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HISTO-ANATOMICAL AND PRELIMINARY PHYTOCHEMICAL RESEARCHES ON *SCUTELLARIA ALTISSIMA* L. (*LAMIACEAE*) SPECIES

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Keywords: *Scutellaria altissima L., histo-anatomy, polyphenols, thin layer chromatography*

ABSTRACT

In this paper, the authors highlight the results of the histo-anatomical analysis on the root, rhizome, aboveground stem and leaf harvested from Scutellaria altissima L. species, alongside the chromatographic fingerprint evidenced by the polyphenol content of the aerial part (Scutellariae altissimae herba). Thin layer chromatogram includes 13 bands due to the polyphenols, which one chlorogenic acid derivative was identified.

INTRODUCTION

Scutellaria altissima L., Somerset skullcap, Tall skullcap, *Lamiaceae* family, is a perennial herbaceous plant, 30–100 cm high, with purple flowers in May–July, spontaneous in Pontic and Mediterranean areas (Ciocârlan 2000).

Scutellaria species contain important active principles, such as: flavonoids (Shang et al. 2010), diterpenoids (Bruno et al. 1996, Dai et al. 2016, Malakov et al. 1996, Shang et al. 2010), phenylethanoid glycosides (Bardakci et al. 2015), essential oil (Formisano et al. 2013), lignan glucosides (Long et al. 2016), polysaccharides (Gaochen et al. 2011, Ye and Huang 2012), carotenoids (Liu et al. 2014).

The extracts from *Scutellaria* sp. exhibit various pharmacological actions: antitumoral and cytotoxic, inducing cell cycle arrest and apoptosis (Boozari et al. 2015, Dai et al. 2016, Gao et al. 2011, Gaochen et al. 2011, Park et al. 2014, Xue et al. 2016), antioxidant (Bardakci et al. 2015, Ye and Huang 2012), antiviral against H1N1 virus (Ji et al. 2015), protective against lipopolysaccharide-induced acute liver injury in mice (Thanh et al. 2015), anti-inflammatory (Cuong et al. 2015) and anti-allergic on inflammation *in vivo* and *in vitro* (Jung et al. 2012), anti-osteoporotic (Long et al. 2016), for the prevention of Alzheimer disease and vascular dementia (Hwang et al. 2011), antifeedant on *Spodoptera littoralis* (Formisano et al. 2013).

In the specialty papers, there are some data concerning *S. altissima* histo-anatomy (de Oliveira et al. 2013) and chemical composition (Shang et al. 2010). The aim of our paper was the histo-anatomical investigation of the root, stem and leaf of the above-mentioned species and the preliminary analysis of the polyphenols content from the aerial parts (*Scutellariae altissimae herba*).

MATERIAL AND METHODS

Histo-anatomical analysis

The vegetal material was harvested from *S. altissima* plants in blossom, in May 2016, from the surroundings of Radovan village (Valea Rea zone), Dolj County (south-west of Romania).

The fixation and preservation of roots, aboveground stems and leaves were achieved in 70% ethanol. Using botanical razor, the cross-sections and longitudinal-radial sections were obtained.

After washing with distilled water, the sections were clarified using 10% sodium hypochlorite solution (Javel water). Then, the clarifying agent was removed by washing with distilled water. Congo red–chrysoidine mixture (Genevese reagent) was used for the staining of sections. Depending on the chemical composition of cell membranes, the reactive induced various stains: pink to red for cellulose and mucilage, 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 (objectives ×4, ×10, ×40) and then photographed using a Sony DSLR-A380 digital system adapted to the microscope.

The description of microscopic sections was accomplished according to some classical authors (Toma & Rugină 1998).

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols was performed on the aerial parts of *S. altissima* species (*Scutellariae altissimae herba*), using a CAMAG (Muttenz, Switzerland) system in the following experimental conditions: stationary phase TLC silica gel 60 F_{254} (Merck, Darmstadt, Germany) 20×10 glass plates pre-washed with chloroform–methanol (1:1, *v/v*); mobile phase chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10:10:1, in volumes); sample – 20% methanolic extract of *Scutellariae altissimae herba*; standards (Merck) – 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin; migration distance 80 mm; sample (1–10 µL) and standards (2 µL) application – CAMAG Linomat 5 semiautomatic system (spray gas nitrogen, dosage speed 150 nL/s, band length 8 mm); detection – CAMAG TLC Scanner 3 photodensitometer, UV 254 nm, without derivatization, deuterium–wolfram lamp, scanning speed 20 mm/s, data resolution 100 µm/step, measurement mode – absorption; winCATS software package (Altemini et al. 2015, Bojić et al. 2013, Gîrd et al. 2014).

RESULTS AND DISCUSSIONS

Histo-anatomical analysis

Root

In cross-section, the root has circular shape and secondary structure due to the presence of libero-ligneous cambium. From the outside towards the inside of the root, the following histological sequence was achieved. A single layer of

rhizodermis, peeled from place to place, shows long absorbent hairs. Primary cortex is well represented, consisting of exodermis, cortical parenchyma and endodermis of primary type. Exodermis is made of a single layer of large polyhedral cells, with suberin-impregnated walls and passage cells opposite to the absorbent hairs. Cortical parenchyma is well represented, consisting of large parenchyma cells, delimiting small intercellular meatuses. Some cells accumulate ergastic substances. From place to place, in the central area of cortical parenchyma, sclerenchyma fibers are observed. Endodermis is made of a single cellular layer with cellulose-impregnated walls and passage cells at some intervals. Conducting tissues are arranged in two concentric rings. Phloem forms a thin, external ring, consisting of sieve tubes, phloem parenchyma and annex cells. Libero-ligneous cambium is placed between xylem and phloem tissues. Xylem tissue forms the internal ring, consisting of some secondary xylem vessels of different sizes, disseminated into the libriform tissue. In longitudinal-radial sections, xylem vessels exhibit reticulate, ringed and spiral thickenings. Primary xylem vessels are pushed toward the center near the medullary parenchyma. Medullary rays are multicellular, uniseriate, cellulosic into the phloem ring, and multicellular, uniseriate, lignified at the xylem ring level. Medullary parenchyma is well developed and lignified (Figures 1–3).

Figure 1. Cross-section through *S. altissima* root: overview (Congo red–chrysoidine staining, ×40).

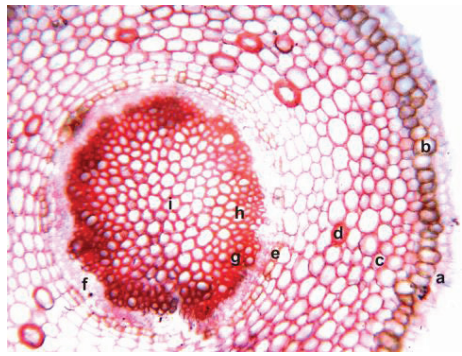

Figure 2. Cross-section through *S. altissima* root: (a) rhizodermis; (b) exodermis; (c) cortical parenchyma; (d) sclerenchyma fiber; (e) endodermis; (f) secondary phloem tissue; (g) secondary xylem vessel; (h) libriform tissue; (i) medullary parenchyma (Congo red–chrysoidine staining, ×100).

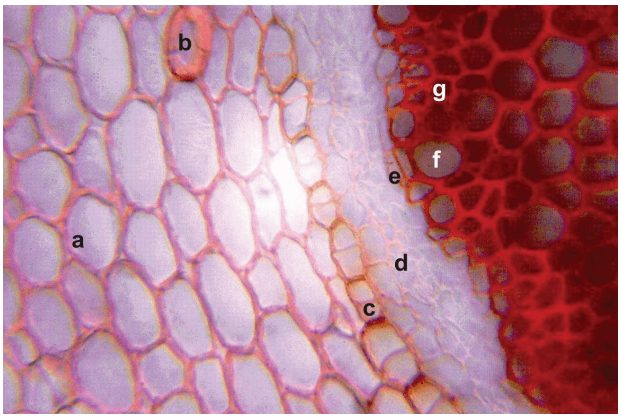

Figure 3. Cross-section through *S. altissima* root: (a) cortical parenchyma; (b) sclerenchyma fiber; (c) endodermis; (d) secondary phloem tissue; (e) libero-ligneous cambium; (f) secondary xylem vessel; (g) libriform tissue (Congo red–chrysoidine staining, ×400).

Aboveground stem

In cross-section, into the upper third, the aboveground stem has quadrate shape with four prominent ribs and secondary structure generated by libero-ligneous cambium. Epidermis consists of heterodiametric cells, with thickened tangential external wall domed and covered by a thin cuticle. Epidermal cells are anterior-posterior flattened, with thin radial walls and thickened tangential external and internal walls. From point to point, we found stomata, multicellular uniseriate tector hairs, sharp at the tip, multicellular oval glandular hairs with small epidermal foot, and long, multicellular, uniseriate glandular hairs with a cupuliform head. Cortex is differentiated in angular collenchyma cords arranged in protruding ribs and inter-costal chlorenchymatic tissue. Conducting tissues are organized into four large collateral-open libero-ligneous fascicles disposed opposite ribs and some small libero-ligneous fascicles placed intercostal and generated by the interfascicular libero-ligneous cambium. Conducting fascicles are flanked at the phloemic pole by one sclerenchyma cap. Libero-ligneous cambium is intra- and inter-fascicular. Phloem tissue is made up of sieve tubes, phloem parenchyma and annex cells. At this level, medullary rays are large, multicellular, pluriseriate, cellulosic. Secondary xylem tissue is made up of xylem vessels of different sizes and well represented libriform tissue. In longitudinal-radial sections, xylem vessels have spiral, ringed and reticulate thickenings. Primary xylem is poorly represented, consisting of some protoxylem vessels accompanied by xylem parenchyma. Xylem vessels are seriate arranged. Large medullary rays are multicellular, pluriseriate and lignified. Medullary parenchyma is well developed, of meatus type (Figures 4–8).

In cross-section, into the lower third, the aboveground stem has circular-ribbed shape and secondary structure generated by libero-ligneous cambium. Histological elements are identical at the cortex level, the difference being determined by the activity of annular libero-ligneous cambium, generating conductive tissues organized in two concentric rings. Through cambium activity results a thin external ring of secondary phloem made of sieve tubes, phloem parenchyma and annex cells, and a thick internal ring of secondary xylem consisting of xylem vessels

scattered into libriform tissue. At the level of xylem tissue, medullary rays are sclerified and lignified (Figures 4–8).

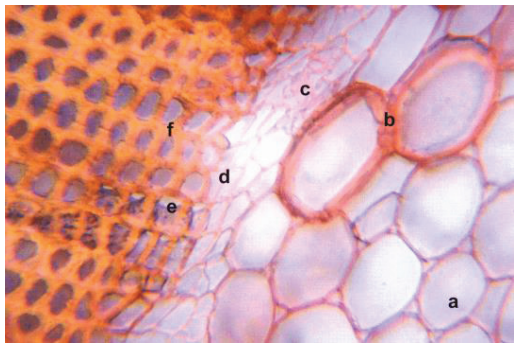

Figure 4. Cross-section through *S. altissima* aboveground stem: (a) cortical parenchyma; (b) sclerenchyma cap; (c) secondary phloem tissue; (d) libero-ligneous cambium; (e) secondary xylem vessel; (f) libriform tissue (Congo red–chrysoidine staining, ×400).

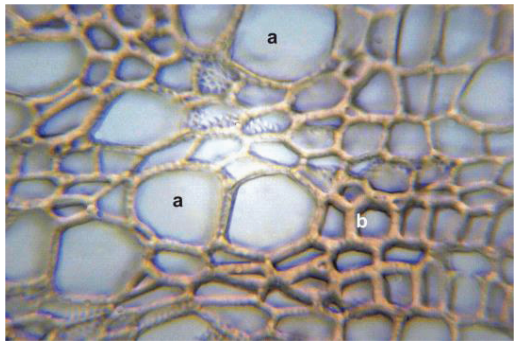

Figure 5. Cross-section through *S. altissima* aboveground stem: (a) secondary xylem vessel; (b) libriform tissue (Congo red–chrysoidine staining, ×400).

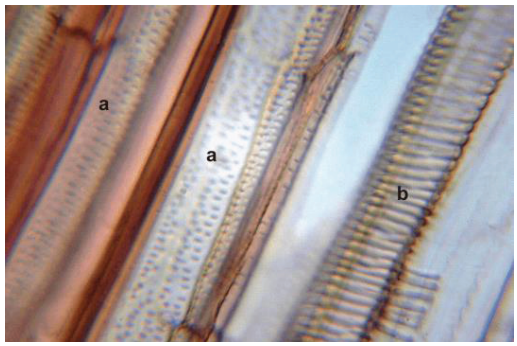

Figure 6. Longitudinal-radial section through *S. altissima* aboveground stem: (a) reticulate xylem vessel; (b) spiral xylem vessel (Congo red–chrysoidine staining, ×400).

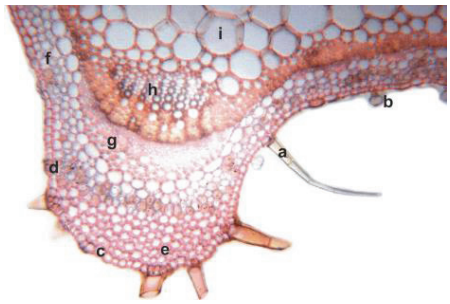

Figure 7. Cross-section through *S. altissima* aboveground stem: (a) tector hair; (b) glandular hair; (c) epidermis; (d) stomatal apparatus; (e) angular collenchyma; (f) chlorenchyma; (g) sclerenchyma cap; (h) libero-ligneous conducting fascicle; (i) medullary parenchyma (Congo red–chrysoidine staining, ×100).

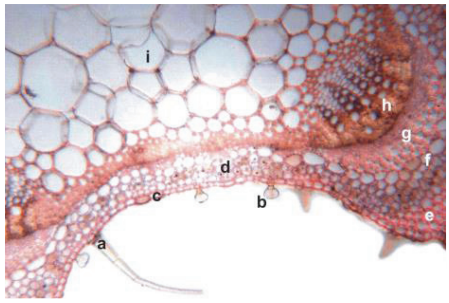

Figure 8. Cross-section through *S. altissima* aboveground stem: (a) tector hair; (b) glandular hair; (c) epidermis; (d) chlorenchyma; (e) angular collenchyma; (f) cortical parenchyma; (g) sclerenchyma cap; (h) libero-ligneous conducting fascicle; (i) medullary parenchyma (Congo red–chrysoidine staining, ×100).

Leaf

Leaf's limb

In cross-section, from the outside to the inside of leaf's limb, the following histological sequence is observed. A single layer of upper epidermis is made of flattened large cells with thickened tangential external and internal walls and thin radial walls. External walls are bulged and covered by a thin cuticle. Mesophyll is organized in a single layer of palisade parenchyma made of small-elongated cells, rich in chloroplasts, as well as of 3–4 layers of lacunose parenchyma consisting of disorderly-arranged small cells with aeriferous spaces. Many small libero-ligneous fascicles, each surrounded by an assimilatory fascicular sheath, are found into the mesophyll. Mesophyll has bifacial dorsiventral structure. Lower epidermis is made of a single layer of tangential elongated cells, with thin radial walls and thickened tangential external and internal walls. At this level, we found stomata and round multicellular glandular hairs with a short leg. The median rib looks as a prominent trough on abaxial side. The outer epidermis is made of small cells, slightly anterior-posterior flattened, with external wall covered with cuticle. At the abaxial pole, in hypodermic position, there are 2–3 layers of angular colenchyma. A single libero-ligneous conducting fascicle is located in the central zone, in a mesophyll mass. Into the libero-ligneous fascicle, xylem vessels are seriate disposed and the

medullary rays are uniseriate, cellulosic. The leaf's limb has hypostomatic bifacial dorsiventral structure (Figure 9).

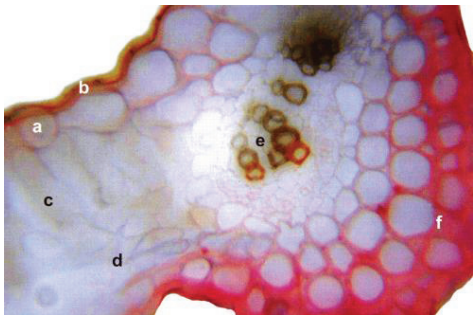

Figure 9. Cross-section through *S. altissima* leaf's limb: (a) epidermis; (b) cuticle; (c) palisade parenchyma; (d) lacunose parenchyma; (e) libero-ligneous conducting fascicle; (f) angular collenchyma (Congo red–chrysoidine staining, ×400).

Petiole

Petiole has corded shape, with two adaxial wings. In cross-section, from the outside to the inside of petiole, the following histological sequence is highlighted. A single epidermal layer consists of heterodiametric small cells, with thin radial walls and thickened tangential external and internal walls. Tangential external wall is bulging and covered by cuticle. From place to place, we found stomata, multicellular, uniseriate, sharp tector hairs and multicellular round glandular hairs with short epidermal foot. Angular collenchyma is organized into 1–2 layers arranged sub-epidermal. Into the fundamental parenchyma are found 4–6 libero-ligneous fascicles with various sizes. In the central area are placed 2–4 large libero-ligneous fascicles and one fascicle in each of the two wings (Figure 10).

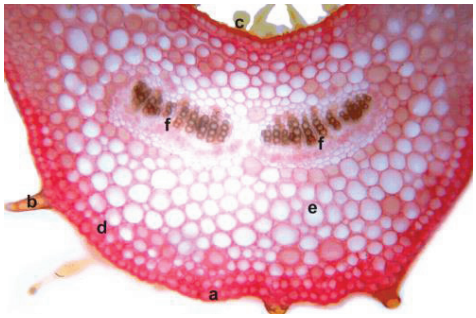

Figure 10. Cross-section through *S. altissima* petiole: (a) epidermis; (b) tector hair; (c) glandular hair; (d) angular collenchyma; (e) fundamental parenchyma; (f) conducting fascicle (Congo red–chrysoidine staining, ×100).

TLC analysis

The experimental data on the preliminary TLC analysis of polyphenols from *Scutellariae altissimae herba* are highlighted in Figures 11–13. Chlorogenic acid derivative (R_f 0.16) was identified in an amount of 122 mg/100 g of dried vegetal product.

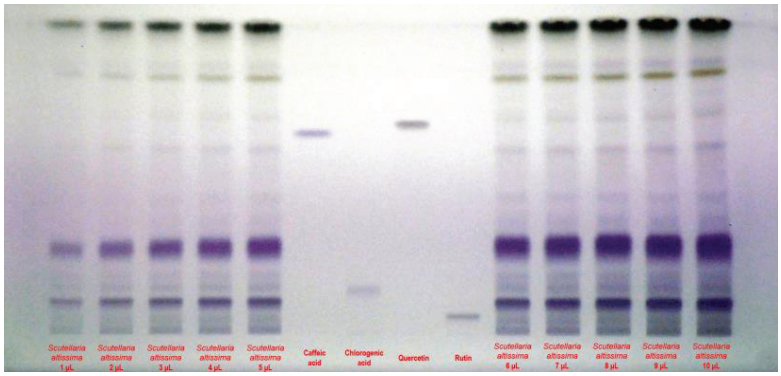

Figure 11. TLC chromatogram of polyphenols from *Scutellariae altissimae herba* methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

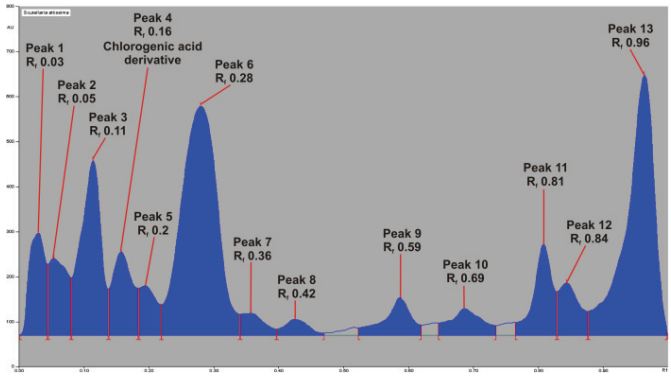

Figure 12. Densitogram of polyphenols (UV 254 nm) separated from *Scutellariae altissimae herba* methanolic extract.

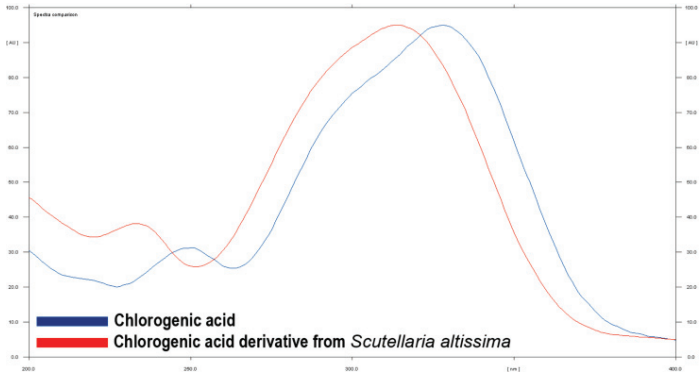

Figure 13. Chlorogenic acid derivative *in situ* UV spectra of standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigation of the root, stem and leaf of *Scutellaria altissima* species and the preliminary TLC analysis of *Scutellariae altissimae herba* polyphenols were made. The root has circular shape and secondary structure due to the presence of libero-ligneous cambium. The aboveground stem has quadrate shape with four prominent ribs (into the upper third), circular-ribbed shape (into the lower third), and secondary structure generated by libero-ligneous cambium. Leaf's limb has hypostomatic bifacial dorsiventral structure. Petiole has corded shape, with two adaxial wings. TLC highlighted 13 bands due to the polyphenols, which one chlorogenic acid derivative was identified.

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METCALFA PRUINOSA (HOMOPTERA: FULGOROIDEA), NEW AND DANGEROUS PEST FOR LAWN IN OLTENIA

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Keywords: *Metcalfa pruinosa Say*, *insect, identifying*

ABSTRACT

Melliferous *cicada Metcalfa pruinosa Say 1830 is a homopter which belongs to Flatidae family, native from the east side of North America, wide-spread both in the United States, Mexico and Cuba. In Europe it was mentioned in 1979 on a number of trees and ornamental shrubs. The high capacity to adapt to the areas with temperate continental climate conditions has made possible its rapid spread across the entire European continent: France (1987), Spain in 1988, Slovenia (1991), Switzerland (1993), Great Britain (1994), Austria (1996), Croatia (2000), Czeck Republic (2001), Greece (2002), Montenegro (2003), Turkey (2004), Hungary (2004), Bulgaria (2006), Serbia (2003), Bosnia Herzegovina (2007), Netherlands (2009), Romania (2009). Starting with May 2016 its presence is signaled on a series of herbaceous and woddy plants from the lawn in Craiova city, having a higher frequency on the ornamental species.*

INTRODUCTION

Metcalfa pruinosa Say 1830, popularly known as plum cicada is an invasive species, native from the south part of Nort America, widespread from here to the United States, Mexico and Cuba.

In Europe, *Metcalfa pruinosa* was signaled for the first time by Zangheri and Donadini (1980), in the north part of Italy, wherefrom was widespread later in the majority of countries: France - Della Giustina (1987), Spain in 1988 (Pons et al. 2002), Cehia 2001 (Lauterer 2002), Slovenia (Sivic 1991), Elveţia 1993 (Jermini et al. 1995), Marea Britanie (Malumphy et al. 1994), Austria (Holzinger et al. 1996), Croaţia (Alma 2000), Grecia 2002 (Drosopoulos et al. 2004), Muntenegru (Hrncic 2003), Turcia (Karsavuran and Guclu 2004), Ungaria (Orosz and Der 2004), Bulgaria (Trenchev et al. 2007), Serbia 2006 (Ljubodrag 2007), Bosnia Herţegovina (Gotlin Culjak et al. 2007, cited by Preda and Skolka 2009). In Romania *Metcalfa pruinosa* has been indentified for the first time by Preda and Skolka (2009).

The adult insect of *Metcalfa pruinosa* Say, 1830, although a homopter, presents a characteristic aspect, despite it can easily be confused with a Lepidoptera insect from Pyralidae family (according to Lauterer, 2002).

The insect body is covered with an obvious serous secretion both in adult and larva stages. The body color varies with different shades from brown to gray.

The above wings present almost straight outer margins with an apex and tornus almost symmetrical with two characteristic blackish spots in the basal part (Figure 1 and 2).

Figure 1. *Metcalfa pruinosa* - adults (orig.)

Figure 2. *Metcalfa pruinosa* adult on *Paulownia tomentosa* leave (orig.).

The body lenght in adults case varies between 7 and 8 mm. Mead, 2004, noting that in the native area this can be smaller (5.5 mm).

Cristina Preda and M. Skolka, 2009, mention that sexual dimorphism is poorly differentiated, so males cannot be distinguished from females.

On host plants the adults can be easily reached by their specific way of disposing on twigs, having a gregarious behaviour (Figure 3 and 4) and by the presence of waxy filaments (Figure 4).

Figure 3. *Metcalfa pruinosa:* group of adults on *Paulownia tomentosa* plants.

Figure 4. *Metcalfa pruinosa,* group of adults on *Hibiscus syriacus* plants.

Figure 5. The arrangement of waxy filaments of *Metcalfa pruinosa* on *Cornus sanguinea.*

The adults lifetime is several weeks, these being present on host plants, depending on climate conditions, from May to October. The larva has a soft body, covered with a waxy white fluff with dense filaments, waxy, which persist for long time on top of the abdomen. Larva has a white to open green color after the waxy layer is outsted and go through five stages, with a body lenght of 4 mm in the last larval stage. After larvae moult some waxy white spots are let and stick to the host plant. From the researches made by Dell Gistina and Navarro (1993), Mead (2004), the melliferous cicada (*Metcalfa pruinosa*) hibernates in the egg stage put in the bark of host plant.

Noteworthy is the fact that the larvae of the same species secret the honey dew which is very appreciated by the bees, but which obstruct the normal processes of photosynthesis, being an open door for phytopathogens installation.

MATERIALS AND METHODS

The research on species *Metcalfa pruinosa* monitoring have been made between May – September 2016, in different lawns from Craiova city (parks and public gardens and also a series of hedges from the blocks neighborhoods), on different ornamental woody or grass species where the presence of pests was signaled.

The observations and determination focused on pest presence recording, identification of development stages and host plant species.

RESULTS AND DISCUSSIONS

Plum cicada or melliferous cicada (*Metcalfa pruinosa)* is systematically included to class Insecta, order *Homoptera,* superfamily *Flugoroidea,* family *Flatidae,* genus *Metcalfa,* species *Metcalfa pruinosa* Say., having several synonyms: *Flata pruinosa* Say 1830, *Ormenis pruisana*, *Poeciloptera pruinosa* (Say 1830).

In 2016, in Craiova city, starting from May, it was observed the presence of pest attack on a series of ornamental plants from parks and public gardens and

also a series of hedges between blocks, which, at the first view, looked like a woolly aphid attack. Following the observations and determination both in the field and laboratory, it was mentioned the presence of species *Metcalfa pruinosa,* known as plum cicada or melliferous cicada.

During the summer it was followed the presence of the pest on different host plants, the attack having different infestation degree very abundant with an intensity enough to produce considerable damage, (figure 6.), the insect presenting a pronounced polifagism.

The pest was observed initially on the twigs and leaves of ornamental shrubs from hedges (*Hibiscus syriacus, Cornus sanguinea, Paulovnia tomentosa*) in parks, public gardens and allays between blocks.

Figure 6. Damage on *Hibiscus syriacus.*

In the green area of Craiova city, the melliferous cicada was signaled on other different plants, too: *Ligustrum vulgare, Philadelphus coronarius.*

The specialized literature mentions the pest presence on more than 200 host plant species, grassy or woody, from spontaneous plants to fruit-trees and ornamental species (Lauterer 2002, Lauterer and Malenovsky 2002a; Pons et al. 2002, Preda and Skolka 2009, 2011, Kim et al. 2011, Gogan et al. 2011).

Considering the population density of *Metcalfa pruinosa* recorded on a numerous group of ornamental plants and the attack aggressivity, required its careful monitoring both in the Craiova city area and other urban or rural settlements from Oltenia.

CONCLUSIONS

The detection of *Metcalfa pruinosa* species on an important number of ornamental plants in parks, public gardens and hedges from the recreational areas inside the blocks neighborhoods, requires the registration as new pest for Oltenia, particularly dangerous and its making in study to limit the attack.

Carefully checking planting material of ornamental plants from both our country and abroad for registration of new green area in urban and rural settlements, in order to detect the pest.

Further research on insect morphology, biology, ecology, host plants, in order to take the most effective measures for pest combat.

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HISTO-ANATOMICAL AND PRELIMINARY PHYTOCHEMICAL RESEARCHES ON *GERANIUM ROBERTIANUM* L. (*GERANIACEAE*) SPECIES

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Keywords: *Geranium robertianum L., histo-anatomy, polyphenols, thin layer chromatography.*

ABSTRACT

The paper presents the histo-anatomical researches on the root, stem and leaf of Geranium robertianum L. species and preliminary analysis of the polyphenols content from the aerial parts (Geranii robertiani herba). One quercetin derivative was identified from 12 specific bands of polyphenolic compounds, in the thin layer chromatogram.

INTRODUCTION

Geranium robertianum L., Herb Robert, Red Robin, *Geraniaceae* family, is an annual or biennial herbaceous plant, up to 50 cm high, which blooms from April to September, as spontaneous and ornamental in Europe, Central Asia and North America (Ciocârlan 2000).

The aerial parts of *Geranium* species contain some useful active principles: essential oil rich in linalool (23%), *γ*-terpinene (14%), *α*-terpineol, D-germacrene, limonene, geraniol and phytol (Pedro et al. 1992, Radulović et al. 2011 & 2012), gallic and ellagic tannin – geraniin (Bate-Smith 1972 & 1982, Gayosso-De-Lucio et al. 2014), proanthocyanidins (Bate-Smith 1982), anthocyanins (Andersen et al. 1995), flavonosides, phenolic acids (Graça et al. 2016).

The active principles from *Geranium* sp. exhibit important pharmacological actions: antimicrobial effect – essential oil (Radulović et al. 2011 & 2012), anti-diarrheal, antihemorragic, cicatrizing, in various wounds, frostbites and burns – tannin (Amabeoku 2009, Bate-Smith 1982), antioxidant and radical scavenging effect, anti-inflammatory, hypoglycemic, cytotoxic – tannin and flavonoids (Akdemir et al. 2001, Graça et al. 2016, Shim et al. 2009), diuretic (Montejano-Rodríguez et al. 2013), antiviral (Li et al. 2008, Serkedjieva and Ivancheva 1999), antiprotozoal on *Entamoeba histolytica* and *Giardia lamblia* (Calzada et al. 2005), repellent against mosquitoes – essential oil (Graça et al. 2016).

In the specialty papers, there are scarce and incomplete data concerning *G. robertianum* histo-anatomy (Salimpour et al. 2009) and chemical composition (Graça et al. 2016). The aim of our paper was the histo-anatomical investigation of the root, stem and leaf of the above-mentioned species and the preliminary analysis of the polyphenols content from the aerial parts (*Geranii robertiani herba*).

MATERIAL AND METHODS

Histo-anatomical analysis

The vegetal material was harvested from *G. robertianum* plants in blossom, in June 2016, from the surroundings of Băile Herculane City, Caraş-Severin County (south-west of Romania).

The fixation and preservation of roots, aboveground stems and leaves were achieved in 70% ethanol. The cross-sections and longitudinal-radial sections were obtained using botanical razor.

After washing with distilled water, the sections were clarified using 10% sodium hypochlorite solution (Javel water). Then, the clarifying agent was removed by washing with distilled water. Congo red–chrysoidine mixture (Genevese reagent) was used for the staining of sections. Depending on the chemical composition of cell membranes, the reactive induced various stains: pink to red for cellulose and mucilage, 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 (objectives ×4, ×10, ×40) and then photographed using a Sony DSLR-A380 digital system adapted to the microscope.

The description of microscopic sections was accomplished according to some classical authors (Toma & Rugină 1998).

Thin-layer chromatography (TLC) analysis

The preliminary analysis of polyphenols was performed on the aerial parts of *G. robertianum* species (*Geranii robertiani herba*), using a CAMAG (Muttenz, Switzerland) system in the following experimental conditions: stationary phase TLC silica gel 60 F_{254} (Merck, Darmstadt, Germany) 20×10 glass plates pre-washed with chloroform–methanol (1:1, *v/v*); mobile phase chloroform–ethyl acetate–toluene–formic acid–methanol (15:20:10:10:1, in volumes); sample – 20% methanolic extract of *Geranii robertiani herba*; standards (Merck) – 0.05% methanolic solutions of caffeic acid, chlorogenic acid, quercetin and rutin; migration distance 80 mm; sample (1–10 µL) and standards (2 µL) application – CAMAG Linomat 5 semiautomatic system (spray gas nitrogen, dosage speed 150 nL/s, band length 8 mm); detection – CAMAG TLC Scanner 3 photodensitometer, UV 254 nm, without derivatization, deuterium–wolfram lamp, scanning speed 20 mm/s, data resolution 100 µm/step, measurement mode – absorption; winCATS software package (Altemini et al. 2015, Bojić et al. 2013, Gîrd et al. 2014).

RESULTS AND DISCUSSIONS

Histo-anatomical analysis

Root

In cross-section, the root has circular shape and secondary structure due to the presence of the two meristematic secondary zones: phellogen and libero-ligneous cambium. In cross-section, from the outside towards the inside of the root,

the following histological sequence was highlighted. Periderm is made of suber, phellogen and phelloderm. Suber consists of 4–5 layers of flattened cells impregnated with suberin. From place to place, suber is exfoliated. Subero-phellodermic cambium is made of a single layer of anterior-posterior flattened cells, with thin walls, the radial slightly undulated. Phelloderm consists of 2–3 cellular layers, with cellulosic thin walls. Phloem forms a thin, external ring, consisting of sieve tubes, phloem parenchyma and annex cells. Between xylem and phloem tissues is placed the libero-ligneous cambium. Into the phloem tissue, medullary rays are cellulosic. Xylem is made of metaxylem vessels of different sizes, disorderly dispersed in a mass of libriform tissue, pushing to the center protoxylem vessels. Metaxylem has reticulate thickenings. Protoxylem, underrepresented, is accompanied by xylem parenchyma and occupies the central area. Medullary rays are wide, multicellular, multiseriate, cellulosic. In the secondary xylem zone, medullary rays have triangular shape with the base placed on libero-ligneous cambium and the top into the root center; they split the functional xylem area into two parts. Medullary parenchyma is absent (Figures 1 and 2).

Figure 1. Cross-section through *G. robertianum* root: overview (Congo red–chrysoidine staining, ×40).

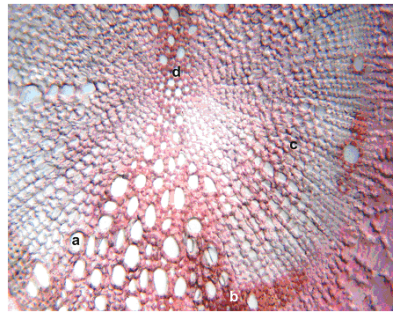

Figure 2. Cross-section through *G. robertianum* root: (a) metaxylem; (b) libriform tissue; (c) medullary ray; (d) protoxylem (Congo red–chrysoidine staining, ×100).

Aboveground stem

In cross-section, into the lower third, the aboveground stem has circular shape and secondary structure generated by libero-ligneous cambium. Epidermis has heterodiametric cells with thickened outer wall covered by a thick cuticle.

Epidermis cells are tangential elongated with thin radial walls and thick tangential external and internal walls. Cortex is made up of two areas. External hypodermic zone consists of 1–2 chlorenchyma layers. The inner zone is well represented, of parenchyma type, with many ursins. Conductive tissues are organized in several large collateral-open libero-ligneous fascicles. Libero-ligneous cambium is intra- and inter-fascicular, and generates secondary conducting tissues which tend to organize in two concentric circles. Phloem tissue is made up of sieve tubes, phloem parenchyma and annex cells. Secondary xylem is made up of metaxylem vessels with different calibers disorderly scattered into well-represented libriform tissue. In longitudinal-radial sections, xylem vessels have ringed, spiral and reticulate thickenings. Primary xylem is poorly represented, consisting of some protoxylem vessels, placed at the base of conducting fascicle, and accompanied by xylem parenchyma. Large medullary rays are multicellular, pluriseriate and cellulosic. Medullary parenchyma is well developed, of meatus type, and accumulate ursins and amyloplasts (Figures 3–8).

In cross-section, into the upper third, the aboveground stem has circular shape and secondary structure generated by intrafascicular libero-ligneous cambium. Epidermis shows small heterodiametric cells with bulging external wall covered by a thick cuticle. Epidermal cells are tangential elongated with thin radial walls and thickened tangential external and internal walls. At this level, we found stomata, many unicellular tector hairs, long and sharp at the tip, and oval glandular hairs with uniseriate epidermal foot. Cortex is made up of two areas. Hypodermic external zone consists of 1–2 layers of clorenchyma. The inner zone is made of 2–3 layers of oval cells, of parenchyma type, accumulating ursins and amyloplasts. Near the cortical parenchyma is a continuous sclerenchyma ring. Conducting tissues are organized into numerous collateral-open libero-ligneous fascicles with different sizes. Libero-ligneous cambium is intrafascicular. Phloem tissue is made up of sieve tubes, phloem parenchyma and annex cells. Secondary xylem is made up of metaxylem vessels with different calibers, placed on a semicircle and accompanied by xylem parenchyma. In longitudinal-radial sections, xylem vessels have ringed, spiral and reticulate thickenings. Primary xylem is poorly represented, consisting of some protoxylem vessels placed at the base of libero-ligneous fascicle and accompanied by xylem parenchyma. Medullary parenchyma is well developed, of meatus type, and accumulate ursins and amyloplasts (Figures 3–8).

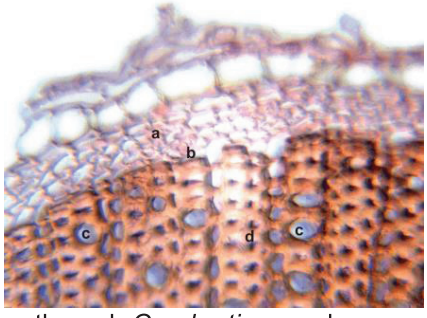

Figure 3. Cross-section through *G. robertianum* aboveground stem: (a) phloem tissue; (b) libero-ligneous cambium; (c) metaxylem; (d) libriform tissue (Congo red–chrysoidine staining, ×100).

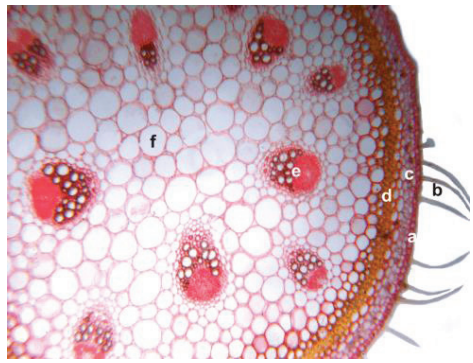

Figure 4. Cross-section through *G. robertianum* aboveground stem: (a) epidermis; (b) tector hair; (c) hypodermis; (d) sclerenchyma ring; (e) collateral-open libero-ligneous conducting fascicle; (f) medullary parenchyma (Congo red–chrysoidine staining, ×40).

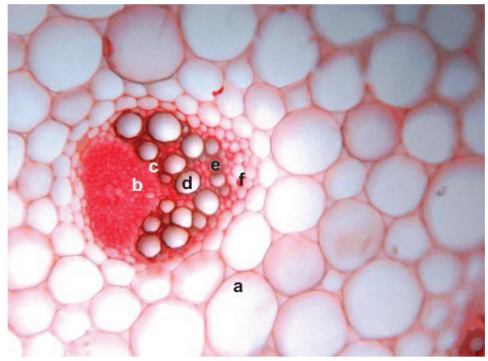

Figure 5. Cross-section through *G. robertianum* aboveground stem: (a) medullary parenchyma; (b) phloem tissue; (c) libero-ligneous cambium; (d) metaxylem; (e) protoxylem; (f) xylem parenchyma (Congo red–chrysoidine staining, ×100).

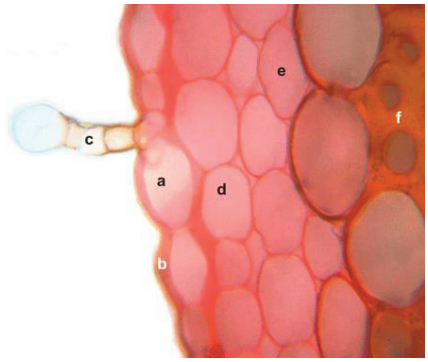

Figure 6. Cross-section through *G. robertianum* aboveground stem: (a) epidermis; (b) cuticle; (c) glandular hair; (d) hypodermis; (e) cortical parenchyma; (f) sclerenchyma ring (Congo red–chrysoidine staining, ×400).

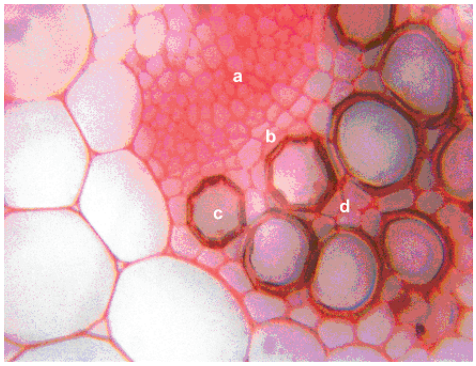

Figure 7. Cross-section through *G. robertianum* aboveground stem: (a) phloem tissue; (b) libero-ligneous cambium; (c) metaxylem; (d) xylem parenchyma (Congo red–chrysoidine staining, ×400).

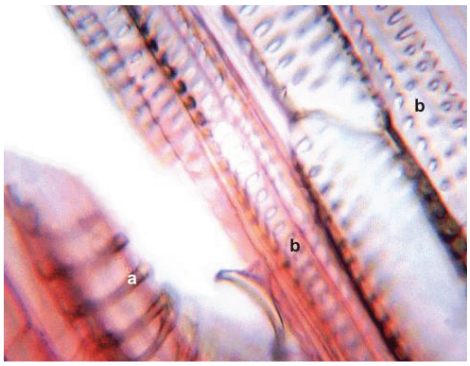

Figure 8. Longitudinal-radial section through *G. robertianum* aboveground stem: (a) metaxylem with ringed thickening; (b) metaxylem with reticulate thickening (Congo red–chrysoidine staining, ×400).

Leaf's limb

In cross-section, from the outside to the inside of leaf's limb, the following histological sequence is evidenced. A single layer of upper epidermis is made of flattened large cells with thickened tangential external and internal walls and thin radial walls. External walls are bulged and covered by a thick cuticle. Mesophyll is organized in 1–2 layers of palisade parenchyma made of small oval cells, rich in chloroplasts, as well as of 3–4 layers of lacunose parenchyma composed of disorderly arranged small cells with aeriferous spaces. Mesophyll contains usins and has bifacial dorsiventral structure. Lower epidermis is made of a single layer of tangential elongated cells, with thin radial walls and thickened tangential external and internal walls. At this level, there are many stomata. Median rib is weak prominent on abaxial side. The outer epidermis consists of small cells, slightly anterior-posterior flattened, with external wall covered with a thin cuticle. At the adaxial pole, there are rare unicellular tector hairs, long and sharp peak. In the central area is located a single libero-ligneous conducting fascicle ordered in a mesophyll mass rich in usins. Leaf's limb has hypostomatic bifacial dorsiventral structure (Figures 9 and 10).

Figure 9. Cross-section through *G. robertianum* leaf's limb: (a) upper epidermis; (b) tector hair; (c) palisade parenchyma; (d) lacunose parenchyma; (e) lower epidermis; (f) libero-ligneous conducting fascicle (Congo red–chrysoidine staining, ×100).

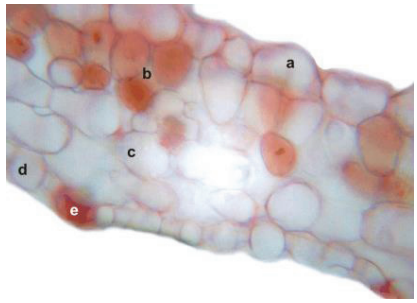

Figure 10. Cross-section through *G. robertianum* leaf's limb: (a) upper epidermis; (b) palisade parenchyma; (c) lacunose parenchyma; (d) lower epidermis; (e) stoma (Congo red–chrysoidine staining, ×400).

TLC analysis

The experimental data on the preliminary TLC analysis of polyphenols from *Geranii robertiani herba* are highlighted in Figures 11–13. Quercetin derivative (R_f 0.63) was identified in an amount of 19.92 mg/100 g of dried vegetal product.

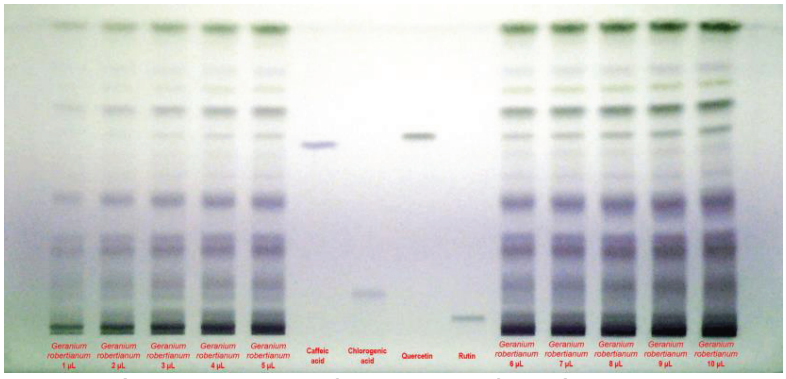

Figure 11. TLC chromatogram of polyphenols from *Geranii robertiani herba* methanolic extract (UV 254 nm, without derivatization). From left to right: first five applications – sample (1–5 µL); subsequent four applications – standards (2 µL); last five applications – sample (6–10 µL).

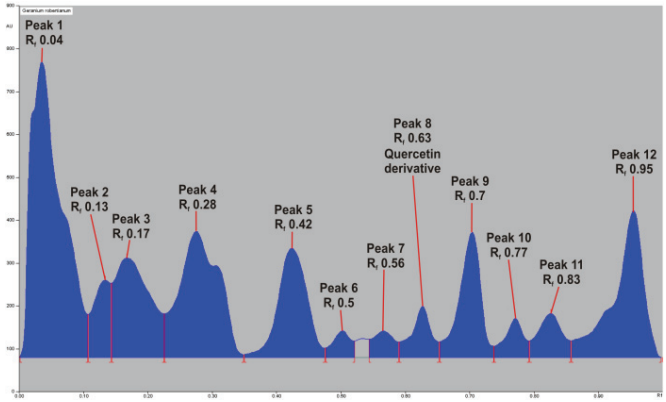

Figure 12. Densitogram of polyphenols (UV 254 nm) separated from *Geranii robertiani herba* methanolic extract.

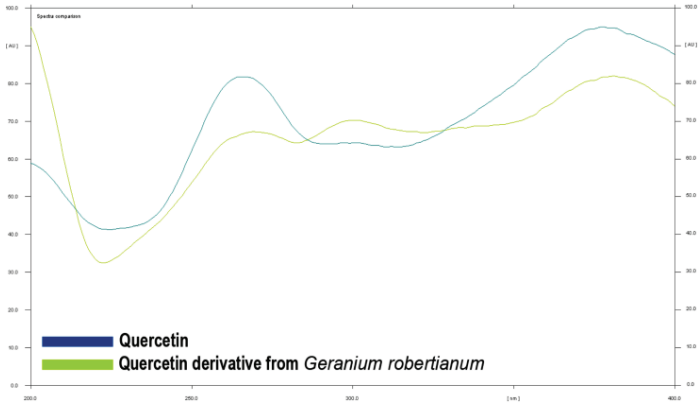

Figure 13. Quercetin derivative *in situ* UV spectra of standard and compound separated from the analyzed sample.

CONCLUSIONS

The histo-anatomical investigation of the root, stem and leaf of *Geranium robertianum* species and the preliminary TLC analysis of *Geranii robertiani herba* polyphenols were highlighted. The root has circular shape and secondary structure due to the presence of two meristematic secondary zones (phellogen and libero-ligneous cambium). The aboveground stem has circular shape and secondary structure generated by libero-ligneous cambium. Leaf's limb has hypostomatic bifacial dorsiventral structure. Using TLC technique, one quercetin derivative was identified from 12 specific bands of polyphenolic compounds.

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BILIRUBIN DYNAMICS IN PATIENTS TESTED AT MEDICAL DIAGNOSTIC CENTER EUROMEDICA CRAIOVA IN 2016

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Keywords: *direct bilirubin, total bilirubin, hyperbilirubinemia, jaundice*

ABSTRACT

Bilirubin is a byproduct of normal hemoglobin breakdown. When old red blood cells are broken down, the hemoglobin within them is converted into bilirubin. The liver then degrades bilirubin into bile and either stores it in the gallbladder or excretes it through the stool. A small amount of bilirubin circulates in the bloodstream. Several diseases involving the liver, gallbladder or red blood cells may cause an increased bilirubin level. When bilirubin builds up in the blood, it may cause jaundice, a yellow discoloration of the whites of the eyes and skin. Jaundice is the most common symptom of hyperbilirubinemia and is typically seen once total bilirubin levels approach 2-3 mg/dl.

INTRODUCTION

Bilirubin is a yellow pigment that is in everyone's blood and stool. Sometimes, because of excess bilirubin, an obstruction or an inflamed liver, the liver cannot process the bilirubin in the body. When the body has too much bilirubin, the skin and the whites of eyes will start to yellow, condition called jaundice.

Bilirubin is a tetrapyrrole (http://emedicine.medscape.com/article/2074068-overview) made in the body when old red blood cells are broken down (http://www.healthline.com/health/bilirubin-blood#Follow-up9). Then bilirubin is removed from the body through the stool (feces) and gives stool its normal color (http://www.webmd.com/digestive-disorders/bilirubin-15434#5).

Approximately 85% of the total bilirubin (BT) produced is derived from the heme moiety of hemoglobin, while the remaining 15% is produced from RBC precursors destroyed in the bone marrow and from the catabolism of other heme-containing proteins. After production in peripheral tissues, bilirubin is rapidly taken up by hepatocytes where it is conjugated with glucuronic acid to produce bilirubin mono- and diglucuronide, which are then excreted in the bile (http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/8452).

Intramolecular hydrogen bonding fixes it in a rigid structure that blocks exposure of its polar groups to aqueous solvents, making it very insoluble in blood (http://www.columbia.edu/itc/hs/medical/pathophys/gi/2008/bilirubinColor.pdf).

Bilirubin was once considered a toxic waste product and has more recently been recognized as a potent antioxidant of physiological importance and a power-

ful immunomodulatory agent (Liu et al. 2008). Bilirubin has also been discovered in plants (Pirone et al. 2009). In the bloodstream, bilirubin circulates in two forms: indirect (unconjugated) bilirubin (BI) and direct (conjugated) bilirubin (BD). BI does not dissolve in water and travels through the bloodstream to the liver, where it is changed into a soluble form (direct). BD dissolves in water and is made by the liver from BI (Iancău, 2009). Total bilirubin (BT) and BD levels can be measured directly from the blood, whereas BI levels is calculated from the total and direct bilirubin measurements (http://en.academic.ru/dic.nsf/enwiki/42678/Bilirubin).

Bilirubin level can be checked with a simple blood test. Since many of the causes of a high bilirubin level are potentially serious, it's important to see if we are concerned about jaundice (http://www.livestrong.com/article/172084-causes-of-elevated-bilirubin-levels/). Jaundice is a complex clinical and laboratory syndrome characterized by a yellow pigmentation of whites, mucous membranes and skin, while increasing serum bilirubin (hyperbilirubinemia) (Taisescu 2010).

Jaundice caused by hyperbilirubinemia is true. Yellowing of the skin may occur after excessive consumption of carrots, pumpkin, administration of drugs, in such circumstances it is false jaundice (Lutan et al. 2004).

Jaundice reflects the imbalance between production and elimination of bilirubin or bilirubin clearance alteration, alteration due to the following mechanisms: hyperbilirubin, reduced hepatic uptake by the cell bilirubin, deficit for bilirubin conjugation captured, decreased excretion hepatocellular, bile flow blockage removal (Vrăbete 2006).

MATERIALS AND METHODS

This paper is based on a retrospective statistical study that includes a lot of patients tested between 01.01.2016 - 07.31.2016 in the Laboratory of medical analyzes Euromedica, Craiova.

Bilirubin is one of the most commonly used tests to assess liver function. In the serum, bilirubin is usually measured as both direct bilirubin (BD) and total bilirubin (BT) (Westwood, 1991).

Method used for serum bilirubin dosage in clinical practice: colorimetric, spectrophotometric method, Jendrassik Grof method, DMSO.

Material: automated analyzers.

In an older child or adult, normal values of BD are from 0 to 0,4 mg/dl and normal values of BT are from 0,3 to 1,0 mg/dl. Normal bilirubin in a newborn would be under 5 mg/dl, but many newborns have some kind of jaundice and bilirubin levels above 5 mg/dl (http://www.healthline.com/health/bilirubin-blood#Follow-up9).

RESULTS AND DISCUSSIONS

The present study has as a data source of analyzes the results included in worksheets, being considered only the results determinations of BD and BT, excluding BI measurements in the study. Individuals belonging to the group in the study were divided by gender (177 females and 140 males), depending on the age group (0-20 years, 21-40 years, 41-60 years, 61-85 years), depending on analysis, BD and BT, and depending on the outcome of the analysis, positive (+) or negative (-) (Table 1). By statistical processing of results from the worksheet it was performed a database that was used in graphical and tabular representation of their interpretation and discussion of the results and drawing conclusions from reading them.

Of the 177 females tested to determine dosage of BD, 60 had a positive result (+), while the rest of the group have registered negative results (-) (Graphic 1). Regarding dosing BT, of the 177 tested, 47 had a positive result (+) and 130 females from the group recorded a negative result (-) (Graphic 2).

Table 1

Dynamics of positive (+) and negative (-) results on determining the levels of direct bilirubin (BD) and total bilirubin (BT) according to sex and age in individuals studied group

Age group	BD				BT			
	Males(140)		Females(177)		Males(140)		Females(177)	
	58(+)	82(-)	60(+)	117(-)	47(+)	93(-)	47(+)	130(-)
Age group 0-20 years	3	8	2	11	4	7	1	12
Age group 21-40 years	9	16	13	35	5	20	6	42
Age group 41-60 years	34	42	22	40	29	47	19	43
Age group 61-85 years	12	16	23	31	9	19	21	33

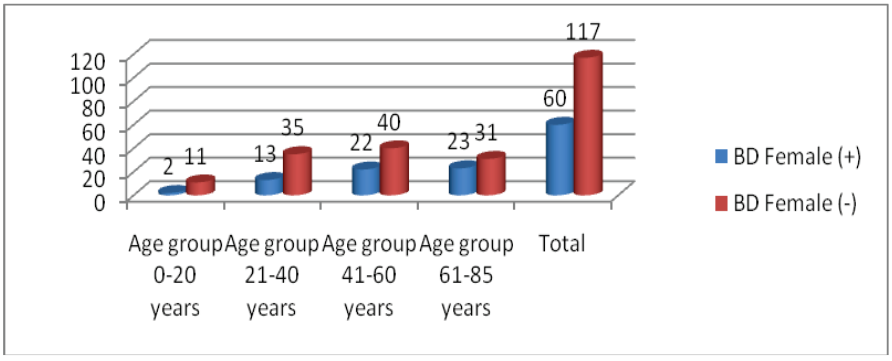
Referring to male patients, out of the 140, 58 had positive result (+) in dosage BD, 82 of them registering negative result (-) of this determination (Graphic 3). Regarding dosing BT, only 47 males out of 140 have been positive (+), 93 were negative (-) (Graphic 4). The distribution by group age indicates that in any of the four groups, patients with positive results (+) are numerically lower compared with patients whose outcome was negative (-) (Graphic 1, 2, 3, 4).

If we consider the distribution by age group and sex of only patients with positive results (+), it is noted that the determination of BD solded in positive results (+) about equal in terms of the number of females and males, 60 females and two fewer males. As for the positive results (+) of the determinations BT, was recorded impairment balanced in both sexes, with a total of 60 patients for each sex (Graphic 5). This time, however, there were differences in the distribution of age groups. Age, is a risk factor that increases susceptibility that results determinations BD and BT levels are higher than normal.

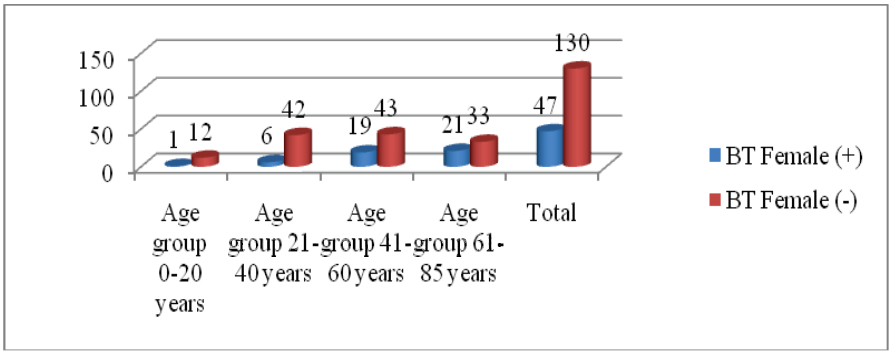
The distribution by age group of the 238 patients with positive results (+) in the study group (Graphic 5,) shows an important impairment of the group 41-60 years, with a share of 56 patients in case of BD and 48 patients in the case of BT. At small difference, followed the group 61-85 years who were registered in 35 patients for BD and 30 patients in case of BT. Less affected was the group 21-40 years with 2 patients for BD and 11 patients for BT. The lowest number of patients was registered in group 0-20 years, with a weight balanced in terms of the number of those with a positive result, 5 patients where both dosing BD and BT. Our results confirm data from literature. In adults, specially over 40 years, high bilirubin may be due to problems with the liver, bile ducts or gallbladder (http://www.healthline.com/health/bilirubin-blood#Follow-up9). Signs include: shows evidence of jaundice, history of drinking excessive amounts of alcoholism,

suspected drug toxicity, exposure to hepatitis-causing viruses (http://emedicine.medscape.com/article/2074068-overview), Gilbert's disease, a genetic disease affecting some families, cirrhosis, scarring of the liver, biliary stricture, part of the bile duct is too narrow to allow fluid to pass, cancer of the gallbladder or pancreas, gallstones (http://www.healthline.com/health/bilirubin-blood#Follow-up9).

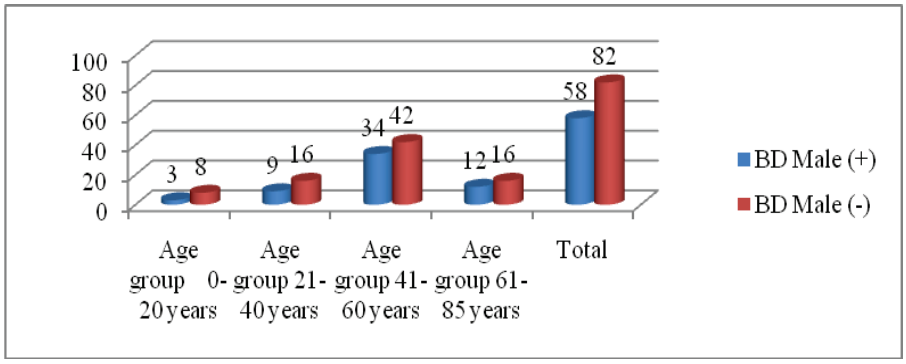
All these signs and problems encountered by adults aged over 20 years, the people who belong to groups 41-60 years and 61-85 years and very little in the age group 0-20 years. Most newborns have some yellowing of the skin or jaundice. This is called physiological jaundice. It is often most noticeable when the baby is 2 to 4 days old. Most of the time it does not cause problems and goes away within 2 weeks (https://medlineplus.gov/ency/article/001559.htm). It is important that an elevated level of bilirubin in a newborn be identified and quickly treated. When severe jaundice goes untreated for too long, it can cause a condition called kernicterus, a type of brain damage that can result from high levels of bilirubin in a baby's blood (http://www.cdc.gov/ncbddd/jaundice/facts.html).The consequences of this damage include mental retardation, learning and developmental disabilities, hearing loss, eye movement problems and death.Tests for bilirubin may also be ordered when someone is suspected of having hemolytic anemia as a cause of anemia (https://labtestsonline.org/understanding/analytes/bilirubin/tab/test).



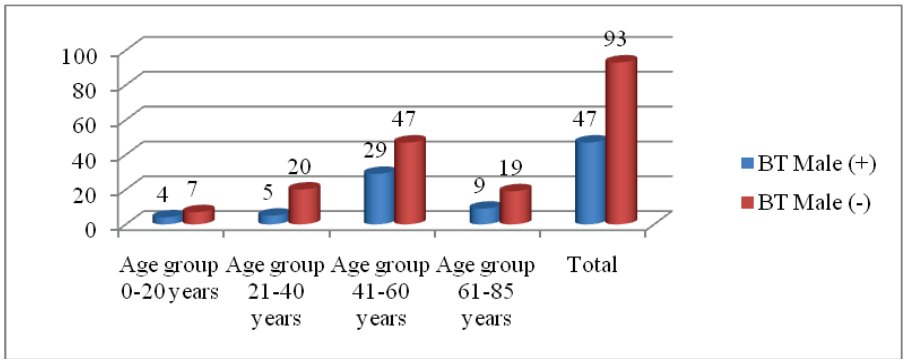
Graphic 1. BD level dynamics at females.



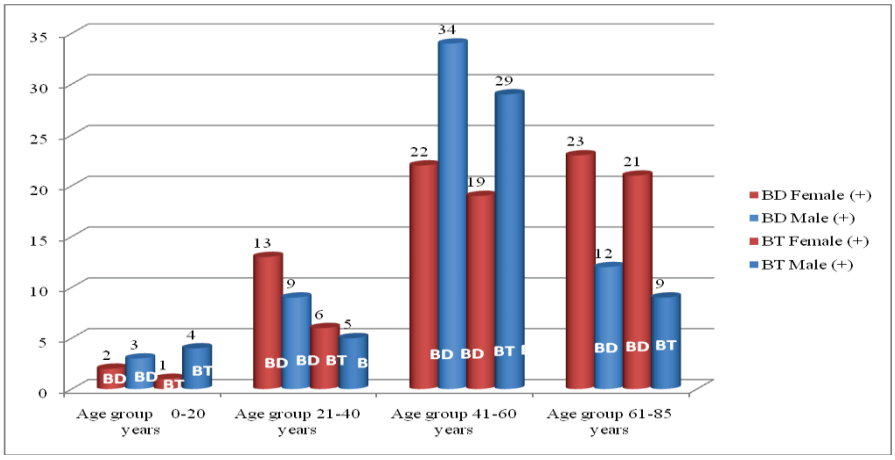
Graphic 2. BT level dynamics at females.



Graphic 3. BD level dynamics at males.



Graphic 4. BT level dynamics at males.



Graphic 5. Bilirubin level dynamics according to sex and age group.

CONCLUSIONS

An interesting aspect observed after analyzing the sample under study is the distribution by age group and sex of patients with bilirubin levels of positive

results. Both values determine BD and BT, have led to positive results about equal in terms of the number of women and men. Differences were recorded after the age group distribution of patients with positive results from the study group. The most significant damage was observed in individuals over 40 years, while least affected were individuals groups with young people aged up to 20 years. Also, the distribution of age groups indicates that any of the four groups, patients with positive results are reduced in number as compared to patients whose outcome was negative. We can conclude thus the importance of patient test values in accurate diagnosis and hence the application of the correct treatment. The consequences that could arise from incorrect diagnosis, and to mistreat are not neglected.

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***http://www.healthline.com/health/bilirubin-blood#Follow-up9

***http://www.webmd.com/digestive-disorders/bilirubin-15434#5

***http://www.columbia.edu/itc/hs/medical/pathophys/gi/2008/bilirubinColor.pdf

***http://en.academic.ru/dic.nsf/enwiki/42678/Bilirubin

***http://www.livestrong.com/article/172084-causes-of-elevated-bilirubin-levels/

***http://www.cdc.gov/ncbddd/jaundice/facts.html

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WATER IN THE HUMAN BODY

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Keywords*: bioengineering, water, biotechnologies, biochemical processes, human body*

ABSTRACT

Many vital processes are carried out thanks to the water, this substance being irreplaceable. Thus, the water is part from: The proper absorption and digestion, The dissolution and transport of substances (minerals, salts, ions, organic compounds, etc.), Biodegradation and disposal of toxic or unspecific substances, Realization of acid-base balance and osmosis, Regulating body temperature, Respiration and cellular nutrition, Creation of a reaction medium for numerous biochemical processes (hydrolysis, hydration, oxidation-reduction, etc.), Ensuring the continuous exchange of substances between the external and internal environment. But the most important function of the water is the body energy production.

INTRODUCTION

We are made of water, almost 65% of everything we stand for as human beings is water. We are born of water which then gradually lose as we get older. This explains that a human embryo is 97% water, at birth the newborn is made up of 90% water, toddler contains 80% water, the same child at school age will have a 70% water, following that in adulthood to remain only 60-65% water by weight.

The percentage of water also differ by gender and body fat percentage. So that women have a higher percentage of fat (placed in the hands, hips and the cellular tissue subcutaneous fat) than men, they will average 5% less water than men of the same age. This is because fat tissue has the ability to retain a very small amount of water, which explains the reduction of the body's water content.

Water is the medium which holds all biological and chemical reactions in the body, resulting in the production of heat and energy metabolism reactions that required life support. For these reactions can take place is necessary that substances to be dissolved in water, otherwise they can not pry into ions and reactions can not occur.

MATERIAL AND METHODS

The water is in the composition of all organs and helps them perform their functions. Blood contains 90% water, muscles 75%, 83% brain, lungs 86%, 83% kidneys, eyes 95%, 22% bones. Kidneys need water in order to filter the blood,

digestive needs water for digestion, skin need water to keep smooth and healthy, and bones to maintain elasticity and strength (Babaev et al., 2010).

Water circulates through the blood and lymph oxygen and nutrients to the organs. Water helps remove metabolic waste by the kidneys, which is impossible in the absence of a quantity of water acting as a conductor. Water participates in the formation and the growth of cells of the body. The water helps in maintaining a constant body temperature by making the body thermoregulation. When the body is overheated (summer or in fever) water removes heat which abound through perspiration, and in winter when the body suffers of cold, water adapts the body to keep warm blood around vital organs, causing capillaries to contract to save water losses (Buzea et al., 2015; Petrescu et al., 2015).

The main source of energy of living matter is made in molecules known as ATP (Adenosine triphosphate). An ATP molecule may change any sunlight heat to a form of energy which may be used by plants, humans and animals. An ATP molecule contains these four vital elements: oxygen, hydrogen, nitrogen and phosphorus (thirteen oxygen atoms, eight hydrogen atoms, five nitrogen atoms and three phosphorous atoms). Of the 28 atoms of an energy molecule ATP, 20 are originating from water. Practically is important how the atoms of the four elements are connected in an ATP molecule (Fig. 1).

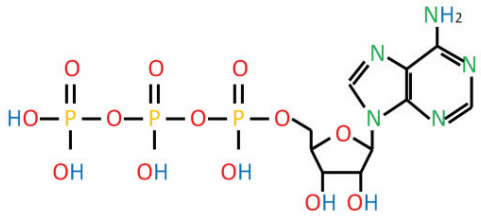

Figure 1. How the atoms of the four elements are connected on one ATP molecule

When water is present at the cellular level in small quantity, the cells (mitochondria) cannot produce enough energy. Then are decrease the number of energy molecules (ATP). The body is getting weaker (emaciated energy), aging, and death. Water loss triggers thirst reflex (that means it has already begun the process of dehydration and body suffer). Sometimes that thirst not works (to be inhibited by factors such as age, hormonal disorders, or a chaotic lifestyle, but that does not mean that the body does not require hydration). In addition dehydrated and tired brain refuses to send appropriate signals for thirst. Therefore it requires some water consumption calculated from time to time whether we are thirsty or not. Serious problem is the loss of water from cells (cellular water loss) with age.

Brain affected. British researchers found that dehydration causes shrinkage of brain size but it returns to normal immediately after consuming a few glasses of water. When we lose water affected the smooth functioning of the brain and nervous system, leading to the emergence of imbalances that we experienced as nervousness, irritability and anxiety, tiredness, exhaustion and inability to concentrate. Because a dehydrated brain can't think or process information correctly then are deducted intellectual performances, short-term memory is also

impaired to a decrease of only 2% of body water. When decreases the hydration level of the body, lungs slow down their activity thus limiting water loss through breathing. Appear difficulty in breathing and organs are not functioning properly oxygenated in damage (dizziness and general bad).

When the water is low in the body appears constipation and decreased urine output. Without water, feces become dry and hard, very difficult to eliminate (manifested by abdominal pain) and release of toxins into the bloodstream. Urine becomes too concentrated, dark and limited as quantity, making kidneys to do not have enough water to remove metabolic wastes. This concentrated urine promotes the crystallizing of molecules of calcium, oxalate and uric acid, ie kidney stones. As the water level drops in the body (dehydration or old age) comes the drying of the skin and mucous membranes. The skin loses its elasticity and firmness, get easy wrinkles and can exfoliate. The feeling of "dry mouth" look red and cracked lips, saliva becomes sticky. Vary the pressure in the eyeballs, eyes can't produce tears, appears the feeling of "sand in the eyes". Headache can occur due to decreased oxygen flow to the brain and its poor irrigation. Dehydration could also be the cause of migraines. It has been shown that the best approach to headache is to drink a glass of water of 10 in 10 minutes for an hour.

Fatigue after meals can occur when there is a deficiency of water in the body. If the digestion process starts on a background deficient water, the blood thickens and for the hydration process it draws water from vital organs (that so they dehydrate) in order to achieve digestion, which explains why, after eating, we feel tired and flabby. If you drink a glass of water before a meal, digestion is without difficulty and we feel energized. When the body is very dehydrated can no longer provide water to the blood circulation, case in which, may occur the state of shock, hallucinations, fainting condition that can culminate with coma and even death.

RESULTS AND DISCUSSIONS

Approximately 2/3 of the total water to a human body is located in the compartment intracellular (water that is kept as a reserve within every human cell). A third is the extracellular water so apportioned: 20% extra vascular sector (Lymph and interstitial fluid) and 8% vascular sector (the water from blood). Water flows freely and quickly between all these compartments in response to changes of the solvents concentrations, to maintaining the balance between compartments (osmosis). In an adult of 70 kg, water is of about 40 l, of which 25 l is intracellular water, extracellular water 15 l; in the total plasma volume is typically 2.75 to 3 l. The water intravascular is at least 85% in the territory of the capillary and venous and arterial within 15%. Of maintaining the volume of arterial water depends greatly the arterial blood pressure, regulation of extracellular fluid volume, and retention of sodium and water.

Sodium is the main extracellular cation and the determining element of achieving an effective osmosis. The intracellular sodium concentration is an average of 15 mmol/l and the extracellular sodium 140 mmol/l. The average daily intake of sodium is 100-200 mmol. Sodium absorption occurs in the small intestine and colon. Body water (intra and extracellular) comes on one side of liquids and foods (1-4 l / day), and on the other side of the oxidation of lipids, carbohydrates and proteins (200-500 g water daily).

Water is eliminated non renal (loss of skin and respiratory) and kidney (via urine formation). These water losses have essential role in the body to remove

toxins that accumulate in the human body permanently (intra and extra cellular). The volume of urine daily intake can equalize with the removal of water. Final urine is the result of filtering of the plasmatic water (levels 180 l/day) and reabsorption (99% of the filtered water is reabsorbed into the renal tubule, of which approximately 66% is reabsorbed in the proximal convoluted tubule, iso-osmotic, non-adjustable; reabsorption adjustable occurs in the collector tube, cortically and medullary). Urine is only 1% of the filtered water (1-2 liters/day). Water losses (skin and respiratory) are 650-850 ml/day.

So, about a third of water with toxins is eliminating through the skin. In addition the skin breathes like the nostrils, so it is necessary not only maintaining skin cleanliness but and its integrity (no burns or tattoos). Sodium is the main extracellular cation, representing the most important force osmotic which maintains the size of the extracellular fluid volume.

Increasing the total amount of sodium in extracellular fluid is followed by an increased volume of the extracellular fluid compartment thereof and its overcharging. Increase blood pressure. Syndromes of swelling (in congestive heart failure, liver cirrhosis, nephrotic syndrome) are medical conditions with increased amount of sodium in the extracellular compartment (Abdul-Razzak et al., 2012; Ajith et al., 2009; Atasayar et al., 2009).

If the total amount of sodium in the extracellular compartment decreases, the volume decreases and resulting depletion of the extracellular compartment volume (extracellular dehydration). In this case the water supply decreases and from the blood, resulting in disorders of the entire body. In case of decrease in the volume of the sector plasma it can observed the acceleration in heart rate, decreased blood pressure in lying or standing, pale skin and cool extremities, decreased urine output, flattening the neck and forearm veins, the blood pressure occlusion. In case of loss of more than 30% of plasma volume, occurs the hypovolemic shock with cyanosis of extremities and neurological signs of cerebral hypoperfusion (drowsiness, confusion, agitation).

Keeping the decrease of the volume of extracellular salt and water (much) longer produces an excessive consumption from the intracellular water reserves. It also produces and decreased interstitial fluid volume. Interstitial sector is partly responsible for skin elasticity and firmness. Such skin aging occurs, and of the cells. With the loss of water from the cells and energy is lost. Without enough water in cells are produced less ATP molecules (even if we have enough mitochondria). Cell aging occurs and a lack of energy. Conversely, in the event of increased plasma sector, there is an increase in blood pressure.

All processes of a human body are and finely controlled by the various hormones produced by glands. In this respect it is important to know as well physio-pathology of various human processes. That happens and with retention or release of sodium and water.

Renin is an enzyme formed and released by the granule cells of the glomus (Ahmed et al., 2011). Renin catalyzes the conversion of angiotensinogen (protein synthesized by the liver) to angiotensin I. Angiotensin I is converted by an converting enzyme into angiotensin II, which is biologically active (Covic et al., 2007).

Angiotensin II promotes salt retention through direct and indirect effects. It directly stimulates sodium reabsorption in the proximal tubule (by stimulating the exchange Na+/H+). Indirectly, it affects sodium balance by stimulating the

production and release of aldosterone from the glomerular zone of the adrenal. Aldosterone induces a sodium reabsorption (and K + secretion) in the distal convoluted tubule and sewer.

Vasopressin, or antidiuretic hormone is regulated primarily by the osmolarity of body fluids, but the vasopressin increases and in volume-depleted states. This promotes water retention and restoration of body fluid volume.

Atrial natriuretic factor is a peptide synthesized by atrial myocytes and released as aresponse to atrial distension. It is effective to increase natriuresis by the action of vasodilating the afferent arteriole with increasing DFG (glomerular filtration flow) and filtered soda task; It has direct inhibitory action on the absorption of soda in the proximal tubule and medullary tube collector. It has inhibitory effect on renin release, ADH (antidiuretic hormone) and aldosterone.

Prostaglandins have vasodilating renal action and induce the removal of sodium and water. They increase the excretion of sodium by increasing the amount of sodium filtered. And all they increase water excretion by antagonizing ADH.

The sympathetic nervous system, the renin-angiotensin-aldosterone system, the natriuretic peptide atrial and the vasopressin represent the four main systems regulators who tailor their activity in response to changes in body fluid volume.

CONCLUSIONS

All processes of a human body are and finely controlled by the various hormones produced by glands. In this respect it is important to know as well physio-pathology of various human processes. That happens and with retention or release of sodium and water. The sympathetic nervous system, the renin-angiotensin-aldosterone system, the natriuretic peptide atrial and the vasopressin represent the four main systems regulators who tailor their activity in response to changes in body fluid volume. Keeping the decrease of the volume of extracellular salt and water (much) longer produces an excessive consumption from the intracellular water reserves. It also produces and decreased interstitial fluid volume. Interstitial sector is partly responsible for skin elasticity and firmness. Such skin aging occurs, and of the cells. With the loss of water from the cells and energy is lost. Without enough water in cells are produced less ATP molecules (even if we have enough mitochondria). Cell aging occurs and a lack of energy. Conversely, in the event of increased plasma sector, there is an increase in blood pressure.

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ABOUT THE VITAMINS C AND E

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ABSTRACT

A Canadian researchers team led by dr. GC Willis, found that heart diseases are caused by hidden forms of scurvy and chronic, and can be cured with high doses of vitamin C without heart surgery and drug treatments costly and with effects devastating side in organic plan. But the process of healing cardiovascular disease with vitamin C is not approved by the medical institutions and the pharmaceutical industry, which earns billions of dollars in surgeries and drugs that are used in allopathic therapies for various cardiac diseases. Canadian scientist discovery was confirmed in the late 80s by Dr. Linus Pauling, Nobel laureate, the most noted human of his time. The medical care developed by Dr. Pauling include vitamin C with added lysine (amino acid nutritional qualities, existing in most plant and animal proteins) and it works cleanly, healing cardiopathy once thought were incurable.

INTRODUCTION

If we consider the statistics established by WHO regarding the annual deaths caused by coronary artery disease, since the discovery made by Canadian researchers in the 50s until today, were billion earthlings which died of cardiovascular disease, that could be cured with vitamin C, if mankind could have benefited by the experimental results of Canadian researchers team. Deaths from heart disease (caused by chronic scurvy) could be avoided by a simple treatment with vitamin C (Willis, 1953-1954, 1957). What are not told to the patients is the fact that open heart surgery fails in 40-50% proportion of cases, since the disease soon recurs after surgery. Solving the most safe and effective known heart disease, it consists of daily administration of a dose of 6000 to 10,000 mg vitamin C (Abdul-Razzak, 2012; Ahmed, 2011; Ajith, 2009; Atasayar, 2009; Babaev, 2010; Buzea, 2015). It was known long ago that vitamin C deficiency in the body weakens human arteries by forming atherosclerotic plaques (infiltration of the walls of the aorta and arteries with fat), which blocks blood circulation. Dr. Pauling and his team found that upon submission of atheromatous plaques within the arteries, often occurs rupture of an weakened artery and resulting blood clots that lead to heart attack or stroke. Scurvy chronic responsible for heart disease, it was

confirmed by cardioretinometry and was healed by daily doses of vitamin C, by Dr. Sidney Bush, from UK. Dr. Sidney Bush noted that building plagues are reduced and disappear following daily doses of up to 10,000 mg vitamin C. Based on these observations he developed a new method of diagnosing coronary disease, called cardioretinometry.

MATERIAL AND METHODS

Free Radicals meet many critical functions in our body, checking blood passing through artery up to the fight against infection. Some free radical signal molecules that is responsible for starting and stopping of genes. Some free radicals, such as nitric oxide and superoxide, are produced in large quantities by our cells immune to "poison" viruses and bacteria. However in less than a second, free radicals may turn against us, we may sicken and we can grow old too fast. Where there is a stroke or a heart attack, or just an inflammatory disease such as arthritis, free radicals are existing or in production. For us to understand what are free radicals must be known human cells, where every day, and every second, is wearing a war between free radicals and antioxidants. As anything in the universe, and the cells are made up from smaller units called atoms. Each atom contains a nucleus that is surrounded by electrons. Two or more atoms may be linked by dividing electrons forming molecules. The biological process of oxidation (the process of training of energy), involve the transfer electrons from an oxygen molecule to the next. For these reasons sometimes may escape one lone electron. An atom remained without one electron (an ion) present in human body, bears the name of a free radical. Free radicals are formed constantly almost everywhere in the body in an amazing proportion. If free radicals spread by all over the body are not caught, may attack and oxidize DNA, genetic material that controls growth and development of cells, promote spiral down premature aging, or may cause many other heavy damage by intoxicating the body (Ha, 2010).

Many people consume network antioxidants - vitamin C, vitamin E, lipoic acid, coenzyme Q10, glutathione, etc because they want to look and feel younger (Buzea, 2015; Petrescu, 2015). Keeping antioxidant use -ie keeping free radicals in check can be one of the most effective ways to slow the aging process. Antioxidants network (network of special antioxidants) work together in the body. Only five are considered the network antioxidants: Vitamin C, Vitamin E, lipoic acid, coenzyme Q10, and glutathione. Vitamin C and E are not produced by our body but must be obtained through food (Abdul-Razzak, 2012; Ahmed, 2011; Ajith, 2009; Atasayar, 2009; Babaev, 2010; Buzea, 2015). When an antioxidant encounters a free radical, the radical is automatically joined to antioxidant molecular structure (El-Gendy, 2009; Enstrom, 2014). Antioxidant becomes a new type of free radical, harmless. This will exempt cells and tissue destructive action of a radically freely got out of control. What really makes the network of antioxidants to be special is that they can "recycle" or regenerate after they were loosened a radically free, widening and more power. For example: When Vitamin C triggers a radically free it becomes weak free radical in the process. This anti-oxidant can be recycled in the form of vitamin C. As an anti-oxidant network saves him on the other side, cycle continues by making sure that the body will keep the correct balance of the antioxidants. This particular scenario - the antioxidant meets a free radical - it take it, becomes a free radical friend - is recycled for another antioxidant network - occurs repeatedly in the body, in the blink of an eye. Research has

estimated that the number knocks oxidative degradation administered daily DNA in each cell is 10,000 and if this figure is multiplied by the millions of cells in the body can be understood the scale of such processes in the human body. Each antioxidant has a unique niche in the cell; for example in the areas of aqueous cell or in the blood, which consists primarily of water, they will act only vitamin C and glutathione. Vitamin C prevents skin cancer, guarding DNA of threat free radicals. In normal amounts Vitamin C provides beautiful skin (Buzea, 2015; Petrescu, 2015). Vitamin C is essential for collagen production cellular glue that holds the body bound and keeps skin young and supple. Vitamin C protects the body against health problems especially in the elderly. Vitamin C is considered the center of the antioxidant network, because the connection between fat-soluble antioxidants and the water soluble. Vitamin C is important for reloading a fat-soluble vitamin E; when it becomes a free radical, it best meets function. Vitamin C (ascorbic acid) is found in the following foods: citrus, green peppers, broccoli, blueberries, strawberries, tomatoes, cabbage (Buzea, 2015; Petrescu, 2015).

The daily minimum requirement of Vitamin C at tissues and cells level for a healthy man is: 90mg/day for men, 75mg/day women, 100 mg/day smokers (Petrescu, 2015). But always need a bigger tank because these amounts could reach all tissues and cells of a healthy organism: about 500-1000 mg/day. When the body is disturbed or ill, quantities needed to repair cells, are much higher 5000-10000 mg/day. Whenever, it is preferable to get our daily requirement of vitamin C only from food. But in tough conditions, when there is a diagnosed disorder or disease, the body having really need large amounts, must intervene urgently with high amounts of vitamin C synthesized.

RESULTS AND DISCUSSIONS

Canada's variety 2 killer, just behind cancer, is heart disease (Hansen, 2014). It's the variety one cause of hospitalization and the price to the Canadian economy is staggering at $18.5 billion per year! What is the first sign of a heart attack? A first sign in those situations means death. Minimum 70 per cent of the heart attacks take place in people with vas blockages that don't seem to be detectable by roentgenogram the foremost relied upon method of assessment used today. On the other hand more than 50% of people with heart attack had normal cholesterol and 33% had no risk factors for cardiovascular diseases.

The natural treatments are made with individualized schemes to avoid the heart disease. Important and needed first step is to determine the health situation. These assessments include blood and urine laboratory testing. Computerized assessment measures stiffness of the major artery and the aorta. Aortic stiffness increases blood pressure and puts a strain on your heart. Moreover the heart frequency variability (which is an important indicator of stress of heart) is also measured (Hansen, 2014). Heart diseases prevention studies indicate that 93% of patients can control their blood pressure with diet alone by consuming more vegetables and fruits and salt reducing. Studies, however, show that only 38% of patients were able to keep this regimen. Weight control also prevents heart disease but like diet, they are hard to keep up. In these situations medical treatment becomes a necessity.

Dr. Linus Pauling, Ph.D., won 2 Nobel prizes in medication and with Dr. M. Rath, M.D., established a cure for heart disease and a way to prevent it (Rath, 1990, 2003). They used high doses of aliment C to strengthen the walls of blood

vessels and stop the injury or breakdown of the walls that results in plaque built up, and narrowing of the blood vessels. They additionally used high doses of essential amino acid, AN amino acid, to stop the excessive build up of plaque on the vessel walls. Taken along, vitamin C and lysine are clinically effective. Dr. Robert Cathcart, M.D. treated over 20,000 patients without one case of cardiopathy developing (Yilmaz, 2006).

Dr. U. Ravnskov, M.D., a great authority in the cholesterol theory, say that all drugs to lowering cholesterol are very dangerous for our health and can shorten our life. All these drugs are action by inhibiting the body's innate ability to produce its own cholesterol. When one blocks the own cholesterol production it is creating significant complications on body healt. Generally, cholesterol is an important life element for our liver, skin, for brain health, for heart, etc. which are making the body's hormones, being particularly important when we age. When the hormone levels decrease our bodies respond with increased levels of cholesterol to supply the basic elements to raise our hormones levels (Ravnskov, 2009).

Meanwhile the world experts in natural medicine claim the high cholesterol as a great risk factor, there are other factors contributing to the development of heart disease that are more significant. Thus with respect to heart disease, more comprehensive approach is needed. Some experts from Harvard have found that inflammation is one of the major causes of heart diseases. Today one knows that measured of blood levels by C-reactive protein (an inflammatory marker), inflammation is a critical indicator in need of investigation.

The need to reduce negative human cholesterol levels started from the need to reduce the risk of cardiac or cerebrovascular accident. Natural medicines have today already a known history of success in preventing and especially treatment of heart disease, offering some alternative safe and effective treatments to classic pills (which after all are just drugs that do more harm to the body than good, dropping more the good cholesterol needed to the body). Realistically most common sign of a heart attack is sudden death unfortunately. Who survived such an attack, however, it was the subject of multiple and difficult tested, treated with a lot of chemical drugs and perhaps unfortunately has had deregulate and one or more surgeries to control the disease. If in some extreme cases surgery and drugs are absolutely necessary today, in most cases they can be replaced by natural methods of treatment (alternative treatments) or when the disease is early (here the prevention can play an important role) or in situations the inflammation did not exceed certain limits.

The alternative (natural) medicine has today more effective and noninvasive treatment options. Therapy Pauling with lysine (an amino acid) and high doses of vitamin C, already has a history officially recognized treatments successful in situations where the disease was early (preventive), and in difficult cases of some patients who already had suffered at least one attack stroke or heart, with one or multiple surgeries, and heavy chemical treatments. Chelation therapy with intravenous injections consisting in an essential amino acid that removes heavy metals from the body, has a success rate globally certified 87%. Another intravenous therapy, plaquex, became a primary treatment for heart disease due to its ability to reduce plaque deposited in the arteries. These alternative treatments are for those with heart failure who can cause fatigue, shortness of breath, angina, and numbness in the limbs.

Existing inflammation in the blood has been unanimously identified as a major cause of heart disease and C-reactive protein (which is measured by a simple blood test) can donate valuable information regarding your risk of a heart attack. An oral or intravenous medication natural can be used to reduce inflammation. Homocysteine and lipoprotein levels can analyze and give useful information on identifying the risk of blood clots. Testing Vital Signs provides a measure of the health of the heart as a score that can identify health of the heart, specifically the aorta (the major artery). This last test computerized non-invasive blood flow also assesses the state of stress and nervous system learned about heart.

In 1953, Dr. G. C. Willis, M.D., (a Canadian doctor) first formulated the principle that all cardiovascular diseases are linked to a deficiency of vitamin C (ascorbic acid) in the body. He found that plaques and inflammation occurring in the blood of patients were due to deficiency of vitamin C in the body. Based on the idea already known that humans can't synthesize their own vitamin C like they do generally animals is needed in many situations a filler daily vitamin C in the body, either naturally (from food) or synthetic (when required doses are higher) (Willis, 1953-1954, 1957).

In the 1980s, medical research led to the theory that heart disease starts with a fracture (or stress) produced in the arterial wall. The human body defends itself by producing single in liver lipoprotein (a) stored as plaque in order to repair the damaged artery wall (These materials they gave a Nobel prize in medicine in 1985). The theory was then supported by German researchers, led by Dr. Beisiegel, which examined post mortem plaques and found only lipoprotein (a) but did not found and cholesterol considered "bad" (LDL cholesterol). Pauling and Rath have understood then that lack of vitamin C (which can't be manufactured by the liver) force liver to produces and uses instead of vitamin C lipoprotein (a) for the reconstruction of the broken blood vessels.

That was understood that chronic heart disease represents only a consequence of chronic scurvy (lack of vitamin C prolonged). The liver produces and stores in its place the plaque to repair the tissue absented. But this thread (reserve) has no elasticity and superior properties of fabrics produced normally with the help of vitamin C. On the other hand, needed reconstruction plaque behaves as a concrete, depositing it in large quantities and obstructing the arteries.

Alternative medicine, herbal, has made available to patients (over time) more effective treatment options therapies used varying in function on the specificity and seriousness of the case. One way general treatment was initiated and then generalized in 1989 (after many successful experiments, clinical and then patented in 1994 in the US) by Dr. Linus Pauling, Ph.D. and Dr. Mathias Roth, MD, the treatment being known as "Pauling Therapy" (Rath, 1990, 2003).

Some researchers of the University of California reports that patients taking daily minimum of 500 milligrams of vitamin C as a dietary supplement, managed everyone to drop naturally (this way) C-reactive protein (CRP) in the blood by 24 percent after about two months of sustained treatment. More recent studies suggest that CRP is a far better predictor of heart disease than cholesterol. C-reactive protein is a real indicator of inflammation in the blood and body, and there is today evidence that large chronic inflammation generates both the risk of heart disease or the occurrence of a stroke, but also an increased risk of producing a shape diabetes, Alzheimer's disease or other serious diseases (Dr. Gladys Block, UC Berkeley professor) (Gladys).

Dr. James Enstrom (University of California) investigated the benefits of the addition of 300 mg vitamin C daily (food supplement) on 11,000 people in over ten years. He showed that this additional minimal amount administered daily (300mg vitamin C), reduces the risk of heart disease by 50% in men and 40% in women (Enstrom, 2014). G.C. Willis doctor found that all those who consumed 1,500mg daily dietary supplement of vitamin C, within a year, managed to remove plaque from arteries, and those who did not receive the treatment with vitamin C have suffered an increase of plaque (Willis, 1953-1954, 1957).

Dr. Sydney Bush has developed a way to test the existence of plaque in the arteries by optometry (photo retina). He could track and assess accurately the existence and plaque thickness deposited in the arteries by this ingenious way of diagnosis. The method was devised in 2002 after being tested on more patients, some of whom were treated daily with vitamin C. The method can accurately predict the amount of vitamin C present in an organism, and additional daily needs.

Vitamin C (ascorbic acid) contributes to a wide range of benefits. Vitamin C is known to perform several critical functions within the body involving such as: detoxification, tissue-building, strengthen the immune system, pain control, and control or kill pathogens. It is known to be helpful for wound and bone healing, healthy skin and eyes, fighting infections, stress management, toxic exposure, and repair damaged tissue of all kinds.

Richard T. Lee, MD, led a team of researchers from Harvard Medical School, in order to test the 880 chemical compounds (all already authorized for use in humans in medicines, supplements, etc.) on their effect on stem cells embryo (in this case collected from mice). All stem cells used were initially treated to emit a certain color green when developed in heart muscle cells, and only one chemical issue (from all 880 tested) managed to promote stem cell activity, which is just vitamin C (Richard).

Vitamin C has been used in tandem with vitamin K3 in order to realize an auxiliary treatment against cancer, because vitamin C is the main antioxidant independent, playing a key role in protecting healthy cells against oxidative stress. When vitamin C is used in combination with other antioxidants such as glutathione and other drugs such as magnesium, sodium and optionally iodine, it can cause considerable damage to cancer cells, without affecting the healthy cells.

Dr Sudhir Kurl of the University of Kuopio, Finland conducted a study of more than 2400 overweight patients who had high blood pressure. It was demonstrated that a low level of vitamin C made the risk of a stroke three times greater (Kunutsor, 2016). If we want to find out more we may read ‘Why Animals Don’t Get Heart Attacks - But People Do!’ by Dr Matthias Rath (Rath, 1990, 2003).

CONCLUSIONS

Our ancestors have undergone a genetic mutation about 50 million years ago, the mutation that caused the final enzyme in the liver vitamin C synthesis to disappear. For this reason the human liver has not made it and was so ordinary. Principal missing enzyme is called GLO (gulonolactone oxidase). In animals it appears and fulfills its function of vitamin C production in the liver. However, there are some animals such as gorillas, chimpanzees and orangutans which share the same mutation GLO and also can’t produce vitamin C. These primates can also have cardiovascular disease, if their food do not donate them enough vitamin C.

Except for humans and primates, all other animals have the three enzymes in the liver which can synthesize vitamin C from glucose (a simple sugar).

Steve Hickey and Hilary Roberts have shown in their book and made the statement "Vitamin C and Tocotrienols can reverse coronary artery disease" (Hickey, 2007).

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THE ROLE OF HLA IN THE NON-ALCOHOLIC FATTY LIVER DISEASE DEVELOPMENT PREDISPOSITION

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Keywords: *non-alcoholic fatty liver disease, human leukocyte antigen, disease susceptibility*

ABSTRACT

*The non-alcoholic fatty liver disease (NAFLD) is now recognized as the most common liver disease in both adults and children globally. Human leukocyte antigens (HLA antigens) represent a genetic marker that is involved in the pathogenesis of this disease, knowing the histocompatibility genes involved in susceptibility to the condition. The aim of our study was to identify the relationship between the liver steatosis and the HLA typing in the NAFLD. We studied 36 patients diagnosed with non-alcoholic fatty liver diseases together with 36 healthy subjects, which all underwent molecular tests for determining the HLA phenotype in order to correlate some histocompatibility antigens with the risk of developing this condition. The DR2 (DR15, DR16) class II HLA antigens had a high frequency in the patients' group. Of the DQ class II HLA antigens, the DQB1*05 and the DQB1*03 antigens showed a higher frequency compared to the healthy controls. The allelic analysis of antigens DQB1*03:01, DQB1*05:02, showed higher values in the patients' subset also. The HLA-DQ antigens expressed the most intense association with the disease, particularly the antigens DQB1*03 and DQB1*05; therefore, they could be considered susceptibility factors for the disease. HLA-DQ antigens had the strongest association with the studied disease. The existence of specific family haplotypes, common to those who developed the disease, might prove the involvement of the HLA polymorphic system in the transmission of the disease together with the characteristic susceptibility haplotype. Subjects carrying susceptibility HLA antigens should be monitored according to well-established protocols.*

INTRODUCTION

The non-alcoholic fatty liver disease (NAFLD) is now recognized as the most common liver disease in both adults and children globally. It exhibits a broad spectrum of diseases, that range from simple steatosis to steatohepatitis (NASH), with progress to advanced fibrosis and cirrhosis. It is estimated that 20-35% of the general population has steatosis and 10% of these will progress to NASH that is associated with a significant increase in the risk for cardiovascular- and liver-related mortality.

The non-alcoholic fatty liver disease represents the pathological accumulation of fat in the liver in the absence of alcohol intake.

The pathogenesis of NAFLD/NASH is complex; however, it is recognized that the increased visceral adiposity plus the insulin resistance with the increased release of free fatty acids play an initial key role in the onset and perpetuation of the liver steatosis. Later events in the liver involve cellular and molecular mechanisms affecting the oxidative stress and lipid peroxidation, decreased antioxidant defenses, early mitochondrial dysfunction, iron accumulation, unbalance of adipose-derived adipokines with a chronic proinflammatory status and gut-derived microbial adducts also.

However, as there are obese and diabetic individuals who do not have NAFLD, and since NAFLD can occur in normal weight individuals with normal glucose and lipid levels (Bacon et al. 1997), there are obviously multiple genetic and environmental factors determining NAFLD development and progression. Initial evidence for a genetic component to NAFLD comes from familial clustering studies (Struben et al. 2000, Willner et al. 2001) and the ethnic variation in NAFLD prevalence (Browning et al. 2004). Various genetic single nucleotide polymorphisms (SNPs) have been investigated in NAFLD including SNPs in the adiponectin (Musso et al. 2008, Tokushige et al. 2009), IL-6 (Carulli et al. 2009), TNF-α (Tokushige et al. 2007), and apolipoprotein E (Sazci et al. 2008) genes among others. Investigated in a “candidate gene” fashion in small cohorts, these studies have yielded somewhat conflicting results in different populations. In 2008 the first genome-wide association scan in a large multiethnic population targeting NAFLD, identified the PNPLA3 (Patatin-like phospholipase domain-containing protein 3, also known as adiponutrin) gene strongly associated with hepatic triglyceride content (Romeo et al. 2008).

Recently new gene polymorphisms, namely APOC3 and PNPLA3, have been identified. They would increase the risk of fatty liver, but at the same time allow further insights into the pathogenesis of this condition.

As the natural course of NAFLD/NASH can be highly variable it is conceivable that gene polymorphisms may increase the risk of fibrosis in subgroup of subjects with fatty liver (Osterreicher et al. 2007). Studies investigating family clustering report that about 20% of NASH subjects might have first degree relatives who are also affected (Willner et al. 2001) and that a high degree of concordance exists in families with respect to NASH and cryptogenic cirrhosis (Struben et al. 2000).

The human leukocyte antigen is another genetic marker that may well be implicated in the pathogenesis of the NAFLD. These histocompatibility antigens were less studied than the gene polymorphism; however, it is believed that HLA might play an important role in determining susceptibility for disease development (Amzolini et al. 2015).

Human leukocyte antigens (HLA antigens) represent a genetic marker that is involved in the pathogenesis of this disease, knowing the histocompatibility genes involved in susceptibility to the condition.

These antigens have been shown to be highly polymorphic and are encoded by a number of genetic loci on chromosome 6 that are recognized as class I genes. These findings were parallel with the identification of a murine homolog HLA system - H2 system.

Class I molecules link foreign protein antigens to form complexes that are recognized by antigen-specific T lymphocytes (T CD8 + cytotoxic lymphocytes).

Class II genes are located in the HLA-D region (DR, DQ, DP) and encodes at least 6 α-chains and 10 β-chains for class II molecules.

The distribution of class II molecules is distinct from that of class I molecules, normally these being found on B lymphocytes, macrophages, antigen presenting cells specialized in lymphoid tissues (dendritic cells) or skin (Langerhans cells) and in the thymus' epithelia. The expression of MHC class II molecules can be induced on many cell types by the lymphokines, especially γ-interferon (IFN-γ). Their importance is manifest in the normal immune function and autoimmunity (Caisan et al. 2007).

HLA class II molecules bind several types of peptides in order to present them to the CD4+ Th lymphocyte, in particular those derived from exogenous proteins that are endocytic degraded and also peptides derived from proteins linked to membranes which are then internalized by phagocytosis or by endocytic mediated receptor, thus entering the endocytic pathway. The peptides produced by the antigens' degradation are loaded into class II molecules in a specialized endosomal compartment, called class II MHC compartment.

The aim of our study was to identify the relationship between the liver steatosis and the HLA typing in the NAFLD.

MATERIAL AND METHOD

Our study group consisted of two subsets of subjects.

The first subset included 36 patients diagnosed with non-alcoholic fatty liver diseases, which underwent molecular tests for determining the HLA phenotype in order to correlate some histocompatibility antigens with the risk of developing this condition.

In all 36 patients allelic HLA testing was performed in order to determine susceptibility alleles with risk of developing non-alcoholic fatty liver disease.

The allele level HLA testing at which was realized by polymerase chain reaction (PCR) sequence specific primers (SSP) molecular techniques - molecular biology technique based on agarose gel electrophoresis.

The second subset consisted of 36 healthy subjects in which we have also studied the frequency of the HLA II antigens compared with data from the literature regarding Caucasians. These healthy subjects were HLA tested by DNA techniques – PCR SSP – also.

The working protocols for the first subset were well established and included protocols for extracting DNA for each subject in the study group and protocols for DNA amplification by SS0-Mr. SPOT molecular technique.

The ARROW BLOOD DNA extraction method was used to extract the DNA from peripheral blood for each patient in the study. The method is based on automatic purifying of DNA from whole blood. The extraction tool kit and instruments use magnetic beads technology for purification of genomic DNA from blood. The magnetic particles are used for capturing DNA in solution. The DNA on the magnetic beads is washed and then eluted from the magnetic beads in the solution for use.

The DNA concentration varied widely in the test subjects, ranging from 30 μg/ml to 90 μg/ml as a maximum, after adjusting the concentration of DNA.

The DNA purity in the study group ranged from 1.94 to 2.01.

In the second step, after the DNA extraction, we conducted the class I and II HLA phenotyping tests in all the patients, through molecular biology techniques – SSO - HISTO SPOT.

The principle of the test is based on 4 stages:

- DNA isolation
- PCR amplifying
- hybridization and detection
- data interpretation

For an optimal test, the DNA should have a concentration of approximately 15 ng/μl and a purity index between 1.5 and 2. Higher levels indicate the presence of RNA; lower levels show protein contamination.

In the study group, the concentration and purity of DNA were normal, thus allowing the determination of HLA alleles in our subjects by the DNA amplification through the SSO (specific sequence oligonucleotide) technique.

The working protocol for the patients' HLA allelic tested was well established and covered the stages of PCR-SSP.

PCR-SSP STAGES

1. The DNA extraction was performed after EDTA blood sampling.

In order to get good results by this technique, one of the decisive factors is the purity of DNA, along with DNA homogeneity in the reaction buffer.

2. The DNA amplification requires the following features of the primers:

- at the 3'-end the primers must contain at least one nucleotide characteristic of for the allele or the group of alleles
- to have a ring template temperature between 60-62ºC
- to have a G/C to A/T *ratio*, although this is not always possible and in fact several primers contain 100% G/C.
- to have a control primer as an internal control of amplification.

3. Electrophoresis in 2% agarose gel.

We used high resolution kits for the DQB1 locus to amplify DNA at the studied patients.

RESULTS AND DISCUSSIONS

In the first study subset, consisting of 36 NAFLD patients we recorded the following frequency of the class II DRB1 DQB1 HLA antigens:

Table 1

The class II HLA antigens' frequency in the patients' subset

HLA antigen	Frequency
DR15	8.3%
DR16	30.55%
DR8	2.7%
DQB1*05	36.11%
DQB1*03	38.88%
DR7	2.7%
DR1	0
DR3	0
DR4	0

The same antigens were determined in the 36 healthy subjects, the results are shown in table 2.

Table 2

The class II HLA antigens' frequency in the healthy subjects' subset

HLA antigen	Frequency
DR15	5.55%
DR16	2.7%
DR8	2.7%
DQB1*05	19.44%
DQB1*03	19.44%
DR7	13.8%
DR1	2.7%
DR3	11.11%
DR4	2.7%

Comparing the results in each study subgroup, we noticed that the DR2 (DR15, DR16) class II HLA antigens had a high frequency in the patients' group. Other HLA-DR antigens – DR1, DR3, DR4 and DR7 – showed a lower frequency compared to the healthy group.

Regarding the DQ class II HLA antigens, the DQB1*05 and the DQB1*03 antigens showed a higher frequency.

Due to the increased incidence of the DQ antigens, in the 36 patients' subset DQB1 locus alleles molecular tests were carried out.

Table 3

The HLA-DQB alleles' frequency in the studied subjects

HLA antigen	Study patients	Healthy controls
DQB1*03:01	38.8%	16.66%
DQB1*05:02	16.66%	11.11%
DQB1*05:01	5.55%	2.7%
DQB1*06:03	13.8%	0

The most frequently recorded antigens were DQB1*03:01 and DQB1*05:02.

The alleles' frequency in the patients' subset was compared with the control of 36 healthy subjects.

The allelic analysis of antigens DQB1*03:01, DQB1*05:02, alleles with relatively high frequency in the general population, showed higher values in the patients' subset.

We noticed the DQB1*05:01 and DQB1*06:03 alleles, that have a low frequency in the population, but were most strongly associated with the development of the susceptibility to disease.

The higher frequency of the HLA antigens in the studied patients compared to the healthy controls may suggest a possible susceptibility to disease associated with HLA antigens in the Caucasian population (One Lambda statistics).

Considering the class II HLA antigens, the DR2 (DR15 and especially DR16) antigen can be considered as showing a positive association with the NAFLD, thus being a susceptibility factor for the disease.

At the same time, the DR1, DR3, DR4 and DR7 antigens were negatively associated to the disease through their low frequency compared to the controls.

The HLA-DQ antigens expressed the most intense association with the disease, particularly the antigens DQB1*03 and DQB1*05; therefore, they could be considered susceptibility factors for the disease.

The alleles' analysis of the DQ locus showed a possible disease susceptibility in the subjects who express the DQB1*03:01, DQB1*05:02, DQB1*05:01 and DQB1*06:03 alleles.

The lowest risk of developing the disease was observed in the patients carrying the DQB1*03:02, DQB1*04:02, DQB1*06:02 alleles.

Table 4

The correlation of HLA antigens with the study subset

Variable	Studied group *vs*. Control group
HLA DQB1*03	<0.01
HLA DQB1*05	<0.01

In this study we evaluated the frequency of class II HLA antigens in a group of subjects with non-alcoholic fatty liver disease.

Despite the international studies, there are a few studies published in Romania regarding the involvement of gene polymorphism and other genetic factors in NAFLD.

There are very few studies at present. In a Turkish study population (Celikbilek et al. 2011), HLA-DQ5 was met with a greater frequency in patients with NAFLD, consistent with our findings.

Multiple factors may be involved in the pathogenesis of non-alcoholic fatty liver disease; the genetic factor is one of them.

Some HLA antigens with increased frequency in patients can be considered as possible susceptibility factors for the disease, alone or in combination with other predisposing factors.

In non-alcoholic fatty liver disease, the existence of genetic factors must be considered, considering the disease being a multiple factorial entity.

Our study was limited by the small number of patients who underwent HLA typing. It is possible that performing this investigation to a greater number of subjects can lead to a conclusion whether the histocompatibility antigens have an important role in determining the susceptibility to NAFLD.

CONCLUSIONS

HLA-DQ antigens had the strongest association with the studied disease.

Susceptibility alleles for non-alcoholic fatty liver disease are DQB1*03:01, DQB1*05:02, DQB1*05:01 and DQB1*06:03 alleles.

Subjects carrying susceptibility HLA antigens should be monitored according to well-established protocols.

Completing the HLA analysis with family studies could bring new data regarding the involvement of HLA antigens in disease susceptibility development.

The existence of specific family haplotypes, common to those who developed the disease, might prove the involvement of the HLA polymorphic system in the transmission of the disease together with the characteristic susceptibility haplotype.

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All authors had an equal contribution in preparing this manuscript and thus share first authorship.

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RARE SPECIES FROM ROMANIA THREATENED BY HABITAT DESTRUCTION: I. ZIZIPHORA CAPITATA L.

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ABSTRACT

The making of present paper started from two reasons: first is because this taxa northern limit is on our country area and thus continues the series of works which aimes the species with limits area on Romania territory and second to draw an warning to the residents where this plant species is found and also to the responsible, because the surfaces in Oltenia where Ziziphora capitata Linnaeus 1753 grows (Valea Rea - Radovan) are in danger due to the zooanthropogenic factor (sheepfolds installation on these surfaces, E_{79} road widening, that crosses this area).

INTRODUCTION

The problem of rare plants and steppe reservation in Oltenia is far from being solved, although it is a great necessity.

Ziziphora capitata L. a plant that is the object of this study is a rare species, included in the Romanian Red Book (Dihoru & Negrean 2009) (Figure 1).

Figure 1. *Ziziphora capitata* L. – inflorescence detail (orig.).

It is found in one of the most representative natural grassland with steppe character in Oltenia – Valea Rea, from the outskirts of Radovan.

Although this area is under Radovan Village administration and is for nature conservation (Sârbu & al., 2007), the ruderalised process is becoming more intensive because of the zoo-anthropogenic factor.

This valley is oriented NE-SW, and is 2 km long and 800 m wide. At the base of the 14 terraces, next to the English oak forest (*Quercus robur*) with ash (*Fraxinus angustifolia* subsp. *oxycarpa*) is found Desnăţui Creek that comes from Sălcuţa Village. The level differnece between the inferior part of the valley and the superior terraces is 30 m. The valley and the terraces are used as grassland (Figure 2).

The soil is leachate chernozem and the temperature and the rainfall are favorable to the development for a vegetation with steppe character.

MATERIAL AND METHODS

The material used to make this paper consisted, on the one hand, in specialized works, that had *Ziziphora capitata* in the floristic list or phytocoenosis composition, herbarium data from the main herbarium in the country (Bucureşti, Cluj Napoca, Iaşi and Craiova), and, on the other hand, the analyze data obtained after the travel done in this area from 2001 to the present.

Figure 2. The use of grasslands (orig.).

The work method is the typical one for this kind of study: many travels has been made during the flowering species period, notations in the clipboard, about the species situation during the 12 years; the cohabiting species, and the influence of the zoo-anthropogenic factor on *Ziziphora capitata* populations.

RESULTS

The need to protect this plant species is supported by the small number of its stations in Romania. After consulting the literarture we can say that certain data about this species chorology are found only form Dolj and Constanţa Counties. (Borza, 1944; Grinţescu, 1961; Buia & Popescu, 1952; Buia, 1959; Buia ET al., 1962; Păun M. & al., 1967; Cârţu, 1968; Beldie, 1979; Popescu et al., 2003, Ciocârlan, 1990, 2000, 2009; Dihoru & Negrean, 2009).

Herbarium data with tis taxa are few. (Figures 3,4):

Herbarium material from Iaşi (I):

Constanţa County, Limanu-Hagieni, 03.VI.1971 Leg. et det. Diaconescu Fl., Tabacaru C. , Teodorescu G., Slonovschi V., (Flora Moldaviae et Dobrogeae Exsiccata), [I 10699].

Dolj County, Radovan, 14.VII.1962, Leg. Buia Al., Cîrţu C., Maloş C., Olaru M., Păun M., (Flora Olteniae Exsiccata), [I 10700].

Dobrogea-Caliacra, Ponti Euxini penes Ecrene, 06.VI.1924. Leg. et det. Panţu Z.C., Solacolu Th., (F.R.E. nr. 598). [I 10701].

Herbarium material from Cluj (CL):

Dolj County, Radovan, 14.VII.1962, Leg. Buia Al., Cîrţu C., Maloş C., Olaru M., Păun M. (Flora Olteniae Exsiccata), [CL 621395].

Limanu, in Hagieni Natural Reseravtion, 03.VI.1971. Leg. Diaconescu F., Tăbăcaru C, Teodorescu G., Slonovski V. [CL 610096].

Mangalia, beyond sulphur springs. 1927. Leg. Borza Al., Det. Zapletalek I. [CL 505317].

Herbarium material from Bucureşti (BUC)

Dodrogea, Constanţa County, west of Mangalia, Başpunar, ?3.VI.2001. Leg. Gavril Negrean. (Dihoru & Negrean 2009).

Hagieni Forest, south-south-east, Cotul Văii, Valea Mare, in saxosis calcareis, 434826N, 282017E, alt. 65 m. 01.VI.2001. Leg. Negrean G. (DIHORU & NEGREAN, 2009).

Herbarium material from Craiova (CRA):

Dobrogea, distr. Caliacra. In declivibus herbosis ad oras Ponti Euxini penes Ecrene, solo argilloso. Alt. approx. 10-50 m.s.m. 06.VI.1924. Leg. et det. Panţu Z.C., Solacolu Th., (F.R.E. nr. 598).

Craiova Region, Segarcea District, Valea Rea, Radovan, Perişor. Alt. approx. 90 m.s.m. 03.06.1955. Leg. Buia Al. & Păun M.

Constanţa District, Hagieni. 15.06.1964. Leg. et det. Zanoschi V.

Oltenia, Dolj County, Radovan, Valea Rea, in xerophile grasslands. Alt. approx. 85 m.s.m. 08.06.2001. Leg. Popescu Gh., Costache I. & Răduţoiu D.

Oltenia, Dolj County, Valea Rea – Radovan Reservation, on terraces 7-12, only on slopes wiyh southern exposition. Alt. approx. 85-90 m.s.m. 29.05.2013. Leg. Răduţoiu D. & Dumitriu D. Det. Răduţoiu D.

Area. At European level is found only in the SE side of the continent: Al, Bu, Gr, ?Hs, It, Ju, Kry, Rm, Rs (E). It is an east-mediterranean element.

Habitat and coenology

Ziziphora capitata is a thermophilic species, heliophilic, that grows on dry soils (Figure 3), in xerophile grasslands (Figure 4) where the vegetation is relatively short.

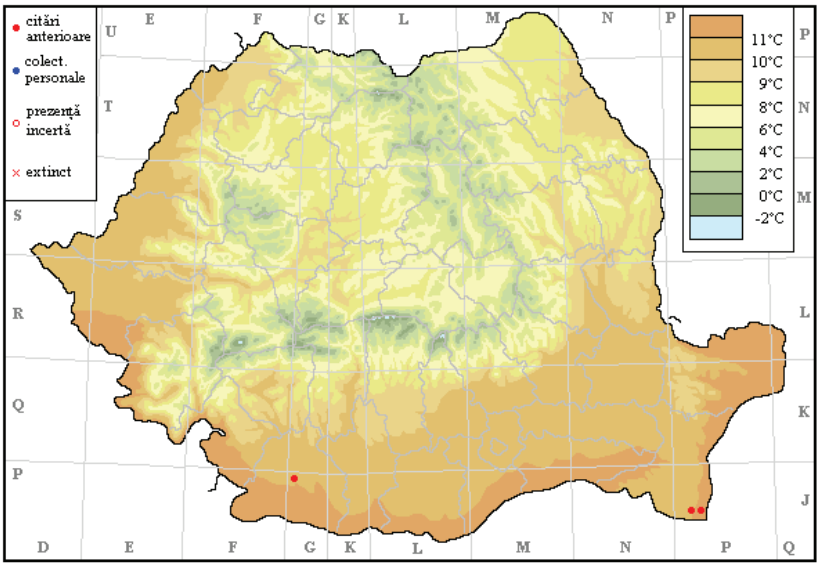

Figure 3. *Ziziphora capitata* area in correlation with the annual average temperature (orig.).

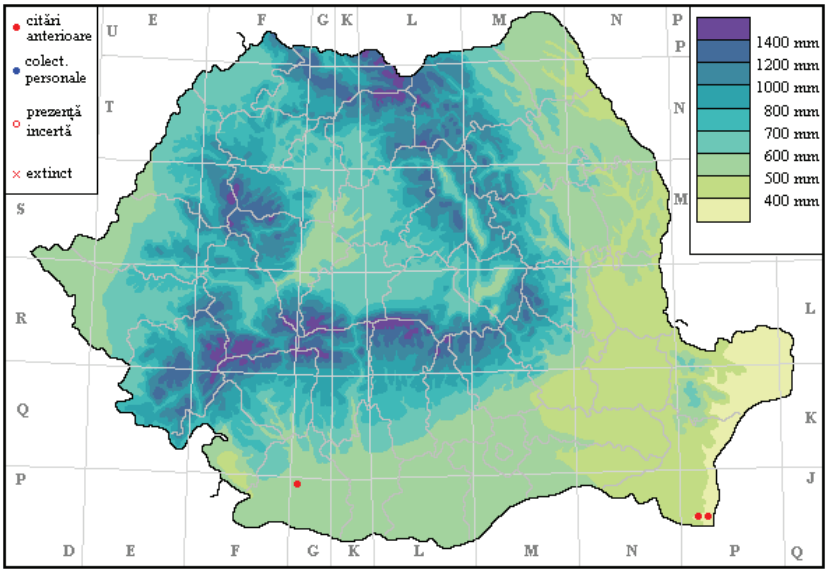

Figure 4. *Ziziphora capitata* area correlated with the average of annual rainfall (orig.).

DISSCUSIONS

The cohabiting plant species in Valea Rea – Radovan are relatively different from Hagieni (Dihoru & Negrean, 2009). At Radovan it grows together with *Aegilops cylindrica*, *Bromus squarrosus*, *Alyssum desertorum*, *Medicago minima*,

Convolvulus arvensis, *C. cantabricus*, *Chondrilla juncea*, *Chrysopogon gryllus*, *Eryngium campestre*, *Achillea collina*, *Arenaria serpyllifolia*, *Dichantium ischaemum* (vegetative stage), *Cardaria draba*, *Daucus carota* (vegetative stage), *Linaria vulgaris*, *Verbascum chaixii* subsp. *austriacum*, *Salvia aethiopis* (vegetative stage), *Bromus tectorum*, *Sisymbrium orientale*, *Poa bulbosa*, *Potentilla recta* and *Teucrium polium* subsp. *capitatum* (Figure 5).

If we compare the data collected by us with those from Hagieni Natural Reservation we will notice that only 3 taxa are in the coenotic environment of *Ziziphora capitata* species: *Chrysopogon gryllus*, *Poa bulbosa* and *Teucrium polium* subsp. *capitatum*. The presence in a high number of the ruderal species in Valea Rea – Radovan point, shows the high ruderalised degree of these grasslands.

Figure 5. The species phytocoenotic ambiance in Valea Rea – Radovan (orig.).

Although it has been tried in many times to cultivate this species in "Al. Buia" Botanical Garden conditions, in Craiova, was noticed that the species perpetuation outside its natural habitat is doomed to failure

CONCLUSIONS

In conclusion we can say that the necessity to protect this plant species and also the surfaces were we find it, is stringent, being known that in our country the plant species is to the northern limit of its area.

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PASPALUM DISCTICHUM IN OLTENIA FLORA

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Keywords: *flora, new taxa, Oltenia*

ABSTRACT

The present paper provides informations about the presence and chorology of Paspalum distichum L. plant species in Oltenia. Nationally is known only from Danube Delta (Letea forest and Rusca channel), where has a very good development and from Caraș-Severin (at the mouth of Nera river into the Danube, place where it forms a small delta). The identification of this taxa presence along the Danube, in other place except those mentioned in the specialty literature make us to believe that in the future this species area will expand on the Romanian Danube bank from the entrance to the mouth into the Black Sea.

INTRODUCTION

Oltenia is a region in Romania, placed in the south-west part of the country. It is geographically delimited by the Danube to the south, Southern Carpathians to the north, Olt river to the east and Cerna river to the west.

The Oltenia vascular flora is quite rich and varied. With the general changing climate conditions new taxa appear that complete the rich flora inventory of this country region.

The study of adventive plant species in Romania, category which includes the plant species presented in this paper, was intensified after 2000, when numerous work papers start to appear bringing valuable informations about the chorology of some adventive species (Anastasiu et al. 2005; Costache & Răduțoiu 2005; Anastasiu & Negrean 2007; Sîrbu C. 2007, 2008; Ciocârlan 2000, 2009; Sîrbu C. et al. 2011; Sârbu I. et al. 2013, Răduțoiu & Stan, 2013) and about the mention of new taxa in our country (Costache & Răduțoiu, 2008; Sîrbu & Oprea 2008a, 2008b, 2010 Sîrbu & Oprea, 2011).

Data regarding the adventive plant species in Oltenia are sporadically (Buia & Popescu 1952; Buia & Păun 1958; Păun 1963). These appear in few work papers targeting the flora and vegetation of some territories (Niculescu 2009, Costache 2011) and in the paper prepared by Răduțoiu D. and Stan I. (2013).

MATERIAL AND METHODS

The working method consisted of field trips to achieve a complete floristic inventory in Oltenia. In one of our field trip was found *Paspalum distichum*.

It was photographed, collected and included in the herbarium from University of Craiova (CRA). Also, there were noticed the cohabiting plant species.

RESULTS AND DISCUSSIONS

The origin regions of this plant species are the tropical ones from America and Africa, in Europe, Australia, Asia, New Zealand and so on being naturalized.

The presence of this plant species in Europe dates from the nineteenth century, from Germany (Sîrbu & Oprea 2011). Later it was metioned from other countries, too: France (Verloove & Reynders 2007), Italy, Portugal, Spain, Turkey, Russia, Albania, Bulgaria, Serbia, Montenegro, Croatia, Great Britain and Ukraine (according to Sîrbu & Oprea 2011).

Valuable information on alien plants is made by Pyšek et al. in 2004.

The first signaled of *Paspalum distichum* L. plant species in Romania is found in the paper published by Roman (1992). He found it in Letea forest. Later, it was identified in other places from Danube Delta, too.

Paspalum distichum L. (Syn. *Paspalum paspalodes* (Michx.) Scribn., *Digitaria paspalodes* Michx.) is known in different names in the Romanian specialty literature. Some authors use the name *Paspalum distichum* L. (Sârbu & al. 2013), approach agreed also by us, while others: *Paspalum paspalodes* (Michx.) Scribn. (Ciocârlan 2000, 2009, Anastasiu & Negrean 2007; Sîrbu & al. 2013).

It was identified by us in Dolj county, on the left bank of the Danube, next to Rastu Vechi locality (Figure 1). It was included in the herbarium from University of Craiova (CRA114396, CRA114397, CRA114398, CRA114398, CRA114400, CRA114401).

Figure 1. *Paspalum distichum* L. from Rastu Vechi locality – Dolj county (orig.).

It is a plant species from Poaceae family popularly known as "water couch grass", that usually vegetate on coastal sands, but also in wetlands and riverbanks. It is a polyploid taxa, what is often multiplied by rhizomes and stolons, rarely by seeds. The overgrowth by rhizomes and stolons was one of the reason why this

plant species is cultivated in Australia and New Zealand on riverbanks, preventing soil erosion. In Europe it seems that it was introduced accidentally (Anastasiu & Negrean 2007).

In the locality where was found, it occupies few square meters surfaces where it grows almost alone. (Figure 2). To the outskirts of these surfaces were found isolated specimens of *Cynodon dactylon*, *Bidens frondosa*, *Polygonum lapathifolium* and *Bidens tripartita*.

Figure 2. Phytocenosis dominated by *Paspalum distichum* on the Danube bank, next to Rastu Vechi locality (orig.).

CONCLUSIONS

In conclusion, we can say that this plant species presence in Oltenia is encouraging on the one hand (if we consider the enrichment of Oltenia floristic inventory), but on the other hand, it compels us to monitor the plant because it can fully replace the other plant species in the places where it installs, with negative consequences for phytodiversity.

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* CRA – acronimul pentru Herbarul Universității din Craiova.

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BENEFICIAL SPECIES FROM BOTANICAL GARDEN CRAIOVA

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Keywords: *botanical garden, beneficial species species, predators species*

ABSTRACT

From our observation regarding the beneficial species made in Botanical Garden ,,A. Buia,, Craiova, we have identified a number of 51 beneficial species. Most of the beneficial insect species identified belonged to the order Coleoptera (7 species) followed by the Diptera (6 species) and Odonata (5 species). Vertebrates beneficial species from Aves class were represented by 14 species and those of the Mamalia class by 3 species. Amphibia class was represented by 2 species and Reptilia class by 4 species.

INTRODUCTION

In this paper we have propose to inventory the beneficial species present in Botanical Garden Craiova ecosystem.

In the ecosystem like botanical gardens pests are not usually a problem because there is an adjustment of populations, natural control, where internal factors of these populations intervene as well external factors. The endogenous factors are represented by the population polymorphism and the exogenous factors are as for other organisms, represented by abiotic factors: physical, chemical, mechanical and biotic ones: predators, parasites, pathogens, also called "natural enemies", antagonists, as well food and competition

In anthropogenic agroecosystems human intervention through chemical methods to limit the pest attacks, resulting in serious disruption of beneficial organisms activity having a negative influence, due to direct exposure to pesticide action and indirect action due to the active substance ingested with the prey (Stan et al. 2013).

Classical biological control (natural enemy introductions) has long served as a paradigm for the role of predators and parasitoids in insect herbivore population dynamics, and it is widely held that there is no fundamental difference between successful biological control and the action of native natural enemies 'natural control' (Hawkins et al. 1999).

MATERIALS AND METHODS

To determine the structure of the beneficial populations were made collection of material using various means and methods: directly by hand from plants or soil, frame metric, soil surveys and soil surface collected with

entomological net, visual inspection, collection with sticky traps for flying insects, analyzing samples with binocular magnifier glass directly in the field or laboratory.

For as little impact on the ecosystem we have preferred to capture images with the camera than to capture live specimens were subsequently removed from their natural environment.

After collecting, the biological material was analyzed and determined using the Identification Manual (Panin 1951, Chatened du Gaetan 1990, Chinery 1998, Godeanu 2002, Zoltán et al. 2010, Bruun et al. 2011).

RESULTS AND DISCUSSION

There were identified a number of 51 useful species (table 1), belonging to 6 classes: ARACHNIDA, INSECTA, AVES, MAMALIA, AMPHIBIA and REPTILIA.

Table 1

Entomofauna and beneficial vertebrate identified during research

No. of species	Class/Order	Species
1	ARACHNIDA/ACARI	*Typhlodromus spp.*
2		*Amblyseius spp.*
1	INSECTA/ORTHOPTERA	*Mantis religiosa*
1	INSECTA/DERMAPTERA	*Forficula auricularia*
1	INSECTA/ NEUROPTERA	*Crisopa carnea Ste.*
2		*Crisopa perla Steph.*
1	INSECTA/ HYMENOPTERA	*Scolia flavifrons*
2		*Vespa crabro*
3		*Bombus terrestris*
4		*Xylocopa violacea*
1	INSECTA/ COLEOPTERA	*Adalia decimpunctata L.*
2		*Adalia bipunctata L.*
3		*Coccinella 7 punctata L*
4		*Carabus cancelatus L.*
5		*Carabus ulrichi L.*
6		*Carabus violaceus L.*
7		*Calosoma sycophanta L.*
1	INSECTA/ DIPTERA	*Syrphus ribesii L.*
2		*Syrphus torvus L.*
3		*Episyrphus balteatus De Geer*
4		*Metasyrphus corollae F.*
5		*Scaeva albmaculata Macq.*
6		*Scaeva pyrastri L.*
1	INSECTA/ ODONATA	*Orthetrum coerulescens*
2		*Lestes dryas*
3		*Libelulla depresa L.*
4		*Libelulla fulva M.*
5		*Calopterix splendens*
1		*Parus parus*

No. of species	Class/Order	Species
2	AVES/	*Parus caeruleus*
3		*Parus major*
4		*Picus viridis*
5		*Picus picus*
8		*Athene noctua*
9		*Hirundo rustica*
10		*Sturnus vulgaris*
11		*Cuculus canorus*
12		*Columbo palumbus*
13		*Streptopelia decaocto*
14		*Luscinia megarhynchos*
1	MAMALIA	*Pipistrellus pipistrellus*
2		*Talpa europaea*
3		*Erinaceus europaeus*
1	AMPHIBIA	*Bufo bufo*
2		*Rana esculenta*
1	REPTILIA	*Lacerta agilis*
2		*Lacerta viridis*
3		*Emis orbicularis*
4		*Natrix natrix*

Figure 1. Beneficial Coccinelidae Beneficial Syrphidae.

Figure 2. Beneficial species from Odonata order.

As it can be observed in fig. no. 3 most beneficial insect species identified belonged to the Order Coleoptera (7 species) followed by the Diptera (6 species) and Odonata (5 species). Beneficial vertebrates from Aves class were represented by 14 species, and those of Mamalia class by 3 species. Amphibia class was represented by 2 species and Reptilia class by 4 species.

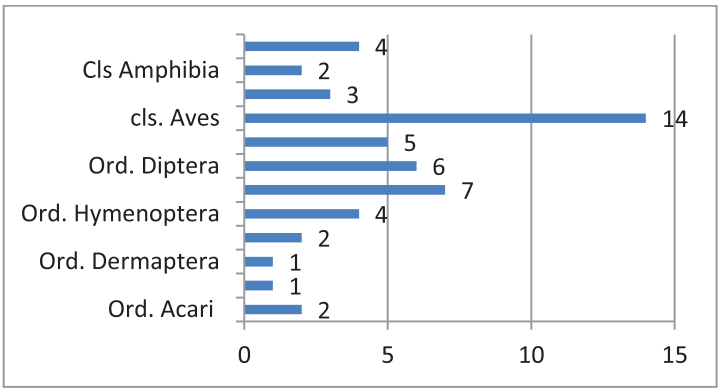

Figure 3. Beneficial fauna structure.

CONCLUSIONS

The ecosystem Botanical Garden from Craiova is an anthropogenic ecosystem but chemical interventions against pests, which directly disturb the predators and parasites organisms activity are very low.

They are limited to chemical treatment to control mosquitoes, ticks and some seasonal defoliators (hairy caterpillar - Hyphantria cunea) or application of treatments only in areas where there has been recorded damage caused by pests (treatments against buxus caterpillar - Cydalima perspectalis).

Thus trophic activity of the predators takes place in conditions almost natural and that's why the population of predators provide a reasonable level of pest control.

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SPATIAL NICHE OVERLAP OF FALCON SPECIES IN EUROPE

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Keywords: *falcon species, spatial niche overlap, niche similarity, environmental niche models*

ABSTRACT

In a classical niche approach, the resource utilization niche is perhaps the most operational approach to study how species differ in their requirements. Environmental niche models (ENMs) are used to describe the ecological tolerances of populations or species. In this paper utilized a series of GIS techniques to assess similarity (based on two indices, D and I) of ENMs (the degree of overlap of niches) for eight falcon species (order Falconiformes) present in Europe. Both niche similarity indices highlight that Falco subbuteo and Falco peregrinus are species that overlap most regarding environmental niche models.

INTRODUCTION

Research on ecological niches has moved in two directions: one of them has been to quantify niches in order to investigate potential competition between similar species at the local scale (this is often termed the Eltonian niche concept) and the other one, more recent and perhaps more important direction in niche approach (called the Grinnellian niche), has been to use GIS-based approaches to estimate spatial niches in order to estimate geographical distributions of species (Krebs 2014).

In a classical niche approach, the resource utilization niche is perhaps the most operational approach to study how species differ in their requirements (Morin 2011). Therefore, species are characterized in terms of their similarity in resource use, important measures used include various estimates of niche overlap and niche width (breadth of resource use) (Morin 2011).

Environmental niche models (ENMs) are used to describe the ecological tolerances of populations or species. The development of ENMs, generated by combining specie occurrence data (i.e. sample localities associated with latitude and longitude coordinates) with environmental GIS data layers, allow to quantify niches at an unprecedented scale (Warren 2008; Franklin 2009; Waren 2010). Ecological niche modeling has been applied to a diverse range of organisms, from aquatic and terrestrial plants, to animals (i.e. fish, beetles, butterflies and vultures) (Townsend 2008).

Conservation biologists have used bird of prey species to attract public support for biodiversity preservation, raise funds, protect and restore ecosystems, considering the fact that top predators have inspired respect and admiration, being

depicted as powerful, majestic, mysterious, beautiful, elusive, dangerous, rare, fierce, and secretive (Sergio et al. 2008; Kruuk 2002; Gittleman et al. 2001).

In this paper we intended to use a series of GIS techniques to assessing similarity of ENMs (the degree of overlap of niches) for eight falcon species (order Falconiformes) present in Europe: kestrel (*Falco tinnunculus*), lesser kestrel (*Falco naumanni*), red-footed falcon (*Falco vespertinus*), hobby (*Falco subbuteo*), saker falcon (*Falco cherrug*), peregrine falcon (*Falco peregrinus*), lanner falcon (*Falco biarmicus*) and Eleonora's falcon (*Falco eleonorae*).

MATERIAL AND METHODS

Environmental niche models (ENMs) for these raptors species were obtained following the steps:

- downloading the polygons with falcons distribution areas from the IUCN website (http://maps.iucnredlist.org).
- extraction of points of presence within these polygons for obtaining data of each species presence in .csv format.
- downloading eighteen environmental climatic variables (ESRI grid format) from Worldclim database (http://www.worldclim.org/) used for prediction of species distributions.
- obtaining maps of species prediction (ENMs) using maximum entropy algorithm (Maxent model -/www.cs.princeton.edu/~schapire/maxent/, Elith et al. 2011) in ASCII format.
- quantification of overlapping spatial niches of species using Maxent and ENMTools 3.1 (http://enmtools.blogspot.ro/).

Measurement of similarity among ENMs is central to comparative analyses. ENMTools software quantifies spatial niche similarity using the two measures introduced by Warren et al. (2008): Schoener's (1968) *D* index and a measure derived from Hellinger distance called *I*. These similarity measures are obtained by comparing the estimates of habitat suitability calculated for each grid cell of a study area using a Maxent-generated ENM, after normalizing each ENM so that all suitability scores within the geographic space sum to 1.

Schoener's D is defined as

$$D\left(p_x, p_y\right) = 1 - \frac{1}{2}\sum\left|p_{x,i} - p_{y,i}\right|$$

where p_{xi} and $p_{y,i}$ are the normalized suitability scores for species *X* and *Y* in grid cell *i*.

I is defined as:

$$I\left(p_x, p_y\right) = 1 - \frac{1}{2}H\left(p_x, p_y\right)$$

$$H\left(p_x, p_y\right) = \sqrt{\sum_i \left(\sqrt{p_{x,i}} - \sqrt{p_{y,i}}\right)^2}$$

Note that *I* is 1 - ''Hellinger's distance'' from probability theory; similarly, *D* is 1 - ''total variation distance''. Both similarity measures range from 0, when species predicted environmental tolerances do not overlap at all (i.e. $p_{x,i}py_{,i} = 0$ for all *i*), to 1, when all grid cells are estimated to be equally suitable for both species (i.e. $p_{x,i} = py_{,i}$ for all *i*).

RESULTS AND DISCUSSIONS

Spatial niche overlap (valued based on two indices presented above) between the eight falcon species was synthesized in the form of matrix, one for each index (Table 1 and 2).

D index

Niche overlap analysis (niche similarity) - based on species potential distribution maps resulting from Maxent algorithm revealed:

- highest niches overlaping (0.779) was between hobby (*Falco subbuteo*) and peregrine falcon (*Falco peregrinus*);

- a high value of overlapping niches (0.762) was also between peregrine falcon (Falco peregrinus) and kestrel (*Falco tinnunculus*).

- the lowest value of overlapping niches (0.199), was recorded between lanner falcon (*Falco biarmicus*) and saker falcon (*Falco cherrug*).

I index

And for this index, the highest overlapping values (0.954) was observed between hobby (*Falco subbuteo*) and peregrine falcon (*Falco peregrinus*). Also the lowest overlapping niches (0.470) after this index was recorded between lanner falcon (*Falco biarmicus*) and saker falcon (*Falco cherrug*).

As we can see, both niche similarity indices indicated that *Falco subbuteo* and *Falco peregrinus* are species that overlap most regarding environmental niche models generated by Maxent algorithm (Fig. 1). This is not surprising considering that these species (to which is added *Falco tinnunculus*) are some of the most widespread in Europe.

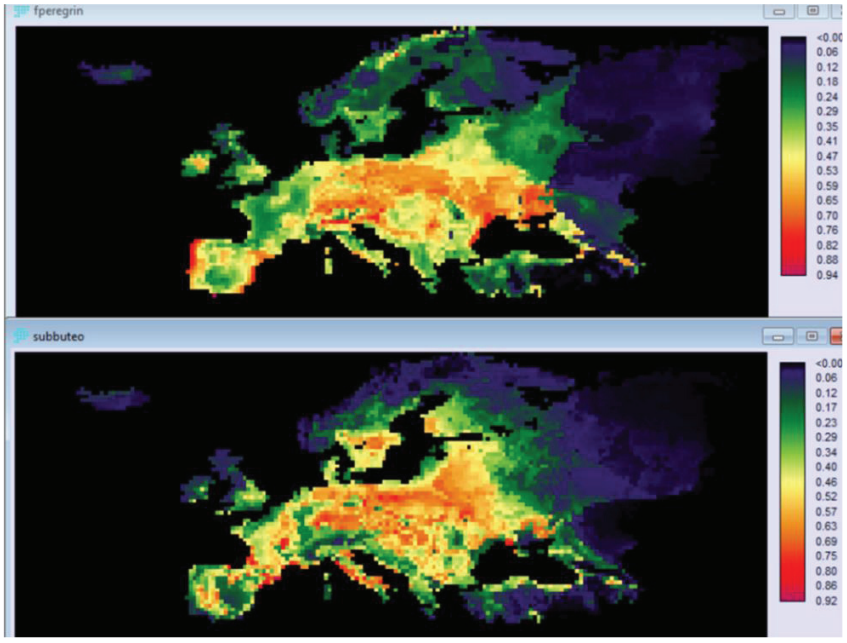

Figure 1. Spatial niche distribution for *Falco peregrinus* (top) and *Falco subbuteo* (down). Both maps of species prediction (ENMs) was obtained by maximum entropy algorithm.

Although both spatial niche overlap indices invoked the same species as the most similar in terms of their spatial niches, mean spatial overlap of *D* index (0.486 (SD:3.30)) differs significantly ($t_{0.05(2),27} = 2.052$; $P < 0.0001$) from the *I* index (0.759 (1SD:0.1460)), the latter index indicating a highier spatial overlap between species. Therefore, the conclusions about spatial niches overlap of falcon species must be drawn carefully, indicated all the time the index used.

CONCLUSIONS

Measurement of similarity among ENMs is central to understand how species coexisted at different spatial scale in nature. After our analysis, we can say that the eight falcon species showed a spatial niche overlap that goes from moderate (*D* index) to high (*I* index), being quite difficult to say that falcons spatial distribution in Europe is generated by environment rather than by interspecific competition.

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Table 1

Matrice of values of niche overlap for analyzed falcon species (*D* index)

Species	*F. biarmicus*	*F. cherrug*	*F. eleonore*	*F. naumanni*	*F. tinnunculus*	*F. peregrinus*	*F. subbuteo*	*F. vespertinus*
F. biarmicus	1	0.199	0.420	0.310	0.243	0.235	0.245	0.207
F. cherrug		1	0.293	0.629	0.419	0.536	0.497	0.597
F. eleonore			1	0.528	0.532	0.470	0.434	0.348
F. naumanni				1	0.519	0.591	0.538	0.559
F. tinnunculus					1	0.762	0.700	0.576
F. peregrinus						1	0.779	0.712
F. subbuteo							1	0.739
F. vespertinus								1

Table 2

Matrice of values of niche overlap for analyzed falcon species (I index)

Species	*F. biarmicus*	*F. cherrug*	*F. eleonore*	*F. naumanni*	*F. tinnunculus*	*F. peregrinus*	*F. subbuteo*	*F. vespertinus*
F. biarmicus	1	0.470	0.723	0.613	0.529	0.529	0.545	0.505
F. cherrug		1	0.597	0.867	0.742	0.828	0.800	0.865
F. eleonore			1	0.787	0.832	0.781	0.747	0.671
F. naumanni				1	0.803	0.861	0.820	0.813
F. tinnunculus					1	0.953	0.920	0.844
F. peregrinus						1	0.954	0.915
F. subbuteo							1	0.929
F. vespertinus								1

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EVALUATION OF BISTREȚ IBA (IMPORTANT BIRD AREA) CONSERVATIVE POTENTIAL

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Keywords: *IBA, conservative potential, potential species richness, mean potential species occurrence, habitat diversity*

ABSTRACT

Important Bird Areas (IBAs) are designated specifically for the biodiversity they contain, in particular for the bird species and populations they support. In the present study we aimed to analyze how we can process data obtained from monitoring the abundances of most common bird species identified within the Bistreţ IBA area for further conservation actions. The main purpose of this paper is to find a way to synthesize the conservation potential of Bistreț IBA area by a series of indices: potential species richness, mean potential species occurrence and habitat diversity. For Bistreț area, aquatic habitats, inland marshes and forest habitats represent landscape units with high conservation value.

INTRODUCTION

Very few areas have escaped the impact of recent developments in agriculture, forestry, fisheries, transport, energy, industry and urbanization, and associated pollution in the last decades, across most of the European continent man's use of land and sea continues to intensify (Heanth & Evans 2000).

Conservation of biological diversity is one of the greatest challenges that humans need to face, given our vast capacity of transformation of the environment (Lopez-Lopez et al. 2007). Birds are one of the best-researched and most reliable indicators of biodiversity loss in Europe (Tucker & Heath 1994; Tucker & Evans 1997). BirdLife's Important Bird Area concept has been developed and applied for over 30 years. Important Bird Areas (IBAs) are designated specifically for the biodiversity they contain, in particular for the bird species and populations they support (Mwangi et al. 2010). Important Bird Areas (IBAs) are priority sites for bird conservation because they regularly hold significant populations of one or more globally or regionally threatened, endemic or congregatory bird species, or highly representative bird assemblages (Heanth & Evans 2000). Considerable effort has been devoted to refining and agreeing a set of simple but robust criteria that can be applied worldwide:

● Places of international significance for the conservation of birds and other biodiversity

● Recognised world-wide as practical tools for conservation

● Distinct areas amenable to practical conservation action

● Identified using robust, standardised criteria

● Sites that together form part of a wider integrated approach to the conservation and sustainable use of the natural environment

Public and private land managers are in need of tools that ably incorporate landscape, species, and habitat relations into their conservation planning processes (Thogmartin et al. 2006). In the present study we aimed to analyze how we can process data obtained from monitoring the abundances of most common bird species identified within the Bistreţ IBA area for further conservation actions. The main purpose of this paper is to find a way to synthesize the conservative potential of Bistreț IBA area by a series of indices: *potential species richness*, *mean potential species occurrence* and *habitat diversity*.

Lake Bisteț is a natural lake situated near the Danube river (Fig. 1), and it is supplied by the Desnutui and Baboia rivers. This is an important breeding and stop-over site for large number of waterbirds (*Phalacrocorax carbo, Phalacrocorax pygmmeus, Pelecanus crispus, Botaurus stellaris, Ardeola ralloides, Ardea purpurea, Anser albifrons*) (Heanth & Evans 2000).

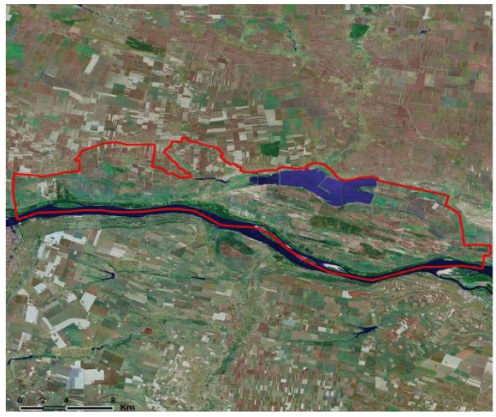

Figure 1. Bistreț IBA area (http://iba.sor.ro/).

MATERIAL AND METHODS

To relate birds data identified within Bistreț area with landscape (habitat) characteristics we utilized a GIS tool named LINK (www.umesc.usgs.gov/). LINK is a set of ArcGIS tools designed to map species–habitat patterns across a landscape. Any species that can be scored against land cover can be modelled with this tool, including birds, repetiles, and mammals. LINK maps values are contained in a matrix to a raster data layer called a source layer to generate indices of potential habitat. The program summarizes indices of potential habitat using a zonal layer, such as a layer delineating counties or management units.

Matrices

A matrix is a table containing a list of individuals, such as species (in Table 1 there are indicated the most common 19 species identified over the last years in this area). Matrices are related to a spatial data layer or a group of spatial data layers called source layers. Each species has a suitability score for each

classification in the related source layer. These scores range in value from 0 (little to no value as habitat) to 100 (prime habitat).

Source Layers

A source layer is a raster-formatted spatial data layer that contains landscape information pertinent to the species listed in the matrix. We utilized a GIS shapefile of habitat coverage (Corine Land Cover - CLC) of study area. The identified Habitats (with CLC code in parenthesis) are: vineyards (221), pastures (231), inland marshes (411), industrial or commercial units (121), non-irrigated arable land (211), discontinuous urban fabric (112), complex cultivation patterns (242), water bodies (512), broad-leaved forest (311), rice fields (213), and transitional woodland-shrub (324) (Table 1).

Zonal Layers

A zonal layer is an ESRI ShapeFile used to divide the landscape into units of comparison. A shapefile of counties within a state or management units within a refuge are examples of zonal layers. We divide area by a grid of 1: 5000.

Spatial products (maps) obtained by LINK software were:

- *potential species richness PSR* (number of species having a matrix score > 0 for the source grid class. The possible range of values is 0 to *n*, the number of species).

- *mean potential species occurrence PSO* (this is a reclassed source layer that shows the average matrix score of the species queried for each source layer class. PSO values are rounded to the nearest integer. Values range from 0 to 100).

- *Simpson's Diversity Index By Zone SDI* (This index measures the diversity of habitats in each zone of a zonal layer).

RESULTS AND DISCUSSIONS

At the level of this area we identified a large number of birds nesting or occurring only in winter or/and passage. Our observations have focused only on a total of 19 species (species most common), for simplicity and ease of understanding the results (Table 1).

Potential species richness PSR had the highest values in broad-leaved forest habitats (9 species) water bodies habitats (14 species) and inland marshes (16 species) (this being an area where aquatic habitats are dominant) (Fig. 2; Fig. 3a).

Mean potential species occurrence PSO also presented the highest values in the three habitats categories mentioned before: aquatic habitats (25 species), inland marshes (21 species) and forest habitats (14 species) (Fig. 2; Fig. 3b).

Simpson's Diversity Index By Zone SDI highlighted zones at Bistreț area showing the highest diversity of habitats (Fig. 3c). Such an approach is particularly useful in order to carry out the management project regarding this area.

CONCLUSIONS

The main benefit of such kind of analysis it that we could compare conservative potential of different zone of area of study or between management units and the surrounding landscape. The three main indices (*PSR*, *PSO* and *SDI*) can be successfully used when we want to obtain data regarding species richness and habitat diversity of an area in a very short time specific. For Bistreț area, aquatic habitats, inland marshes and forest habitats represent landscape units with high conservation value, future management actions at this area must take into account the specific requirements of them.

Table 1

Relative score (abundance) for bird species identified within the Bisteț IBA area

Species	Order	Habitat										
		1	2	3	4	5	6	7	8	9	10	11
Podiceps cristatus	Podicipediformes	0	0	10	0	0	0	0	80	0	0	0
Ixobrychus minutus	Ciconiiformes	0	0	40	0	0	0	0	50	0	0	0
Nycticorax nictycorax	Ciconiiformes	0	0	50	0	0	0	0	20	0	0	0
Egretta garzetta	Ciconiiformes	0	0	60	0	0	0	0	10	1	0	0
Ardea purpurea	Ciconiiformes	0	0	50	0	0	0	0	50	8	0	0
Ardea cinerea	Ciconiiformes	0	0	40	0	0	0	0	60	7	0	0
Ciconia nigra	Ciconiiformes	5	3	4	0	4	0	5	0	40	0	0
Anas querquedula	Anseriformes	0	0	20	0	0	0	0	40	0	0	0
Aythya ferina	Anseriformes	0	0	8	0	0	0	0	25	0	0	0
Aythya nyroca	Anseriformes	0	0	2	0	0	0	0	30	0	0	0
Haliaetus albicilla	Falconiformes	0	0	20	0	0	0	0	50	40	0	0
Circus aeruginosus	Falconiformes	1	5	60	0	1	0	3	28	0	0	1
Accipiter gentilis	Falconiformes	1	1	1	12	1	1	1	1	100	1	0
Buteo buteo	Falconiformes	10	15	1	1	28	0	30	20	30	1	17
Aquila pomarina	Falconiformes	0	20	0	0	0	0	0	0	0	0	0
Falco subbuteo	Falconiformes	12	10	4	3	20	1	12	4	20	0	2
Burhinus oedicnemus	Charadriiformes	15	0	0	0	0	0	0	0	0	0	2
Sterna albifrons	Charadriiformes	0	0	20	0	0	0	0	0	0	0	0
Strix aluco	Strigiformes	0	0	0	0	0	0	0	0	27	0	0

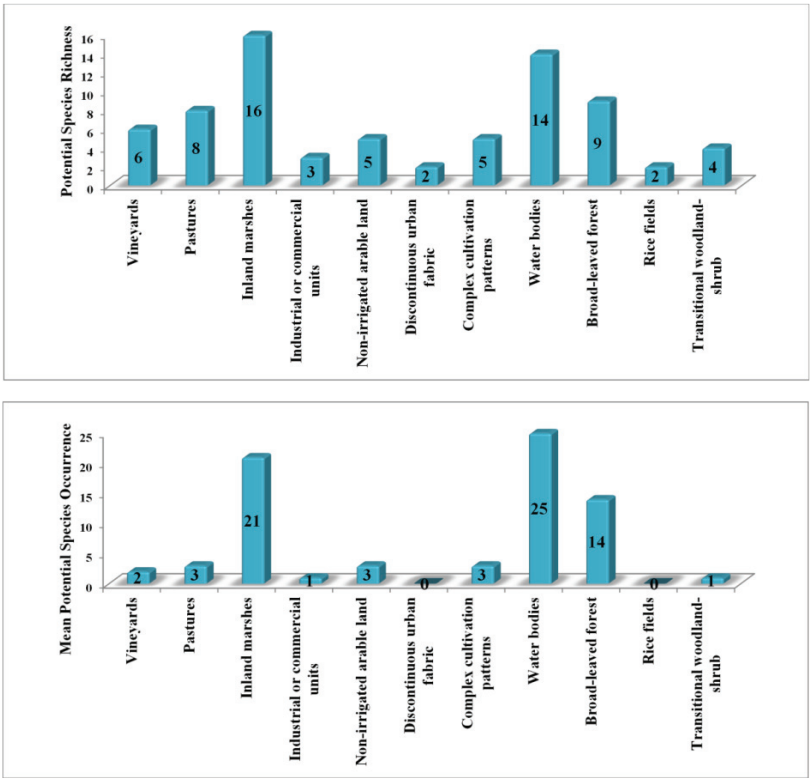

Figure 2. Values of *Potential species richness PSR* (top) and *Mean potential species occurrence PSO* (down) for different categories of habitats present in the study area.

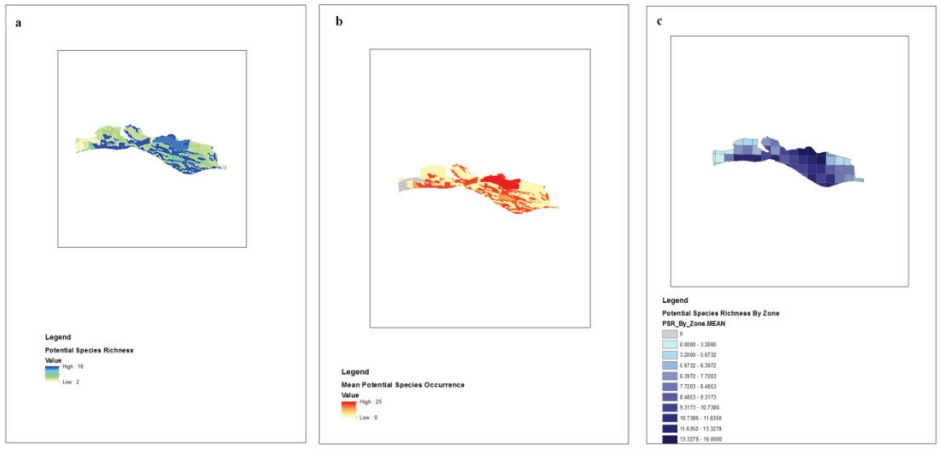

Figure 3. *Potential species richness PSR* (a), *Mean potential species occurrence PSO* (b), and *Simpson's Diversity Index SDI* (c) summarized by a zonal grid of 1: 5000 scale.

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ANTHROPOGENIC THREATS ON SOME INSECT SPECIES PROTECTED UNDER THE IUCN RED LIST IN THE PROTECTED AREA SLVOSTEPA OLTENIEI

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Keywords: *anthropogenic threats, insect, protected area*

ABSTRACT

From our observation regarding the protected area Silvostepa 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. As part of the management plan of the protected area Silvostepa Olteniei, during our research we have to identify the major anthropogenic threats for these protected species. Following our studies we have identified several anthropogenic threats, among them: intensive farming, forestry exploitation without replanting or natural recovery, the use of biocidal, hormones and chemical, collection of individuals.

INTRODUCTION

There is no doubt that human civilization has had a negative impact on biodiversity, particularly since the industrial revolution. Overfishing and hunting, the destruction of habitats through agriculture and urban sprawl, the use of pesticides and herbicides, and the release of other toxic compounds into the environment have all taken their toll, particularly on vertebrates. The World Conservation Union (IUCN; Gland, Switzerland) now includes more than 16,000 entries in its Red List of Threatened Species: 5,624 vertebrates, 2,101 invertebrates and 8,390 plants. The number of documented extinctions since 1500 AD is now 784 species and the IUCN estimates that extinction rates are now 50 to 500 times higher than previous rates calculated from the fossil record (Baillie et al. 2004).

Recognising the growing pressures on invertebrates is important, as local declines lead to global extinctions. This loss is signifycant as each species is distinct, fascinating and beautiful, and part of the network of life that makes up our global ecosystem. There are additional more utilitarian reasons why we must stem the tide of invertebrate decline. Though their importance to human wellbeing is rarely recognised, invertebrates form the basis of many of the critical services that nature provides. Terrestrial invertebrates are primarily threatened by agriculture and logging (both affecting 31% of threatened species; followed by infrastructure development (28%). Agriculture (including tree plantations) is a threat to many species due to new areas of previously natural or non-intensively managed habitat being converted to high intensity agriculture (Foden et al. 2009).

In anthropogenic agroecosystems human intervention through chemical methods to limit the pest attacks, resulting in serious disruption of beneficial organisms activity having a negative influence, due to direct exposure to pesticide action and indirect action due to the active substance ingested with the prey. (Stan C., Tuca O., Mitrea I. 2013).

The second most common threat is climate change; effects are felt by species in several different ways. Saproxylic beetles, for example, depend on tree species as their habitat and it is therefore difficult to predict the mobility of those beetles in the face of a changing climate (Nieto and Alexander 2010).

MATERIALS AND METHODS

As part of the management plan of the protected area Silvostepa Olteniei, during our research we have to identify the major anthropogenic threats for some insected species protected under the IUCN red list. In order to determine the major anthropogenic threats for these inseted species we had to identify and locate the habitats of these species in the protecetd area Siulvostepa Olteniei.

Following our studies we have identified a number of three species belonging to Coleoptera order (*Carabus hungaricus Fabr., Cerambyx cerdo L., Lucanus cervus L.*) and one specie belonging to Lepidoptera order (*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, Perişor, Târnava, Mărăcinele, Ştiubeiului, Verbicioara and Pleniţa. The other beetle species has been located on several meadows near: Radovan, Vela, Bucovicior, Tencănău, Vârvoru de Jos, While the lepidoperan species has been observed on the sunny banks of lake Ciutura as well the rivers and Terpeziţa.and Desnăţui.

RESULTS AND DISCUSSION

Following our studies we have several anthropogenic threats, among them: intensive farming, forestry exploitation without replanting or natural recovery, the use of biocidal, hormones and chemical, collection of individuals.

For each species protected under the IUCN red list we had identified the major anthropogenic threats.

For *Carabus hungaricus* Fabr, 4013 we have identified four major anthropogenic threats:

A02.01 Intensive Farming

Intensive farming or intensive agriculture also known as industrial agriculture is characterized by a low fallow ratio and higher use of inputs such as capital and labour per unit land area.

Intensive crop agriculture is characterised by innovations designed to increase yield. Techniques include planting multiple crops per year, involves increased use of fertilizers, plant growth regulators, pesticides and mechanization, controlled by increased and more detailed analysis of growing conditions, including weather, soil, water, weeds and pests. In this area in the early ninties irrigation network was abandoned, forest shelter belts were destroyed. Without forest vegetation and regular periodic floods, summer droughts have become more frequent and winter winds emphasizes this effect.

On the agricultural land from this protected area, intensive farming practices lead to the restriction of the species habitats.

A02.03 - replacing pasture with arable land

Although in this protected area agricultural areas have a very good representation because the soil in the area is suitable to sustainable agriculture in some localities: Radovan, Vela, Bucovicior, Tencănău, Vârvoru de Jos, these area have expanded affecting some of the area meadows. This impact leads to restriction of the species habitats, namely the reduction of the species livestock.

A07 - The use of biocidal products, hormones and chemicals

B03 – Forestry Exploitation without replanting or natural recovery

Generally, in the forests of this area wood is extracted only by local for domestic use. This practice leads to the fragmentation of the species habitat

For *Cerambyx cerdo* L, 1088 we have identified four major anthropogenic threats:

B02.04 - removal of dead trees or decaying wood

Eliminating rotting trees and shrubs of the forest ecosystem, ie forests Întorsura, Perişor, Târnava, Mărăcinele, Ştiubeiului, Verbicioara and Pleniţa, lead to the extinction of species habitat

B03 - Forestry exploitation without replanting or natural recovery

Generally, in the forests of this area wood is extracted only by local for domestic use. This practice leads to the fragmentation of the species habitat

B04 - The use of biocidal products, hormones and chemicals (in the forest)

Applying pesticide products, hormones and various chemicals cause mutations, deaths of individuals with genetic, biological and environmental implications on short and long term.

F03.02.01 - collection of animals (insects, reptiles, amphibians).

Collection of the individuals leads to the reduction of species livestock.

For *Lucanus cervus* L 1083 we have identified four major anthropogenic threats:

B02.04 - removal of dead trees or decaying wood

Eliminating rotting trees and shrubs of the forest ecosystem, ie forests Întorsura, Perişor, Târnava, Mărăcinele, Ştiubeiului, Verbicioara and Pleniţa, lead to the extinction of species habitat

B03 - Forestry Exploitation without replanting or natural recovery

Generally, in the forests of this area wood is extracted only by local for domestic use. This practice leads to the fragmentation of the species habitat

B04 - The use of biocidal products, hormones and chemicals (in the forest) Applying pesticide products, hormones and various chemicals cause mutations, deaths of individuals with genetic, biological and environmental implications on short and long term.

F03.02.01 - collection of animals (insects, reptiles, amphibians).

Collection of the individuals leads to the reduction of species livestock.

For *HyLes hippophaes* Esp. 1077 we have identified four major anthropogenic threats:

A07 - The use of biocidal products, hormones and chemicals

Applying pesticide products, hormones and various chemicals cause mutations, deaths of individuals with genetic, biological and environmental implications on short and long term.

F03.02.01 - collection of animals (insects, reptiles, amphibians).

Collection of the individuals leads to the reduction of species livestock.

CONCLUSIONS

Following our studies we have identified several anthropogenic threats.

For the saproxylic beetles *Cerambyx cerdo* L. and *Lucanus cervus* L. we have identified as major anthropogenic threats: removal of dead trees or decaying wood, forestry exploitation without replanting or natural recovery, the use of biocidal products, hormones and chemicals collection of the individuals leads to the reduction of species livestock.

For *Carabus hungaricus* Fabr. we have identified as major threats: intensive farming, replacing pasture with arable land, the use of biocidal products, hormones and chemicals collection of the individuals leads to the reduction of species livestock.

For *Hyles hyppophae* Esp. We have identified as major threats: the use of biocidal products, hormones and chemicals collection of the individuals leads to the reduction of species livestock.

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NON-ALCOHOLIC FATTY LIVER DISEASE IN PATIENTS WITH TYPE 2 DIABETES

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Keywords: *non-alcoholic fatty liver disease, obesity, dyslipidemia*

ABSTRACT

This paper aims to assess the incidence of non-alcoholic fatty liver in patients with type 2 diabetes. Most patients present dyslipidaemic nonalcoholic steatosis, dyslipidemia representing alongside obesity and diabetes main risk factors for NAFLD (Angulo P). The lipid profile is most frequently associated with hypertriglyceridemia. Ultrasonographic changes typically associated with the following parameters: BMI, blood sugar, lipid, transaminase ratio AST / ALT <1, GGT levels are predictors of nonalcoholic hepatosteatosis (Eiji Miyoshi, Yoshihiro Kamada). Given the existence of same primary mechanism of action, insulin resistance, and the similarity of the main risk factors in metabolic syndrome include hepatosteatosis is justified (Ford ES, Giles WH, Dietz WH).

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD - hepatosteatosis) and nonalcoholic steatohepatitis (NASH) is the evolutionary stages of a metabolic disease that affects the liver, whose main risk factors are represented by central obesity, diabetes and dyslipidemia. NAFLD is histologically characterized by excessive accumulation of lipids in the liver, in the absence of alcohol (Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH, Liou I, Kowdley KV).

MATERIAL AND METHODS

The studied group included 110 patients with type 2 diabetes who were registered Ambulatory Diabetes Nutrition & Metabolic Diseases Philanthropy Craiova Municipal Hospital. As a working method we used the following: age diabetes, personal history, waist circumference, body mass index, blood pressure measurement, blood sugar, lipidogram, uric acid, liver function tests, viral markers, transabdominal sonography. I mention that liver biopsy was a difficult procedure because patients have not accepted this procedure.

RESULTS AND DISCUSSIONS

Of the 110 patients, 43 (39.09%) were female and 57 (60.90%) men.

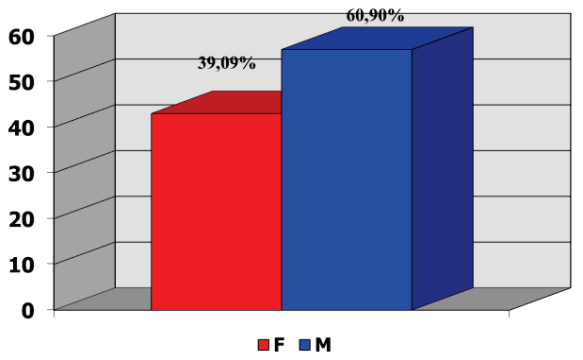

Figure 1. Distribution by gender of studied patients.

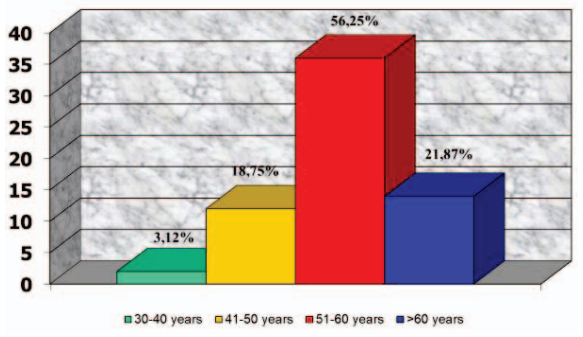

Figure 2. Age decades of studied patients.

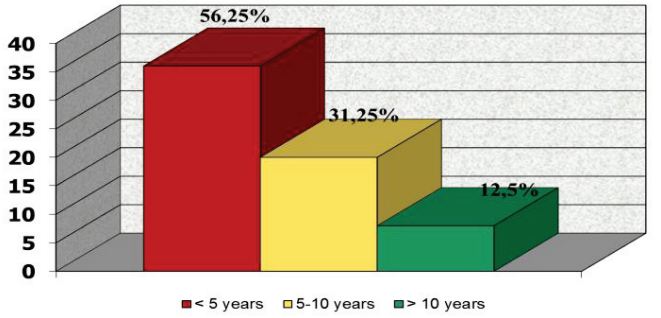

Figure 3. Diabetes duration.

Studying the incidence of non-alcoholic fatty liver we noted its presence in a percentage of 75.45% (83 patients).

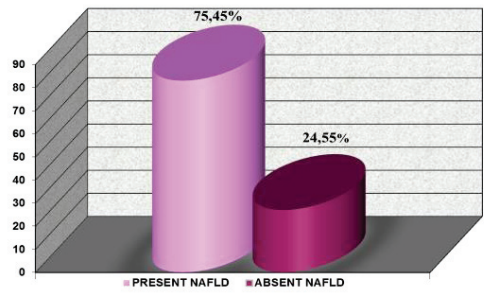

Figure 4. Non alcoholic fatty liver disease (NAFLD) incidence.

Calculating BMI patients in whom we identified non-alcoholic fatty liver disease, 10 of them (12.04%) were found with normal body weight, 29 patients (34.93%) are overweight and 71 patients (53.03%) with the obesity of various degrees. In these patients we measured waist circumference and we found abdominal obesity in all patients (100%).

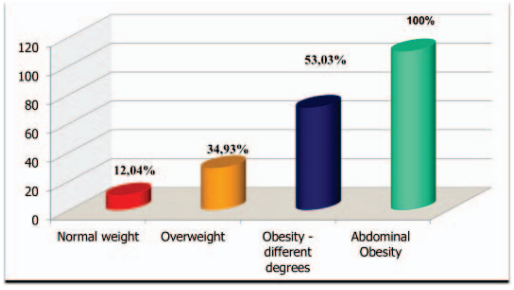

Figure 5. Body mass index (BMI) in patients with non-alcoholic fatty liver disease.

14 patients (16.86%) diagnosed with non-alcoholic fatty liver had also hyperuricemia.

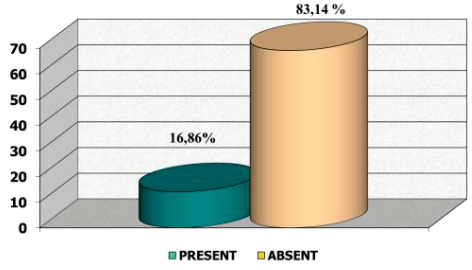

Figure 6. The incidence of hyperuricemia in patients with non-alcoholic fatty liver disease.

The patients diagnosed with NAFLD was investigated in the presence of lipid abnormalities. Isolated hypertriglyceridemia was associated in 27 patients

(32.53%) and mixed dyslipidemia in 48 patients (57.83%). Hypertension was present in 67 patients (80.71%) diagnosed with liver steatosis.

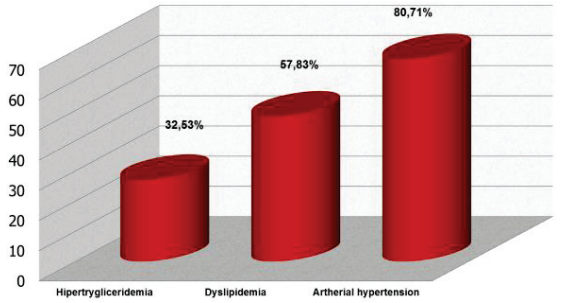

Figure 7. NAFLD association with hypertension and impaired lipid metabolism.

CONCLUSIONS

There was an increased frequency of NAFLD in patients with type 2 diabetes. This frequency is probably underestimated, not having availability to perform a liver biopsy. We observed the association of NAFLD with DM and the others components of MS. NAFLD incidence is increased in overweight patients that obesity, especially abdominal obesity. Hyperuricemia, component of metabolic syndrome, was also seen.

Alterations in lipid metabolism were encountered in a high percentage.

NAFLD can be estimated as hepatic manifestation of metabolic syndrome.

Non-alcoholic fatty liver is commonly associated with incidence of type 2 diabetes is increased in patients with overweight that obesity, especially abdominal obesity. Hyperuricemia was also found, but a low percentage by comparison with lipid metabolism abnormalities present in over 50% of patients diagnosed with liver steatosis. HTA was frequently associated with non-alcoholic fatty liver.

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CLIMATIC EVOLUTIONS IN THE EARLIEST SPRING (2016) IN THE SOUTH-WEST OF ROMANIA

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Keywords*: climate warming, late hoar frosts, early spring.*

ABSTRACT

The spring of 2016 was early and came after the warm winter of 2015-2016. As a consequence of the warm month, the vegetation began to develop starting with February. The hoar frosts of March have been destructive. Although air temperature has continued to increase in April, the cooling from April and the late spring hoar frosts have caused significant damages. In April, the vegetation had some advanced stages of development and, consequently, the hoar frost of the morning of 27th April was extremely destructive. The paper analyses the climatic macroprocesses, which caused climatic evolutions in the earliest spring of all the history of climate observations, registered in 2016. The paper is useful to specialists, PhD candidates, master graduates and to all people interested in climate evolution.

INTRODUCTION

The analysis of the climatic conditions of the spring 2016 in the South-West of Romania is a continuation of the extended studies on the regional climate variability (Marinică et al. 2015, Marinică Andreea Floriana et al. 2015). ***The year of 2015 reached the first climatic record of global average temperature ≥ 1.0°C than the global average of the last century and the entire period of weather records from 1880-1899***. As a consequence of the increase of warm winters in Romania, the frequency of liquid precipitation during winter has increased too. Regionally, the manifestations of climate disruptions are specific to each distinct climatic region. Most of the winters of the last 26 years have been warm.

RESULTS AND DISCUSSIONS

Indexes of spring arrival in Oltenia in 2016 were comprised between 356.2°C in Voineasa and 635.5°C in Dr. Tr. Severin. Their mean for the entire region of Oltenia was 520.3°C. The highest values of these indexes (absolute climate records), exceeding 600.0°C were registered in the extreme South-West of the region, where the advections of warm air were more intense and frequent. *The*

deviations of spring indexes were comprised between 201.7% in Voineasa and 321.3°C in Drăgășani, and the percentage deviations between 75.8% in Calafat in the extreme South-West and 130.6% in Voineasa Intramountainous Depression. These high percentage deviations led to a classification of spring arrival types of excessively early for the entire region of Oltenia. The excessively early spring arrival was also confirmed by the mean deviation of spring arrival index for the entire region. The highest deviations were registered in the high area of Oltenia, a common situation for temperature increases, showing that high areas are warming faster than low areas. ***The analysis of spring arrival indexes in the interval 1998-2016*** shows that in 2016 the indexes of spring arrival exceeded with high differences all the indexes registered in the last 19 years (1998-2016) at most of the meteorological stations (82.4%) excepting three meteorological stations: Calafat with a maximum of 610.0°C, Apa Neagră with a maximum of 563.8°C and 404.1°C in Voineasa, all these records being registered in 2002. In 2016 the mean of spring arrival indexes calculated for the entire region was 520.3°C being the highest of this interval and the only one exceeding 500.0°C. It should be noted the early spring arrivals in 2010 and 2012 whose average spring arrival calculated for the entire region differs only in a tenth of degree (339.3°C and 339.4°C) and the increasing and generalization tendency of excessively early spring arrivals. ***The spring of 2016 holds the record for the absolute climatic record of the earliest spring arrival of the history of climatic observations in Romania,*** not only in Oltenia, because in the West of the country, Moldova and East of Muntenia the temperature values in the interval 1 February-10 April 2016 were higher than in Oltenia (as shown above). The same type of spring arrival was registered in the whole Europe and even in the Northern Hemisphere. Regarding the spring of 2016, WMO (World Meteorological Organization) showed that, according to data published by NASA, the spring of 2016 has been the earliest in the history, ever recorded in Northern Hemisphere and the warmest.

Climatic conditions in March 2016. ***Thermal regime of March 2016.*** *Monthly means of air temperature* were positive in all days and were comprised between 4.6°C in Voineasa and 8.3°C in Dr. Tr. Severin, and the monthly mean was 2.3°C. Their deviations from the multiannual monthly means were comprised between 2.1°C in Băilești and Bâcleș and 2.7°C in Caracal and Tg. Logrești, and according to Hellmann criterion leads to a classification of warm month for the entire region. Monthly mean deviation from the normal, calculated for the entire region, was 2.3°C, which confirms the classification of warm month (W).

The sums of daily positive means calculated for the entire month were comprised between 143.7°C in Voineasa and 258.4°C in Dr. Tr. Severin with an average for the entire region of 205.7°C. The percentage ratios of these sums of the values of spring arrival index were comprised between 37.0% in Plovragi and 41.8% in Apa Neagră, with a mean for the entire region of 39.6%, being the highest contributions to the spring arrival index. *Monthly maximum temperature values* were registered in the last day of the month and were comprised between 19.8°C in Voineasa and 25.4°C in Calafat, and their mean for the entire region was 22.5°C. *Weather warming in March 2016* (with maximum temperature values ≥ 10.0°C) occurred in the intervals: 1-3 March, 5-10 March, 17-21 March and 26-31 March amounting 20 warm days. There were 6 days in which the mean of daily minimum values calculated for the entire region was ≤ 0°C. As a consequence of air temperature drop, there has often been hoarfrost locally and sparsely which

damaged the early fruit trees, especially the almond tree, apricot tree and peach tree, whose flowers were almost entirely destroyed. The vegetation was developing since the last decade of February. *On ground surface, most of the monthly maximum temperature values* were registered on 31 March and were comprised between 26.5°C in Apa Neagră and 42.2°C in Dr. Tr. Severin, and their mean for the entire region was 33.1°C. *On ground surface, most of the monthly minimum temperature values* were registered on 17 March and were comprised between -6.4°C in Polovragi and -0.1°C in Caracal, and their mean for the entire region was -3.4°C.

Pluviometric regime of March 2016*. Monthly quantities of precipitation* were comprised between 83.5 l/m^2 in Tg. Jiu and 142.8 l/m^2 in Craiova, and their mean for the entire region was 10.3 l/m^2, equal with the mean of May. The percentage deviations of monthly quantities of precipitation from the normal were comprised between 88.8% in Apa Neagră and 353.3% in Craiova leading to classifications of pluviometric time type from excessively rainy at all meteorological stations in Oltenia (Table no. 1). The percentage deviations of the mean of monthly quantities of precipitation calculated for the entire region was 137.9%, confirming the classification of excessively rainy month for the entire region. *The maximum quantity of precipitation registered in 24 hours* was 49.8 l/m^2 in Craiova.

Climatic conditions in April 2016. *Thermal regime of April 2016.* *Monthly air temperature means* were comprised between 10.9°C in Voineasa and 15.0°C in Dr. Tr. Severin, and *their deviations from the multiannual means* were comprised between 2.5°C in Bechet in the extreme South of the region and 3.9°C in Drăgășani, leading to the classification of thermal time type of warm month (W) to all meteorological stations in Oltenia. The highest deviation of the monthly temperature mean was registered in Parâng (4.7°C). *Air temperature mean for the entire region* was 13.6°C, and its deviation from the normal was 3.2°C, which confirms the classification of warm month for the entire region.

The monthly minimum air temperatures were registered on 27 April and were comprised between -2.4°C in Voineasa and 2.5°C in Bâcleș in Mehedinți Hills, and their mean for the entire region was -0.2°C. This was the only day in April 2016 in which the minimum temperature mean calculated for the entire region was negative. In the morning of 27 April there was hoarfrost on extended areas and locally frost in the air, and the vegetation was in advanced stages of development. Many crops have been damaged: sunflower, bean, pumpkin, grape offspring, corn, potatoes, field tomatoes, acacia flowers from areas located in the lower part of valleys where the extremely cold air lingers etc. The apiculture was also affected. ***The hoarfrost in the morning of 27 April was the most destructive and latest in the spring of 2016 and represented an extremely intense climatic risk.*** The number of days with minimum temperatures ≤ 0°C in April was between 0 in Dr. Tr. Severin, Băilești, Caracal, Craiova, Slatina, Bâcleș, Drăgășani and Rm Vâlcea and 3 in Apa Neagră and Voineasa, and in the mountainous area, 4 in Parâng, but the hoarfrost has also occurred in areas where the minimum temperature was ≤ 1.5°C and the sky was clear at night. *Monthly maximum air temperatures* were registered on 18 April and were comprised between 28.2°C in Voineasa and 33.1°C in Calafat in the extreme South-West of Oltenia, and their mean for the entire region was de 29.1°C. *On ground surface, minimum temperatures* were registered on 27 April and were comprised between -2.9 °C in Polovragi and 3.3°C in Caracal and Slatina, and their mean for the entire region was 0.2°C. ***Air temperature drop in April,***

late hoarfrost on 27 April and frosted ground in some area in that morning represent the climatic anomaly of April 2016 and an extremely destructive climatic and agroclimatic risk.

Pluviometric regime of April 2016. Monthly quantities of precipitation registered in April 2016 were comprised between 23.5 l/m^2 in Băilești and 62.6 l/m^2 in Slatina, and the percentage deviations from the normal were comprised between -52.4 in Băilești in Oltenia Plain and 47.6 in Drăgășani, leading to classifications of pluviometric time type from excessively droughty (ED) in Oltenia Plain (in Băilești) and Tg. Jiu Subcarpathian Depression and very rainy in Vâlcea Hills and in Drăgășani (table no. 1). Monthly precipitation mean calculated for the entire region was 53.9 l/m^2, and its percentage deviation from the normal was -3.6% leading to the classification of normal pluviometric month overall for the entire region.

Climatic conditions in May 2016. ***Thermal regime of May 2016.*** Monthly air temperature means were comprised between 11.6°C in Voineasa and 16.8°C in Calafat, and their deviations from the multiannual means were comprised between -1.2°C in Bechet and Craiova and -0.3°C in Apa Neagră and Tg. Jiu leading to classifications of thermal time type at all meteorological stations from cool (CO) in Oltenia Plain in Bechet, Băilești, Craiova and in Getic Piedmont in Slatina, and normal (N) in the rest of the region. *Monthly minimum air temperatures* were registered in the interval 17-19 May and were comprised between 0.5°C in Voineasa and 7.2°C in Calafat, and their mean fir the entire region was 3.8°C. *Monthly maximum air temperatures* were comprised between 26.3°C in Polovragi and 31.6°C in Calafat, and their mean for the entire region was 28.9°C. *Summer days*[1] at all meteorological stations in Oltenia were registered in the intervals 21-24 May and 26-31 May, and *tropical days* were on 30 May. *On ground surface most of the monthly minimum temperatures* were registered in the interval 17-19 May and were comprised between 1.0°C in Polovragi and 8.8°C in Caracal, and their mean for the entire region was 5.1°C. Monthly maximum temperatures on ground surface were registered in the interval 27-30 May and were comprised between 33.2°C in Apa Neagră and 60.4°C in Dr. Tr. Severin, and their mean for the entire region was 44.8°C.

Pluviometric regime of May 2016. Monthly quantities of precipitation were comprised between 62.2 l/m^2 in Caracal and 160.4 l/m^2 in Polovragi, and their percentage deviations from the multiannual means were comprised between 0.4% in Dr. Tr. Severin and 76.1% in Bechet, leading to classifications of pluviometric time type at all meteorological stations in Oltenia from normal (N) on restricted areas in the extreme West in Dr. Tr. Severin and in the East in Olt Couloir in Caracal and Rm. Vâlcea and excessively rainy in the South of Oltenia Plain in Calafat and Bechet and in Polovragi Subcarpathian Depression (table no. 1).

Seasonal climatic conditions in the spring of 2016. ***Seasonal thermal regime in the spring of 2016.*** *Seasonal average temperatures* were comprised between 9.0°C in Voineasa Intramountainous Depression and 13.3°C in Dr. Tr. Severin, and their deviations from the multiannual means were comprised between 1.2°C in Băilești and 1.9°C in Drăgășani and Tg. Jiu leading to classifications of pluviometric time types for all the spring season of warmish (WS) at all meteorological stations in Oltenia.

[1] Summer day = day with air temperature ≥ 25.0°C; tropical day = day with air temperature ≥ 30.0°C

Table 1

Quantities of precipitation registered in the spring of 2016 (Σ), compared to the normal values (N) for the period 1901-1990), deviation (%) and pluviometric time type according to Hellmann criterion (CH) (ED = excessively droughty, VD = very droughty, D = droughty, LD = little droughty, N = normal, LR = little rainy, R = rainy, VR = very rainy, ER = excessively rainy).

Meteorological Station	Hm	March 2016				April 2016			
		ΣIII	**N**	Δ%	**CH**	**ΣIV**	N	Δ%	**CH**
Dr. Tr. Severin	77	98.7	49.3	100.2	**ER**	54.2	56.5	-4.1	**N**
Calafat	66	85.6	38.1	124.7	**ER**	26.6	47.3	-43.8	**VD**
Bechet	65	89.1	36.3	145.5	**ER**	58.0	48.6	19.3	**LR**
Băileşti	56	93.0	38.3	142.8	**ER**	***23.5***	49.4	***-52.4***	**ED**
Caracal	112	96.9	35.7	171.4	**ER**	33.0	45.1	-26.8	**D**
Craiova	190	**142.8**	31.5	**353.3**	**ER**	48.0	43.1	11.4	**LR**
Slatina	165	103.2	37.5	175.2	**ER**	**62.6**	47.4	32.1	**VR**
Tg. Logreşti	262	101.5	37.9	167.8	**ER**	56.0	49.9	12.2	**LR**
Drăgăşani	280	115.2	37.4	208.0	**ER**	59.2	40.1	**47.6**	**VR**
Apa Neagră	250	120.1	63.6	***88.8***	**ER**	59.1	76.4	-22.6	**D**
Tg. Jiu	210	***83.5***	43.8	90.6	**ER**	31.6	64	-50.6	**ED**
Polovragi	546	99.9	50.9	96.3	**ER**	56.0	70.4	-20.5	**D**
Rm. Vâlcea	243	78.7	36.8	113.9	**ER**	72.6	58.5	24.1	**R**
Parâng	1585	95.8	53.0	80.8	**ER**	114.1	86.3	32.2	**VR**
Mean Oltenia		100.3	42.2	137.9	**ER**	53.9	55.9	-3.6	N
Meteorological Station	Hm	May 2016				Spring 2016			
		ΣV	N	Δ%	**CH**	**ΣR**	N	Δ%	**CH**
Dr. Tr. Severin	77	81.0	80.7	***0.4***	**N**	233.9	186.5	25.4	**R**
Calafat	66	103.6	60.8	70.4	**ER**	215.8	146.2	47.6	**ER**
Bechet	65	103.2	58.6	**76.1**	**ER**	250.3	143.5	74.4	**ER**
Băileşti	56	91.6	70.1	30.7	**VR**	208.1	157.8	31.9	**VR**
Caracal	112	***62.2***	61.4	1.3	**N**	***192.1***	142.2	35.1	**VR**
Craiova	190	89.0	60.6	46.9	**VR**	279.8	135.2	107.0	**ER**
Slatina	165	75.4	64.8	16.4	**LR**	241.2	149.7	61.1	**ER**
Tg. Logreşti	262	92.8	73.4	26.4	**R**	250.3	161.2	55.3	**ER**
Drăgăşani	280	78.6	69.7	12.8	**LR**	253.0	147.2	71.9	**ER**
Apa Neagră	250	121.7	108.8	11.9	**LR**	300.9	248.8	20.9	**R**
Tg. Jiu	210	100.4	85.3	17.7	**LR**	215.5	193.1	11.6	**LR**
Polovragi	546	**160.4**	103.9	54.4	**ER**	**316.3**	225.2	40.5	**VR**
Rm. Vâlcea	243	105.6	97.3	8.5	**N**	256.9	192.6	33.4	**VR**
Parâng	1585	139.2	114.8	21.3	**R**	349.1	254.1	37.4	**VR**
Mean Oltenia		100.3	79.3	26.5	**R**	254.5	155.2	42.6	**VR**

(Sourced: processed data from Oltenia MRC Archive)

Seasonal pluviometric regime in the spring of 2016. *Seasonal quantities of precipitation* were comprised between 192.1 l/m^2 in Caracal and 316.3 l/m^2 in Polovragi, and their percentage deviations from the normal values were comprised between 11.6% in Tg. Jiu Subcarpathian Depression and 107.0% in Craiova, leading to classification of seasonal pluviometric time types of little rainy (LR) in Tg. Jiu Subcarpathian Depression to exceedingly rainy (ER) in Oltenia Plain in Calafat, Bechet and Craiova and in Getic Piedmont in Slatina, Tg. Logreşti and Drăgăşani. The mean of seasonal quantities of precipitation calculated for the

entire region was 254.5 l/m^2, and its percentage deviation from the normal was 42.6% designating a very rainy spring (VR) overall.

CONCLUSIONS

The mean of spring arrival indexes for the whole Oltenia region was 520.3°C. The highest values of these indexes (absolute climatic records), exceeding 600.0°C were registered in the extreme South-West of the region, where the advections of warm air were more intense and frequent. The highest percentage deviations were registered in the high area of Oltenia, a common situation for temperature increases, showing that high areas are warming faster than low areas. Consequently, the spring of 2016 was an excessively early spring, rainy and warm overall, but marked by a high climatic variability. The spring followed a very warm winter, in which only January was a normal thermal month, and the vegetation was developing since the second decade of February, and the species of early fruit trees (almond tree, apricot tree) blossomed in the last pentad of February.

In March there were 6 days in which the mean of daily minimum values for the entire region was ≤ 0°C. As a consequence of air temperature drop, there has often been hoarfrost locally and sparsely (15 mornings) which damaged the early fruit trees, especially the almond tree, apricot tree and peach tree, whose flowers were almost entirely destroyed. March was an excessively rainy month in the whole region.

April was a warm month overall, but the intense weather cooling in the night of 26/27 April 2016 caused *monthly minimum air temperatures* which were comprised between -2.4°C in Voineasa and 2.5°C in Bâcleș in Mehedinți Hills, and their mean for the entire region was -0.2°C. This was the only day in April 2016 in which the minimum temperature mean calculated for the entire region was negative, and consequently an intense hoarfrost occurred on extended areas, being extremely destructive. In areas where the thermal minimum value on ground surface was negative there was frost on ground surface and in the air.

Warm winters an early spring arrivals represent an extremely serious climatic and agroclimatic risk, due to the fact that plants are thermophiles and reactive to weather warming, starting their vegetation early, and late spring hoarfrost catch them in very advanced stages of development causing highly damaging effects. The same type of variability of biological and vegetal processes are encountered in all biotopes and, consequently, the risk of early springs is generalized causing much more wide damages for the whole environment.

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SCIENCE PARK FOR SUSTAINABLE DEVELOPMENT AND CLIMATE CHANGE IN EAST SERBIA

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ABSTRACT

Science Parks support a knowledge-based economy by bringing together academic, business and governmental organizations into one physical location, and sustaining interrelationships between these groups. They are recognized as a solution to the complex problems of economic development, unemployment, job creation and new business development. Author is presenting a theoretical framework for development of "Knowledge Exchange Center" and consequently the Science Park for Sustainable development and Climate Change in East Serbia, on the foundation of International PhD in The Economics and Management of Natural Resources, developed at LUM Jean Monnet, Italy in collaboration with several Universities from Europe, USA, China and Russia. The project should also involve higher education institutions in the Region, neighboring countries Romania and Bulgaria as well as regional and local authorities, business community, NGOs, international development agencies. This concept should become an instrument for promoting the role of business-science partnerships in utilizing technologies for sustainable development and climate change mitigation and adaptation, with priority in sustainable energy, environmental protection, and agriculture and food security.

INTRODUCTION

Science parks are seen as a solution to the complex problems of economic development, under-employment, job creation, and new business development (W. Clark, 2003). They are a means of supporting a knowledge-based economy where the most critical input for economic growth is knowledge (OECD 1996). Presently there are over 400 science parks worldwide. Most of them are in the USA, Japan and China. The most famous science park is Silicon Valley, USA, originally known as Stanford University Science Park, dating back in 1950.

Committee of the Regions (COR) and the European Commission(EC) organised the European Innovation Ecosystems conference on 'Good governance and effective support for smart specialisation' in January 2016, which concluded that Science parks and areas of innovation play an important role in translating and implementing European policies and ideas in different cities and regions, and interact with clusters and business incubators to multiply the efficiency of these policies on a local and national level. As intermediary organizations, science and technology parks (STPs) network interacts with clusters and business incubators to multiply the efficiency of these policies on a local and national level.

EU experience from Spain shows that location in an STP increases the likelihood of cooperation for innovation, and the intangible benefits of cooperation with the main innovation partner, due mainly to a more diverse relationship (Vásquez-Urriago et al, 2016).

It has already been known, that there needs to be a regional approach with a strong community support and pivotal role of university or research centre (W. Clark, 2003).

Universities have an important role in knowledge-based economy and economic growth since they provide excellent human resources to society through education, as well as in promoting innovation and entrepreneurship by acting as an external source of knowledge for innovation (Fukugawa 2013).

Various regional experiences show that technology-based firms receive significant knowledge spillover from university research, measured by the improvement in the rate of return innovation, patents and patent productivity (Chan, 2010; Chunlei 2014; Mindruta, 2013).

Science park for sustainable development and climate change in East Serbia

The basic concepts of our project idea for "Science Park for Sustainable Development and Climate Change in East Serbia" presented at the 2014 UNESCO-WTA International Workshop held in Daejeon Metropolitan City, Republic of Korea (http://uw.wtanet.org/), are outlined in the Beijing Forum on New and Emerging Technologies and Sustainable Development, held in Beijing, China, from 15 to 17 April 2002 :

"New and emerging technologies in fields of information technologies, telecommunications, environment, clean energy, health, materials and transportation technologies are imposing a new social and ethical responsibility on the scientific and technological community to direct applications of these new developments in ways that strengthen efforts for achieving the objectives and goals of sustainable development.

They typically accomplish this by bringing together academic, business and governmental organizations into one physical location, and supporting interrelationships between these groups through incentives established by governmental policies. Since academic institutions tend to draw technically qualified personnel to a particular region, locations immediately near these institutions become prime candidates for such parks.

While a wide range of technologies might be appropriate for sustainable development efforts, it may be appropriate for the Science and Technology Park to become more narrowly focused. It may be appropriate for the proposed entity to focus on clean energy technologies, eco-environment protection, cleaner production technologies, water efficiency technologies, agriculture, health issues, or other topics of immediate concern to developing countries. An analysis is to be conducted to explore the advantages and disadvantages of such an approach, based upon market analyses, commercialization potential, existing technological factors, etc."

Furthermore, Science Park for Sustainable development has to provide the analysis of fundamental social, economic and ecological factors, as well as the engagement of stakeholders and political factors at local and national level.

Climate change, as a complex interaction between climatic, environmental, economic, political, institutional, social, and technological processes is becoming

increasingly important factor for sustainable development, and climate change mitigation and adaptation measures have to be included as an important part of this project.

BACKGROUND

Basic Facts about Serbia

The Republic of Serbia is a democratic state with population of around 7,186,860, and territory of 88,407 km2, border length of 2,364.4 km and location (according to Greenwich): Between 41°53' and 46°11' of North latitude and 18°49' and 23°00' of East longitude.

The capital of Serbia is Belgrade. With a population of 1,639,121, it is the country's administrative, economic and cultural centre. In terms of administrative and territorial division, the Republic of Serbia is divided into provinces, regions, administrative areas, the City of Belgrade cities and municipalities.The territorial organization of Serbia includes five regions (Belgrade region, Vojvodina region, Sumadija and western Serbia region, eastern and southern Serbia region and Kosovo-Metohija region). They include the City of Belgrade as a separate territorial unit established by the Constitution and law, and 29 administrative areas, 23 cities, 28 urban municipalities, 150 municipalities, 6,158 villages and 195 urban settlements.

The ethnic composition of the population of the Republic of Serbia is very diverse, which is a result of the country's turbulent past. The majority of the population of Serbia are Serbs, but another 37 ethnicities also live on its territory. All citizens have equal rights and responsibilities and enjoy full ethnic equality. The official language in Serbia is Serbian and the script in official use is Cyrillic, while the Latin script is also used. In the areas inhabited by ethnic minorities, the languages and scripts of the minorities are in official use, as provided by law. The main religion of Serbia is Christian Orthodox, the faith of the Serbian people. Beside the Christian Orthodox population, there are also other religious communities in Serbia: Islamic, Roman Catholic, Protestant, Jewish and others. The Government of the Republic of Serbia defined the state administration affairs that shall be run by the competent Ministries out of their seats, within the districts as regional centers of state authority. The Republic of Serbia is divided into 29 districts.

EAST SERBIA - TIMOK REGION

The Timok Region is located in the East of Serbia, bordering with Romania on the North and Bulgaria on the East. The Timok Region is situated at the intersection of two Pan European Corridors: Corridor Nr. 10 (Serbia) and Corridor Nr. 4 (Bulgaria). With the area of 7,497 km² Timok Region corresponds to two Serbian districts (Bor and Zaječar), and consist of the Municipalities of Zajecar, Knjazevac, Boljevac, Sokobanja, Bor, Negotin, Majdanpek and Kladovo, with population of around by 285 000 people, or some 3 % of the total number of Serbia’s inhabitants. Five out of eight are border Municipalities with EU which is now becoming a clear advantage.

Territory of the Timok Region is mainly highland, with about 45% of the total territory under arable land. The most developed fields are cattle breeding, crop husbandry, fruit growing and viticulture. Forests cover about 300,000 hectares – about 40% of the total area of Timok region, or as much as 11.3% of the total

forest fund of The region is rich in copper and gold. Two large hydroelectric power systems, Đerdap I and Đerdap II, are built on the Danube.

Timok Region is a region with some areas of preserved nature and excellent natural potentials:

- Rivers: the Danube, the Crni Timok and Beli Timok, the Pek
- Baths and thermo-mineral waters: Sokobanja, Brestovacka banja, Gamzigradska banja and excellent potentials in the villages of Nikolicevo and Rgošte
- Lakes and Reservoirs: Grliško, Đerdapsko, Bovan, Rgotsko and Sovinac.
- Mines: copper (Bor and Majdanpek), anthracite, coal…
- Protected natural resources: National park "Đerdap", Nature Park Stara planina

According to Regional development Agency of East Serbia (RARIS http://www.raris.org/) there is a long list of constraints for municipalities in the Timok Region: institutional weakness of existing economic development structures; absence of a comprehensive and coherent vision for sustainable economic development; information on the availability and use of both natural and human resources and the potentials and constraints for local /regional economic development are highly deficient; centralized system of public finances and the shortage of financial resources at regional and municipal levels; infrastructure, research and development investment are not put in place now in the region; lack of entrepreneurship and business knowledge and skills.

As a result of these constraints, the Timok Region is not just lagging in comparison with the regions in Europe, but is also behind the most of the regions in Serbia. This deficiency leads to diminished life prospects for the people of the Region.

Negative birth rate and population decline due to migrations in last decades, limited employment opportunities, inefficient health care service and lack of educational opportunities make the situation intolerable. This disparity in regional development is damaging the national economy as well, since the Region is not meeting its potential. Although the Government of Serbia has began some policy adjustments by creating relevant strategies (Poverty Reduction Strategy, National Investment Plan, Strategies for agricultural and tourism development, Strategy for stimulation and development of foreign investments etc), scale of development disparity between Serbia's regions is not fully recognized.

National Strategy Sustainable Development of Serbia (NSOR) has been adopted at government level in 2008. NSOR defined a vision of Serbia as a country with knowledge based economy, efficient use of natural and produced resources, with a wealth of educated people and preserved environment, historical and cultural heritage, and equal opportunities for all its citizens.

Although strategies of sustainable development have been presented at government level, there is a lack of professional and organizational knowledge of internationally recognized campaigns for the sustainable development of cities and towns in local communities. There is also inadequate coordination among possible implementing subjects including general public, municipal officers, NGOs, professional association and, more importantly, among legislators and key-decision makers in local communities.

Regrettably, more than 8 years after the adoption of NSOR, there is still a general lack of awareness about sustainability at all levels in Serbia. Some of our research data (Paunkovic, 2014) has revealed that less than 5% of the employees in local municipalities actually involved in sustainable development programs and actions were able to explain the concept of sustainability in common terms. General public and students (high school and university), are even less informed, despite proclamations about UN "Decade of education for sustainability" (UN DESD). People in East Serbia are still not adequately about the importance of environmental protection, waste management, and other important ecological problems and the number of people actually adopting sustainable life style is despondently very low.

DISSCUSSION

Our project idea is to develop Science Park for Sustainable development and Climate Change in East Serbia on the foundation of International PhD in The Economics and Management of Natural Resources that has been developed at LUM Jean Monnet, in collaboration with the Megatrend, Belgrad (Serbia), China Three Gorges University (CTGU) - China, Louisiana Tech University - USA and Saint Petersburg State Forest Technical University; Russia. This PhD partnership has been in operation for number of years and has a number of associated partner institutions in other countries (ALUM - Adriatic Linked University Network). The project will also involve Higher education institutions in the Region : Technical Faculty, Bor and Copper Rresearch Institute, Bor, as well as regional and local authorities, business community, NGOs, international development agencies.

The initial steps in this project development will include:

- learning from the experience of existing Science and Technology Parks,
- identifying appropriate sustainable technologies,
- estimate the financial needs necessary to establish the project, and to sustain its future efforts,
- explore potential opportunities for partnership with existing Science parks.

A few policies to foster the development of the Science Park for Sustainable Development and Climate Change in East Serbia will have to include: international economic and technical cooperation measures; measures for the attraction of talent, entrepreneurship support, protection of intellectual property rights.

When these analyses are completed, a development plan for the Science Park will include: the location of the park and coordinating partners; the nature of facility; technical focus; funding arrangements; financial viability; and short, medium and long term goals.

This plan should then become an instrument for promoting the role of business-science partnerships in utilizing technologies for sustainable development, taking into consideration complex environmental, social, organizational and cultural factors for particular Region.

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ENVIRONMENTAL PROTECTION THROUGH GREEN ENERGY

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Keywords: *Environmental protection, Green energy, Wind power, Hydropower, Pumped-storage*

ABSTRACT

Environmental protection through the implementation of green energy has become a daily reality. In the entire planet they were introduced especially in recent years various sources of green energy. The process started hard, but finally managed to accelerate the implementation of new green energy sources, albeit major obstacles have emerged. The most difficult obstacle met in worldwide but and in our country, was the inconstant green energy products. This study proposes some solutions designed to solve this unpleasant aspect of inconstant production of green energy. The basic idea refers to the construction of hydroelectric specially designed to represent a true energy buffer. A hydroelectric old (adequately equipped) or a new one built according to this principle, can become a true buffer energetic, able to take energy generated by the turbines at full capacity (energy otherwise lost by unable to be stored or taken over by the national network) and a national system restore when green energy sources produce less.

INTRODUCTION

Every day, the planet produces carbonic acid gas that's free to the earth's atmosphere and which is able to still be there in 100 years time. This augmented content of carbonic acid gas and increases the heat of our planet. One answer to heating is to exchange and retrofit current technologies with alternatives that have comparable or higher performance, however don't emit carbonic acid gas.

By 2050, minimum of one third of the global energy has to be came from stars (solar), wind, and different renewable resources. Who says that? Even "British Oil" and "Royal Dutch Shell" two of the world's largest oil corporations. Global climate changes, increment of planet population, and fuel depletion, mean that renewables ought to play an even bigger role within the future than they are doing it now (Pineda & Bock 2016).

All new energies need to have no desagreable consequences such as for example the fossil fuels or nuclear energy. Real planetary alternative energy sources need to be renewable and are thought to be "free" energy sources. These need to have decreased carbon emissions, compared to conventional energy

sources. It may be included: Biomass Energy, Wind Energy, Solar Energy, Geothermal Energy, Hydroelectric Energy, Tidal Energy, Wave Energy, (Petrescu F.I., Petrescu R.V., 2011; 2012).

Nuclear fission energy was virtually a necessary evil. With all its risks, he managed to stop the increasing of energy crisis of humanity until the advanced technology has allowed us the transition to alternative energy.

Nuclear fusion energy will be the most powerful energy source for mankind when it will be implemented (Petrescu & Petrescu 2014). Although great advances have been made in this direction, the nuclear fusion power plants did not yet built. Because it is not known when they will be operative in large quantity, should be required to equip us in advance with green energy farms.

Most that are easy to be built and used now are the wind farms and the solar ones (Ramenah & Tanougast 2016).

Their great technical problem is to have times when they produce less, or do not produce anything.

MATERIAL AND METHODS

Our country has managed rapid introduction of wind farms to generate on average about 25-30% of Romania's energy needs. Then wind turbines construction was abruptly halted on the ground that they can have small or long periods, when they do not give energy. In these times when the wind does not blow, the amount of energy that turbines would have to provide, needs to be produced otherwise. For not were built yet and reactors 3 and 4 at Cernavoda (which could operate from small or medium capacity, and provide more energy to our country when there is no wind) was sometimes necessary to supplement the energy production with coal.

The two existing reactors in the area generate more energy when needed but can’t replace all the energy produced by the wind when necessary. This is a real problem for us, which must to be solved right now.

A solution to this problem would be the completion of reactors 3 and 4 at Cernavoda and start their operation.

Another elegant solution that would allow even further additional wind energy sources is to build a particularly energetic system in area, a symbiotic one. This paper will try to present one such system.

Hydropower was used since ancient times for many kinds of watermills or has been used as a renewable energy source to irrigation and to operate various mechanical devices (Sabău 2015), (Sabău & Iovan 2015).

A known method for produces energy (electric energy) for supply high energy demands is to moving and storing water between reservoirs at different elevations. This method is named pumped-storage.

At the times with low energy demand, the excess generation capacities are used to pump water into a reservoir upper positioned.

In the moments when the demand becomes greater, water is released back into a lower positioned reservoir by a turbine (see the Fig. 1).

Such systems can be positioned everywhere in our country, in any great existing hydropower. But the need for symbiosis with existing windmills requires the construction of such a system even in Dobrogea area.

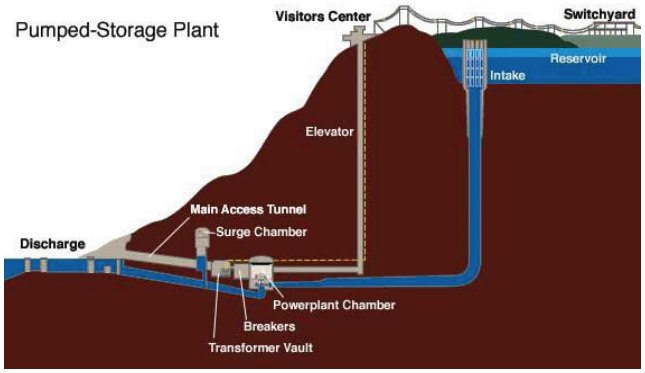

Figure 1. Diagram of the TVA pumped storage facility at Raccoon Mountain Pumped-Storage Plant
Source: https://en.wikipedia.org/wiki/Pumped-storage_hydroelectricity#/media/File:Pumpstor_racoon_mtn.jpg

It can build such a hydropower plant in that area with the great advantage to be constantly supplied with water pumped even further by the surplus electricity generated by wind (that otherwise would be lost in vain).

For a better understanding of the ideas, we will present below, very briefly, special technical characteristics of a windmill (Dubău 2015).

Electric power generated by wind is proportional to the cube of the wind speed.

A windmill is set to function optimally for a small or medium wind speed. If the speed of wind in the area increases 10 times for example, one single windmill will produce wind power such as a normal production given from the 1000 windmills (El-Naggar & Erlich 2016).

Obviously, this surplus energy cannot be picked up by any electric network and is lost. There is thus a large amount of energy produced but not used. If this energy could be used to act the pumps which lift water to a storage energy system, it would solve two problems simultaneously. Once, it would use the extra energy produced, which is lost otherwise. Second would store energy, that is then used in periods of high consumption, or when the wind stops beating.

RESULTS AND DISCUSSIONS

On September 4, 2013, in Romania has surpassed the psychological threshold of 2 GW of renewable energy produced by wind, solar and biomass. Thus, given that the total energy production was 7 GW, result that the proportion of green energy sources in our country is about one third.

As it knows, the wind sector produces the largest amount of renewable energy, with a daily average of 1,900 MW, followed by photovoltaics branch, only 100 MW. On the other hand, the biomass sector came to a halt, the production being constant, only 34 MW.

Data released by Transelectrica shows that on 4 September 2013 the wind energy production, representing 27% of the total, has been leading in the ranking of energy sources, followed by energy from coal, nuclear, hydro and the one based on hydrocarbons.

According to the legal provisions in force, the energy from renewable sources is received with priority in the network, and a such as energy production as

big as the one recorded in September 4 2013, would have caused difficulties in the national energy system. As such, National Power Control stops delivering networked energy in hydro and coal sector when green energy production is high and population demand is low.

In other words, when wind energy is very high (when strong wind) energy production on coal and hydro are limited and even stopped temporarily. But the inverse problem (when not too windy and the demand is high from population and industry) is more difficult to solve.

Usually in such situations are utilized at maximum capacity all hydro and coal plants.

A more viable solution would be to introduce into the national power grid yet two nuclear fission reactors.

But another important solution would be the introduction of hydro energy storage systems, as it has already been described previously.

It is estimated that in the Romanian energy system would require investments of several billion Euros to ensure confidence in the system takeovers of a significant amount of intermittent energy.

The system described in presented article it would cost much less our country. Another advantage of proposed system is that it would use only additional green energy (otherwise lost) as buffer energy, through water temporarily stored at a certain height. No oil, no coal (no hydrocarbons) in use, no pollution!

Compensation schemes currently in use are polluting, difficult to handle, and fail to compensate for green energy fluctuations of more than 2 GW (installed power).

The new proposed scheme has the advantage of being able to operate on a longer period (longer periods when the wind does not blow). If the amount of stored water is higher, or the height of the lifted water is higher, such a system could work for a longer period of time without being refueled. If it want such a system to replace total green energy wind for a much longer period, it will be necessary to build more such schemes.

As a lifeline if it would like to remain driven pumps and on windless period, it is possible, but this time they must to be powered from another source. This source of electrical power could come from existing nuclear plants (which would work then at full capacity) from photovoltaic panels, from a solar farm complementary, or by burning hydrogen extracted from water (through modern methods, with nanocells, to not have big yield losses), (Muthumeenal et al., 2016).

A large hydropower with pumped-storage built somewhere on Danube or on the Danube-Black Sea channel, near the wind farms, could store and give energy for a long period of time (for a week or maybe two) without wind alimentation. For possible windless periods longer than a month would be needed three or four such centers. It would be possible to build such a central solution in floors, with several levels of water lift and storage. Today appeared very large windmills that have an installed power very much improved compared to conventional models.

Instead of a wind national park with 1000-2000 windmills, we may build today a modern wind park with the same power having only 100 windmills, each with an installed capacity much higher than the older models. Wind energy is practically inexhaustible. We use the wind! Capture its energy and transform into

electricity, use, or store it. Installing wind farms is obvious only in areas where winds are often and hard.

CONCLUSIONS

1. The wind farms are reliable, economical, sustainable, friendly and affordable.

2. Installing wind farms is obvious only in areas where winds are often and hard.

3. Even in these areas there may be sometimes shorter or longer break, without wind. That does not mean to give up the use of wind energy. Obviously in these moments the energy must be supplied by other sources. One can use more wind farms built in totally different areas, so if one of them is no longer in the wind, in exchange for the other to have continuous activity. It can use in the same area, wind energy parks combined with solar farms. We still can use the fission nuclear power, accepting it as a necessary evil.

4. But a smart way to build green energy areas is to put a wind farm together with a storage hydropower.

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ENVIRONMENTAL PROTECTION THROUGH NUCLEAR ENERGY

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Keywords: *environmental protection, green energy, nuclear energy, nuclear fusion, renewable energy*

ABSTRACT

Environmental protection through the implementation of green energy has become a daily reality. In the entire planet they were introduced especially in recent years various sources of green energy. The process started hard, but finally managed to accelerate the implementation of new green energy sources, albeit major obstacles have emerged. The most difficult obstacle met in worldwide but and in our country, was the inconstant green energy products. This study proposes some solutions designed to solve this unpleasant aspect of inconstant production of green energy. The basic idea refers to the construction of nuclear power plants specially designed to represent a true energy buffer. Some nuclear power plants may become a true buffer energetic, able to work to a minimum capacity when the green (wind power) energy it is produced normal (when the energy generated by the turbines is at full capacity) and may work to an increased capacity when the wind stops.

INTRODUCTION

Every day, the planet produces carbonic acid gas that's free to the earth's atmosphere and which is able to still be there in 100 years time. This augmented content of carbonic acid gas and increases the heat of our planet. One answer to heating is to exchange and retrofit current technologies with alternatives that have comparable or higher performance, however don't emit carbonic acid gas.

In the years 1970-1980 it was provided for a serious crisis of energy resources. Hydrocarbon-based energies were polluting and increasingly fewer (endangered). Cars and large enterprises (large energy consumers) proliferated permanent. It was urgently needs of a new energy source. In these dramatic circumstances was introduced fission nuclear energy, as a necessary evil. Nuclear fission power plants have secured a new large amount of energy needed by the blue planet (Petrescu & Petrescu 2011).

They presents great advantages but also some disadvantages.

Nuclear fission energy has managed to take over the existing energy deficit and to leave time for major oil companies to discover new deposits of oil,

natural gas and shale gas. Nuclear fission energy is generally cheap and safe. The fission nuclear energy uses a fuel that existed in large quantities on the planet (uranium) but he begins to decrease (as well as the hydrocarbon). The thorniest issue at the nuclear fission plant is that both, used fuel (enriched uranium) and residual products are radioactive (and so dangerous to humans). Nuclear fission energy was virtually a necessary evil. With all its risks, he managed to stop the increasing of energy crisis of humanity until the advanced technology has allowed us the transition to alternative energy. Nuclear fusion energy will be the most powerful energy source for mankind when it will be implemented. Although great advances have been made in this direction, the nuclear fusion power plants did not yet built. Because it is not known when they will be operative in large quantity, should be required to equip us in advance with green energy farms.

Environmental protection through the implementation of green energy has become a daily reality. In the entire planet they were introduced especially in recent years various sources of green energy. The process started hard, but finally managed to accelerate the implementation of new green energy sources, albeit major obstacles have emerged. The most difficult obstacle met in worldwide but and in our country, was the inconstant green energy products. All new energies need to have no desagreable consequences such as for example the fossil fuels or nuclear energy. Real planetary alternative energy sources need to be renewable and are thought to be "free" energy sources. These need to have decreased carbon emissions, compared to conventional energy sources. It may be included: Biomass Energy, Wind Energy, Solar Energy, Geothermal Energy, Hydroelectric Energy, Tidal Energy, Wave Energy, or Nuclear Energy, (Petrescu & Petrescu 2015).

Most that are easy to be built and used now are the wind farms and the solar ones. But their great technical problem is to have times when they produce less, or do not produce anything.

The basic idea of this paper refers to the construction of nuclear power plants specially designed to represent a true energy buffer. Some nuclear power plants may become a true buffer energetic, able to work to a minimum capacity when the green (wind power) energy it is produced normal (when the energy generated by the turbines is at full capacity) and may work to an increased capacity when the wind stops.

Nuclear fusion power could not yet be made, but their season is fast approaching. The advantages of nuclear fusion energy are enormous.

Used fuel (hydrogen or water) is not radioactive. Of course this is not the first isotope of hydrogen or normal water, as a fusion reaction between two protons are made extremely difficult (only to enormous temperatures, in stars). Usually it uses the second isotope of hydrogen (Deuterium, which is the nucleus with one proton and one neutron) or heavy water (a molecule containing an atom of oxygen and two atoms of Deuterium). Water is found everywhere, so the fuel needed for fusion reaction is infinite, cheap, easy to find, friendly and non-toxic or radioactive. The technology for producing heavy water from water today is well planned.

The products resulting from fusion reactions are a large amount of energy and helium (an inert gas). So without radioactive wastes (as at the nuclear fission). The reaction itself is much easier to control (De Ninno et al., 2002).

MATERIAL AND METHODS

Current nuclear power is only a transition way to the thermonuclear energy based on light nuclei fusion.

The main particularity of fusion reaction is the prevalence of the fuel used (Deuterium). It can be obtained very simply from water ordinary. Deuterium has been extracted from water for the first time in 1931 by Harold Urey. Even at that time some small electrostatic linear accelerators have been indicated the fact that D-D reaction (the fusion between two Deuterium nuclei) is an exothermic reaction. Today we already know that not only the second isotope of hydrogen-Deuterium (a Deuterium nucleus contains two nucleons, one proton and one neutron) produces fusion energy, but and the third (heavy) isotope of hydrogen-Tritium (a Tritium nucleus contains three nucleons, one proton and two neutrons) can produce energy by nuclear fusion.

If we try to use and some more heavier isotopes of hydrogen the fusion reaction would be even and more simple, but these isotopes can be obtained very difficult today. Even the third isotope of hydrogen, Tritium can't be obtained directly (as Deuterium) but only by nuclear reactions between two Deuterium nuclei. The only first reaction is possible just between two nuclei of Deuterium, from which can be obtained, either a Tritium nucleus plus a proton and energy, or an isotope of helium with a neutron and energy (see relationships 1-2), (Petrescu F.I., 2012).

$$_1^2D+_1^2D->_1^3T+1MeV+_1^1H+3MeV=_1^3T+_1^1H+4MeV \quad (1)$$

$$_1^2D+_1^2D->_2^3He+0.8MeV+^1n+2.5MeV=_2^3He+^1n+3.3MeV \quad (2)$$

Once the Tritium has been obtained the fusion can occur between a nucleus of Deuterium and one of Tritium (see the expression 3), from which can be obtained an atom of helium with a neutron and high energy. Such a fusion reaction is preferable but to obtain it we need first of a reaction between two Deuterium nuclei.

$$_1^2D+_1^3T->_2^4He+3.5MeV+^1n+14MeV=_2^4He+^1n+17.5MeV \quad (3)$$

A nucleus of Deuterium may produce and another nuclear reaction between a nucleus of Deuterium and an isotope of helium (see the relation 4).

$$_1^2D+_2^3He>_2^4He+3.7MeV+_1^1H+14.7MeV=_2^4He+_1^1H+18.4MeV \quad (4)$$

For make these reactions to occur, should that the deuterium nuclei to have enough kinetic energy to may overcome the electrostatic forces of rejection due to the positive tasks of protons from the nuclei (Petrescu F.I. and Petrescu R.V., 2014).

Deuterium fuel is delivered in heavy water, D_2O.

To obtain Tritium we need first of a reaction between two Deuterium nuclei.

Tritium may be obtained in the laboratory and by the following reaction (relation 5).

$$_3^6Li+^1n-->_1^3T+_2^4He+4.6MeV \quad (5)$$

The third element in Mendeleev's table (lithium) is found in nature in sufficient quantities. Neutrons needed to produce the reaction 5 (with lithium), appear from the second and from the first + third reaction. This means that besides deuterium (heavy water), need to be added and lithium.

Raw materials to start fusion are Deuterium and lithium. All shown fusion reactions shown produce finally energy and He. He is a gas (an inert element). Because of this, fusion reaction is clean, and far superior to nuclear fission.

Hot fusion works with very high temperatures. In cold fusion, we need accelerate the deuterium nuclei, in linear or circular accelerators.

Final energy of accelerated deuterium nuclei should be well calibrated for a positive final yield of fusion reactions (more mergers, than fission).

Electromagnetic fields needed to maintain the plasma (cold or warm), should be and constrictors (especially at cold fusion), to close more together the nuclei. Getting the necessary (huge) temperature for hot fusion is still difficult, and for that reason we must focus now on cold nuclear fusion.

We need to bomb the fuel with accelerated deuterium nuclei. The fuel will be made from heavy water and lithium. The optimal proportion of lithium will be tested. It's obligatory to keep fuel in the plasma state, to achieve a strong ionization of the fuel, so instead of Deuterium atoms to obtain Deuterium nuclei (positive ions), which can be accelerated by electromagnetic fields.

RESULTS AND DISCUSSIONS

The smallest radius between Deuterium and Tritium is the radius of Deuterium nucleus (relations 6), (Halliday D. and Robert R., 1966).

Deuterium A=2 $A^{1/3}$=1.259921 --> R_D=1.8268855223476E^{-15} [m] (6a)

Tritium A=3 $A^{1/3}$=1.44224957 --> R_T=2.0912618769457E^{-15} [m] (6b)

Now we must calculate the minimum distance between two particles which need to meet together. This distance is just the Deuterium nucleus diameter, d_{12D} (expression 7).

$d_{12D}=2R_D=2x1.8268855223476E^{-15}[m]=3.6537710446952E^{-15}[m]=3.653771E^{-15}[m]$ (7)

The energy potential which reject two particles each other, can be obtained with the following expression (8), (Halliday D. and Robert R., 1966).

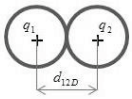
$U=E_p=q_1q_2/(4\pi\varepsilon_0 d_{12})=(1.602E^{-19})^2/(4\pi 8.8541853E^{-12}x$
$x3.653771E^{-15})=6.3128464855E^{-14}[J]=6.3128464855E^{-14}$ (8)
$x6.242E^{18}[eV]=3.94E^5[eV]=3.94E^2[keV]=394[keV]$

This is the expression of the potential energy between two adjacent particles (electrostatic potential energy), which should be the energy with that a particle needs to be accelerated before to collide (relationships 8 or 9, Fig. 1), This electrostatic potential energy must to be the same with the (final) kinetic energy of motion translational of accelerated particle $E_p=1/2mv^2$.

$$\begin{cases} U = E_p = \dfrac{1}{4\pi\cdot\varepsilon_0}\cdot\dfrac{q_1\cdot q_2}{d_{12}} = \dfrac{q_1\cdot q_2}{8\pi\cdot\varepsilon_0\cdot R} \\ E_p = \dfrac{1}{2}m\cdot v^2 \end{cases} \quad (9)$$

The radius of Deuterium at rest (without motion, static), was determined in Fig. 1 according to the following relationships (6a) and (10), (Petrescu & Calautit 2016).

$$\begin{cases} R_D = r_0\cdot A^{1/3} \\ r_0 = 1{,}45E-15[m] \text{ the average radius} \\ \text{of a nucleon fixed} \\ \text{A} = \text{the atomic mass} \end{cases} \quad (10)$$



$$d_{12D} = 2 \cdot R_D = 2 \cdot 1.8268855223476 \cdot 10^{-15} [m] =$$
$$= 3.6537710446952 \cdot 10^{-15} [m] =$$
$$\approx 3.653771 \cdot 10^{-15} [m]$$

Fig. 1. Two adjacent particles of Deuterium.

One obtains first the needed speed value (v) of the accelerated particle required for fusion (expression 11), (Petrescu & Calautit 2016).

$$v = 691664.8602 [\text{m/s}] \tag{11}$$

With expression 12 it may determine now with high accuracy the radius of a Deuteron or any other elementary moving particle, as a function of its velocity, v, and its rest mass, m_0, (Petrescu & Calautit 2016).

$$R = \sqrt{\frac{10}{8}} \cdot \frac{h \cdot \sqrt{c^2 - v^2} \cdot \sqrt{c^2 - \frac{v^2}{2} - c \cdot \sqrt{c^2 - v^2}}}{\pi \cdot m_0 \cdot c^2 \cdot v} \tag{12}$$

Potential energy of a Deuteron in movement has the below value (13):

U=Ep=6.01333E-10 [J]= 3753521838 [eV]= 3753521.838 [KeV]= 3753.521838 [MeV]= =3.753521838 [GeV] (13)

CONCLUSIONS

1. The wind farms are reliable, economical, sustainable, friendly and affordable.

2. The basic idea refers to the construction of nuclear power plants specially designed to represent a true energy buffer. Some nuclear power plants may become a true buffer energetic, able to work to a minimum capacity when the green (wind power) energy it is produced normal (when the energy generated by the turbines is at full capacity) and may work to an increased capacity when the wind stops. Nuclear fission power plants have secured a new large amount of energy needed by the blue planet.

3. The time of nuclear fusion power plants is approaching with quick steps.

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ISSUES CONCERNING FLOODS AND FLOODING FROM WATERCOURSES MANGALIA PLATEAU

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Keywords: *water, pollution, flooding, damage, amplitude*

ABSTRACT

Mangalia Plateau, semi-arid climatic conditions and topography tabular nature produce flash floods and floods. Maximum flow rates and runoff, floods most dangerous, usually occur in summer, when there are the most intense rainfall. The average duration of floods on temporary courses of hours is very rapid pace of growth (3-8 hours) with flood heights of 1.5-2.5 m and speeds of 5-7 m/s. The purpose of the paper is to present a study on floods in Mangalia Plateau products and the impact these have on the environment in all its components: water, air, soil, buildings, human activity. For these aspects we used data on flash floods, provided by ABADL Dobrogea Litoral, Constanța National Agency for Environmental Protection, Water Management System Constanța. The data were the basis of this study are the result of investigations and research conducted under the national integrated monitoring of the environmental situation, involving a large number of specialized institutions under the coordination of the Ministry of Environment, Water and Forests. Data collected were placed in tables and processed to yield graphics that then we have interpreted and analyzed. After studies we can conclude that Mangalia Plateau rivers can have very different behaviors, a series of precipitation with floods give low flow, but the same type of precipitation can lead to catastrophic flow, causing extensive damage and loss of life.

INTRODUCTION

In Mangalia Plateau, semi-arid climatic conditions and under the influence of a tabular relief character, heavily fragmented meandering valleys, to set up a network of groundwater in different repositories, a number of hydrographical bodies typical of their flowing regime with intermittent, and some marine estuaries (Posea 2005). Too, Ujvary (1972), noted that "the surprise is greater in Southern Dobrogea, where inside karst valleys leak is observed only in case of exceptional rainfall." In other words, in terms of hazard hydrological character, the degree of hazard is due to the phenomenon of violence and suddenness of production.

MATERIAL AND METHODS

For these aspects we used data on flash floods, provided by ABADL Dobrogea Litoral, Constanța National Agency for Environmental Protection, Water Management System Constanța. Data collected were placed in tables and processed to yield graphics that then we have interpreted and analyzed.

RESULTS AND DISCUSSIONS

Amid a climate semiarid meet the following rivers flow recorded in Mangalia Plateau (Dobrogea Litoral Water Basin Administration 2012).

Table 1

Levels and highs flows in Mangalia Plateau rivers

Hydrometric station	Stream	Location	Hazard rate (cm)	Year	Maximum level (H max, cm)	Maximum flow (Qmax m^3/s)
Biruința	Urlichioi Valley	Oltinei Plateau	250	2002	384	54,3
Biruința	Biruința Valley	Oltinei Plateau	350	2012	282	85,6
Albești	Albești Valley	Negru Vodă Plateau	250	2007	293	117,5

Source: Dobrogea Litoral Water Basin Administration,2012

The figures in Table 1 lead to the idea that floods, although a constant presence in the geographical landscape of South Dobrogea Plateau are rather causing damage to local small scale.

This emerges from the 2012 report of the Commission of Civil Protection Constanta County. This report states that "during 2012, Constanta county was faced with special problems, caused mainly by large-scale flooding."

In 2012 there have been two distinct events and negative consequences were quite limited in nature. Furthermore, based on effects recorded significant anthropogenic causes and not just violence phenomena, and two of the five dead must be placed on behalf of a hazard geomorphology (the collapse of a bank of earth) and no flood or flooding.

The data tabulated in table 1 allow comparison of absolute maximum monthly flow hydrometric station Albești annual average of maximum flow recorded every month. At the station recorded maximum values of the two parameters in the warm season, while the rest of the year are extremely low flow rates. Since the South Dobrogea Plateau is not a question of great waters spring due to snowmelt, the situation must be blamed on rainfall and surface characteristics active. Albeşti station absolute values recorded rainfall and hydrological highlights the diversity of South Dobrogea Plateau. The largest liquid flow is recorded at Albești, where they exceeded 100 m^3/s for three times, two of overruns in May.

In terms of hydrological hazard, it should pay attention to that absolute maximum values greatly exceed the average situation, both the corresponding month and annual scale. Thus, there are cases where absolute maximum flow exceeded more than 10 times the annual average, the record being recorded in Albeşti (153.0 m^3/s in 2012 to 9.4 m^3/s, respectively from the annual average maximum flow for May).

Also, in terms of hydrologic hazard, the maximum flow mode of production is significant. This comes amid a chronic shortage of water, especially on small

water courses, but possibly expanding into larger pools. In 2011, Albeşti River drainage had only 12 days, only 21 days in 2013 and in 2012 was dry all year. For example, the maximum flow rates of 2011 and 2012 were 0.149 m^3/s, respectively, 0.675 m^3/s (figure 1).

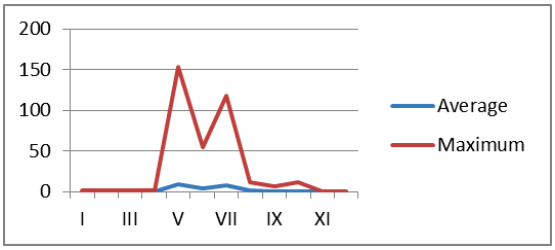

Figure 1. Regime annual average yearly maximum monthly flow (medium) and absolute maximum values (max) fluid flow hydrometric station Albeşti (Source: Own determination).

In this context, the occurrence of catastrophic flow, accompanied by spectacular growth level, has a special significance. In Mangalia Plateau such events rainfall occurs regardless of general feature of the given year, the excess or dry, and apparently independent of geographical position in the region and could trigger any area. The common feature of floods in South Dobrogea Plateau is increasing very fast, sometimes sudden flow. For example, I chose some record flood flow rates and special forms recorded in Albeşti hydrometric station.

Maximum flow from hydrometric station Albeşti,Albeşti River, was recorded during the floods of 30 June 2012 and had a value of 153 $m^3/$ s(figure 2).

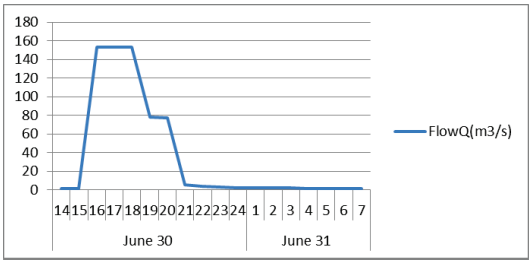

Figure 2. Evolution liquid flows on the river Albesti, at Albesti hydrometric station, during the flood of 30-31.06.2016 (Source: Own determination).

Increased water flow during flood occurred as a result of torrential rains which fell during the 124.2 mm of water, in June 30.

The flood occurred amid extremely low flow rates in the previous months, March and April, but have been deprived of leakage. Another element of this flood, as well as the most flood the Dobrogea is growing very fast time. For flood of 30 June 2012, it was about 30 minutes, the flood peak is reached at 16 pm (figure 2). According to the measured flow rate of 153 m^3/s was maintained for about 2 hours, after which the flood begins to shrink. Decreasing to levels recorded between 19,20 and 30 pm hours and return to normal on this river flow is achieved around 23 pm.

Another flood that occasioned achieve extreme river flows Albeşti dates from May 13 to 15, 2010, after a period of 19 months in a row, at the hydrometric station Albeşti no longer recorded higher flow rates of 0.6 m^3 / s (Dobrogea Litoral Water Basin Administration 2010).

The flood was caused by generalized rainfall. Large quantities were fallen, but have not reached extreme levels: average flow of 24.1 mm was recorded on May 13 and 4.0 mm on May 14; while at Mangalia 1 mm on May 13 and 16.8 mm on May 14.

In these circumstances, the maximum flow during the flood was reached at 17.00 and was 102.4 m^3/s (figure 3).

The flow rate oscillated around this value for about 2 hours, then began to decline relatively slowly, with several levels. Due to large retention basin and precipitation generalized, overall it was great flood flow recorded at 40 hours after the peak flood still having an annual amount that exceeded many records or 2.77 m^3/s.

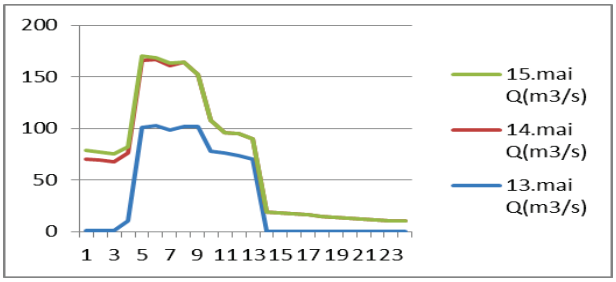

Figure 3. Evolution liquid flows on the river Albești, Albești hydrometric station, during the flood of 13-15.05.2010 (Source: Own determination).

CONCLUSIONS

The importance of these revenues in terms of hydrologic hazard lies in the following aspects:

- Demonstrate that Mangalia Plateau floods can occur at short intervals;
- Compared to other income demonstrates that, for approximately rainfall characteristics similar Mangalia Plateau rivers can have very different behaviors;
- There is a series of precipitation give flood with low flow and precipitation leading to catastrophic flow in a short period of time. In this case people may be taken aback producing the damage and loss of life.

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STUDY ON THE IALOMIȚA RIVER WATER QUALITY DOWNSTREAM OF SLOBOZIA.IMPLICATIONS FOR ECONOMIC AND TOURISM ACTIVITIES

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Keywords: *water, pollution, industry, human activities, tourism activities*

ABSTRACT

Human activities in the catchment Ialomita, the amount of waste, urban and industrial wastewater, harm strong pressure on water quality, destroying parts of flora and fauna and water quality. The purpose of this paper is to present a study area over Ialomita river water quality on the lower, downstream Slobozia. The data were the basis of this study are the result of investigations and research conducted under the national integrated monitoring of the environmental situation, involving a large number of specialized institutions under the coordination of the Ministry of Environment, Water and Forests: Water Basin Administration Buzău-Ialomița, National Environmental Protection Agency Ialomița, Water Management System Buzău-Ialomița. Data collected were placed in tables and processed to yield graphics that then we have interpreted and analyzed. Analyzing the average values of the main indicators of water quality on the river Ialomita, in the period 2010-2015, it is estimated that in terms of quality it is in a process of improvement, because the values are declining in some indicators. In 2015, the category of quality indicators and sections obtained was as follows: Category III: ph (section Ţăndărei) CBO5 (all sections); Category II: CCOMn, fixed residue, chlorides (all sections) and NH4 (sections Cosâmbeşti and Ţăndărei); Category I: dissolved O_2, NO_3, NH_4 and pH (upstream from Slobozia). Improve water quality in the lower Ialomita river can be achieved by exploiting the high efficiency of wastewater treatment plants discharging wastewater, reducing the amount of pollutants emissary, but through a rational use of water from the river, knowing that Ialomita river has low flow in summer, favoring the decline in self-purification.

INTRODUCTION

In the field of waters and environmental protection a priority is ensuring a clean and healthy environment for all residents, amid specific legislation harmonization with the EU in order to accelerate the European integration process. Enhancing environmental performance can not be conceived without effective management of environmental factors, by providing resources of good quality water for the population, industry, agriculture, modernization of alarm and warning the population, triggering an investment process for execution of protection against floods and dangerous meteorological phenomena, creating a system of household and industrial waste management.

Ialomita River is an environmental factor affected by pollution, especially due to the activities of the chemical and petrochemical, agricultural activities, activities of the food industry, textile, tourism activities. To all this is added the expansion of urban areas and infrastructure. The county Ialomita drainage works were carried out to eliminate the deficit of water in the soil and excess moisture from soil-irrigation systems or drainage.

MATERIAL AND METHODS

The data were the basis of this study are the result of investigations and research conducted under the national integrated monitoring of the environmental situation, involving a large number of specialized institutions under the coordination of the Ministry of Environment, Water and Forests: Water Basin Administration Buzău-Ialomița, National Environmental Protection Agency Ialomița, Water Management System Buzău-Ialomița.

Data collected were placed in tables and processed to yield graphics that then we have interpreted and analyzed.

RESULTS AND DISCUSSIONS

Hydrographic factors

Ialomița river crossing in the study, Bărăgan Plain, the dominant fields and meadows stretched tabulated. From a climate perspective, the fall in temperate-continental climate with hot summers, little rainfall, which sometimes presents showers, relatively cold winters, marked sometimes by strong blizzards, but also frequent periods of warming, causing discontinuities repeated of snow (Geography of Romania, 2005).

The main features of the geographical unit basins studied are: water resources have a complete representation by surface water (rivers, lakes) and groundwater; hydrographic network consists of valleys with drainage permanent and temporary drain of valleys called "Vaiuga" who are born and ends on the field; lakes located in Plain Bărăganului are located in the valleys; availability of irrigation drainage channels which cover approximately 80% of the area studied were built in the years 1950-1990. In the studied region only river allochthonous is Ialomița that has a small slope and flow of silt suspended high of 3790000 tons/year which led intensive processes alluvial, which led to the formation of wide meadows, with a strong asymmetry. The river has a power pluvio-nival, average monthly maximum flow recorded in April-May, and the lowest in September-October.

Since flows are reduced during the warm season, this reduction the decline in self-purification and favors keeping pollutants in amounts which often exceeded the permissible limit (Haiduc Iovanca, 1996).

For the study, during 1950-2015, there were a maximum flow at Slobozia station in May with a value of 64.7 m^3/s and a minimum flow station registered Coşereni 20.7 m^3/s in October(Water Basin Administration Buzau Ialomita,2010).

Socio-economic factors. In the study, Ialomița river runs through the administrative territory of two cities, Slobozia and Țăndărei city and 10 municipalities: Bucu, Ograda, Cosâmbești, Mărculești, Sudiți, Săveni, Mihail Kogălniceanu, Gura Ialomiței, Vlădeni, Giurgeni.

Agriculture is the branch with the greatest impact on the area considered making a mark directly or indirectly on the pollution level, the geographical

landscape as a whole. Edafic with potentially higher soil characterized almost permanent softness, mainly those in group cernozems, the plain is particularly favorable crop, especially cereals.Following are places pastures, vineyards and orchards.Enjoying a very important fodder base, livestock farming has always been a core both in private households and in agribusiness complex.

Industrial activities focus mainly on exploiting agricultural resources or processing. The main branch is the food industry, represented by sub-branches: industrialization and processing of milk (Slobozia) sugar industry (Ţăndărei), oil industry (Slobozia, Ţăndărei), the meat industry and meat products (Slobozia).

The main sources of pollution in the studied area are the city Slobozia and Ţăndărei discharging insufficiently treated or untreated in Ialomiţa river.

In Slobozia main economic objectives have a negative impact on the environment by eliminating specific products, and in particular the Ialomita river water quality are: Chemgas (chemical industry), Constrif(building materials), Furniture, Viprod, Prodesign (processing industry furniture), Expur, Diamonds and Boromir (food industry). These objectives may be added in the agriculture and other units, such as silos, or in agro-food: Avicola, Ceres.

Ţăndărei in the city, the main economic activities are: Ultex SA (oil industry), Beta SA (sugar industry), AGFD SA (starch industry), Ceram Material Construct SRL (machine-building materials).

Collection, purification and distribution of water to households and businesses is done by SC Apatermo SA, which receives wastewater and treats them prior to discharge into the river Ialomiţa.

Water quality indicators

Ialomiţa river water quality assessment in the present work was done by processing and interpretation of data resulting from the analysis of water samples collected (monthly average concentration), 2010-2015, in the sections I control: Slobozia, Cosâmbeşti, Ţăndărei. Processing values obtained allowed assessment Ialomita river water quality by comparing them with the limits of STAS 4706/1988.

Slobozia section upstream of the discharge, pre-treated industrial wastewater, industrial platform from the west, and is discharged downstream from wastewater treatment plant on the city and the industrial platform Chemgas. Therefore, Cosâmbeşti section presents an important role in assessing the Ialomita river water quality(1996-2015, Yearbook county on the state of the environment).

Water quality also influences the tourist activity in the area. In the study area are a number of tourist attractions: Hermes Holiday Park, Monastery Slobozia, Heroes Monument Slobozia, National Museum of Agriculture in Slobozia, Ialomiţa County Museum, Episcopal Cathedral of Slobozia, Sfinţii Voievozi Monastery, Ionel Perlea Memorial House, Strachina Lake, Hagieni Monastery. Water quality is one of the environmental indicators that contribute to motivation for a tourist stay of people.

Overall assessment of the Ialomiţa river water quality was done by analyzing groups of indicators relating to: pH, oxygen regime indicators of mineralization, specific indicators of contamination.

1. The concentration of hydrogen ions

The pH range within 24 hours and can be raised by day, when the chlorophyll assimilation processes consume large amounts of CO_2 in the water and lower at night when the plant is assimilation stops breathing intense and releasing large amounts of CO_2.

PH wastewater can be acid or alkaline a cause of disturbance of the biological balance of the receptor basin, preventing the normal process of self-purification.

Ialomița river is classified as III quality STAS 4706/88 providing a pH between 6.5 to 9.0 values to the rivers in this category.

Noting pH in water collection sections of Section Slobozia-Ţăndărei were following values presented in figure 1.

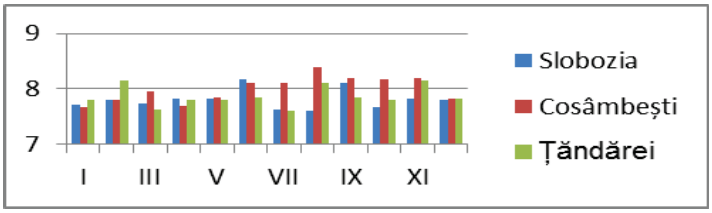

Figure 1. Change in average monthly pH gauging the main points on the river Ialomița, 2010-2015.
Source: Own determination

Analyzing the average monthly change in pH to the main points hydrometer (fig. 1) revealed that it varies between a minimum of 7.60 Ţăndărei station in July and a maximum of 8.38 Cosâmbeşti station in August.

Depending on the values recorded, the 3 sections studied fall into I the category of water quality.

The main pollution sources for this section are: Chemgas, Urban, Expur.

2. Indicators of the oxygen

A) The concentration of dissolved oxygen depends on the water temperature, air pressure, the content of oxidizable substances and microorganisms. Decreasing the amount of oxygen in the water leads to loss of freshness of its character, giving it a fad character and making it undrinkable. It also reduces the natural water self-purification capacity favoring the persistence of pollution any unintended consequences. STAS 4706/88 provides that the concentration of dissolved oxygen for rivers in category III quality does not fall below 4 mg/l. In section studied, the dissolved oxygen concentration values are shown in figure 2 (National Environmental Protection Agency Ialomița,2010).

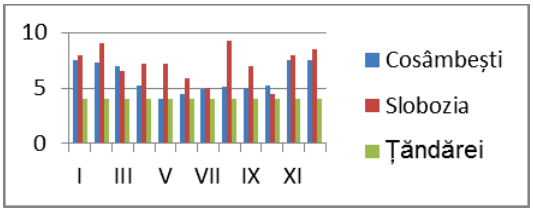

Figure 2. Dynamics of average monthly amount of dissolved ox.ygen, the Ialomița river, the main points hydrometric 2010-2015.
Source: Own determination

Analyzing the data presented in table 2 and fig. 2 notes that the highest values, with a maximum of 9.2 mg/l in August, recorded at Slobozia and lowest

values of 4 mg/l occur in the station Ţăndărei. Low levels of oxygen are caused by the discharge into the river Ialomița industrial wastewater from ULTEX Țăndărei starch GLUCOSE Ţăndărei, Ţăndărei APATERMO.

B) Biochemical oxygen demand

CBO5 represent the amount of oxygen consumed by microorganisms in the biodegradation of pollutants in the water in the first 5 days. CBO5 the maximum permissible provided by STAS is 12 mg/l.

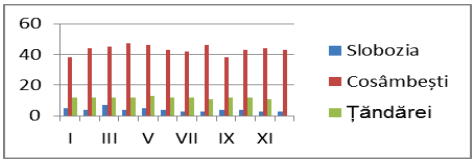

Figure 3. Dynamics of average monthly indicator CBO5 the main sources of pollution on the river Ialomița, 2010-2015.
Source: Own determination

Data analysis (figure 3) it can be seen that the variation is very high CBO5 between points of observation.

Thus, the lowest values are found in Slobozia, between 3 and 4.8 mg/l, the quality category II; Cosâmbeşti recorded at values of 38-47 mg/l and to Sandar, values between 0-12 mg/l, framing section in grade III.

C) Chemical oxygen

Increasing organic matter in water or their appearance at one time synonymous with water pollution by germs that usually accompany them.This organic substances in water for a long time favors the persistence of germs, including those pathogens. To determine oxygen consumption degradation of all such substances, water is introduced into evidence a strong chemical oxidant, KMnO4 and oxygen consumed in the chemical process is CCOMn. STAS permissible value for CCOMn is 25 mg/l. For Ialomița river, this indicator has frequent exceedances, and sometimes 100%. The average frequency goes up from 80% to 100% Slobozia and Țăndărei.

The study carried out in sections studied for the period 2012-2015, average CCOMn values are presented in figure 4.

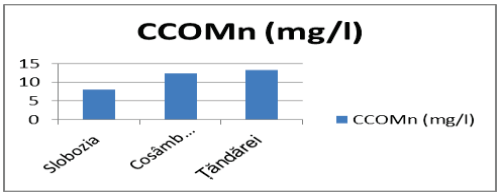

Figure 4. Dynamics CCOMn average during 2010-2015, the Ialomița river, the main gauging stations.
Source: Own determination

After processing the data is observed that higher values are found laŢăndărei, 13.34 mg/l. All the 3 sections control, fall in the second category of water quality.

3. Indicators mineralization

Mineralization indicators in the category included fixed residue and chlorides.

a) Fixed residue is all organic and inorganic substances dissolved in the water and which are not volatile at the temperature of 105 ^{0} C. The maximum allowable value of STAS for fixed residue is 1200 mg/l. For the three mean values of the control sections of the fixed residue are shown in figure 5.

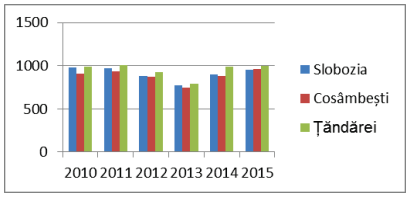

Figure 5. Dynamics of average fixed residue, the Ialomita river at major stations hydrometric 2010-2015.
Source: Own determination

From data processing is observed that the values are in the range 745.3 mg/l to Cosâmbeşti in 2013 (minimum) and 1006.9 mg/l Țăndărei in 2011 (maximum). Thus, we see that the maximum permissible limit was not exceeded (2010, Report on the use of water in normal hydrological periods and wastewater discharge water balance).

a) Chlorides in water coming from the ground or as a result of pollution from human or animal origin when the concentration varies with time. For Ialomița river, this indicator is irrelevant because there were frequent exceedances of the maximum permissible limit of STAS, 400 mg/l for Class III water quality(2010, Progress report on execution of the works of water supply and wastewater treatment of utilities).

In recent years there have been declines in this indicator, but more than 50% of the values exceed 300 mg/l (figure 6) and most exceed a maximum for class I, 250 mg/l.

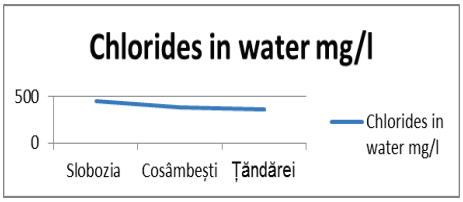

Figure 6. Dynamics of average indicator chlorides, Ialomita River, the main hydrometric stations in 2010-2015.
Source: Own determination

4. Indicators of specific contaminants

Of these, the most important for the section of the study are ammonium (NH4) and nitrate (NO3), because they come from nitrogenous fertilizer industry (Chemgas Slobozia) and from livestock.

If nitrates, STAS provides for rivers in category II quality a maximum of 30 mg/l and category III are not standardized. Nitrates can have a direct effect on the body by blocking hemoglobin to methemoglobin formation, but also an indirect decrease in overall body strength and favoring infections of respiratory and digestive(Teodoreanu 2004). May also constitute a factor for the development of algae or other organisms aquatic plant. For the 3 hydrometric stations in 2010-2015, mean ammonia are shown in figure 7.

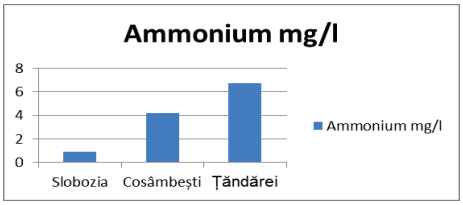

Figure 7. Average dynamics of ammonia, the Ialomita river, the main hydrometric stations in 2010-2015.
Source: Own determination

CONCLUSIONS

Analyzing the average values of the main indicators of water quality on the Ialomița river, in the period 2010-2015, it is estimated that in terms of quality it is in a process of improvement, because the values are declining in some indicators. Ialomița River is part of watercourses classified as III quality with outstanding contaminations several types of pollutants.

On its lower course it is affected by discharges of waste water from sources of contamination in the sector, but already released water intake and tributaries Prahova and Sărata, contributing to changing water chemistry resulting quality classification in this category.

In 2015, the category of quality indicators and sections obtained was as follows:

- Category III: ph (section Ţăndărei) CBO5 (all sections)
- Category II: CCOMn, fixed residue, chlorides (all sections) and NH4 (sections Cosâmbeşti and Ţăndărei)
- Category I: dissolved O_2, NO_3, NH_4 and pH (upstream from Slobozia)

Compared to the period before 2000, there is an improvement in water quality in the lower Ialomita river, this decrease being due to work on some pollution sources and increasing the efficiency of wastewater treatment discharged into the river.

In the study period, 2010-2015, the control sections, shows the following:

- Stock declining indicators: CCOMn, ammonium nitrate, fixed residue;
- Values increasing dissolved oxygen;
- Constant values on pH and chlorides.

Recommendations

Improve water quality in the lower Ialomița river can be achieved by exploiting the high efficiency of wastewater treatment plants discharging wastewater, reducing the amount of pollutants emissary, but through a rational use of water from the river, knowing that Ialomita river has low flow in summer, favoring the decline in self-purification.

Ialomita river to improve water quality recommend the following:

- Ensure for Slobozia, a program providing for more rational use of water, especially since the town lies on a small river flow;
- The need to extend the treatment plant for wastewater flow discharged by industrial units have skyrocketed and connection of new units due to the sewage network;
- If the wastewater will be made by the two-stage mechanical and biological sludge can be used in agriculture. Physico-chemical indicators will be considered under the Ialomita river discharge in the normal range;
- Correlation of city development with the expansion and completion of the water supply and sewerage;
- Study the arrangement of Ialomița river;
- By exploiting the high efficiency of the treatment plant will greatly reduce the concentration of pollutants, leading to improved water quality Ialomita river;
- By taking the necessary measures at the point of waste water upstream from Slobozia, Ialomița river water quality would improve and, over time, could be classified in a higher quality.

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THE BIOREMEDIATION POTENTIAL OF *ATRIPLEX SAGITTATA* BORKH. FOR SOIL HYDROCARBON POLLUTION

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Keywords: *Atriplex sagittata, hydrocarbons, bioremediation, biomass, pigments.*

ABSTRACT

Atriplex sagittata plants of similar sizes were grown on soil experimentally polluted with diesel oil (0.5%). Control pots with no plants and plants grown on non-polluted soil were also set up. Soil total oil and grease content was monitored over 60 days and plant biomass and leaf pigment concentration were determined for evaluating the phytotoxic effect.
During the first half of the period, average pollutant biodegradation was similar in all samples. Subsequent removal rate was higher for pots containing A. sagittata, showing a selective degradation of petroleum fractions. The overall removal rate was 24% higher.
Pigment loss was 20% in plants subjected to pollution, with individual variation. However, plants grown on polluted soil had, on average, a 61% lower biomass, suggesting a limited tolerance to hydrocarbons and, thus, a limited bioremediation potential for this species.

INTRODUCTION

Bioremediation is process of ecological reconstruction, by which natural populations (especially microbiota) are used to biodegrade various pollutants, thus lowering their concentration in a selected environment (Atlas 1981, Doboș & Puia 2010).

Soil pollution is a growing problem in many parts of the world and has a significant impact on crop productivity, biodiversity and human health. One of the most frequent and dangerous forms of soil pollution is due to petroleum hydrocarbon spills. Such spills are caused by various industrial activities involving the refining, transportation and use of petroleum products (Alboiu 2008).

While there are many mechanical, physical and chemical means of removing hydrocarbon pollutants, they also involve significant costs. This is why finding appropriate natural remediation methods is important.

The primary hydrocarbon degraders in both terrestrial and aquatic environments are bacteria, with various species and strains being able to oxidize specific hydrocarbons to acids, alcohols or cetones (Atlas 1981, Hazen 2010, Korda et al 1997, Okoh 2006). Various environmental factors can enhance or inhibit microbial biodegradation (especially temperature, oxygen and nutrients), meaning that their knowledge and control are essential in designing remediation strategies (Atlas 1981, Doboș & Puia 2010, Okoh 2006).

Thus, *in situ* bioremediation techniques usually involve accelerating the metabolic activity of indigenous hydrocarbon microbiota, through nutrient addition and/or bioamplification by inserting selected microorganisms in the polluted environment (Alboiu 2008, Ball 2002).

However, indigenous or introduced vegetation can significantly contribute to pollutant removal, a process called *phytoremediation.* This can be done through various physiological processes, including phytovolatilization, phytostabilization, phytoaccumulation, phytodegradation and, mostly, through providing oxygen and nutrients to local hydrocarbon-oxidizing microbiota (Ball 2002).

Searching for common, hardy plant species, able to contribute to natural hydrocarbon biodegradation is, thus, an important task. The aim of the current paper is to assess the bioremediation potential of *Atriplex sagittata* Borkh., a common herbaceous species.

Atriplex sagittata Borkh. (syn. *Atriplex nitens* Schkuhr., *Atriplex acuminata* Waldst. et Kit., *Atriplex hortensis* subsp. *nitens* (Schkuhr.) Pons) is a member of the Amaranthaceae family (Chenopodioideae subfamily).

Native to Central and Western Asia and Eastern Europe, it is widespread throughout Northern Eurasia. 30-250 cm tall, with deep roots, erect stem and triangle-shaped leaves (with a characteristic white-silver lower leaf surface) and elongated inflorescences. It is a ruderal plant, able to grow in dry or halophile environments or in urban areas (Mandák & Pyšek 1998, Mandák & Pyšek 2001).

MATERIAL AND METHODS

Six mature *Atriplex sagittata* plants of similar size were collected and nine pots containing soil, the plants and/or the pollutant were set up as follows:

- Three control pots (A1-3), contained soil, experimentally polluted by mixing 0.5% (v/v) diesel oil (previously incubated at 50°C, for 5-6 hours, in order to eliminate volatile fractions).
- Three pots (B1-3), containing polluted soil (with 0.5% weathered diesel oil) and *A. sagittata* plants.
- Three pots (C1-3) containing non-polluted soil and *A. sagittata* plants as a control for determining the phytotoxic effect of the pollutant.

All pots were kept at natural temperature and sunlight. The experiment lasted for 60 days, during which soil samples were periodically taken for hydrocarbon content analysis.

A gravimetric Total Oil and Grease (TOG) determination method was adapted from existing literature (Nwaogu et al. 2008, Popoviciu 2012). Soil samples were oven dried at 50°C, for 5-6 hours. Extraction was done using chloroform (Ezekiel et al., 2011, Kalėdienė et al 2003) – 10 mL for each 5 g of soil sample.

The liquid extract was incubated for 5-6 hours at 50°C, to evaporate the solvent and the TOG (in parts-per-million) was calculated from the weight difference.

At the end of the experiment, whole plants were collected and dry biomass was determined after oven drying at 105ºC, for 48 hours.

Leaf material was collected separately, for determining pigment concentration, as a measure of plant health. 0.1 g of each leaf were grounded in 10

ml acetone (80%). After filtering, the extract was analysed through spectrophotometry, at specific wavelengths (470, 647 and 663 nm).

The concentrations of chlorophyll a, chlorophyll b and carotenoids (total xantophyll+carotin) were calculated using the trichromatic equations of Lichtenthaler & Buschmann (2001).

RESULTS AND DISCUSSIONS

The TOG concentration dropped significantly during the first two weeks in all pots containing *A. sagittata* plants. In control pots, the evolution of TOG was highly variable, ranging from a significant initial decrease to a 30-day lag (Figure 1). After 30 days, the hydrocarbon concentration was similar (average 2,867 ppm in control pots, versus 3,067 ppm in plant-containing ones). Thus, the plant had little contribution to the biodegradation of light hydrocarbon fractions (easier to oxidize).

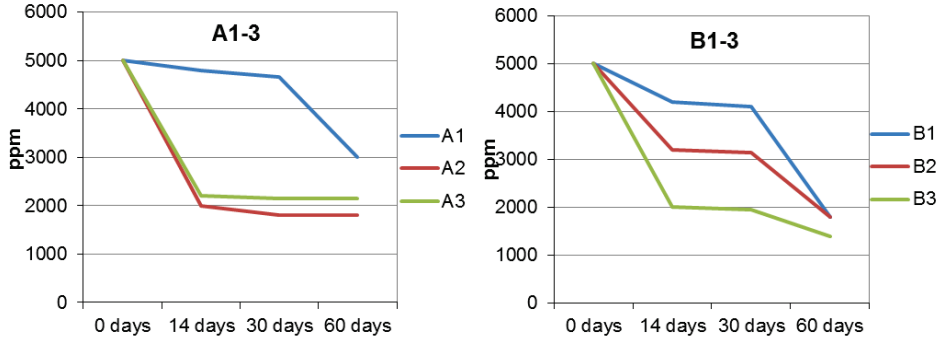

Figure 1. TOG concentration evolution (ppm) in control and plant-containing pots.

However, the second stage of the experiment showed an accelerated hydrocarbon removal rate in all pots containing *A. sagittata*. After 60 days, the average TOG concentration was 1,667 ppm, versus 2,317 ppm in control soil, meaning a 66.67% versus 53.67% overall removal rate. This indicates *Atriplex* plants enhanced the biodegradation of heavy hydrocarbon fractions, probably through rhizospheric stimulation.

Significant differences were found regarding pigment concentration (especially for xantophylls and carotin) among different plants. On average, plants in experimentally-polluted pots had a 20% lower chlorophyll concentration (for both chlorophylls) and an over 46% lower carotenoid concentration compared to plants in non-polluted soil (Figure 2).

The chl a : chl b ration was similar in all plants analyzed (0.573-0.576). However chl a : carotenoid ration varied significantly (2.1-11.7, with higher values on polluted soil).

Differences in dry biomass at the end of the experiment were extremely significant. All plants started with similar biomasses. After 60 days of growth on soil experimentally polluted with 0.5 diesel oil, *A. sagittata* plants in C1-3 pots had a 61.2% lower biomass than those grown on non poluted soil (2.1 g versus 5.7 g average dry biomass values).

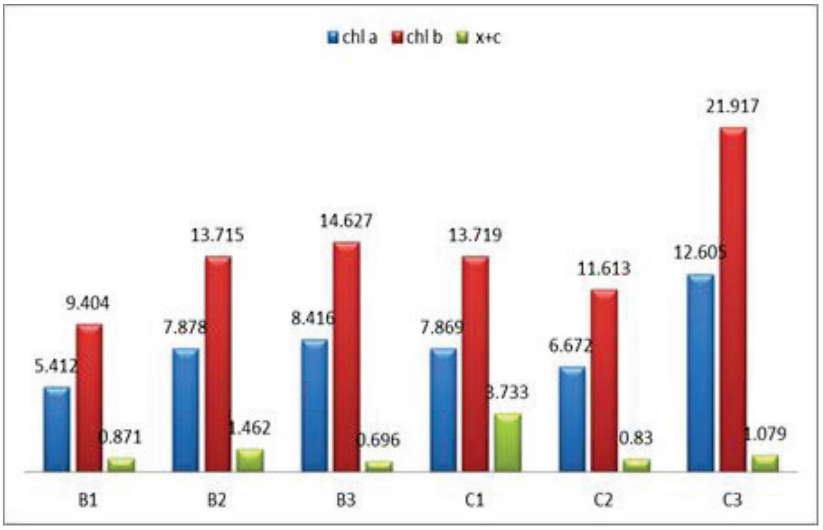

Figure 2. The concentration of photosyhthetic pigments (chlorophyll a, chlorophyll b, xantophyll, carotin) in *Atriplex sagittata* leaves (µg/g).

Table 1

Dry biomass of *Atriplex sagittata* plants grown on diesel oil-polluted and non-polluted soil.

Plant	Biomass
B1	1.12 g
B2	2.50 g
B3	3.01 g
C1	5.70 g
C2	1.19 g
C3	10.21 g

Various hydrocarbon phytoremediation experiments involving native herbaceous plants in various parts of the world have given various results.

Liužinas et al. (2003) experimented on species like *Calamagrostis epigejos, Carex hirta, Elytrigia repens, Leymus arenarius, Poa compressa, Artemisia campestris, Cirsium arvense, Convolvulus arvensis, Tanacetum valgare, Tussilago farfara*, showing that some can tolerate up to 5,000 ppm pollutant, with no phytotoxic effects, due to their deep root system.

Telysheva et al. (2011) used common agricultural and pasture species, finding significant differences among non-tolerant species, like *Medicago sativa* or *Sinapis alba* (with over 2/3 drops in biomass at 1% soil hydrocarbon concentration) and tolerant/degradative species, like *Zea mays* (60% hydrocarbon decomposition in one month, at 5% pollution).

Mirabilis jalapa, a common ornamental plant is able to tolerate up to 10,000 ppm, and degrade 41-63% of soil pollutant, but only over long periods of time (Peng et al 2009).

Some Poaceae (*Zea mays*, *Festuca arundinacea*) also have hydrocarbon-degradative abilities (Zand et al. 2010), with 1/3 TOG drops in one month, (similar to the results of the current experiment) and with minor biomass loss, even at 30,000-35,000 ppm.

However, it should be noted that specific local soil conditions can affect hydrocarbon biodegradation. For instance, in another experiment, *Zea mays* did not have significant impact on soil hydrocarbon content even at 0.5% pollution and 100-150 days of growth (Dominguez-Rosado & Pichtel 2004).

Diab (2008) showed that common agricultural species (*Vicia faba, Zea mays*, *Triticum aestivum*) can significantly stimulate the growth of hydrocarbon-oxidant microbiota in their rhizosphere, however, this was translated in only 13-30% TOG loss over 60 days. As an interesting aspect, each species had an affinity towards a specific hydrocarbon class (saturated, aromatic, asphaltenes etc.).

CONCLUSIONS

As a conclusion, the current experiment showed some long-term hydrocarbon bioremediation potential for *Atriplex sagittata.* While overall TOG removal after 30 days was similar to that in control pots, there was a difference in the biodegradation rate during the subsequent phase.

While pigment concentration indicated a good health state in plants, biomass differences were highly significant. Biomass and, especially root biomass, is known to be a key factor in long-term bioremediation processes, regardless of the actual physiological mechanism involved in biodegradation.

A poor growth at moderate soil pollution indicates a limited bioremediation potential for this species.

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THE BIOREMEDIATION POTENTIAL OF HORDEUM MURINUM L. FOR SOIL HYDROCARBON POLLUTION

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Keywords: *Hordeum murinum, hydrocarbons, bioremediation, biomass, pigments*

ABSTRACT

Hordeum murinum plants were experimentally grown on diesel oil (0.5%) polluted soil, with two series of control pots (polluted soil with no plants and plants grown on non-polluted soil). Over a 49 days experimental period, soil total oil and grease content was monitored. The final concentration of leaf pigments and dry plant biomass were also determined.
Average TOG removal at the end of the experiment was 50% in pots with H. murinum and 22.25% in soil with no plants.There were individual differences concerning pigment concentration and dry biomass, however, they were not due to the presence of the pollutant. Average final biomass was similar on polluted and non-polluted soil (4.29 g versus 4.56 g). These values indicate no significant phytotoxic effect on H. murinum plants at this pollution level and that the species has a potential for hydrocarbon bioremediation.

INTRODUCTION

Bioremediation is an ecological reconstruction process, employing various types of organisms, in order to biodegrade, or enhance natural decomposition of various pollutants. Its purpose is to offer a natural, cheap and non-invasive method of dealing with pollution effects (Atlas 1981, Doboș & Puia 2010).

Soil pollution is a problem affecting many regions and vith various negative effects on human activities, especially on agriculture. Pollutants reduce crop output and, often, cause health hazards for humans and livestock. Among the most common and noxious pollutants are petroleum hydrocarbons (Alboiu 2008).

There are many methods to remove hydrocarbon pollutants, many of them employing high costs and complex technical equipment. The alternative is using natural hydrocarbon degraders.

The main hydrocarbon degraders in any type of environment are microorganisms. Several groups of bacteria can break hydrocarbon chains and oxidize them to alcohols, acids and cetones (Atlas 1981, Das & Chandran 2011, Hazen 2010, Korda et al 1997, Okoh 2006).

Since there are many environmental factors regulating this process, there are ways to enhance the biodegradation rate of natural microbiota, by supplying nutrients and oxygen, for instance, a process calle *biostimulation* (Alboiu 2008, Atlas 1981, Ball 2002, Das & Chandran 2011, Doboș & Puia 2010, Okoh 2006).

A natural way of stimulating local bacteria is by taking advantage of their relationship with certain plant species. Plants can enhance soil oxygenation, provide nutrients, but also have their own means of lowering pollutant concentration in soils (volatilization, stabilization, accumulation, or degradation), collectively known as *phytoremediation* (Ball 2002).

Not all plant species have such abilities, thus making the search for potential phytoremediators, resistant and able to grow extensively under local conditions an important task for researchers.

The species considered in this experiment was *Hordeum murinum* L. (mouse barley, wild barley, barley grass) is a member of the Poaceae family (Triticae tribe). A native of Central and Soth Europe, North Africa, Caucasus and West Asia, it extensively grows as an annual weed, on croplands and non-croplands. Due to the large number of seeds and hardiness, it became invasive in many parts of the world.

Growing up to 30 cm high, with 10 cm spikes, it prefers warm and dry conditions (Eslami & Afghani 2009).

MATERIAL AND METHODS

Six mature *Hordeum murinum* individuals (similar in size) were collected from the coastal area in Constanța. Plants were grown in pots as follows:

- Three pots (A1-3) were set as control, containing soil with no plants; the pollutant (0.5% v/v diesel oil, weathered by 5-6 hour incubation at 50°C) was mixed into the soil.
- Three pots (B1-3), contained *H. murinum* plants grown on polluted soil (0.5% weathered diesel oil).
- Three pots (C1-3) contained *H. murinum* plants grown on non-polluted soil.

Experimental pots were all kept at natural sunlight and temperature. The experiment was conducted for 49 days, with periodical collection of soil samples, for determining hydrocarbon content. At the end of the experiment, leaf fragments were collected for determining the concentration of photosynthetic pigments, while the rest of the plants were dried for biomass analysis.

Hydrocarbon content in soil samples (precisely, the Total Oil and Grease – TOG) was determined gravimetrically. 5 g of dry soil (dried 5-6 hours at 50°C) were mixed in 10 mL chloroform; the extract was poured in preweighed recipients and left for 5-6 hours at 50°C, in order to evaporate the solvent. TOG was weighed and expressed as parts-per-million (ppm; Kalėdienė et al 2003, Nwaogu et al. 2008, Ezekiel et al., 2011, Popoviciu 2012).

For pigment analysis, 0.1 g of leaf tissue from each plant were extracted in 10 mL acetone solution (80%) and filtered. Analysis was done by spectrophotometry, at 470, 647 and 663 nm. The concentration of chlorophyll a, chlorophyll b and carotenoids (xantophyll+carotin) were determined using the equations of Lichtenthaler & Buschmann (2001).

For determining dry biomass, plant material was dried 48 hours at 105ºC and weighed.

RESULTS AND DISCUSSIONS

A decrease in TOG concentration was found in all experimental pots. Both pots with and without *H. murinum* plants had a similar soil hydrocarbon content

after the first week (an average of 1,200 ppm representing the residual hydrocarbon left after natural volatilization; Fig. 1).

However, in the control pots, the decrease was low and, after 3 weeks, TOG stabilized to an average of (1,000-933 ppm). In the plant-containing pots (B1-3), degradation continued down to an average concentration of 600 ppm, at the end of the experiment (Fig. 1).

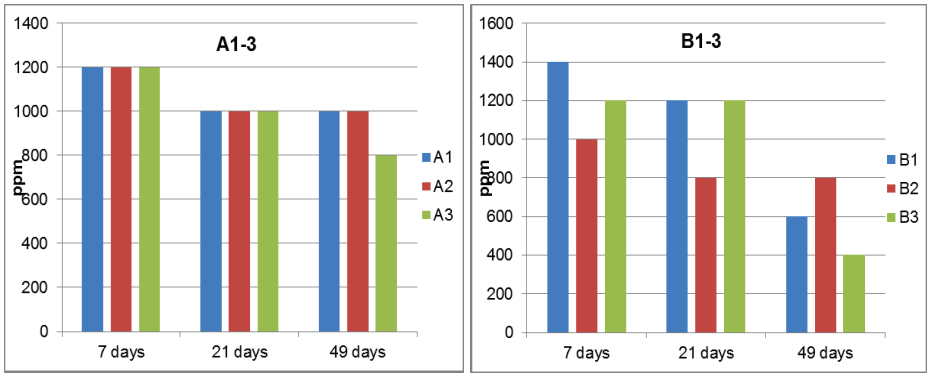

Figure 1. TOG concentration evolution (ppm) in control and plant-containing pots.

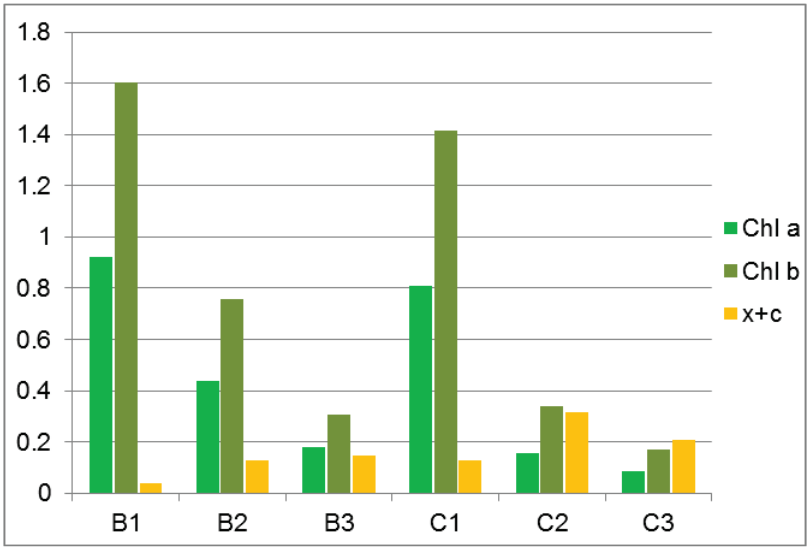

Figure 2. Photosyhthetic pigments concentration (chlorophyll a, chlorophyll b, xantophyll+carotin) in *H. murinum* leaves (µg/g).

Thus, *H. murinum plants* had a contribution to soil hydrocarbon degradation, including some of the heavier fractions.

Concerning photosynthetic pigments, there were differences among *H. murinum* individuals, differences that were not due to soil pollution. On average, total chlorophylls amounted for 1.40 µg/g for plants grown on polluted soil and 0.99

μg/g for those on non-polluted soil. Carotenoids were found in higher concentration on non-polluted soil (0.22 μg/g) than on polluted one (0.10; Fig. 2).

While the chl a : chl b ratio had similar values for all individuals examined was similar in all plants analyzed (0.46-0.58). Differences were found in chl a : carotenoid ratio (average higher values on polluted soil, but significant differences within the same group: 0.4-25.5)

Regarding biomass, differences among individuals were not due to soil pollution. The average was 4.29 g on polluted soil, compared to 4.56 g in non-polluted pots (Table 1).

Table 1

Dry biomass of *Hordeum murinum* plants grown on diesel oil-polluted and non-polluted soil

Plant	Biomass
B1	4.00 g
B2	5.60 g
B3	3.27 g
C1	7.30 g
C2	3.66 g
C3	2.73 g

Various Poaceae species have proven remarkable abilities for hydrocarbon tolerance and degradation, due, most probably, to their ability to supply oxygen to rhizospheric microbiota.

Calamagrostis epigejos, Elytrigia repens, Leymus arenarius, Poa compressa for instance, are able to withstand medium hydrocarbon pollution (5,000 ppm) without phytotoxic effects (Liužinas et al. 2003).

Apart from resistance, many species are able to stimulate soil hydrocarbon-degrading bacteria, thus contributing to the removal of certain hydrocarbon fractions (Diab 2008).

However, this does not necessarly translate into an effective reduction of soil TOG. There are other determinant local factors that impact on phytoremediation efficiency. For instance, *Zea mays* had a 16.8% hydrocarbon removal over at 2,330 ppm pollution in the experiment of Diab (2008), over 30% with low phytotoxic effects, even at 30,000 ppm (Zand et al. 2010), 60% degradation at 5,000 ppm (Telysheva et al. 2011) or negligible effects at 5,000 ppm, even after 100-150 days experimental period (Dominguez-Rosado & Pichtel 2004). The same was found in other related monocots, such as *Cyperus rotundus* (Basumatary et al. 2012, Efe & Okpali, 2012)

In the current experiment, the average TOG removal during the studied period (42 days) was 50% in pots containing *H. murinum*, compared to 22.25% in soil with no plants. This indicates a high potential for hydrocarbon phytoremediation.

Similar average biomass values indicate there was no effect of diesel oil pollution on plant growth, at the selected concentration. It should be noted that

biomass is a crucial factor in all biodegradation processes (especially, root biomass; Vaziri et al. 2013).

Pigment concentration is a good indicator of plant health. While there were differences between studied individuals, they were not due to soil pollution, but to other individual factor. Thus, a 0.5% (v/v) diesel oil pollution had no significant phytotoxic effects on *H. murinum* plants.

CONCLUSIONS

At the end of the experimental period, pots with *Hordeum murinum* plants showed a 50% decrease in TOG, compared to only 22.25% due to natural microbial biodegradation. This indicates a significant contribution of the selected plants to enhancing soil hydrocarbon removal.

Concerning the phytotoxic effect, while differences in leaf pigment concentration and final dry biomass were observed, they were not due to diesel oil pollution, since differences were higher between individuals form the same group (grown on polluted/non-polluted soil) than between the two groups.

On average, the final dry biomass 4.29 g on polluted soil, versus 4.56 g for control plants.

The lack of a significant effect on pigment synthesis and plant growth at the selected hydrocarbon concentration, together with effective TOG removal suggest a potential for soil hydrocarbon phytoremediation using *H. murinum*.

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