al., 2017). The herbarium has been inscribed in INDEX HERBARIUM and includes a huge number of plants coming from private donations and from foreign collections.

The aim of this article is to present the state of this collection, to describe the species, the total number of *Plantago* specimens (48 species), together with the date when they were collected, their location, the botanist who gathered each exemplar and their conservation degree.

MATERIALS AND METHODS

The study material was represented by the 231 plates present in the above mentioned Herbarium and belonging to the *Plantago* genus.

The plates were grouped by species, harvest year, the place where they were harvested and by the specialist who gathered them. Table number 1 renders a small part from the database.

Table 1

The inventory of *Plantago* genus from INCDS Bucharest's Al. Beldie Herbarium (excerpt)

Plate no.	Drawer no.	Herbarium/ Botanic collection/ Institution	Species	Harvest date	Harvest place	Collected/ Determined by:	Conservation degree (1..4)
48	17	Bucharest Polytechnics Herbarium, Silviculture Faculty, Botanic Laboratory	<i>Plantago montana</i> L.	1942.06.21	Bucegi, V. Jepilor	Beldie Alexandru	1
48	55	ICEF Forestry Research and Experimentation Institute	<i>Plantago lanceolata</i> L.	1938.06.06	Mures	S. Pascovschi	1
48	59	ICEF Forestry Research and Experimentation Institute	<i>Plantago lanceolata</i> L.	1936.08.09	Tulcea, Nisipuri	Haralamb P. Cretzoiu	1
48	105	Bucharest Polytechnics School Herbarium, Botanic Laboratory	<i>Plantago arenaria</i>	1931.07.12	Dobrogea	C. C. Georgescu	2
48	112	Bucharest Polytechnics School Herbarium, Botanic Laboratory	<i>Plantago indica</i> L.	1935.06.28	Tecuci	C. C. Georgescu si P. Cretzoiu	1
70	13	Herbarium Normale editum ab I. Dorfler	<i>Plantago ramosa</i> (Gilib.) Asch.	1909.07.01	Austria Stadlau	L. Keller	1
70	33	Bucharest Polytechnics Herbarium, Silviculture Faculty	<i>Plantago media</i> L.	1943.07.01	Bucegi, Poiana Costilei	Al. Beldie	1
70	48	ICEF	<i>Plantago media</i> L.	1935.05.26	Stoiceni	At. Haralamb	1
70	75	ICEF	<i>Plantago media</i> L.	1933.01.01	Parang	At. Haralamb, J. Neuwirth	1

RESULTS AND DISCUSSION

Plantago is a genus of the Plantaginaceae family, Lamiales order that has approximately 265 species. The small plants usually have a dense tuft of basal leaves and long, leafless stalks bearing a terminal spike of small flowers (Beldie, 1979).

The leaves are sessile but have a narrow part near the stem which is a pseudo-petiole. They have three or five parallel veins that diverge in the wider part of the leaf. Leaves are broad or narrow, depending on the species. The inflorescences are borne on stalks typically 5–40 cm tall and resemble a short cone or a long spike, with numerous tiny wind-pollinated flowers.

The species of this genus present in the above mentioned collection are as follows: *Plantago lanceolata* L., *Plantago media* L., *Plantago major* L., *Plantago montana* L., *Plantago indica* L., *Plantago maritima* L., *Plantago gentianoides*, *Plantago atrata*

Hoppe, *Plantago major* L. var. *intermedia* (Gilib) Beck., *Plantago gentianoides* Sm., *Plantago cornuti* Gouan, *Plantago tenuiflora* W.et K., *Plantago schwarzenbergiana* Schur., *Plantago serpentina* All., *Plantago psyllium* L. , *Plantago media* L. var. *urvilleana* Rapin, *Plantago columnae*, *Plantago arenaria*, *Plantago argentea* Chaix, *Plantago carinata* Schrad., *Plantago coronopus* L., *Plantago lagopus*, *Plantago maxima* Juss., *Plantago minor*, *Plantago alpina*, *Plantago altissima* L., *Plantago sulubata*.

The most widespread *Plantago* species present in this herbarium are: *Plantago lanceolata* L. (53), *Plantago media* L. (48), *Plantago major* L (22), *Plantago maritima* L (8) and *Plantago coronopus* L. (2).

Plantago lanceolata is a rosette-forming perennial plant, with leafless, silky, hairy flower stems (10–40 cm). The basal leaves are lanceolate, spreading or erect, scarcely toothed with 3-5 strong parallel veins narrowed to a short petiole. The flower stalk is deeply furrowed, ending in an ovoid inflorescence of many small flowers, each with a pointed bract. Each flower can produce up to two seeds. Flowers can reach 4 millimetres (calyx green, corolla brownish), with 4 bent back lobes with brown midribs and long white stamens (https://en.wikipedia.org/wiki/Plantago_lanceolata).

Plantago media grows in damp grassy meadows up to an altitudes of 2000 m. A slender stalk of between 5 and 50 cm develops from a basal rosette of finely-haired leaves. Delicate pink-white flowers are borne between May and September. *Plantago media* is hermaphrodite and is pollinated by wind or insects, particularly bees. (https://en.wikipedia.org/wiki/Plantago_media).

Plantago major is an herbaceous perennial plant with a rosette of leaves that can reach 15–30 cm in diameter. Each leaf is oval-shaped, 5–20 cm long and 4–9 cm broad, rarely growing up to 30 cm long and 17 cm broad, with an acute apex and a smooth margin; there are five to nine conspicuous veins. The flowers are small, greenish-brown with purple stamens, produced in a dense spike 5–15 cm long on top of a stem 13–15 cm tall (rarely to 70 cm tall) (https://en.wikipedia.org/wiki/Plantago_major).

Plantago maritima is a herbaceous perennial plant with a dense rosette of stemless leaves. Each leaf is linear, 2–22 cm long and under 1 cm broad, thick and fleshy-textured, with an acute apex and a smooth or distantly toothed margin; three to five veins can exist. The flowers are small, greenish-brown with brown stamens, produced in a dense spike 0.5–10 cm long on top of a 3–20 cm stem. (https://en.wikipedia.org/wiki/Plantago_maritima).

Plantago coronopus produces a basal rosette of narrowly lance-shaped leaves up to 25 centimeters long that are toothed or deeply divided. The inflorescences grow erect to about 4 to 7 cm in height. They have dense spikes of flowers which sometimes curve. Each flower has four whitish lobes each measuring about a millimeter long. *Plantago coronopus* mainly grows on sandy or gravelly soils close to the sea. https://en.wikipedia.org/wiki/Plantago_coronopus

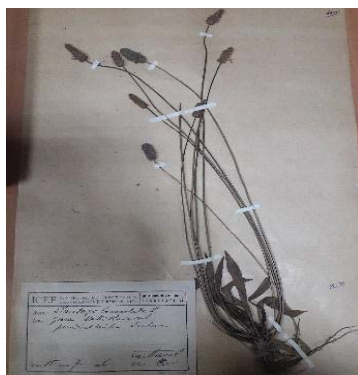


Figure 1. *Plantago lanceolata*



Figura 2. *Plantago media*



Figure 3. *Plantago major*

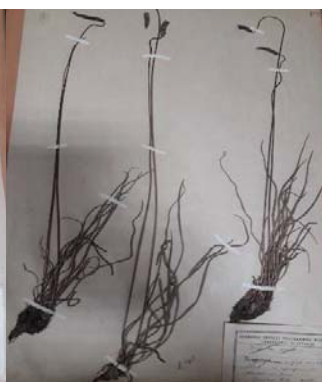


Figure 4. *Plantago maritima*

The plant's harvest year. The plants were gathered in a time period ranging between 1838 and 1963. The oldest plants of this genre are *Plantago major* L., collected in 1838. The periods in which most plants were gathered were 1930-1949 (Figure 1).

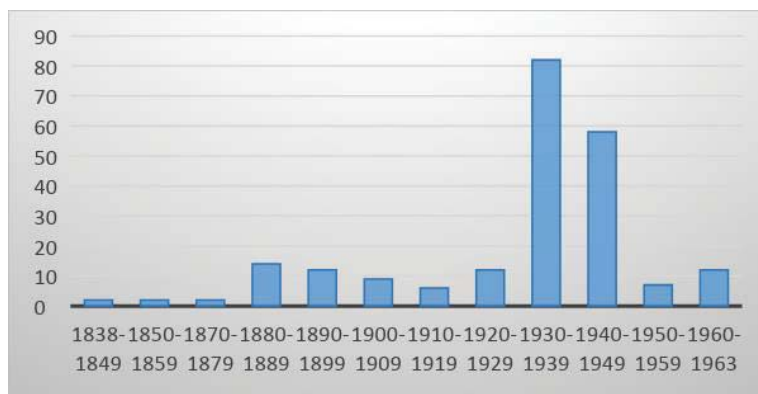


Figure 5. Harvesting periods of *Plantago* plants from INCDS Herbarium

The harvesting place of most species (*P. lanceolata* L., *P. media* L., *P. major* L., *P. montana* L., *P. indica* L., *P. maritima* L., *P. gentianoides*, *P. altrata* Hoppe) is generally represented by high mountain areas: Bucegi Mountains (Babele, Baba Mare, Valea Ialomitei, Varfu Omu, Valea Jepilor, Varfu Doamnei, Piatra Arsa), Parang Mountains, Retezat Mountains, or near cities from our country (Bucuresti, Borsa, Buzau, Braila, Cheia, Cluj, Constanta, Dolj, Sibiu, Hunedoara, Suceava, Tecuci, Tulcea, Valcea). Various species of this genus were also harvested from abroad: Austria, Italy, Switzerland, Russia, Sweden, Holland, France.

The people who gathered the plants are renowned Romanian specialists (Al. Beldie, P. Cretzoiu, C. Georgescu, A. Haralamb, M Ciuca, I Morariu, I. Prodan, Ov. Cosma), M. Iacobescu), as well as foreign botanists (Wolff, Becker, Richter, Heuser, Keller, Guzzino, Rosenberg, Petta, Lojos).

CONCLUSIONS

"Alexandru Beldie" Herbarium, contains more than 40,000 plates, from which 231 plates belong to the *Plantago* genus.

In regard with the harvesting period, by analyzing Figure 5, it can be observed that they were gathered in a long period of time, almost 125 years, from 1838 until 1963. The maximum number of gathered plants from this long period of time is recorded during 1930-1949 and were gathered by renowned Romanian and foreign botanists (Al. Beldie, P. Cretzoiu, C. Georgescu, A. Haralamb, M Ciuca, I Morariu, I. Prodan, Ov. Cosma, M. Iacobescu). The plants were gathered from Romanian mountain areas (Bucegi, Parang, Retezat) or near cities from our country (Bucuresti, Borsa, Buzau, Braila, Cheia, Cluj, Constanta, Dolj, Sibiu, Hunedoara, Suceava, Tecuci, Tulcea, Valcea), as well as from some European areas (Austria, Italy, Switzerland, Russia, Sweden, Netherlands, France). The plants are in a good conservation degree and are essential in many research and science domains.

REFERENCES

- Beldie A. 1979, Flora Romaniei - determinant ilustrat al plantelor vasculare. Editura Academiei RSR. vol. II, p. 125.
- Cântar I.C., Dincă M., 2017, The characteristics of plant species from *Arabis* type present in Al. Beldie Herbarium from I.N.C.D.S. Bucharest. Annals of West University of Timișoara, ser. Biology, vol. 20 (2), p.115-122.
- Crișan V., Dincă L., Oneț C., Oneț A., 2017, Collection species from *Potentilla* genus. Natural Resources and Sustainable Development, pag. 27-34.
- Dincă L., Vasile D., Voiculescu I., 2017, The characteristics of plants from *Hieracium* genre present in Alexandru Beldie Herbarium from I.N.C.D.S. Bucharest". Lucrări științifice USAMV Iași, seria Horticultură 60 (1), p. 39-48.
- Dincă L., Enescu R., Oneț A., Laslo V., Oneț C., 2017, Plant species from Al. Beldie herbarium - *Veronica* genre - short description. Natural Resources and Sustainable Development, pag. 43-50.
- Dincă M., Dincă L., Vasile D., 2017, A short description of *Androsace* genre plants present in Alexandru Beldie Herbarium from I.N.C.D.S. Bucharest. Currents Trends in Natural Sciences, 6 (12), p.16-24.
- Enescu R., Dincă L., 2017, Description of plant species of *Ornithogalum* genus present in Al. Beldie Herbarium from "Marin Drăcea" Bucharest N.I.R.D.F. Journal of Horticulture, Forestry and Biotechnology, Volume 21(3), p. 89-95.
- Vasile D., Dincă L., Andreica A., Voiculescu I., 2016, Herbarul Alexandru Beldie - o colecție de plante și o importantă bază de date pentru specialiști. Revista de Silvicultură și Cinegetică, nr. 39, p.114-119.

*** https://en.wikipedia.org/wiki/Plantago_lanceolata.
*** https://en.wikipedia.org/wiki/Plantago_media.
*** https://en.wikipedia.org/wiki/Plantago_major.
*** https://en.wikipedia.org/wiki/Plantago_maritima.
*** https://en.wikipedia.org/wiki/Plantago_coronopus

**THE INFLUENCE OF RISK ELEMENTS TO THE SOIL QUALITY
AT THE FORMER MINING AREAS IN SLOVAKIA**

Demková Lenka^{1*}, Bobuřská Lenka, Árvay Július²

¹University of Prešov, Department of Ecology, 17. november 1, 081 16, Prešov, Slovakia

²Slovak Agricultural University in Nitra, Department of Chemistry, Tr. A. Hlinku 2, 949 76 Nitra

*Correspondence author. E-mail: lenka.demkova@unipo.sk

Keywords: soil pollution, nutrients, soil enzymes, former mining area

ABSTRACT

Among human activities, mining is responsible for environment contamination in huge areas of the globe. Soils polluted by risk elements lose their fertility and the toxicity effects are manifested in plants, animals, and micro fauna. Nutrients content (Ca, Na, K, Mg) and the activity of soil enzymes (urease, acid and alkaline phosphatase, FDA and β -glucosidase) was determined in soil samples from two types of mining bodies – heaps of waste material and the open mines. Nutrient content analysis was carried out on an Agilent ICP-OES spectrometer 725 and the soil enzyme activity was determined according to valid methodologies under laboratory conditions. The aim of the study was to determine the content of the nutrients as well the activity of soil enzymes at different mining bodies and evaluate the influence of soil pollution to the soil characteristics. Soil quality has been different in terms of soil enzyme activity in the evaluated sampling sites because different enzymes react differently to environmental stress. The values of Ca, Na and K were higher at the heaps of the waste material comparing open mines.

INTRODUCTION

Bad soil quality at the former mining areas, especially near mining bodies, is a long-term serious environmental problem (Guo et al. 2017). The high content of the risk elements reduces soil fertility and ultimately endangers the health of the ecosystem and the humans (Xiao et al. 2017). The area of Slanske vrchy hills, near the Zlata Bana village (ZB) is characterized by a high number of mining bodies, especially open mining pits and the heaps of mining waste material. It poses a threat to the surrounding environment, due to the release of hazardous elements - heavy metals, into the soil, water and air. Nizna Slana village is under the constant influence of the risk elements coming from the tailing pond situated above the village. The sludge of a dusty consistency is transmitted by a wind to the big distances and interferes whole slope under the tailing pond and the village laying below. It has been repeatedly shown that soils with the high volume of the risk elements has low values of nutrients and are not valuable for agricultural purposes (Fazekašová and Bobuřská 2012). Additionally, it has been shown that enzyme activity responds very quickly to the environmental stress (Hinjosa et al. 2008), that's why it is used as relative stable, very sensible biochemical indicator of soil pollution. According to Šarapatka (2002), enzymatic activity is closely related to important soil characteristics, making it an ideal indicator of soil quality. In addition, soil enzymes response to the presence of the risk elements in soils much faster than other chemical or physical soil parameters (Nannipieri et al. 2002). Urease belongs to the group of hydrolases enzymes that catalyze hydrolysis of urea to carbon dioxide

and ammonia (Hasan 2000). Soil phosphatases catalyse the decomposition of organic phosphorus and play a key role in mineralization processes. Phosphatases are divided into acidic and alkaline, and their occurrence is influenced by the soil reaction (Kumar et al. 2011). FDA is conducted with a wide range of enzymes such as lipases, esterase and proteases (Gaspar et al., 2001). β -glucosidase enters the carbon pathway as a hydrolase. The high content of toxic elements in the soil has an inhibitory effect on its activity (Wang et al., 2008). The aim of the study was to compare the nutrient content (Na, Mg, Ca, K) in the soils of two former mining villages (Zlata Bana, Nizná Slana) to determine and compare soil enzyme activity among villages and to determine the relation between nutrients, soil enzymes and risk elements.

MATERIAL AND METHODS

Two former mining areas were selected for research activities. In Zlata Bana village (ZB), the mining activities focused to the gold mining started in the 16th century. Subsequently were stopped and the attempts to the restoration during the years 1730-1861, and again in 1989 were not successful. Nizna Slana (NS) is an old mining village focused to the iron and precious metals mining. Mining activities were developed during the 12th and 13th century, when mining specialists from Germany were invited to the village. Iron mining was stopped in 2008.

Soil samples (0-10 cm) were sampled during the summer in 2017 from 11 sampling sites in the Nizna Slana (NS), and 11 sampling sites in the Zlata Bana (ZB) village. Soil samples (500 g) were stored in the plastic bags, transported to the laboratory conditions and air-dried. Subsequently, the soil samples were sieved (<2mm), homogenized and stored to the analyses (-20°C). The soil reaction (pH) was measured by InoLab pH 720-WTW in the solution of 5g and 25mL of a 0.01M CaCl₂. The activity of acid (KF) and alkaline phosphatase (ZF) was determined according to Grejtovski (1991), urease activity (URE) according to Khaziev (1976), soil β -glucosidase activity (BG) according to Eivazi and Tabatabai (1988) and FDA according to Green et al. (2006). The nutrient content as well as the content of the risk elements was determined with an axial plasma configuration of Agilent ICP-OES 725 (Agilent Technologies Inc., Santa Clara, CA, USA). All statistical operations were performed in STATISTICA 12 (© TIBCO Statistica™).

RESULTS AND DISCUSSIONS

A total content of the risk elements determined at the sampling sites from two former mining villages and the limit values for each element set by Act. No. 220/2004 Coll. of Laws are listed in Figure 1. Average values of Cd, Fe, Pb and As exceed the limit value in both villages. The limit value of Cu was exceeded only in the case of NS. Serious pollution by As and Fe was determined in the sediments (Brehuv et al. 2007) and atmospheric deposition (Hančulák et al. 2011) in Nizna Slana.

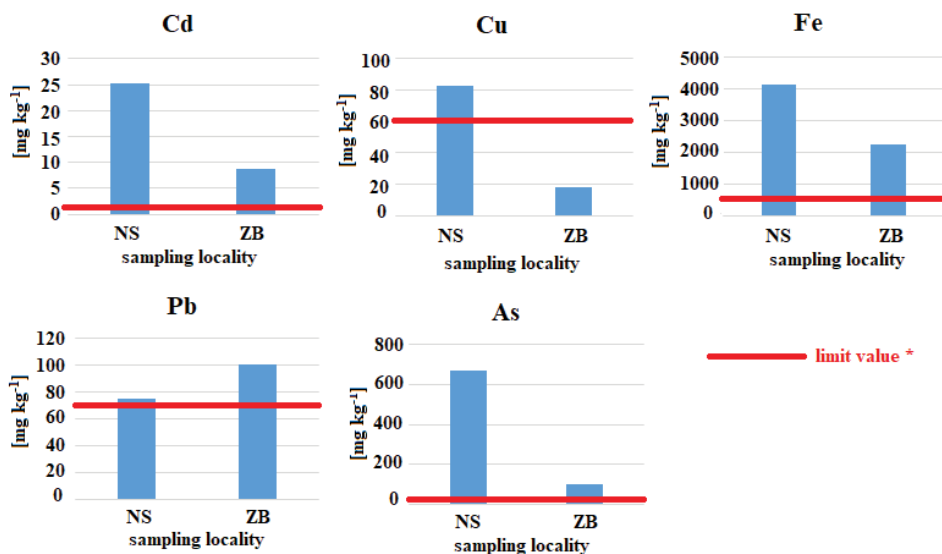


Figure 1 Average values of the risk elements determined in the Nizna Slana (NS) and Zlata Bana (ZB) village, and the limit value set by the *Act No. 220/2004 Coll of Laws.

The values of soil enzymes and soil pH are listed in Table 1.

Table 1
Values of the nutrients, soil enzymes and pH determined in the sampling sites in Nizna Slana (NS) and Zlata Bana (ZB) villages.

Soil properties		NS Min-max (average±st.deviation)	ZB
Ca	mg kg ⁻¹	308-3605 (1304±1142)	1069-79973 (20520±21267)
Na		211-736 (398±182)	120-436 (219±102)
K		1012-9534 (3224±2658)	3211-7517 (4863±1215)
Mg		631-4191 (1631±1146)	2414-24514 (9638±6233)
URE	(mg NH ₄ ⁺ -Ng ⁻¹ 24 ⁻¹)	0.07-0.73 (0.36±0.23)	0.06-0.83 (0.49±0.21)
ACP	(mg P g ⁻¹ 3h ⁻¹)	29.1-241 (99.5±66.1)	9.95-248 (106±79.1)
ALP	(mg P g ⁻¹ 3h ⁻¹)	28-169 (68.3±46.3)	18.1-213 (100±63.2)
FDA	(µg FS / g soil h)	0-57.4 (12.6±18.8)	0.00-51.6 (12.6±18.8)
BG	(µg p NP / g soil h)	11.7-158 (91.5±53.4)	6.56-364 (114±103)
pH	-	3.3-7.42 (114±103)	4.72-7.54 (6.73±0.99)

Soil pH values ranged between 3.30 – 7.42 and 4.72-7.54 in NS and ZB, repeatedly. According to the Čurlík and Šefčík (1990) classification, soil samples in NS and ZB were determined as extremely acid to alkaline. It has been repeatedly demonstrated that soils with high levels of toxic elements use to reach low pH values (Alloway 2010).

In the study of Franc et al. (2016), who determined the content of the nutrients in the sludge of the tailing pond, the average values of Ca, Mg and K varied between 4110-4560 mg kg⁻¹, 6610-6900 mg kg⁻¹, 10310-12510 mg kg⁻¹, repeatedly. Comparing our results, only values of Ca in ZB village reached a higher value. It has been

demonstrated several times that a high degree of contamination of the natural environment uses to negatively affected by the activity of soil enzymes (Wang et al., 2008). Comparing villages, the lowest values of soil enzymes were determined in ZB, which was (as it is shown in the Figure 1) higher polluted.

Spearman's correlation relationship (Table 2) shows a significant positive correlation between soil pH and Ca, and soil pH and Mg. Ca and Mg also gave significant positive correlation between themselves. Taylor et al (2002) determined a negative correlation between soil pH and soil enzymes. In our case, pH gave negative correlation only with ACP and ALP. In the study focused on the assessment of the soil properties in the metal bordered region (Angelovičová et al. 2014), positive correlation between K and soil urease activity was determined, what wasn't confirmed in our study.

Correlation relationships between risk elements and soil properties are listed in Table 3. Soil pH gave significant positive correlation with Cd, Cu and Fe. High values of Cd, Cu, Fe, and As significantly positively influenced the level of Mg. Potassium (K) gave a significant positive correlation with Cu and Fe. As and Pb gave a negative correlation with all evaluated soil enzymes. The level of URE and ACP was negatively influenced by the risk elements. It has been repeatedly shown, that accumulation of the risk elements in soils reduce the content of microbial biomass, limiting the functional diversity of ecosystem (Wang et al., 2008).

Table 2

Correlation relationships between nutrients, soil enzymes and soil pH.

	Ca	Na	K	Mg	URE	ACP	ALP	FDA	BG
pH	0.59*	-0.39	0.02	0.66*	0.23	-0.34	-0.03	0.05	0.25
Ca		-0.33	0.02	0.88**	-0.32	-0.46	-0.27	-0.27	0.64**
Na			0.32	-0.38	-0.48*	-0.16	-0.12	-0.20	-0.07
K				0.08	-0.18	0.07	0.27	0.13	-0.08
Mg					-0.19	-0.35	-0.19	-0.14	0.56*
URE						0.28	0.11	0.27	-0.38
ACP							0.78**	0.77**	-0.08
ALP								0.82**	-0.16
FDA									0.04

*p<0.05; **p<0.01

Students t-test (Table 4) was used to determine significant differences in soil pH and soil enzymes between sampling villages. Significantly highest values of Ca and Mg were determined in ZB. In the soil samples from NS significantly highest values of Na were assessed. There were found no differences between soil enzyme activity between villages. Soil pH reached significantly highest values in ZB comparing NS.

Table 3

Correlation relationship between soil enzymes, soil pH and risk elements.

	Cd	Cu	Fe	Pb	As
pH	0.53*	0.55*	0.49*	-0.15	0.37
Ca	0.48*	0.21	0.41	-0.02	0.34
Na	-0.28	-0.36	-0.21	0.20	-0.23
K	0.36	0.52*	0.49*	-0.03	0.12
Mg	0.77**	0.45*	0.67**	0.27	0.53*
URE	-0.05	-0.08	-0.21	-0.36	-0.13
ACP	-0.29	-0.01	-0.21	-0.27	-0.38
ALP	-0.07	0.09	0.09	-0.16	-0.26
FDA	-0.04	0.12	0.09	-0.17	-0.16
BG	0.14	0.07	0.11	-0.12	-0.08

*p<0.05; **p<0.01

Table 4

The results of t-test expressing statistically significant differences in nutrient, soil enzyme activity and pH between the evaluated former mining villages.

	factor	df	f	p
Ca	Between villages	1	5.07	0.039*
Na		1	6.24	0.023*
K		1	2.79	0.114
Mg		1	10.1	0.005**
URE		1	1.20	0.289
KF		1	0.03	0.856
AF		1	1.18	0.293
FDA		1	1.29	0.272
GLUK		1	0.26	0.616
pH		1	17.8	2.e-10 ^{-5**}

*p<0.05; **p<0.01

CONCLUSIONS

High values of the risk elements in the soils of the former mining area negatively affected soil quality and health. Serious pollution by As, Cd, and Fe was confirmed in both villages. An activity of soil enzymes was negatively influenced by the risk elements. Comparing villages, higher values of the risk elements in NS have been proven by lower soil enzyme levels and lower values of soil pH. The level of Ca and Mg significantly positively correlated with soil pH, additionally, Mg significantly correlated with all evaluated risk elements except Pb.

ACKNOWLEDGMENT

The research was supported by the project of Ministry of the Education, Science, Research and Sport of the Slovak republic VEGA 1/3026/2018.

REFERENCES

- Act. No. 220/2004 Coll of Laws. On the protection and use of agricultural land. National Council of the Slovak Republic, Bratislava.
- Alloway J. B., 2010, Heavy Metals in Soils: Trace Metals and Metalloids in Soils and their Bioavailability. Springer, Dordrecht, Heidelberg and New York, pp. 368.
- Angelovičová L., Bobuřská L., Fazekašová D., 2014. Chemical and biological soil properties as the indicators of the soil environment influenced by heavy metals. Acta universitatis Prešovensis Folia Oecologica, 11, 11-19. (in Slovak).

Brehuv J., Špaldon T., Šestínová O., Slančo P., Hančuľák J., Bobro M., 2007, Contamination of the water and sediment load from the drainage basin of the Slaná river by influence of former and present mining activities. *Acta Facultatis Ecologiae*, 16(1), 91-100.

Čurlík J., Šeřčík P., 1990, Geochemical atlas of Slovak republic, part V. Soils, MŤP, Bratislava, pp. 99.

Eivazi F., Tabatabai M. A., 1988, Glucosidases and galactosidases in soils. *Soil Biology and Biochemistry*, 2, 601-606.

Fazekášová D., Bobuľská L., 2012, Soil Organisms as an Indicator of Quality and Environmental Stress in the Soil Ecosystem. *Životné prostredie*, 46, 103 – 106.

Gaspar M. L., Cabello M. N., Pollero R., 2001, Fluorescein diacetate hydrolysis as a measure of fungal biomass in soil. *Current microbiology*, 42(5), 339-344.

Green V. S., Stott D. E., Diack M., 2006, Assay for fluorescein diacetate hydrolytic activity: optimization for soil samples. *Soil Biology and Biochemistry*, 38, 693-701.

Grejtovski A., 1991, Influence of soil improvers on enzymatic activity of heavy alluvial soil. *Plant Soil Environment*, 37, 289-295. (in Slovak).

Guo L., Zhao W., Gu X., Zhao X., Chen J., Cheng S., 2017, Risk assessment and source identification of 17 metals and metalloids on Soils from the Half-Century Old Tungsten Mining Areas in Lianhuashan, Southern China. *International Journal of Environmental Research and Public Health*, 14(12), 1475.

Hančuľák J., Fedorová E., Šestínová O., Špaldon T., Matík M., 2011, Influence of iron ore works in Nižná Slaná on the atmospheric deposition of heavy metals. *Acta Montanistica Slovaca*, 16(3), 220-228.

Hasan H. A. H., 2000, Ureolytic microorganisms and soil fertility: A review. *Communication in Soil Science and Plant Analysis*, 31(15-16), 2569-2589.

Hinjosa M. B., Carreira J. A., Rodrigues-Moroto J. M., Garcia-Riuz R., 2008, Effects of pyrite sludge pollution on soil enzyme activities: ecological dose-response model. *Science of Total Environment*, 396, 89-99.

Khaziev F. K., 1976. Soil enzyme activity (in Russian). Nauka, Moskva, pp. 180.

Kumar S., Chaudhuri S., Maiti S. K., 2011, Phosphate activity in natural and mined soil – A review. *Indian Journal of Environmental Protection*, 31(11), 955-962.

Nannipieri P., Kandeler E., Reggiero P., 2002, Enzyme activities and microbiological and biochemical processes in soil. In Burns R. G. & al. (eds.) *Enzymes in the Environment, Activity, Ecology and Applications*. New York, Marcel Dekker, pp. 1-33.

Šarapatka B., 2002, Possibilities of utilization of enzyme activity as an indicator of the productivity and quality of the system. *Biological indicators of soil quality*. Brno, MZLU, pp. 62-71.

Taylor J. P., Wilson B., Mills M. S., Burns R. G., 2002. Comparison of microbial numbers and enzymatic activities in surface soil and subsoils using various techniques. *Soil Biology and Biochemistry*, 34(3), 387-401

Wang Y. P., Li Q. B., Shi J. Y., Lin Q., Chen X. C., Wu W., Chen Y. X., 2008, Assessment of microbial activity and bacterial community composition in the rhizosphere of a copper accumulator and a non-accumulator. *Soil Biology and Biochemistry*, 40(5), 1167-1177.

Xiao R., Wang S., Li R., Wang J. J., Zengqiang Z., 2017, Soil heavy metal contamination and health risks associated with artisanal gold mining in Tongguan, Shaanxi, China. *Ecotoxicology and environmental Safety*, 141, 17-24.

**RESEARCH ON THE EVOLUTION OF SOME RISK FACTORS
AND STRESS IN THE DEALU BUJORULUI VINEYARD IN THE CONTEXT
OF THE LIKELY CLIMATE CHANGE**

Enache Viorica^{1*}, Donici Alina¹, Tabaranu Gabriel¹

¹Research and Development Station for Viticultural and Winemaking Bujoru

*Correspondence author E-mail enacheviorica57@gmail.com

Keywords: *climate risk, vine, air temperature, precipitation*

ABSTRACT

The research was carried out between 1980 and 2016 and its objective is to establish the trend of risk and stress factors in the Bujoru vineyard ecosystem. Viticulture is sensitive to climate change, therefore depending on the evolution of the weather the most efficient technological measures that minimize the effect of climate change must be established in the future. For the Bujoru vineyard ecosystem, drought is a frequent phenomenon, with long periods of time without quality rainfall followed by short periods of torrential rain, over the average quantity, not entirely able to be harnessed. Evolution of precipitation marks a cyclical trend of about 7-8 years during which rainy years alternate with droughty years. The precipitation deficit is especially occurring during the winter and at the beginning of the vegetation period (April to May). The air temperature during the vegetation period shows a trend of growth until 2003, followed by a decrease until 2014, followed by a growth trend. The frequency of tropical days (days with $T_{max} > 30\text{ }^{\circ}\text{C}$) shows a rising trend during the vegetative period and the number of days during winter ($T_{max} < 0\text{ }^{\circ}\text{C}$) shows a decreasing trend.

INTRODUCTION

Since the beginning, agriculture has enjoyed a remarkably stable climate, but now, since the 1980, we are facing with a global warming trend. Expansion and intensity of extreme weather phenomena reduces the production annually, and for the sustainable conservation of natural resources in agriculture it is necessary to provide a scientific basis for all actions and measures to prevent and mitigate the consequences. Global climate change associated with increased pollution, deforestation, etc. have led to an increase in the dry and aridity process. Global climate change is one of the major concerns of our century - a complex area where it is necessary to improve knowledge and understanding to take immediate and accurate action. Analysis of climatic data over long periods has shown a climate change trend. Simulations with complex global climate models have shown that the phenomenon is determined by both natural and anthropogenic factors (IPCC, 2007). The climate risk, by acting outside normal limits according to the bioclimatic requirements of the vine, causes violent destruction, eventually leading to "partial or total loss of biological capacity". In determining climatic risk, the climate risk threshold of the vine is also considered. Any significant climatic change has repercussions on the vineyard ecosystem (Alexandrescu I.C., 1994). The wine-growing plants in Moldova and especially in the South are increasingly affected by the climate changes that have occurred in the last decade. A changing climate is one of

the major environmental and socio-economic problems facing sustainable wine-growing and production in the next century.

MATERIAL AND METHODS

The research was carried out in the experimental field of the Statiunea de Cercetare Dezvoltare pentru Viticultură și Vinificație Bujoru between 1980 and 2016. Data were analyzed and processed on:

- average air temperature over the vegetation period, minimum and maximum annual temperature;
- number of days in the winter with maximum air temperature $< 0\text{ }^{\circ}\text{C}$;
- number of days with maximum air temperature $> 30\text{ }^{\circ}\text{C}$ (tropical days);
- the annual and vegetative season;
- number of days with rainfall during the vegetation period;
- number of days with rainfall during the vegetation period;
- the global thermal heat balance, active and useful/actual.

RESULTS AND DISCUSSIONS

During the vegetation period, the mobile average indicates a tendency of increase in the air temperature by the year 2003, followed by a tendency of decreasing in the air temperature until 2014, followed by a growth trend (fig. 1). In general, the average air temperature shows an increasing trend from 1980 to 2016.

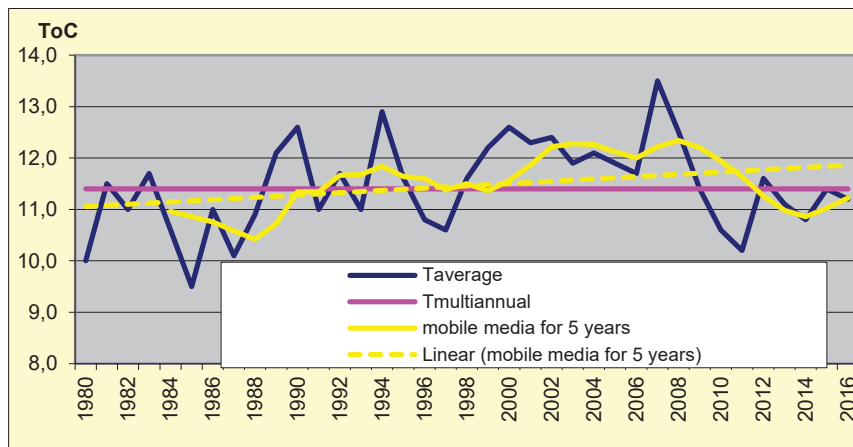


Figure 1 Air average temperature during the vegetation period at RDSVW Bujoru

In terms of minimum air temperatures, over the 27-year period minimum temperatures below $-16,0\text{ }^{\circ}\text{C}$ and only 10 years with T_{min} higher than $-16,0\text{ }^{\circ}\text{C}$ (Figure 2) have been recorded. The maximum air temperature above $40\text{ }^{\circ}\text{C}$ was recorded in 2007 and 2012, the remainder of the period is generally above $35\text{ }^{\circ}\text{C}$ excluding 1982, 1984, 1991 and 1997. In 29 years out of 37 were recorded temperatures below freezing ($<0,0\text{ }^{\circ}\text{C}$) during the vegetation period (Figure 3). The year 1988 ($-1,5\text{ }^{\circ}\text{C}$), 2014 ($-0,2\text{ }^{\circ}\text{C}$) and 2000 with the minimum temperature of $-10,0\text{ }^{\circ}\text{C}$ on May 1 and T_{max} of $28,0\text{ }^{\circ}\text{C}$ (daily amplitude of $38,0\text{ }^{\circ}\text{C}$) were highlighted.

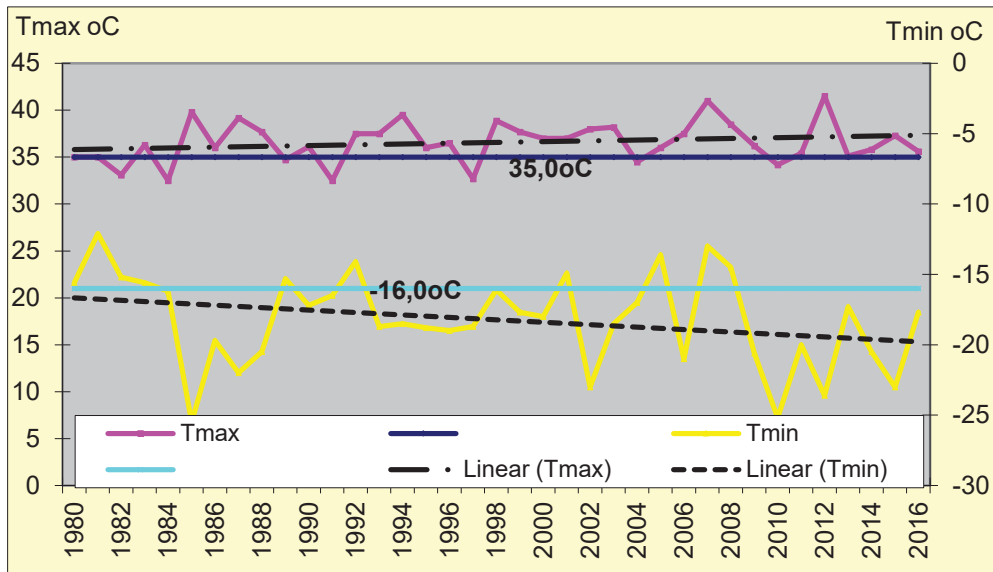


Figure 2 Minimum temperatures and annual maximum temperatures evolution at the Dealu Bujorului vineyard

In the Dealu Bujorului vineyard, the multiannual average rainfall (1980-2016) is 462,1 mm, with 293,8 mm during the vegetation period (with a minimum of 103,1 mm in 1986 and a maximum of 484,3 mm in 1991). Evolution of precipitation marks a cyclic tendency of approximately 7-8 years in which the rainy interval alternates with the dry interval (Figure 5).

Relative to the number of days with $T_{max} > 30\text{ }^{\circ}\text{C}$, all the years analyzed were favorable to the vine culture. The trend shows an increase in the number of tropical days, from 1980 to 2016 (Figure 4).

The five-year moving average which is consistently expressing the climate trend suggests that there has been an increasing trend in annual rainfall over the last period (Enache Viorica et al., 2017).

There is an uneven distribution of rainfall over the year, with periods of drought framed by short periods of abundant rainfall and often torrential. Rainfall deficiency occurs especially during the winter and early vegetation period (April to May) with repercussions on the occurrence of vegetation phenophases. Frequently, in June-July there are torrential rains that are not fully exploited, leading to an increase of the rainfall deficit. In 7 years out of the 10 with precipitations lower than 250 mm during the vegetation period, $T_{max} > 35\text{ }^{\circ}\text{C}$ was recorded and in 17 years out of 37 annual precipitation of less than 450 mm and $T_{max} > 35\text{ }^{\circ}\text{C}$ were recorded.

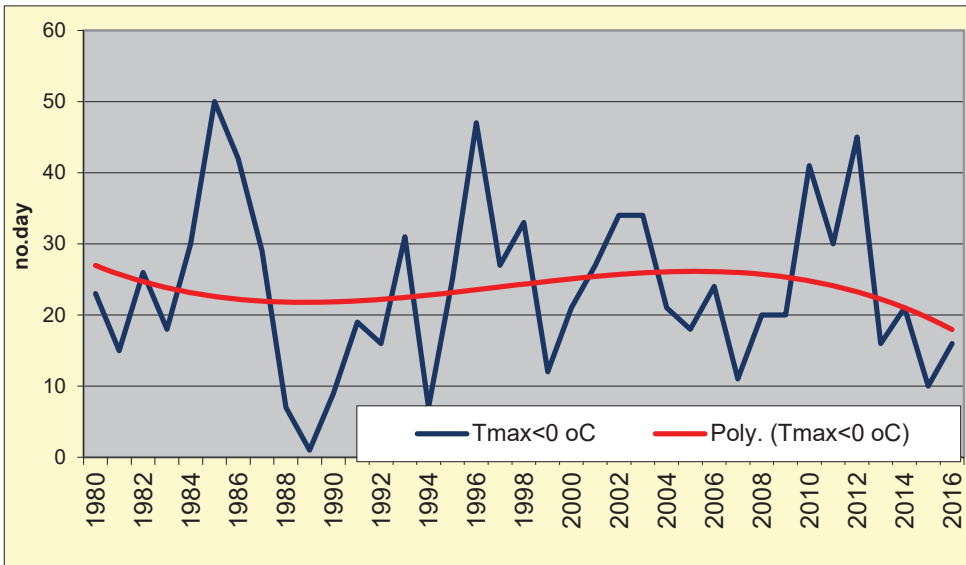


Figure 3 Number of winter days with the minimum air temperature $< 0\text{ }^{\circ}\text{C}$

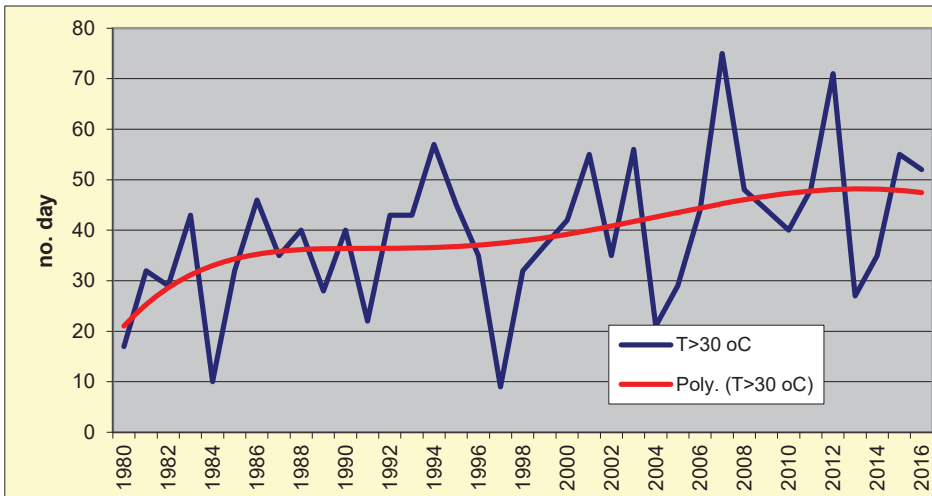


Figure 4 Number of days with the air minimum temperature $> 30\text{ }^{\circ}\text{C}$ (tropical days)

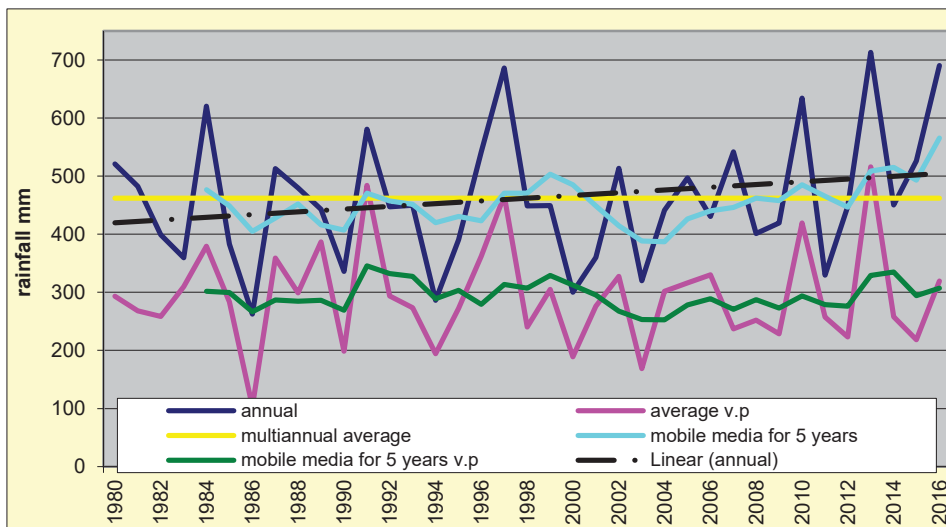


Figure 5 Multiannual average rainfall during the vegetation period

The number of days with rainfall of less than 5 mm predominates over the vegetation period, followed by precipitation between 5-10 mm and over 10 mm (Figure 6). In recent years the number of days with precipitation < 5 mm is increasing.

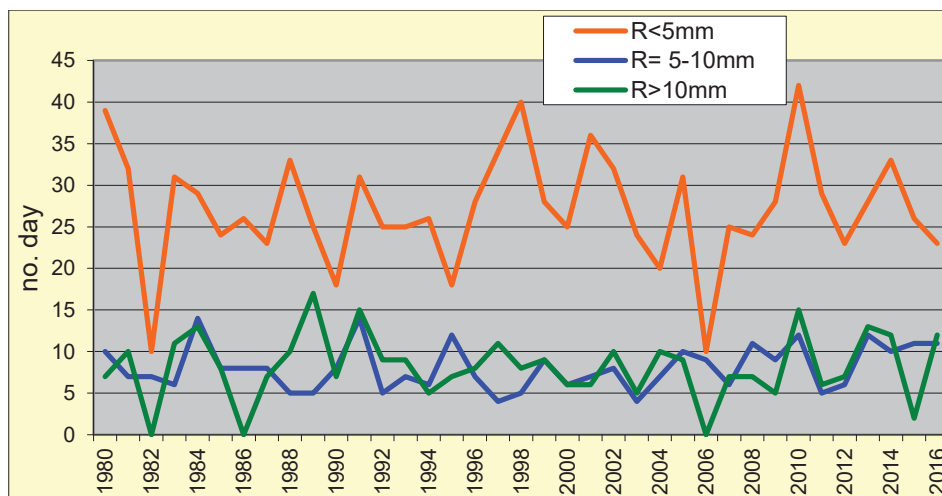


Figure 6 Days with rainfall during the vegetation period

Drought is a frequent phenomenon for the Bujoru Vineyard ecosystem. In order to clarify this phenomenon, it is necessary to know not only the annual rainfall, but the interval of the year in which the precipitations were absent or in insufficient quantities. Between 1980 and 2016 there were long periods without useful rainfall followed by short periods of surplus precipitation, which were totally untapped. In July and August 2001, 10,0 mm and 0,8 mm precipitations were recorded, followed by torrential rains in September 2001. The same aspect is recorded in 2007 in July, when in 30 days there

were only 1,0 mm and in August there were extreme rain events (torrential rains). Insufficient precipitations were also recorded in 2011 (5,2mm / September), 2002 (4,2mm / May), 1994 (9,3 / May), 1988 (7,0 / July), 1986 (7,4 mm / May), 1985 (0,1mm / April) and 1982 (0,2mm / July). During the rest of the time there are months when the rainfall is recorded in small quantities, well below the multiannual average of the respective month. The global heat thermal balance, active and useful heat balance have been calculated for the 1980-2016 vegetation period to measure the caloric resources of the wine-growing zone. We observe a random evolution of the amount of global temperature, active and useful, with an increasing trend from 1980 to 2016. Significant deviations of the active and useful temperatures were recorded in 1994, 2007 and 2012 (Figure 7).

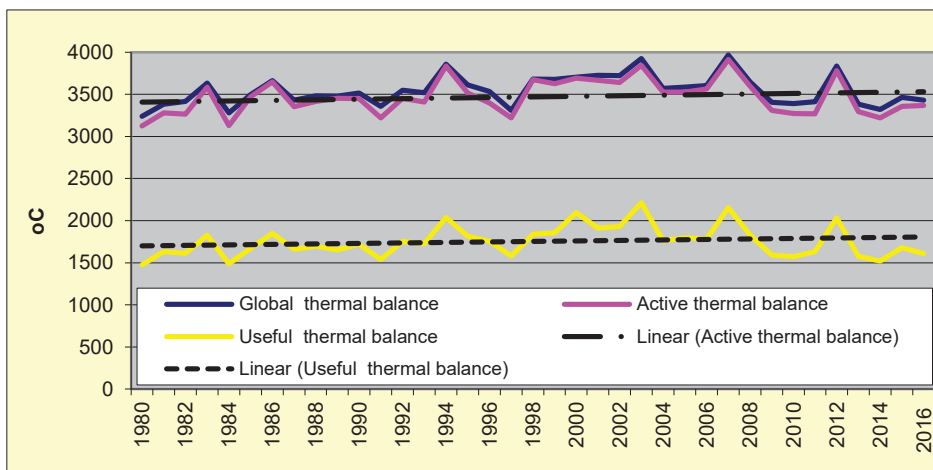


Figure 7 Global heat thermal balances, active and useful

CONCLUSIONS

Analyzing the evolution of some risk and stress factors between 1980 and 2016 in the Dealu Bujorului vineyard, it can be observed that:

- average air temperature has a growth trend from 1980 to 2003, then a slight decrease until 2014, followed by a growth trend;
- Maximum air temperature above 40 °C has been recorded in 2007 and 2012;
- Temperatures below the freezing threshold (<0,0 ° C) during the vegetation period have been recorded in 29 years out of 37 analysed, with 2000 being recorded when -10 ° C on May 1 and Tmax of 28,0 ° C (daily amplitude of 38 °C).
- The number of tropical days is increasing from 1980 to 2016;
- The five-year rolling average that consistently expresses the climate trend suggests that lately there has been a tendency of increased annual precipitation but with uneven distribution over the year, with periods of drought fraught with short periods of heavy rain and often torrential;
- In recent years the number of days with precipitation <5 mm is increasing;
- Global, active and useful heat balance/thermal balance is showing an increasing trend from 1980 to 2016.

REFERENCES

Alexandrescu I.C., Oslobeanu M., Jianu L., Pituc P., 1994 - Mică enciclopedie de viticultură, Editura Glasul Bucovinei, Iasi, pp 712

Enache Viorica, Donici Alina, Tabaranu Gabriel, 2017 - Trend of the evolution of daily precipitation in the condition of probable climatic changes in the Dealul Bujorului vineyard, Lucrări științifice, Anul L, vol. 60(1), Seria Horticultura, Editura Ion Ionescu de la Brad, 97-102

IPCC, 2007 - Climate change, 2007 - The physical science basis. Contribution of working Group I., the fourth assessment rapport of the IPCC, Cambridge UP.

**ASPECTS REGARDING THE DESERTIFICATION PROCESS
IN OLTENIA REGION, ROMANIA**

Gavrilescu Elena¹, Marinică Ion², Popescu Simona Mariana^{1*},
Ghermec Cristian³, Ghermec Olimpia³

University of Craiova, Faculty of Horticulture, Craiova

²University of Craiova, Faculty of Science, Craiova

³University of Craiova, Faculty of Mechanical Engineering, Dr. Tr. Severin

* Correspondence author. E-mail: popescu_simona83@yahoo.com

Keywords: *drought, desertification, heat waves, anthropogenic impact*

ABSTRACT

The climate warming process has progressed from one year to the next, not only in Oltenia or Romania but on the whole planet, which has led to the increase of droughts, their extension and their duration. As a result, desertification processes in vulnerable areas have widened and expanded. The summer of 2017 was excessively warm with long drought intervals and excessively high temperatures. The summer heat peaked at the beginning of August when absolute thermal peaks were reached and exceeded at many weather stations in the country, and in Oltenia there were 10 meteorological stations that recorded temperature records. The paper examines the climatic conditions and desertification processes in Oltenia, which have occurred in this part of the country and the impact on the environment.

INTRODUCTION

The anthropic impact on the climate, especially in the last century, was particularly great that it is considered to be a global, regional and local modifying factor, and human action on the environment has become a climatogenic factor. The climate data of the last century have revealed, in addition to a progressive general warming of the atmosphere (highlighted at a planetary level) a reduction in rainfall which have become limiting factors for the growth, development and productivity of agricultural crops in certain geographical regions and at the same time very restrictive factors for the allocation and use of water resources. The year 2015 was the first year in which the global average temperature exceeded by 1 ° C, the average of the last century, followed by 2016 with a higher exceedance than in 2015, and 2017 is expected to be the hottest on our planet in the first three years, with extreme weather phenomena (climatic extremes), according to the WMO Provisional Statement of Climate Status.

The United Nations Conference on Environment and Development – UNCED has defined desertification as a land degradation in arid, semiarid and dry sub-wet areas resulting from the action of several factors, including climatic variations and human activity. Droughts are complex risk climatic processes with slow manifestations that affect and engage (depending on their duration and intensity) a diverse number of components of the geographical environment.

For this study we chose the time span between 2007-2017, during which the dry summer periods in the region of Oltenia, Romania had a large spatio-temporal expansion and in some years they began even in the spring or occurred after dry winters. During

droughts, the flow of the rivers and the Danube decreases, the level of groundwater decreases considerably and many fountains dry. Therefore, serious problems arise from the water supply of the localities, the crops dry in some areas and suffer static forces which cause premature rupture, and the grains become shabby, with little content of pungent substances or become unfit for consumption. This paper is part of an extensive series of studies on climate change in Oltenia and its impact on the environment (Marinică, 2006; Marinică and Marinică 2014).

MATERIAL AND METHODS

The phenomena of desertification and land degradation are closely related to drought and drought phenomena. For the study of drought phenomena, the following criteria and methods can be used: analysis of non-periodic variations of precipitation and deviations of precipitation quantities (monthly, seasonal and annual); the frequency of the dry rainfall type and its spatio-temporal expansion, according to the Hellman criterion applied to the monthly and annual quantities (the criterion also sets the percentage deviations of the annual and half-yearly amounts from normal), use of Walter Lieth climates, use of standardized rainfall index (ISP). In this paper we mainly used the Hellman criterion, and as a database we used the records from the meteorological stations in Oltenia with long strata of meteorological observations (some over 120 years). We have compiled a series of graphs necessary for comparative analysis in the study of these complex phenomena and we have used the published statistical data to assess the effects of drought periods.

RESULTS AND DISCUSSIONS

Aspects of increasing summer air temperature. Of many aspects, we will analyze the increase in temperature during summer. In June, due to the Earth-Sun geometry, the day's duration reaches the maximum of 15 hours and 32 minutes at 20.VI, and the summer solstice takes place at 21.VI. Day length reaches 15 hours and 1 minute on May 22nd and therefore at 30.VI there are a total of 40 days where the duration of the day is ≥ 15 hours (at 1.VI the day's duration is 15 hours and 18 minutes, and at 30.VI, 15 hours and 28 minutes). As a result of the long daylight and sunshine angle of nearly 90° , the sunstroke is maximum and causes the air temperature to rise. The general circulation of the atmosphere over this period of time determines the frequent occurrence of tropical warm air adventures in North Africa and the intensification of air heating. Heat waves occur in June more frequently after the 15th, but generally during the hot season, the waves of heat can occur even from the first decade of April. The analysis of the maximum monthly temperature values recorded in June in the studied range shows that they were between 32.5°C at Polovragi and 41.3°C at Calafat with the average for the entire Oltenia region of 36.4°C , being the highest overall average of monthly temperature peaks in June of all history of climate observations for a period of 11 years (Tabel 1). There were 8 absolute temperature climates for June: Calafat 41.3°C , 41.1°C at Bechet and Bailești, 39.7°C at Caracal, 39.1°C at Dr. Tr. Severin, 38.9°C at Slatina, 38.8°C in Craiova and 25.9°C at Parâng Peak in the mountain area.

Table 1

Maximum temperature values recorded in the summer months in Oltenia in the period 2007-2017 (Tmax (°C), date = AAAA/ZZ, Hm = altitude of the weather station (m), the values marked with * are absolute thermal absolutes for those meteorological stations)

Meteorological Station	June			July		August	
	Hm	Tmax	data	Tmax	data	Tmax	data
Dr. Tr. Severin	77	39.1*	2007/26	42.2	2007/24	41.3*	2017/5
Calafat	66	41.3*	2007/26	44.3*	2007/24	42.2*	2017/5
Bechet	65	41.1*	2007/26	44.2*	2007/24	41.2*	2012/24;26
Băilești	56	41.1*	2007/26	44.0*	2007/24	40.5	2012/24
Caracal	112	39.7*	2007/26	41.9	2007/24	40.5	2012/24
Craiova	190	38.8*	2017/29	42.6*	2007/24	40.8	2017/5
Slatina	165	38.9*	2017/29	41.4*	2007/24	39.6	2012/7;24
Băcleș	309	38.6	2007/26	43.2*	2007/24	38.3	2017/5
Tg. Logrești	262	35.4	2017/29	39.4	2007/24	38.3*	2017/5
Drăgășani	280	37.1	2007/26	40.6	2007/24	38.7	2012/24;25; 2017/4;5
Apa Neagră	250	35.6	2013/19	40.6	2007/24	38.8*	2015/12
Tg. Jiu	210	36.6	2017/29	40.7*	2007/24	39.6*	2017/5
Polovrași	546	32.5	2017/29	36.5	2007/24	36.5*	2012/25
Rm. Vâlcea	243	35.6	2007/26	39.8	2007/24	40.7*	2012/7
Voineasa	573	33.4	2016/22	36.4	2007/24	36.7*	2012/24
Parâng	1585	25.9*	2010/13	28.4*	2007/24	28.3*	2017/5
Media Oltenia		36.4	2007	40.4	2007	38.5	2012

Source: data from the ANM archive

At the pluviometric point Cujmir (Mehedinți County) was recorded the maximum value of 42.0 °C on 26th of June 2007 (Marinică I., Marinică Andreea Floriana 2016), equalling the absolute climatic record of the last century recorded on 29.VI.1938 in Oravița. Most of the records were recorded in June 2007 during the most intense heat wave in June of all the history of the climate recorded between 18-27.VI.2007. **June 2007 was the hottest summer beginning of the last 136 years.** In June 2017, five maximum temperature values were recorded, two of which were absolute records for the respective meteorological stations in Craiova and Slatina (Tabel 1). Of the other years, we notice June 2013 with a maximum of 35.6 °C in the area of the sub-Carpathian depressions at Black Water and June 2010 with the absolute climatic record at Parâng Peak. In Romania, between 29.VI-1.VII.2017, there was an intense heat wave, with the peak of 1.VII.2017, when the highest temperatures were recorded in the history of the observations, at the date of 1 July. The Danube level at Bechet declined by 6 cm from the previous day on 1.VII.2017 and it was then decided that the Bechet-Oreahovo ferry would run only the day (07-21 hours).

Spatial-temporal expansion of dry time in June. As a result of high temperatures and low or no rainfall over long periods of time there has been intense drought that has caused major damage to agriculture and animal husbandry and not only in these economic areas. During this time, 79 months / dry weather at the meteorological station was recorded in June, according to Hellmann's criterion, a 44.9 %. Three months with spatial-temporal expansion over 90 % were registered: June 2007 and 2012 with 93.3 % and June 2017 expanding by 100 % (total drought) (Fig 1). The graph of the variation of the spatio-temporal expansion of dry time has an increasing trend with a significant growth rate of 0.5809. The driest month was June 2017 with the average for the entire region was 25.2 l/m², followed by June 2012 with an average of 38.0 l/m². The lowest monthly precipitation was in 2017: 2.2 l/m² in Craiova, 3.4 l / m² at

Dr. Tr. Severin, 4.0 l/m² at Calafat, 4.2 l/m², 6.3 l/m² at Băilești and 9.8 in Băcleș, and in 2012: 3.0 l/m² in Băilești, 7.2 l/m² in Craiova and 9.2 l/m² in Calafat.

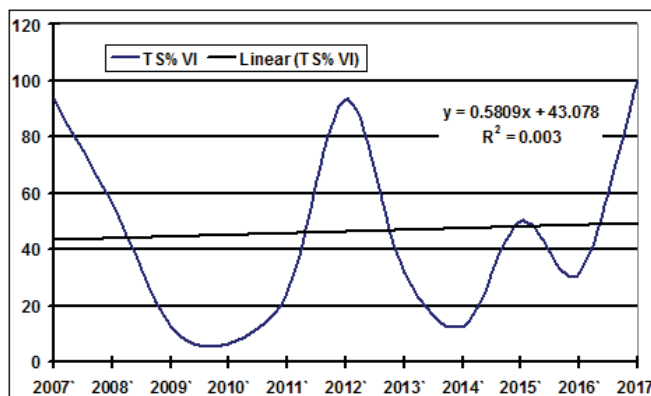


Figure 1. The variation of the space-time extension of drought in June. (Source: processed data from the ANM archive)

In July, the duration of the day is maintained ≥ 15 hours until 22.VII when the first decrease is recorded for 1 minute under 15 hours, and on 31.VII the duration of the day is 14 hours and 40 minutes, the decrease in the duration of the day the whole month is only 47 minutes. So in the last month of spring and in the first two months of summer, for 62 days, between 22.V-22.VII the duration of the day is ≥ 15 hours.

In the analyzed range, the maximum temperature values were between 36.4 °C at Voineasa and 44.3 °C at Calafat, and of those recorded at eight meteorological stations (50 % of the Oltenia meteorological stations) became absolute records for July being the largest of all weather observations: 28.4°C at Parâng Peak, 40.7°C at Tg. Jiu, 41.4°C at Slatina, 41.9°C at Caracal 42.6°C at Craiova, 43.2°C at Băcleș, 44.0°C at Băilești, 44.2°C at Bechet and 44.3°C at Calafat. Their average for the whole region was 40.4 °C being the largest of all summer but also in the history of the climate. All the monthly thermal peaks of the studied range were recorded in 2007 during the most intense heat wave in the Romanian climate history produced in the period 15-24.VII.2007, and the value of 44.3 °C from Calafat is the absolute thermal maximum for the entire country of July, 24.VII.2007 being the first day of July when in Romania the air temperature reached and exceeded 44.0 °C (Tabel 1).

Spatial-temporal expansion of dry time in July.

In July, there was a 78-month / dry-time meteorological station, according to Hellmann's criterion, a 44.3 % temporal extension. The hottest month was July 2007 with the average for the entire region was 20.2 l/m², and the registered quantities were due to rainfall that came on 31.VII.2007, followed by July 2015 with the average of 26.2 l/m². The lowest monthly rainfall was in 2007: 3.2 l/m² la Caracal, 6.6 l/m² at Drăgășani, 8.5 l/m² at Băilești and 9.8 l/m² at Rm. Vâlcea; in 2010: 9.8 l/m² at Caracal; in 2015: 1.6 l/m² at Băilești, 6.8 l/m² at Calafat, l/m² at Bechet, 8.8 l/m² at Caracal and 9.0 l/m² at Rm. Vâlcea. Four months were recorded with a time-span extension of the drought: July 2012 with 73.3 % expansion, July 2013 with 81.3 % July 2007 expanding by 93.8 % and 2015 with 100 % expansion (total drought). The graph of the variation of the spatio-temporal expansion of dry time has a slightly decreasing trend with an insignificant coefficient of

decline of -0.2818, due to rainfall in 2017 recorded in the period 2-3.VII.2017 and followed by intense drought to 6. X.2017.

In August, the duration of the day lasts ≥ 14 hours until the 15th and on 16th, the night duration begins to exceed 10 hours and as a result the nights gradually cool down, allowing for a good enough rest, (tropical nights become uncommon) although the days are stagnant in some years until September 15 or even after, although the polar climate front migrates quite quickly southward, bringing behind him increasingly hot air masses. Decreasing the duration of the day in August is 1 hour and 20 minutes being the first decrease of the duration of the day > 1 hour, during the year. As a result, the monthly average air temperature for the entire region in Oltenia recorded the first decrease in the year, compared to July, with a modest value of 0.5 °C. However, the absolute absolute maximum temperature of air in Romania was recorded at 10.VIII.1951 in Râmnicelu commune at the Ion Sion agricultural farm (in Bărăganul Brăilei), at 44.5 °C, not surpassed until now. During the studied period, the monthly temperature peaks were between 36.5 °C at Polovragi and 42.2 °C at Calafat, and their average for the whole region was 38.5 °C, the second highest value in the summer. 10 absolute temperature records have been recorded at the meteorological stations: Parâng, Drăgășani, Polovragi, Voineasa, Tg. Logrești, Apa Neagră, Tg. Jiu, Rm. Vâlcea, Bechet and Dr. Tr. Severin, and at Craiova, Slatina and Dragasani meteorological stations were the highest in the last 72 years (Tabel 1). August 2017 being the hottest month of the last 72 years, and the most intense heat wave was recorded between 1-6.VIII.2017.

Spatial-temporal expansion of drought in August.

In August there were 96 months / weather station of dry weather, being the largest of all summer, according to Hellmann's criterion, a 54.5 % temporal extension. The driest months were August 2008 with the average for the whole region was of 9.7 l/m², august 2011 with the average of 16.5 l/m² and august 2012 with the average of 24.5 l/m². During this period (2007-2017), there were 30 months station / meteorological with precipitation values ≤ 10 l/m², which confirms that August is the driest month of the summer, in which the desertification processes are amplified. Four months of spatial-temporal expansion of the drought were recorded: August 2008 with 100 % expansion (total drought), August 2013 with expansion of 81.3 %, July 2007 expanding by 93.8 % and 2015 with 100 % expansion (total drought), August 2001 expanding by 93.8 %, August 2012 with 87.5 % and August 2016 expanding by 81.3 %.

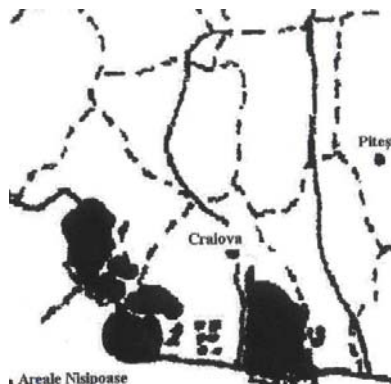


Figure 2. Sandy Areas in Oltenia (after Marinica and Marinica, 2016)

Monthly precipitation averages in August for the whole period considered (2007-20017) ≤ 30.0 l/m² were recorded in the area of Dr. Tr. Severin, Calafat, Bechet, Bailești Caracal, that is where the temperatures were the highest and the sandy soils

very vulnerable to the desertification processes (Fig. 2). The processes of aridization and desertification were intense throughout the southern half of Oltenia and especially in the area called ,*Oltenia' s Sahara*".

CONCLUSIONS

During the studied period (2007-2017), the climatic heating in Oltenia continued and even intensified, recording 24 hot thermal temperatures with a local character and one for the whole country during the summer. The above shows the intensification of the heat and drought in the summer, and the field observations as well as our documentation have shown that the processes of desertification during the summer have been accentuated and extended in all counties. All of this is due primarily to the intensification of climatic warming but also to intensive land use. In the sandy area, in some years the "migratory" sand dunes occur due to the wind intensification. We report that among the most destructive actions is the use of herbicides in agriculture.

REFERENCES

Marinică, Ion. 2006. Fenomene climatice de risc în Oltenia, Editura Autograf MJM, Craiova, 386 p;

Marinică I., Marinică Andreea Floriana. 2014. Considerations on Desertification Phenomenon in Oltenia, Forum Geografic. Studii și Cercetări de Geografie și Protecția Mediului. Vol. XIII, 2, Universitatea din Craiova, pp. 136-147.

Marinică I., Marinică Andreea Floriana. 2016. Variabilitatea climatică în Oltenia și schimbările climatice, Edit. Universitaria, Craiova.

FLOWERS AND FRUITS VARIABILITY OF
Convolvulus arvensis L. WEED

Ionescu Nicolae^{1*}

^{1*}Agricultural Research and Development Station Pitești
Correspondence author. E-mail: nicolaeionescu50@gmail.com

Keywords: *Convolvulus arvensis*, corolla, capsule, seeds, variability

ABSTRACT

*The species is a perennial dicotyledonous that stifles serious crop plants. In the world is considered among the top of 10 weeds like importance. It's spread occurs both by seeds and by rhizomes. The morphological characteristics of the plant and specially the reproductive showed a trend continues, depending on the ecotype existing at a time. Corolla average diameter measured 27 mm, the capsule was 6.5 mm high, 4.4 mm wide and formed 3 seeds each. The seeds have a length of 3.1 mm and 2.0 mm width. Among the characters studied were established both positive and some negative correlations. Positive correlations were obtained between the capsule size ($r = 0.421^{***}$) and between the seed size ($r = 0.621^{***}$). An-significant negative correlations were between the corolla diameter and capsule length ($r = -0.068$) and between the number of seeds/capsule and seed length ($r = -0.174$). A very low positive correlation was found between the capsule diameter with capsules width ($r = 0.024$). In these circumstances weed was adapted into agriculture field by forming larger dimensions of capsules and seeds, in contrary of flowers corolla diameter.*

INTRODUCTION

Weed control has entered in a new phase: on the one hand herbicides is restricted for environmental protection (Stone et al, 2005), and on the other hand existing species evolves (Parnell & Curtis, 2012). Adapting to new conditions (Gianoli, 2004) can be demonstrated by observing the variability trend as many morphological characters (Black et al, 1994). It was found that a weed with reproductive traits (Angiosperm Phylogeny Group 2003) as high variability, has managed to adapt better to the ecology of crop (Carine & Robba, 2010). The current trend requires control measures (Boldt & Sobhian, 1993), accepted (Wiese et al, 1996; Wiese et al, 1997), diversified and complex (Coombs et al, 2004).

A weed known in agricultural fields is *Convolvulus arvensis* L. (pro syn *C. minor* A.L. Juss., bindweed, field bindweed, lesser bindweed, field morning glory, CONAR code Bayer). The plant grows in different environmental conditions and blooms in midsummer ((Weaver & Riley, 1982). Flowers 1-3 in axillary cymes inflorescence, have short stems. Corolla funnel-shaped, pentamerous, with 5 radial pubescent bands but not divided into distinct lobes, with small bands. The color of the petals is white, sometimes pinkish and reddish. Bands of petals are pink or white. Corolla diameter is 10-25 mm. Five stamens are inserted to the base of the corolla tube, their filaments flattened at the base and rounded at the top, about 1 cm long. Style with 2 oblong stigmas and ovary 2-locular, both white (Weaver & Riley, 1982). The ovary is surrounded by a circle of yellow-orange

ring of nectar, for pollinator insects. Fruits are capsules 4-valve, hairless, brown- yellow, globular, peak with style rudiments. Capsule height surpasses customary width. In capsule form seed dark-brown to black, sub-ovate. The 1-4 pieces are rounder- thinner with rough surface and arched back side. The seeds were 2.5-4.5 mm long, 2.0-3.5 mm wide and 2.0-3.0 mm thick.

The aerial part of the plant is not recommended in animal feed (Austin, 2000; Meng et al, 2002). But in terms of medicinal, plant contains a number of promising compounds (Kaur & Kalia, 2012). Thus, the rhizome containing resin (*convolvulin*), alkaloids (*cuscohygrins* and *calystegins*), lipids (*butyric acid* forms) and flavonoids (*rutin*). The roots and the air parts have flavonoids (7) and *coumarin* (5). Green part of the plant contains steroids (3) and phenolic acid compounds (9) (Schultheiss et al, 1995; Todd et al, 1995). The seeds are rich in lipids (7 acids such as oleic, arachidic, lioleic, linolenic, stearic, palmitic and behenic) (Kaur & Kalia, 2012).

The weed is native to Europe and Asia and prefer both winter cereals and spring crops. System "convolve" felt like a weed cover the entire acreage, then climbs on crop plants. By performing measurements in different conditions of vegetation, it might bring some new elements to describe this species of weed. In the present study we analyzed the corolla diameter, length and width of capsule, number of grains per capsule, length and width of the grains formed.

MATERIAL AND METHODS

Measurements were performed in August on *Convolvulus arvensis* plants, last three years. They were chosen from more areas with winter wheat and maize crops. The areas were located in the resort region. 100 plants of *C.arvensis* were measured diameter of flowers corolla (the cups) completely open. The basal portion of the plant was harvested by a single capsule completely mature. Of 100 capsules were measured: height, width and the number of formed seed. Seeds were measured by length and width.

Morphological characters were analyzed by histograms (*polygons frequency, %*). In the method were used class intervals, and in the number of seeds per capsule, absolute values were used. Study has highlighted several aspect and namely: i) modal values with highest frequency, ii) variability within ranges of characters studied, iii) the specifics of each character of the ecotype of the plant analyzed.

Among the analyzed characters were established some correlations, by which they could observe and study their tendencies within ecotype. The expression values was used Excel.

In the statistical calculation of all the values obtained was used analysis of variance (ANOVA test) i.e. on the ranges of variation. Statistical parameters were calculated using formula: $\bar{a} = \frac{\sum x}{n}$, unde \bar{a} = media of determinations, iar x = values obtained, S^2 (variance) = $\frac{1}{n-1} \left[\sum x^2 - \frac{(\sum x)^2}{n} \right]$, S (standard error) = $\sqrt{S^2}$, $S\%$ (variation coefficient) = $\frac{S}{\bar{a}} \cdot 100$. Finally it was developed a summary of the data from characters variability of *C.arvensis* flowers, fruits and seeds established by determinations.

RESULTS AND DISCUSSIONS

Variability of *C.arvensis* corolla diameter. Cime forming by plant with large flowers, are visible in the crops. Corolla diameter is accustomed 1.0- 2.0- 2.5- 3.0 cm. Determinations resulting were values between of 17 and 35 mm. Corolla average diameter was 27 mm- figure 1. The color of weed corolla is white- rose- Figure 2. Dominant between the diameter of *C.arvensis* were the corolla of 26-28 mm (33 %), followed by those with 29 to 31 mm (24 %). Corolla with lower values and with higher value were 3 % and 1 % respectively.

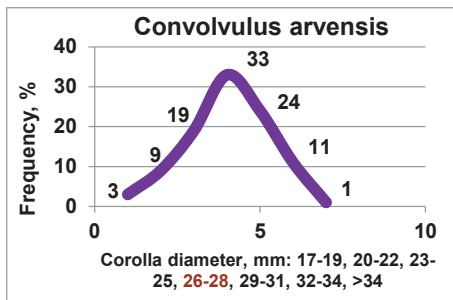


Figure 1. Evolution of corolla diameter

Figure 2. *C.arvensis* corolla aspect

Variability of capsules and seeds. After fecundation the capsules are formed. Data from literature shows values of dimensions of the capsule between 50-70 mm in height and 50-60 mm in width. By measuring the height of the capsule from bottom to the top were found some differences- Figure 3. Limit values ranged between 4.3 and 8.4 mm. The modal value ranged from a maximum length of 6.7-7.2 mm (42 %), followed by those with 6.1-6.6 mm (33 %). Capsules shorter of 4.3-4.8 mm had a frequency of 1 %, while the longest between 7.3-8.4 mm were a total of 5 %. Width capsules showed values between 3.6 and 6.6 mm. The modal value was 4.6-5.0 mm (33 %), followed by those with 4.1-4.5 mm (21 %) and 5.1-5.5 mm (21 %). Capsules with greater widths constituted 6 % of total- Figure 4.

C.arvensis capsule form a variable number of seeds, between 1 and 4 (rarely found a higher number). In the case of capsules analysis found 1-4 seeds/ capsule. The highest frequency had 4 seeds/ capsule (37 %), followed by the 3 seeds (32 %), 2 seeds/ capsule were 29 % and 2 % with one seed/ capsule- Figure 5. Appearance and color of seeds are specific- Figure 6.

Seed size are different, the usual length is of 3-4 mm. In other determinations found seeds 2.5-4.5 mm long, 2.0-3.5 mm width and 2.0-3.0 thickness. Their overall shape is ellipsoid, with bush skin, dark brown to black. Measurements have demonstrated that *C.arvensis* seed are between 2.0 and 4.9 mm. The highest frequency had a seed of 3.0-3.4 mm (36 %), followed by those with 3.5-3.9 mm (23 %), and those with 2.5-2.9 mm (22 %). Seeds for long, 4.5-4.9 mm constituted only 1 % of total- Figure 7. Seed width was between 1.0 and 3.0 mm. The most frequent ones had 1.9-2.1 mm (32 %), followed by 16 % of those with 1.6-1.8 mm and 2.5-2.7 mm respectively. Variability of this character was quite high, with discontinue values- Figure 8.

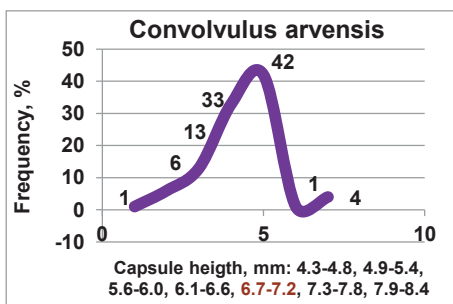


Figure 3. Evolution of capsula height

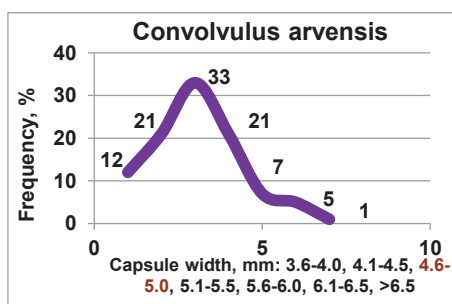


Figure 4. Evolution of capsula width

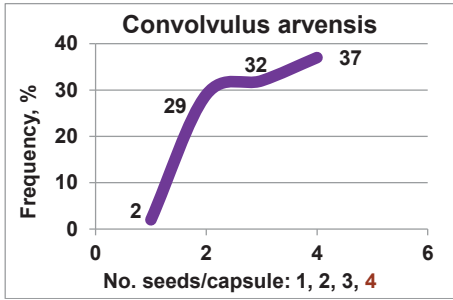


Figure 5. Evolution of seeds no. from capsula Figure 6. Seeds and capsules of *C.arvensis*

Correlations between the main characters. The correlation between the diameter of the corolla and capsule dimensions (length and width) were an-significant: negative for capsule length, $r = -0.068$, and positive for capsule width, $r = 0.024$. These links show that weed make capsules somewhat larger, indifferent of capsule diameter- Figure 9 and Figure 10.

Between length and thickness of the *C.arvensis* capsule was established a significant and positive correlation ($r = 0.421^{***}$). Capsules, how they are longer, their thickness is much higher. The correlation is supported by the conditions that they encounter in the crops- Figure 11.

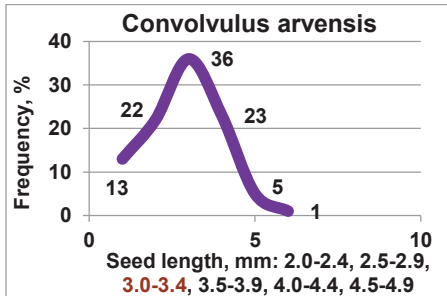


Figure 7. Frequency of seed length

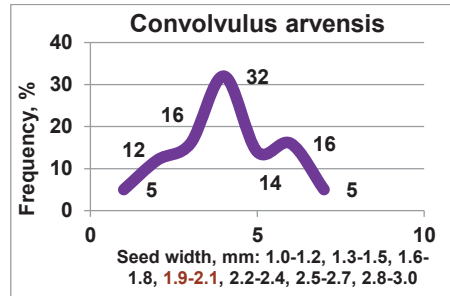


Figure 8. Frequency of seed width

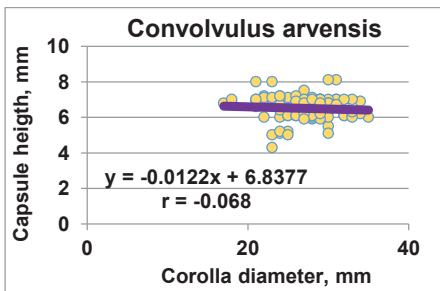


Figure 9. Correlation between corolla diameter and capsula height

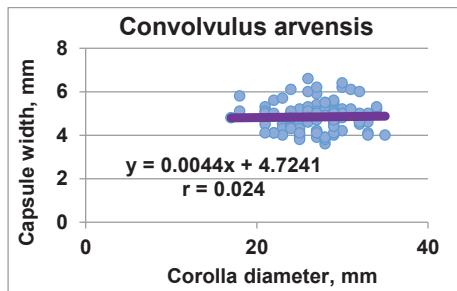


Figure 10. Correlation between corolla diameter and capsula width

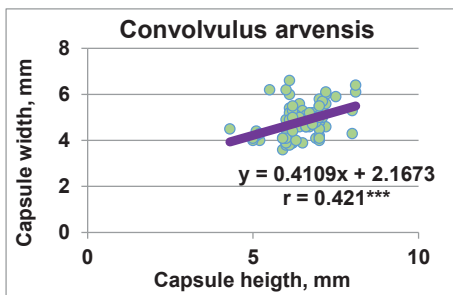


Figure 11. Correlation between height and width of capsule

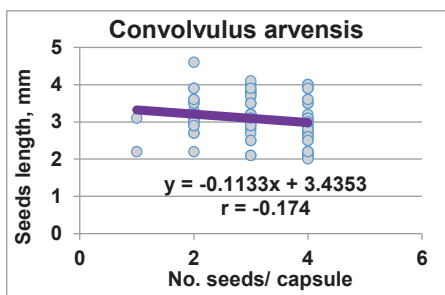


Figure 12. Correlation between no. seeds/ capsula and seeds length

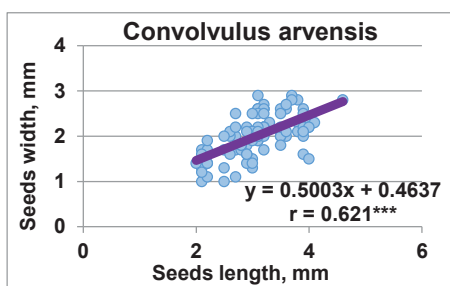


Figure 13. Correlation between length and width of weed seeds



Figure 14. Seeds of *C. arvensis* weed

Between the number of seeds per capsule and seed length achieved a negative relation, quite obvious: $r = -0.174$ - Figure 12. This demonstrates that in capsules with a greater number of seeds, they have slightly less length. Between seed size (length and width) achieved a very favorable correlation ($r = 0.621^{***}$), which shows that as the crop conditions are better, the plant forms better seed size- Figure 13. Appearance and color of *C. arvensis* seeds are shown in Figure 14. Proportion of brown and black seeds was quite equal.

From a synthetic table of correlations between various characters emerged negative links between the number of seeds per capsule and size of seeds- Table 1. Positive correlations were obtained between the width of the capsule with the number of seeds per capsule ($r = 0.493^{***}$) and with the seeds length ($r = 0.296^{**}$).

Table 1

Correlations between differing characters of <i>C. arvensis</i> weed flowers and fruits						
Character	Corolla diameter	Capsule height	Capsule width	No. seeds/ capsule	Seeds length	Seeds width
Corolla diameter	1	-0.068	-0.024	0.060	-0.067	-0.014
Capsule height		1	0.421***	0.033	0.378***	0.245*
Capsule width			1	0.493***	0.296**	0.039
No. seeds/capsule				1	-0.174	-0.528 ⁰⁰⁰
Seeds length					1	0.621***
Seeds width						1
DL 5 % = 0.19 DL 1 % = 0.25 DL 0.1 % = 0.32						

Statistical analysis of the corolla, capsule and seed variability. Were calculated for each character analyzed: the average (\bar{a}), the variance (s^2), the standard error of the mean (s) and the coefficient of variation (CV, %). Statistical estimates made have

highlighted the characteristic values of *Convolvulus arvensis* ecotype. The values obtained were characteristic. Thus flowers corolla measured on average 27.08 mm, capsule measured 6.503 mm in height and 4.369 mm wide. The capsules were formed 3.04 grains which measured 3.091 mm long and 2.008 mm width- Table 2.

Table 2

Indices	Corolla diameter, mm	Capsule		No. seeds/ capsule	Seeds	
		Height, mm	Width, mm		Length, mm	Width, mm
Media, \bar{a}	27.08	6.503	4.369	3.04	3.091	2.008
Variance, s^2	13.43	0.366	4.824	0.6347	0.4708	0.2098
Standard error, s	3.6644	0.605	2.196	0.7967	0.6862	0.4581
Coef. of var., %	13.53	9.30	50.26	26.21	22.20	22.81

CONCLUSIONS

A common species that cause significant damage is *Convolvulus arvensis* L. weed. Plant is widespread in southern territory, because the existing cross-type is very well suited in wheat and maize. To control it through its management it is good to know as many morphological characters. It was found that a species that express variability broadest possible help in finding the most suitable method of control. Morphological variability, especially reproductive being less known, may express existing cross-type in these conditions- Table 3.

The corolla of the flowers was something larger, formed capsules apparent with bigger height and width. The number of grains in a normal capsule was 1 to 4 pieces. The dimensions of the length and width of grain showed a lower than normal. Weight of a thousand kernels (WTK) of *C. arvensis* each 10 capsules ranged between 3.33 and 6.47 g.

Table 3

Values of corolla and fruits characters variability, CONAR weed		
Characters	Literature	Research
Corolla diameter, mm	10 – 30	17 - 35
Capsule	Height, mm	5.0 – 7.0
	Width, mm	5.0 – 6.0
No. seeds/ capsule	2 - 4	1 - 4
Seeds	Length, mm	4.0 – 5.0
	Width, mm	2.0 – 3.0
Weight of a thousand kernels, WTK, g	-	3.33 – 6.47

REFERENCES

- Angiosperm Phylogeny Group, 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. Botanical Journal of the Linnean Society, 141(4): 399-436.
- Austin D.F., 2000. Bindweed (*Convolvulus arvensis*, *Convolvulaceae*) in North America- from medicine to menace. Bulletin of the Torrey Botanical Club, 127(2):172 -177.
- Black I.D., Matic R., Dyson C.B., 1994. Competitive effects of field bindweed (*Convolvulus arvensis* L.) in wheat, barley and field peas. Plant Protection Quarterly, 9(1):12-14.
- Boldt P.E., Sobhian R., 1993. Release and establishment of *Aceria malherbae* (Acari: *Eriophyidae*) for control of field bindweed in Texas. Environmental Entomology, 22(1):234-237.
- Carine M. A. and Robba L., 2010. Taxonomy and evolution of the *Convolvulus abatus* complex (*Convolvulaceae*). Phytotaxa: 1- 14 1.

Coombs E. M., et al., Eds., 2004. Biological Control of Invasive Plants in the United States. Corvallis: Oregon State University Press: 1- 151.

Gianoli E., 2004. Plasticity of Traits and Correlations in Two Populations of *Convolvulus arvensis* (Convolvulaceae) Differing in Environmental Heterogeneity. International Journal of Plant Science, 165(5): 825-832.

Kaur M., Kalia A.N., 2012. *Convolvulus arvensis*- a useful weed. International Journal of Pharmacy and Pharmaceutical Sciences, 4(1): 38- 40 (ISSN 0975-1491).

Meng x.l., Riordan N.H., Casciari J.J., Zhu Y., Zhong J., Gonzales M.J., Miranda-Massari J.R., Riordan H.D., 2002. Effects of a high molecular mass *Convolvulus arvensis* extract on tumor growth and angiogenesis. Health Science Journal, 21: 323- 328.

Parnell J. and Curtis T., 2012. Webb's An Irish Flora. Cork University Press. ISBN 978-185918-4783.

Schultheiss P.C., Knight A.P., Traub-Dargatz J.L., Todd F.G., Stermitz F.R., 1995. Toxicity of field bindweed (*Convolvulus arvensis*) to mice. Vet Hum Toxicol, 37(5):452-454.

Stone A.E., Peeper T.F. and Kelley J.P., 2005. Efficacy and acceptance of herbicides applied for field bindweed (*Convolvulus arvensis*) control. Weed Technology, 19:148–153.

Todd F.G., Stermitz F.R., Schultheis P., Knight A.P., Traub-Dargatz J., 1995. Tropane alkaloids and toxicity of *Convolvulus arvensis*. Phytochemistry, 39: 301- 303.

Weaver S.A. & Riley W.R., 1982. The biology of Canadian weeds. 53. *Convolvulus arvensis* L. Canadian Journal of Plant Science, 62:461-472.

Wiese A.F., Salisbury C.D., Bean B.W., Schoenhals M.G., Amosson S. 1996. Economic evaluation of field bindweed (*Convolvulus arvensis*) control in a winter wheat-fallow rotation. Weed Science, 44:622-628.

Wiese A.F., Bean B.W., Salisbury C.D., Schoenhals M.G, Amosson S., 1997. Economic evaluation of field bindweed (*Convolvulus arvensis*) control. Weed Science, 45(2): 288-295.

**ASSESSMENT AND CHARACTERIZATION OF MICROBIAL COMMUNITIES
IN SALT AFFECTED SOIL**

Mihai Constanța¹, Crăciță Ana Gabriela², Paraschiv Maria^{3,4},
Cîmpeanu Carmen¹, Constantin Carmen^{1,3*}

University of Agronomic Sciences and Veterinary Medicine of Bucharest

² OMV Petrom S.A., Upstream – Domestic Assets – Laboratory RO/ICPT Campina

³ National Institute of Research and Development for Biological Sciences Bucharest

⁴ Research Center for Advanced Materials, Products and Processes – University Politehnica of
Bucharest, Romania

* *Correspondence author. E-mail: manolecarmen2000@gmail.com*

Keywords: *halophytes, microorganisms, saline*

ABSTRACT

This paper presents the population of micro-organisms found in the saline soil with a certain electrical conductivity.

The soil samples were dried, and ground. After this procedure, the soil was revitalized with peptone water, then incubated 12 hours (overnight) at 31 °C. The culture media used were: nutrient agar: yeast extract, peptone, agar; YPG agar: yeast extract, peptone, glucose, agar supplemented with chloramphenicol. For the identification of osmotolerant microorganisms, these media were also used with different NaCl concentrations ranging from 5 ± 15 %. The soil samples were inoculated in Petri dishes and incubated for 7 days at 35 °C for bacteria and 28 °C for fungi. After incubation, the number of bacteria and fungi in each soil sample was calculated by the UFC determination technique (colony forming units).

INTRODUCTION

There are many studies that state that more than 5 % of agricultural land worldwide is affected by salinity (Etesami 2018, Almeida Machado & Serralheiro, 2017, Shrivastava & Kumar 2015, Pitman & Läuchli 2002). Along with salinity, the composition of the soil is modified. Within it, the microbial communities which are involved in processes and biogeochemical cycles (Lima do Nascimento et al. 2016, Stefanis et al. 2013). Microbial communities in the saline soils are an indicator to the functionality of ecosystem services. However, the literature about the microbial diversity and composition is scarce (Zhao et al. 2018). Playing an important role through mineralization of organic matter, it is recommended to maintain high microbial activity (Yan et al. 2015).

Thus, the aim of this study was to evaluate the microbial population of soils with known electrical conductivity. This study was needed for a broader research that involved the use of halophyte plant species in order to improve soil quality.

MATERIAL AND METHODS

For this experiment, six soil samples with different salinity was taken. The evaluation, identification and characterization of microbial diversity in three repetitions were performed using an adapted method (Shafkat et al. 2017).

Soil samples were taken from a depth of 40 cm, in southeastern Romania (Figure 1).



Figure 1. The soil sampling area

To revitalize and enrich the microorganisms, peptone water and NaCl solution in 1: 5 and 1:10 dilutions were added to soil samples and then incubated for 12 hours (overnight) at 31 °C.

After incubation, the samples of the soil were inoculated on culture media (nutrient agar - NA and YPG agar). Sowed Petri dishes were incubated for 7 days at 35 °C for bacteria and 28 °C for fungi.

After incubation, the number of bacteria and fungi in each soil sample was calculated by the CFU determination technique (colony forming units), which allows to determine the number of viable cells in a sample, in the form of colony-forming units, in Petri dishes on selective culture media, above mentioned.

$CFU = N \times c \times 10$, where

CFU = number of viable cells/1ml sample

N = average of the colonies counted from the same dilution

c = dilution reverse

10 = coefficient reference to 1ml.

In the experiments performed, the macroscopic examination was performed visually, considering the characteristics of the bacterial colonies developed on the culture medium. Also, the microbial culture characters the shape, the color, and the texture of the colony were analyzed.

The EC was realized using an adapted method (Rayment and Higginson, 1992).

RESULTS AND DISCUSSIONS

The soils selected for this study had different EC values, and contained different microbial composition and viability (Table 1).

Table 1

Results of growth in NA and YPG media containing 5 % (w/v), 10 % (w/v),
and 15 % (w/v) NaCl

Sample- Dilution	Nutrient Agar (5 % NaCl) CFU	Nutrient Agar (10 % NaCl) CFU	Nutrient Agar (15 % NaCl) CFU	YPG (5 % NaCl) CFU	YPG (10% NaCl) CFU
P _{1a} – 1:5	395.6	90.5	x	31.5	x
P _{1b} – 1:10	730	69.5	x	10.5	x
P _{2a} – 1:5	199.5	20	x	44	x
P _{2b} – 1:10	9	x	x	21	x
P _{3a} – 1:5	353	142	x	69	x
P _{3b} – 1:10	59.5	52.5	x	21	x
P _{4a} – 1:5	146	27	x	47	x
P _{4b} – 1:10	135	x	x	19	x
P _{5a} – 1:5	590	x	x	5	x
P _{5b} – 1:10	439	x	x	7	x
P _{6a} – 1:5	522	x	x	3	x
P _{6b} – 1:10	327.5	236	x	x	x

x - the appearance of salt crystals

All the samples showed growth in NA and YPG media containing 5 % (w/v), NaCl. But failed to grow in media containing 10 % (w/v) and 15 % (w/v) NaCl. The results are similar who those obtained by Shafkat et al. (2017) in media containing 8 % (w/v) and 10 % (w/v) NaCl.

Bacterial growth in 10 % NaCl supplemented media reached the stationary phase at 24 hours incubation with fewer bacteria. Concentration of 5 % NaCl in the growth media contributes to a faster growth of bacteria and the cells have reached the stationary phase after 48 hours. This may be due to the fact that an increase in salt content restrains the growth and development of bacteria without affecting the composition (Zhang et al. 2015).

The negative control (medium without NaCl) showed the total absence of microorganisms.

Through macro and microscopic observations of colonies, several typea of bacteria and fungi have been identified.

Thus, the highest percentage in the microflora of the studied soil has *Bacillus* genus with a total of 4 species, followed by *Staphylococcus*, *Enterococcus* and *Micrococcus*.

Morphology and phenotypic characteristics suggest that the bacterial isolate is a moderately halotolerant member of the genus *Bacillus*. The isolated cells are thin, short strands, 0.3-0.5mm wide and 1.3-1.9 mm long. The colonies are creamy, smooth and circular. Strictly aerobic, non-flagellated, non-motile, long, straight or slightly curved in the middle, with rounded heads.

Bacterial colonies of the genera *Staphylococcus*, *Enterococcus* and *Micrococcus* dispersed on the culture medium formed rounded colonies, embossed with nearly round yellow and light creamy edges. There were creamy, smooth, low convex and circular / slightly irregular bacterial colonies on NA medium containing 10 % NaCl.

Microscopically, the cells appeared in the form of coils, diplococi, isolated or in pairs, short chains, immobile.

The microbial population increased in inverse proportion to the chloride concentration, salt crystals making their appearance (Figure 2).



Figure 2. The occurrence of salt crystals on medium with 10-15 % NaCl

Also, different fungi species were isolated and identified at different concentrations of NaCl. Thus, the presence of *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Mucor* genus has been revealed (Figure 3).

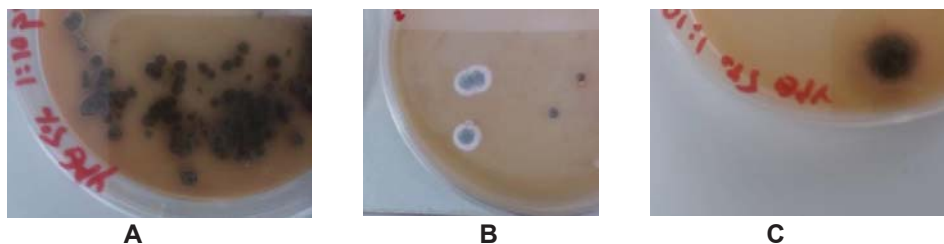


Figure 3. Colonies of *Cladosporium* sp. (A), *Penicillium* sp. (B), *Alternaria* sp. (C)

To assess the microbial population, soil samples with electrical conductivity between 4000 and 11000 mS cm⁻¹ were used. According to the classification (Canfora et al. 2014), the soil is saline (the Electrical Conductivity is greater than 4 dS m⁻¹).

CONCLUSIONS

With regard to microbiological soil assessment, it can be concluded that most important bacteria and fungi groups can live in concentrations up to about 5 % salt and that many groups are physiologically active even at much higher concentrations. Also, due to the total absence of colonies in salt-free environments, isolated and identified bacteria and molds can be classified as moderately halotolerant. Different fungal species were isolated and identified at various concentrations of NaCl. These results indicate a significant presence of the strains of *Aspergillus* sp., *Penicillium* sp., *Cladosporium* sp., *Alternaria* sp., and *Mucor* sp.

The highest share in the soil microflora studied was *Bacillus* genus with a total of 4 species, followed by the genus *Staphylococcus* sp., *Enterococcus* and *Micrococcus* sp.

ACKNOWLEDGMENT

These researches were carried out under the financing contract no. 56 / 05.09.2016 Project ID P_40_308, Clean technologies for processing and / or capitalizing fuel-efficient materials – CleanTech.

REFERENCES

Almeida Machado R. M., and Serralheiro R. P., 2017, Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization, *Horticulturae*, vol. 3, 1-13.

Canfora L., Bacci G., Pinzari F., Lo Papa G., Carmelo Dazzi, and Benedetti A., 2014, Salinity and bacterial diversity: to what extent does the concentration of salt affect the bacterial community in a saline soil? *PLoS One*, vol. 9, 1-15.

Hassan Etesami, 2018, Using halotolerant bacteria for farming in salt affected soils, *Acta Scientific Agriculture*, vol. 2, 1-2.

Lima do Nascimento Paula Gracielly Morais, Beatriz Letícia Silva da Cruz, Andréa Mirne de Macêdo Dantas, Francisco Cláudio Lopes de Freitas, Márcia Michelle de Queiroz Ambrósio and Rui Sales Júnior, 2016, Microbial communities in soil cultivated with muskmelon under different management systems, *Revista Brasileira de Ciencia Solo*, vol. 40, 1-15.

Rayment G. E., Higginson F. R., 1992, Australian laboratory handbook of soil and water chemical methods, Melbourne, Inkata Press. (Australian Soil and Land Survey Handbooks, vol. 3.

Shafkat S. R., Romana S., Nafisa T., 2017, Isolation and identification of halotolerant soil bacteria from coastal Patenga area, *BioMedCentral Research Notes*, vol.10, 1-6.

Stefanis C., Alexopoulos A., Voidarou C., Vavias S., Bezirtzoglou E., 2013, Principal methods for isolation and identification of soil microbial communities, *Folia Microbiol (Praha)*, vol. 58, 61-68.

Zhang Y., Cao C., Guo L., Wu Q., Cui Z., 2015, Soil properties, bacterial community composition, and metabolic diversity responses to soil salinization of a semiarid grassland in northeast China, *Journal of Soil and Water Conservation*, vol. 70, 110-120.

Pooja Shrivastava and Rajesh Kumar, 2015, Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation, *Saudi J Biol Sci.*, vol. 22, 123–131.

Pitman M. G., and Läuchli A., 2002, *Salinity: Environment - Plants - Molecules, olecules* , Kluwer Academic Publishers, Printed in the Netherlands, 3–20.

Yan N., Marschner P., Cao W., Zuo C., Qina W., 2015, Influence of salinity and water content on soil microorganisms, *International soil and water conservation research*, vol. 3, 316-323.

Zhao S., Liu J. J., Banerjee S., Zhou N., Zhao Z. Y., Zhang K., Tian C. Y., 2018, Soil pH is equally important as salinity in shaping bacterial communities in saline soils under halophytic vegetation, *Scientific Reports*, vol. 8, 1-11.

**THE NEW PEST *CYDALIMA PERSPECTALIS* (WALKER, 1859)
(LEPIDOPTERA: CRAMBIDAE) IN AREA OF CENTRAL OLTENIA.**

Mitrea Ion^{1*}, Stan Raluca²

University of Craiova, Faculty of Horticulture;

²PhD University of Craiova

*Correspondence author. E-mail: mitreion@gmail.com;

Keywords: *Cydalima perspectalis*, monitoring, *Buxus*, Oltenia.

ABSTRACT:

Cydalima perspectalis Walker, 1859 (Lepidoptera: Crambidae) is a dangerous pest for the *Buxus sempervirens* shrub, which gives it this status is the spread rate, being present on almost the entire European continent. In recent years, it has also spread to Romania in several parts of the country. In the Oltenia area it is observed both in the urban and rural areas, and the degree of defoliation is different from one area to another. A small defoliation rate was encountered in Craiova, Sărari area with a percentage of 20 %, and the highest in Dobrun village of 100 %, finally leading to the death of the host plant.

INTRODUCTION

Cydalima perspectalis (Walker, 1859), known as the hairy caterpillar of the buxus, is an invasive species originating in East Asia: China, Japan, Korea, India.

This pest is part of the Order: Lepidoptera, Family: Crambidae, accidentally introduced into Europe from China.

So far, the general view is that, the main route of introduction is the international trade of *Buxus* plants, and the rapid spread of *Cydalima perspectalis* is most likely caused by the repeated supply of infested plants (Leuthardt et al., 2010; Van der Straten & Muus 2010).

In Europe, it was first reported in 2007 in Germany (Krüger, 2008; Korycinska, & Eyre, 2009), then in the Netherlands (Muuš et al., 2009), Switzerland (EPPO, 2008); Käppeli, 2008), France (Feldtrauer et al., 2009), Great Britain (Mitchell, 2009), Austria (Rodeland, 2009), Liechtenstein (Slamka, 2010), Italy (FEI, 2010), Belgium (Castells et al. (2011), the Czech Republic (Šumpich, 2011) Hungary (Sáfián & Horváth, 2011), Slovenia (Matjaž Jež in Sáfián & Horváth, 2011), Turkey (Hizal et al., 2012) Croatia (Koren & Črne, 2012), Russia (CABI/EPPO, 2012, Matsiakh, 2014), Slovakia (Pastoralis et al., 2013), Denmark (Hobern, 2013), Spain (Pérez-Otero et al., 2015), Bosnia-Herzegovina (Ostojic et al., 2015), Greece (Strachinis et al. 2015), Serbia (Konjević et al., 2015), Albania (Mariotti, 2017), quoted by Raineri et. al., 2017).

In Romania, this pest was first reported in 2010 in three different locations in Bucharest (Iamandei, 2010, quoted by Fora & Pošta, 2015). On the territory of Romania was also present: in the north-west part of Bucharest (Székely et al., 2011), Timișoara (Vîrteiu, 2014 and Fora & Pošta, 2015), in other localities in Timiș county (Gugea & Vîrteiu, 2017), Botosani (Balan & Corduneanu, 2014), Constanța (Skolka & Zaharia,

2014), Cluj (Bunescu & Florian, 2015), Arad (Don et al., 2016), Satu Mare (Katonea et al., 2016) and Salaj (Katonea et al., 2016, quoted by Gugea & Virteiu, 2017.)

Because of the very high spreading rate, this species is likely to be present in other areas of the country in addition to the above mentioned, the data not yet published.

METHOD AND MATERIAL

The study on the attack and spread of the species *Cydalima perspectalis* Walker on the *Buxus sempervirens* was carried out between July and September 2018.

The first step was achieved by visual inspection and observation of attack on the host plant, then counting and collecting both larvae and adults.

Larvae were collected by hand, and an entomological mesh was used to collect the adults. (figure 1).



Figure 1. Collection of larvae (left) and entomological mesh (right) (original).

The collected biological material was transported to the Entomology Laboratory of the Faculty of Horticulture, the determined larvae belonging to the species *Cydalima perspectalis* (Figure 2).



Figure 2. Growth box (left) and insectarium (right) (original).

RESULTS AND DISCUSSIONS

Buxus sempervirens is one of the most cultivated ornamental species, but in recent years it faces a dangerous pest, *Cydalima perspectalis* Walker.

Infestation symptoms include feeding damage on the leaves of the shoot edges by the larvae, which can leave only leaf skeletons and the epidermis behind them. (Leuthardt and Baur, 2013, quoted by I. Strachinis et al., 2015). Larvae can completely defoliate the host plant and finally lead to its death.

In Craiova, the first attack was detected in July 2018 in the Botanical Garden due to the high defoliation degree of this shrub, and after visual inspection the first larvae were discovered, and attacks were also found in other public areas: in the Red Valley neighborhood in front of a block and on Știrbei Vodă Boulevard in front of the Art Gallery, and in September in the Beethoven Special Technological High Court.

Other attacks being reported in Olt County in Dobrun commune, in a private yard and in Balș on Station Street.

In August it was also reported in Drănic commune, Dolj County.

The degree of attack on *Buxus sempervirens* shows the presence of the *Cydalima perspectalis* pest at least one year before being reported.

After the first stage of the study, reporting the presence of larvae and the identification of the attacked shrubs, were carried out the second step that of larval collection of different ages (young and mature).

After the species was reported, a number of observations were made regarding the degree of attack on the host plant in order to apply the control measures (Table 1).

Table 1
Spread of the species *Cydalima perspectalis* and the degree of attack on the species *Buxus sempervirens*.

Date	Location	Collection point	Attack rate (%)
04.07.2018	Craiova, Dolj	Botanical garden	70 %
05.07.2018	Dobrun, Olt	Private yard	100 %
14.07.2018	Dobrun, Olt	Private yard	70 %
09.08.2018	Craiova, Dolj	Street Sărarilor, nr. 33	20 %
15.08.2018	Craiova, Dolj	Boulevard Știrbei Vodă	50 %
14.08.2018	Balș, Olt	Station street	80 %
17.08.2018	Drănic, Dolj	Private yard	50 %
04.09.2018	Craiova, Dolj	În the yard of Special Technological Beethoven high school	90 %

Percentages between 20-100 % indicate the degree of defoliation of the host plant, the lowest degree of attack of 20 % represents a minimal attack of this pest registered on *Buxus* plants from Sărari Street, no. 33, Craiova, and the one of 100 % was recorded on *Buxus* plants in a private yard in Dobrun, Olt, (Figure 3).



Figure 3. Total defoliation of *Buxus sempervirens* (original).

We mention that in this location besides the total defoliation, it has been observed that the larvae have also been fed with the bark, causing the death of the host plant (Figure 4).



Figure 4. *C. perspectalis* larvae feeding on bark (original).

We later found that the above mentioned percentages changed, the lack of chemical control increased the degree of attack and the spread of the species at several points, for example in the Botanical Garden, initially the attack was found near the lake, then on 04.09.2018 all existing *Buxus* bushes were attacked by *Cydalima perspectalis*.

Recognition of the species can be done taking into account the external morphology of the stages of development. Thus, adults can easily be identified with white iridescent wings, and their edges are dark brown with a discoidal spot on the front of the wings (Fora & Pošta, 2015).

Adults of *Cydalima perspectalis* have a wingspan of about 4 cm (Leuthardt and Baur, 2013, quoted by Fora & Pošta, 2015). The body is white with the exception of the head and the last segment of the abdomen, which are brown (Mally & Nuss, 2010), (Figure 5, left.)

Occasionally, melanic forms appear which are completely brown, with the exception of the white discoidal spot on the forewings (Korycinska & Eyre 2011, Sáfián & Horváth 2011, quoted by Szekely et al., 2011) (Figure 5, right).

After mating, the females laid eggs on the leaves of the host plant in groups of 5-20, coated with a translucent jelly (Leuthardt and Baur, 2013, quoted by Fora & Pošta, 2015), initially being pale yellow, but close hatching, the cephalic capsule of the larva can be observed (Fora & Pošta, 2015) .



Figure 5. Adult general appearance *Cydalima perspectalis* (left) and melanic adult general appearance (right) (original)

Larvae are light green, characterized by longitudinal black (thick) and white (thin) stripes and black dots outlined in white on the dorsal side of the body and a bright black head (Figure 6), and in the last larval stage they can reach a length of up to 4 cm. (Szekely et al., 2011, Bella, 2013).

Larvae feed mainly on leaves, but may also attack the bark of the host plant, and young larvae prefer leaves with high alkaloids (older leaves), under natural conditions, young larvae tend to feed on old leaves from the bottom of shrubs (Figure 7), on a strong attack the shrub dries. (Leuthardt and Baur, 2013, quoted by Fora & Pošta, 2015)



Figure 6. *Cydalima perspectalis* – larva (original).



Figure 7. Attack produced by larvae on mature leaves (left) and on old leaves at the bottom of shrubs (right) (original).

Pupae are between 1.5 to 2.0 cm long, initially are green with dark stripes on the dorsal surface, towards the end of the growth period can be noticed a dark pattern corresponding to the marginal line of the adult wings (figure 8). They are concealed in a cocoon of white silk spun among the leaves and twigs (Korycinska and Eyre, 2009, quoted by Fora & Pošta, 2015).



Figure 8. Pupal stage of *Cydalima perspectalis* (original)

CONCLUSIONS

Results from research on *Cydalima perspectalis* and damage caused in the central area of Oltenia reveal that this pest spreads very rapidly, leading to the destruction of the *Buxus sempervirens* ornamental species of public or private gardens and gardens.

The degree of defoliation may be from a minimum of 100 %, leading to the death of the host plant in a short time, in the absence of control measures.

Particular importance should be attached to species monitoring, and biology must be further studied in order to observe the dynamics of the pest in terms of effective treatments to combat this species.

REFERENCES

Bunescu H., Florian Teodora, 2016, The Box Tree Moth, *Cydalima perspectalis* Walker (Lepidoptera: Crambidae: Spilomelinae) a New Invasive Species in Cluj Area (Romania), *University of Agricultural Sciences and Veterinary Medicine, Faculty of Agriculture, Cluj-Napoca, Romania*: 62-65.

Fora & Poșta, 2015, *Cydalima perspectalis* Walk. (Lepidoptera: Crambidae), a dangerous pest of *Buxus sempervirens* in Timis County, Romania, *Journal of Horticulture, Forestry and Biotechnology*, Vol. 19 (3), USAMVB Timișoara, 26-32.

Hızal et. al., 2012, The new pest *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) in Turkey, *Journal of Animal And Veterinary Advances* 11 (3); 400-403.

Gugea I., Vîrteiu Ana-Maria, 2017, Remains *Cydalima perspectalis* (Lepidoptera: Crambidae) an invasive species dangerous for landscaping in western Romania?, *Research Journal of Agricultural Science*, 49 (4), 135-141.

Strachinis I., Kazilas C., Karamaouna F., Papinikolaou N.E., Partsinevelos G.K. and Milonas P.G., 2015 First record of *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae) in Greece, *Hellenic Plant Protection Journal* 8: 66-72.

Leuthardt F.L.G., Billen W., Baur B., 2010: Spread of the box-tree pyralid *Diaphania perspectalis* (Lepidoptera: Pyralidae) in the region of Basel - a pest species new for Switzerland. *Entomo Helvetica* 3:51- 57.

Székely L., Dincă V. & Cristian M. ,2011 *Cydalima perspectalis* (Walker, 1859), a new species for the Romanian fauna (Lepidoptera: Crambidae: Spilomelinae), *Bul.inf. Entomol.*, 22: 73-78.

Mally R. & Nuss M. (2010) Phylogeny and nomenclature of the box tree moth, *Cydalima perspectalis* (Walker, 1859) comb. n., which was recently introduced into Europe (Lepidoptera: Pyraloidea: Crambidae: Spilomelinae). *European Journal of Entomology* 107: 393-400.

Van der Straten Marja J. & Muus Tymo S.T., 2010, The box tree pyralid, *Glyphodes perspectalis* (Lepidoptera: Crambidae), an invasive alien moth ruining box trees, *Proc. Neth. Entomol. Soc. Meet. - VOLUME 21*, 107-111.

Raineri et. al., 2017, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera, Crambidae) and the threats for the Nature 2000 Habitat 5110 in Liguaria (NW-ITALY), *Boll. Mus. Ist. Biol. Univ. Genova*, 79, 215-236.

Pérez-Otero R. et. al., 2014, *Cydalima perspectalis* Walker, 1859 (Lepidoptera, Crambidae): una nueva amenaza para *Buxus* spp. en la Península Ibérica, *Archivos Entomológicos*, 10: 225-228.

Salvatore Bella, 2013, The Box Tree Moth *Cydalima perspectalis* (WALKER, 1859) Continues to spread in southern Europe: New records for Italy (Lepidoptera Pyraloidea Crambidae), *Redia*, XCVI, 51-55.

Skolka M., R. Zaharia, 2014, *Cydalima perspectalis* (Lepidoptera: Crambidae) – new invasive species in Constanța, International Zoological Congress of "Grigore Antipa" Museum 19-22 November 2014, Book of Abstracts, Bucharest, Ed. Medialux, București, 181-182.

Koren Toni & Črne Mitja, 2012 The First Record of the box tree moth, *Cydalima perspectalis* (WALKER, 1859) (Lepidoptera, Crambidae) in Croatia, *NAT. CROAT. VOL. 21 No 2* , 507–510.

**STUDIES ON THE MONITORING AND CONTROL OF THE SPECIES
METCALFA PRUINOSA SAY (HOMOPTERA: FLATIDAE)**

Mitreă Ion^{1*}, Stan Raluca²

University of Craiova, Faculty of Horticulture;

²PhD University of Craiova

*Correspondence author. E-mail: mitreion@gmail.com;

Keywords: *Metcalfa pruinosa*, chemical control, monitoring.

ABSTRACT

Metcalfa pruinosa Say 1830, an invasive species of North American origin, has expanded its area to almost the entire European continent, being also a polyphagous species.

Over the years has been tried to combating the specie through biological control over the mass appearance of the populations of *Metcalfa pruinosa*, but also chemical combating.

The monitoring and control of this species was carried out in the Botanical Garden of Craiova.

Following the chemical control we performed, we found that the most effective treatment is Nurelle D 50/500 EC.

INTRODUCTION

Metcalfa pruinosa Say 1830, also known as melliferous cicada, belongs to the Flatidae family, being of North American origin, an invasive species extending from the southeast of Canada (Ontario - Quebec) to Florida (Mead, 2004, quoted by Preda & Skolka, 2009)

In Europe, it was first reported in 1979 (Zangheri & Donadini, 1980), France 1986 (Della Giustina, 1987), Spain 1988 (Pons et al., 2002), Slovenia 1990 (Sivic, 1991) (1994), Switzerland 1993 (Jermini et al., 1995), Croatia 1993 (Maceljiski et al., 1995), Austria 1996 (Holzinger et al., 1996), Czech Republic 2001 (2004), 2003 (Karsavuran & Guclu, 2004), Hungary 2004 (Penzes et al. 2005), Bulgaria 2004 (Trenchev et al., 2007), Serbia 2006 (Mihajlović, (2009, quoted by Preda & Skolka, 2009).

In Romania it was first reported in Constanta in 2009 (Preda & Skolka, 2009), then in Timisoara (Gogan et al., 2010), Bucharest (Chireceanu & Gutue, 2011) Craiova (Mitreă, 2016).

MATERIAL AND METHOD

The study was conducted between July and September 2018, so we could identify the attacked plants by *Metcalfa pruinosa* in the area of green spaces in Craiova (Botanical Garden, Nicolae Romanescu Park, private gardens, on the streets and in front of the blocks).

In addition to identifying host plants, we made a series of observations and determinations to observe population densities and the degree of attack on plants.

To control the pest, chemical treatments were performed using four insecticides with different chemical bases (anthranilamides, ketoenols, chloronicotins, organophosphorus).

RESULTS AND DISCUSSIONS

Polyphagous species, *Metcalfa pruinosa*, hosts over two hundred species of plants, from spontaneous plants to fruit trees and especially ornamental plants (Pons et al., 2002).

Following the studies we have identified a number of 105 host plants from which 80 plants are identified in the botanical garden and 25 in Nicolae Romanescu Park. This pest is present in most of the green areas in Craiova, parks, private gardens or in front of blocks attacking ornamental plants isolated or cultivated as living fences.

Metcalfa pruinosa has a characteristic appearance, with gregarious behavior, it can easily be recognized by the specific layout of the bark, branches of trees or shrubs, stem, leaves and inflorescences (Figure 1).



Figure 1. The arrangement of branches and leaves of adults and larvae of *Metcalfa pruinosa*.(original)

This polyphagous planthopper species has become a serious pest in agriculture and public green space by forming dense populations which subsequently cause severe damage in orchards, vineyards, ornamentals and urban areas (Zangheri and Donadini 1980; Girolami and Camporese 1994, quoted by Strauss, 2009).

Large infestations of *M. pruinosa* may weaken the host plants by excessive phloem sucking and honeydew production which supports the growth of sooty moulds on affected plants, resulting in reduced fruit quality and leaf photosynthesis but also in quality damage in ornamentals (Strauss, 2009).

The secretion of sweet substances favors the development of various types of funguses, especially from Fam. Capnodiaceae (Della Giustina and Navarro, 1993, quoted by Preda & Skolka, 2009), named *Capnodium salicinum*, known as fumagin, being a black film, found on the leaves and inflorescences of several wood species such as **Forsythia x intermedia* Vahl, **Buxus sempervirens* L., **Berberis vulgaris* L., *Fraxinus excelsior* L., **Liriodendron tulipifera* L., **Magnolia tripetala* L., **Broussonetia papyrifera* L., **Philadelphus coronarius* L., (Figure 2).



Fig. 2. Leaves covered with *Capnodium salicinum* on *Fraxinus excelsior* L., **Philadelphus coronarius* L., **Magnolia tripetala* L., **Liriodendron tulipifera* L. (from left to right).(original)

This fungus is feeding by colonizing the dew layer produced by the cicada and does not directly affect the plant tissues, their damage is indirect because the black layer prevents the light to reaching on leaves, thus diminishing the photosynthesis process.

Regarding the economic impact of this species, *Metcalfa* can cause debilitation of plants affected by various factors (for example, late frost) (Preda & Skolka, 2010), as well as the aesthetic, thus decreasing the ornamental value of the plants attacked.

The limitation of the species spreading in urban green spaces is difficult to achieve (Girolami and Mazzon, 1999, quoted by Preda & Skolka, 2009). In the Czech Republic Sumithion used 0.1 % concentration to combat it (Lauterer, 2002, cited by Preda & Skolka, 2009).

Difficulties during chemical combat can be encountered due to three factors:

a) marked polyphagia leads the species to be almost omnipresent and able to pass easily, due to the remarkable mobility of nymphs and adults, from spontaneous vegetation to cultivated plants;

b) the pronounced scatter of egg hatching, which takes about two months and which causes a prolonged coexistence of different stages of development;

c) the substantial waxy layer covering and protecting the juvenile stages. (Pieri, 2007).

Based on these considerations, we proposed to test the biological efficacy of four insecticides (Coragen 20 SC 1.5 ml / 1 liter of water, Movento 100SC 0.05 ml / 0.5 water, Calypso 480 SC 0.5 ml / 5 l water and Nurelle D 50/500 EC 3 ml / 0.5 l water) used against mobile forms (adult and larvae) of the species *Metcalfa pruinosa* found on various host plants in the Botanical Garden.

Insecticides have a systemic effect on mobile stages (adults and larvae) that are affected by contact and ingestion.

Application of insecticides was carried out on 17.07.2018 with a spray atomizer. Observations on the number of adults and live larvae/plant were performed both before treatment and at 24, 48, 72 hours, 7 days and 14 days after treatment.

The results obtained after applying the treatments are presented in Table 1.

Following the treatments and the monitoring of their effect on the *Metcalfa pruinosa* pest, we found out that of the four insecticides used, the best results were recorded on the Nurelle D 50/500 EC product, the effect of which could be observed from 24 hours to its application and has been maintained so far.

The products of Coragen 20 SC, Movento 100 SC and Calypso 480 SC had a low effect in the first 72 hours, and promising results were recorded on the woody plants.



Figure 4. *Pyracantha coccinea* Roem. before and after treatment.(original)

For biological control worldwide, studies have been conducted on the introduction of parasitoid chimenopters from Fam. Drynidae, mainly the parasitic wasp *Neodrynus typhlocybae* (Strauss,2009).

Further releases have been undertaken in the canton of Ticino (Coldrerio and Carasso) in Switzerland (Jermeni et al. 2000), in western Slovenia close to the Italian border (Nova Gorica and Volčja Draga) (Žežlina et al. 2001), in Istria in Croatia (Poreč) (Ciglar et al. 1998; B. Barić, pers. comm.), and in southern France (Malausa et al. 2003), as well as in Catalonia and Valencia in Spain (A. Soto Sanchez, pers. comm., quoted by Strauss,2009).

Promising results were obtained both in Italy, France and Slovenia (Girolami and Conte, 1999; Villani and Zandigiacomo, 1999; Malausa et al., 2000; Žežlina et al., 1998, quoted by Strauss, 2009).

Table 1.

Influence of application of insecticides on the number of mobile forms (adults and larvae) existing on the host plant

Nr. crt.	Host plant name	Number of mobile forms (adults and larvae) on the host plant before and after treatment					
		Before treatment	After 24 h	After 48 h	After 72 h	After 7 days	After 14 days
Nurelle D							
1	* <i>Pyracantha coccinea</i> Roem.	37 A 80 L	1 A	0	0	0	0
2	<i>Iris</i> sp. L.	8 A 180 L	0	0	0	0	0
Coragen							
1.	<i>Acer platanoides</i> L.	75 A 80 L	67 A 80 L	50 A 80 L	45 A 75 L	55 A 70 L	40 A 28 L
2.	<i>Cephalaria uralensis</i> (Murray) Roem.&Schult	5 A 56 L	5 A 50 L	2 A 30 L	2 A 25 L	8 L	12 A 2 L
3.	<i>Amorpha fruticosa</i> L.	15 A 45 L	15 A 45 L	8 A 25 L	1 A 20 L	1 A 10 L	8 A
4.	* <i>Iris</i> sp. L.	45 A 130 L	45 A 80 L	25 A 65 L	20 A 40 L	30 A 45 L	15 A 20 L

Nr. crt.	Host plant name	Number of mobile forms (adults and larvae) on the host plant before and after treatment					
Movento							
1.	<i>Salvia</i> sp. L.	2 A 15 L	1 A 20 L	2 A 18 L	2 A 15 L	7 A 25 L	10 A 32 L
2.	* <i>Hosta plantaginea</i> (Lamarck) Ascherson	40 L	1 A 45 L	4 A 50 L	2 A 50 L	7 A 50 L	8 A 25 L
3.	<i>Cephalaria uralensis</i> (Murray) Roem.&Schult	8 A 40 L	8 A 37 L	10 A 30 L	14 A 28 L	50 A 35 L	70 A 15 L
Calypso							
1	* <i>Rudbeckia fulgida</i> Aiton	10 A 45 L	8 A 40 L	7 A 37 L	8 A 35 L	12 A 25 L	15 A 20 L
2	<i>Prunus cerasifera</i> Ehrh.	5 A 50 L	5 A 40 L	3 A 27 L	3 A 20 L	2 A 6 L	1 A
3	<i>Amorpha fruticosa</i> L.	15 A 40L	13 A 35 L	10 A 22 L	10 A 20 L	6 A	3 A

Legend: h- hours; A - Adults; L – Larvae.

Also in the literature are mentioned different natural enemies consuming *M. pruinosa*, especially from Fam. Coccinellidae (Coleoptera) e.g. *Coccinella septempunctata* (L., 1758), Miridae (Hemiptera) and Chrysopidae (Neuroptera), as well as various bird species (Barbattini et al., 1991, Greatti et al. 1994, quoted by Strauss, 2009).

CONCLUSIONS

Throughout the study, we met a total of 105 host species in the Craiova area.

In the chemical control, the best result was recorded at the Nurelle D 50/500 EC, the results still being maintained.

Because of the colonization of as many host species in the Oltenia area, it is necessary to carry out new treatments for the reduction of the *M. pruinosa* population and the damage caused.

Studies on chemical control of the species should be continued to find the best product so as to reduce the spread of this pest.

REFERINCES

Chireceanu Constantina, Gutue C., 2011, *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae) Identified in a new south eastern area of Romania (Bucharest area), Romanian Journal of Plant Protection Vol. 4, 28-34.

Preda Cristina, Skolka M., 2009, First record of a new alien invasive species in Constanța -*Metcalfa pruinosa* (Homoptera: Fulgoroidea), *Lucrările Simpozionului Mediul și agricultura în regiunile aride*, Prima ediție, 141-146.

Pieri F., 2007, L'introduzione accidentale di insetti esotici: il caso del flatide neartico *Metcalfa pruinosa* (say) (Homoptera- Fulgoroidea), Università di Pisa, Facoltà di Agraria: 20- 35.

Gogan Alina, Grozea Ioana, Virteiu Ana Maria and Baghina N., 2013, Flatid plant hopper (*Metcalfa pruinosa* Say) a new dangerous insect pest of ornamental and agricultural plants in Romania, *Journal of Food, Agriculture & Environment* Vol.11 (3&4) : 2065 - 2070.

Strauss G., 2011, Environmental risk assessment for *Neodryinus typhlocybae*, biological control agent against *Metcalfa pruinosa*, for Austria, European Journal of Environmental Sciences, Vol. 2, No. 2, 102-109.

Grozea et al. 2011, *Metcalfa pruinosa* Say (insecta: homoptera: flatidae): A new pest in Romania, African Journal of Agricultural Research Vol. 6(27) , 5870-5877.

Mitrea I. 2016, *Metcalfa pruinosa* (Homoptera: Fulgoroidea), new and dangerous pest in lawn of Oltenia, Annals of Unifersity of Craiova, Vol:XXI (LVII), 573-578.

Pons X. et al. 2002, *Metcalfa pruinosa* (Say) (Homoptera: Flatidae), ¿una plaga potencial de plantas ornamentales en espacios verdes urbanos de Cataluña?, Bol. San. Veg. Plagas, 28: 217-222

**THE COLLECTION OF ROSES FROM THE BUCHAREST
“DIMITRIE BRÂNDZĂ” BOTANICAL GARDEN.
PAST, PRESENT AND FUTURE.**

Negulici Marius^{1*}

¹Bucharest Botanical Garden – Bucharest University

*Correspondence author e-mail: marius.negulici@gmail.com

Keywords: roses, botanical garden, Bucharest, Romania.

ABSTRACT

The present paper aims to present some aspects from the Bucharest “Dimitrie Brandza” Botanical Garden rose collection history.

Throughout time, the rosary, much like the Botanical Garden, went through several radical changes. Data gathered showed that, before the second World War, the rose collection was located where today we find the Green Houses. There was also a significant change in variety and number of rosebushes, with the current collection hosting more than 4000 rosebushes and more than 200 varieties.

INTRODUCTION

Roses through the gentleness of the petals, but also through the scent of aromas, from fruits to spices, have always fascinated generations throughout time; it is common knowledge that when someone receives flowers, the primary reaction is to smell the perfume. Roses also occupy an important place in any landscaping design; there are almost no parks or gardens that do not have roses that, in combination with other plant species, especially in lawns, offer a wonderful painting, whether planted in singles, arches or massive. Roses have delighted and attracted visitors to enjoy their beauty and marvel at the atmosphere created by both color and perfume, for years and years.

The founding of the Botanical Garden is mainly due to the initiative of Dr. Carol Davila, former head of the School of Medicine and Pharmacy, which preceded the current Faculty of Medicine. Since 1855, when the school was known as the Mihai Vodă Surgery School, he insisted that Știrbei Vodă would establish a Botanical Garden. With all the events of 1859 with the Unification of the Principalities under a single ruler, it was decided that the works to establish a botanical garden would begin in 1860, when the Cotroceni Botanical Garden was inaugurated on November 5th (Ionescu, 1902).

According to documents found in the Botanical Museum, the Rosary was founded in 1886 (Fig. 1.), after the return of the Botanical Garden in Cotroceni. Between 1874-1886 (Fig. 2.) the garden was relocated near Vasile Șuțu Palace (Diaconescu 1981).



Fig. 1. The period (1874-1886) when the garden was relocated (The Museum of Botanical Garden)

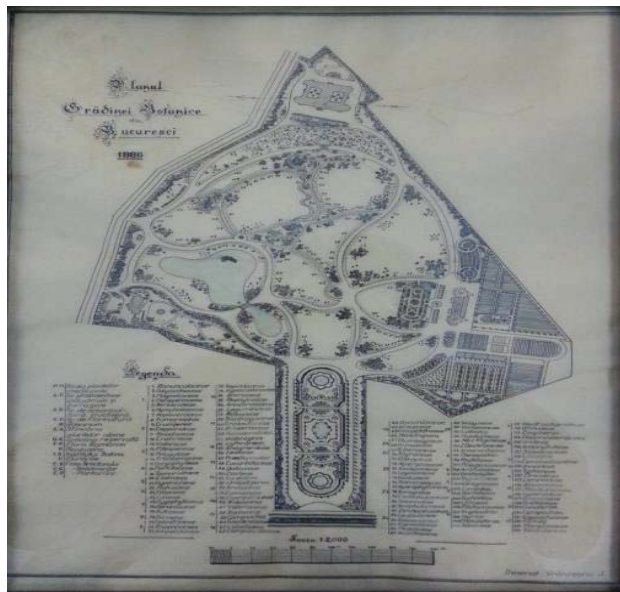


Fig. 2. The Botanical Garden plan in 1886 (The Museum of Botanical Garden)



Fig. 3. Aspects from the Rosary before the Second World War
(The Museum of Botanical Garden)

The first data on the Botanical Garden rosary refers to the way it was organized and its location within the premise. The place where it was originally designed and setup is the place where today we find the Green Houses (Fig.3.); back then the rosary hosted 200 varieties of roses. The photos found in the Botanical Museum show that there was an

important collection of roses grafted in the trunk, but also important varieties of thea hybrids, floribunda, polyantha, etc. (Ionescu, 1902).

During the bombing of World War II in the Botanical Garden, 96 bombs were dropped destroying almost everything in their path. The holes resulting from the bombing were, unfortunately, filled especially with debris from the rubble of the Capital, causing a poor soil quality, a problem which still persists in certain areas. (Săvulescu, 1961; Tarnavschi, 1961).

With the construction of the new greenhouses between 1976-1978 (Fig.4.), the Rosary was moved and restored by planting over 100 new varieties on to the place where it is still stands today, with the help of Liuba Tipa, who was the coordinator (Diaconescu 1981).



Fig. 4. The Botanical Garden rosary in 1978
(Photo L. Țipa)

An important step in the past few years happened in 2008 when the Botanical Garden management started rebuilding the rosary, by rehabilitating the aisles, constructing arches and bringing in and planting about 900 new roses, representing about 100 new varieties (Fig 5,6.).

At present the rosary covers an area of approx. 0.7 ha and includes about 4000 rosebushes, of which 200 varieties and 7 specie (*Rosa arvensis* Huds., *Rosa galica* L., *Rosa jundzillii* Besser., *Rosa alba* L. *Rosa sempervirens* L. *Rosa wichurana* Crép., *Rosa virginiana* Mill.).

On the main avenue, from the gate on the left, immediately after the "Mediterranean plants" sector, we see the group of species from the Fabaceae family (*Cercis siliquastrum* L., *Cladastris lutea* Koch., *Gymnocladus dioica* (L.) C. Koch.), "History of Roses" (Fig.7.), which attempt to present 30 years of variety evolution. It is mainly composed of *Rosa galica* L. and varieties such as 'Rose de Recht', 'The Fairy', 'Crimson Glory', 'Masquerade', 'Peace', 'Queen Elizabeth', 'Super Star', 'Nina Weibul', 'Pascali' 'Mister Lincoln'.

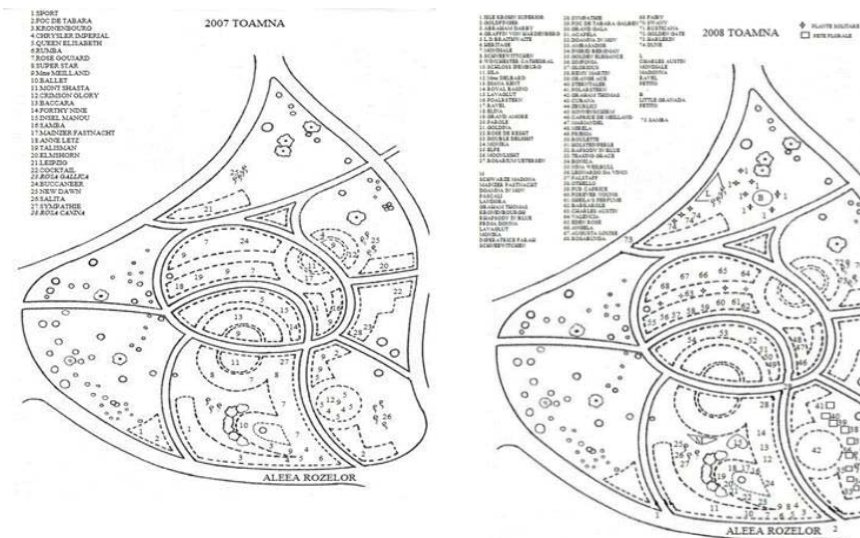


Fig. 5. Rosary plan 2007-2008 (orig.)

If we visit the Rosary on the main aisle, in front of the exhibition greenhouses (Fig.8.), we are greeted on the left by a mini-collection of English roses that is distinguished by the way the flowers (peony) are opened, by the shape of the rosebushes and by their perfume ('Abraham Darby' which has peach fragrance). Next, along the alley, we find the 'Chrysler Imperial' group that dazzles us with the fragrance that reminds us of grandmother's garden, followed by 'Queen Elizabeth'. On the right we find a red and yellow 'Foc de Tabără'. It is the beginning of a Japanese-style arrangement in which we find varieties such as 'Grand Gala', 'Doamna în Mov', 'Acapella', 'Remy Martin' and 'Sterntaler'.

Still on the left side, we have 'Madame Delbard' in dark red color, and next to the lavender bushes we have a huge heart made of about 50 red roses of the 'Lavaglut' variety. Moving along on the aisle we have some new varieties that were planted in 2016 like 'Chartreuse de Parme', 'Heather Austin', 'Pat Austin', 'Paul Gaugain', 'Pierre Arditi'. Immediately we pass under an archway of roses from the 'Sympathie' variety and find on the left several varieties from the old collection like 'Baccara', 'Mount Shasta' or 'Peace'. On the right we have a special kind called 'Caprice de Meilland' which impresses us through the delicacy of pink petals and its sweet fragrance. We walk along the alley and on the left and we find a variety called 'Rhapsody in Blue', whose fragrance reminds us of grandmother's perfume.



Fig.6. Aspects from the rehabilitation of the aisles and the construction of arches (orig.)

If we visit the Rosary on the main aisle, in front of the exhibition greenhouses (Fig.8.), we are greeted on the left by a mini-collection of English roses that is distinguished by the way the flowers (peony) are opened, by the shape of the rosebushes and by their perfume ('Abraham Darby' which has peach fragrance). Next, along the alley, we find the 'Chrysler Imperial' group that dazzles us with the fragrance that reminds us of grandmother's garden, followed by 'Queen Elizabeth'. On the right we find a red and yellow 'Foc de Tabără'. It is the beginning of a Japanese-style arrangement in which we find varieties such as 'Grand Gala', 'Doamna în Mov', 'Acapella', 'Remy Martin' and 'Sterntaler'.



Fig. 7. Some aspects from the “History of Roses” (orig.)

Still on the left side, we have 'Madame Delbard' in dark red color, and next to the lavender bushes we have a huge heart made of about 50 red roses of the 'Lavaglut' variety. Moving along on the aisle we have some new varieties that were planted in 2016 like 'Chartreuse de Parme', 'Heather Austin', 'Pat Austin', 'Paul Gaugain', 'Pierre Arditi'. Immediately we pass under an archway of roses from the 'Sympathie' variety and find on the left several varieties from the old collection like 'Baccara', 'Mount Shasta' or 'Peace'. On the right we have a special kind called 'Caprice de Meilland' which impresses us through the delicacy of pink petals and its sweet fragrance. We walk along the alley and on the left and we find a variety called 'Rhapsody in Blue', whose fragrance reminds us of grandmother's perfume.

One of the next steps for the collection, will be to create a special area honoring the late great breeder Dr. Ștefan Wagner, but also setting up a rose nursery for grafting.



Fig 8. Aspects from the rosary (orig.)

CONCLUSIONS

- The first documents about the Rosary sector date back to 1886, which shows that it was established after the return of the Botanical Garden in Cotroceni;
- The documents show that between 1886 and 1944 there was an important collection of roses, which consisted of about 200 varieties and was situated on the site where the Exhibition Green Houses are today;
 - During the period 1976-1978 the Rosary sector was moved, with the construction of the Exhibition Green Houses where it is still today;
 - In the period between 2008 and 2010 the reorganization and enrichment of the present collection was 80 % redone by introducing 150 new varieties, represented by 2500 rosebushes;
 - Among the projects for rehabilitation and improvement of the landscape aspect we can mention:
 - The "History of Roses" - the attempt to present through the varieties of roses, how they evolved under the "guidance" of specialists over time, from the year 1840 ('Rose de Rescht') until 2001 with 'Parole';
 - The archway - Variety of roses of different colors arranged in arches that form a multicolored aisle;
 - Rehabilitation of brick paths to complete the painting offered by the variety of colors;
 - Now there are more than 200 varieties represented by 4000 rosebushes covering an area of 0.7 ha;

REFERENCES

Diaconescu V. 1981. Grădina Botanică din București — 120 de ani de activitate neîntreruptă, *Acta Botanica Horti Bucurestiensis* 1979-1980, pp 3-17.

Ionescu G.M. 1902. Istoria Cotrocenilor, Lupescilor (Sf. Elefterie) și Grozăvescilor, pp. 7-8.

Săvulescu T. 1961. Gânduri cu prilejul centenarului Grădinii Botanice din București. *Acta Botanica Horti Bucurestiensis*, pp 7-8.

Tarnavski I.T. 1961. Grădina Botanică din București la împlinirea unui veac de existență. *Acta Botanica Horti Bucurestiensis*, pp 9-22.

HYPERURICEMIA AND ITS CORRELATION WITH METABOLIC SYNDROME

Olaru Mariana Luminița^{1*}

¹University of Craiova, Biology Department, Al.I.Cuza 13, 200 585, Craiova

*E-mail: luminitaolaru@yahoo.com

Keywords: *hyperuricemia, metabolic syndrome, triglycerides*

ABSTRACT

Metabolic syndrome, also known as syndrome X or dysmetabolic syndrome, refers to a cluster of metabolic conditions that can lead to heart disease. The underlying causes of metabolic syndrome include overweight and obesity, physical inactivity, genetic factors and getting older.

Hyperuricemia is commonly associated with obesity, glucose intolerance, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. The resemblance of the metabolic syndrome and hyperuricemia has led to the suggestion that hyperuricemia is a part of the metabolic syndrome.

This study shows serum uric acid is markedly associated with metabolic syndrome and its components, in particular serum triglycerides, HDL-C and serum glucose.

INTRODUCTION

Metabolic syndrome is a combination of metabolic abnormalities that pose an increased risk of cardiovascular disease including: insulin resistance, hyperinsulinemia, hypertriglyceridemia, HDL-cholesterol lowering and hypertension.

(http://rmj.com.ro/articles/2010.3/RMR_Nr-3_2010_Art-3.pdf)

The contribution to the establishment of appropriate terminology was brought by the World Health Organization (WHO) which in 1998 launched its first definition. According to the WHO, metabolic syndrome is glucose intolerance (IGT) or diabetes mellitus and / or insulin resistance plus two or more of the components: high blood pressure > 140/90 mmHg; serum triglycerides > 150 mg/dl and/or low HDL cholesterol < 35 mg/dl in men and < 39 mg/dl in women; abdominal obesity: hip ratio > 0,90 in men and > 0,85 in women, BMI > 30 kg/m²; microalbuminuria > 20 µg minimum.

Approximately 20 %-30 % of the population in industrialized countries has metabolic syndrome.

(<https://www.webmd.com/heart-disease/guide/metabolic-syndrome#3>)

The "National Cholesterol Education Program Adult Treatment Panel III" identifies metabolic syndrome as a constellation of clinical elements associated with an increased risk of developing type 2 diabetes and atherosclerotic cardiovascular disease (Ramos et al. 2001).

Any three of the following traits in the same person meet the criteria for the metabolic syndrome (Alberti 2009): abdominal obesity white race (CA-CA ≥ 94 cm in men / ≥ 80 cm in women); serum triglycerides ≥ 150 mg/dl; HDL-cholesterol < 40 mg/dl in men and < 50 mg/dl in women; blood pressure of 130/85 or above; glycemia ≥ 100 mg/dl.

There are authors who used two definitions of metabolic syndrome (Chinali et al. 2008) one for adults with both supporters and opponents and the second, pediatric

definition used in children and adolescents. For these, in the period of growth and development, it is impossible to choose a single threshold value for a variable considered risk factor (Daniels 2008).

Regardless of how it is defined, metabolic syndrome is a significant predictor of diabetes. Among its components, impaired fasting glucose is generally considered to be the most predictive predictor of diabetes mellitus (Cheung et al. 2007).

The metabolic syndrome, which is a cumulative risk factor that causes cardiovascular disease and diabetes (abdominal obesity, dyslipidemia, hypertension, altered glucose tolerance) is well defined in adults and its frequency is estimated at about 25 % of the population.

(http://rjp.com.ro/articles/2011.3/Pedia_Nr-3_2011_Art-9.pdf)

The concept of metabolic syndrome, though controversial, continues to gain acceptance, as a major health problem in the world.

Each component of the metabolic syndrome is actually a factor that can cause increased cardiovascular risk, but also moderate expression of metabolic syndrome components is a higher risk for cardiovascular disease than their isolated presence.

Further elements of the metabolic syndrome not found in the defined criteria (proinflammatory and prothrombotic status, insulin resistance) are determinants of increased cardiovascular risk.

(<http://www.umfiasi.ro/scoaladoctorala/tezedoctorat/teze%20doctorat/rezumate%20preda%20maria%20ecaterina.pdf>)

Excess fat in the stomach area is a greater risk factor for heart disease than excess fat in other parts of the body, such as on the hips. The risk for metabolic syndrome increases with age is highest in the population over 65 years.

(<https://www.nhlbi.nih.gov/health-topics/metabolic-syndrome>)

Hyperuricemia, the major etiologic factor in gout is an excess of uric acid in the blood (<http://chemocare.com/chemotherapy/side-effects/hyperuricemia-high-uric-acid.aspx>).

Because is an increasingly common medical problem not only in the advanced countries, but also in the developing countries, hyperuricemia may play a role in the development and pathogenesis of a number of metabolic, hemodynamic, and systemic pathologic diseases, including metabolic syndrome, hypertension, stroke, and atherosclerosis (Billiet et al. 2014).

In recent years there has been a renewed interest in hyperuricemia and its association with a number of clinical disorders other than gout, including hypertension, atherosclerosis, cardiovascular disease, and chronic kidney disease. It has been described that hyperuricemia is associated with metabolic syndrome components (Conen et al. 2004, Schachter 2005), such as obesity, glucose intolerance, dyslipidemia, hyperglycemia and hypertension (Chen et al. 2007, Billiet et al. 2014).

The resemblance of the metabolic syndrome and hyperuricemia has led to the suggestion that hyperuricemia is a part of the metabolic syndrome (Liou et al. 2006).

MATERIAL AND METHODS

The purpose of our study was to investigate the prevalence of hyperuricemia and the association between uric acid levels and the various metabolic syndrome components.

The present paper is based on a retrospective statistical study comprising a group of patients tested between December 2017 and June 2018, who presented themselves at the Medical Analysis Laboratory Priority Medical, Craiova during the aforementioned period for the determination values of serum uric acid concentrations. Uric acid (UA) determinations values were also correlated with serum triglycerides (TRI),

serum HDL cholesterol (HDL-C) and serum glucose (GLU) determination values of the same patients.

The values of these indices were determined with the Biosystems A15 automatic analyzer. Method used for serum triglycerides, HDL cholesterol, glucose and uric acid dosage in clinical practice is the spectrophotometric method.

The present study has as a data source of analyzes the results included in worksheets. In the final analysis were included a total of 117 subjects, men (M) and women (W), aged between 21 and 85 years.

Individuals in the study were divided according to clinical criteria, normal or pathological results of triglycerides (TRI), HDL cholesterol (HDL-C), glucose (GLU) and uric acid (UA) determinations, epidemiological criteria, gender (80 men subjects and 37 women subjects), and age groups (21-40 years, 41-60 years, 61-85 years of the patients (reviewed in the analysis report of each patient).

Through the statistical processing of the results from the analysis bulletins a data base was developed which was used for their graphical and tabular representation, interpretation and discussion of the results, as well as draw conclusions by reading them.

RESULTS AND DISCUSSIONS

In an adult, normal values of triglycerides (TRI) are under 150 mg/dl, and normal values of glucose (GLU) are from 70 to 105 mg/dl.

According to their serum levels of HDL cholesterol (HDL-C), the participants were categorized into 3 groups, as follows: low risk (LR) - HDL-C > 60 mg/dl, average risk (AR) - HDL-C from 40 to 60 mg/dl and high risk (HR) - HDL-C < 40 mg/dl.

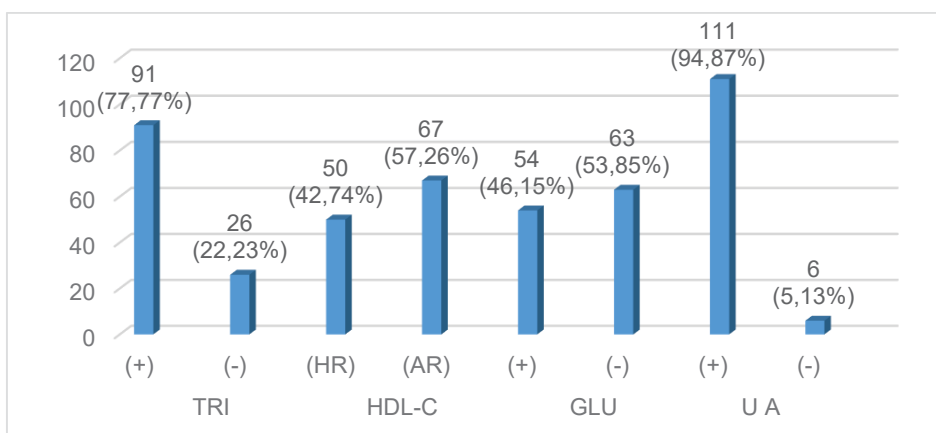
Normal values of uric acid (UA) were also categorized into 2 groups, as follows: men < 7 mg/dl, women < 5,7 mg/dl.

An analysis of the subjects' distribution according to the positive (+) and negative (-) results of the determinations (Table 1, Graphic 1), showed an imbalance in the uric acid (U A) values, the number of patients with positive values, 111 (94,87 %), being much higher of the negative one, 6 patients (5,13 %).

The distribution of subjects according to the positive and negative results of triglyceride (TRI) determinations indicates that a large number of individuals 91 (77,77 %) showed positive values (+), the remaining, 26 (22,23 %), having negative values (-) (Table 1, Graphic 1).

Table 1
Gender dynamics of positive (+) and negative (-) values of triglycerides (TRI), HDL cholesterol (HDL-C), glucose (GLU) and uric acid (UA)

TRIGLYCERIDES				HDL-CHOLESTEROL				GLUCOSE				URIC ACID			
(+)		(-)		(HR)		(AR)		(+)		(-)		(+)		(-)	
M	W	M	W	M	W	M	W	M	W	M	W	M	W	M	W
63	28	17	9	31	19	49	18	29	25	51	12	74	37	6	0
91 (77,77 %)		26 (22,23 %)		50 (42,74 %)		67 (57,26 %)		54 (46,15 %)		63 (53,85 %)		111 (94,87 %)		6 (5,13 %)	



Graphic 1. The dynamics of positive (+) and negative (-) values of triglycerides (TRI), HDL cholesterol (HDL-C), glucose (GLU) and uric acid (UA)

Concerning HDL-C risk group assignment revealed that of the 117 investigated individuals none of them were in the low-risk group. In contrast, the number of average-risk (AR) individuals, 67, recorded the highest percentage (57,26 %), the remaining 50 being the high-risk (HR) individuals, 42,74 % (Table 1, Graphic 1).

Regarding serum glucose (GLU), 63 individuals (53,85 %) were in normal range (-), compared with 54 individuals (46,15 %) who had elevated blood glucose levels (+) (Table 1, Graphic 1).

In conclusion, of the 111 patients (94,87 %) with hyperuricemia, 91 of them (77,77 %) had hypertriglyceridemia, 60 patients (42,76 %) were at high risk of HDL-C, and 54 individuals (46,15 %), had hyperglycemia.

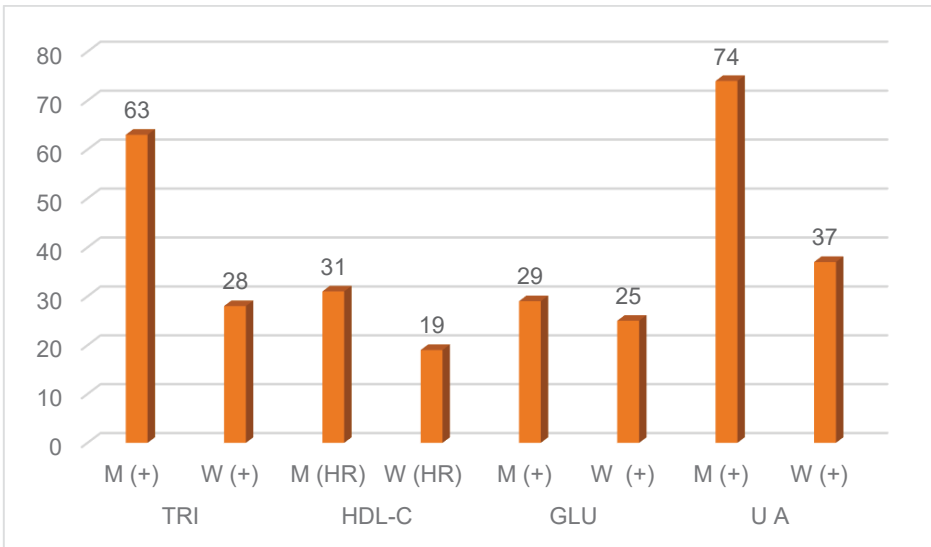
Hyperuricemia has been correlated with three components of metabolic syndrome (hypertriglyceridemia, high risk HDL-C, hyperglycemia) (Table 2).

The results of this study show a significant correlation between hyperuricemia and hypertriglyceridemia.

Regarding the gender distribution of patients with positive values of the four indices (Table 2, Graphic 2), women occupy the highest percentage of all determinations, except for hypertriglyceridemia, which is approximately equal to men and women.

Table 2
The dynamics by gender and the age group of the patients number with positive values (+) of triglycerides (TRI), HDL cholesterol (HDL-C), glucose (GLU) and uric acid (UA)

Age group	TRIGLYCERIDES		HDL-CHOLESTEROL		GLUCOSE		URIC ACID	
	M (+)	W (+)	M (HR)	W (HR)	M (+)	W (+)	M (+)	W (+)
	21-40 years	25 (39,68 %)	1 (3,57 %)	9 (29,03 %)	1 (5,26 %)	3 (10,35 %)	3 (12 %)	24 (32,44 %)
41-60 years	29 (46,03 %)	12 (42,86 %)	9 (29,03 %)	8 (42,10 %)	15 (51,72 %)	10 (40 %)	34 (45,94 %)	15 (40,54 %)
61-85 years	9 (14,29 %)	15 (53,57 %)	13 (41,94 %)	10 (52,64 %)	11 (37,93 %)	12 (48 %)	16 (21,62 %)	19 (51,35 %)
TOTAL	63 (78,75 %)	28 (75,67 %)	31 (38,75 %)	19 (51,35 %)	29 (36,25 %)	25 (67,56 %)	74 (92,5 %)	37 (100 %)

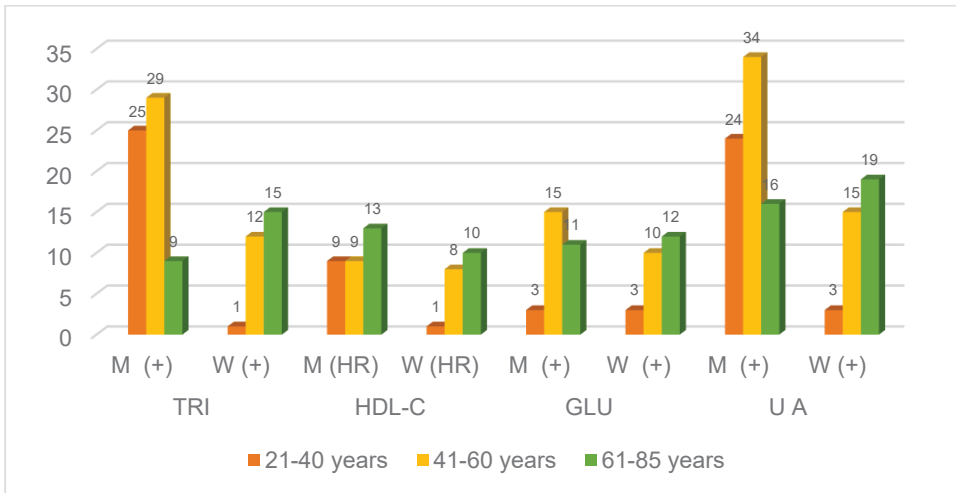


Graphic 2. Gender dynamics of the patients number with positive values (+) of triglycerides (TRI), HDL cholesterol (HDL-C), glucose (GLU) and uric acid (UA)

In individuals of the study group, hyperuricemia was met in the proportion of 92,5 % in men and in the maximum percentage of 100 % in women (Table 2).

On the contrary, a study by chinese researchers showed that hyperuricemia was more common in men (19,07 %) than in women (3,42 %) (Chen et al. 2007).

High differences were observed in the age group and gender distribution of the same subjects with positive (+) values determinations (Table 2, Graphic 3).



Graphic 3. The dynamics by gender and the age group of the patients number with positive values (+) of triglycerides (TRI), HDL cholesterol (HDL-C), glucose (GLU) and uric acid (UA)

In the 41-60 age group, hyperuricemia occurs in approximately equal proportions in both sexes, 45,94 % in men, and 40,54 % in women. In the 21-40 age group, hyperuricemia occurs in a higher percentage in men, 32,44 %, compared with only 8,11 % in women. The situation is reversed in the age group 61-85 years, where the higher percentage is for women, 51,35 %, compared with only 21,62 % for men (Table 2).

Conen et al. 2004 showed the same results. The prevalence of a serum uric acid level in men was 35,2 % and the prevalence of a serum uric acid level was 8,7 % in women. Serum uric acid was strongly related to serum triglycerides in men as well as in women.

According to a study of Chinese researchers the contribution of uric acid as an additional component of the metabolic syndrome in middle-aged men seems to be insignificant (Liou et al. 2006).

The situation is similar in patients with hypertriglyceridemia. Thus, in the 41-60 age group, hypertriglyceridemia occurs in approximately equal proportions in both sexes, 46,03 % in men, and 42,86 % in women. In the 21-40 age group, hypertriglyceridemia occurs in men, 39,68 %, versus only 3,57 % in women. The situation is reversed in the age group 61-85 years, where the higher percentage is for women, 53,57 %, compared with only 14,29 % for men (Table 2).

With regard to HDL-C, the age group 21-40 years is at high-risk only 5,26 % of women, the remaining 29,03 % being men. In the other two age groups the situation is reversed, the percentage of women being higher for both - the 41-60 age group, 42,10 % women and 29,03 % men and for the age group 61-85 years – 52, 64 % women and 41,94 % men (Table 2).

These correlations were similar to those in the literature.

Not only collectively, but also individually, hypertension, obesity, dyslipidemia, hyperglycemia, and insulin resistance are positively correlated with serum levels of uric acid (Billiet et al. 2014).

The research data of Schachter 2005 and Chen et al. 2007, showed the same results and indicated that serum triglyceride was markedly associated with hyperuricemia. Hyperuricemia and hypertriglyceridemia are suggested to be associated with insulin resistance syndrome.

Buşu et Stolear studied the impact of hyperuricemia on metabolic syndrome components. A direct proportional correlation of hyperuricemia and hypertriglyceridemia has been observed in the study group.

Also, HDL-C values were significantly lower in these patients (<http://library.usmf.md:8080/jspui/bitstream/123456789/255/1/SINDROMUL%20METABOLIC%20C5%9EI%20HIPERURICEMIA.pdf>).

In the study of Chen et al. 2007, uric acid was negatively correlated with serum HDL-C, but this association was not evident among women.

Differences were also observed with respect to the positive (+) values of serum glucose measurements. Women occupy a smaller percentage in the 41-60 years age group, 40 %, compared to 51,72 % in men. The 21-40 age group recorded approximately equal percentages, 12 % for women and 10,35 % for men, whereas in the 61-80 age group, women recorded much higher values, 48 %, against men, 37,93 % (Table 2).

Yoo et al. 2005 reported that hyperglycemia was a remarkable risk factor for hyperuricemia. Serum uric acid concentration was found to be independently correlated with insulin resistance and the risk factors of metabolic syndrome (Yoo et al. 2005).

Hyperuricemia predicts the development of hypertension, obesity, and type 2 diabetes mellitus (Billiet et al. 2014).

CONCLUSIONS

Metabolic syndrome is a cluster of conditions: increased blood pressure, high blood sugar, excess body fat around the waist, and abnormal cholesterol or triglyceride levels, that occur together, increasing risk of heart disease, stroke and diabetes.

Hyperuricemia, the excess of uric acid in the blood is commonly associated with obesity, glucose intolerance, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease.

The resemblance of the metabolic syndrome and hyperuricemia has led to the suggestion that hyperuricemia is a part of the metabolic syndrome.

Hyperuricemia is an increasingly common medical problem not only in the advanced countries, but also in the developing countries.

This study shows serum uric acid is markedly associated with metabolic syndrome and its components, in particular serum triglycerides, HDL-C and serum glucose.

REFERENCES

- Alberti K.G., Eckel R.H., Grundy S.M., Zimmet P.Z., Cleeman J.I., Donato K.A., Fruchart J.C., James W.P., Loria C.M., Smith S.C. Jr., 2009, Harmonizing the metabolic syndrome. *Circulation* 120(16):1640-5. Available online at <https://www.ncbi.nlm.nih.gov/pubmed/19805654>
- Billiet Laura, Doaty Sarah, Katz J.D., Velasquez M.T., 2014, Review of Hyperuricemia as New Marker for Metabolic Syndrome. *ISRN Rheumatology*, Article ID 852954. Available online at <https://www.hindawi.com/journals/isrn/2014/852954/>
- Chen L.Y., Zhu W.H., Chen Z.W., Dai H.L., Ren J.J., Chen J.H., Chen-Qian L., Zheng L.F., 2007, Relationship between hyperuricemia and metabolic syndrome. *J Zhejiang Univ Sci B*; 8(8):593–598. Available online at <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1934956/>
- Cheung B.M., Wat N.M., Man Y.B., Tam S., Thomas G.N., Leung G.M., Cheng C.H., Woo J., Janus E.D., Lau C.P., Lam T.H., Lam K.S., 2007, Development of diabetes in chinese with the metabolic syndrome. *Diabetes Care*, 30(6):1430-6. Available online at <https://www.ncbi.nlm.nih.gov/pubmed/17337491>
- Chinali M., de Simone G., Roman M.J., Best L.G., Lee E.T., Russell M., Howard B.V., Devereux R.B., 2008, Cardiac markers of preclinical disease in adolescents with the metabolic syndrome: the strong Heart Study. *J. Amer. Coll. Cardiol*; 52:932–938. Available online at <http://www.onlinejacc.org/content/accj/52/11/932.full.pdf>
- Conen D., Wietlisbach V., Bovet P., Shamlaye C., Riesen W., Paccaud F., Burnier M., 2004, Prevalence of hyperuricemia and relation of serum uric acid with cardiovascular risk factors in a developing country. *BMC Public Health*; 4(1):9. Available online at <https://www.ncbi.nlm.nih.gov/pubmed/15043756>
- Daniels S.R., 2008, Metabolic syndrome and cardiovascular abnormalities in Children. *J. Am Coll. Cardiol.*, 52(11):939-40. Available online at <https://www.ncbi.nlm.nih.gov/pubmed/18772066>
- Liou T.L., Lin M.W., Hsiao L.C., Tsai T.T., Chan W.L., Ho L.T., Hwu C.M., 2006, Is Hyperuricemia Another Facet of the Metabolic Syndrome?, *Journal of the Chinese Medical Association*, Volume 69, Issue 3, pp 104-109. Available online at <https://www.sciencedirect.com/science/article/pii/S1726490109701868>

Ramos F., Baglivo H.P., Ramirez A.J., Sánchez R., 2001, The metabolic syndrome and related cardiovascular risk. *Current Hypertension Reports*, 3: 100-106. Available online at <https://www.ncbi.nlm.nih.gov/pubmed/11276389>

Schachter M., 2005, Uric acid and hypertension. *Curr Pharm Des*; 11(32):4139–4143. Available online at

<https://www.ncbi.nlm.nih.gov/pubmed/16375735>

Yoo T.W., Sung K.C., Shin H.S., Kim B.J., Kim B.S., Kang J.H., Lee M.H., Park J.R., Kim H., Rhee E.J., Lee W.Y., Kim S.W., Ryu S.H., Keum D.G., 2005, Relationship between serum uric acid concentration and insulin resistance and metabolic syndrome. *Circ J.*; 69(8):928–933. Available online at

<https://www.ncbi.nlm.nih.gov/pubmed/16041161>

***<http://chemocare.com/chemotherapy/side-effects/hyperuricemia-high-uric-acid.aspx>

***<http://library.usmf.md:8080/jspui/bitstream/123456789/255/1/SINDROMUL%20METABOLIC%20C5%9E1%20HIPERURICEMIA.pdf>

***http://rjp.com.ro/articles/2011.3/Pedia_Nr-3_2011_Art-9.pdf

***http://rmj.com.ro/articles/2010.3/RMR_Nr-3_2010_Art-3.pdf

***<http://www.jmedar.ro/pdf/vol12/iss2/JMA12-2-09Gligor.pdf>

***<http://www.umfiasi.ro/scoaladoctorala/tezedoctorat/teze%20doctorat/rezum%20preda%20maria%20ecaterina.pdf>

***<https://www.nhlbi.nih.gov/health-topics/metabolic-syndrome>

***<https://www.webmd.com/heart-disease/guide/metabolic-syndrome#3>

**STUDY ON KNOWLEDGE AND BEHAVIOR
TOWARDS ORAL HYGIENE RULES**

Olimid Diana^{1*}

¹University of Craiova, Department of Biology and Environmental Engineering,
A. I. Cuza Street, No. 13, Craiova

*Correspondence author e-mail: olimiddiana@yahoo.com

Keywords: *oral cavity, hygiene methods, prevention*

ABSTRACT

The aim of this study was to evaluate the knowledge and attitudes of 347 subjects regarding the oral health and hygiene practices.

Regarding the age, of the 347 subjects, 63.11 % were female. The female patient group was divided into age groups. The analysis showed that the majority of patients were under 49 years of age, 33.79 % belonged to the 30-39 years of age group, 26.48 % to the 18-29 years of age group, and 18.72 % to the 40-49 years of age group. The lowest number of patients belonged to the 60-69 years of age group.

Regarding the reason for presenting to the dentist, only 25.36 % of the subjects presented for a prophylactic examination, respectively 29.68 % of the female subjects, and only 17.96 % of the male subjects. Pain was the main symptom that caused the presentation to the dentist's office, respectively in 71.42 % of cases.

INTRODUCTION

The oral cavity is the first part of the digestive tube and contains the teeth, the gum (gingiva) surrounded by periodontium and alveolar bone and the tongue. The soft structures and the inner tissue of the cheeks are lined with mucous membrane. The oral cavity plays roles in taste because taste buds are present on it, in the qualitative control of the food, in mastication, salivation, swallow and speech act (Papilian V., 2014).

A proper oral hygiene is necessary for a fresh breath, healthy teeth and gum. The oral hygiene is the practice of keeping the oral cavity clean to prevent dental diseases. These pathologic conditions include dental caries, gingivitis, periodontitis (Vlad R.S., 2003).

MATERIALS AND METHODS

The study was conducted on a group of 347 subjects (male and female) aged 18 to 69 years. The study was performed into a private dental care practice for a period of 6 months, between October 2017 and March 2018.

Oral hygiene assessment was made by using a questionnaire with the following aspects:

- the age and the gender;
- the cause to visit the dentist (prophylactic exams or pathologic conditions);
- the knowledge of the proper teeth brushing technique;
- the use of the dental floss;
- the use of the mouthwash.

RESULTS AND DISCUSSIONS

Regarding the age, of the 347 subjects, 63.11 % were female. The female patient group was divided into age groups. The analysis showed that the majority of patients were under 49 years of age, 33.79 % belonged to the 30-39 years of age group, 26.48 % to the 18-29 years of age group, and 18.72 % to the 40-49 years of age group. The lowest number of patients belonged to the 60-69 years of age group (table 1; figure 1).

The distribution of the male subjects according to the age group showed the same tendency, 60,15 % of them were under the age of 49.

The obtained results show that females are more interested than men in maintaining dental health. Also, the addressability to the dentist is higher for young people, due to the interest in the esthetic aspect of teeth and facial expression. This aspect is very important at the moment, given that the integration into society and the way we are perceived by the others also depends on the physical appearance. The way a person speaks, smiles and the self-esteem determines the attitude of others towards her.

At young ages, the esthetic aspect may be more important than dental health, but the fact that youngsters present to the dentist is a first step because he can provide advice (counsel) so that the patient start to be aware of the importance of maintaining hygiene and health of the oral cavity.

Table 1

The repartition of the cases according to the gender

The gender	No. of cases	Percentage
Women	219	63,11%
Men	128	36,89%

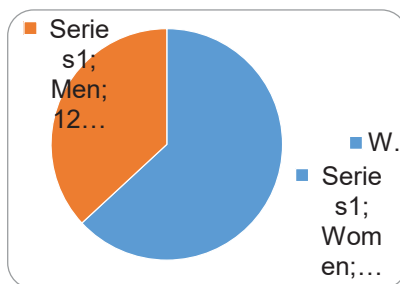


Figure 1. The repartition of the cases according to the gender

Table 2

The repartition of the cases according to the age group

Age group	18-29	30-39	40-49	50-59	60-69
No. of cases	58	74	41	27	19
Percentage	26,48 %	33,79 %	18,72 %	12,33 %	8,68 %

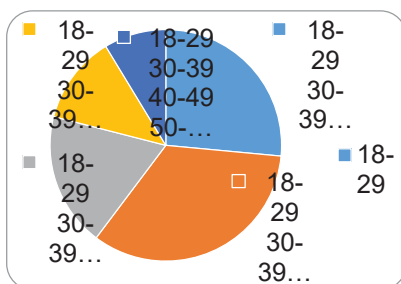


Figure 2. The repartition of the cases according to the age group

Regarding the reason for presenting to the dentist, only 25.36 % of the subjects presented for a prophylactic examination, respectively 29.68 % of the female subjects, and only 17.97 % of the male subjects (table 3, 4, figure 3, 4).

The result is according to the general tendency to call a physician only in the case of a pathological condition manifested by symptoms such as pain or gingival bleeding. Pain was the main symptom that caused the presentation to the dentist's office, respectively in 71.43 % of cases (table 5, figure 5).

Table 3

The repartition of the female subjects according to the cause of dental exam

The cause of the exam	No. of cases	Percentage
Prophylactic exam	65	29,68 %
Pathologic condition	154	70,32 %

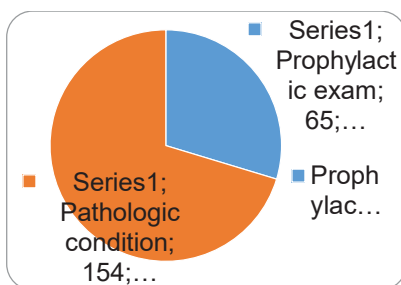


Figure 3. The repartition of the female subjects according to the cause of dental exam

Table 4

The repartition of the male subjects according to the reason of the dental exam

The reason of the exam	No. of cases	Percentage
Prophylactic exam	23	17,97 %
Pathologic condition	105	82,03 %

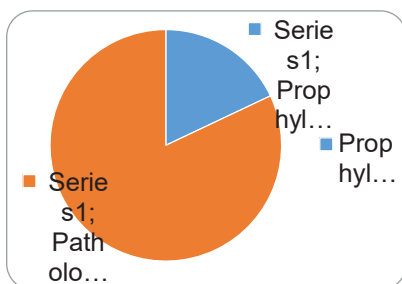


Figure 4. The repartition of the male subjects according to the reason of the dental exam

Table 5

The repartition of the cases according to the main symptom associated to the pathology condition

Symptom	No. of cases	Percentage
Pain	185	71,43 %
Gum bleeding	34	13,13 %
Others	40	15,44 %

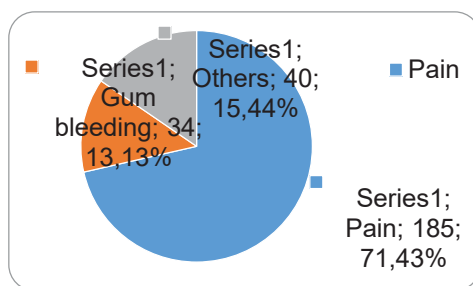


Figure 5. The repartition of the cases according to the main symptom associated to the pathological condition

The repartition of the cases according to the proper teeth brushing technique showed that 69,86 % of women reported the correct technique, while only 57,81 % of men knew the right movements and their order (table 6).

Table 6

The distribution of the cases according to the correct technique

The proper brushing technique	No. of cases	Percentage
Women	153	69,86 %
Men	74	57,81 %

Dentists recommend that teeth be cleaned by brushing with fluoride toothpaste at least twice a day. This is essential for preventing tartar build-up which causes the dental injuries (caries).

The best way to remove the bacterial plaque deposited on dental surfaces is gingival-dental brushing. Even if brushing is associated with other practices of maintaining oral hygiene (scaling, professional brushing, subgingival curettage and

curettage of soft areas on the surface of the dental root, the local application of antiseptic and antibiotic substances), the essential condition to prevent gum damage is the daily removal of the bacterial plaque through dental brushing (Vlad R.S., 2003).

If the patient does not understand this and does not get the correct dental brushing technique, all the secondary procedures initiated by the dentist will be doomed to failure. Prophylactic exams should be done every six months.

For home dental brushing, the best brushes are the ones made of natural hair (pork or camel hair), soft or medium consistency plastic brushes, those with high consistency risking to unnecessarily injure the gums and produce over time gingival retraction or loss of dental hard substance.

According to many authors, gingival-dental brushing should be performed after each meal or snack, so at least 3-5 times a day. It is recommended a correct brushing in the morning, when waking up and in the evening, before bedtime.

The dentist is required to inform the patient about the correct brushing technique, and when fully mastered by the patient, the brushing time should not exceed 5 minutes.

Correct dental brushing will begin with one of the arches, either the maxillary or the mandibular one. Only after brushing is completed at one of the dental arches, brushing will be performed at the other one. The end of the toothbrush should be held at an angle of 45° to the longitudinal axis of the teeth, and in this position vertical movements will be made only from the gum to the tooth, otherwise risking traumatizing the gum (WHO, 2013).

For each group of teeth, 10 vertical movements will be performed for cleaning the gingival-dental ditch, the gingival edge and dental surfaces. Vertical movements will be performed on dental groups, for lateral surfaces, both on the exterior and on the inside of the arches. For the occlusal surfaces that perform the crushing and trituration of food, the brushing will be horizontal combined with brush rotations, in order to effectively remove food debris accumulated in the ditches and beaks of these dental surfaces (Mahnoush N. D., 2007).

After use, the toothbrush should be washed under a strong jet of water and then shaken vigorously to remove water.

The brushes should be changed after a period of time ranging from 3 to 6 months due to their degradation and impregnation with organic debris and bacteria.

It is necessary to spend at least three minutes brushing each time, but no more than 5 minutes.

It should be brushed also the surface of the tongue. Tongue cleaning removes bacteria, food particles, fungi as *Candida* and dead cells.

A study made in Bucharest, in two schools, on a group of 46 students aged 10 to 12 years showed that the entire group had a moderate degree of oral hygiene. Almost 40 % of children had a number of streptococci colonies over the limit and a high risk for dental caries (Funieru C. et al., 2015).

Over the last decade, the supply of electric dental brushes has increased. However, these brushes in most cases perform only a rotating, beneficial, but not sufficient movement.

Electrical brushing is especially indicated for young children, lazy people, non-cooperating people, either due to a physical disability in the upper limbs, or because of mental illness and for people who wear fixed orthodontic appliances.

An important step to maintaining the dental hygiene is flossing. This technique is necessary to removing food and plaque between teeth and along the gum line where the toothbrush can't reach.

The evaluation showed that 39,72 % of women and 24,21 % of men use the dental floss for a proper hygiene of the interdental spaces (table 7).

Table 7

The repartition of the cases according to the use of the dental floss

The use of dental floss	No. of cases	Percentage
Women	87	39,72 %
Men	31	24,21 %

Flossing should be done once a day, preferably before bedtime.

It is recommended to use enough floss to enable easy use, usually 5 centimeters. Floss is then wrapped around the middle finger or index finger and supported the thumb on each hand. It is then held tightly and moved up and down between each tooth. It is important to floss under visible areas by curving the floss around each tooth. The gum bleedings are normal at the first usage of floss (Vlad R.S., 2003).

Between two brushings, for hygiene it is enough to rinse the mouth vigorously with water or various antiseptic solutions.

This practice can't replace the teethbrushing. The most effective antiseptic solution is clorhexidine which is absorbed on the dental surface where kills the bacteria and stops the development of the bacterial plaque. The antiseptic efficacy remain until 8 hours after use (Mahnoush N. D., 2007).

Fluoride mouthwash and toothpaste are not recommended for small children younger than six years because they may swallow the rinse. Too much fluoride may be toxic to infants (Marques L. A. et al., 2008).

The distribution of the cases showed that 7,76 % of women have never used the mouthwash, 38,81 % have used occasionally and 53,43 % have used every day. For the male subjects the percentages were 16,40 %, 49,21 % and 34,37 % (table 8).

Table 8

The distribution of the cases according to the use of the mouthwash

The use of the mouthwash	Women		Men	
	No. of cases	Percentage	No. of cases	Percentage
Every day	117	53,43 %	44	34,37 %
Occasionally	85	38,81 %	63	49,22 %
Never	17	7,76 %	21	16,41 %

A study published in 2011 in West Indian Medical Journal evaluated the dental health knowledge and attitudes of primary school teachers toward developing dental health education. The 75 % of respondent were female. 28,9 % of participants had received training in health education and only 6,9 % in dental health education. 92 % of teachers identified irregular tooth brushing as an important cause of dental disease, only 65 % and 53 % respectively identified the amount and frequency of sugar consumption as important causative factors.

85 % of the teachers believed that gum disease is caused by bacterial infections and 67 % thought it was caused by lack of vitamins. Most of the subjects thought that proper tooth brushing (90 %), visiting the dentist (89 %) and eating a good diet (79 %) would prevent gum disease.

Teachers were aware that fluoride toothpaste helped prevent caries. 97 % of all agreed that dental education should be included in the school curriculum.

The most important obstacles to the implementation of a health promotion program are the lack of material resource (77 %), lack of adequate training (70 %), and lack of support from local administration (47 %) (Ramroop V. et. al., 2011)

The association between oral hygiene and oral cancer or pharynx was studied in a hospital based case control study in the metropolitan area of Sao Paulo, Brazil, from 1998 to 2000. The information on risk factors were obtained through interviews.

The risk of oral cancer was higher among subjects who reported regular gingival bleeding during tooth brushing. Patients who reported never attending dental visits had a risk of 2,5 compared to those who attended annual dental exam. Subjects who used mouthwash more than once a day showed a 3-fold increase in the risk of oral cancer compared to those who never used them. In Brasil, many mouthwash products contain as much as 27 % alcohol, which is a risk factor for oral cancer. (Marques L. A. et. al., 2008).

The treatment of dental disease is expensive in both developed and developing countries and exceeding the cost of treating cardiovascular disease, cancer and osteoporosis (Sheiham A., 2001).

Some people used different forms of teeth cleaning tools. Indian medicine has used the neem tree and its products. A number of plants are used as chewing sticks in West Africa such as the lime tree, the orange tree, the roots of senna, neem, nutmeg (Muhhamad S. and Lawal M. T., 2010).

CONCLUSIONS

The main goals of the prophylactic measures are removal of the bacterial plaque from the teeth and gingival areas adjacent to the dental surfaces and stimulation of blood circulation in the gingiva.

The women, especially the youngest, are more preoccupied than men to keep the oral hygiene, to do frequent dental examinations and more interestedly about the facial appearance.

The parental involvement in promotion of the dental health education in schools is essential.

The prophylactic measures should be applied from the early years of life which is why the parents have a great responsibility to teach children to eat healthy foods and to learn the hygiene rules.

The implication of the dental profession could be an important part in oral health promotion.

Dental diseases influence the self-esteem and are expensive to treat, so their prevention is very important.

It is the responsibility of the national health authorities to provide educational programmes.

REFERENCES

Funieru C., Sfeatcu Ruxandra Ionela, Funieru Elena, Răescu Mihaela, Ivan Loredana, Dumitrache Adina Mihaela. 2015. Studiu asupra factorilor de risc ai cariei dentare la un lot de școlari din Municipiul București. Romanian Journal of Stomatology. Vol. 61, issue 2, pp. 156-160.

Mahnoush N. D. 2007. Afecțiunile gingivale și paradontoza pe înțelesul tuturor. Ed. M.A.S.T. pp. 103-125.

Marques L.A., Eluf-Neto J., Figueiredo R.A.O., de Gois J. F., Kowalski L. P., de Carvalho M. B., Abrahao M., Wunsch V. 2008. Oral health, hygiene practices and oral cancer. Rev. Saude Publica, 42(3):471-9.

Muhhamad S., Lawal M. T. Oral Hygiene and the use of plants. Scientific Research and Essays. Vol. 5(14), pp. 1788-1795, 18 July 2010. Available online at <http://www.academicjournals.org/SRE>. ISSN 1992-2248.

Papilian V. 2014. Anatomia omului, vol. 2, Splahnologia, ed. a 12-a, Edit. ALL, București, pp. 4-38.

Ramroop V., Wright D., Naidu R. 2011. Dental health knowledge and attitudes of primary school teachers toward developing dental health education. West Indian Medical Journal. Vol. 60, no. 5. Oct. 2011. Print version. ISSN 0043-3144. pp. 576-80.

Sheiham A. 2001. Dietary effects on dental diseases. Public Health Nutrition. 4:569-91.

Vlad Roxana Speranța. 2003. Dinții și bolile lor. Grupul Editorial Corint, București, pp. 47-85.

*** WHO. 2013. Oral Health Surveys: Basic Methods. 5-th ed. WHO Press. Geneva. Available online at <http://www.who.int>.

SUSTAINABILITY CONSTRAINS OF HYDROPONIC HELOPHYTE BIOFILTERS IN RECIRCULATED AQUACULTURE

Popa Radu^{1,2}, Cimpoiașu Vily Marius^{2*}

1 - University of Southern California, Department of Biological Sciences, 3616, Trousdale Parkway, Los Angeles, CA, 90089, rpopa@usc.edu

2 - University of Craiova, Faculty of Horticulture, Biology and Environmental Engineering Department, Frontier in Biology and Astrobiology Research Center, A.I. Cuza Street, no 13, RO 200585, Craiova, Romania

*Correspondence author e-mail: vilycimpoiasu@yahoo.com

Keywords: *Biofilter, Recirculated aquaculture, Hydroponic, Sustainability, Helophite plants, Wetland.*

ABSTRACT

Wastewater treatment adds large costs to Recirculated Aquaculture Systems (RAS), often rendering intensive aquaculture operations financially unsustainable. Emergent marshland plants (called helophytes), used in the form of Hydroponic Helophyte Biofilters (HHB) are proposed as a straightforward means to lower the cost of water treatment in RAS. We analyzed main constrains in the sustainability of RAS/HHB systems. We discuss the cost of recirculating water through HHB systems; criteria to select the best helophytes, consequences of evaporative loss, and optimal ratios between RAS and HHB. Calculations have been made for a theoretical RAS with 20 kg catfish m⁻³ grown in temperate continental conditions and HHB with cattail at a density of 70 kg m⁻². We show that the cost of maintaining water quality in a RAS/HHB system with catfish and cattail in temperate climate are sustainable and can reach less than 20 % from revenue.

INTRODUCTION

Some of the most promising food production pathways in modern agriculture are hydroponics and Recirculated Aquaculture Systems (RASs). These technologies have high biomass yield, yet are expensive and polluting. Water treatment technologies that can be connected with RAS include filtration, settling, aeration, sterilization, nitrification, denitrification and hydroponics. In this paper we analyze the merits of Hydroponic Helophyte Biofilters (HHBs) when connected to RAS (Fig. 1). Emergent helophytes are plants that live in marshes or lake-edge environments, have perennating plant organs in soil or mud below the water level, and aerial parts (stems, leaves, flowers) above the water level. What recommends helophytes for hydroponic filters is their fast growth, hardiness, simple propagation from over-wintering buds and tolerance of the submersed parts to low O₂. What recommends HHBs for RAS is their high biofilter potential, culture simplicity, high buffer capacity and potential for automation. The pollutants targeted in this study are organic carbon (C_{org}), nitrogen (N) and phosphorus (P). Unlike in aquaponics, the purpose of HHBs is not to grow another crop, but to lower the cost of water treatment by using a natural filtration system.

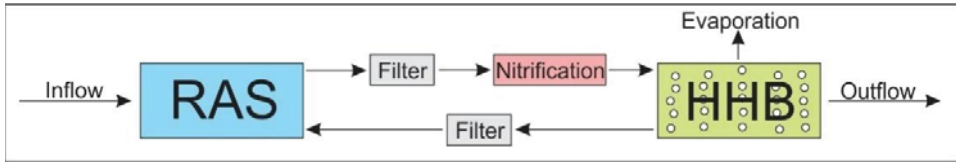


Fig. 1. A Recirculated Aquaculture System (RAS) with a Hydroponic Helophyte Biofilter (HHB). A filter upstream from the HHB reduces the particulate organic load. A nitrification unit converts part of ammonia (NH_4^+) to nitrate (NO_3^-) and increases the HHB efficiency, because many plants prefer up-taking N-NO_3^- to N-NH_4^+ .

The theoretical RAS/HHB model analyzed in this study is a 100 m^3 circular tank with 100 m^2 surface area and 1 m deep, used to farm catfish in a temperate climate. The system is active 8 months per year, populated in April with 2-12 inch fingerlings and harvested in Nov., when the HHB stops functioning and fish reached harvestable size (400-500 g) and stopped growing. The HHB section is a $1,000 \text{ m}^2$ and 14 cm shallow water raceway with 0.5 % slope, planted with hydroponic cattail (i.e. 100 L of water m^{-2} and 70 kg of wet weight (WW) plant biomass m^{-2}). Water flows at $100 \text{ m}^3 \text{ h}^{-1}$ and fresh water is added from an external source at a rate of 10 m^3 per day.

This paper analyzes: (1) the cost of recirculating water in RAS/HHB systems; (2) the best helophytes for HHBs; (3) consequences of increased evaporative loss in HHBs; (4) principles of pairing up fish species with helophytes; and (5) optimizing ratios between RAS and HHB subunits.

RESULTS AND DISCUSSIONS

1) The cost of recirculating water in RAS/HHB systems

Lowering the pumping head is expected to decrease water recirculation costs. Most clarifier/decanter tanks from conventional RAS filters are 1.5-2.5 m tall, while HHBs are shallow (20-30 cm) relative to the fish tank (Fig. 2). The most common types of water pumps in aquaculture are: airlift, radial and axial pumps. Air lift pumps (used in some small systems and aquaria) work with low heads, but are little efficient. Centrifugal (or radial flow) pumps are the most common in aquaculture, but best suited for raising water at high elevation. Axial flow (impeller or propeller) pumps are highly efficient when large volumes of water are moved at low head. They are frequently employed at clearing up flooded fields. For RAS/HHB systems with very large water tanks or ponds (such as $\geq 1,000 \text{ m}^3$) axial pumps are the best choice.

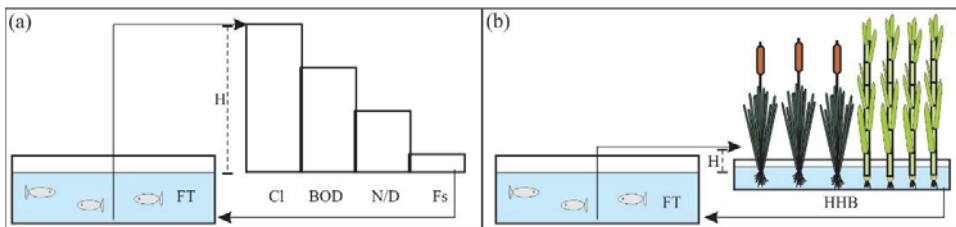


Fig. 2 Differences in pumping head (H) between: (a) RAS with conventional filters; and (b) RAS with HHB filter. FT = fish tank. Cl = Clarifier/decanter. BOD = bioreactor for mineralizing dissolved organic carbon. N/D = nitrification/denitrification bioreactors. Fs = filters. $H = 1.5\text{-}2.5 \text{ m}$ in (a) and $0.2\text{-}0.3 \text{ m}$ in (b).

The cost of recirculating water in RAS can be derived as follows:

$$C_P = \frac{V * \rho * H * g * P_{eff} * HPM * Months * E_{\$}}{T_t * 3600 * 1000} \quad (\text{Eq.1})$$

where:

C_P = cost of pumping water in a full growth cycle of a RAS system [\$];

V = volume of water the fish tank [L];

ρ = density of water [kg/L];

H = pumping head [m];

g = gravitational acceleration (9.81 m s⁻²);

P_{eff} = the pump's efficiency (%);

HPM = hours of pumping per month [24*30 = 720 hours];

Months = months in the fish growth cycle;

$E_{\$}$ = price of electricity = \$ 0.12 kWh⁻¹; and

T_t = turnover time of the water from the fish tank [h].

In these calculations, we assumed that 2,000 kg of fish are harvested, having \$2,000 total retail value, and that an impeller pump with 80% efficiency is used to recirculate water. Based on Eq.1 the water circulation costs are \$301 per growth season (i.e. 15.5 % from retail) in the case of a conventional RAS with 2 m head, and \$45 (= 2.25 % from retail) in a RAS/HHB system with 0.3 m head.

2) Best helophytes for HHBs

Here, selection criteria include: suitability to hydroponics, plant density, growth rate, and withstanding low O₂, nutrient deprivation and nutrient imbalance. HHB plants can be selected from species of bulrush (*Scirpus spp.*), cattail (*Typha spp.*), reeds (*Phragmites spp.*), papyrus (*Cyperus spp.*), *Eleocharis*, swamp grass (*Cladium spp.*), wild rice (*Zizania spp.*), flowering rush (*Butomus spp.*), yellow flag (*Iris spp.*) and sweet flag (*Acorus spp.*). Helophytes vary greatly in their suitability for HHB. For example, *Phragmites* and most bamboos have more biomass density than cattail, but are richer in ligno-cellulose, and are more expensive to trim. Papyrus, grows fast, has large biomass density, it is less woody and easy to trim, yet does not grow well in temperate conditions. Bulrush and sweet flag have non-woody texture and are easy to trim, but the biomass density is low. Some helophytes have commercial value that may lower RAS/HHB costs. For example, wild rice seeds are a valuable commodity, cattail rhizomes can be used to make bio-ethanol, cattail leaves can be fed to animals and reed biomass can be composted.

With regards to biofilters, *Typha angustifolia* and *T. latifolia* are some of the best studied helophytes. These plants reach a density of 21-30 stems m⁻² or 5.6 kg WW m⁻² in wetlands (Coon et al. 2000; Urban et al., 1993), but can be maintained at 150-220 stems m⁻² or 40-100 kg WW m⁻² in hydroponic cultures. The geographic distribution of cattails is broad, from tropical to subarctic with annual mean temperature ranging from 6°C to 28°C (Davis, 1991; Newman et al., 1996). Densely packed cattail in hydroponic cultures do not require special props (important for low infrastructure costs). Cattails withstand well nutrient deprivation (Miao and Sklar, 1998; Lorenzen et al., 2001). The hydroponic medium for fastest cattail growth contains: 43 ppm K; 20 ppm Ca; 9.76 ppm Mg; 28.8 ppm N-NO₃⁻; 2.8 ppm N-NH₄⁺ and 3.1 ppm P-PO₄³⁻ (Richardson et al., 1990; Davis, 1991; Newman et al., 1998). Cattails prefer up-taking N-NO₃⁻ relative to N-NH₄⁺, a N:P ratio of about 10:1 and P is biofiltered better in positive Eh conditions (DeLaune et al., 1999; Li et al., 2010). A BOD bioreactor is not necessary in a cattail HHBs and nitrification and aeration increase the biofilter efficiency. The NH₄⁺/NO₃⁻ ratio influences

plant biomass and root exudates significantly (Wu et al., 2016). Retaining nitrogen depends on phosphorus availability (Urban et al., 1993; DeBusk et al., 1994; Rutchey and Vilchek, 1994).

3) Consequences of HHB evaporative loss

Evaporation from the HHBs influences the net water loss and salinity. Our RAS/HHB dynamic model (Fig. 1) has 4 reservoirs (fish tank, filters, nitrification and HHB) and 5 flows (RAS inflow, RAS/HHB flow, HHB/RAS flow, HHB outflow and evaporation). In RAS about 10 % of the fish tank volume is replaced daily with clean fresh water from an external source (i.e. 2,400 m³). Evaporative water loss is highly variable, and depends on many factors such as temperature, humidity, wind, light intensity, the type of plant, vegetal biomass and the growth state of the plant. Hydroponic cattail at a density of 70 kg/m², noon temperature 22-25°C and 45 % atmospheric humidity) evaporates 3.6-8.3 L m⁻² d⁻¹. At a yearly average of 2 L m⁻² d⁻¹ the net evaporative water loss of a 1,000 m² hydroponic system in temperate conditions is approximately 480 m³ yr⁻¹. Agricultural water costs are estimated to 0.01 \$ m⁻³, but the price of water varies widely. Hence, the cost of water loss cannot be accurately predicted and has to be determined in each area.

4) Matching helophyte species with fish species

The following criteria are important here: fish sensitivity to pollutants, biofilter thresholds by the plants and temperature range for fish and plants.

Some species of fish, such as carp and catfish, are more tolerant to pollutants, while trout and salmon are more sensitive. Chemical context will also influence toxicity. For example, the acute toxicity of nitrite varies between < 1 ppm to > 10 ppm depending on chloride. Un-ionized ammonia (UIA), one of the most toxic pollutant in RAS (Knepp and Arkin, 1973; Tomasso et al., 1980; Hargreaves and Tucker, 2004) is a fraction of the total ammonium dissolved in water (<https://www.svl.net/2016/01/unionized-amonia-calculator/>). The UIA abundance depends on pH and temperature. The recommended limit for UIA is 0.005 ppm (EU aquaculture guidelines). At > 0.02 to 0.2 ppm UIA is potentially toxic, 0.3 ppm is the toxic threshold for rainbow trout and 1 ppm UIA is lethal to most fish. A good HHB system is one that can maintain the UIA below 0.005 ppm at reasonable cost.

The concentration below which removal of a pollutant no longer occurs is labelled C_{min}. The cattail C_{min}(PO₄³⁻) can be as low as 0.0012-0.025 ppm, with lowest value in aerated conditions. The cattail C_{min}(NH₄⁺) varies between 0.036-0.1026 ppm. One key requirement for an efficacious HHB system is: C_{min} < C_{tox}, where: C_{tox} is the recommended limit of a pollutant in a given system. The broadleaf cattail's C_{min}(NH₄⁺) is approximately 0.036 ppm at pH 8 and 20°C (Dyhr-Jensen and Brix, 1996), i.e. < 0.002 ppm UIA. Efficient nitrogen uptake occurs when the N:P g:g ratio is close to 10:1. Hence, lowering the UIA to 0.005 ppm requires a minimum concentration of 0.05 ppm P-PO₄³⁻. This can be easily obtained by adding a little soluble phosphate mineral to the system (e.g. apatite, hydroxyapatite, struvite).

Successful RAS/HHB systems requires matching fish with plants and local climate. Most temperate plants slow down growth in the fall. Because during the fish production cycle the fish stock are abundant and the pollutants are also abundant and harder to biofilter, the HHB plants have to be growing till the fish harvest time. Not many plants do this. Climate / fitness diagrams such as the one from Fig. 3 are used select HHB plants. Based on this diagram, the best fish and plant species for temperate climate are catfish (or carp) and cattails or temperate reeds.

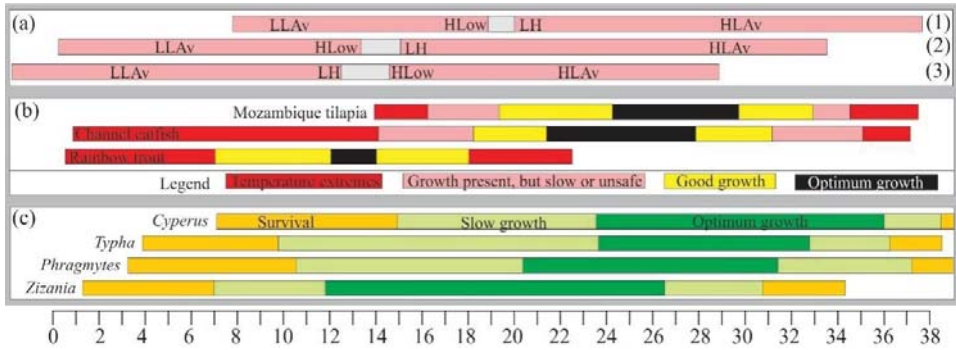


Fig. 4 Example of climate/fitness diagrams used to match the best fish and helophyte plants with a geographic location. (a) Local climate conditions in three geographic areas: (1) Hot southern and subtropical climate; (2) Temperate continental; and (3) Cold temperate and alpine. (b) Growth conditions for various fish. (c) Growth conditions for various marshland plants. LLAv = lowest lunar average temperature. HLA = Highest Lunar Average temperature. LH = Lowest high lunar average. HLA = Highest Low Lunar Average.

5) Size ratio between RAS and HHB

The sustainability of RAS/HHB requires that the HHB section is as large as necessary to withstand pollution peaks, yet not oversized. Calculations are based on: biomass and growth of the fish and plants; production of pollutants; safe levels for the pollutants; water circulation, the biofilter' efficiency and temperature.

1st. Experimental HHB systems are constructed, with similar water:plants proportions to the model RAS/HHB. The following have to be known: the surface area, amount of plants, water volume and flow rate. Temperature control and aeration are necessary, and physical-chemical parameters should be measurable.

2nd. Water is spiked with pollutants at similar concentration with values expected in fish tanks 12 hours after feeding. Temperature is maintained at desired control value that is between the low and high monthly average. Pollutants are monitored. A reasonable goal for the final BOD is 3-7 mg/L O₂ (Farooqi et al., 2008). A desirable goal for UIA is 0.005 ppm; cattail can reach < 0.002 ppm UIA.

3rd. The reaction rate constant is determined for each pollutant at a Control temperature (e.g. $k_T(\text{BOD}) \text{ h}^{-1}$; $k_T(\text{UIA}) \text{ h}^{-1}$, etc.). To do this, the evolution of the pollutants is graphed, with time on the OX axis and $-\ln(C(t)/C_0)$ on the OY axis. The linear part of the graph is used to calculate the rate constant:

$$k_{20} = [-\ln(C(t)/C_0)] / t \quad \text{Eq.2}$$

where: k_{20} = the biofiltration rate constant at 20°C for the given experimental system (h^{-1}); $C(t)$ = the pollutant concentration after time t ; C_0 = the initial concentration of the pollutant; and t = time (days).

The k_{20} varies significantly between habitats, the hour of the day, wetland communities, HHB designs and HHB management, and will deviate from linearity toward the ends of the concentration range of a species. Eq.2 should not be used to calculate k_T in non-linear parts of the graph.

4th. The rate constant is calculated at other temperatures as well:

$$k_T = k_{20} \cdot (\theta^{(T-20)}) \quad \text{Eq.3}$$

where: k_T = calculated reaction rate at the desired temperature (T); θ = the temperature coefficient for the rate constant, e.g. $\theta(\text{BOD}) = 1.06$ and $\theta(\text{NH}_4^+) = 1.048$; k_{20} = reaction rate constant at the experimentally measured temperature (here 20°C); and T = desired temperature (°C);

The biological rate constants depart from chemical predictions near the margins of a species' temperature range. Hence, k_T values should only be calculated for temperatures that are close to the measurement T (e.g. +/- 3°C). Control measurements have to be made at various points (e.g. at 5°C intervals) to cover the temperature range predicted for the entire growth season.

5th. The detention time (the time water should remain in the HHB in order to reach a desired level) is:

$$t_{det} = \frac{-\ln\left(\frac{C}{C_0}\right)}{k_T} \quad \text{Eq.4}$$

where: t_{det} = detention time (days); C_0 is the pollutant concentration entering in the HHB system (mg/L); and C is the desired pollutant concentration exiting the system (mg/L).

For sustainability purposes it is important to set up reasonable concentration targets. For example, the recommended limit for a fish/pollutant pair may be $C_{tox} = 0.005$ ppm. Yet even though cattails $C_{tox} < 0.002$ ppm, it is best to calibrate the system to reasonable values, rather than impose unnecessary burden on the HHB's size.

6th. The necessary HHB surface area is calculated from:

$$A_s = \frac{Q_{avg} t_{det}}{\eta \cdot d_w} \quad \text{Eq.5}$$

where: A_s = the necessary surface area for the HHB flow bed (m^2); Q_{avg} = average daily flow through the biofilter system (m^3/day); t_{det} = estimated detention time (days); η = the effective porosity of the medium (the proportion of the non-solid volume to the total volume of materials in the biofilter); and d_w = active depth of the biofilter system (m).

We used Eqs. 2-5 to estimate efficient RAS:HHB size ratios in a RAS/HHB system. The model system has a RAS fish tank (100 m^3 of water; 100 m^2 ; maximum fish load 20 kg of catfish m^{-3} and 1 hr. water turnover time) and a cattail-HHB unit (up to 1,000 m^2 ; 70 kg of plants m^{-2} ; 14 cm water depth; 57 % porosity; and up to 800 m^3 of water). The fish are fed with 3 % of their body mass per day. The feed contains 41 % crude protein and 6.6 % nitrogen (i.e. 1.98 g of N $\text{kg}_{fish}^{-1} \text{day}^{-1}$). Based on an estimated ammonium excreted by catfish of approximately 0.75 $\mu\text{mol h}^{-1} \text{g weight}^{-1}$ (Garcia et al., 2012), 14 % of the feed nitrogen was not retained by the fish and ends up in water. Assuming that most of the nitrogen released was in dissolved form (ammonium and urea), the 100 m^3 RAS system has produced 2,000 kg x 3 % x 14 % = 84 kg of N day^{-1} . This is equivalent to 8,400 g N / 100,000 L water / 24 = 3.5 ppm N-NH₄⁺ h^{-1} for a flow rate of 100 $\text{m}^3 \text{h}^{-1}$. Nitrification is used to produce a solution with approximately 32 ppm N-NO₃⁻ h^{-1} and 0.32 ppm N-NH₄⁺ h^{-1} (i.e. 0.012 ppm UIA h^{-1} at pH 8 and 20°C).

Using experimental systems with proportions between water and cattail similar to the model RAS/HHB we have obtained $k_{20}(\text{N-NO}_3^-) = 1.29 \text{ d}^{-1}$ and $k_{20}(\text{NH}_4^+) = 0.94 \text{ d}^{-1}$. The surface area needed to lower N-NO₃⁻ from 32 ppm to 0.58 ppm at 20°C is 93 m^2 while lowering the N-NH₄⁺ from 0.32 ppm to 0.132 ppm (i.e. 0.005 ppm UIA) requires 28.3 m^2 . The 0.58 ppm target value for N-NO₃⁻ was selected to reach the recommend safe level 0.005 ppm UIA should all nitrate be reduced to ammonium. Correcting for

temperatures as low as 10°C (consistent with night temperatures in late fall of temperate continental climate) the safe surface area of the cattail-HHB needed to control N-related toxicity is 166 m².

CONCLUSIONS

We analyzed requirements and costs of an HHB system using broadleaf cattail (*Typha latifolia*) and narrowleaf cattail (*Typha angustifolia*) for RAS wastewater treatment. System specifications were analyzed based on costs and benefits. These include the cost of the HHB infrastructure, costs of recirculating water, consequences of evaporation from the HHB, criteria for selecting the best halophyte plants and filtration efficiency by the HHB. The target fish in this study is channel catfish and the target chemical is un-ionized ammonia (putatively the most toxic pollutant in recirculated aquaculture). We found the following parameters for the RAS/HHB system: 100 m² of RAS with 100 m³ of water, populated with 20 kg of catfish m⁻³, 100 m³ h⁻¹ water flow rate and 190 m² of HHB with 14 cm deep hydroponic culture and 70 kg of cattail m⁻². We assume a yearly revenue from fish of \$ 2,000 and an HHB biofilter with EPDM rubber liner and concrete margins with 20 years life span and 30 \$m⁻² investment cost and 1.5 \$m⁻² yearly usage costs. The HHB system costs have been estimated to \$249 for HHB infrastructure usage, \$45 for water recirculation, \$1 for fresh water to compensate evaporation, \$24 for fresh water input and \$100 in labor. According to our model, the cost of maintaining water quality in a RAS/HHB system with catfish and cattail in temperate climate can be brought as low as 20 % from revenue. Comparative to aquaponics, the surface area planted with hydroponic cultures in a RAS/HHB is approximately five times smaller. Revenue sources from HHB can come from example from *Zizania* seeds, cattail leaves use as fodder and biomass from reeds, bamboo and papyrus used to make compost.

REFERENCES

- Coon, W.F., J.M. Bernard, and F.K. Seischab. 2000. Effects of a cattail wetland on water quality on Irondequoit Creek near Rochester. New York, US Geol. Surv., Water-Resources Investigations Report. 00-4032.
- Davis S.M. 1991. Growth, decomposition, and nutrient retention of *Cladium jamaicense* Crantz and *Typha domingensis* Pers. In the Florida Everglades. Aquatic Botany. 40(3): 203-224.
- DeBusk, W.F., K.R. Reddy, M.S. Koch, and Y. Yang. 1994. Spatial distribution of soil nutrient in a northern Everglades marsh: Water conservation area 2. SSSA J. 58(2): 543-552.
- DeLaune, R.D., A. Jugsujinda and K.R., Reddy. 1999. Effect of root oxygen stress on phosphorus uptake by cattail. J. Plant. Nutrition. 22(3): 459-466.
- Farooqi, I.H., F. Basheer and J. Chaudhari 2008. Constructed wetland system (CWS) for wastewater treatment. Proceedings of Tall 2007: The 12th World Lake Conference 1004-1009.
- Hargreaves J.A. and C.S. Tucker, 2004, Managing ammonia in fish ponds, Southern Regional Aquaculture Center, SRAC Publication No. 4603.
- Dyhr-Jensen K. and H. Brix, 1996, Effects of pH on ammonium uptake by *Typha latifolia* L. Plant, Cell and Environment. 19(12): 1431-1436.
- Garcia, L. D. O., Braun, N., Becker, A. G., Loro, V. L., and Baldisserotto, B. 2012. Ammonia excretion at different life stages of silver catfish. Acta Scientiarum. Animal Sciences, 34(1), 15-19.
- Knepp G.L. and G.F. Arkin. 1973. Ammonia toxicity levels and nitrate tolerance of channel catfish. The Progressive Fish-Culturist. 35(4): 221-224.

Li, S., J. Lissner, I.A. Mendelssohn, H. Brix, B. Lorenzen, K.L. McKee and S. Miao. 2009. Nutrient and growth responses of cattail (*Typha domingensis*) to redox intensity and phosphate availability. *Ann. Bot.* 105(1): 175-84.

Lorenzen, B., H. Brix, I.A. Mendelssohn, K.L. McKee, and S.L. Miao. 2001. Growth, biomass allocation, and nutrient use efficiency in *Cladium jamaicens* and *Typha domingensis* as affected by phosphorus and oxygen availability. *Aquatic Botany.* 70(2): 117-133.

Newman, S., J.B. Grace and J.W. Koebel. 1996. Effects of nutrients and hydroperiod on *Typha*, *Cladium*, and *Eleocharis*: Implications for Everglades restoration. *Ecol. Applic.* 6(3): 774-783.

Newman, S., J. Schuette, J. B. Grace, K. Rutchey, T. Fontaine, K. R. Reddy, and M. Pietrucha. 1998. Factors influencing cattail abundance in the northern Everglades. *Aquatic Botany* 60(3): 265-280.

Miao, S. L., and F. H. Sklar. 1998. Biomass and nutrient allocation of sawgrass and cattail along a nutrient gradient in the Florida Everglades. *Wetlands Ecol. Manage.* 5(4): 245-263.

Richardson, J. R., W. L. Bryant, W. M. Kitchens, J. E. Mattson, and K. R. Pope. 1990. An evaluation of refuge habitats and relationships to water quality, quantity, and hydroperiod. Boynton Beach, Fla.: A.R.M. Loxahatchee National Wildlife Refuge.

Rutchey, K., and L. Vilchek. 1994. Development of an Everglades vegetation map using SPOT image and the global positioning system. *Photogramm. Eng. Remote Sens.* 60(6): 767-775.

Tomasso, J.R., C.A. Goudie, B.A. Simco and K.B. Davis. 1980. Effects of environmental pH and calcium on ammonia toxicity in channel catfish, *Transactions of the American Fisheries Society*, 109(2): 229-234.

Urban, N.H., S.M. Davis and N.G. Aumen. 1993. Fluctuations in sawgrass and cattail densities in Everglades Water Conservation Area 2A under varying nutrient, hydrologic, and fire regimes. *Aquatic Botany* 46(3-4): 203-223.

Wu, H., K. Xu. X. He and X. Wang. 2016. Removal of nitrogen by three plant species in hydroponic culture: plant uptake and microbial degradation. *Water, Air and Soil Pollution.* 227: 324.

EVALUATION OF NOISE LEVELS IN AN INDUSTRIAL AREA OF CRAIOVA CITY, ROMANIA

Popescu Simona Mariana^{1*}, Căpruciu Ramona¹

¹University of Craiova, Faculty of Horticulture, Craiova

* Correspondence author. E-mail: popescu_simona83@yahoo.com

Keywords: *noise, pollution, urban and industrial area.*

ABSTRACT

In the present society, noise in cities is considered a health problem and it has been well studied worldwide. Noise is unwanted sound and acts on the whole body because the auditory sensation reaches the central nervous system through which it affects other organs. This paper presents the evaluation of the noise level in an industrial area of the city of Craiova, Romania. The noise level measurements were carried out under normal conditions of activity within the objective investigated with BRUEL & KJAER 2238 sound meter, in good weather conditions, during day, evening and night hours in week days between March and June, 2017. The results of the study revealed that the noise level was not exceeded in any of the 2 points of interest: access gate and workshops area and no necessary actions are required to protect the area.

INTRODUCTION

Alongside air, water and soil, noise pollution is becoming a major concern for the modern society. Noise is unwanted sound and when it exceeds a certain amount that causes harm to human activities or animal life it becomes an environmental pollutant.

Among the most important sound characteristics regarding pollution is the intensity determined by the amount of energy transported by the sound wave which penetrates per second a surface unit perpendicular to the sound propagation direction.

Nowadays noise in cities is considered a health problem affecting quality of life in urban areas and it has been well studied worldwide (Zannin et al. 2002; Guedes et al. 2011; Frei et al., 2014). The response of the human ear to sound is dependent on the frequency of the sound. Noise acts on the whole body because the auditory sensation reaches the central nervous system through which it affects other organs and produces stress, fatigue, decreased or loss of auditory capacity, mental instability (Schwela et al. 2005 Babisch et al. 2005). Very high noise levels can cause damage to buildings, appliances and instruments.

The present study was conducted to show the noise level measurements carried out in an industrial area of the city of Craiova, Romania. The measured noise levels were classified according to the environmental legislation in effect for the country.

MATERIAL AND METHODS

The study was conducted in the S-E part of Craiova city, where an industrial company operates its activity which is the production of industrial electric motors and generators, heavy duty power transformers, railway and urban vehicles, manufacture of metal structures and parts of metal structures, manufacture of containers and similar

products of steel. Right next to the company, in the close vicinity, there is also the shopping mall of the city. The noise level measurements were carried out under normal conditions of activity within the objective investigated with BRUEL & KJAER 2238 sound meter, provided with a weighing circuit A, C and L, response "slow, fast", software BZ 7126 and microphone ZC 0030. All the noise measurements were taken in good weather conditions, without rain or strong wind, between March and June 2017 in week days, the average of the measurements being presented in this study.

Table 1

Measuring periods for noise	
Period of one calendar day	Time interval (local time)
day	13.00
evening	19.00
night	22.00

The noise measurements were performed at the functional limit of the company in 2 measuring points located on the cardinal directions of the source in daytime, evening and night mode (Table 1), as follows:

- Point no. 1 - in front of the access gate (which is very close to the access gate of the mall also) - in direction N;
- Point no. 2 – in front of the workshops of the company – in direction S.

The spatial distribution of noise pollution in urban areas depends on various parameters like construction density, open spaces, the shape and physical position of buildings, the type of passages and population distribution (Ariza-Villaverde et al., 2014).

RESULTS AND DISCUSSIONS

The noise sources within the company are represented by: transport, loading and unloading of raw materials and finished products, the metal workshop and by the other production activities. The company's specific noise sources are discontinuous. Other noise in the area is caused by heavy traffic and the flow of people reaching the shopping mall.

Maschke, 1999 suggests that 65 dB (A) is the sound level limit for noise exposure of a population in an urban environment. In other countries, for example India, the day time ambient noise standard of Leq is 75 dB (A) and that for night time is 70 dB (A) for industrial area (Hunashal and Patil, 2012). For Romania the allowable noise levels, for urban areas, according to the national regulations is 65 dB (A) - equivalent continuous sound level.

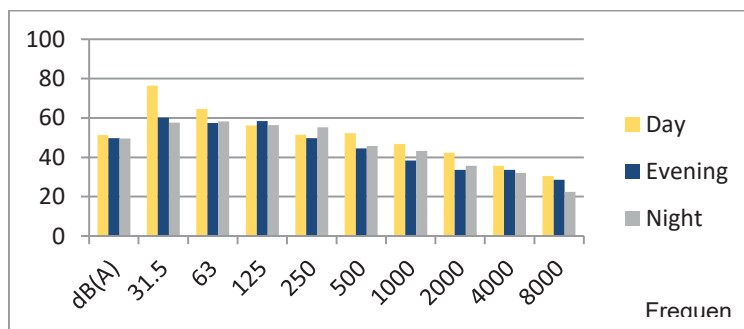


Figure 1. The level of noise measured at the access gate, N direction

The results obtained for the access gate measurement point (Fig. 1) revealed that the noise level exceeded (76.4 dB) only one time the legal limit of 64.5 dB (A), recorded during daytime for the frequency of 31.5 Hz. In the rest of the cases the noise level measured in daytime, evening and night was below the limit and no exceeding frequencies were recorded.

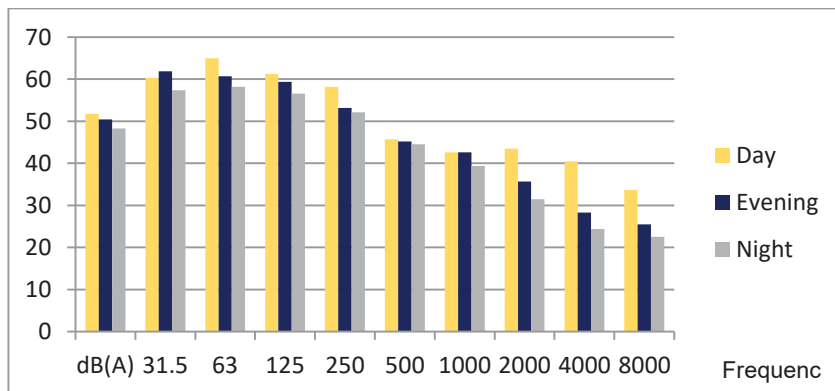


Figure 2. The level of noise measured at the workshops, S direction

Regarding the noise level measured at the workshops area (Fig. 2) there were recorded values over 60.0 dB (A) but no exceeding frequencies were recorded during the selected hours in daytime, evening and night respectively.

CONCLUSIONS

The results of the measurements evaluated in this study revealed that the level of the noise in the studied area was not exceeded in any of the 2 points where the measurements were carried out, namely the access gate and workshops area respectively.

No special facilities and equipment are required to protect the area because: the buildings of the company's workshops are of brick structure, without openings, with sealed metal doors, that represent an important factor for noise mitigation. In the work area staff are provided with noise protection materials.

Nevertheless, if the noise is not controlled, in the future the frequency of noise complaints will show an increase because of rapid increase in population, city life activities and industrialization.

REFERENCES

- Ariza-Villaverde, A.B., Jiménez-Hornero, F.J., Gutiérrez De Ravé, E., 2014. Influence of urban morphology on total noise pollution: Multifractal description. *Sci. Total Environ.* 472, 1–8.
- Babisch W, Beule B, Schust M, Kersten N, Ising H. 2005. Traffic noise and risk of myocardial infarction. *Epidemiology*;16(1):33–40.
- Frei, P., Mohler, E., Röösli, M., 2014. Effect of nocturnal road traffic noise exposure and annoyance on objective and subjective sleep quality. *Int. J. Hyg. Environ. Health* 217 (2–3), 188–195.
- Guedes ICM, Bertoli SR, Zannin PHT. 2011. Influence of urban shapes on environmental noise: a case study in Aracaju Brazil. *Sci Total Environ*; 412:66–76.

Hunashal Rajiv B., Patil Yogesh B. 2012. Assessment of noise pollution indices in the city of Kolhapur, India. *Procedia - Social and Behavioral Sciences* 37, 448 – 457.

Maschke C. 1999. Preventive medical limits for chronic traffic noise exposure. *Acustica*; 85(1999):444–8.

Schwela D, Kephelopoulos S, Prasher D. 2005. Confounding or aggravating factors in noiseinduced health effects: air pollutants and other stressors. *Noise Health*; 7:41–50.

Zannin PHT, Diniz FB, Calixto A, Barbosa W. 2002. Environmental noise pollution in the city of Curitiba, Brazil. *Appl Acoust*; 63:351–8.

CHROMIUM BIOACCUMULATION IN THREE POACEAE SPECIES

Dan Răzvan Popoviciu^{1*}, Bogdan-Ștefan Negreanu-Pîrjol²,
Ticuța Negreanu-Pîrjol²

¹„Ovidius” University of Constanța, Faculty of Natural Sciences and Agricultural Sciences,
Constanța, Romania

²„Ovidius” University of Constanța, Faculty of Pharmacy, Constanța, Romania

* Correspondence author. E-mail: dr_popoviciu@yahoo.com

Keywords: *Hordeum murinum*, *Leymus racemosus*, *Lolium perenne*, *bioaccumulation*, *chromium*.

ABSTRACT

Chromium concentration was determined, through HR-CS-AAS, in tissular and rhizospheric soil samples from three Poaceae species growing in Romanian ruderal and coastal areas: *Hordeum murinum* L., *Leymus racemosus* ssp. *sabulosus* (M. Bieb.) Tzvelev and *Lolium perenne* L. Biological Accumulation Coefficients (BAC) were calculated.

The highest average concentration was found in *L. racemosus* (22.21 mg/kg) higher than the 1.5 mg/kg “standard reference” levels, but below the hyperaccumulation threshold (1,000/300 mg/kg) With 0.11-0.54 average BAC, all selected species can be considered as moderate accumulators, with the highest values in *L. racemosus*.

Results show that *L. racemosus*, although not an accumulator under natural conditions, has a potential for Cr phytostabilization, or enhanced phytoextraction experiments.

INTRODUCTION

Research in heavy metal phytoaccumulation is a growing field, due to its multiple applications. Industrial expansion leads to an increasing demand in heavy metal mining and processing and, thus, to increasing soil pollution issues, affecting biodiversity, crops and, indirectly, human health. This in turn requires new and non-invasive soil remediation techniques.

On the other hand, identifying new metal ores, or extracting metal from subeconomic ones are also priorities.

For all these, knowledge of natural metal accumulators is of great importance. While heavy metals usually induce various types of toxic effects in plants (especially at root level), some species are able to cope with this by means of exclusion, root sequestration or general bioaccumulation (accumulating, in specific tissues, metal concentrations higher than in the surrounding soil).

The extreme version of bioaccumulation is called “hyperaccumulation” (100-1,000 times more metal ions than in normal vegetation). The practical application of plant metal bioaccumulation and hyperaccumulation include phytoprospection, phytoremediation (phytostabilization and phytoextraction) and phytomining (Rascio & Navari-Izzo 2011, Tang et al. 2012).

The aim of this research was to assess chromium bioaccumulation in three common Poaceae species growing in Romanian marine coastal areas and not only.

Chromium occurs (either trivalent or hexavalent) in minerals such as chromite, crocoite, bentorite, tarapacaita, vauquelinite etc. In normal soils, its concentration ranges

between 10-50 mg/kg. Industrial applications are variate: special alloys, electroplating, pigment industry etc. Tanning industry is a major source of Cr pollution (40 % of known cases; Oliveira 2012).

Cr is not an essential nutrient for plants. It is usually stored in vacuoles of root cells. Phytotoxic effects include an inhibition of root growth, water absorption, growth of stems and leaves, proper development of photosynthetic apparatus, a lower productivity and a lower seed viability (Shanker et al. 2005). Cr phytoaccumulators include *Amaranthus dubius*, *Brachiaria decumbens*, *Convolvulus arvensis*, *Dyera costulata*, *Genipa americana*, *Ipomoea aquatica*, *Pennisetum purpureum*, *Pluchea indica* (Oliveira 2012).

The three species investigated include two ruderal grasses and one specific to sandy areas.

Hordeum murinum ssp. *murinum* L. (barley grass, wall barley) is an annual, tuft-forming, species, with a short stem (~30 cm), glabrous green leaves, compound terminal (green or purplish) spikes. A native of Western Eurasia and North Africa, is currently found worldwide. Its uses include foraging, turf and culinary uses (in China; Jacobsen & von Bothmer 1995).

Leymus racemosus ssp. *sabulosus* (M. Bieb.) Tzvelev (mammoth wildrye) is a perennial, tuft-forming species, with extensive rhizomes, long, pubescent stem (~100 cm), long, glaucous green leaves (20-40 cm) and long, terminal compound spikes (15-30 cm). A native of Central Asia, it is currently found in many temperate areas. In Romania, it only grows on littoral sands. Uses include dune stabilization or mine tailing revegetation (St. John et al 2010).

Lolium perenne L. (perennial ryegrass) is a perennial, tuft-forming grass, with short rhizomes, 30-100 cm stems, narrow, dark green leaves and narrow, terminal compound spikes. A native of Eurasia and North Africa, it currently grows worldwide. Its uses include foraging, turf and erosion control (Ogle et al. 2008).

MATERIAL AND METHODS

Plant material was collected from marine beaches and surroundings in Constanța, Romania. Samples from aboveground organs (stems and leaves) and rhizospheric soil were collected from different areas of the “Trei Papuci” and “Modern” beaches, from three individuals for each species.

Organ samples were cut into small pieces and, together with soil samples, oven-dried for 3 days at 80°C. 0.25 g of each sample were digested overnight in 5 mL concentrated HNO₃ and boiled 1 hour at 150 °C. 2 mL H₂O₂ (30 %) were added, followed by 2-hour boiling. The solution was made up to 50 mL with distilled water, adding CaCl₂ (0.5 %) and NH₄Cl (2 %) (Shanker et al. 2004, Popoviciu et al. 2016, Popoviciu et al. 2017).

Analysis was done with a HR-CS Atomic Absorption Spectrometer (ContrAA700, Analytik Jena AG), with air-acetylene flame at 357 nm wavelength.

Resulting metal concentrations were expressed as mg/kg and used to calculate the biological accumulation coefficients (BAC; Nazir et al. 2011, Obasi et al. 2013):

$$BAC = [Cr]_{Shoot}/[Cr]_{Soil}.$$

RESULTS AND DISCUSSIONS

Average tissular metal concentrations are shown in Fig. 1, while soil chromium levels are shown in Fig. 2 (1.9 mg/kg minimal detection limit of the device). Fig. 3 shows average BAC values for each studied species.

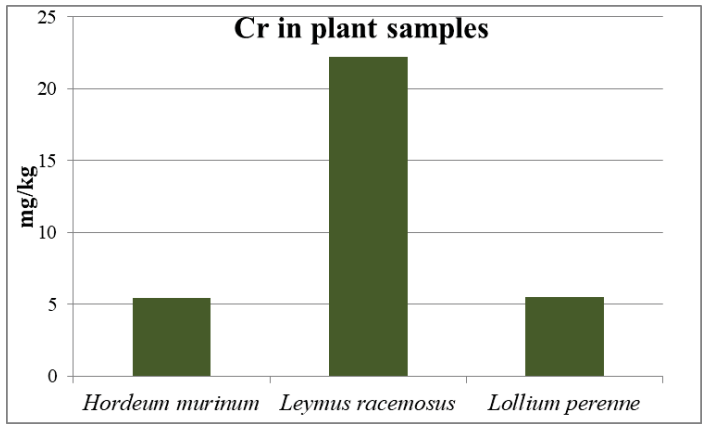


Figure 1. Average chromium concentrations in aboveground organs of selected Poaceae species (mg/kg; dry weight).

Normally, plant tissues have low Cr concentrations. The „standard reference plant” (an average model of known species) contains 1.5 mg/kg Cr (Van der Ent et al. 2013). All three species had tissular chromium levels higher than this standard (Fig. 1). The highest average concentration was found in *L. racemosus* (22.21 mg/kg – not constant, however; the highest value found in one individual was 32.08 mg/kg).

Soil concentrations ranged mostly between 22.68-55.08 mg/kg (Fig. 2), i.e. normal soil Cr levels (Oliveira 2012).

Most authors define Cr hyperaccumulation as starting from 1,000 mg/kg tissular concentration, with no toxic effects, with some proposing an alternate value of 300 mg/kg (Rascio & Navari-Izzo 2011, Van der Ent et al. 2013). According to these thresholds, none of the three Poaceae species is a hyperaccumulator under normal soil conditions.

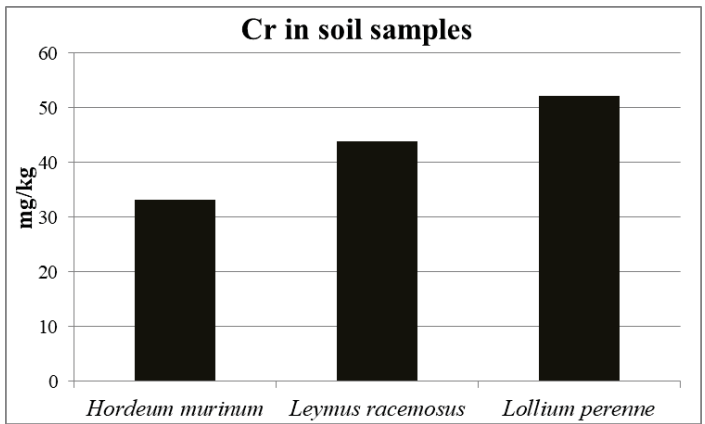


Figure 2. Average chromium concentrations in rhizospheric soil for selected Poaceae species (mg/kg; dry weight).

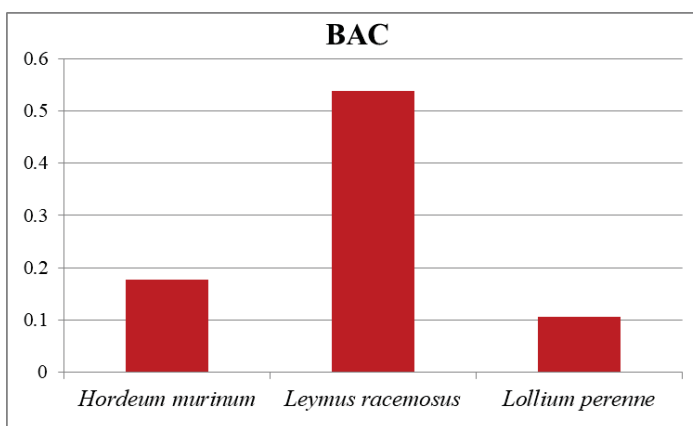


Figure 3. Average chromium biological accumulation coefficients for selected Poaceae species.

Biological accumulation coefficients indicate whether a certain species concentrates more metal ions in aboveground organs than in surrounding soil. BAC can be variable – influenced by local factors, such as heavy metal levels in soil or the availability of certain nutrients. However, it is extremely useful in assessing the usefulness of a plant for phytoextractive purposes (Rascio & Navari-Izzo 2011, Van der Ent et al. 2013).

Some authors (Sekabira et al., 2011) propose a scale ranging from non-accumulating plants ($BAC < 0.01$) to low accumulators (0.01-0.1), moderate accumulators (0.1-1) and high accumulators/hyperaccumulators (> 1). According to this scale, *H. murinum* and *L. perenne* would be classified as low to moderate accumulators (average BAC of 0.18, respectively, 0.11), while *L. racemosus* would fall within the upper range of moderate accumulation (0.54 average, 0.79 highest value).

Other Poaceae, such as oat, sorghum or wheat, are known to show a much lower Cr accumulation than the three studied species (López-Luna et al. 2009). However, due to a low translocation to upper organs, their phytoextractive potential is low. *L. racemosus* seems to be the only one suitable to further experimenting, for chemically-enhanced accumulation, since it is known that factors such as a lower pH or various chelators can improve the process (Amaria & Brahim 2016).

CONCLUSIONS

The three Poaceae species studied had tissular chromium concentrations above average, while metal levels in surrounding soil were within the normal range. The highest concentrations (although not constant) were found in *Leymus racemosus*. This species also had an average BAC higher than 0.5, while the other two had BAC below 0.2.

None of the species is a high accumulator, according to acknowledged tissular concentration thresholds or BAC values. *L. racemosus* might prove suitable for certain phytostabilization or even phytoextraction purposes, but only after further laboratory research.

REFERENCES

- Amaria B., Brahim L., 2016, Cr (III) and Cr (VI) absorption and translocation of *Atriplex halimus* L., International Journal of Biosciences, 9 (6), 234-246.
- Jacobsen N., von Bothmer R. 1995, Taxonomy in the *Hordeum murinum* complex (Poaceae), Nordic Journal of Botany, 15, 449-458.
- López-Luna J., González-Chávez M.C., Esparza-García F.J., Rodríguez-Vázquez R., 2009, Toxicity assessment of soil amended with tannery sludge, trivalent chromium and hexavalent chromium, using wheat, oat and sorghum plants, Journal of Hazardous Materials, 163, 829-834.
- Nazir A., Malik R.N., Ajaib M., Khan N., Siddiqui M.F., 2011, Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi, Pakistan Journal of Botany, 43 (4), 1925-1933.
- Obasi N.A., Akubugwo E.I., Kalu K.M., Ugbogu O.C., 2013, Speciation of heavy metals and phyto-accumulation potentials of selected plants on major dumpsites in Umuahia, Abia State, Nigeria, International Journal of Current Biochemistry Research, 1 (4), 16-18.
- Ogle D., Engle S., Shewmaker G., 2008, Plant guide for perennial ryegrass (*Lolium perenne* L. ssp. *perenne*), USDA-Natural Resources Conservation Service, Aberdeen, ID Plant Materials Center, http://plants.usda.gov/plantguide/pdf/pg_lopep.pdf.
- Oliveira H., 2012, Chromium as an environmental pollutant: insights on induced plant toxicity, Journal of Botany, 2012, doi: 10.1155/2012/375843.
- Popoviciu D.R., Negreanu-Pîrjol T., Bercu R., 2016, Copper, chromium, zinc and manganese accumulation in three common Brassicaceae on the Romanian littoral, Revista de Chimie, 67 (4), 670-673.
- Popoviciu D.R., Negreanu-Pîrjol B.Ş., Bercu R., Negreanu-Pîrjol T., 2017, Chromium bioaccumulation in some herbaceous species from Constanța littoral area (Romania), SGEM 2017: 17th International Multidisciplinary Scientific GeoConference, 17 (61), 1183-1189.
- Rascio N., Navari-Izzo F., 2011, Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Science, 180, 199-181.
- Sekabira K., Oryem-Origa H., Mutumba G., Kakudidi E., Basamba T.A., 2011, Heavy metal phytoremediation by *Commelina benghalensis* (L) and *Cynodon dactylon* (L) growing in urban stream sediments, International Journal of Plant Physiology and Biochemistry, 3(8), 133-142.
- Shanker A.K., Cervantes C., Loza-Tavera H., Avudainayagam S., 2005, Chromium toxicity in plants, Environment International, 31, 739-753.
- Shanker A.K., Djanaguiraman M., Sudhagar R., Chandrashekar C.N., Pathmanabhan G., 2004, Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO 4) roots, Plant Science, 166, 1035-1043.
- St. John L., Ogle D.G., Stannard M., Pavek P., 2010, Plant guide for mammoth wildrye (*Leymus racemosus*), USDA-Natural Resources Conservation Service, Aberdeen, ID Plant Materials Center, http://plants.usda.gov/plantguide/pdf/pg_lera5.pdf.
- Tang Y.T., Deng T.H.B., Wu Q.H., Wang S.Z., Qiu R.L., Wei Z.B., Guo X.F., Wu Q.T., Lei M., Chen T.B., Echevarria G., Sterckeman T., Simonnot M.O., Morel J.L., 2012, Designing cropping systems for metal-contaminated sites: a review, Pedosphere, 22 (4), 470-488.
- Van der Ent A., Baker A.J.M., Reeves R.D., Pollard A.J., Schat H., 2013, Hyperaccumulators of metal and metalloid trace elements: Facts and fiction, Plant and Soil, 362 (1-2), 319-334.

CHROMIUM BIOACCUMULATION IN THREE COMMON WOODY SPECIES

Dan Răzvan Popoviciu^{1*}, Rodica Bercu¹, Ticuța Negreanu-Pîrjol²

„Ovidius” University of Constanța, Faculty of Natural Sciences and Agricultural Sciences,
Constanța, Romania

²„Ovidius” University of Constanța, Faculty of Pharmacy, Constanța, Romania

* *Correspondence author. E-mail: dr_popoviciu@yahoo.com*

Keywords: *Ailanthus altissima*, *Robinia pseudoacacia*, *Salix alba*, *bioaccumulation*, *chromium*

ABSTRACT

Chromium levels were determined (through HR-CS-AAS) in tissular samples and rhizospheric soil from three common woody species: Ailanthus altissima (Mill.) Swingle, Robinia pseudoacacia L., Salix alba L., also calculating Biological Accumulation Coefficients (BAC).

The highest average Cr content was found in R. pseudoacacia (15.38 mg/kg, but with high individual variations), higher than “standard reference” (1.5 mg/kg), lower than hyperaccumulation thresholds (1,000/300 mg/kg) All selected species were moderate accumulators (with average BAC of 0.12-0.44).

Results show a limited accumulation potential and phytoextractive value in the three selected species.

INTRODUCTION

Heavy metals are an important resource for industry and not only. They are also common pollutants, affecting agriculture, livestock, human health and biodiversity. While high levels of heavy metals in soil are toxic to most plants, some species are able to tolerate and even accumulate them in their organs.

Phytoaccumulation in specific aboveground organs, or metal sequestration at root level are two strategies employed for preventing the intoxication of more sensitive tissues. In extreme cases, some plants are able to accumulate 100-1,000 times more metal ions than the surrounding vegetation – the so-called “hyperaccumulation” (Rascio & Navari-Izzo 2011, Tang et al. 2012).

Potential applications include phytoprospection (metal ore-indicating plants), phytoremediation (cleaning polluted soils by phytostabilization at root level or phytoextraction and cropping), or even phytomining subeconomic metal ores.

This is why searching for naturally bioaccumulating species and enhancement methods is of great scientific importance. The aim of this study (part of a wider screening effort of local flora) was to determine natural chromium accumulation potential in three woody species widely found in Romania.

Chromium (as trivalent or hexavalent ions) is found in chromite, crocoite, bentorite, tarapacite, vauquelinite and other minerals. Soils usually contain 10-50 mg/kg Cr ions. It is used in metallurgy (for alloys), electroplating, dye industry, or tanning industry (a major source of chromium pollution; Oliveira 2012).

For plants, Cr is a non-essential element. It usually accumulates in root cell vacuoles. At high concentrations, it affects proper root development and water absorption. It also hinders general plant growth and productivity, photosynthesis and

seed germination (Shanker et al. 2005). However, some species are known to be Cr bioaccumulators: *Amaranthus dubius*, *Brachiaria decumbens*, *Convolvulus arvensis*, *Dyera costulata*, *Genipa americana*, *Ipomoea aquatica*, *Pennisetum purpureum*, *Pluchea indica* (Oliveira 2012).

The three species studied in this research are trees and shrubs commonly growing as ornamental, spontaneous, or even invasive in many temperate areas.

Ailanthus altissima (Mill.) Swingle (tree-of-heaven, Chinese sumac), from the Simaroubaceae family is native to North-Central China. A medium tree, (up to 18-30 m), with taproot and large pinnated-compound leaves. It is dioecious. An invasive species, it grows in many parts of the world, on variate soil types, various climates (mostly warm/temperate, including arid climates, but not particularly resistant to frost). Vegetative reproduction (through root sprouts) is one of the causes of its invasivity. It is cultivated for ornamental, soil stabilization or, sometimes, medicinal purposes (Kowarik & Säumel, 2007).

Robinia pseudoacacia L. (black locust, false acacia, Chinese scholar tree), from the Fabaceae family, is a medium tree (up to 30 m), with gray/dark-brown bark, pinnated-compound leaves, spiny stipules, white flowers forming pendulous racemes, dark brown legume fruits. Native to Southeastern USA, it is found worldwide. It tolerates variate soil types, but not frost and shade. It is cultivated for ornamental purposes, soil nitrogen enrichment and apiculture (Sitzia et al. 2016).

Salix alba L. (white willow), from the Salicaceae family, is a medium tree or shrub, native to Northern Eurasia, and common in most temperate areas. It has dark grey bark, yellow-orange on new branches, silver-grey, lanceolate leaves and catkins for both male and female flowers. It is dioecious, it reproduces by seeds and twigs. It prefers wetland areas, or other moist areas moisture. It is cultivated in some areas for wood, ornamental or medicinal purposes (Houston Durrant et al. 2016).

MATERIAL AND METHODS

Plant material (branch fragments) was collected from coastal ridges adjacent to “Trei Papuci” and “Modern” beaches, Constanța, Romania (triplicate samples per species, from different areas). Adjacent soil samples were also collected for comparison.

Samples were fragmented into small pieces and oven-dried (3 days, 80°C). 0.25 g of each sample were left overnight in 5 mL concentrated HNO₃ and boiled in oven (1 hour, 150°C). 2 mL of 30 % H₂O₂ were added and the samples were boiled again, for 2 hours. Samples were diluted to 50 mL with distilled water, also adding 0.5 % CaCl₂ and 2 % NH₄Cl (Shanker et al. 2004, Popoviciu et al. 2016, Popoviciu et al. 2017).

Metal concentrations were determined by using a HR-CS Atomic Absorption Spectrometer (ContrAA700, Analytik Jena AG), with air-acetylene flame (357 nm wavelength) and expressed as mg/kg.

Subsequently, the biological accumulation coefficients were calculated, based on plant and soil metal concentrations (BAC; Nazir et al. 2011, Obasi et al. 2013):

$$BAC = [Cr]_{Shoot}/[Cr]_{Soil}.$$

RESULTS AND DISCUSSIONS

Average tissular metal concentrations are shown in Fig. 1 shows tissular Cr concentrations in plant samples (average values) and Fig. 2 shows average Cr concentrations in soil samples (1.9 mg/kg is the minimal detection limit for the device used). Fig. 3 shows BAC average values for each of the three species species.

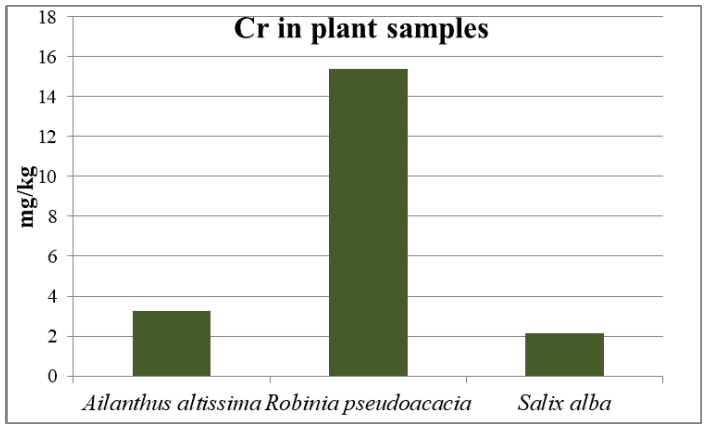


Figure 1. Average chromium concentrations in branch tissues of selected species (mg/kg; dry weight).

Most plants accumulate and store only low amounts of chromium. For comparison, a so-called „standard reference plant” (a theoretical model based on average values in worldwide flora) would contain 1.5 mg/kg (Van der Ent et al. 2013). Among the three species investigated, *A. altissima* and *S. alba* had tissular Cr levels similar to the „reference plant”. The average for *R. pseudoacacia* was higher, but only due one single individual (with 46.14 mg/kg).

Soil concentrations ranged between above detection limit to 67.25 mg/kg (Fig. 2), with high variations, but mostly within the normal range (10-50 mg/kg; Oliveira 2012). The highest Cr levels in plant tissue were encountered in a black locust individual growing on the soil with the lowest Cr concentration.

Cr hyperaccumulation is considered to start from 1,000 mg/kg concentration in aboveground organs, with no significant toxic effects. An alternative value is 300 mg/kg (Rascio & Navari-Izzo 2011, Van der Ent et al. 2013). Both are much higher than those found in this study.

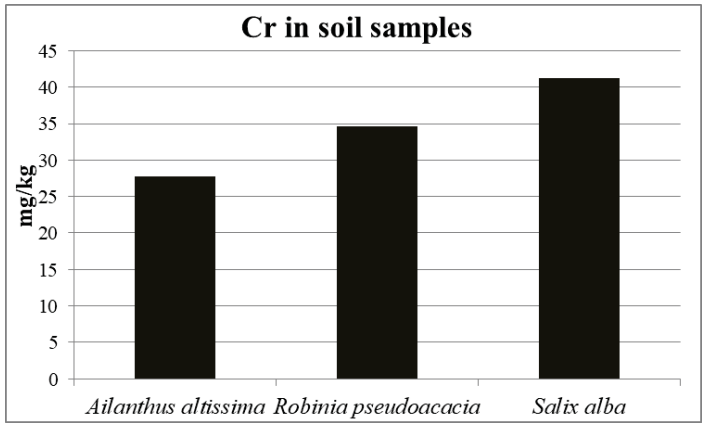


Figure 2. Average chromium concentrations in rhizospheric soil for selected species (mg/kg; dry weight).

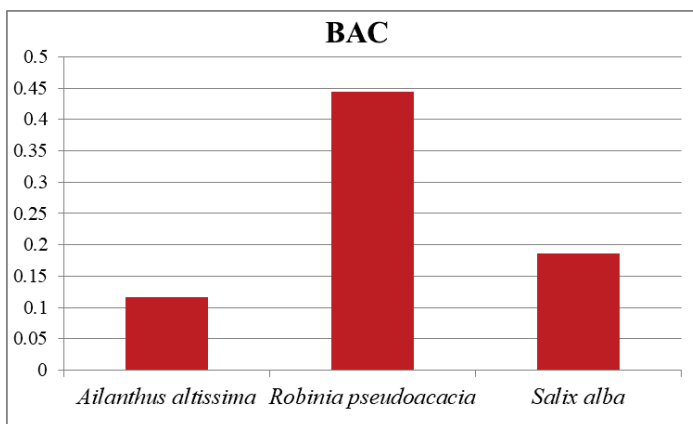


Figure 3. Average chromium biological accumulation coefficients for selected species.

Biological accumulation coefficient (BAC) values are useful for identifying plants that naturally concentrate more metal ions than in the surrounding environment and store them in harvestable organs. It should be noted that BAC for one species is often can be variable, mostly due to soil factors, such as metal concentration or nutrients (Rascio & Navari-Izzo 2011, Van der Ent et al. 2013).

According to their BAC, plants can be classified in non-accumulators (BAC<0.01), low accumulators (0.01-0.1), moderate accumulators (0.1-1) and high accumulators/hyperaccumulators (>1; Sekabira et al., 2011). Thus, *A. altissima* and *S. alba* are at the lower limit between low and moderate accumulation. *R. pseudoacacia* ranks higher (BAC=0.44), but only due to one isolate accumulation event – on soil with extremely low Cr levels.

Such results are to those obtained on some Poaceae of agricultural interest (oat, sorghum, wheat; López-Luna et al. 2009), or various ruderal herbaceous species (Amaria & Brahim 2016, Popoviciu et al. 2017) and do not indicate any valuable phytoextractive potential).

CONCLUSIONS

The three species investigated had tissular chromium levels close to the normal average. In the case of *R. pseudoacacia*, the average value was higher due to a single accumulation event, on soils with very low Cr content, which does not indicate a valuable trait.

BAC values indicated low to moderate accumulation. Thus, none of the three species is a valuable phytoextractor, on soils with normal Cr concentrations and under normal conditions. For eventual phytostabilization properties (root sequestration), further investigations are required.

REFERENCES

- Amaria B., Brahim L., 2016, Cr (III) and Cr (VI) absorption and translocation of *Atriplex halimus* L., International Journal of Biosciences, 9 (6), 234-246.
- Houston Durrant T., de Rigo D., Caudullo G., 2016, *Salix alba* in Europe: distribution, habitat, usage and threats, in San-Miguel-Ayanz J. & al. (eds.), European Atlas of Forest Tree Species, Publications Office of the European Union, Luxembourg, pp. 168.

Kowarik I., Säumel I., 2007, Biological flora of Central Europe: *Ailanthus altissima* (Mill.) Swingle, Perspectives in Plant Ecology, Evolution and Systematics., 8, 207-237.

López-Luna J., González-Chávez M.C., Esparza-García F.J., Rodríguez-Vázquez R., 2009, Toxicity assessment of soil amended with tannery sludge, trivalent chromium and hexavalent chromium, using wheat, oat and sorghum plants, Journal of Hazardous Materials, 163, 829-834.

Nazir A., Malik R.N., Ajaib M., Khan N., Siddiqui M.F., 2011, Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi, Pakistan Journal of Botany, 43 (4), 1925-1933.

Obasi N.A., Akubugwo E.I., Kalu K.M., Ugbogu O.C., 2013, Speciation of heavy metals and phyto-accumulation potentials of selected plants on major dumpsites in Umuahia, Abia State, Nigeria, International Journal of Current Biochemistry Research, 1 (4), 16-18.

Oliveira H., 2012, Chromium as an environmental pollutant: insights on induced plant toxicity, Journal of Botany, 2012, doi: 10.1155/2012/375843.

Popoviciu D.R., Negreanu-Pîrjol T., Bercu R., 2016, Copper, chromium, zinc and manganese accumulation in three common Brassicaceae on the Romanian littoral, Revista de Chimie, 67 (4), 670-673.

Popoviciu D.R., Negreanu-Pîrjol B.Ş., Bercu R., Negreanu-Pîrjol T., 2017, Chromium bioaccumulation in some herbaceous species from Constanța littoral area (Romania), SGEM 2017: 17th International Multidisciplinary Scientific GeoConference, 17 (61), 1183-1189.

Rascio N., Navari-Izzo F., 2011, Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Science, 180, 199-181.

Sekabira K., Oryem-Origa H., Mutumba G., Kakudidi E., Basamba T.A., 2011, Heavy metal phytoremediation by *Commelina benghalensis* (L) and *Cynodon dactylon* (L) growing in urban stream sediments, International Journal of Plant Physiology and Biochemistry, 3(8), 133-142.

Shanker A.K., Cervantes C., Loza-Tavera H., Avudainayagam S., 2005, Chromium toxicity in plants, Environment International, 31, 739-753.

Shanker A.K., Djanaguiraman M., Sudhagar R., Chandrashekar C.N., Pathmanabhan G., 2004, Differential antioxidative response of ascorbate glutathione pathway enzymes and metabolites to chromium speciation stress in green gram (*Vigna radiata* (L.) R. Wilczek. cv CO 4) roots, Plant Science, 166, 1035-1043.

Sitzia T., Cierjacks A., de Rigo D., Caudullo G., 2016, *Robinia pseudoacacia* in Europe: distribution, habitat, usage and threats, in San-Miguel-Ayanz J. & al. (eds.), European Atlas of Forest Tree Species, Publications Office of the European Union, Luxembourg, pp. 166-167.

Tang Y.T., Deng T.H.B., Wu Q.H., Wang S.Z., Qiu R.L., Wei Z.B., Guo X.F., Wu Q.T., Lei M., Chen T.B., Echevarria G., Sterckeman T., Simonnot M.O., Morel J.L., 2012, Designing cropping systems for metal-contaminated sites: a review, Pedosphere, 22 (4), 470-488.

Van der Ent A., Baker A.J.M., Reeves R.D., Pollard A.J., Schat H., 2013, Hyperaccumulators of metal and metalloid trace elements: Facts and fiction, Plant and Soil, 362 (1-2), 319-334.

STATUS OF FOREST HABITATS THE CONSERVATION IDENTIFIED IN SEVERAL PROTECTED AREAS FROM OLTENIA NATURA 2000 SITES (I)

Răduțoiu Daniel¹, Dinulescu Laura Gabriela²

1 - University of Craiova, Faculty of Horticulture, Biology and Environmental Engineering Department A.I. Cuza Street, no 13, RO 200585, Craiova, Romania e-mail: radutoiudaniel@yahoo.com
2 - University of Craiova, Master student, Biodiversity and Conservation of Ecosystems

Keywords: *conservation status, forest habitats, NATURA 2000, Oltenia, Romania.*

ABSTRACT

The present paper renders the results of our research concerning the conservation status of the forest from two NATURA 2000 sites located in Oltenia: ROSCI0202 Oltenia Forest Steppe and ROSCI0039 Ciuperceni - Desa. Within these sites, there are two habitats: 91M0 Pannonian-Balkan turkey oak – sessile oak forests, 91I0 Euro-Siberian steppic woods with Quercus spp.*

The investigation of the conservation status of these habitats was carried out by assessing their conservation status at EU level, namely by including them into one of the four categories - “favourable”, “unfavourable-inadequate”, “unfavourable-bad”, and “unknown” - according to the stated attributes for each habitat, to the threats of destruction and to the dangers that may lead to their destruction or disappearance.

INTRODUCTION

In order to know the conservation status of the habitats within a certain protected area it is required to assess this matter from the viewpoint of the surface occupied by those habitats, of their structure and functions and of their future perspectives.

One of the main issues regarding protected areas consists in their difficult management in the framework of different ownership types.

The measures stipulated by the *Habitats* Directive aim at maintaining or at restoring to a favourable conservation status the natural habitat types, as well as the wild fauna and flora species of community interest (Article 2.2. of the *Habitats* Directive/Council Directive 92/43 EEC).

In order to achieve an objective assessment, the specialised literature data has been harmonised with field information collected by the author and subsequently processed in the laboratory.

Data concerning the study of the habitats within these two areas is scarce in the papers published up to the present (Răduțoiu, 2015), whereas information regarding flora and vegetation is present in numerous studies that deal with the two aspects at the level of certain areas (Buia, 1959; Păun, 1966, 1967, 1985; Cârțu, 1968; Cârțu, 1971; Popescu, 1988; Dihoru and Negrean, 2009).

The two habitat types under analysis offer optimum development conditions to many species of community interest, such as the Scarlet Peony - *Paeonia peregrina*, the Stag Beetle - *Lucanus cervus*, *Carabus hungaricus*, the Greater Capricorn Beetle - *Cerambyx cerdo*, etc.

MATERIAL AND METHODS

The reference values for the favourable status must be assessed according to scientific principles. Their ascertainment for the favourable state is not simple, even if these concepts are not new and they are tackled in a number of scientific papers (Soule and Orians (eds.), 2001; Primack, 2008).

They can differ from those stated in the management plan of the area, because the level of knowledge, understanding and maintenance of conservation measures for habitats may vary from one year to another.

The assessment of the conservation status is more accurate when the knowledge level increases and the volume of collected data is more important. The first such evaluation of the conservation status of a habitat type must be regarded as the starting point in the gradual process of scientific understanding improvement and of increasing the quantity of available data (for example, as a result of the monitoring in accordance with the provisions of Article 11, the *Habitats Directive*).

The conservation status of a natural habitat is considered "favourable" if the natural area of the habitat, as well as the extent of the surfaces covered by the habitat are stable or increasing and if the perspectives show a future progress without significant changes in the existing policy and management.

The unfavourable conservation status falls into two classes: "unfavourable-inadequate" - for those situations when a change at the level of policies or of the management is necessary in order to restore the habitat type or the species to a favourable conservation status, but there is no danger of extinction in the predictable future (e.g.: 50 – 100 years); "unfavourable-bad" - for those situations when the habitat type or the species are threatened with extinction in the foreseeable future (e.g.: 50 – 100 years).

The parameters used to evaluate the conservation status of a habitat are the natural distribution range, the area covered by the habitat, the specific structure and functionality of the habitat, and the future prospects that are associated with it (Combroux and Schwoerer, 2007).

The codes and the description of the habitat types correspond to NATURA 2000 and the Romanian classification system (Doniță et al., 2005; Doniță et al., 2006, Gafta and Mountford, 2008).

A colour-coding system was adopted for the graphic representation of the four conservation states (following the guidance document issued by the European Commission: *Assessment and reporting under Article 17 of the Habitats Directive: Reporting formats for 2007-2012 period*): red for "unfavourable-bad" (abbreviation U2); orange for "unfavourable-inadequate" (U1); green for "favourable" (FV); grey for "unknown" (XX).

Specialised field guides were used in order to identify all the species within the analysed habitats (Beldie, 1977, 1979; Ciocârlan, 2009; Sârbu et al., 2013).

The vegetation of these habitats was classified according to the specialized Romanian literature (Sanda et al. 1980, Sanda et al. 2001, Sanda, 2002).

The *Oltenia Forest Steppe* site (Ro: *Silvostepa Olteniei*) is located in the western part of Oltenia Plain, i.e. within the Desnățui Plain, at the contact with the Getic Tableland.

The site consists of six forest plots that cover 9,297 ha (ROSCI0202). 91E0* extends on a surface of 108.4 ha, i.e. 1.2 % of the total site area, 91M0 – 6,487 ha, i.e. 70.7 % of the site, and 91I0* - 975.4 ha, i.e. 10.5 % of the entire site. The territorial boundaries of the six areas that compose the site are marked by the irregular outline of the forest plots, which separates them from the surrounding agricultural land.

The site overlaps the *Poiana Bujorului* Reserve within Plenița Forest.

The *Ciuperceeni-Desa* site is located in the Danube Alluvial Plain and it covers a surface of 40,853 ha (ROSCI0039). 9110* extends over 204.26 ha (i.e. 0.5 % of the area), 91F0 – 40.85 ha (0.1 % of the site), and 92A0 – 1,225.59 ha (3 % of the total area).

RESULTS AND DISCUSSIONS

Following the objective assessment of the field reality during multiple years (2012-2018), it can be stated that we achieved a correct description of the two analysed habitats within the two areas, as well as an adequate framing of the vegetation type characteristic to each habitat and an evaluation of their conservation status that is as accurate as possible.

91M0 Pannonian-Balkan turkey oak – sessile oak forests

This habitat has the most important range in the Oltenia Forest Steppe site.

Information concerning the phytocoenoses of these forests is mentioned by Păun (1966), Mariana Cârțu (1971) for the Amaradia basin, Cârțu D. (1968) for the area between the Jiu - the Desnățui - Craiova, Popescu Gh. (1988). They vegetate on soils with seasonal oscillations of humidity (excessively moist during spring and dry during summer).

The association composition highlights the constancy of some species for the recognition of the order *Quercetalia cerris*. These include *Potentilla micrantha*, *Lychnis coronaria*, *Vincetoxicum hirundinaria*, *Lithospermum purpuro-caeruleum*, etc.

The tree layer, which is 10 to 15 meters high, consists of the two recognition species of this vegetation, but with different proportions, Turkey oak or Hungarian oak being dominant. Besides them, *Quercus polycarpa* has a sporadic presence.

The shrub layer, well developed in some phytocoenoses consists of numerous species: *Acer campestre*, *A. tataricum*, *Crataegus monogyna*, *Rosa canina*, *Ligustrum vulgare*, etc.

Certain rare species within the spontaneous flora of Romania (*Acanthus balcanicus* and *Paeonia peregrina*) were identified in the floristic composition of some surfaces covered by this habitat.

Turkey oak represents a valuable wood, with rapid growth, being excellent as firewood. This importance becomes even more obvious if we consider that Turkey oak and Hungarian oak are marginally demanding species to the environmental conditions of the plain, being able to grow in conditions that are unsuitable to other tree species with narrower ecologic amplitude (Răduțoiu, 2008).

This habitat was identified within the settlements of Radovan, Perișor, Mârza, Vârvoru de Jos, Criva, Castele Traiane, Plenița, Verbița, Verbicioara, Seaca de Pădure, Geblești, between Carpen and Bucovicior, as well as between Bucovicior and Vela.

The shrubs edified by *Prunus spinosa* with *Crataegus monogyna* are present on the edge of the 91M0 habitat.

Following the numerous field trips conducted in the study-area, on the surfaces covered by this habitat, we can state that the conservation status of the 91M0 habitat - *Pannonian-Balkan turkey oak - sessile oak forests* within ROSCI0202 Oltenia Forest Steppe protected area is favourable (Figure 1, Table 1).

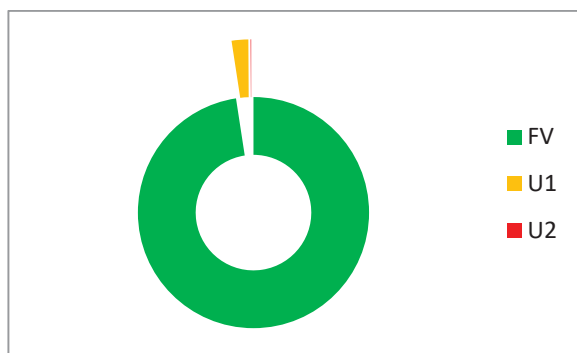


Fig. 1. Conservation status class structure for the 91M0 habitat

9110* Euro-Siberian steppic woods with *Quercus* spp.

The typical vegetation of this habitat within the two studied areas belongs to *Aceri tatarici – Quercetum roboris* Zólyomi 1957 (Syn.: *Aceri tatarico-Quercetum pubescentis* Bârcă 1973).

In the Ciupereni-Desa site, the tree layer displays a good consistency (with 65-80 % coverage) and an obvious stratification. The upper layer, which is 12-14 meters high, is reached by *Quercus robur* and *Carpinus betulus*, whereas the secondary height level belongs to *Acer tataricum*, *Pyrus pyraeaster* and *Tilia tomentosa*. The shrub layer consists of a high number of species, but the coverage they realise shows low percent values. The regeneration stratum is discontinuous and its presence is not significant, the development of the sapling being affected by the grass layer. The regeneration through root and stump offshoots is more active. The herbaceous layer is dominated by a typically mesophilic flora that includes *Arum orientale*, *Corydalis bulbosa* ssp. *marschalliana*, *Viola canina*.

Table 1.

Assessment of the conservation status for the 91M0 habitat

Parameters	Indicators	Observations
Natural distribution range		The area occupied by this habitat in the site is located in the plain region of Oltenia and in the continental one.
Covered surface		The surface of 6,693.84 ha covered by this habitat is largely identical to that occupied at the moment of designation; major disturbances have not been registered.
Structure and functionality		The unfavourable-inadequate status corresponding to ca. 156 ha is due to the introduction of allochthon species: <i>Robinia psudacacia</i> , <i>Gleditsia triacanthos</i> , <i>Pinus nigra</i> .
		The unfavourable-bad status corresponding to ca. 6 ha was assigned to those surfaces on which the part of allochthon species exceeds 70 %.
Future perspectives		Future perspectives are good, as long as the appropriate forestry works are complied with.
CONSERVATION STATUS		

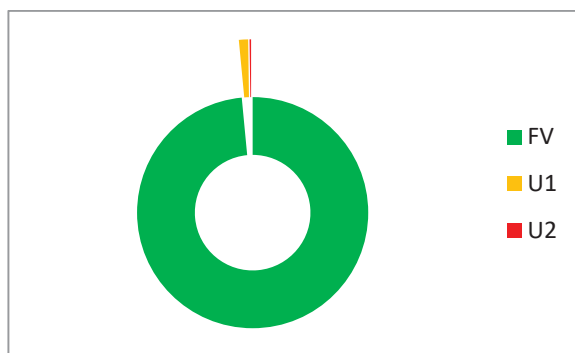


Fig. 2. Conservation status class structure for the 9110* habitat

Table 2.

Assessment of the conservation status for the 9110* habitat

Parameters	Indicators	Observations
Natural distribution range	FV	The surface occupied by this habitat in the two areas is located in the plain region of Oltenia and in the continental one.
Covered surface	FV	The surface is largely identical to that occupied at the moment of the designation of the sites; major disturbances have not been registered.
Structure and functionality	U1	The regeneration stratum is discontinuous and its presence is not significant, the development of the sapling being affected by the grass layer.
	U2	The overgrazing conducted on certain areas within the Radovan and Perișor forests.
Future perspectives	FV	Only biotic and abiotic threats exist, but their intensity could be diminished.
CONSERVATION STATUS	FV	

CONCLUSIONS

The analysis of the conservation status corresponding to the forest habitats within the two sites located in Oltenia shows that the 91M0 habitat has very good conservation status on more than 95 % of its surface and the same status corresponds to ca. 80 % of the area of the 9110* habitat.

The "unfavourable-bad" conservation status displays very low percent values in almost all habitats, with the exception of the 91M0 habitat. Among the main causes that led to the degradation of those surfaces, there are to be mentioned the illegal clearings and the grazing inside the forests. In the absence of urgent measures, the surfaces occupied by these habitats within the two areas might significantly diminish in the near future.

REFERENCES

- Beldie Al. 1977. Flora României. Determinator ilustrat al plantelor vasculare. Vol. I. Edit. Acad. Române, București, 406 pp.
- Beldie Al. 1979. Flora României. Determinator ilustrat al plantelor vasculare. Vol. II. Edit. Acad. Române, București, 412 pp.
- Buia Al. 1959. Plante rare pentru flora R.P.R. existente în Oltenia. Bul. Com. Ocrot. Monum. Nat.: 13-42. Edit. Acad. Române, București.
- Cârțu D. 1968. Contribuții la flora Olteniei. Bul. Ști. Univ. din Craiova, 10: 63-70.
- Cârțu Mariana. 1971. Aspecte privind vegetația pajiștilor de luncă din Bazinul Amaradiiei. Studii și cercetări. C.C.S.E. Craiova. Intreprinderea Poligrafică Oltenia, Craiova: 93-98.
- Ciocârlan V. 2009. Flora ilustrată a României. Pteridophyta et Spermatophyta. 1041 pp. Edit. Ceres, București.
- Combroux, I., Schwoerer, Ch. 2007. Evaluarea statutului de conservare a habitatelor și speciilor de interes comunitar din România-Ghid metodologic, 51 pp. și anexele. Edit. Balcanic, Timișoara.
- Dihoru Gh. & Negrean G. 2009. Cartea roșie a plantelor vasculare din România. 630 pp. Edit. Acad. Române. București.
- Doniță, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S., Biriș I.-A. 2005. Habitate din România, Edit. Tehnică Silvică București.
- Doniță, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S., Biriș I.A. 2006. Habitatele din România. Modificări conform amendamentelor propuse de România și Bulgaria la Directiva Habitate (92/43/EEC), Edit. Tehnică Silvică, București.
- Gafta, D., Mountford, O. (coord.), 2008. Manual de interpretare a habitatelor Natura 2000 din România, Edit. Risoprint, Cluj-Napoca.
- Păun 1966. Materiale pentru flora și vegetația Raionului Balș. Lucr. Șt. Inst. Agron. T. Vladimirescu” Craiova. Edit. Agro-Silvică, București. 6: 35-67.
- Păun M. 1967. Vegetația Raionului Balș. Regiunea Oltenia. Com. Bot. București. Societatea de Științe Naturale și Geografie din R.P.R. București. 6: 121-127.
- Păun M. 1985. Quercu-Fagetă Br.-Bl. Vlieg. 37 în Oltenia. Contrib. Bot.: 121-130. Cluj-Napoca.
- Popescu Gh. 1988. Phytocoenological consideration on the Quercus cerris L. and Q. frainetto Ten. forest of Oltenia. Rev. Roumanie Biol. Ser. Biol. Veg. 33: 75-91.
- Primack R. 2008. A Primer of Conservation Biology. Fourth Edition. Sinauer Associates, Sunderland, MA. 349 pp.
- Răduțoiu D. 2008. Flora și vegetația Bazinului Cernei de Olteț. 407 pp. Edit. Sitech. Craiova.
- Răduțoiu D. 2015. The conservation status of grassland habitats belonging to protected areas from Oltenia 'Natura 2000' site, Romania. Not. Sci. Biol. 7 (4): 430-434.
- Sanda V. & al. 1980. Cenotaxonomia și corologia grupărilor vegetale din România. Stud. Com. (24) Supliment Științele Naturii Muzeul Brukenthal: 171 pp. Sibiu.
- Sanda V., Popescu A. & Stancu Daniela Ileana 2001. Structura cenotică și caracterizarea ecologică a fitocenozelor din România. 359 pp. Edit. CONPHIS, București.
- Sanda V. 2002. Vademecum cenostructural privind covorul vegetal din România. 331 pp. Edit. Vergiliu. București.
- Sârbu I., Ștefan N. & Oprea A. 2013. Plante vasculare din România. Determinator ilustrat de teren. Edit. VictorBVictor, București, 1320 pp.
- Soulé M. E., Orians G. H., 2001 - Conservation biology research: its challenges and contexts. In: M. E. Soulé & G. H. Orians (eds), Conservation biology. Society for Conservation Biology, Island Press, Washington, pp. 271-285.

*** Habitats Directive 92/43/EEC – Concl Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (OJ L 206, 22.7.92).

*** ROSC10202 - Formularul standard Natura 2000 "Silvostepa Olteniei".

*** ROSC10039 Formularul standard Natura 2000 "Ciuperceni-Desa"

STATUS OF FOREST HABITATS THE CONSERVATION IDENTIFIED I N SEVERAL PROTECTED AREAS FROM OLTENIA NATURA 2000 SITES (II)

Răduțoiu Daniel¹, Dinulescu Laura Gabriela²

1 - University of Craiova, Faculty of Horticulture, Biology and Environmental Engineering Department A.I. Cuza Street, no 13, RO 200585, Craiova, Romania e-mail: radutoiudaniel@yahoo.com
2 - University of Craiova, Master student, Biodiversity and Conservation of Ecosystems

Keywords: *conservation status, forest habitats, NATURA 2000, Oltenia, Romania.*

ABSTRACT

The present paper renders the results of our research concerning the conservation status of the forest from two NATURA 2000 sites located in Oltenia: ROSCI0202 Oltenia Forest Steppe and ROSCI0039 Ciuperceni - Desa. Within these sites, there are three habitats: 91E0 Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae), 91F0 Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia*, along the great rivers (*Ulmion minoris*) and 92A0 *Salix alba* and *Populus alba* galleries.*

The investigation of the conservation status of these habitats was carried out by assessing their conservation status at EU level, namely by including them into one of the four categories - "favourable", "unfavourable-inadequate", "unfavourable-bad", and "unknown" - according to the stated attributes for each habitat, to the threats of destruction and to the dangers that may lead to their destruction or disappearance.

INTRODUCTION

In order to know the conservation status of the habitats within a certain protected area it is required to assess this matter from the viewpoint of the surface occupied by those habitats, of their structure and functions and of their future perspectives.

One of the main issues regarding protected areas consists in their difficult management in the framework of different ownership types.

The measures stipulated by the *Habitats* Directive aim at maintaining or at restoring to a favourable conservation status the natural habitat types, as well as the wild fauna and flora species of community interest (Article 2.2. of the *Habitats* Directive/Council Directive 92/43 EEC).

In order to achieve an objective assessment, the specialised literature data has been harmonised with field information collected by the author and subsequently processed in the laboratory.

Data concerning the study of the habitats within these two areas is scarce in the papers published up to the present (Răduțoiu, 2015), whereas information regarding flora and vegetation is present in numerous studies that deal with the two aspects at the level of certain areas (Buia, 1959; Păun, 1967; Dihoru and Negrean, 2009).

MATERIAL AND METHODS

The reference values for the favourable status must be assessed according to scientific principles. Their ascertainment for the favourable state is not simple, even if

these concepts are not new and they are tackled in a number of scientific papers (Soule and Orians (eds.), 2001; Primack, 2008).

They can differ from those stated in the management plan of the area, because the level of knowledge, understanding and maintenance of conservation measures for habitats may vary from one year to another.

The assessment of the conservation status is more accurate when the knowledge level increases and the volume of collected data is more important. The first such evaluation of the conservation status of a habitat type must be regarded as the starting point in the gradual process of scientific understanding improvement and of increasing the quantity of available data (for example, as a result of the monitoring in accordance with the provisions of Article 11, the *Habitats Directive*).

The conservation status of a natural habitat is considered "favourable" if the natural area of the habitat, as well as the extent of the surfaces covered by the habitat are stable or increasing and if the perspectives show a future progress without significant changes in the existing policy and management.

The unfavourable conservation status falls into two classes: "unfavourable-inadequate" - for those situations when a change at the level of policies or of the management is necessary in order to restore the habitat type or the species to a favourable conservation status, but there is no danger of extinction in the predictable future (e.g.: 50 – 100 years); "unfavourable-bad" - for those situations when the habitat type or the species are threatened with extinction in the foreseeable future (e.g.: 50 – 100 years).

The parameters used to evaluate the conservation status of a habitat are the natural distribution range, the area covered by the habitat, the specific structure and functionality of the habitat, and the future prospects that are associated with it (Combroux and Schwoerer, 2007).

The codes and the description of the habitat types correspond to NATURA 2000 and the Romanian classification system (Doniță et al., 2005; Doniță et al., 2006, Gafta and Mountford, 2008).

A colour-coding system was adopted for the graphic representation of the four conservation states (following the guidance document issued by the European Commission: *Assessment and reporting under Article 17 of the Habitats Directive: Reporting formats for 2007-2012 period*): red for "unfavourable-bad" (abbreviation U2); orange for "unfavourable-inadequate" (U1); green for "favourable" (FV); grey for "unknown" (XX).

Specialised field guides were used in order to identify all the species within the analysed habitats (Beldie, 1977, 1979; Ciocârlan, 2009; Sârbu et al., 2013).

The vegetation of these habitats was classified according to the specialized Romanian literature (Sanda et al. 1980, Sanda et al. 2001, Sanda, 2002).

The *Oltenia Forest Steppe* site (Ro: *Silvostepa Olteniei*) is located in the western part of Oltenia Plain, i.e. within the Desnățui Plain, at the contact with the Getic Tableland.

The site consists of six forest plots that cover 9,297 ha (ROSCI0202). 91E0* extends on a surface of 108.4 ha, i.e. 1.2 % of the total site area, 91M0 – 6,487 ha, i.e. 70.7 % of the site, and 91I0* - 975.4 ha, i.e. 10.5 % of the entire site. The territorial boundaries of the six areas that compose the site are marked by the irregular outline of the forest plots, which separates them from the surrounding agricultural land.

The *Ciuperceni-Desa* site is located in the Danube Alluvial Plain and it covers a surface of 40,853 ha (ROSCI0039). 91I0* extends over 204.26 ha (i.e. 0.5 % of the area), 91F0 – 40.85 ha (0.1 % of the site), and 92A0 – 1,225.59 ha (3 % of the total area).

RESULTS AND DISCUSSIONS

Following the objective assessment of the field reality during multiple years (2012-2018), it can be stated that we achieved a correct description of the three analysed habitats within the two areas, as well as an adequate framing of the vegetation type characteristic to each habitat and an evaluation of their conservation status that is as accurate as possible.

91F0 Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia*, along the great rivers (*Ulmion minoris*)

The vegetation of this habitat is well known in Romania and in Oltenia (Popescu et al. 2001). Nevertheless, its phytocoenoses classification is rather difficult. The main association encountered in these areas is *Querco robori-Fraxinetum* Zölyomi 1931.

The research conducted upon this type of vegetation led to the conclusion that the phytocoenoses analysed by us display common and, at the same time, intermediary elements with the descriptions realised by certain botanists (Roman, 1974; Păun, 1985, Popescu et al. 2001).

Consequently, we include the phytocoenoses in the association *Querco robori-Fraxinetum* Zölyomi 1931 (Popescu et al. 2001), considering that the definition corresponds to the presence of the species *Fraxinus angustifolia* subsp. *oxycarpa*, *F. excelsior*, installed on the place of the former forests of *Quercus robur*, because of the moisture excess (Pop, 1968; Roman, 1974; Păun, 1985).

At national level, these forests are tackled differently (Doltu et al., 1980), depending on the vegetation layer or the area where they have been located.

The association does not cover an important area (40.85 ha). Within these forests, the water table is shallow, the soil being saturated with water almost the entire year.

The relief type is characteristic for the high alluvial plain and the forest type is represented by the mixed foliage riparian forest. The soils are of alluvial type, with mollisols and gleysols.

On the surfaces that are always waterlogged, the ash tree species (*Fraxinus angustifolia* and *Fraxinus excelsior*) are accompanied by *Alnus glutinosa*. *Quercus robur* is absent from these studied areas. As the altitude of the alluvial plain increases and the ground is drier, the European Oak displays abundancy-dominance indices of 1-2.

The *Ulmus* species are present in all surveyed areas, their corresponding abundancy-dominance indices being low.

During the drought periods, when the alluvial plain is not flooded and the ground is not saturated with water, numerous species that are typical of the *Alno-Ulmion* alliance and of the *Salicetalia purpureae* order appear within the phytocoenoses of the association; this demonstrates the common ecology and the sindynamic connections between the associations that are subordinated to them.

The tree layer is dominated by *Fraxinus angustifolia*, *Fraxinus excelsior*, accompanied by *Quercus robur*, *Ulmus glabra*, *Ulmus minor*, *Populus alba*, *Populus canescens*.

The shrub layer is composed of the following species: *Crataegus monogyna*, *Acer campestre*, *Cornus mas*, *C. sanguinea*, *Viburnum lantana*, *Ligustrum vulgare*, etc.

Within the herbaceous layer, *Ranunculus ficaria* ssp. *bulbilifer*, *Scilla bifolia* ssp. *drunensis*, *Viola odorata*, *Anemone nemorosa*, *A. ranunculoides*, *Isopyrum thalictroides*, *Veronica chamaedrys*, *Polygonatum latifolium*, *Ornithogalum pyrenaicum*, and *Carex divulsa* are abundant during the vernal season; subsequently, during the summer season, the layer gradually changes and it consists of *Brachypodium sylvaticum*, *Dactylis glomerata*, *Geum urbanum*, *Galium aparine*, *Agrimonia eupatoria*, *Cruciata laevipes*, *Scutellaria hastifolia*, etc.

Analysing the humidity demands of the species located in the surveyed areas, it can be noticed the very good ratio of the mesophytes and of the mesohygrophytes. This fact explains once again the variations of the hydrological regime typical of these surfaces. The hygrophilous species prevail during the flood (in areas where *Quercus robur* is nearly absent or it has poor representation), whereas the mesophilic ones are dominant on higher surfaces, where humidity is low.

Within the Ciupereni – Desa protected area, the conservation status of this habitat is good to moderate (Fig. 1, Table 1).

92A0 *Salix alba* and *Populus alba* galleries

This habitat comprises vegetation located on alluvial soils, with the water table near surface. Obvious changes take place in the floristic composition of the phytocoenoses because of the annual flood.

The vegetation of this habitat belongs to the *Salici – Populetum* association (Tx. 1931) Meyer-Drees 1936.

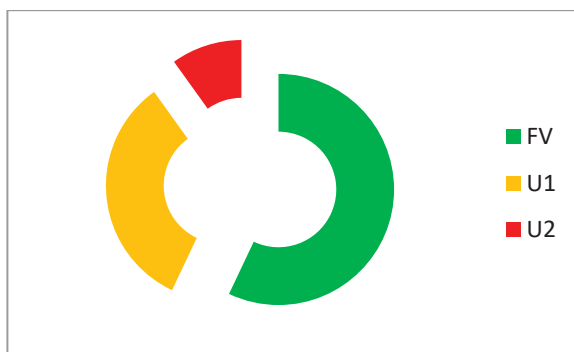


Fig. 1. Conservation status class structure for the 91F0 habitat

Table 1.

Assessment of the conservation status for the 91F0 habitat

Parameters	Indicators	Observations
Natural distribution range		The area occupied by this habitat in the sites is located in the plain region of Oltenia and in the continental one.
Covered surface		The surface in the site is identical. The changes registered in certain areas can be corrected if the interventions are prompt.
Structure and functionality		More than 10 % of the surface of this habitat falls into unfavourable-bad category, because of illegal clearings and of grazing.
Future perspectives		There are human-induced threats that can be diminished if the forestry standards are complied with.
CONSERVATION STATUS		

The willow and poplar forests are frequent on alluvial soils, along watercourses, near water bodies or where water temporarily stagnates.

We shall not conduct a detailed characterization of this association, as it is very well known; only its particular features within the researched area will be mentioned.

The coverage degree within the phytocoenologic surveys that we conducted varies between 85 and 100 %. An obvious stratification is to be noticed within these phytocoenoses: the first layer consists of a large part of species characteristic to the alliance, the order and the class *Salix alba*, *Populus alba*, as well as of species for their recognition; the second layer comprises *Rubus caesius*, *Amorpha fruticosa*, *Vitis sylvestris*, *Crataegus monogyna*, *Ligustrum vulgare*; the third layer consists of herbaceous species. The latter layer is strongly influenced by the size and the frequency of recent floods.

Because of the complex combinations realised by the wooden species within the floristic composition of this association, the cenotaxonomic description and classification of these phytocoenoses differs from one author to another.

The conservation status of this habitat within the Ciuperzeni-Desa protected area, where it was identified and analysed, is good to moderate.

This type of habitat, which represented the natural vegetation of the Romanian steppe and forest steppe in the past, has favourable conservation status within the two areas (Figure 2, Table 2).

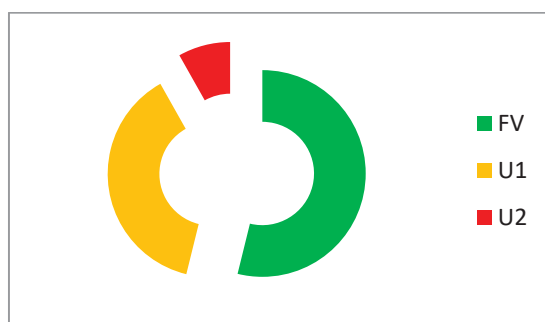


Fig. 2. Conservation status class structure for the 92A0 habitat

Table 2.

Assessment of the conservation status for the 92A0 habitat

Parameters	Indicators	Observations
Natural distribution range		The area occupied by this habitat in the sites is located in the plain region of Oltenia and in the continental one
Covered surface		The surface is largely identical to that occupied by the habitat at the moment of site designation; major disturbances have been registered in certain places.
Structure and functionality		More than 8 % of the surface of this habitat falls into unfavourable-bad category, because of illegal clearings and of grazing.
Future perspectives		There are human-induced threats that must be stopped in order to preserve the surface occupied by this habitat.
CONSERVATION STATUS		

91E0* Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (*Alno-Padion*, *Alnion incanae*, *Salicion albae*)

The woodlands with willows grouped in the *Salicion albae* alliance are located along the rivers and their main tributaries. The species vegetate on hygro-mesophilous, alluvial soils or on those rich in organic substances. Among the characteristic species

there are to be mentioned *Salix alba*, *Calystegia sepium*, *Humulus lupulus*, *Populus alba*, *Symphytum officinale*, etc.

In the Oltenia Forest Steppe protected area, the habitat includes alluvial forests edified by the European ash (*Fraxinus excelsior*) and arborescent galleries including tall willow and poplar trees (*Salix alba*, *Salix fragilis*, *Populus nigra*), situated along water courses, at low altitudes (e.g. the settlements of Radovan and Ciutura, near Fântânele reservoir – in the area locally known as the *Tail of the Lake*, along the Baboiași stream, at the edge of Verbița village and in Carpen settlement).

Certain invasive species (*Ambrosia artemisiifolia*, *Cannabis sativa* ssp. *Spontanea*) can disturb the habitat.

Moreover, periodic cleaning measures are required in order to remove the waste accumulated through illegal deliberate disposal near the water or following high water periods when waste material is carried by the overflowing water and left on riverbanks.

The vegetation of this habitat belongs to the association *Salicetum albae* Issler 1924 (Syn. *Salicetum albae-fragilis* Issler 1926 em. Soo 1957)

The phytocoenoses encountered in the study area are present under the shape of longitudinal stripes along both banks of the main rivers. Sometimes, forests edified by European ash (*Fraxinus angustifolia*) are to be found near them (e.g. Radovan – along the Desnățui stream). On rare occasions, the phytocoenoses of this association form dense riverside coppices, as in the case of Ciuperceni – Desa site.

The upper layers, edified by wooden species, maintain their consistency regardless the climatic conditions during the year, whereas the grass layer undertakes a strong negative influence from the size and the frequency of the floods.

Besides the species that compose the cenotic nucleus of this association, there were also identified other species from neighbouring phytocoenoses: *Urtica dioica*, *Rubus caesius*, *Myosoton aquaticum*, *Lythrum salicaria*, etc.

The 91E0* habitat is characterised by moderate-poor status (Fig. 3, Table 3).

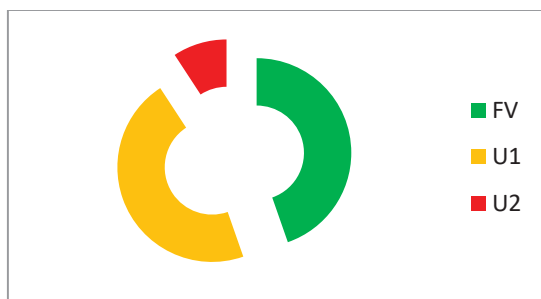


Fig. 3. Conservation status class structure for the 91E0* habitat

Table 3.

Assessment of the conservation status for the 91E0* habitat

Parameters	Indicators	Observations
Natural distribution range		The area covered by this habitat in the sites is located in the plain region of Oltenia and in the continental one.
Covered surface		The surface is largely identical to that occupied by the habitat at the moment of site designation; major disturbances have been registered in certain places.
Structure and functionality		More than 10 % of the surface of this habitat falls into unfavourable-bad category, because of illegal clearings and of grazing.
Future perspectives		There are human-induced threats that can be diminished if the forestry standards are complied with.
CONSERVATION STATUS		

In "Oltenia Forest Steppe", this habitat is located only at the level of Radovan, Perișor and Geblești settlements. In Radovan, the shrub layer, which covers limited surfaces, is dominated by Downy Oak (*Quercus pubescens*) and it is represented by small trees on the grasslands edified by *Festuca valesiaca*.

Their physiognomy is given by the European Oak (*Quercus robur*). Secular trees belonging to the species have been identified in some locations (e.g. Radovan and Perișor).

CONCLUSIONS

The analysis of the conservation status corresponding to the forest habitats within the two sites located in Oltenia shows that the 92A0 and the 91F0 habitats are characterised by good to moderate conservation status, whereas the "unfavourable-bad" conservation status corresponds to about 10 % of the 91E0* habitat.

The "unfavourable-bad" conservation status displays very low percent values in almost all habitats. Among the main causes that led to the degradation of those surfaces, there are to be mentioned the illegal clearings and the grazing inside the forests.

REFERENCES

- Beldie Al. 1977. Flora României. Determinator ilustrat al plantelor vasculare. Vol. I. Edit. Acad. Române, București, 406 pp.
- Beldie Al. 1979. Flora României. Determinator ilustrat al plantelor vasculare. Vol. II. Edit. Acad. Române, București, 412 pp.
- Buia Al. 1959. Plante rare pentru flora R.P.R. existente în Oltenia. Bul. Com. Ocrot. Monum. Nat.: 13-42. Edit. Acad. Române, București.
- Ciocârlan V. 2009. Flora ilustrată a României. Pteridophyta et Spermatophyta. 1041 pp. Edit. Ceres, București.
- Combroux, I., Schwoerer, Ch. 2007. Evaluarea statutului de conservare a habitatelor și speciilor de interes comunitar din România-Ghid metodologic, 51 pp. și anexele. Edit. Balcanic, Timișoara.

- Dihoru Gh. & Negrean G. 2009. Cartea roșie a plantelor vasculare din România. 630 pp. Edit. Acad. Române. București.
- Doltu M.I. Popescu A., Sanda V. 1980. Conspectul asociațiilor vegetale lemnoase din România. Stud. și Comunic. Ști. Nat. Muz. Brukenthal, Sibiu, 24: 315-362
- Doniță, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S., Biriș I.-A. 2005. Habitate din România, Edit. Tehnică Silvică București.
- Doniță, N., Popescu, A., Paucă-Comănescu, M., Mihăilescu, S., Biriș I.A. 2006. Habitatele din România. Modificări conform amendamentelor propuse de România și Bulgaria la Directiva Habitate (92/43/EEC), Edit. Tehnică Silvică, București.
- Gafta, D., Mountford, O. (coord.), 2008. Manual de interpretare a habitatelor Natura 2000 din România, Edit. Risoprint, Cluj-Napoca.
- Păun M. 1967. Vegetația Raionului Balș. Regiunea Oltenia. Com. Bot. București. Societatea de Științe Naturale și Geografie din R.P.R. București. 6: 121-127.
- Păun M. 1985. Quercu-Fagetea Br.-Bl. Vlieg. 37 în Oltenia. Contrib. Bot.: 121-130. Cluj-Napoca.
- Pop I. 1968. Flora și vegetația Câmpiei Crișurilor. Interfluviul Crișul Negru-Crișul Repede. 280 pp. Edit. Acad. Române. București.
- Popescu G., Răduțoiu D., Gămănesci Gh. 2001. The flora and the vegetation of the Jilț Basin, between the localities Negomir and Turceni, Gorj County. Acta Horti Bot. Bucurest. 29: 171-197.
- Primack R. 2008. A Primer of Conservation Biology. Fourth Edition. Sinauer Associates, Sunderland, MA. 349 pp.
- Răduțoiu D. 2015. The conservation status of grassland habitats belonging to protected areas from Oltenia 'Natura 2000' site, Romania. Not. Sci. Biol. 7 (4): 430-434.
- Roman N. 1974. Flora și vegetația din sudul podișului Mehedinți. 222 pp. Edit. Acad. Române, București.
- Sanda V. & al. 1980. Cenotaxonomia și corologia grupărilor vegetale din România. Stud. Com. (24) Supliment Științele Naturii Muzeul Brukenthal: 171 pp. Sibiu.
- Sanda V., Popescu A. & Stancu Daniela Ileana 2001. Structura cenotică și caracterizarea ecologică a fitocenozelor din România. 359 pp. Edit. CONPHIS, București.
- Sanda V. 2002. Vademecum cenostructural privind covorul vegetal din România. 331 pp. Edit. Vergiliu. București.
- Sârbu I., Ștefan N. & Oprea A. 2013. Plante vasculare din România. Determinator ilustrat de teren. Edit. VictorBVictor, București, 1320 pp.
- Soulé M. E., Oriens G. H., 2001 - Conservation biology research: its challenges and contexts. In: M. E. Soulé & G. H. Oriens (eds), Conservation biology. Society for Conservation Biology, Island Press, Washington, pp. 271-285.
- *** Habitats Directive 92/43/EEC – Concil Directive 92/43/EEC on the conservation of natural habitats and of wild fauna and flora (OJ L 206, 22.7.92).
- *** ROSC10202 - Formularul standard Natura 2000 "Silvostepa Olteniei".
- *** ROSC10039 Formularul standard Natura 2000 "Ciuperceni-Desa"

**HOMEOPATHIC EFFECTS OF SODIUM CHLORIDE HIGH DILUTIONS
ON WHEAT SEED GERMINATION AND SEEDLING GROWTH**

Rîndașu Ileana ¹, Delian Elena ^{1*}, Stănică Florin ¹

¹University of Agronomical Sciences and Veterinary Medicine of Bucharest, 59 Marăști Blvd, District 1, Bucharest, Romania

*Corresponding author email: elenadelian@yahoo.com

Keywords: Germination indices; Seedlings Vigor; *Triticum aestivum*; Homeopathic remedies

ABSTRACT

Germination and early seedlings growth are decisive stages for a good establishment of the plant and implicitly for obtaining a suitable crop, given that especially in these phases different stressors can have a major negative impact. With a view to seeds invigoration, a laboratory experiment has been done by using wheat (*Triticum aestivum* L.), cv. Glosa and some homeopathic remedies were used for seeds priming, during 24 hrs: V1. NaCl (*Natrum muriaticum*) – D6; V2. NaCl (*Natrum muriaticum*) - CH30; V3. NaCl (*Natrum muriaticum*) - CH200; V4- hydro-priming; V5- non-priming. The obtained results emphasized that the homeopathic remedies had a significant influence on some germination indicators, as mean germination time and mean daily germination ($P < 0.05$). Also, seedlings growth was positively affected by the homeopathic priming treatments. According to our results, the homeopathic remedies may be recommended for further studies as promising alternatives for seeds invigoration.

INTRODUCTION

Homeopathy, a form of complementary medicine, as one of the most controversial subjects in therapeutics was developed by the German physician Samuel Hahnemann (1755–1843) (Swayne, 2000), based on the principle of similitude (like cures like). This method refers to preparations of substances which administrated to healthy subjects determine the manifestation of symptoms, clinical signs and pathological states (see review Ernst, 2002) and when administrated to sick individuals determine the cure of symptoms. Even if many claims that principles on which homeopathy are based are “scientifically implausible” (Lancet, 2005) according to conventional science, homeopathic medicine remain popular (Relton et al., 2017) and there is emerging evidence for its effects *in vitro* and *in vivo* activity (Betti et al., 2010).

Due to population and income growth, as well as rapid urbanization, the global food demand in 2050 is projected to grow by at least 60 percent above 2006 levels (FAO, 2016). Thus, at a globally scale, the way that this requirement can be met is to increase the crop yields (Thirumdas, 2018), to achieve food security under limited arable areas, also to improve the sustainability of agriculture, while reducing its environmental impact (Edmondson et al., 2014). Wheat, as one of the most important crop plants worldwide, still remains one of the primary sources of food for human consumption (FAO, 2016). In the same time it is a suitable model plant for physiological studies and not only.

Taking into consideration that in commercial agriculture, rapid and uniform seed germination and seedling emergence are important determinants of successful stand

establishment (Rajjou et al., 2012), germination indices can be appreciated as an effective screening tool in the early stages of plant establishment and also based on their values the physiological traits of a plant at later stages may be surmised (Aflaki et al. 2017). Previous studies have been focused on seed priming as a tool to seeds invigoration by using different practices (Paparella et al., 2015) or physical methods (Araújo et al., 2016), including those related to plasma (Dobrin et al., 2015, cited by Thirumdas, 2018).

The science of Homeopathy has great potentials and could give a new direction that requires attention of the researchers in alternative agriculture (Singhania et al., 2014). To respond to some basic study questions concerning the specificity of homeopathic preparations, healthy plants seem to be a useful tool. Regarding the homeopathic potencies on germination and seedlings growth, wheat is on the first position as an experimental plant model (Betti et al., 2010; Brizzi et al., 2011). There were evaluated the effect of Silver nitrate on seedlings growth; influence of *Arsenicum album* potencies on seeds germination rate and speed of germination; the specific effect of copper sulphate, ferrous sulphate and lead nitrate were also tested with the wheat germination model. The homeopathic potencies effects have been noticed almost in the case of all researches, even if there were used high dilutions, far beyond the Avogadro number (see review Majewsky et al., 2009). Also the positively influence of potentised homeopathic medicine, *Arsenicum album* and *Baryta carbonica* on germination, growth and photosynthetic activity of pea seedlings (*Pisum sativum* L.) was noticed by Panda et al. (2013).

Therefore, the present study was done with a view to use some homeopathic remedies for wheat seeds invigoration.

MATERIAL AND METHODS

Experimental conditions

The experiment has been done at the Faculty of Horticulture of USAMV Bucharest, in laboratory conditions (e.g. a 12-h photoperiod with 150 $\mu\text{moles photon m}^{-2} \text{s}^{-1}$ quantum irradiance, 20°C, 70 % relative humidity).

Biological material and seeds priming treatments

Seeds wheat (*Triticum aestivum* L.) cultivar Glosa were exposed to different priming treatments: V1. NaCl (*Natrum muriaticum*) – D6; V2. NaCl (*Natrum muriaticum*) - CH30; V3. NaCl (*Natrum muriaticum*) - CH200; V4- hydro-priming; V5- non-priming. Four replicates of 25 seeds placed on a filter paper in 9 cm Petri dishes were used, in a factorial experiment based on a completely randomized design. First of all, the seeds were sterilized by sodium hypochlorite 0.5 % for 5 minutes, and after that three times rinsed with distilled water. Then, seeds were submitted to imbibition during 24 hours, in distilled water as well as in different homeopathic remedies as it were mentioned above. The second day, after the seeds air drying they were arranged on Petri dishes and 5 ml distilled water was applied into the dish, as well as on the lid filter paper.

Germination indicators

After 24 hours, during 7 days, the germinated seeds were counted at the same hour every day. A seed was considered as germinated if the radicle protruded the tegument and had 2 mm length. By using these data the following germination indicators have been calculated: cumulative germination percentage (%); electrolytes leakage during seeds imbibition ($\mu\text{S cm}^{-1} \text{g}^{-1}$); final germination percentage (FGP) (%); mean germination time (MGT) (days); mean daily germination (MDG)(number of germinated seeds per day) (Delian and Lagunovschi-Luchian, 2015).

Seedlings vigor

On the seventh day, seedlings were measured for the length of roots and shoots. The following indicators have been established by the following formula: *Seedlings vigour index (SVI)* = seedling length (cm) × final germination percentage (%). *Radicle vigour index (RVI)* = radicle length (cm) × final germination percentage (%). *Shoot vigour index (SVI)* = shoot length (cm) × final germination percentage (Patil et al., 2012, cited by Delian and Lagunovschi-Luchian, 2015).

Statistical analysis

Statistical analysis of the obtained results was done by two-way analysis of variance (ANOVA). To evaluate pairwise significant differences between treatments, means values of the obtained indicators were compared by T test and the differences were taken as significant when P value was ≤ 0.05 .

RESULTS AND DISCUSSION

For all homeopathic remedies (HR) used seeds started to germinate at 1 day after sowing (DAS) (33 % of V2), while in the case of V4 and V5 no germination was noticed. Beginning from the second day, a faster germination, with remarkable higher values were registered especially for V2 and V3, a trend that has been later maintained. As we can see in Figure 1, the germination start of hydro-primed (V4) and non-primed seeds (V5) was registered on the second day, with major differences during the germination period, between these variants, in favor of V4. However, germination percentage was close to about 95 %, at 5 days after sowing.

The positive influence of homeopathic remedies can be due to a better water absorption during imbibition period on the one hand (a mean of 51.12 % for V1, V2 and V3, as compared with V4 – 46.98 %), and on the other, possibly an earlier induction of metabolic processes, when the strict germination began. In addition, cellular mechanisms can be triggered based on the imprint memory due to proteins, transcription factors and epigenetics changes induced in primed seed (Chen and Arora, 2013). A faster imbibition after 24 h of imbibition as compared the hydro-priming was also noticed by Mahakham et al. (2017), when rice seeds were primed with homeopathic remedies (AgNPs10 and AgNPs20).

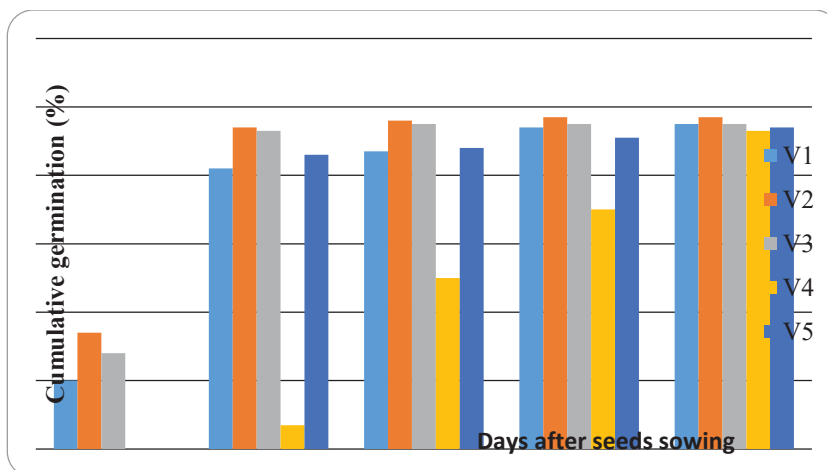


Figure 1. Cumulative germination percentage of wheat seeds under different priming treatments

It can be also mentioned that electrolytes leakage values during imbibition period was lower in the case of V3, as against the hydro-priming (Figure 2), a physiological feature with a major impact on the following germination stages, including those related to seedlings vigor.

These benefits of homeopathic remedies were also reflected into the final germination (FGP) (%) data (Figure 3), with the highest values for V2 (97 %) and the lowest ones for V4 (93 %), even if from the statistical view point, the differences are not significant between variants ($p > 0.05$).

Data presented in Figure 4 show that the mean germination time (MGT) was significantly reduced by homeopathic remedies, from 3.52 days (hydro-primed seeds), to 1.68 days in the case of V3. A lower value was also noticed for V2 (1.69 days) and moreover, even in the case of V1 MGT was significantly lower as against hydro-primed or non-primed seeds.

In addition, regarding the mean daily germination (MDG) data (Figure 5), there can be observed the same trends, with higher and closely values for V3 and V2 (around 39.00) , followed by V1 (22.70). As was expected, the lowest value was registered in the case of V4 (18.60).

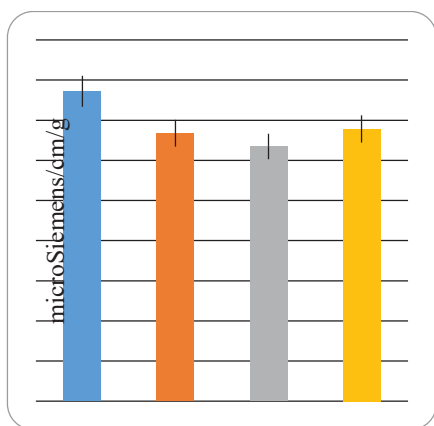


Figure 2. Electrolytes leakage after wheat seeds imbibition period

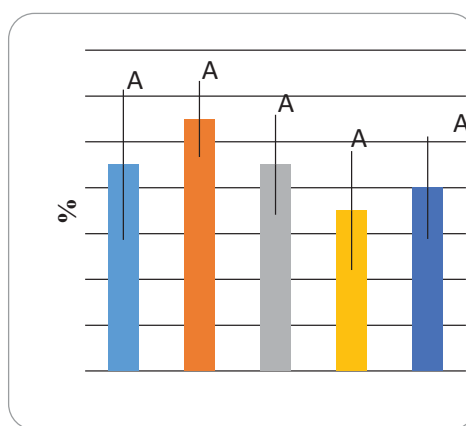


Figure 3. Final wheat seed germination percentage (mean ± SE) (n=4)

Seedlings weight was not significantly influenced in general by the seeds priming treatments, as we can see in Figure 6.A. and Figure 6.B. when shoots and roots fresh weight data were compared with the control value V5, (uppercase letters), or in the case of shoots and roots dry weight (lowercase letters) as against the control. Exception was registered for shoots dry weight, which was significantly higher in the case of V1 (0.007983 g) as against V5 (0.00555 g). The sprouts and roots mass was heavier for both parameters, when compared with the control V5.

Therefore, germination indices can be considered effective screening tools in the early stages of plant establishment. This idea is supported by previous publications, which suggest that physiological traits of a plant at later stages may be surmised by evaluating germination indices.

As we mentioned above, the homeopathic remedies had little effect on the germination speed, while the growth parameters have been influenced. In Figure 7 it can be observed that the seedlings length have been significantly higher, as against the hydro-primed samples.

The sprouts length was about two times higher for the treated samples (especially V1 and V3) as compared with the hydro-primed seeds. Also, root to shoot ratio (R/S) had different values (1.27 – for the control -V5 and 0.86 for the homeopathic treatments samples (V2)).

Dobrin et al. (2015) obtained remarkably differences concerning these parameters when the cold plasma treatment was applied to wheat seeds (being 0.88 ± 0.016 for the untreated seeds and reaching 1.2 ± 0.005 for the treated samples). The authors' explanation was that this improvement might be due to increased wettability observed for the treated seeds. The cold plasma treatment of seeds has a synergistic effects i.e. it acts as antimicrobial agent (ability to kill wide range of microbes) and enhance the seed germination and plant growth (see review Thirumdas, 2018).

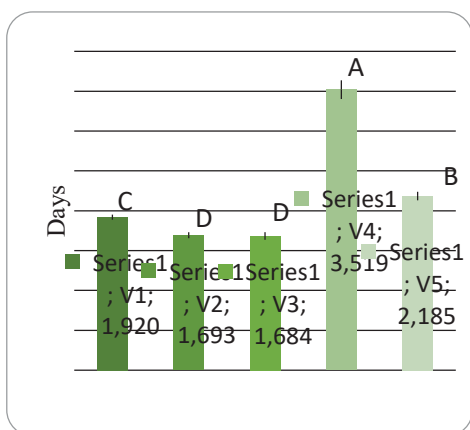


Figure 4. Mean germination time (mean ± SE) (n=4)

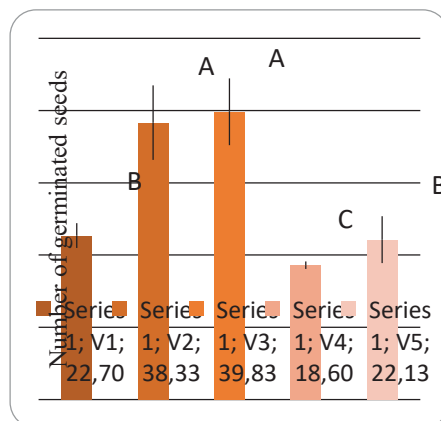


Figure 5. Mean daily germination (mean ± SE) (n=4)

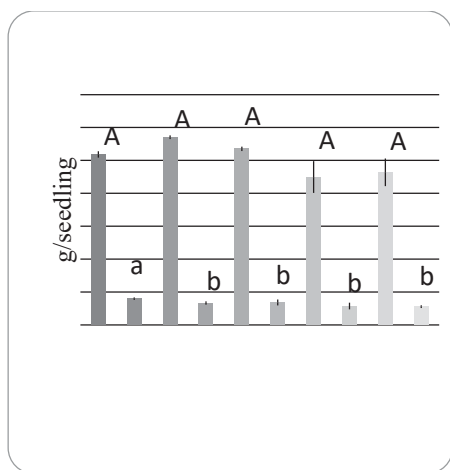


Figure 6.A. Shoot fresh weight (FW) and shoot dry weight (DW) (mean ± SE) (n=4)



Figure 6.B. Root fresh weight (FW) and root dry weight (DW) (mean ± SE) (n=4)

The homeopathic remedies action mechanism on seeds germination and seedlings growth has not yet been fully elucidated.

Some explanations have recently been done by Mahakham et al. (2017) after the obtained results of the silver nanoparticles (AgNPs) that enhanced seed germination of rice aged seeds. They proposed that nano-priming determined some physiological changes in seeds such as: creation of nanopores that permit a better water uptake, rebooting reactive oxygen species (ROS)/antioxidant systems, an easier wall loosening due to hydroxyl radicals generation, and also a faster starch hydrolysis thanks to nanoparticles catalytic effects.

In fact, there are some similarities between nanoparticles and enzymes; therefore, they are able to mimic natural enzymes activity, as it was noticed by Wei and Wang (2013). In addition, Panda et al. (2013) emphasized the influence of potentised homeopathic medicine, *Arsenicum album* and *Baryta carbonica* on germination, growth and photosynthetic activity of pea seedlings (*Pisum sativum* L.). The effects of three different centesimal potencies (8CH, 32CH, 202CH) of both the medicine were: an increase of plumule and radicle length, with the increase in potency. Also, the photosynthetic pigments content (chlorophyll, carotenoids, and pheophytin) showed similar results. A stimulating effect was noticed on growth as well as on photosynthetic activity, at the higher potency, as compared to untreated one, even after the dilution of drug beyond the Avogadro's number.

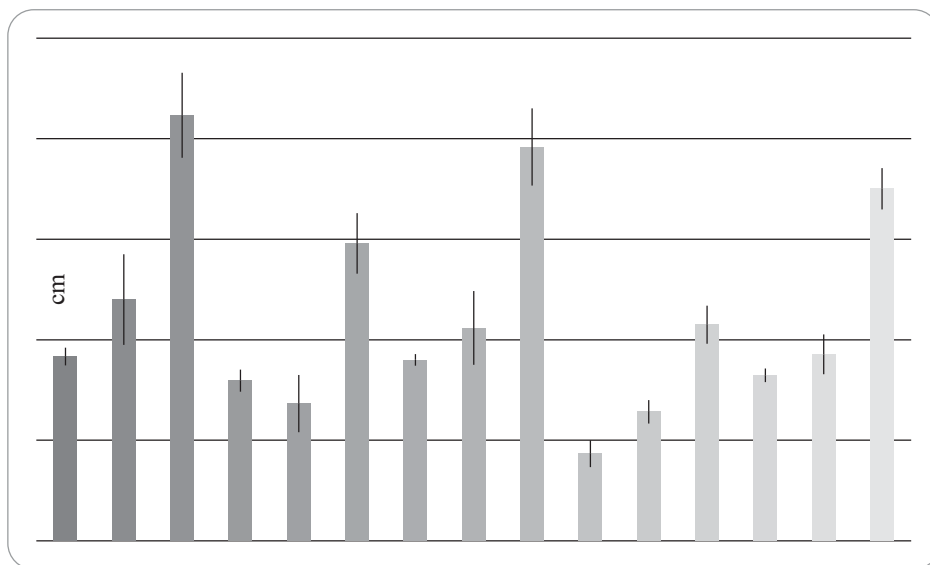


Figure 7. Shoots, roots and seedlings length (means \pm SE) (n=4)

CONCLUSIONS

Homeopathic remedies should be a promising alternative for wheat seeds invigoration. Further studies are necessary in order to recommend the more appropriately seeds priming treatments.

REFERENCES

- Aflaki, F., Sedghi, M., Pazuki, A., Pessarakli, M. 2017. Investigation of seed germination indices for early selection of salinity tolerant genotypes: A case study in wheat. *Emirates Journal of Food and Agriculture*. 29(3): 222-226.
- Araújo, S.S., Paparella, S., Dondi, D., Bentivoglio, A., Carbonera, D., Balestrazzi, A. 2016. Methods for Seed Invigoration: Advantages and Challenges in Seed Technology. *Front Plant Sci*. 7: 646.
- Betti, L., Trebbi, G., Zurla, M., Nani, D., Peruzzi, M., Brizz, M. 2010. A Review of Three Simple Plant Models and Corresponding Statistical Tools for Basic Research in Homeopathy. *The Scientific World Journal*. 10: 2330–2347.
- Brizzi, M., Elia, V., Trebbi, G., Nani, D., Peruzzi, M., Betti, L. 2011. The Efficacy of Ultramolecular Aqueous Dilutions on a Wheat Germination Model as a Function of Heat and Aging-Time. *Evid Based Complement Alternat Med*. : 696298. doi:10.1093/ecam/nep217.
- Chen, K., Arora, R. 2013. Priming memory invokes seed stress tolerance. *Environ. Exp. Bot*. 94: 33-45.
- Dobrin D, Magureanu, M, Mandache, N.B., Ionita, M.D. 2015. The effect of non-thermal plasma treatment on wheat germination and early growth. *Innovative Food Science & Emerging Technologies*. 29: 255- 260.
- Delian, E., Lagunovschi-Luchian, V. 2015. Germination and vigour of primed *Daucus carota* L. seeds under saline stress conditions. *Romanian Biotechnological Letters*, 20 (5):10833-10840.
- Edmondson, J. L., Davies, Z. G., Gaston, K. J., Leake, J. R. 2014. Urban cultivation in allotments maintains soil qualities adversely affected by conventional agriculture. *J. Appl. Ecol*. 51: 880–889.
- Ernst, E. 2002. A systematic review of systematic reviews of homeopathy. *Br J Clin Pharmacol*.54(6): 577–582.
- FAO 2016. Food and Agriculture Organization of the United Nations. Rome. The State of Food and Agriculture. Climate Change, Agriculture and Food Security. ISBN 978-92-5-109374-0.
- Lancet, 2005. The end of homoeopathy. 366(9487): 690.
- Mahakham, W., Sarmah, A.K., Maensiri, S., Theerakulpisut, P. 2017. Nanoprimering technology for enhancing germination and starch metabolism of aged rice seeds using phytosynthesized silver nanoparticles. *SCIENTIFIC REPORTS* | 7: 8263.
- Majewsky, V., Arlt, S., Shah, D., 4, Scherr, C., Ja'ger, T., Betti, L., Trebbi, G., Bonamin, L., Klocke, P., Baumgartner, S. 2009. Use of homeopathic preparations in experimental studies with healthy plants. *Homeopathy*. 98:228–243.
- Panda, S.S., Mohanty, S.S., Dhal, N.K. 2013. Effects of potentised homeopathic medicines on the germination, growth and photosynthetic activity of *Pisum sativum* L. *Recent Research in Science and Technology*. 5(4): 11-14.
- Paparella, S., Araújo, S.S., Rossi, G., Wijayasinghe, M., Carbonera, D., Balestrazzi, A. 2015. Seed priming: state of the art and new perspectives. *Plant Cell Rep*. 34: 1281-1293.
- Patil, J.G., Ahire, M.L., Nikam, T.D. 2012. Influence of Plant Growth Regulators on in Vitro Seed Germination and Seedling Development of *Digitalis purpurea* L. *The Asian and Australasian Journal of Plant Science and Biotechnology*, 6 (Special Issue 1):12-18.
- Rajjou, L., Duval, M., Gallardo, K., Catusse, J., Bally, J., Job, C., Job, D. 2012. Seed germination and vigor. *Annu. Rev. Plant Biol*. 63:507–533.

Relton, C., Cooper, K., Viksveen, P., Fibert, P., Thomas, K. 2017. Prevalence of homeopathy use by the general population worldwide: a systematic review. Homeopathy. <http://dx.doi.org/10.1016/j.homp.2017.03.002>.

Singhania, P.K., Singhania, A. 2014. Homoeopathy in agriculture. RAHMANN G&AKSOY U (Eds.) Proceedings of the 4thISOFAR Scientific Conference. 'Building Organic Bridges', at the Organic World Congress, 13-15 Oct., Istanbul, Turkey (eprint ID 23727).

Swayne, J. 2000. International Dictionary of Homeopathy. Edinburgh: Churchill Livingstone.

Thirumdas, R. 2018. Exploitation of Cold Plasma Technology for Enhancement of Seed Germination. Agri Res & Tech: Open Access J. 13(2): 555874.

Wei, H., Wang, E. 2013. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. Chemical Society Reviews. 42(14):6060-6093.

**RESEARCHES ON THE EVOLUTION OF VINE MOTH IN THE VINEYARD
OF S.D. BANU MARACINE**

Stan Catalin¹, Tuca Ovidiu¹, Ciupeanu Eleonora Daniela¹

University of Craiova, Faculty of Horticulture, Biology and Environment Protection Department
* E-mail: st_catalin2000@yahoo.com

Keywords: *vineyard, vine moth, key pest*

ABSTRACT

This paper presents the researches regarding the evolution of the vine moth during 2017, in the vineyard of S.D. Banu Mărăciine.

Climatic conditions were favorable for the development of the vine moths species, in the case of Lobesia botrana species there were recorded 3 flying peaks that belonged to so many generations. In the case of the species Eupoecilia ambiguella and Sparganotis pileriana, there were respectively 2 or 1 maximum annual flying, belonging to 2 or 1 generation.

Due to the fact that the vine plantation is newly established, on an uncultivated land area (formerly occupied with spontaneous vegetation), the pests reserve including the moths of the vine was reduced.

INTRODUCTION

Within the vineyard plantation, usually only a few pests have a great economic influence. These are the species that meet year after year, which are called key species, requiring mandatory treatments without which the production obtained cannot be based on the quantitative and qualitative parameters accepted according to the rules in force worldwide (Mary Louise Flint & van den Bosch R.

Vine moths are considered by most specialists to be the most dangerous pests of grapevines, along with mites in the key pest group. (Stan C. 2007).

Among the species of moths found in Oltenia, the most dangerous and for which protection measures are taken, is the green moth Lobesia botrana Den et Schiff. (Mitrea I. et al. 2003).

The prognosis of the grape moth is made by recording the flight curve with the pheromone traps: ATRABOT E 7, for Lobesia botrana, a trap for 3 ha or 2 for 5 ha; ATRAPIL E 9 - Sparganotis pileriana; ATRAMBIG Z9 - Eupoecilia ambiguella (Roșca I. et al., 2000).

The economic damage threshold is reached when are recorded 100 catches / butterflies / trap / week or when 5-7 % of the inflorescences are attacked in the case of the GI generation, or 5-7 % of the total grapes in the case of the GII generation and the GIII generation (Stoian Elisabeta 2001).

MATERIAL AND METHODS

Research on the evolution of moths in the vine was carried out in 2017, in the vineyard plant at S.D. Banu Mărăciine. The plantation was set up in 2016 with different grape varieties (Tamaioasa, Merlot, Feteasca n., Cabernet sauvignon).

The species of vine moths for which the experiments were set during the research period were: *Lobesia botrana* Den et Schiff., *Eupoecilia ambiguella* Hb. and *Sparganotis pilleriana* Den et Schiff. Monitoring the evolution of moth generation was based on catches recorded on atraBOT pheromone traps to capture males of the *Lobesia botrana* Den et Schiff species., atraAMBIG to capture males of the species *Eupoecilia ambiguella* Hb. and atraPil for catching specimens of *Sparganotis pilleriana* Den et Schiff. (Fig.1) Pheromone traps were produced by the Romanian Raluca-Ripan Chemistry Institute Cluj-Napoca. The pheromone traps were installed at the end of April in early May, the norm being 2 traps/ ha. The trap reading was performed over a 7-day period, the captured butterflies being recorded and removed with plastic tweezers to avoid displacement of the adhesive, the replacement of the synthetic pheromone-impregnated capsules and trap adhesive parts was made at a range of 4-5 weeks.



Fig. 1. Feromonal trap atraBot

RESULTS AND DISCUSSIONS

The evolution of the moths in 2017 in the vineyard plantation of SD Banu Mărăcine is correlated with specific climatic conditions, the predominant species being *Lobesia botrana* Den et Schiff. Adults of the hibernate generation of *Lobesia botrana* Den et Schiff. have been identified since April 13, 2002, the population level (relative to the number of captured specimens) being moderate. The last butterflies were captured at the end of September. Table nr.1.

Adults of the hibernating generation of *Eupoecilia ambiguella* have been identified since 04.05.2002 and the population level has been very low and did not require a warning for treatments. Adults of the hibernate generation of *Sparganotis pilleriana* appeared since 29.06.2002, the population level was very low and did not require a warning for treatments.

Table nr.1

Evolution of vine moths in the vineyard at SD Banu Mărăcine

Year 2017					
Lobesia botrana		Eupoecilia ambiguella		Sparganotis	
Date	Nr. captured males	Date	Nr. captured males	Date obser	Nr. captured males
13.04	1	13.04	-	13.04	-
20.04	5	20.04	-	20.04	-
27.04	11	27.04	-	27.04	-

04.05	32	04.05	1	04.05	0
11.05	71	11.05	5	11.05	0
18.05	125*	18.05	47	18.05	0
25.05	92	25.05	11	25.05	0
01.06	56	01.06	6	01.06	0
08.06	14	08.06	3	08.06	0
15.06	18	15.06	1	15.06	0
22.06	52	22.06	0	22.06	0
29.06	195*	29.06	0	29.06	2
06.07	111	06.07	0	06.07	12
13.07	81	13.07	0	13.07	31
20.07	76	20.07	0	20.07	42
27.07	41	27.07	0	27.07	54
03.08	68	03.08	0	03.08	59
10.08	89	10.08	12	10.08	63
17.08	141*	17.08	29	17.08	27
24.08	89	24.08	57	24.08	19
31.08	42	31.08	12	31.08	3
07.09	37	07.09	4	07.09	1
14.09	25	14.09	0	14.09	0
21.09	13	21.09	0	21.09	0
28.09	3	28.09	0	28.09	0
05.10	1	05.10	0	05.10	0
12.10	0	12.10	0	12.10	0
19.10	0	19.10	0	19.10	0

* = warning of treatments

The climatic conditions of 2017 were favorable for the development of the vine moths species, in the case of *Lobesia botrana* species there were recorded 3 flying peaks that belonged to so many generations.

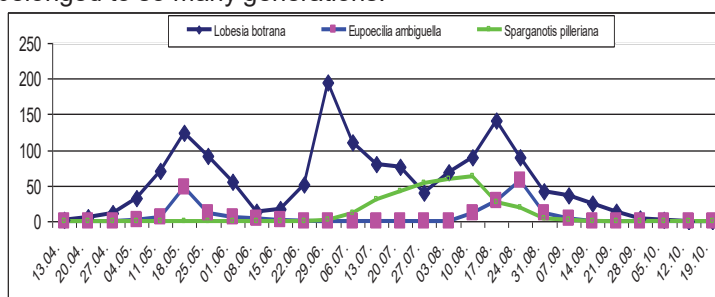


Fig. 2. The dynamics of vine moth flight in correlation with the climatic conditions at SD Banu Mărăciine in 2017

For the hibernating generation (G1), maximum flight (125 butterflies / trap) was recorded in the second decade of May (18.05). The second generation (GII) showed a

maximum flight (195 butterflies / trap) in the third decade of June (29.06), and for the third generation (GIII), the maximum flight (141 butterflies / trap), was recorded in the second decade of August (17.08.). In the case of the species *Eupoecilia ambiguella* and *Sparganotis pilleriana*, there were respectively 2 or 1 maximum annual flying, belonging to 2 or 1 generation respectively.

CONCLUSIONS

The vineyard plantation of SD Banu Mărăcine is a young plantation set up on a terrain that was previously occupied by natural grasslands and spontaneous bushes (*Crataegus monogyna*, *Prunus spinosa*, *Rosa canina*) so that the biological reserve of vine moths as well as other vines specific pests are very diminished. Besides, there are no vineyards left in the area, which are being overwhelmed by the current owners of the land. This justifies the small number of catches recorded in the pheromone traps for all species of vine moths studied.

REFERENCES

Mary Louise Flint & van den Bosch R., 1981, Introduction to integrated pest management. The Philosophy of Integrated Pest Management pp.107-119. Plenum Press, New York.

Mitrea I., et al., 2003, Combaterea integrată a bolilor, dăunătorilor și buruienilor la vița de vie. Lucrările Simpozionului Științific Internațional 70 de ani ai Universității agrare de stat din Moldova, Seria horticultură, Silvicultură, Protecția Plantelor, pg. 231, Chișinău

Roșca I. et al., 2000, Combaterea integrată a bolilor și dăunătorilor culturilor agricole, Editura Didactică și Pedagogică R.A., București.

Stan, C., 2007, Cercetări privind combaterea integrată a principalilor dăunători ai plantațiilor de viță de vie din Oltenia. Teză de doctorat, Univ. din Craiova, 2007.

Stoian Elisabeta, 2001, Principalele pesticide omologate pentru combaterea dăunătorilor viței de vie. Rev. Sănătatea plantelor nr. 3/2001.

**RESERACHES ON THE INTEGRATED VINE GREEN MOTH CONTROL
(*Lobesia botrana* Den et Schiff.) IN THE VINEYARD
OF S.D. BANU MARACINE**

Stan Catalin¹*, Ivascu Alexandra²

¹, University of Craiova, Faculty of Horticulture, Biology and Environment Protection Department,

², MA student, Master Program: Ecological Management of Natural Resources (MERN)

* Correspondence author. E-mail: st_catalin2000@yahoo.com

Keywords: *vineyard, vine moth, integrated control*

ABSTRACT

*This paper presents the researches regarding the integrate control of the vine green moth (*Lobesia botrana* Den et Schiff.) during 2017, in the vineyard of S.D. Banu Mărăcine.*

The frequency of the vine green moth attack in the year 2017 in the untreated control variant has been recorded with an average of 19.5 %. As a result of the treatments, the average frequency of the attack was considerably reduced: 3.2 % for V5, 2.7 % for V6, 2.2 % for V4, 1.7 % for V3 and 1 % for variant V2. The efficacy of treatments was 94.8 % for V2, 91.5 % for V3, 88.9 % for V4, 86.2 % for V6 and 83.7 % for V5.

INTRODUCTION

Vine moths are considered by most specialists to be the most dangerous pests of grapevines, along with mites in the key pest group. (Stan C. 2007).

Among the species of moths found in Oltenia, the most dangerous and for which protection measures are taken, is the green moth *Lobesia botrana* Den et Schiff. (Mitrea I. et al. 2003).

The prognosis of the grape moth is made by recording the flight curve with the pheromone traps: ATRABOT E 7, a trap for 3 ha or 2 for 5 ha (Roșca I. et al., 2000).

The economic damage threshold is reached when are recorded 100 catches/butterflies/trap/week or when 5-7 % of the inflorescences are attacked in the case of the GI generation, or 5-7 % of the total grapes in the case of the GII generation and the GIII generation (Stoian Elisabet 2001).

MATERIAL AND METHODS

Research on the integrated control of the the vine green moth was carried out in 2017, in the vineyard plant at S.D. Banu Mărăcine. The plantation was set up in 2016 with different grape varieties (Tamaioasa, Merlot, Cabernet Sauvignon, Feteasca neagra, Chardonay).

Monitoring the evolution of moth generation was based on catches recorded on atraBOT pheromone traps to capture males of the *Lobesia botrana* Den et Schiff species. Pheromone traps were produced and purchased from the Romanian Raluca-Ripan Chemistry Institute in Cluj-Napoca. Pheromone traps were installed at the end of April. The trap reading was performed over a 7-day period, the captured butterflies being recorded and removed with plastic tweezers to avoid displacement of the adhesive, and

the replacement of the synthetic pheromone-impregnated capsules and trap adhesive parts was made at a range of 4-5 weeks.

The experiments were performed according to the randomized block method, each variant consisting of 100 vines (placed on a row), leaving one row between the variants and applying the treatments using the Solo 546 atomizer. For the biological control of the pest *Lobesia botrana* Den et Schiff. two species of *Trichogramma dendrolimi* Mats and *Trichogramma embriophagum* Htg were used by the use of oofage parasitoids of the genus *Trichogramma*. For each species, three usage rules were tested: 150,000, 200,000 and 300,000 trichograms / ha / launch respectively.

RESULTS AND DISCUSSIONS

Establishing a warning term for treatments was based on biological, ecological and phenological criteria. For this purpose, the lower biological threshold of the vine green grape moth (*Lobesia botrana* Den et Schiff.) $T_0 = 12^{\circ} \text{C}$, adult flight curve, the host plant phenology as well as the environmental and biological data.

Treatments were performed, with products of Group III and IV toxicity, in order to protect the useful fauna of the vineyards, with an important role in maintaining biochemical balance. In order to increase the effectiveness of the treatments, they were complexed with fungicides, while combating the phytopathogenic agents of the vine. As well it was taken to account the pest's biological reserve, the cultivated variety and the weather conditions during the development and multiplication of the pest.

The dynamics of the vine green moth flight (*Lobesia botrana* Den et Schiff.) comprised 3 peaks corresponding to the three generations and for each there has been applied a treatment.

Launchers with parasitoids were applied after the adult maximum flight record, after five to seven days, depending on the climatic conditions of each year. The method of launching the oofage parasitoids requires planting white platelets with eggs of the Mediterranean flour moth (*Anagasta kuehniella* Zell.) parasite by *Trichogramma* spp. The platelets are installed by hanging on shoots, at different heights each plate contained about 500 parasite eggs.

The frequency of vine green moth attack in the untreated control variant in 2017 has recorded an average value of 19.5 % (Table 1). As a result of the treatments, the average frequency of the attack was considerably reduced: 3.2 % for V5, 2.7 % for V6, 2.2 % for V4, 1.7 % for V3 and 1 % for variant V2.

Table nr.1

Biological efficacy of products used in integrated vine green moth control

Nr. var.	Treatment variant	Attack Frequency (F %)					Efficacy (E %)						
		GI	GII	GIII	Average	Dif. Mt	Signif. dif.	GI	GII	GIII	Average	Dif. Mt	Signif. dif.
V1	Untreated control variant Mt	19	18,5	21	19,5	0	Mt	0	0	0	0	0	Mt
V2	GI- Vicienon 50 WP, 1 kg/ha	0,5	1	1,5	1	-18,5	000	97,3	94,5	92,8	94,8	94,8	xxx
	GII- Fastac 10 EC, 0,075 l/ha												
	GIII- Rimon 10 EC, 0,5 kg/ha												
V3	GI- Talstar 10 EC, 0,2 l/ha	1	1,5	2,5	1,7	-17,8	000	94,7	91,8	88,1	91,5	91,5	xx
	GII- Calypso 480 SC 0,1 l/ha												
	GIII- Dipel ES, 1 l/ha												
V4	GI- Bulidock 025 EC, 0,3 l/ha	0,5	3	3	2,2	-17,3	000	97,3	83,7	85,7	88,9	88,9	xx
	GI- Foray 48B (Biobit XL) 0,1 %												
	GIII- <i>T. dendrolimii</i> , 300000/ha/trata.												
V5	GI- Calypso 480 SC, 0,1 l/ha	1	4,5	4	3,2	-16,3	00	94,7	75,6	80,9	83,7	83,7	x
	GII- 10 trap atraBOT/ha												
	GIII- <i>T. dendrolimii</i> , 200000 tricog. / ha/trata. + 8 trap/ha												
V6	GI- Dipel ES 1 l/ha + 8 trap/ha	2	3,5	2,5	2,7	-16,8	00	89,4	81,1	88,1	86,2	86,2	x
	GII- <i>T. embryophagum</i> , 300000trich/ ha/treatment												
	GIII- <i>T. embryophagum</i> , 300000 trich /ha/treatment												

E %- attack efficacy

F % - attack frequency

G I – first generation

G II – second generation

G III –third generation

The differences, compared to the untreated control variant, of the attack average frequency, recorded during the research period, for all variants were negative, very significant negative difference were presented in the variants: V2, V3, V4.

The average efficacy of the treatments has recorded a value of 94.8 % for variant V2 (treatment with different chemicals for the 3 generations), 91.5 % for V3 (treatment with PI pyrethroid synthesis for GI continued arthropod metalloprotein inhibitor for GII and biological product for GIII), 88.9 % at V4 (treatment with PI pyrethroid synthesis for GI biologic product for GII and oophage wasps from *Trichogramma* genus for GIII), 86.2 % at V6 (treatment with biological product for GI oophage wasps of the genus *Trichogramma* for GII and GIII) and 83.7 % to V5 (treatment with arthropod metamorphosis inhibitor for GI *Trichogramma* oophage wasps combined with mass trapping for GII as well for GIII *Trichogramma* oophage wasps combined with mass trapping).



Fig. 1. Pheromonal trap for capture male butterflies (*L. botrana*)

CONCLUSIONS

Integrated vine moths control involves combining preventive methods of control with the application of biological and chemical control measures to limit moth populations below the economic threshold of pests (P.E.D.).

It is noteworthy that V2 in which chemicals were used has produced the best results, but very good results have also been obtained in V3 and V4 variants where first-generation chemicals combined with biological products have been used for the following generations.

Variants V5 and V6 in which we only used biological products have shown weaker results, but given that these products are not polluting for the viticultural ecosystem and for grapes, we recommend using these variants of treatment in any integrated moth control scheme of vines.

REFERENCES

Mitrea I., et al., 2003, Combaterea integrată a bolilor, dăunătorilor și buruienilor la vița de vie. Lucrările Simpozionului Științific Internațional 70 de ani ai Universității agrare de stat din Moldova, Seria horticultură, Silvicultură, Protecția Plantelor, pg. 231, Chișinău

Roșca I. et al., 2000, Combaterea integrată a bolilor și dăunătorilor culturilor agricole, Editura Didactică și Pedagogică R.A., București.

Stan, C., 2007, Cercetări privind combaterea integrată a principalilor dăunători ai plantațiilor de viță de vie din Oltenia. Teză de doctorat, Univ. din Craiova, 2007.

Stoian Elisabeta, 2001, Principalele pesticide omologate pentru combaterea dăunătorilor viței de vie. Rev. Sănătatea plantelor nr. 3/2001.

**MONITORING OF NITRATES CONTENT OF GROUNDWATER
AND SURFACE WATER OF CURTEA DE ARGEŞ MUNICIPALITY,
ARGEŞ COUNTY**

Tudor Denisa Elena^{1*}, Bucur Doru Gabriel²

¹Agency for Payments and Intervention in Agriculture Argeş

²Office for Pedological and Agrochemical Researches Argeş County

* *E-mail:* denisaelenatudor@yahoo.com

Keywords: *nitrate, monitoring, water, vulnerability, pollution*

ABSTRACT

This study shows nitrate concentrations in surface waters and groundwater of Curtea de Argeş Municipality said under current legislation vulnerable to nitrate pollution of agricultural origin.

The research took place in the seasons of summer and autumn of 2016, by sampling water from wells, springs and the two reservoirs located on the Argeş River, for their analysis of physical and chemical and assessing progress nitrate content after a period of about three months.

INTRODUCTION

Nitrates are compounds of nitrogen and are part of the natural cycle, thereof may develop a potentially toxic to living organisms where the values exceed a certain level as critical.

Global concerns regarding the content of nitrates in surface waters and groundwater have increased in recent decades. This is due to the adverse manifested on the human body after consumption of water with high concentrations of nitrates (Proca et al. 2009).

Most research in the field have concluded that the accumulation of these contaminants in soil and water sources are caused mainly by practicing inadequate agricultural purposes (Sánchez Pérez et al. 2003, Lawniczak et al. 2016).

In Romania, the law (Law no. 458/2002, Law no. 311/2004) establishes a maximum concentration allowed in drinking water of 50 mg/L nitrate and the ammonium and nitrite 0.5 mg/L.

By common Order no. 1552/743 of 2008 Curtea de Argeş Municipality was included as part of the areas vulnerable to pollution by nitrates from agricultural sources.

MATERIAL AND METHODS

In 2016 a study was conducted to assess the content of nitrate in groundwater bodies and the surface held in two stages, with sampling of water in 41 strategic locations (mainly wells) plotted in Figure 1. Water samples were collected and from lakes Curtea de Argeş and Zigoneni (samples number 18 and number 41).

With the support of a powerful multi-parameter device (HANNA) have obtained information from the moment regarding the nitrate content of water samples collection.

The first stage (Series 1) was conducted in the third decade of July and consisted of water sampling of the 41 points, selected to cover the entire surface investigated as possible.

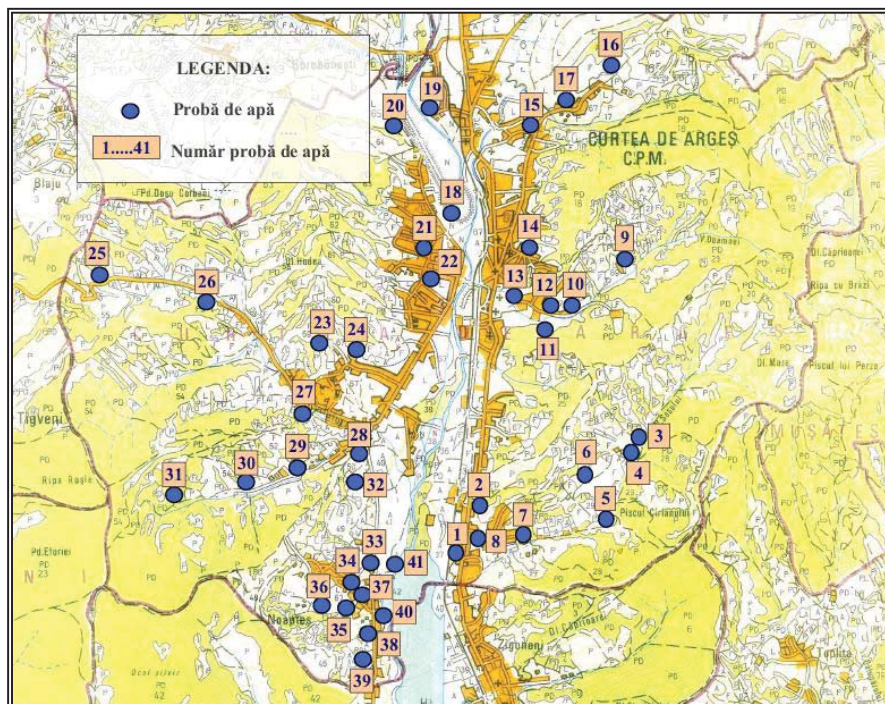


Figure 1. The location of sampling of water from the Curtea de Argeș Municipality

The second stage of harvesting (Series 2) water samples held in October, water sampling points being made of the same monitoring.

Known as the relationship between nitrate concentrations in surface waters and groundwater in relation to other biotic and non-biotic factors, research has involved the summary of the natural knowledge that characterizes Curtea de Argeș Municipality.

RESULTS AND DISCUSSION

Geomorphologically, Curtea de Argeș Municipality is looming on the southern tip of Subcarpathians Argeș (Muscelele Getice), in contact with Getic Piedmont and includes much of the same name inter-hill depression. It comprises a depression area with meadows and terraces lacy aprons parasitized train meandered shaped by the old course of the Argeș River and a hilly area with long slopes predominantly oriented east and west and interfluvial narrow ridges.

It occupies an area of approx. 75 km² and has a population of 33.000 inhabitants.

The depth of water tables is relative to topography. On hills, the heights of the groundwater table has at depth of 30-40 meters, and in the valleys is 1-5 meters.

The climate is temperate-continental with an average temperature of 9 °C and the average precipitation of 730 mm. The rainfall is during May to July. Dry intervals are from January to March and September to December.

Forests occupy about 25 % of the perimeter of the municipality and are predominantly composed of beech (*Fagus sylvatica*) mixed with oak (*Quercus Petraea*), hornbeam (*Carpinus betulus*) and ash (*Fraxinus excelsior*).

The slopes subject to erosion and high peaks are characterized by *regosols* (Tudor et al. 2013). Some secondary peaks, rather narrow evolved *luvisols*. Meadows are characterized by „young soils” - type *fluvisols*. *Eutricambosols* have a pretty wide spatial distribution, growing in some sectors floodplain, terraces, aprons and frequently on slopes. The Argeş River terraces have also developed *pelosols*, constructed entirely of fine pedogenetic material, and the glacis are characterized by *preluposols*. Some sectors have evolved *phaeozems* slope, well matured soils, with a deep and rich in humus surface horizon.

For reasons of pedological, is showed that 12 % of the agricultural area presents high vulnerability to pollution by nitrates; 16 % of the average present moderate vulnerability and 72 % reduced vulnerability to pollution by nitrates.

As a result of water samples from different sources, have been determined concentration of nitrate (NO₃) and other physical and chemical parameters such as: the content of ammonium (NH₄), the acidity of the water (pH) and water content of oxygen, is indicated at the same time and the depth of sampling (Table 1).

By the interpretation of the data obtained from the first stage of the research, the percentage showed that of the 41 bodies of analyzed water, 83 % had nitrate concentrations below 25 mg/L, 12 % had levels between 25 and 50 mg/L and only 5 % had levels between 50 and 75 mg/L.

In the second step of monitoring, was a trend toward water purification in that the number of water bodies, in the gap nitrate concentrations 0-25 mg/L up to 90 %, registering only 5 % of between 25 and 50 mg/L. However, the percentage of wells remained above the threshold of 50 mg/L.

Regarding water bodies that exceed the maximum level for consumption, there was one aspect. While in the well number 29 the nitrate concentration increased in July-October at 66.12 mg/L to 71.26 mg/L, and well number 33 values were raised from 40.03 to 56.01 mg/L NO₃, in the well with the number 4 to the other way round.

Table 1

Physical and chemical analyzes of water samples collected in July and October of 2016 in the Curtea de Arges Municipality

No.	Series 1	Series 2	Series 1/Series 2				
	NO ₃ (mg/L)	NO ₃ (mg/L)	NH ₄ (mg/L)	Water temperature °C	pH	Dissolved oxygen (mg/L)	Harvesting depth
1	24.56	17.13	0.30/0.25	13.6/15.2	7.0/7.1	4.3/3.8	2.5/2.6
2	0.33	12.80	2.36/0.11	11.9/12.0	6.5/6.5	8.8/3.7	8.7/3.7
3	19.87	3.39	0.39/0.30	11.5/12.6	6.7/6.9	1.2/4.2	2.0/2.5
4	74.08	43.10	6.82/4.01	11.4/14.0	7.1/7.6	4.2/3.8	2.1/1.9
5	3.84	4.19	2.16/3.65	11.5/12.3	6.6/7.4	2.9/3.0	3.8/3.7
6	4.45	1.48	0.87/0.35	13.9/15.5	6.6/7.1	3.5/2.9	1.8/1.5
7	6.90	14.66	0.40/0.40	12.0/13.3	7.2/7.5	1.5/3.4	3.8/3.7
8	24.24	11.46	0.38/0.29	11.7/11.9	6.7/6.9	1.6/3.5	6.1/4.4
9	30.04	10.23	0.85/0.31	11.1/11.8	7.1/7.0	3.0/2.7	2.4/2.4
10	18.58	1.66	0.53/0.09	12.9/11.7	7.0/7.3	1.0/3.3	2.5/3.6
11	12.41	7.40	0.85/0.40	12.1/12.2	7.3/7.4	5.1/6.2	3.1/4.3
12	8.64	5.05	0.27/0.17	11.6/11.5	7.5/7.2	6.8/5.4	4.1/3.5
13*	18.26	8.69	0.33/0.24	15.8/11.7	6.1/7.3	5.3/6.0	-
14	14.22	11.96	0.16/0.40	11.0/11.3	7.1/7.2	4.0/5.6	1.8/2.0
15	12.19	5.82	0.22/0.18	11.7/13.0	6.3/6.3	1.7/4.1	3.4/3.2
16	1.09	1.19	0.26/0.09	13.7/15.4	7.0/7.2	1.9/2.9	1.1/1.5
17	1.64	1.59	1.50/0.34	11.5/15.6	7.0/7.7	4.9/3.6	4.1/0.7

18**	5.34	11.62	0.06/0.02	12.4/9.3	6.8/8.1	9.4/8.8	-
19	13.66	10.14	0.84/0.28	17.4/18.5	7.3/7.1	2.0/3.1	1.6/1.6
20	6.79	6.74	4.26/1.33	11.4/11.3	7.1/7.3	1.4/3.8	0.1/0.8
21	47.67	10.62	0.37/0.18	11.5/12.3	7.2/7.4	4.5/5.9	3.0/3.3
22	6.26	5.70	0.33/0.16	18.5/12.8	7.0/7.1	4.7/4.2	2.7/2.5
23	2.14	4.42	0.09/0.12	13.4/14.2	7.5/7.5	3.0/2.9	3.2/3.3
24	9.47	10.12	1.83/0.95	11.6/13.0	7.4/7.3	2.8/2.3	5.1/4.9
25	0.92	1.24	0.10/0.06	11.8/13.3	7.3/7.4	1.9/2.8	3.4/2.4
26	7.09	8.11	3.22/2.35	14.7/13.3	7.4/7.4	3.9/3.4	2.0/2.1
27	13.45	9.28	0.49/0.16	11.7/12.7	7.2/7.2	5.1/2.0	3.3/2.5
28	6.95	13.02	1.32/0.40	13.6/14.2	7.2/7.6	2.4/3.7	1.3/0.8
29	66.12	71.26	0.18/0.10	10.8/12.2	7.0/7.1	2.9/3.5	4.8/4.7
30	49.16	33.63	0.34/0.92	13.6/14.3	7.0/7.1	2.5/4.7	1.3/1.2
31	9.30	9.06	0.35/0.09	12.7/14.5	6.9/7.0	2.8/5.0	2.8/2.5
32	10.95	15.34	0.18/0.07	12.4/13.4	7.0/7.2	6.8/5.0	2.5/2.0
33	40.03	56.01	0.26/0.09	12.3/13.3	7.0/6.9	5.6/3.6	1.3/1.5
34	11.64	6.29	0.28/0.17	13.7/13.4	6.6/6.9	3.9/5.2	2.7/1.2
35	11.09	18.97	0.22/0.12	11.4/12.0	6.5/6.8	2.3/2.1	2.9/4.4
36	10.80	10.07	0.16/0.42	11.6/13.0	7.1/7.2	3.3/2.9	4.0/3.6
37	16.81	22.08	0.26/0.15	11.2/11.9	6.9/7.1	4.9/4.6	3.8/3.1
38	3.56	5.66	0.18/0.09	11.6/11.7	7.1/7.3	4.1/3.5	7.2/6.2
39	6.47	20.16	0.29/0.07	12.1/14.1	6.7/6.7	4.0/3.2	3.4/2.9
40	7.87	13.46	0.22/0.13	10.9/11.6	6.7/6.8	4.1/3.9	5.1/4.6
41***	2.80	3.76	0.08/0.07	12.6/10.0	7.9/8.0	7.4/5.4	-

* sample taken from a spring coastal

** sample taken from the lake Curtea de Argeş

*** sample taken from the lake Zigoneni

In this latter source of water, nitrate concentrations have decreased significantly in the fall by 42 %, going from 74.08 mg/L NO₃ - value recorded in July - at 43.10 mg/L NO₃ in October.

The same was observed in the water body number 21, where the accumulation of nitrates were recorded in summer season 47.67 mg/L, so that later in the autumn, to fall by 78 % to a concentration of only 10.62 mg/L NO₃.

Similarly, it reveals considerable reduction of nitrate concentrations in the body of water and the number of 9 to 30 mg/L NO₃ to 10 mg/L NO₃.

The remaining water bodies monitored maintained at relatively safe for consumption.

With regard to the ammonium content in water, they have been identified in the summer values below 0.5 mg/L in 70 % of samples (compared to 85 % in the fall), between 0.5 mg/L and 1 mg/L in 10 % (compared with 5 % recorded in the autumn) and above 1.0 mg/L to 6.8 mg/L to 20 % of the samples taken in July (compared to 10 % in the fall).

Other analytical data were interpreted according to the Order 161/2006 of M.M.G.A. on quality standards for surface waters.

With regard to the water temperature, it has quite variable. In summer values have ranged 10.8 °C and 18.5 °C, and in autumn they were between 9.3 °C and 18.5 °C. The water temperature is not standardized.

The two sets of samples had pH values between 6.3 and 8.8, weak acid to moderate alkaline. Quality standards set for drinking water pH 6.5-8.5.

Dissolved oxygen in water is classified into five quality categories. The analysis reflected that in summer one sample (2.4 %) is a Class I quality with over 9 mg/L O₂, two samples of water (4.9 %) class II quality (between 7.0 to 8.9 mg/L O₂), six samples (14.6 %) grade III (between 5.0 to 6.9 mg/L O₂), ten samples (24.4 %) grade IV (between 4.0 to 4.9 mg/L O₂), and the more samples, the number of 22 (53.7 %) in grade V (below 4.0 mg/L O₂).

In the autumn a single sample of water (2.4 %) is graded Class II; 9 samples (22 %) in Class III; 5 samples (12.2 %) in Class IV; 26 samples (63.4 %) in grade V of quality.

CONCLUSIONS

Inclusion of Curtea de Argeş Municipality as part of the areas vulnerable to pollution by nitrates from agricultural sources, determined that in the year 2016 to conduct a study to assess the content of nitrate in groundwater bodies and surface.

By the interpretation of the resulting data, about 50 % of waters studied in two stages (Figure 2) contain nitrate concentrations below 10 mg/L.

It was found that in some waters monitoring points nitrate suffered considerable fluctuations in a relatively short time (about three months).

It also noted a downward trend in nitrate concentrations in autumn compared to the summer. One hypothesis is that the first sample of evidence has been collected in full vegetative season and wetter than autumn. This season is conducive to fertilization phase of agricultural perimeters, nitrates consumed by plants with rainwater or irrigated, were subjected to leaching through the soil profile to groundwater.

In any case, to reduce the risk of water pollution and stabilize concentrations of nitrate in water consumption at optimal levels, is recommended to apply the rules of the Local Action Plan against pollution caused by nitrates from agricultural sources. Household level recommended proper management of wastewater, domestic waste and standardizing chemical and organic fertilizers.

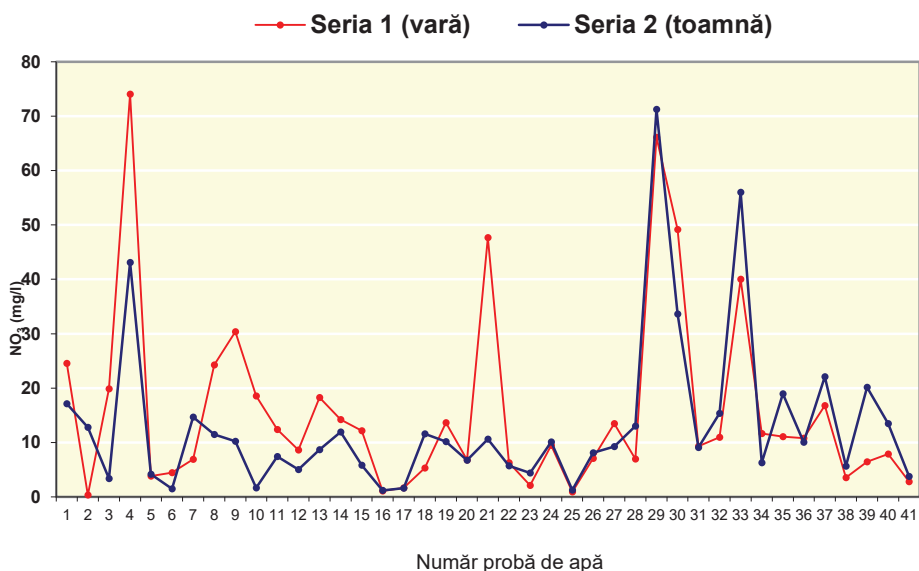


Figure 2. The variation of the nitrate concentrations in the water sources of the Municipality of Curtea de Argeş in the two series of monitoring.

REFERENCES

Lawniczak A. E., Zbierska J., Nowak B., Achtenberg K., Grzeškowiak A., Kanas K., 2016, Impact of agriculture and land use on nitrate contamination in groundwater and running waters in central-west Poland. *Environmental Monitoring and Assessment*. Vol. 188, No. 3, pp. 1-17

Proca C., Micu D., Danielescu C., Manea F., 2009, Case Study of the Risk Assessment of Nitrates on Human Health in the West Side of Romania. In: Simeonov L.I., Hassanien M.A (eds) *Exposure and Risk Assessment of Security C: Environmental Security*. Springer, Dordrecht, pp. 509-516.

Sánchez Pérez J., Antiguada I., Arrate I., Garcia-Linares C., Morell I., 2003, The influence of nitrate leaching through unsaturated soil on groundwater pollution in an agricultural area of the Basque country: a case study. *Science of The Total Environment*. Vol. 317, Issues 1-3, pp. 173-187.

Tudor D. E., Bucur D. G., Creangă I., 2013, Studiu pedologic și agrochimic elaborat în vederea realizării și reactualizării Sistemului Județean și Național de Monitorizare sol-teren pentru Agricultură, teritoriul Curtea de Argeș, Arhiva Oficiului de Studii Pedologice și Agrochimice Argeș.

*** Legea nr. 458/2002 privind calitatea apei potabile; - M.O. nr. 552/iulie 2002.

*** Legea nr. 311/2004 pentru modificarea și completarea Legii nr. 458/2002 privind calitatea apei potabile; - M.O. nr. 582/30.06.2004.

*** Ordinul nr. 161/2006 pentru aprobarea Normativului privind clasificarea calitatii apelor de suprafata in vederea stabilirii starii ecologice a corpurilor de apa - Ministerului Mediului si Gospodarii Apelor (M.M.G.A.).

*** Ordinul nr. 743 din 12 decembrie 2008 pentru aprobarea listei localităților pe județe unde există surse de nitrați din activități agricole.

**CONTRIBUTIONS TO THE STUDY OF THE DISTRIBUTION
OF SOME INSECTS SPECIES LISTED ON IUCN RED LIST
ON THE PROTECTED AREA SILVOSTEPA OLTENIEI**

Țuca Ovidiu¹, Stan Catalin¹, Ciupeanu Călugăru Eleonora Daniela

University of Craiova,

* Correspondence author. E-mail: ovidiu_tuca@hotmail.com

Keywords: *Carabus hungaricus* Fabr., *Cerambyx cerdo* L., *Lucanus cervus* L., *Hyles hippophae* Esp.

ABSTRACT

This paper presents the researches regarding the main features and the distribution of the endangered insects species, from the protected area Silvastepa Olteniei.

From our observation regarding the protected area Silvastepa Olteniei, we have identified a number of four insect species protected under the IUCN red list: Carabus hungaricus Fabr., Cerambyx cerdo L., Lucanus cervus L. and Hyles hippophae Esp.

In the Protected Natural Area, taking into account the habitat requirements, we have identified two saproxylic species and located their habitat in some forests from the protected area: Întorsura, Perișor, Târnavă, Mărăcinele, Știubeiului, Verbicioara and Plenița. The other beetle species has been located on several meadows near: Radovan, Vela, Bucovicior, Tencănuș, Vârvoru de Jos. Regarding the lepidopteran species, it was observed on the sunny and vegetated shores of Lake Ciutura, the Desnățui and Terpezița.

INTRODUCTION

The degradation of natural ecosystems continues unabated, threatening the long-term survival of many species around the world (Pimm et al.2014). In response, many conservation efforts have emerged to stop and reverse this degradation, among which the establishment of protected areas is considered one of the most effective (Hannah et al.2007, Andam et al. 2008, Jenkins and Joppa 2009).

Worldwide there are more than 200,000 protected areas (Juffe-Bignoli et al.2014), many of which have been established for the conservation of particular species of interest. Yet, their establishment and successful management may entail additional benefits beyond the long-term survival of the species of interest, including the conservation of entire biodiversity pools and the provision of ecosystem services such as carbon sequestration (Vina and Liu 2017). It has been demonstrated (Tanaka and Tanaka 1982, Moeed and Mead 1985, Neumann 1979) that Coleoptera may be used as indicators of overall community structure, sometimes referred to as 'indicators of habitat quality'.

All functional groups of communities, ie. detritivores, herbivores, predators etc. are represented within this order, and the dynamics of sampling are better understood than with many other.

MATERIAL AND METHODS

Research regarding the distribution of the endangered insects species was carried out in 2017, in protected area Silvastepa Olteniei.

For the inventory of coleopteran species, several outcomes are needed on the ground. The most efficient method is generally the transept method. Movement is done on forest roads without a compact canopy or along the slit. The transits have a length of 500 m and a width of 20 m, and between the ends of two neighboring transects there must be a distance of 25 m. In case of habitats with small surface the transitions may be shorter and if the species has a very low density may be longer. If the surface of the habitat permits, five transects are made.

For our researches we have used the manual collection, directly from the habitat, of individuals, a method commonly used in faunistic studies and only in addition to research into the diversity and ecology of populations.

A positive aspect of this method is that it allows the collection of individuals in the target group without directly affecting other invertebrates. It is the simplest method for determining presence/absence data, while also allowing individuals to be easily identifiable on the field

RESULTS AND DISCUSSIONS

Geographically, the area is located in the western part of the Oltenia Plain, more precisely in the Desnatuiu Plain, in contact with the Piedmont Getica Platform. The land is generally horizontal, with more or less deep depressions and short slopes. The altitude of the terrain varies between 60 m and 230 m. Groundwater is located on terraces over 10m deep, approaching the surface only in the meadow.

The forest vegetation is specific to the phycoclimatic areas of the forest and the forest steppe. There is a predominance of cereals, garnishes, cere-garnites, which account for more than 80 % of forests, pedunculate oak, oak, russian oak and quartzine mixtures. The proposed site consists of 6 polygons, representing 6 forest bodies (the forest areas of Caprioara-Tencanau-Maracine, Tarnava-Intorsura, Perisor, Plenita and Verbicioara) and Seaca-Stiubei. Following our researches in the protected area Silvestea Olteniei, we have identified four endangered insects species listed on I.U.C.N. Red List: *Carabus hungaricus* Fabr., *Cerambyx cerdo* L., *Lucanus cervus* L. and *Hyles hippophae* Esp.

From the beetle species, two are saproxylic and has been identified and located their habitat in some forests from the protected area: Întorsura, Perișor, Târnavă, Mărăciunele, Știubeiului, Verbicioara and Plenița. The other beetle species has been located on several meadows near : Radovan, Vela, Bucovicior, Tencănuș, Vârvoru de Jos, While the lepidoperan species has been observed on the sunny banks of lake Ciutura as well the rivers and Terpezița and Desnățui (Tuca & al 2016).

Carabus hungaricus is a beetle species native to the Palearctic. The hungarian ground beetle's length is 22-28 mm. Black, and the head is densely dotted, the elytron densely granulated, slightly grossy. It has a wing cover with 3 rows of small pit, otherwise almost completely smooth. The last part of the lower mandibular palp is slightly shorter than the last before one. In the Protected Natural Area, taking into account the habitat requirements of the species and the observations on it, it is estimated that there are stable populations in the investigated distribution areas. The species was identified on several meadows near: Radovan, Vela, Bucovicior, Tencănuș, Vârvoru de Jos., *Cerambyx cerdo* L. The adult has a body length of 28-50 mm, being the largest species in the Cerambycidae family in our country. The female is larger than the male. The body is brownish or black-brown. The Pronot is glossy, with discoidal wrinkles. The abdomen is glossy, with rare puberty, excluding the last sternit, who has a thick puberty. The apode and eucephalus larvae is whiteish-yellowish, with a small head and reddish, and on the dorsal part of the body there are oval shaped chitinous plates, with a body length of 80-90 mm.

Specific information. In the Protected Natural Area, taking into account the habitat requirements of the species and the observations on it, it is estimated that there are stable populations in the investigated distribution areas.

The species was identified in the forests and forests of the forests: Intorsura, Perișor, Târnava, Mărăcinele, Știubeiului, Verbicioara and Plenița.

Lucanus cervus L. The stag beetle is one of the most spectacular looking beetle. The giant antler-like mandibles are used in courtship displays, and wrestling with other males. Although rather fearsome in appearance, the mandibles cannot be closed with any force. You are more likely to be nipped sharply by the female stag beetle, a smaller insect than the male that lacks the huge jaws. The stag beetle, superficially, appears black all over but, in certain lights, it can be seen to have dark maroon or brown wing cases. The impressive mandibles also have a reddish sheen to them. The wing cases are glossy; the head and thorax are a dull black.

In the Protected Natural Area, taking into account the habitat requirements of the species and the observations on it, it is estimated that there are stable populations in the investigated distribution areas. The species was identified in the forests and forests of the forests: Intorsura, Perișor, Târnava, Mărăcinele, Știubeiului, Verbicioara and Plenița.

Hyles hippophaes Esp. The adult has a robust body, covered with a brown pubescence, the wings are brown, the front wings have a darker oblique transverse band, the macules on the hind wings are reddish orange.

Adults of this species have fairly large variations in coloration, in fact the color of adults depends much on temperature. Thus the heat causes a more reddish coloration, while the hotter temperatures cause a darker coloration.

Specific information. In the Protected Natural Area, taking into account the habitat requirements of the species and the observations on it, it is estimated that there are stable populations in the investigated distribution areas. The species was observed on the sunny and vegetated shores of Lake Ciutura, the Desnățui and Terpezița.

CONCLUSIONS

Following our researches in the protected area Silvoștepa Olteniei, we have identified four endangered insects species listed on I.U.C.N. Red List: *Carabus hungaricus* Fabr., *Cerambyx cerdo* L., *Lucanus cervus* L. and *Hyles hippophae* Esp.

In the Protected Natural Area, taking into account the habitat requirements of the species and the observations on it, it is estimated that there are stable populations in the investigated distribution areas.

In the Protected Natural Area, we have identified all the coleopteran species in the forests: Intorsura, Perișor, Târnava, Mărăcinele, Știubeiului, Verbicioara and Plenița.

Regarding the lepidopteran species, it was observed on the sunny and vegetated shores of Lake Ciutura, the Desnățui and Terpezița.

REFERENCES

Andam, K. S., P. J. Ferraro, A. Pfaff, G. A. Sanchez-Azofeifa, and J. A. Robalino. 2008. Measuring the effectiveness of protected area networks in reducing deforestation. *Proceedings of the National Academy of Sciences* 105:16089–16094.

Hannah, L., G. Midgley, S. Andelman, M. Araujo, G. Hughes, E. Martinez-Meyer, R. Pearson, and P. Williams. 2007. Protected area needs in a changing climate. *Frontiers in Ecology and the Environment* 5:131–138.

Jenkins, C. N., and L. Joppa. 2009. Expansion of the global terrestrial protected area system. *Biological Conservation* 142:2166–2174.

Joppa, L. N., and A. Pfaff. 2009. High and far: biases in the location of protected areas. *PLoS One* 4:e8273.

Juffe-Bignoli, D., et al. 2014. Protected planet report 2014. UNEP-WCMC, Cambridge, UK.

Moeed, A. & Meads, M.J. 1985: Seasonality of pitfall trapped invertebrates in three types of native forest. Orongorongo Valley, New Zealand. *New Zealand Journal of Zoology* 12: 17-53.

Neumann, F.G. 1979: Beetle communities in eucalypt and pine forests in North-eastern Victoria. *Australian Forest Research* 9: 277-293.

Pimm, S. L., C. N. Jenkins, R. Abell, T. M. Brooks, J. L. Gittleman, L. N. Joppa, P. H. Raven, C. M. Roberts, and J. O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* 344:1246752.

Tanaka, L.K & Tanaka, S.K 1982. Rainfall and seasonal changes in arthropod abundance on a tropical oceanic island. *Biotropica* 14: 2, 114-123.

Tuca, O., Stan, C., Ciupeanu Călugăru, Eleonora Daniela. 2016. Anthropogenic threats on some insect species protected under the IUCN red list in the protected area Slvostepa Olteniei. *Analele Univ. din Craiova seria Biol., Horticol., T.P.P.A., I. Med; Vol . XXI (LIX): 643 – 647.*

Vina, A., Liu, J. 2017. Hidden roles of protected areas in the conservation of biodiversity and ecosystem services. *Ecosphere* Volume 8 Issue 6 e01864

STUDY ON FREQUENCY AND CAUSES OF ANEMIA

Vlădulescu Carmen^{1*}, Olaru Luminița Mariana²

^{1,2}University of Craiova, Faculty of Horticulture

*Corresponce author: E-mail: carmen_vldl@yahoo.com

Keywords: *anemia, etiology, hemoglobin, hemoleucogram.*

ABSTRACT

This paper presents the study of the incidence of anemia cases. Both anamnesis and physical examination provide important data for anemia frames. The anamnesis has as major objectives: age of anemia according to laboratory data, patient profession, eating habits, alcohol abuse, drugs, the coexistence of chronic diseases and their treatment, performing pretreatment anti-anemic treatments, the existence of blood loss, etc. Anemia is not a disease itself, but a symptom or syndrome that always has one or more causes.

INTRODUCTION

Anemia is defined as the decrease in the values of the parameters that estimate the amount of erythrocytes in the circulation: their number, their volume (Ht) and their content in Hb. Anemia is not a disease itself, but a symptom or syndrome that always has one or more causes that need to be detected and if possible removed before initiating specific therapy.

Clinical signal that suggests the existence of anemia is the flaking of the skin and mucous membranes. Pallor is also present in conditions accompanied by increase in plasma volume (hyperhydration, pregnancy, splenomegaly, macroglobulinemia). Since red blood cells, in these situations, remain normal, they are called pseudoanemia or relative anemia.

Hemoleucogram is the primary investigation that is mandatory in all cases where anemia is suspected. The quantitative assessment of circulating erythrocytes allows the assessment of the degree of anemia.

After Hb values, anemia can be classified as follows: mild anemia Hb 10-12 g/dl; moderate anemia Hb 7-10 g/dl; severe anemia Hb 5-7g/dl; anemia Hb<5 g/dl.

In investigating anemia, the following are mandatory:

- complete blood count - provides information on hemoglobin, hematocrit, erythrocyte counts, if anemia is associated with leukopenia and / or thrombocytopenia;

- determining the number of reticulocytes - helps to divide into two major categories anemias: regenerative and hypo/aregenerative. The regenerative ones are generally peripheral (posthemorrhagic, haemolytic), hypo/aregenerative ones are generally central cause by affecting medullary production (aplastic anemia or iron, vitamin B12 and folic acid deficiency anemia) the appearance of peripheral blood smear - provides information on erythrocytic morphology, hemoglobin loading, the presence of erythrocytes precursors (erythroblasts in peripheral blood) or atypical cells.

MATERIAL AND METHOD

A number of 133 patients was taken into study, who have been presented to the specialized private clinic for various investigations the study lasted 3 months.

Of the approximately 133 patients, we chose a group of 42 heteromorphic patients - women and men, with age between 22 and 78 years old, to whom we found hemoglobin changes in a first stage. The study was conducted on a group of 42 patients, 18 men (42.9 % of the total) and 24 women (57.1 % of the total). For each case, hemoglobin values were determined.

Later, we searched for further investigations to maintain the suspicion of a diagnosis of anemia and allow its classification in one of the many known anemias.

Hemoleucogram is the first stage of the laboratory diagnosis.

Harvesting is done by venous puncture, using special hemoleucograms, dry anticoagulants type Na₂EDTA or K₃EDTA and 2 ml of venous blood is harvested.

The complete hemogram includes: determination of hemoglobin, determination of hematocrit (percentage of mass of erythrocytes in blood volume) and number of hematites/mm³ (Manole Gh., 2004).

RESULTS AND DISCUSSIONS

The results obtained were corroborated with the age group of the patient, sex and background and hemoglobin values as follows:

Of the group of 42 patients, 35 came from urban areas (83.3 %) and 7 from rural areas (16.7 %).

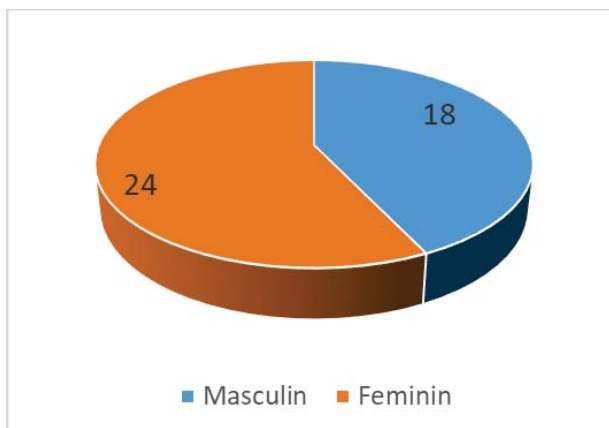


Figure 1. Distribution of cases by gender

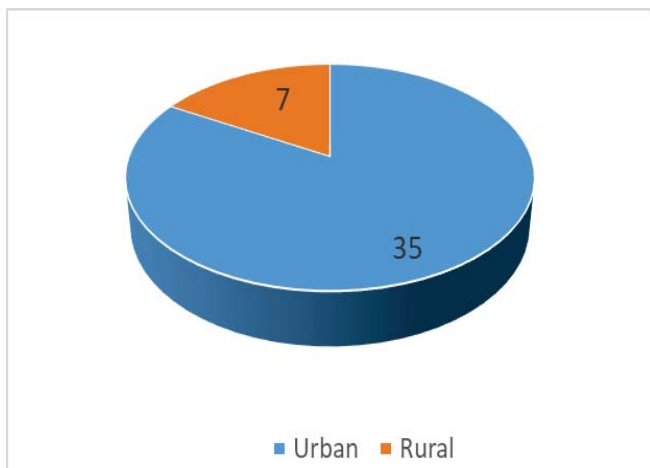


Figure 2. Distribution of cases according to the patient's home environment

It can be seen that the incidence of these anemias was higher in patients from urban areas, which is explained by their increased addressability to the doctor.

The distribution of cases by hemoglobin showed that:

- 1 patient had values between 6-7 g/100 ml;
- 5 patients had values between 7-8 g/100 ml;
- 5 patients had values between 8-9 g/100 ml;
- 12 patients had values between 9-10 g/100 ml;
- 8 patients had values between 10-11 g/100 ml;
- 7 patients had values between 11-12 g/100 ml;
- 4 patients had values between 12-13 g/100 ml.

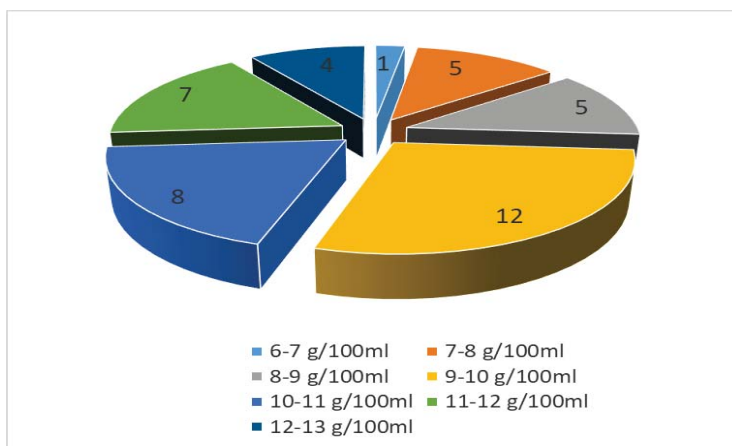


Figure 3. Case distribution based on hemoglobin value

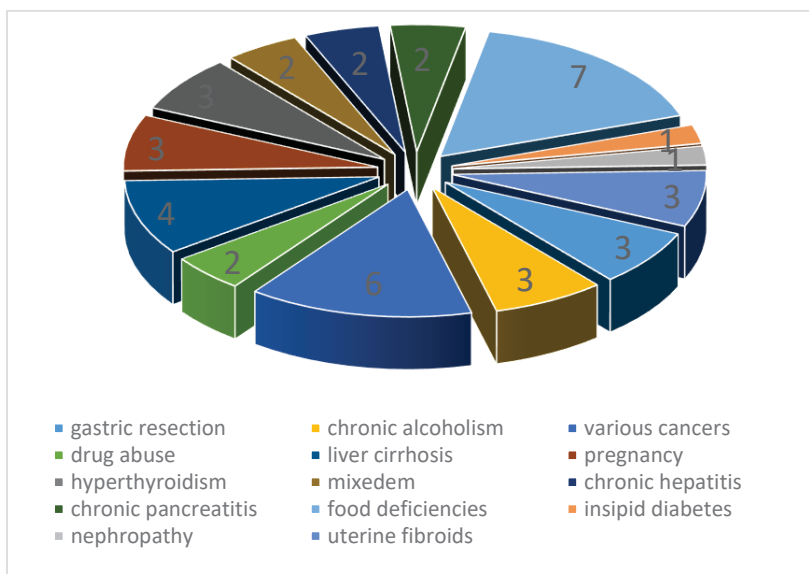


Figure 4. Case distribution according to pre-existing pathology

The study group, consisting of 42 patients, had a history of various personal pathologies. For 3 patients, anemia was associated with pregnancy, this being the most common form of anemia present during pregnancy. In other 3 cases, anemia was caused by prolonged bleeding caused by the presence of a benign tumor, respectively uterine fibroids.

CONCLUSIONS

Anemia has a very high incidence in the general population and can be determined by a number of particularly serious conditions, even cancers. Therefore, it is very important that every person performs at regular intervals, at 6 months, analyzes such as blood counts, which allow a rapid diagnosis.

Anemia is very common among pregnant women. Once anemia is determined, it is very important to determine the cause and therapeutic course. Due to hemodilution in pregnancy, anemia is determined at hemoglobin levels below 11 mg/% (Vlădăreanu R., 1999).

This should be treated given the loss of blood that occurs at birth or cesarean, which can aggravate a pre-existing anemia. Tests for anemia are reimbursed by the National Health House (www.cnas.ro), it may be prescribed by the family doctor himself.

REFERENCES

Manole Gh., Galatescu E.M., Mateescu M., 2004; Laboratory tests. Ed. Coresi, Bucharest, pp. 162-169.

Vlădăreanu Radu, 1999; Medical illnesses associated with pregnancy. Ed. InfoMedica, Bucharest, pp. 71-80.

*** www.cnas.ro

**THE EFFECTS OF *CANNABIS SATIVA* FOLIAR TISSUES ON SOIL
ORGANIC MATTER BIODEGRADATION AND OTHER SOIL CHEMICAL
PROPERTIES**

Wogiatzi Eleni¹, Gougoulas Nikolaos^{1*}, Giannoulis Kyriakos¹

¹Department of Agronomy Technology, Technological Educational Institute of Thessaly, 41110 Larissa, Greece

*Correspondence author. email: ngougoulas@teilar.gr

Keywords: *Cannabis*; soil chemical properties; soil organic matter biodegradation

ABSTRACT

The effects of leaf Cannabis on soil organic matter biodegradation and on soil chemical properties in an incubation experiment 15 weeks were studied. The air dried cannabis plant tissues incorporated at five different rates (0, 0.2, 0.4, 0.6 and 0.8 g per 50 g of soil mixture with 9.74g of manure) resulted increase in organic carbon mineralization, nitrogen nitrate and exchangeable potassium. The levels of the available forms of P and Mn was increased at the two higher rates of the added cannabis. The level of the available forms of Cu and Zn did not show statistically significant differences in comparison with the control at the end of the incubation period. Also, the addition of leaf Cannabis resulted in a increase in total forms of Na, K, P and Mn. The results of this study indicated that the leafs of Cannabis could be applied to the soil without negative effects on the soil chemical and biological properties.

INTRODUCTION

The addition of organic materials on the soil, affects biological activity of microflora and the soil composition. When applying of organic fertilizers in the soil, dominate microbial nutrition (Riber et al. 2014). In biological agriculture is mandatory to replace chemicals with natural additives. Between them, and the plant debris for their role in the soil fertility (Gravanis et al. 2005).

Our previous studies have shown, the effect of oregano and basil on degradation of organic fertilizers, and it was found that the presence of foliar tissues of oregano, act a slowing effect on degradation of organic fertilizer, while the presence of basil, accelerate it (Chouliaras et al. 2007, Gougoulas et al. 2010).

The *Cannabis sativa* plant, contain over 60 different cannabinoids, with delta-9-tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD) being the most prominent among them (Fairbairn and Liebmann 1974, Hillig and Mahlberg 2004).

The objectives of this research were to study in vitro, if the application of foliar tissues of the cannabis dry matter into soil has an effect on soil organic matter and on soil chemical properties.

MATERIAL AND METHODS

The collection of leaves *Cannabis sativa* took place at the end of the growing season from the experimental farm of Technological Educational Institute of Thessaly, Larissa, Greece.

Incubation experiment: In this study, 9.74 g of manure containing 3 g of organic matter, obtained from the farming establishments of TEI of Thessaly, was added to 50 g of air-dried soil that was poor in organic matter, derived from the same region (Table 1). Into 50 g of this soil plus 9.74 g of the manure, 0, 0.2, 0.4, 0.6 and 0.8 g of air-dried and well milled of leaf tissues *Cannabis sativa* were added. Thus an experimental unit is constituted by 50 g of soil, 9.74 g of manure and a variable amount of *Cannabis sativa*. In the incubator, the treatments kept at 28 °C for a period of 15 weeks and were prepared in four replicates. During the first three weeks of the incubation period, the moisture was maintained at two-thirds of field capacity, but for the next three weeks the soils were left to dry. This process was repeated until the end of the incubation period according to (Wu and Brookes, 2005) they reported that the alternation of drying and rewetting soil samples enhances mineralization of both soil biomass organic matter and non-biomass organic matter. At the end of the incubation period, soil samples were analyzed.

Methods of analyses: Samples were analyzed using the following methods which are referred by (Page et al. 1982).

Organic matter was analyzed by chemical oxidation with 1 mol/l $K_2Cr_2O_7$ and titration of the remaining reagent with 0.5 mol/l $FeSO_4$.

Exchangeable forms of potassium and sodium were extracted with 1 mol/l CH_3COONH_4 and measured by flame photometer.

Organic phosphorus was measured after mineralization by combustion of the sample and subtraction of the mineral phosphorus amounts, which had previously been estimated in the laboratory. The mineral amounts were extracted with 1 mol/l H_2SO_4 and all forms were measured by spectroscopy.

Both ammonium and nitrate nitrogen were extracted with 0.5 mol/l $CaCl_2$ and estimated by distillation in the presence of MgO and Devarda's alloy, respectively.

Available P forms (Olsen P) was extracted with 0.5 mol/l $NaHCO_3$ and measured by spectroscopy.

Available forms of Cu, Zn, and Mn were extracted with DTPA (diethylene triamine pentaacetic acid 0.005 mol/l + $CaCl_2$ 0.01 mol/l + triethanolamine 0.1 mol/l) and measured by atomic absorption.

For the determination of total metals 1 g of material, digestion at 350 °C + 10 ml HNO_3 + 5 ml $HClO_4$. According to the method described by (Varian, 1989), the samples were analyzed by Atomic Absorption (Spectroscopy Varian Spectra AA 10 plus), with the use of flame and air-acetylene mixture.

Statistical analysis: Data analysis was made using the MINITAB (Ryan et al. 2005) statistical package. Analysis of variance was used to assess treatments effect. Mean separation was made using Tukey's test when significant differences ($P=0.05$) between treatments were found.

Table 1
Chemical properties of soil samples, manure and leaf *Cannabis* used in the experiment

Property	Soil	*Manure	<i>Cannabis sativa</i> (Air dried material)
Texture	Sandy Loam		
pH	7.94 ± 0.3	8.35 ± 0.33	
EC, dS/m	0.29 ± 0.05	2.97 ± 0.16	
Organic matter (%)	0.79 ± 0.05	30.8 ± 1.4	
CaCO ₃ (%)	7.58 ± 0.6		
N -Total (g/kg)	1.21 ± 0.06	11.22 ± 0.61	25.76 ± 1.1

N-NH ₄ ⁺ (mg/kg)	39.2 ± 3.8		
N-NO ₃ ⁻ (mg/kg)	98.4 ± 8.2		
K-exchangeable (mg/kg)	219.6 ± 6.7		
K-Total (g/kg)	4.66 ± 0.22	19.23 ± 0.98	16.79 ± 0.80
Na-exchangeable (mg/kg)	112.3 ± 4.6		
Na-Total (g/kg)	0.28 ± 0.01	8.20 ± 0.39	0.16 ± 0.02
CEC (cmol/kg)	20.7 ± 1.3		
P -Olsen (mg/kg)	11.6 ± 3.3		
P -Total (g/kg)	0.36 ± 0.09	6.97 ± 0.39	3.33 ± 0.25
Cu -DTPA (mg/kg)	0.72 ± 0.06		
Zn -DTPA (mg/kg)	0.73 ± 0.08		
Mn -DTPA (mg/kg)	2.15 ± 0.11		
Cu -Total (mg/kg)	10.13 ± 0.48	29.27 ± 1.39	4.78 ± 0.23
Zn -Total (mg/kg)	37.45 ± 2.68	198.5 ± 9.02	27.9 ± 1.27
Mn -Total (mg/kg)	522 ± 37.3	75.3 ± 4.14	42.6 ± 2.14
Fe-Total (mg/kg)		5290 ± 289	129.0 ± 7.59
Mg-Total (mg/kg)			8748.0 ± 460.4

* Digested manure four months; Electrical conductivity, (EC) and soil pH is determined in (1:5) soil/water extract; Data represent average means and SE deviation. (n)=4.

RESULTS AND DISCUSSIONS

Decomposition of organic matter was increased with the increase of the leaf Cannabis concentration in soil, as compared with the control (soil plus manure). In particular, the decomposition of organic matter in soil where the three higher rates of the leaf cannabis were incorporated, were higher from 28.8 to 73.03 %, compared to the control (Figure 1). However, was not significantly affected by the smaller quantity addition of leaf cannabis.

The addition of dry plant tissues of Cannabis in the mixture (soil plus manure) increased nitrate nitrogen in comparison with the control, at all treatments at the end of the incubation period, while greater increase was observed with the addition of the highest rate. Contrast, ammonium was decreased by the addition of dry plant tissues of cannabis (Table 2).

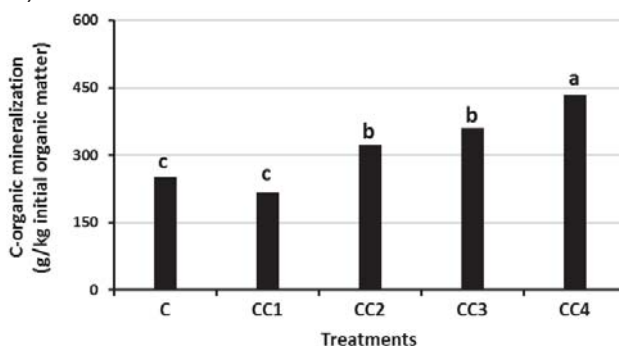


Figure 1. Effect of dry leaf Cannabis added rates on soil organic C mineralization.

Columns in each characteristic of the graph with the same letter do not differ significantly according to the Tukey's test ($P=0.05$). C, control (soil plus manure); CC1, CC2, CC3 or CC4, control and cannabis 0.2, 0.4, 0.6 or 0.8g, respectively.

The available forms of phosphorus and manganese was increased by the addition of dry leaf tissues Cannabis in the two highest rates, and it was not significantly affected by the addition lower rates, of dry leaf tissues of Cannabis (Table 2). Furthermore, available forms of Cu and Zn by the addition of Cannabis in the mixture (soil plus manure), did not show statistically significant differences in comparison with the control at the end of the incubation period, at all treatments (Table 2).

Table 2

Effect of Cannabis dry matter concentration on soil available forms of elements

Treatments	Available forms			DTPA		
	P-Olsen	N-NH ₄ ⁺	N-NO ₃ ⁻	Cu	Zn	Mn
	(mg/kg soil)					
C	197.2b	93.8a	138.0d	0.95a	7.06a	2.28b
CC1	211.5b	16.7c	222.0bc	0.95a	6.59a	2.37b
CC2	204.3b	43.1b	234.2b	0.95a	7.19a	2.98ab
CC3	243.4a	32.3bc	269.2b	0.86a	6.47a	3.12a
CC4	242.9a	51.7b	317.5a	0.95a	6.95a	3.33a

For each chemical property of soil mixtures, columns of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). C, control (soil plus manure); CC1, CC2, CC3 or CC4, control and leaf Cannabis 0.2, 0.4, 0.6 or 0.8 g, respectively.

The analysis of variance performed for the organic phosphorus and exchangeable form of potassium the data indicated that the organic phosphorus and exchangeable potassium was significantly increased by the added cannabis at the end of the incubation period at all rates (Table 3). However, exchangeable Na was not significantly affected by the addition of dry plant tissues Cannabis. In particular, the exchangeable form of sodium increased only from the highest rate of the added cannabis by 13.3 % in compared to the control. Electrical conductivity increased at the end of the incubation period from the two highest rates of dry plant tissues of the added Cannabis. In particular, the greatest increase was caused by the highest rate of the added cannabis by 53.6 % in compared to the control (soil plus manure), without any risk of soil salinity. This increase is due in conditions highest intensity biodegradation of soil organic matter. Contrary, the addition of the higher rate of Cannabis reduced the pH of the soil in compared to the control (soil plus manure) at the end of the incubation period. The decrease of soil pH at the end of the incubation period, probably is due to the stronger decomposition of soil organic matter, and in the oxidation of the N-NH₄⁺ to N-NO₃⁻ (Table 3).

Table 3

Effect of Cannabis dry matter concentration on soil chemical properties

Treatments	Exchangeable forms		Organic	EC	pH
	Na	K	P	Extract (1:5) soil/water (dS/m)	
	(mg/kg soil)				
C	173.6b	698.1e	361.8c	0.82c	7.39a
CC1	173.6b	772.2d	617.2a	0.79c	7.42a
CC2	181.2b	867.4c	707.1a	0.90bc	7.38a
CC3	185.2ab	920.3b	673.4a	0.96b	7.36a
CC4	196.8a	1036.6a	647.0a	1.26a	7.28b

For each chemical property of soil mixtures, columns of table with the same letter do not differ significantly according to the Tukey's test (P=0.05). C, control (soil and manure); CC1, CC2, CC3 or CC4, control and leaf Cannabis 0.2, 0.4, 0.6 or 0.8 g, respectively.

The addition of dry plant tissues Cannabis in the mixture (soil plus manure) increased total form of phosphorus at the end of the incubation period at comparison to the control, at all rates (Table 4), while, total forms of sodium, potassium and manganese was increased from the addition of the two highest rates of dry plant tissues Cannabis. However, total forms of copper and zinc was not significantly affected by the addition dry plant tissues Cannabis at the end of the incubation period at comparison to the control.

Table 4

Effect of Cannabis dry matter concentration on soil total forms of elements

Treatments	Total forms					
	Na	K	P	Cu	Zn	Mn
	(g/kg soil)			(mg/kg soil)		
C	0.422b	5.74b	1.131b	19.22a	64.21a	643.4b
CC1	0.437b	6.11b	1.414a	18.75a	61.78a	631.7b
CC2	0.444b	6.32ab	1.461a	19.34a	66.64a	655.9b
CC3	0.489a	6.67a	1.459a	19.66a	62.89a	698.3a
CC4	0.498a	6.98a	1.433a	19.42a	67.56a	687.8a

For each chemical property of soil mixtures, columns of table with the same letter do not differ significantly according to the Tukey's test ($P=0.05$). C, control (soil plus manure); CC1, CC2, CC3 or CC4, control and leaf Cannabis 0.2, 0.4, 0.6 or 0.8 g, respectively.

CONCLUSIONS

Four different rates of leaf Cannabis biomass were applied into a soil amended with manure, and after an Incubation experiment 15 weeks the effects on the soil chemical properties were attested. These results confirm that, the leaf Cannabis biomass it is a valuable material for soil amendment as improves soil chemical and biological properties, while could be applied as an acceleration agent for soil organic matter biodegradation.

REFERENCES

- Chouliaras N., Gravanis F., Vasilakoglou I., Gougoulias N., Vagelas I., Kapotis T., Wogiatzi E., 2007, The effect of basil (*Ocimum basilicum* L.) on soil organic matter biodegradation and other soil chemical properties. *Journal of the Science of Food and Agriculture*, 87(13), 2416-2419.
- Fairbairn J.W., Liebmann J.A., 1974, The cannabinoid content of *Cannabis sativa* L grown in England. *Journal of Pharmacy and Pharmacology*, 26(6), 413-419.
- Gougoulias N., Vagelas I., Vasilakoglou I., Gravanis F., Louka A., Wogiatzi E., Chouliaras N., 2010, Comparison of neem or oregano with thiram on organic matter decomposition of a sand loam soil amended with compost, and on soil biological activity. *Journal of the Science of Food and Agriculture*, 90(2), 286-290.
- Gravanis F. T., Chouliaras N., Vagelas I. K., Gougoulias N., Sabani P., Wogiatzi, E., 2005, The effect of oregano as an alternative soilborne pathogen control, on soil organic matter biodegradation and other soil chemical properties. In *The BCPC International Congress*, UK (pp. 105-108).
- Hillig K.W., Mahlberg P.G., 2004, A chemotaxonomic analysis of cannabinoid variation in *Cannabis* (Cannabaceae). *American Journal of Botany*, 91(6), 966-975.
- Page A.L., Miller R.H., Keeney D.R., 1982, *Methods of Soil Analysis. Part 2, Chemical and Microbiological properties*. Agronomy (9), ASSSA, Mad. Wisc. USA.

Riber L., Poulsen P.H., Al-Soud W.A., Hansen L.B.S., Bergmark L., Brejnrod A. and Sørensen S.J., 2014, Exploring the immediate and long-term impact on bacterial communities in soil amended with animal and urban organic waste fertilizers using pyrosequencing and screening for horizontal transfer of antibiotic resistance. *FEMS microbiology ecology*, 90(1): 206-224.

Ryan B.F., Joiner B.L., Cryer J.D., 2005, *MINITAB Handbook: Updated for release 14*, 5th edition. Brooks/Cole-Thomson Learning Inc., Kentucky, KY.

Varian M., 1989, *Flama Atomic Absorption Spectroscopy. Analytical Methods*. Varian Australia. Publ. NO: 85-100009-00.

Wu J., Brookes P.C., 2005, The proportional Mineralisation of Microbial Biomass and Organic Matter caused by air-drying and rewetting of a grassland soil. *Soil Biology & Biochemistry*, 37, 507-515.