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CHEMICAL COMPOSITIONS OF AQUATIC MACROPHYTES FROM THE DANUBE – ROLE IN BIOMONITORING AND BIOREMEDIATION

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ABSTRACT. The aim of this investigation was to provide the data on the status of ecological conditions referring to a possible chemical contamination of water and littoral by analysing the content of nutrients and heavy metals in tissue of dominant aquatic macrophytes (mostly *Potamogeton* species) of nine littoral sites of the Danube (Serbia). Also, our aim was to define the role of the macrophytic vegetation in remediation of pollutants, nutrients and heavy metals, in particular, from water and sediments. Sampling sites were selected to assess the effect of anthropogenic activities upon the quality of the Danube water and therefore upon plant world.
An elevation of the Danube organic load was confirmed by nitrogen (N) and potassium (K) content in plant tissue. In general, P concentrations were rather low, amounting mainly about 250 mg/100g dry matter (0.25%).
Purification efficacy and the degree of uptake and accumulation of studied heavy metals relied upon the investigated species and sampling site. Fe accumulation in all the studied species was extremely high (about 2% in *Potamogeton* species). The linear regression points to increase in Mn concentrations at downstream sites (approaching the hydroelectric power station). The distribution of plant tissue Pb and Cd pointed to an elevation of water and littoral pollution at downstream sites. A significantly elevated contamination with heavy metals, of downstream sites, is due to the metallurgical complex-steelworks, wastewaters from power station, municipal and rural localities along the Danube stream in Serbia.

KEY WORDS. Macrophytes, Danube, nutrients, heavy metals, bioaccumulation, bioindication

INTRODUCTION

A continuous biological monitoring of chemical parameters of water and littoral is an essential element needed to plan a sustainable development of the Danube natural resources. In addition to the chemical parameters of water, also the assessment of abundance and distribution of aquatic biota gives reliable data on water and littoral chemical load. The aquatic macrophytes deserve fully attention in the biological monitoring of the ecology of aquatic ecosystems due both to the temporal distribution of macrophytic communities and their structural distribution (species number, population density) that are important indicators of a general ecological status prevailing in aquatic ecosystems (Janauer, 2001). Vascular hydrophytes are known for their biofiltration properties, accumulating various toxic substances like pesticides, phenol derivatives, and heavy metals. Therefore, numerous recent investigations into aquatic ecosystems have been focused at the possibilities of purification of water and littoral zones by employing aquatic vegetation to remediate nutrients, heavy metals, and other pollutants (DeBusk et al., 2001). Efficacious and economically sound application of phytoremediation as a non-invasive technique to a complex, long term, and expensive purification processes (detoxification, filtration) of contaminated areas provides more reasons for the investigations which will define the role of macrophytes in complex pathways of cycling of nutrients and heavy metals in aquatic ecosystems. The metal accumulation represents a direct participation of aquatic macrophytes in this cycling while their indirect effect is slowing of stream flow and acceleration of sedimentation of dispersed particles together with metal ions (St Cyr et al., 1994).

The purpose of this investigation was to provide the data on the status of ecological conditions referring to a possible chemical contamination of water and littoral by analysing the content of nutrients and heavy metals in tissue of dominant aquatic macrophytes (mostly *Potamogeton* species) of nine littoral sites of the Danube (Serbia). Also, our aim was to point to the role of the macrophytic vegetation in remediation of pollutants, nutrients and heavy metals, in particular, from water and sediments.

MATERIAL AND METHODS

Plant material:

Samples of aquatic plants were collected at their maximum development stage from nine selected sites along the Danube bank in summer 2003. Sampling were done from the boat. The plants were rinsed several times in tap water to remove the adherent periphyton and detritus. After final rinsing in distilled water, material was dried and prepared for analyses following Standard methods for the examination of water and wastewater APHA (1995). Before chemical analysis plant material was dried at 100°C and milled. Total N concentration in the dry matter was determined by standard microkjeldahl method (Nelson and Sommers, 1973). The concentrations of P, K and Na, and heavy metals were determined after dry ashing at 450°C and treatment with HCl. Then phosphorus was assayed spectrophotometrically by

ammonium-vanadate-molybdate method, potassium and sodium by flame emission spectrometry and heavy metals by atomic absorption spectrophotometry.

Data analysis was done using multiple interval test (Duncan), and tasting was done for the level of significance of p=0.05.

Study area:
Investigated sites at the left river bank:

1. Bezdan (Baračka river branch at 1426.5 river km). Analysed plant species: *Ceratophyllum demersum* L. and *Trapa natans* L.
2. Beograd (Jojkićev Dunavac at 1168.5 river km). Analysed plant species: *Potamogeton pectinatus* L. and *Potamogeton natans* L.

Investigated sites at the right river bank:

3. Smederevo (downstream of iron processing plant, at 1115 river km). Analysed plant species: *Potamogeton perfoliatus* L., *Potamogeton lucens* L. and *Potamogeton gramineus* L.
4. Kostolac (at 1094 river km). Analysed species: *Potamogeton pectinatus* L.
5. Ram (at 1078 river km). Analysed species: *Potamogeton pectinatus* L.
6. Veliko Gradište (at 1058 river km). Analysed species: *Potamogeton natans* L. and
Potamogeton pectinatus L.
7. Tekija (at 955 river km). Analysed species: *Potamogeton natans* L. and *Potamogeton perfoliatus* L.
8. Mihajlovac (at 872 river km). Analysed species: *Elodea canadensis* Michx. and *Potamogeton lucens* L.
9. Radujevac (at 850 river km). Analysed species: *Potamogeton lucens* L. and *Potamogeton pectinatus* L.

The investigated sites Smederevo, Kostolac, Ram, Veliko Gradište, and Tekija are situated along the accumulation Đerdap I while the site Mihajlovac along the accumulation Đerdap II. Radujevac is the last populated place at the Danube in Serbia. Sampling sites were selected to assess the effect of anthropogenic activities upon the quality of the Danube water and therefore upon plant world, e.g. Smederevo (downstream of the metallurgical complex-steelworks), Kostolac (downstream of power station), and Beograd (stream receiving municipal wastewaters).

RESULTS AND DISCUSSION

Content of nutrients, heavy metals, and other pollutants in substratum relies greatly upon the decomposition of organic matter while an important role in detoxification of bottom and littoral belongs to rooted submersed, floating, and emergent species due to their roots, i.e. rhizomes which uptake conspicuous amounts of soluble elements.

Concentration of macronutrients and Na in tissue of aquatic macrophytes

Nitrogen (N) concentrations in dry plant matter were about 2%, varying in relation to the investigated site (Fig. 1). Concentrations exceeding 3% were recorded in *P. natans* and *E. canadensis* at site 7 and 8. The obtained linear regression points to the elevation trend in N content in plant tissue at downstream sites that shows an elevated organic load of water and mud of the Danube. The increase of N accumulation might be the result of an increased nutrient content in water and, consequently, increased eutrophication. In discussing these results the antagonistic effect of ions originating from plant uptake process have to be taken into account. A significant effect of Cl^-, K^+, SO_4^{2-} and PO_4^{3-}concentrations upon N uptake was reported (Kastori, 1998). An elevation of the Danube organic load was also confirmed by potassium (K) content in plant tissue (Fig. 1). K content variability as related to the investigated site was greater than N variability. In other words, the recorded concentrations ranged between 861mg/100g dry matter (0.86%) in *P. perfoliatus* at site 7 (Tekija) up to 3276 mg/100g, i.e. 3374 mg/100g in *P. lucens* and *E. canadensis* at site 8 (Mihailovac). These two species were also characterized by the highest phosphorus (P) accumulation of about 0.35% (Fig. 2). In general, P concentrations were rather low, amounting mainly about 250 mg/100g dry matter (0.25%). The lowest recorded P concentrations were obtained with *P. pectinatus* at site 6. Rather lower macronutrient concentrations in *P. natans* also contribute to the conclusion that this site is less loaded with organic substances. Rooted aquatic macrophytes in most part satisfy their demands for P from the sediment, therefore hindering biomonitoring since the limiting factor of their spatial distribution is not only nutrient concentration, but, also, water depth, i.e. light regimen and pH. Despite this, P is frequently used as a critical element in controlling of water nutrient load. In recent investigations and projects, the possibility of utilization of aquatic plants in remediation of P, present in wastewaters pouring into streams, has been discussed. P amount remediated by aquatic plants from aquatic environment relies upon plant species and the amount of P in water, i.e. substratum while, most frequently, the main P source is upper mud layer (Kim and Geary, 2001).

Sodium (Na) concentrations were rather high, amounting mainly about 600mg/100g dry matter (0.6%). The highest Na (exceeding 1%) was recorded in *P. lucens* at site 8 (Fig. 2).

Our results also show that the only test species *T. natans* exhibited the lowest macronutrient accumulation at site 1. The species *E. canadensis* showed however, considerable accumulation of N, K, and P, in particular. The nutrient accumulation in *Potamogeton* species relied upon the investigated site to a great extent.

Concentration of heavy metals in tissue of aquatic macrophytes

Elevated concentrations of heavy metals in the environment and, consequently, in plant tissue have no visible impact upon vegetation in most part due to specific physiological mechanisms developed by plants (Boudou and Ribeyre, 1997; Matagi

et al., 1998). Therefore, the phytoremediation (uptake and accumulation of nutrients and heavy metals) is the important biotic factor employed in outlining the program of utilization of macrophytic species in purification of polluted aquatic ecosystem (Karpiscak et al., 2001). Chemical status of heavy metals plays an important role in remediation since certain oxide forms of Pb, Zn, Ni, Ag, and Cd are hardly available to plants, making their presence in plant tissue unclear despite their presence in the environment.

The obtained results show that the purification efficacy and the degree of uptake and accumulation of studied heavy metals relied upon the investigated species and sampling site. The analysed macrophytic species mostly showed the highest Fe then Mn accumulation. The accumulation degree of Cu, Ni, Pb, and Cd was significantly lower while their concentration ratios relied upon plant organ.

Fe accumulation in all the studied species was extremely high. *C. demersum* exhibited the highest accumulation since the average recorded concentration amounted even 50 500 $\mu g \cdot g^{-1}$, i.e. more than 5% (Fig. 3). At the same site (Site 1), another species, *Trapa natans*, exhibited high Fe (4.6%). Therefore, this site is undoubtedly contaminated with chemicals. Fe accumulation in *Pectinatus* species was also high (about 2%). The species *P. pectinatus* showed a distinguishable Fe content of 33 880 $\mu g \cdot g^{-1}$ of dry plant matter at site 5. According to the linear regression, a decrease of Fe content in plant tissue at downstream sites was evident. But, by comparing the recorded Fe concentration values with those reported for the Danube-Tisza-Danube canal complex (Pajević et al., 2003), an elevation of Fe concentration is evident. The same conclusion may be drawn when Fe data on *Nymphoides peltata* of several Danube sites are considered (Pajević et al., 2005). Mn accumulation in plant tissue however, was completely different (Fig. 3). The linear regression points to increase in Mn concentrations at downstream sites (approaching the hydroelectric power station). In general, high Mn concentrations were recorded in plant tissue while among the investigated plants, the species *E. canadensis* and *C. demersum* showed highest values (32 778 and 21 667 $\mu g \cdot g^{-1}$).

The concentration of Cu and Ni relied upon the investigated site. Nevertheless, their elevation in plant tissue at downstream sites was evident (Fig. 4). Ni was somewhat higher than Cu concentration, distinguishing the species *Elodea canadensis* that showed extremely high accumulation of both elements. Higher concentrations of these two micronutrients recorded in plant tissue point to the chemical contamination of water and littoral. By comparing these results with data reported both for the DTD canal complex (Pajević et al., 2003) and aquatic ecosystems of the vicinity of the Novi Sad City (Pajević et al., 2002), a somewhat higher accumulation at the downstream sites in particular, is evident. Chemical contamination is undoubtedly the result of the river traffic, wastewaters of the surrounding arable areas, and municipal wastewaters. The presence of Pb and Cd in all the analyzed plant species showed an evident chemical contamination of water and littoral (Fig. 5). A high Pb (17 $\mu g \cdot g^{-1}$) was recorded in *C. demersum* at site 1. Also, a high Pb accumulation (about 10 $\mu g \cdot g^{-1}$) was obtained with *Potamogeton*

species. Cd concentrations in plant tissue were less than 2 $\mu g \cdot g^{-1}$ with the exception of the site 8 characterized by higher Cd accumulation in both analysed species (*E. canadensis, P. lucens)* (Fig. 5). The distribution of Pb and Cd at the investigated sites points to an elevation of water and littoral pollution at downstream sites. A significantly elevated contamination with heavy metals, of downstream sites, is due to the metallurgical complex-steelworks and power station. Chemical contamination of water and littoral led to an extremely high accumulation of Fe and Mn (up to ten times higher than average values in plants), as well as Pb and Cd in plant tissue while also elevated concentrations of Cu and Ni were evident.

In summary, the health of the Danube at the investigated sites is seriously degraded due to an extremely high chemical pollution of the stream, emphasizing the importance of the aquatic macrophytes in bioindication and remediation of pollutants.

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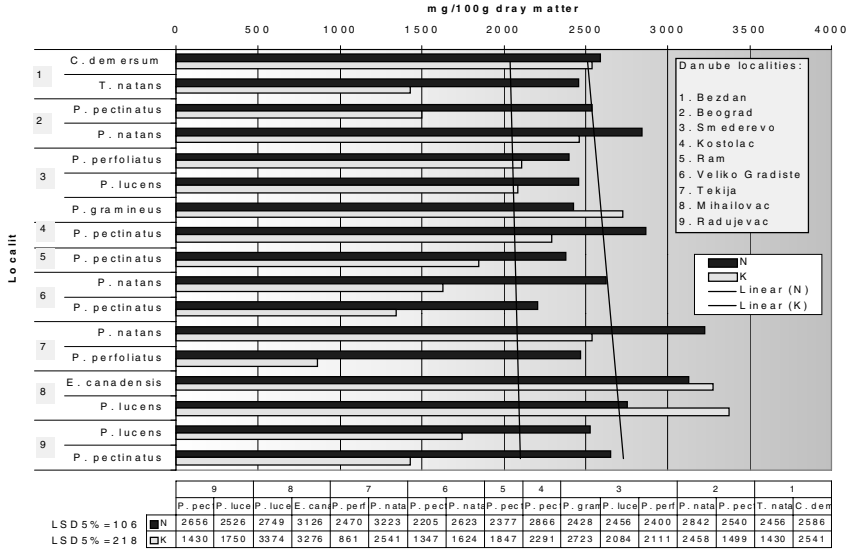


Fig.1. Nitrogen and potassium concentrations in aquatic macrophytes according to sampling site

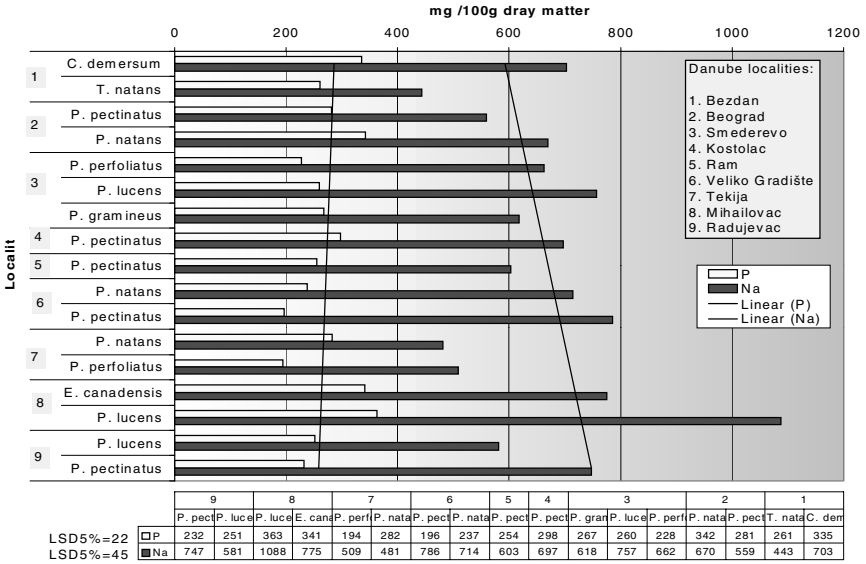
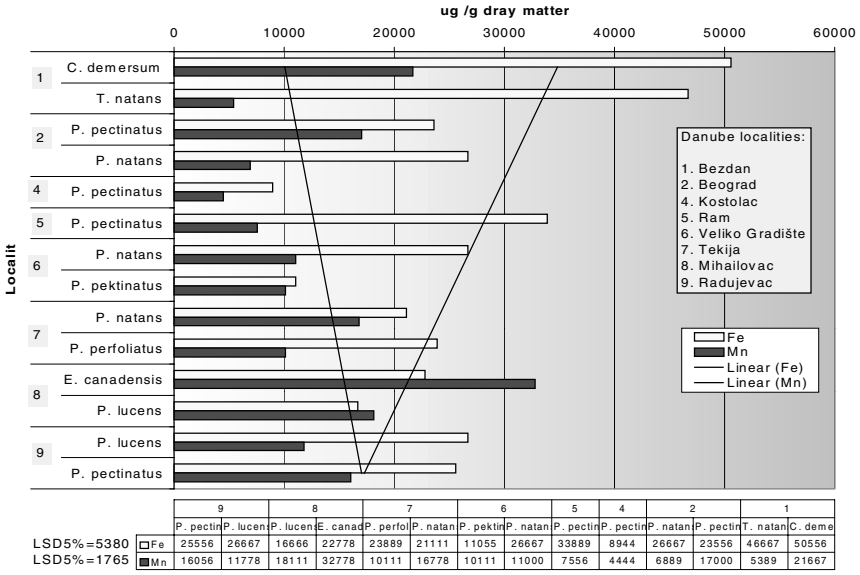
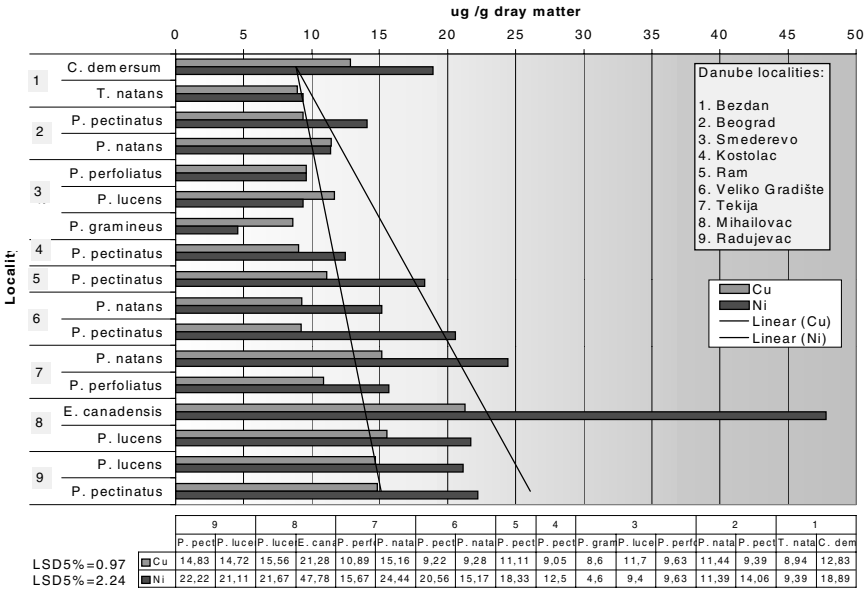


Fig.2. Phosphorus and sodium concentrations in aquatic macrophytes according to sampling site



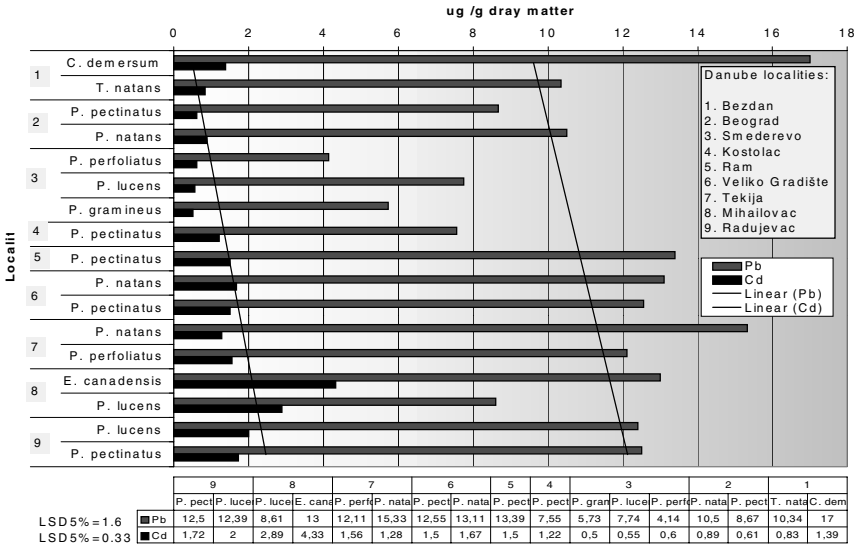
		9		8		7		6		5	4	2		1	
		P. pectin	P. lucen	P. lucen	E. canad	P. perfol	P. natan	P. pektin	P. natan	P. pectin	P. pectin	P. natan	P. pectin	T. natan	C. deme
LSD5%=5380	Fe	25556	26667	16666	22778	23889	21111	11055	26667	33889	8944	26667	23556	46667	50556
LSD5%=1765	Mn	16056	11778	18111	32778	10111	16778	10111	11000	7556	4444	6889	17000	5389	21667

Fig.3. Iron and manganese concentrations in aquatic macrophytes according to sampling site



		9		8		7		6		5	4	3			2		1	
		P. pect	P. luce	P. luce	E. cana	P. perf	P. nata	P. pect	P. nata	P. pect	P. pect	P. gram	P. luce	P. perf	P. nata	P. pect	T. nata	C. dem
LSD5%=0.97	Cu	14,83	14,72	15,56	21,28	10,89	15,16	9,22	9,28	11,11	9,05	8,6	11,7	9,63	11,44	9,39	8,94	12,83
LSD5%=2.24	Ni	22,22	21,11	21,67	47,78	15,67	24,44	20,56	15,17	18,33	12,5	4,6	9,4	9,63	11,39	14,06	9,39	18,89

Fig.4. Copper and nickel concentrations in aquatic macrophytes according to sampling site



		9		8		7		6		5	4	3			2		1	
		P. pect	P. luce	P. luce	E. cana	P. perf	P. nata	P. pect	P. nata	P. pect	P. pect	P. gram	P. luce	P. perf	P. nata	P. pect	T. nata	C. dem
LSD5%=1.6	Pb	12,5	12,39	8,61	13	12,11	15,33	12,55	13,11	13,39	7,55	5,73	7,74	4,14	10,5	8,67	10,34	17
LSD5%=0.33	Cd	1,72	2	2,89	4,33	1,56	1,28	1,5	1,67	1,5	1,22	0,5	0,55	0,6	0,89	0,61	0,83	1,39

Fig.5. Lead and cadmium concentrations in aquatic macrophytes according to sampling site