MONITORING THE VEGETATIVE ORGANS' STRUCTURE OF SEVERAL SPECIES FROM THE CRIŞURI BASIN ECOSYSTEM (NV ROMANIA – E HUNGARY), IN ORDER TO IDENTIFY A POSSIBLE PHARMACEUTICALS AND METABOLITES POLLUTION

Petruş-Vancea Adriana[*], Papp Zsuzsanna[**], Fodor Alexandrina[*], Sándor Zsuzsanna[**], Cupsa Diana[*], Györe Károly[**], Györéné Cseres Ildikó[**], Józsa Vilmos[**], Bíró Janka[**], Petrehele Anda Ioana Gratiela[**]

[*]University of Oradea, Faculty of Science, Universitatii Str., no 1, 410087, Oradea, Romania, adrianavan@yahoo.com
[**]Institute for Fisheries, Aquaculture and Irrigation, 5540 Szarvas, Anna-liget 8, Hungary

Abstract

This study presents a monitoring of vegetative structure of some species from the Crisuri Rivers basin ecosystem, in order to identify a possible accumulation of pharmaceuticals and metabolites in this area. The research was part from a complex project which was carried out to survey the pharmaceuticals in water, sediment, fishes and macrophytes with the working knowledge of lie of the lands. Basic chemical constituents (pH, oxygen, nitrite, nitrate, ammonia, phosphates) were determined by standard methods. Analysis of the pharmaceutical compounds has supposed the screenings of the different antibiotics with ELISA tests. The concentrations of the most abundant antibiotics (e.g. oxitetracycline, and basic nitrofuran compounds) were studied by HPLC with fluorimetric detection. Non steroid inflammatory (ibuprofen, ketoprofen, naproxen, diclofenac and indomethacin) was analysed by GS-MS method in gas-chromatographic laboratory. Dates of plants structure was corroborated with chemical dates. At the end of research, we concluded that the pollution in Crisuri Rivers Basin is not so high as to affect the structure of plant vegetative organs from the aquatic ecosystem.

Keywords: macrophytes structure, pollution, Crişuri Basin, pharmaceuticals.

INTRODUCTION

The structure of plant organs is the result of complex internal and external factors, during the ontogeny and phylogeny of plants. There abiotic factors, edaphic, biotic and anthropogenic affects plant body (Botnariuc and Vădineanu, 1982).

Along with factors independent of human action, anthropogenic factors, of which pollution has drastic consequences on the structure and functioning of the default plant organs, namely vegetable bodies, according to its nature (Varshney and Garg, 1980; Viskari, 2000).

Discharges resulted from punctual or diffuse sources are most of the times complex mixtures that may contain unknown compounds which can act together determining the growth or decline of the toxic effects over ecosystems. Biotests using bacteria, algae, macrophytes, invertebrates and fish are utilized on a large scale in evaluating the impact of chemical substances over aquatic ecosystems (Cioplan, 2005). During monitoring, macrophytes are chosen for the study because they are easy to sample and respond to certain impact categories. This analysis of the bioaccumulation implies two major difficulties in identifying the impact of toxic substances over the ecosystems they reach (because most often, the events they induce have an episodic character, and toxic substances may produce effects at very low concentrations). In those areas where it know or suspect as possible the occurrence of contaminants, monitoring their concentration in different organism groups can be a useful technique (Cioplan, 2005).

There are numerous studies for identifying the content of hard metals (Kapitonova, 2002; Wang et al, 2002**;** Adeyeye, 2005; Ali et al, 2008; Jiang and Wang, 2008; Bonanno and Giudice, 2010; Hadad et al, 2010; Stingu et al, 2011; Galfati et al, 2011; Big et al, 2012; Marian et al, 2012) or detergents (Borner et al, 2005) in the superior plants near rivers, or study the anatomy of plant from endanged ecosystem (Grigore et al, 2012), but there are no researches connected to identifying pharmaceutical products, at this type of vegetal material. Pollution with hard metals like lead and the accumulation in superior species (*Phragmites australis* and *Cyperus rotundus*) or in hydrophytes (*Eichhornia crassipes*) near some industrial areas has been investigated by Ali et al (2008). Transversal sections over plant organs have been examined for the elementary analysis of abundant crystals that appeared in the polluted plants' aerenchyma. High levels of lead registered reflect the urban and industrial sewage influence and have demonstrated the utility of these superior plant species as biosensors in lead pollution. This complies with other authors results (Schneider and Rubio, 1999), who showed that *Eichhornia* is an excellent bio-absorbent for lead. Mazen and El-Maghraby (1998), helped by microanalysis X rays, indicated the presence of three hard metals (Cd, Pb and Sr), connected by crystal Ca oxalate. Keller B. et al (1998) have shown that *Phragmites* species are, generally, considered as filters, accumulating metals, with possible toxic effects over the ecosystem. They are being used efficiently in bio-repairing water and hydro-soils (Abe et al, 1997).

At plants, organisms that cannot move by themselves away from the polluted area, morphanatomical changes are frequently an expression of the physiological adapting to different environment factors (Barnes et al, 1997). The wideness of the morphoanatomical and physiological adaptive changes is related to that vegetal species tolerance degree.

In our researches we wanted to identify the possible histoanatomical changes of the vegetative organs belonging to some species from the Crişuri Basin.

MATERIALS AND METHODS

Crişuri Basin contains the following main rivers: Barcăul, Crişul Repede, Crişul Negru and Crişul Alb which get together two by two on the territory of the Hungarian Republic, forming one course which confluates with Tisa. The Crişuri Rivers, especially Crişul Alb, spring from the gold deposits area in Apuseni and have a flow direction from east to west, Crişul Alb and Crişul Negru confluating into the Crişul Dublu, and the reuniting waters with Crişul Repede, forming Crişul Triplu. The sampling plant material used in the studies of plant biology were taken into account the standard rules for collecting plant material (Andrei and Paraschivoiu, 2003), being established fixed sampling points, locations that were followed every move made in the field; the choice specimens that were taken vegetative organs which were the subject of research in biology plant has tried, wherever possible, to identify individuals with an medium stem size from the population. Spatial and temporal heterogeneity (structural and functional) to ecological systems, interest in this research, was an important aspect in carrying out the sampling program. Swamp plants were not identified at all sampling points, depending on their altitude. An identical situation was found for submerged plant.

In Table 1 are presented plant species collected depending on sample point. After harvesting, the plants of water, either from boat or foot, depend on location, respectively depth of the river, plants or vegetative organs that have been washed. Cutting roots, rhizomes, stems or leaves taken from the swamp plant was achieved by mechanical fixing of their in marrow shock. Plant material was conserved in 70% alcohol, and sections were set up to put the blade in Petri dishes with tap water, brought to laboratory temperature. Sections were colored with 'Red Congo' 3%. For each experimental variant were taken and examined under a microscope every 30 sections per sample. Made preparations were examined under the optical microscope Leitz brand, Webster M, with eye-10X and 10X - 40X objectives. The representative images were photographed with a digital camera adapted to the microscope and saved on computer.

RESULTS AND DISCUSSION

Trapa natans was found in places like: Szarvas-down, Szarvas-upper and Gyula, and cross sections applied in vegetative organs of this species showed no abnormalities related to their structure. Monarch type root (Fig. 1 A) present a rich xylem; the stolon (Fig. 1 B), another vegetative body submerged in water in this species, fundamental parenchyma, aerenchyma and vascular bundle showed normal cell conformity; foliar limb (Fig. 1 C) has large deposits of air, at the nervure level.

Table 1

Plant preserved in August 2011, for histoanatomy study, according to sample point and codes.

No	Location	River	GPS coordinates	Sample	
				Species	**Sample Code**
1	Szarvas-down, HU	CRISUL TRIPLU	N 46° 53'56,0" E 20° 32' 0,0"	*Trapa natans*	111PTr
2	Szarvas-upper, HU	CRISUL TRIPLU	N 46° 53'11,0" E 20° 30' 3,0"	*Trapa natans* *Typha* sp.	112PTr 112PTy
3	Bekes-down A, HU	CRISUL DUBLU	N 46° 48' 09,0" E 21° 08' 9,0"	*Typha* sp.	113PTy
4	Bekes-down B, HU	CRISUL DUBLU	N 46° 48' 0,0" E 21° 08' 10,0"	*Typha* sp.	114PTy
5	Bekes-upper, HU	CRISUL DUBLU	N 46° 47' 51,9" E 21° 08' 12,94"	*Sagittaria latifolia* *Salix alba*	115PSg 115PSx
6	Szeghalom-down, HU	BARCAU	N 47° 15' 00,0" E 21° 09' 10,7"	*Sagittaria latifolia* *Salix alba*	115PSg 115PSx
7	Szeghalom-upper, HU	BARCAU	N 47° 17' 53,5" E 22° 14' 45,5"	*Typha* sp. *Phragmites* sp.	117PTy 117PPh
8	Marghita, RO	BARCAU	N 47° 19' 30,2" E 22° 23' 13,54"	*Typha* sp.	118PTy
9	Abram, RO	BARCAU	N 47° 19' 30,2" E 22° 23' 13,54"	*Potamogeton natans*	119PPt
10	Körösladány, HU	CRISUL REPEDE	N 46° 56' 53,28" E 21° 04' 22,49"	*Sagittaria latifolia* *Typha* sp.	1110PSg 1110PTy
11	Santion (under Oradea), RO	CRISUL REPEDE	N 47° 04' 52,5" E 21° 48' 26,2"	*Butomus umbellatus* *Potamogeton natans* *Sagittaria latifolia* *Typha* sp.	1111PBu 1111PPt 1111PSg 1111PTy
12	Fughiu (above Oradea), RO	CRISUL REPEDE	N 47° 03' 38,1" E 22° 02' 32,1"	*Typha* sp.	1112Pty
13	Urvind, RO	CRISUL REPEDE	N 47° 03' 44,6" E 22° 16' 54,11"	*Potamogeton natans* *Salix alba*	1113PPt 1113PSx
14	Bratca, RO	CRISUL REPEDE	N 46° 35' 26,8" E 22° 36' 01,3"	*Salix alba*	1114PSx
15	Gyula, HU	CRISUL NEGRU	N 46° 42' 08,5" E 21° 19' 02,6"	*Trapa natans* *Typha* sp.	1115Tr 1115Ty
16	Tinca- down, RO	CRISUL NEGRU	N 46° 46' 19,3" E 21° 57' 27,9"	*Salix alba*	1116PSx
17	Tinca- upper, RO	CRISUL NEGRU	N 46° 46' 04,6" E 21° 55' 59,6"	*Salix alba* *Populus* sp.	1117PSx 1117PPo
18	Beius, RO	CRISUL NEGRU	N 46° 39' 35,0" E 22° 20' 39,1"	*Typha* sp. *Salix alba*	1118PTy 1118PSx
19	Saliste de Vascau, RO	"0" point	N 46° 26' 01,1" E 22° 33' 19,3"	*Rubus* sp. *Rumex* sp.	1119PRb 1119PRu
20	Chisinau-Cris, RO	CRISUL ALB	N 46° 31' 34,7" E 21° 30' 26,8"	*Salix alba*	1120PSx
21	Ineu-down, RO	CRISUL ALB	N 46° 25' 38,0" E 21° 19' 47,3"	*Salix alba*	1121PSx
22	Ineu - upper, RO	CRISUL ALB	N 46° 25' 53,9" E 21° 51' 36,6"	*Salix alba*	1122PSx
23	Varfurile, RO	CRISUL ALB	N 46° 17' 31,2" E 22° 30' 48,0"	*Salix alba*	1123PSx

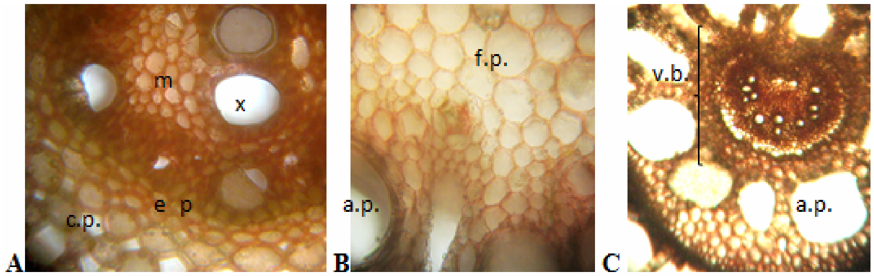

Fig. 1. *Trapa natans*: A (400x) – plant roots from Szarvas-down, Hu (central cylinder); B (400x) – stolon preserved from Gyula, Hu; C (100x) – plant foliar limb from Gyula, Hu (a.p. – aerial parenchyma; c.p. – cortical parenchyma; e – endodermis; f.p. – fundamental parenchyma; m – marrow; p –pericycle; v.b.- vascular bundle; x – xylem).

One of the most common species in this habitat type, *Typha* sp., being found in the meadow, both in Hungary at Szarvas-upper, Bekes-down A, B, Szeghalom- upper, Körösladány, Gyula, and in Romania at Marghita, Sântion, Fughiu and Beiuş we identified a primary root structure, typical for this species, with normal tissue layers identified under the optical microscope (Fig. 2 A), as well as the foliar limb, with vascular bundle (Fig. 2 B) normal embedded in a rich aeriferous tissue. Hadad et al (2010) have identified morphological responses of *Typha domingensis*, to the content of heavy metals from industrial wet area.

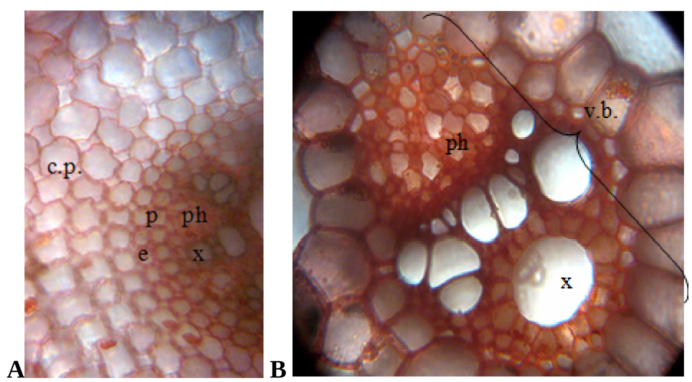

Fig. 2. *Typha* sp.: Root of plant from Beiuş, Ro B (A -100x) – vascular bundle of leaf plant from Szeghalom- upper, Hu (B – 400x) (c.p. – cortical parenchyma; e – endodermis; p – pericycle; ph – phloem; v.b. – vascular bundle; x – xylem).

Sagittaria latifolia was present in Bekes-upper, Szeghalom-down, Körösladány points from Hungary, and just in Sântion (Hungarian border town), in Romania. Root had rizodermis, exodermis, cortical parenchyma, central cylinder composed from normal cells (Fig. 3 A), and the leaf petiole

(Fig. 3 B) was rich in aeriferous tissue, normally structured. Also, *Pragmites* sp. roots which was still submerged in the riverbed as the other species studied, not show structural signs of toxic products accumulation or be affected by any harmful factor (Fig. 3 C).

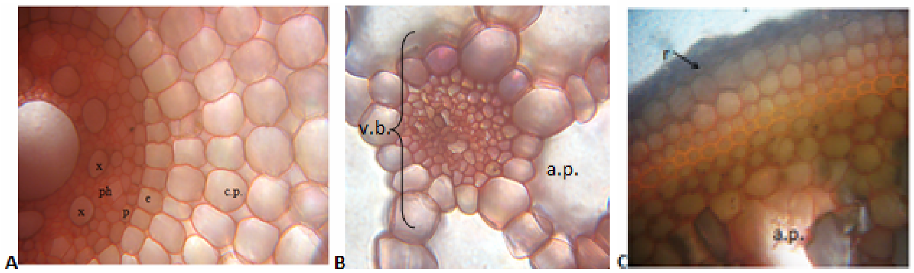

Fig. 3. *Sagittaria latifolia*: Root (A – 200x) and petiole (B - 400x) of plant preserved from Körösladány, Hu; *Phagmites* sp. roots of plant preserved from Szeghalom-upper, Hu (C - 200x) (a.p. – aerial parenchyma; c.p. – cortical parenchyma; e – endodermis; p – pericycile; r – rizodermis; ph – phloem; x – xylem; v.b. – vascular bundle).

Stems of *Potamogeton natans* looks normal, with a rich aeriferous tissue (Fig. 4 A) - specifically to the aquatic species - in this tissue was anchored vascular bundles, protected by springs sclerenchyma (Fig. 4 B), without being noticed abnormal tissues. As the leaf foliar limb of this species (Fig. 4 C), we not reported presence of abnormal structured cells or of foreign bodies or deposits.

Also vegetative organs of *Butomus umbellatus* were normal structure (Fig. 5 A and B).

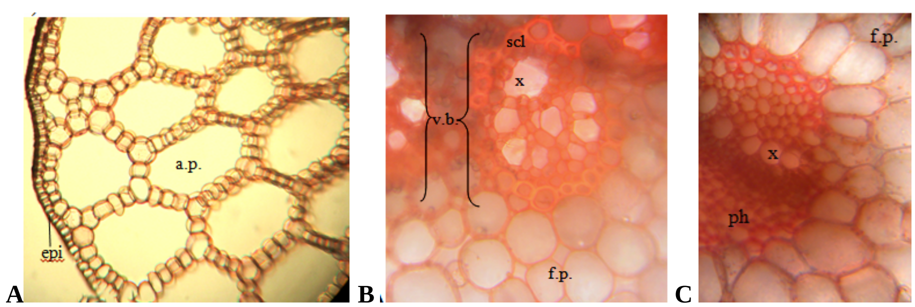

Fig. 4. *Potamogeton natans:* Stem structure of *Potamogeton natans* plant, preserved from Abram, Ro (A – 100x) and Sântion, Ro (B- 400x), and foliar limb structure, with vascular bundle detail of plant preserved from Abram, Ro (C – 400X)(aerial parenchyma; epi – epidermis; f.p. – fundamental parenchyma; ph – phloem; scl – sclerenchyma; v.b. – vascular bundle; x-xylem).

To the *Rumex* sp. roots structure, no abnormal tissues were reported, this was a typical primary root (Fig. 6 A). At the level of foliar limb nervure, hypodermic collenchyma was specific for this vegetative organ (Fig. 6 B).

The roots of *Salix alba*, a perennial species, found both in the plains and the hills, studied in many sampling points (points 5, 6, 14, 17, 18, 20-23) were normally structured (Fig. 7 A and B).

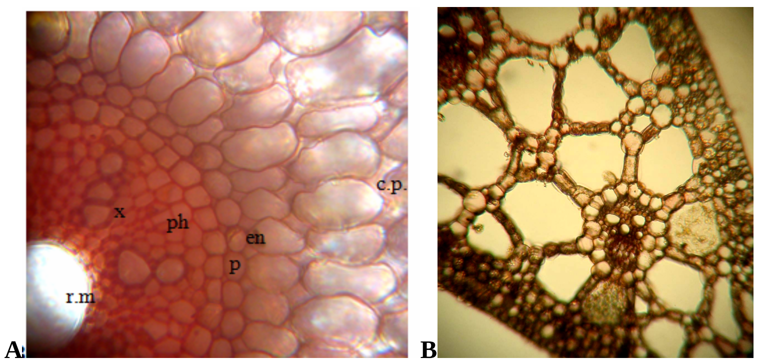

Fig. 5. *Butomus umbellatus:* Root (A - 200x) and foliar limb (B - 100x) of plant preserved from Sântion, Ro (a.p. – aerial parenchyma; c.p. – cortical parenchyma; en – endodermis; p – pericycle; ph – phloem; r.m. – resorbed marrow; v.b. – vascular bundle; x – xylem).

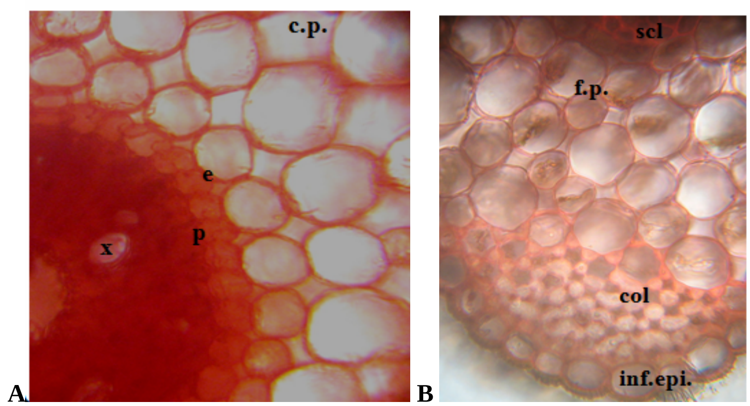

Fig. 6. *Rumex* sp.: Root structure (A – 400x) and foliar limb structure (B - 400x) of plant preserved from Săliştea de Vaşcău, Ro (col – collenchyma; c.p. – cortical parenchyma; e – endodermis; f.p. – fundamental parenchyma; inf.epi - inferior epidermis; p – pericycle; scl – sclerenchyma; x - xylem).

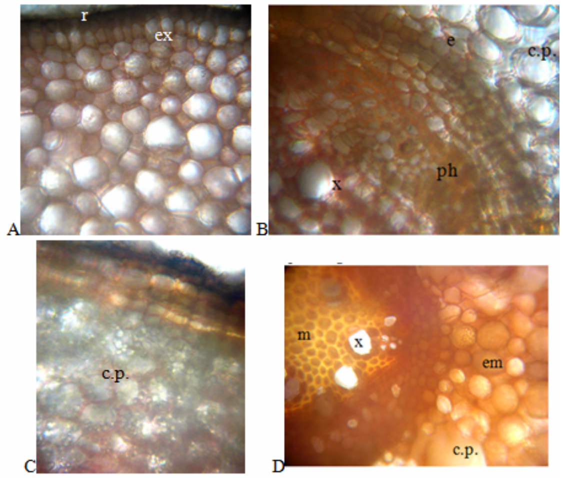

Fig. 7. Root structure of *Salix alba* preserved from Vârfurile, Ro (A - 200x) and Beiuş, Ro (B – 200x); Root structure of *Rubus* sp. preserved from Săliştea de Vaşcău, Ro (C - 200X); Root structure of *Populus* sp. preserved from Tinca - upper, Ro (200x) (D)(c.p. –cortical parenchyma; e – endodermis; em – secondary root emergency were provided by pericycle; ex – exodermis; ph – phoem; m – marrow; r – rizodermis; x - xylem).

The anatomy of the blackberry (*Rubus* sp.) roots, of some samples collected from the control, in place Săliştea de Vaşcău (RO) (considered without pollution because geographical position) it was not identified pathological changes (Fig. 7 C). Cortical parenchyma present starch deposits, was a typical secondary structure of root, specific for a perennial plant. Following numerous monitoring of blackberry (*Rubus* sp.) and nettle (*Urtica dioica*) foliar limb structure, made in 2008-2009 period, at samples coming from Crişul Negru River - from its source and to the border with Hungary - the authors mentioned previously concluded that they did not found relevant structural changes, which suggest that the harmful action of polluting factors (Petruş-Vancea et al, 2010).

In Figure 7 D it can see a mature primary structure of poplar (*Populus* sp.) roots, with aeriferous tissue in cortical parenchyma, with the emergence of secondary roots, but no abnormal elements, in histoanatomical terms, all such cellular changes are occurring physiological, normal for such structure. For anatomically point of view, specific wetlands plants have noted a low level of mechanical tissue and growth of tissue parenchymal mass. Vascular bundle had variable quantity, depending on the species. In our project, three groups of antibiotics, eg tetracycline, sulphomethazine and nitrofuran metabolites were monitories in macrophytes in the Crişuri Basin (Sandor et al, 2012) and the measured values in confirm that these compounds can be taken up by different plants either from sediment or from water, but this accumulation was not observed in the vegetative organ structural level, revealed by optical microscopy.

CONCLUSIONS

In the test points proposed by the project, there were reported no changes, defects or abnormalities in the anatomical structure of vegetative organs (roots, rhizomes, stems and leaves) of plants grown in direct contact with Crişuri Basin rivers. In all species studied - regardless of where taken, both in Hungary and in Romania - were identified structural aspects of normal vegetative organs.

Acknowledgments

This work was evaluated in the frame of European Union Project "PHARMARIVER" No.0901/086/2.2.2 with the support of Hungary-Romania Cross-Border Cooperation Programme 2007-2013.

REFERENCES

1. Abe K., Y. Ozaki, N., K., 1997, Introduction of fiber plants to plant bed filter systems for waste water treatment in relation to resource recycling. Soil Science and Plant Nutrition 43, pp. 35–43.
2. Adeyeye E., I., 2005, Distribution of major elements (Na, K, Ca, Mg) in the various anatomical parts of fadama crops in Ekiti State, Nigeria. Bull. Chem. Soc. Ethiopia 19(2), pp.175-183.
3. Ali M., A., Mohamed, H., F., Amer W., M., 2008, Biophysical measurements of lead in some bioindicator plants. Romanian J. Biophys. 18(1), pp. 57–66.
4. Andrei M., Paraschivoiu R., M., 2003, Microtehnică botanică. Editura Niculescu, Bucureşti, 165 p.
5. Big C., L., Lăcătuşu R., Damian F., 2012, Heavy metals in soil-plant system around Baia Mare City, Romania. Carpathian Journal of Earth and Environmental Sciences 7(3), pp. 219 – 230.
6. Barnes V.B., Zak D., R., Denton S., R., Spurr S., H., 1997, Forest ecology,Wiley, New York, 774 p.
7. Bonanno G., Giudice R., L., 2010, Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. Ecological Indicators 10(3), pp. 639-645.
8. Borner G.H.H., Sherrier D., J., Weimar T., Michaelson L., V., Hawkins N., D., MacAskill A., Napier J., A., Beale M., H., Lilley K., S., Dupree P., 2005, Analysis of detergent-resistant membranes in *Arabidopsis*. Plant Physiology 137, pp. 104–116.
9. Botnariuc N., Vadineanu A., 1982, Ecologie. Editura Didactica şi Pedagogică, Bucureşti, 439 p.
10. Cioplan O., 2005, Monitoringul integrat al sistemelor biologice. Editura Ars Docenti, 294 p.
11. Grigore M.N., Toma C., Zamfirache M., M., Boşcaiu M., Olteanu Z., Cojocaru D., 2012, Ecological anatomy in halophytes with C4 photosynthesis: discussing adaptative features in endangered ecosystems. Carpathian Journal of Earth and Environmental Sciences 7(2), pp.13 – 21.

12. Hadad H., R., Mufarrege M., M., Pinciroli M., Di Luca G., A., Maine M., A., 2010, Morphological response of *Typha domingensis* to an industrial effluent containing heavy metals in a constructed wetland. Archives of Environmental Contamination and Toxicology 58(3), pp. 666-675.
13. Jiang X., Wang C., 2008, Zinc distribution and zinc-binding forms in *Phragmites australis* under zinc pollution. Journal of Plant Physiology 165(7), pp. 697-704.
14. Galfati I., Bilal E., Beji Sassi A., Abdallah H., Zaier A., 2011, Accumulation of heavy metals in native plants growing near the phosphate treatment industry, Tunisia. Carpathian Journal of Earth and Environmental Sciences 6(2), pp. 85 – 100.
15. Kapitonova O., A., 2002, Specific anatomical features of vegetative organs in some macrophytes specie under condition of industrial pollution. Russian Journal of Ecology 33(1), pp. 59-61.
16. Keller B., Lajtha K., Cristofor S., 1998, Trace metal concentration in the sediments and plants of the Danube Delta. Romania Wetlands 18, pp. 42–50.
17. Marian M., Peter A., Mihaly-Cozmuta L., Bakatula E., 2012, Increased survival chances of the species *Quercus petraea* in terms of pollution with cd and cu by using microbiota-bentonite systems. Carpathian Journal of Earth and Environmental Sciences 7(1), pp. 231 – 237.
18. Mazen A., O. El-Maghraby, 1998, Accumulation of cadmium, lead and strontium, and a role of calcium oxalate in water hyacinth tolerance. Biologia Plantarum 40, pp. 411–417.
19. Sandor Z.J., Papp Z., G., Fodor A., Cupşa D., Jozsa V., Györe K., Petruş A., Petrehele A., Biro J., 2012, Occurrence of some antibiotic residues in the aquatic ecosystem of Körös River Basin. In: Proceeding of Balwois Conference on Water, Climate and Environment, Balwois 2012 - Ohrid, Republic of Macedonia - 28 May, 2 June 2012. http://balwois.com/2012/USB/papers/947.pdf (accesed in 09.09.2012).
20. Schneider I., Rubio J., 1999, Sorption of heavy metal ions by the nonliving biomass of fresh water macrophytes. Environ. Sci. Techn. 33, pp. 2213–2217.
21. Stingu A., Stanescu I., Volf I., Popa V., I., 2011, Hyperacumulation of cadmium in maize plant (*Zea mays*). Cellulose Chem. Technol. 45, pp. 287-290.
22. Varshney C., K., Garg K., K., 1980, Significance of leaf surface characteristics in plant responses to air pollution. Water, Air, and Soil Pollution 14, pp. 429-433.
23. Viskari E.-L., 2000, Epicuticular wax of norway spruce needles as indicator of traffic pollutant deposition. Water, Air and Soil Pollution 121, pp. 327-337.
24. Wang Z-Q., Jiang X.-Y., Wang C-H., 2002, Effects of Pb, Cd, and Zn on oxidative stress and antioxidative ability in *Phragmites australis*. The Chinese Journal of Process Engineering, 2(6), pp. 558-563.