Characterization of Heavy Metals Distribution at the Level of Biotic Compartments of the Dambovnic River

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Dâmbovnic River, a tributary of the Neajlov River, has undergone over time al different loads of heavy metals (Cr, Cd, Pb, Mn) from industrial wastewater discharges from nearby settlements. Another source of pollution is the significant quantities of heavy metals that are carried downstream when stronger spills cause resuspension of bottom sediments of upstream lakes. It is known that the highest concentrations of heavy metals in river are found in the bottom sediments. (See also previous article), but the fate of these metals and the impact on the ecosystem are still unknown. The main purpose of this paper is to clarify as much as possible, the impact of heavy metals such as Cr, Cd, Mn have both on submerged and anchored vegetation and on benthic macroinvertebrates in the Dâmbovnic catchment. According to the analyzed results submerged/floating species (Cladophora and Lemna minor) presents a Cd bioconcentration factor greater than one, much larger than the anchored species (where F_{bc}<1), suggesting the potential they have in terms of phytoremediation techniques of aquatic systems which capture industrial effluents with high concentrations of metals. The transfer of chromium from the lower to the upper parts of the Typha, Juncus and Potamogeton is less (Typha sp 0.06 - 0.25; Juncus sp. 0.05-0.09), indicating that, in the event of Cr contamination of aquatic sites, risk transfer of this to primary consumers is reduced. For benthic macroinvertebrates Cr concentration factors are below par, showing that the metal does not focus on the benthic macroinvertebrates, the main compartment that stores this metal is the sediment. For Mn, the dynamics spatial of averages, seasonal of tissue concentrations of benthic macroinvertebrates is characterized by highest values at station D4, for all taxonomic groups which were analyzed.

Keywords: heavy metals, vegetation, benthic macroinvertebrates, Dâmbovnic River

Heavy metals (Pb, Cr, Zn, Ni) accumulated in sediment and in biocenosis leading to the so-colled tertiary pollution, due to the resolubilisation when pH decreases. [1].

Studies that followed the takeover of heavy metals by biocenosis shows that it is difficult to estimate the influence of industrial wastewater on heavy elements content of different aquatic organisms tissues due to the natural variability of their concentration in the fine/mud fraction [4].

Heavy metals can cause severe problems in aquatic ecosystems due to their persistence, toxicity and tendency to accumulate in fish and shellfish tissues [14].

While these constituents have been studied extensively and have been identified standardly, their chemical reactions in environment and their general effects on aquatic life are still complex and poorly understood (see [18]).

It is well known that the fate and the transport of metals in environment are governed by metals interactions and reactions with water, sediments and aquatic organisms [6, 11, 14] and that these metals are also affected by environmental conditions as well as the flow rate [15]. Contaminated sediments have been investigated by many researchers, see for example the papers [2-3] and [9], which demontrated the importance of particle size distribution in relation to the transport of chromium, silver, lead, zinc, copper and other constituents. Since high concentrations of heavy metals are typically associated with solids and thus carried in aquatic environment, many researchers have discussed the role of the grain size and of the organic matter [2, 7, 16-17].

The toxicological impact of metals on environment has also been investigated extensively. Toxicological evaluations often use aquatic bioassays, where the effect of a contaminant on a receiver (eg. aquatic or plants) is assessed. However toxicological testing procedures are relatively complex, often including the need to define a variety of physical and chemical parameters [6, 10, 18-20].

Quantification of metal accumulation may also provide a perspective on the evolution, transport and accumulation of metals in aquatic environment. High concentrations of metals in the vicinity of the river provides the confirmation that the transport or the exchange process exists between the river and the adjacent vegetation near the shore. Transport routes with impact on vegetation may include surface water discharges, underground sources flow, possible direct inputs from river through water immersion or porous. Given the relationship between the analyzes of vegetation and sediment analyzes and the trends that the results of the plants present, a suggestion would be to consider the use of analyzes of terrestrial plants as a screening tool to identify the stretches of water that have high concentrations of heavy metals [5].

In this paper is presented the analysis of heavy metals in Dâmbovnic River (in control sections D1-D5) in the comartments: vegetation and benthic macroinvertebrates [13]. Metals for which complete analysis has been performed and thus have allowed an integrated analysis are Cd, total Cr (Cr^{+3}, Cr^{+6}) and total Mn (Mn^{+2}, Mn^{+6}).

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Species	Class	Code	Stations	Subsamples	Observations
Cladophora sp.	Cladophorales	**Cl**	D2c D3 D4 D5	Whole plant	Submerge species, fixed substrate
Lemna minor	Alismatales	**Lt**	D3 D4 D5	Whole plant	Floating species, present in areas with stagnant water Grow in freshwater, nutrient reach, with pH- between 5-9 (optimum 6.5-7.5), at temperatures between 6 - 33 °C.
Potamogeton sp.	Alismatales	**Po**	D2a D2c D3 D4 D5	Lower part (root) Upper part (strain, leaves, flowers and combinations)	Emergent aquatic species
Juncus sp.	Poales	**Jn**	D2a D2c D5	Lower part (root) Upper part (strain, leaves, flowers and combinations)	Species located aquatic-terestrial interface
Typha sp.	Poales	**Ty**	D1 D2a D2c D3 D4 D5	Lower part (root) Upper part (strain, leaves, flowers and combinations)	Emergent species, present in shallow water (maximum 80 cm)

Table 1
PLANT SPECIES SELECTED FOR ANALYSIS, IN WHICH SECTIONS HAVE BEEN FOUND, THE MAIN FEATURES OF THE HABITAT

Experimental part

Preparation of samples

Data synthesis was very difficult to vegetation due to the temporal and spatial variability (in the monitoring sections) of plants species. Data analysis is difficult because the processes of acquisition and transport of metals in this section depend on the level of exposure, the time of exposure, the parameters specific to each species and individual variations (table 1).

Benthic macroinvertebrates showed large biomass variations in stations, so we grouped them using trophic spectrum as criterion. It was necessary to bring together over several months, the analyzes performed on seasons. All these elements are shown in table 2.

To determine the metal ion content of biological samples (plant tissue and benthic macroinvertebrates) according to the method of atomic absorption spectrophotometry, the universal solvent used (used as a blank for determination) is represented by a solution of 0.2 % HNO_3 concentration. Preparation of the biological material included drying in an oven at 60°C for 2 days, and grinding (600 rpm). For the actual mineralization in the digestion flask was weighed about 0.2000 g biological material to which was added 5 mL HNO_3 65% ultrapure, samples enclosed in a capsule were heated in the microwave at 180°C for 20 min.

After the cooling of the tubes, the samples are transferred to a 25 mL volumetric flask and make up to the mark with HNO_3 3% (v/v).

Flask content was filtered through cellulose acetate filters with a pore diameter of 0.45μm, held beforehand in the solution of HNO_3 3% (v/v) for at least 1-2 h.

Results and discussions

Analysis of the distribution of cadmium in the Dâmbovnic River compartments

Vegetation

Analyzing the results of cadmium determination of vegetation samples taken from control stations D1-D5 in the period of study, it appears that the level of this metal varies between 0.0042 and 0.96 μg Cd/g dry matter and only six values exceed, accidentally, 1 μg/g dry matter. It was also observed that there is a difference in concentrations that this element achieve in various vegetative parts, roots are characterized, in general, by higher concentrations compared with strains, regardless of the species type: aquatic or riparian. This can be explained by the fact that the degree of exposure of the vegetative parts is different, the roots being in direct contact with sediments, which is caracterized by high levels of concentration of the element as compared with water (aproximatively three orders of magnitude) [12].

Cadmium concentrations in the roots vary in a relatively narrow range (0.33-0.66 mg Cd/g dry matter), with one exception – Juncus sp., for wich there is a higher value: 0.86μg Cd/g dry matter. It is noted that in virtually all cases (with one exception) the cadmium level in root tissue is

Taxa/taxonomic groups	Localization	Code	Stations	Trophic spectrum	Seasonal grouping
Oligochaeta	Phylum: Annelida Cls: Clitellata	**Oli**	D2 D3 D4 D5	Geofage (decaying detritus)	**W (winter):** November-December 2008; January-February 2009; **S (Spring):** March-May 2009; **S (Summer):** June-August 2009; **A (Autumn):** September-November 2009
Chironomidae	Phylum: Arthropoda Cls: Insecta	**Ch**	D2 D3 D4 D5	Detritofage, fine organic matter	
Ephemeroptera şi Trichoptera	Phylum: Arthropoda Cls: Insecta	**ET**	D2 D3 D4	Detritofage, coarse organic matter	
Hirudinea	Phylum: Annelida Cls: Clitellata	**H**	D3 D4 D5	Hematofage	

Table 2
MACROINVERTEBRATES TAXA SELECTED FOR ANALYSIS OF HEAVY METALS, STATIONS, GROUPING AND THE SEASONS WHEN THEY WERE ANALIZED

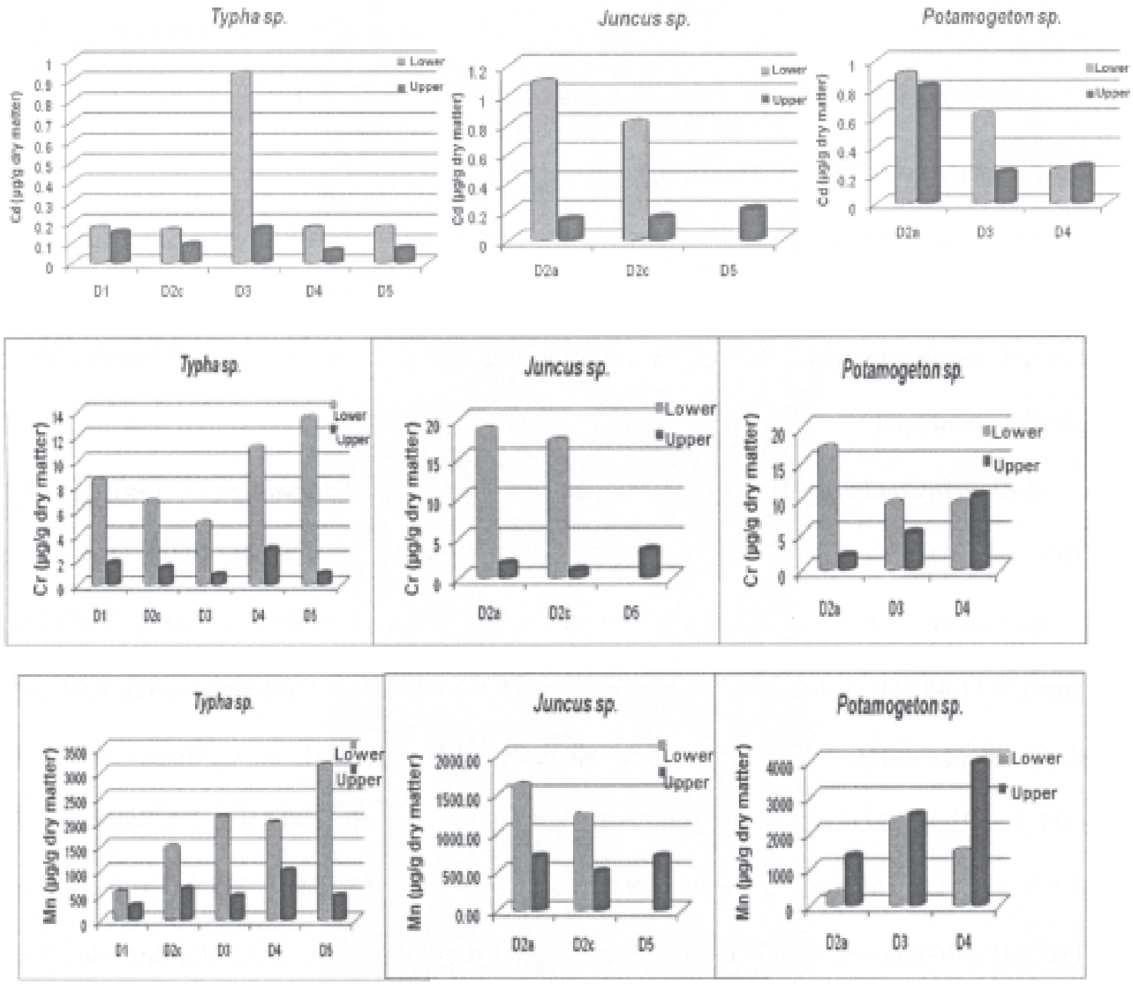

Fig. 1. The average concentrations of Cd, Cr, Mn (g/g dry matter) in the upper and lower parts of the species anchored in sediment, in the Dâmbovnic River stations

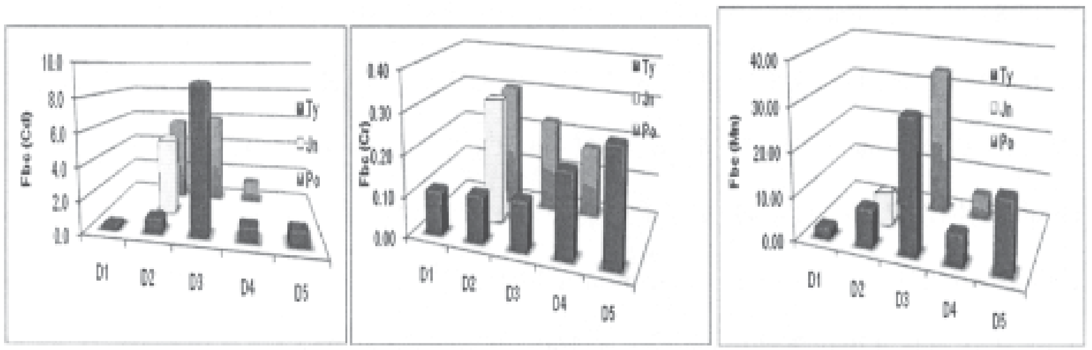

Fig. 2. Bioconcentration factors of cadmium, chromium and manganese calculated for plants species anchored in sediment of the Dâmbovnic River stations

greater than the upper portions, having concentrations ranging from 0.11-0.48 µg Cd/g dry matter.

For species Juncus sp. and Potamogeton sp. it was oberved decrease of Cd concentrations in roots, from upstream stations (D1, D2) to downstream stations (D4, D5), which is in perfect agreement with the dynamic of cadmium in the sediments (fig. 1).

Values of bioconcentration factors in roots of the species anchored in sediments are above par (0.3-9), indicating a process of Cd bioconcentration in the roots. The highest values of this factor are recorded in D3 station. (fig. 2).

Calculating the ratio MS/MR (metal concentration in the top / metal concentration in root) yielded valuable information regarding the internal transport of metal ions (table 3).

In most cases, for the three selected species (Typha, Juncus and Potamogeton), (with one exception - Potamogeton sp. in D4) transfer factors are below par. The biggest factors were obtained for the spacies Potamogeton sp., then for Typha sp. (0.18-0.88) and Juncus sp. (less than 0.2).

Analyzing submerged and floating species, which are in interaction with water (Cladophora and Lemna minor) observed that the concentrations dynanics in plant tissue is similar to the concentrations in the water for Cladophora species (fig. 3), reaching a maximum in D4 station. For these species the Cd concentrations reached higher levels (>0.3 µg/g dry matter) than in the upper parts of the anchored species (<0.3µg/g dry matter).

Bioconcentration factor for these species submerged/ floating is above par (fig. 4), much higher than the anchor (which have F_{bc}<1), suggesting the potential they have regarding the phitoremediation techniques of the aquatic systems which captures industrial effluents with high metal concentrations.

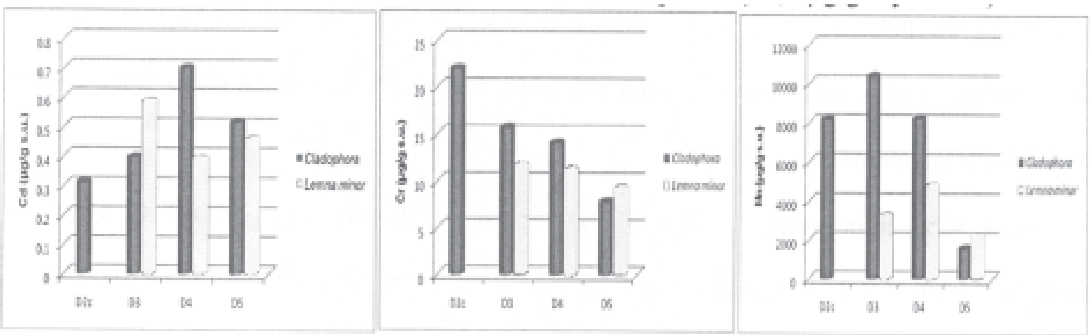

Fig. 3. The average concentrations of cadmium (μg/g dry matter) for floating and submerger plant species, in the Dâmbovnic River stations

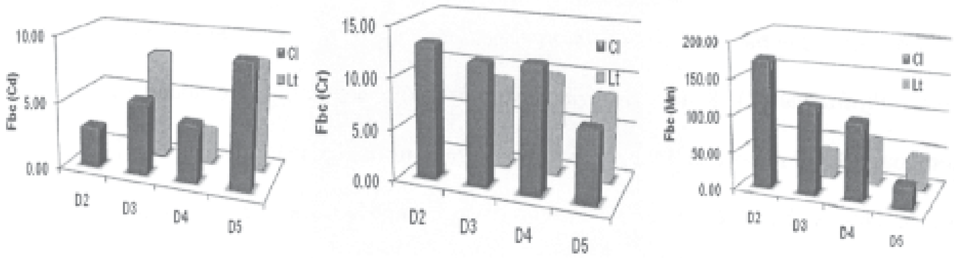

Fig. 4. Cd (μ/g) bioconcentration factors for submerged and floating plant species, in Dâmbovnic River stations

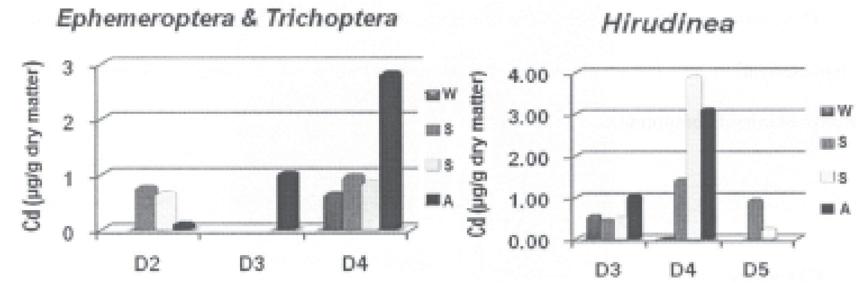

Fig. 5. Seasonal average concentrarions of Cd (μg/g dry matter) for main taxa, in the Dâmbovnic River stations, for 2008-2010

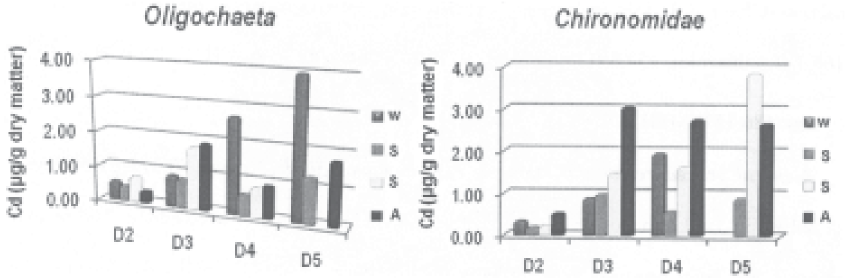

Fig. 6. Average bioconcentration factors of Cd for the major taxonomic groups of benthic macroinvertebrates, in the Dâmbovnic River stations

Macroninvertebrates

Graphical representation of the Cd concentrations, for macroinvertebrates, shows an increasing trend in the level of this metal from upstream to downstream for nearly all taxonomic groups included in the analysis (fig. 5).

Spatial dynamics is characterized by the highest values of tissue concentrations of Cd in D4 station for Ephemeroptera and Hirudinea and in D5 station for Oligochaeta and Chironomidae. Station D4 presents the highest values of Cd for lotic system both in sediments and water, which has been linked to potential emission of oil in this area. The dynamics of the concentrations of heavy metals in the compartment is however affected, by the substrate heterogeneity, which has not been covered by the qualitative method of sampling of the benthos and, on the other hand, by the method of assembly of the samples in order to obtain a sufficient amount of biomass to be done the chemical determination.

Calculation Cd bioconcentration factors for the major taxonomic groups of benthic macroinvertebrates led to above par value (fig. 6), indicating that the macro-invertebrates in the basin perform a bioconcentration of this metal.

For the stations on the Dâmbovnic River similar values were obtained of the ratio of the tissue concentration of cadmium in macroinvertebrates and its concentration in sediments, about 10, except D2 station, which have values below 5. Compared with obtained factors for plant species,those calculated for macroinvertebrates are slightly higher, which means that benthic macroinvertebrates species focus to a greater extent this element.

Distribution of chromium in the Dâmbovnic River compartments

Vegetation

Levels of Cr in plant tissues are an order of magnitude higher than in the case of cadmium, enrolling on the range from 6.34 to 21.72 μg Cr/g dry matter for roots and whithin the range from 1.38 to 12.38 μg Cr/g dry matter for upper parts.

Although the selected species (Typha, Juncus, Potamogeton) are not in all the control stations, the general trend observed is the decrease in their concentrations in the roots from the station D1 to D3. For Typha species the concentrations increase again in stations D4 and D5. Potamogeton species have the same dynamics of Cr concentrations at the roots level as that obtined for abiotic compartments, water and sediment (fig. 1).

For chromium, bioconcentrations factors calculated for species which interact and intensively exchange with

Species	Station	MS/MR (Cd)	MS/MR (Cr)	MS/MR (Mn)
Typha sp.	Dâmbovnic Lake (D1)	0.882	0.211	0.519
	Suseni exit Lake(D2c)	0.563	0.205	0.423
	Slobozia (D3)	0.183	0.159	0.227
	Roata (D4)	0.353	0.259	0.509
	Vadu Lat (D5)	0.412	0.066	0.156
Juncus sp.	Suseni Lake (D2a)	0.130	0.096	0.431
	Suseni ieşire Lake (D2c)	0.188	0.056	0.413
Potamogeton sp.	Suseni Lake (D2a)	0.911	0.110	4.476
	Slobozia (D3)	0.355	0.561	1.077
	Roata (D4)	1.130	1.132	2.694

Table 3
TRANSFER FACTORS OF Cd, Cr, Mn FROM LOWER TO UPPER PARTS FOR AQUATIC PLANTS ANCHORED IN SEDIMENT, IN DÂMBOVNIC RIVER STATIONS, FOR 2008-2010

sediment were below par (fig. 2), which indicate that there is not a bioconcentration process for these species, but one of bioacumulation.

It is interesting that chromium transfer from the lower to the upper parts of Typha, Juncus and Potamogeton species is still below par (Typha sp 0.06 - 0.25; Juncus sp. 0.05-0.09), indicating that, for a contamination of aquatic sites with Cr, the transfer risk of this element to primary consumers is reduced. Only Potamogeton species has a transfer coefficient slightly higher than one in station D4, exactly asin the case of Cd (table 3).

For floating and submerged species (Cladophora and Lemna minor) were obtained values of tissue concentrations approximately an order of magnitude higher than those of Cd. Also, there was a decrease in concentrations of Cr from plant material of these species from upstrem to downstream stations (fig. 3), a perfect dynamics according to that obtained for the abiotic compartiments (paticularly water), which reveals intense interactions between floating/submerged species and water.

Based on these results it was possible to calculate the bioconcentration factors for the two species in control stations on Dâmbovnic River where they were found (fig. 4), which have been shown above par, unlike those calculated for the species anchored in geological substrate. Compared with bioconcentration factors of these plant species for Cd (<8), the Cr are slightly higher (between 7-13), indicating a high potential to retain chromium.

Bioconcentration factors do not vary significantly from one area to another, suggesting that the stationary state has been reached for these species. Also, the differences between the two floating species are small, Cladophora showing slightly higher values.

Macroinvertebrates

A first inspection of tissue concentrations values of chromium for the major taxonomic groups of benthic macroinvertebrates revelas high levels by almost an order of magnitude than those of cadmium (fig. 7).

Spatial dynamics of tissue average concentrations of chromium present higher values in station D4, for Ephemeroptera and Hirudinea, similar dynamics recorded for chromium concentration in sediments. Concentration factors of this metal in the benthic macroinvertebrates in Dâmbovnic River stations are below par (fig. 6). So this metal does not concentrate in the benthic macro-invertebrates, the main compartment that stores this metal in water being the sediment.

Analysis of manganese distribution in Dâmbovnic River comparments

Vegetation

Because manganese is a trace element for primary producers, its levels are much higher in plant tissues compared with cadmium and chromium (fig. 1). Thus, the manganese concentration in plant tissues of plant species anchored in sediments exceeds by approximately two orders of magnitude the concentration of chromium and approximately four orders of magnitude that of cadmium, in the same plant species.

Also, similar to the behaviour of cadmium and chromium, manganese accumulates more at the bottom of the plant, which is in contact with sediment, with one exception, found in Potamogeton species.

Common species selected to highlight the interaction with the substrate (Typha, Juncus and Potamogeton) has a spatial dynamics more obvious than that recorded for Cd and Cr (fig. 1). Thus, the concentrations of Mn in root

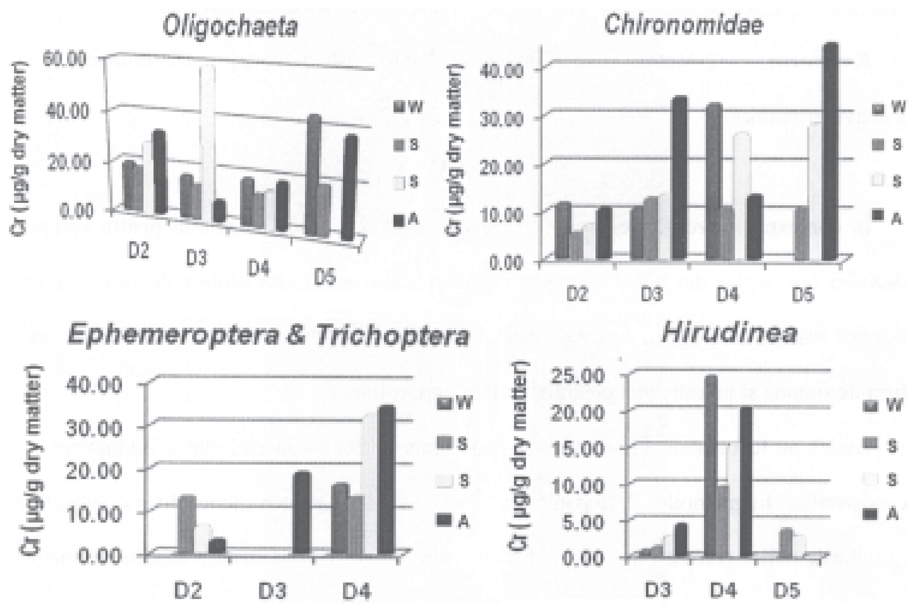

Fig. 7. Seasonal mean concentrations of chromium (µg/g dry matter) for main taxa, in Dâmbovnic River stations, for 2008-2010

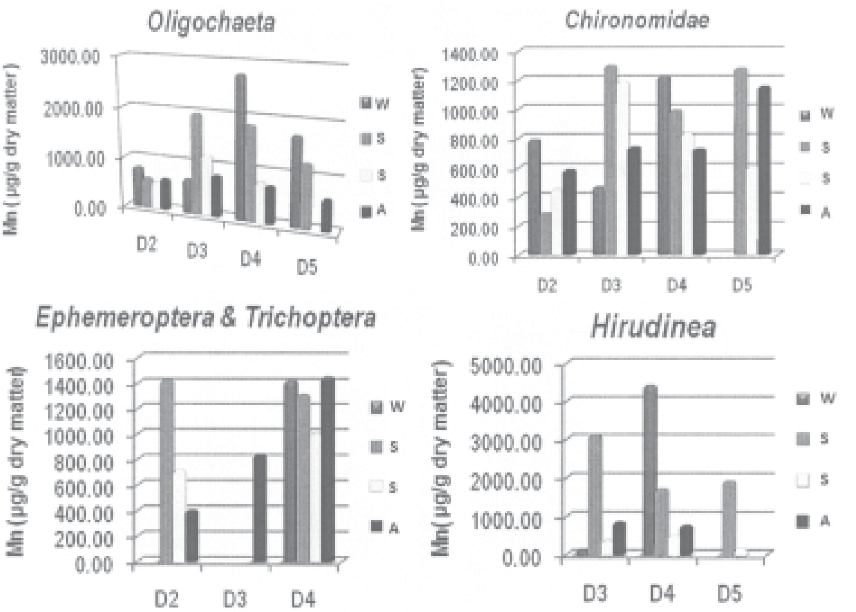

Fig. 8. Seasonal averages of Mn concentrations (µg/g dry matter) for main taxa, in Dâmbovnic River stations, for 2008-2010

system of plants increase from the upstream to the downstream for Typha and Potamogeton species, with the difference that the latter has a maximum in station D3. The absence of these species in some stations make, however, difficult the data interpretation. For Typha sp., the variation of manganese concentration in root follows the trend recorded for its dynamics in sediment.

Bioconcentration factor of manganese in root calculated for analyzed common species is above par and reaches the highest values at station D3 (fig. 2). Mn and Cd are the metals for which was revealed the bioconcentration process in roots.

As in the case of cadmium and chromium, the transfer factor of manganese from the roots to the upper plant parts is below par, except Potamogeton species, for which was obtained an above par transfer factor in all stations where it was sampled. (table 3). This species is the only one, moreover, who also presented above par transfer factors for the other metals analyzed, but only in station D4.

Tissue concentrations of Mn determined for floating/ submerged plant species (Cladophora and Lemna minor) have the highest values of all species in the catchment (fig. 3), the order of mg/g dry matter. If we calculate the ratio of tissue concentrations of manganese and those from water, it is found that the bioconcentration factor of manganese for aquatic submerged and floating plant species is considerable, the tens and hundreds (fig. 4). These data highlight the special ability of taking the metal from water by Cladophora species.

Macroinvertebrates

As aquatic plant species, for animal tissue, Mn concentrations are much higher than the other metals (Cd şi Cr), with orders of magnitude (fig. 8), due to the physiological role of this element, which is a constituent of metallo-enzymes and enzymes activator. Mn is found in high concentrations in tissues rich in mitochondria material, where it forms stable complexes with ATP and phosphate ion. Spatial dynamics of seasonal averages of tissues concentrations of benthic macroinvertebrates is characterized by the highest values in station D4, for all analyzed taxonomic groups. Station D4 (Roata) is the one which registered the highest values of Mn concentrations in sediments, which highlights the strong interaction between substrate (sediment) and biocenosis.

Bioconcentration factors calculating using the average values of tissues concentrations of Mn and its concentrations in sediments had values above par, as for Cd (fig. 6). This feature indicates, as for aquatic plants, the ability of macroinvertebrates to store this element. Bioconcentration factors are higher in station D3, similar to plant species anchored in sediments.

Conclusions

An overview of the results obtained so far allow us the assertion that the key factors influencing the behaviour of nutrients and heavy metals in the Dâmbovnic catchment (emission, transport, transformation) are both anthropogenic and natural nature and have different contribution, depending on type/nature of flows.

Another important retention process has been highlighted by this study and which also involves the compounds transformation is taking over of the heavy metals by primary producers. There have been highlighted the concentration process of heavy metals in aquatic plant roots.

The conversion of chemical compounds affect to a great extent the flows of heavy metals in the area. The most common transformation is the synthesis of organic compounds by primary producers.

Concentration of a compound/element, determined at a time in the tissues of a plant species, is the result of taking that compound by a plant for a longer time. Therefore, the concentration fluctuations of the compound/element recorded at relatively small intervals of time for the same species in a station are rather the result of individual variation, not of the temporal dynamics of the element, for a short time, in abiotic compartment which the plant interact with. It is obvious that for biotic compartments, the analysis of averages values of the compound/element sought is more meaningful than an exhaustive analysis of all data, unmediated.

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Manuscript received: 13.11.2012