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To cite this article: Ecaterina Marcu *et al* 2020 *IOP Conf. Ser.: Earth Environ. Sci.* **616** 012065

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Bioaccumulation of Heavy Metals in Perennial Wetland Vegetation Components from a Sector of the Arges River

Ecaterina Marcu¹, Irina-Elena Ciobotaru¹, Cristina Maria¹,
Alexandru Anton Ivanov¹, Iasmina-Florina Burlacu¹, Naimah Ibrahim²

¹National Institute for Research and Development in Environmental Protection,
Bucharest, Romania

²School of Environmental Engineering, Universiti Malaysia Perlis (UniMAP), Perlis,
Malaysia

E-mail: cristina.maria@incdpm.ro

Abstract. The potential bioaccumulation of hazardous transitional heavy metals in components of the aquatic environment (such as water column, sedimentary matter, plants) constitutes one of the concerns in environmental protection engineering. This experimental investigative study used systematic measurements of the presence of selected heavy metals (cadmium, chromium, copper, lead, nickel and zinc) in components of the aquatic environment to give an indication of their availability and their potential bioaccumulation in the perennial wetland plants. Through environmental quality data processing, this study allowed both the assessment of interphase partitioning constants of these metals throughout the analyzed aquatic environment and the estimation of their cumulative toxicity coefficient. The data analysis highlighted the different potential degrees of dangers caused by metals as pollutants and the synergistic way in which they act on the aquatic environment.

1. Introduction

In later years, more and more attention is given to the assessment of anthropic pollutants in the environment. One of the types of pollutants often detected in aquatic ecosystems is the heavy metals group. These metals have various properties being subjected to different partitioning typologies between the biotic and abiotic components of the aquatic ecosystem, some being actively adsorbed and even concentrated in the biotic components [1]. The accumulation of such metals in plants can also be transferred toward herbivores and insects consuming the plant mass and being later consumed by other superior niche species from the general ecosystem thus potentially propagating higher levels of heavy metals. Not all heavy metals pose a risk at low concentrations, some such as Zn, Cu, Cr being, in low levels, essential to the metabolism of plant and animal life, but at higher levels disrupt the same metabolism functions. Other heavy metals such as Pb and Cd are toxic toward life even at relative low values [2]. This study target area was constituted on Arges River where previous investigations showed a general good status with a few exceptions for general water quality [3] and heavy metal levels in fish tissue [1, 2].

This study takes into account heavy metals levels determined for water, sediment and plant tissues in a sector of the Arges River. The Bioconcentration Factor (*BCF*) [4], Translocation Factor (*TF*) [5], Individual Toxicity Unit (*TU_i*) [6, 7], Mixt Toxicity Index (*MTI*) [8] and the Similarity Index (λ) [9] are few of the tools that may be employed to evaluate the obtained raw quality data for water,



sediment and plants. This data assessment tools provide an objective way to highlight potential environmental problems in the studied ecosystem.

2. Experimental

2.1. Sampling

The studied ecosystem is situated South of Bucharest on the Arges River in the area of 1Decembrie-Copaceni localities. 5 sampling points (depicted in figure 1) were selected roughly 500 m apart on a sector of about 2 km. Water was sampled in previously decontaminated borosilicate laboratory bottles and acidified to 0.5% (v/v) with concentrated Nitric Acid. Plant samples were collected and stored in new polyethylene bags. The sampled plants were members of the *Phragmites* genus, comprising the most spread local wetland perennial species. The sediments were sampled with a paddle and stored in plastic airtight containers. All samples were transported to the laboratory for processing in the same day.



Figure 1. General sampling area (44.267371 N; 26.069540 E).

2.2. Materials and methods

The metal content was determined with a High Resolution Continuous Source Atomic Absorption Spectrometer ContrAA 700 from Analytik Jena, using the flame module and oven modules were appropriate. The spectrometer was calibrated using CRMs and the dilutions were prepared using class A volumetric flasks and an Eppendorf Multipette E3x digital dispenser. The required ultra-pure water of 18.2 M Ω ·cm resistivity (at 25 °C) was obtained from a Millipore Simplicity UV water purification system.

The water samples for total metal content analysis were checked for a pH < 2 and stored at room temperature for 3 days to allow leaching of the metals from the traces of particulate matter present. The stabilized samples were analyzed according to current methods and practices.

Plant samples were rinsed in double-distilled water and partitioned into main parts such as roots, stem and leaves and separately dried, first at room temperature and then at 50°C in a Binder ED53 natural convection oven, until sufficiently brittle. The dried plant samples were then further fragmented by hand and milled with a Retsch RM200 electric grinder mill with a ceramic mortar and pestle until the samples were quantitatively passed through a 200 μ m sieve. The remnant humidity was measured with a Radwag Partner MA210R moisture analyzer and the powdered plant samples were subjected to digestion with concentrated nitric acid under reflux conditions for 2 hours at 150°C using a Velp Scientifica DK6 digester. The digested samples were filtered and quantitatively brought to volume in class A volumetric flasks for analysis.

Sediment samples were dried at room temperature and manually milled with a ceramic mortar and pestle to coarsely disintegrate them without crushing the small pebbles and stones. Then the samples

were granulometrically separated using a vibratory sieve shaker Fritsch Analysette 3 Spartan and incremental smaller mesh sieves. The sediment samples fraction $< 63 \mu\text{m}$ were collected, their remnant humidity determined, and subjected to microwave pressured digestion with *Aqua Regia* mixture of concentrated Nitric and Hydrochloric acids of 1:3 (v/v) ratio using a Berghof MWS-4 digestion oven with DAK 100 vessels with pressure monitoring caps. The main step of the temperature program comprises a plateau of 30 minutes at 200°C . The digested samples were filtered and quantitatively brought to volume in class A volumetric flasks for analysis.

All reagents were of adequate purity to ensure sufficiently low blank values for each analyzed element and applied technique.

2.3. Experimental data processing based on theoretical models

Heavy metals are an important category of hazardous pollutants omnipresent in the environment. Characterized by a high chemical and biological stability, heavy metals can not only change their chemical species (valence) but also their character nature (organic or inorganic). The main issues associated with the persistence of heavy metals in the environment are the bioaccumulation/bioconcentration capacity and the cumulative toxic effects manifested on the ecosystem components.

The bioaccumulation/bioconcentration capacity depends on the heavy metals concentrations present in the aquatic environment, on the chemical properties of these metals, on the biota exposure time to these concentrations, on the diffusion rates in different parts of the plant, on the partition coefficients of the metals ions in the water-sediment-biota system. The bioaccumulation/bioconcentration factor (*BCF*) or the heavy metal partition constant between receptor and donor was estimated by means of the ratio of the equilibrium concentrations of the contaminant in the aquatic organism / plant and water / sediments [4]:

$$BCF = \frac{C_{i,aquatic\ organism/ plant}}{C_{i,water/sediment}} \quad (1)$$

Heavy metal transfer phenomena between the component parts of an organism / plant were estimated based on the translocation factor (*TF*) [5]:

$$TF = \frac{BCF_{stem/leaf}}{BCF_{root}} \quad (2)$$

The individual toxicity of metals has been intensively studied over time, but the results of these studies are limited and sometimes incorrect when extrapolated to a complex ecosystem where there is a mixture of toxic pollutants. Due to such a reason, several rules and cumulative indices have been developed to characterize the joint toxic effect of a mixture of environmental pollutants [6]. In this study, three of these evaluation concepts were assessed, namely: individual unit toxicity (*TU_i*), mixture toxicity index (*MTI*) and compound similarity index (λ).

The individual unit of toxicity of component *i* of a mixture

$$TU_i = \frac{C_i}{EC_{50,i}} \quad (3)$$

Where: C_i is the concentration of component *i* in a mixture of *n* compounds; $EC_{50,i}$ is the concentration of pollutant *i* which causes a negative and observable effect in 50% of the organisms tested.

The toxicity index of a mixture of pollutants [8] can be evaluated using the following relationship:

$$MTI = 1 - \frac{\log M}{\log M_0} \quad (4)$$

Where the parameters M and M_0 are calculated with relations (5) and (6):

$$M = \sum_{i=1}^n TU_i \quad (5)$$

$$M_0 = \frac{M}{TU_{i,\max}} \quad (6)$$

The similarity index of the pollutants (λ), used for the evaluation of the cumulative toxicity effects of the pollutant mixtures [9], can be evaluated using the following relationship:

$$\sum_{i=1}^n (TU_i)^{1/\lambda} = 1 \quad (7)$$

All the data concerning bioaccumulation/bioconcentration factors (BCF), translocation factors (TF), individual units of toxicity (TU_i), mixture toxicity index (MTI) and metal similarity index (λ) were processed using Microsoft Office Excel and are presented in graphical or tabular form.

3. Results and Discussions

3.1. Bioconcentration Factors (BCF)

The bioconcentration factors (BCF) are defined by the heavy metals concentrations measured in different parts of the plant (root, stem, and leaves) divided by the heavy metals concentrations measured in the water, under quasi-equilibrium conditions. The resulted $BCFs$ are presented in figure 2. The plot reveals that *Phragmites australis* accumulated large amounts of all the investigated metals. This result is proved by the fact that all the values of $\text{Log}_{10}(BCF)$ are greater than 2 [6]. The bioaccumulation degree decreases in the following order $Zn > Cd > Cr > Ni > Pb > Cu$.

The significant accumulation of metals in *Phragmites australis* is leading to the following conclusions: i) on the one hand, the selected parts of the plant can be good bio-indicators in assessing the metal pollution of the studied wetland and, on the other hand, ii) the fact that these metals are found in easily bio-assimilable forms in the studied area [10].

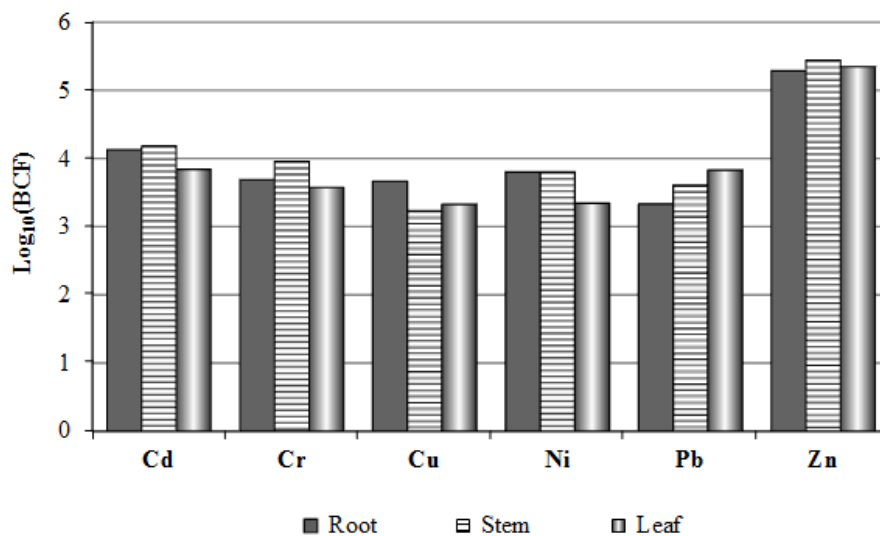


Figure 2. Variability of heavy metal bioconcentration factors BCF (in logarithmic terms) in different parts of the *Phragmites australis*.

Figure 3 reveals the following trend: Cu, Cd and Ni were accumulated in a higher percentage (38-52%) in roots; Cr, Ni and Zn were rather accumulated in stem (36-44%); Pb, Cd and Zn were accumulated in a significant percentage in leaves (34-46%). The heavy metals accumulation mechanism involves three stages: adsorption in the roots, transport and storage in the above ground parts of the plants [5].

It is also observed that the roots have a greater capacity to absorb/accumulate most of the heavy metals which means that *Phragmites australis* may help to mitigate the contaminated areas, and can reduce the negative effects on the biota living in wetlands. As a potential bio-remediation technique, care must be given to the periodical and partial removal of the contaminated plants to prevent the release of the accumulated metals back in the ecosystem through dead organic matter decomposition processes.

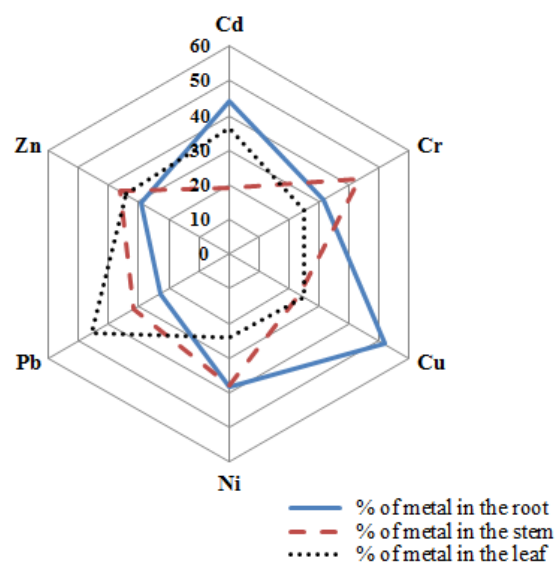


Figure 3. Predominant accumulations of heavy metals in the different parts of *Phragmites australis*.

3.2. Translocation Factors (TF)

For a certain metal, the values of the “translocation” or “transfer” factors (*TF*), for radicular (via the roots) transfer and for foliar (via the leaves) transfer, are obtained by division of the stem-*BCF*, or of the leaf-*BCF* to the root-*BCF*. The obtained results are presented in figure 4. As the plot reveals, the metals are irregularly distributed in the plant, the metals diffusion in the plant being strongly depend on the nature of the metal [11].

Phragmites australis shows the translocation capability of metals from the roots rather to the stem and less towards leaves. The metal translocation (migration) from the roots to the stem is significant for Cr, Ni, and Zn ($TF = 1.5-2.1$), while metal migration from the roots to the leaves is significant only for Pb ($TF = 3.2$). The low *TFs* (lower than 1) for Cd and Cu could indicate that *Phragmites australis* does not retain these metals in the roots but uses them for its internal metabolic processes [10].

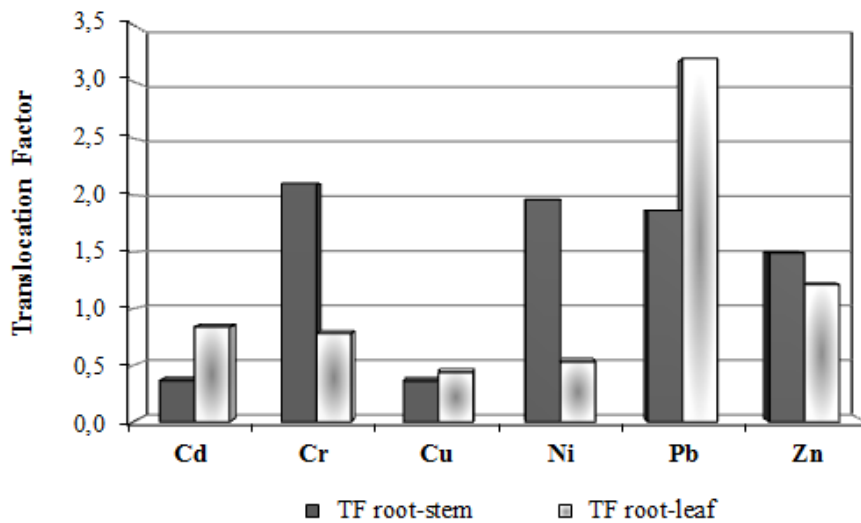


Figure 4. Variability of heavy metals translocation factors from the root toward the other parts of *Phragmites australis* (located above the ground).

3.3. Indices to evaluate the cumulative toxicity of the heavy metals mixture

In order to assess the toxicity of complex mixtures of heavy metals on the aquatic biota, and to identify the possible interactions among pollutants, three indices from literature have been used. These indices of cumulative toxicity allow evaluating in a simple yet intuitive way the heavy metals concomitant action against a common receptor in the aquatic environment (which is, in our study, *Phragmites australis*).

3.3.1. The individual and the cumulative toxicity index (M). The obtained values of individual toxicity indices (TU_i) are presented in table 1 for every metal “ i ”. They have been evaluated by dividing the metal “ i ” average concentration in the water (C_i), to the ($EC_{50,i}$) of that metal. EC_{50} index is known in the literature as being the concentration of a specific metal (pollutant) which causes inhibition of the biological activity of 50% of the members of a tested population. The evaluated individual toxicity indices (TU_i) are presented in table 1 and were calculated by dividing the average metal concentration i measured in water (C_i), to the specific concentration of the same metal that causes inhibition of biological activity in proportion of 50% when acts individually (EC_{50}).

All (TU_i) are much lower than 1, which indicates that the metal concentrations in the water have not reached the maximum dangerous threshold. If the cumulative toxicity index (M) is evaluated using the (TU_i) indices with the relation (5), the resulted value is $M = 0.07$ (table 1). Such a lower than 1 value indicated that the considered heavy metals present in the investigated environment have a synergistic action on the analyzed receptor (*Phragmites australis*) [6].

3.3.2. Toxicity index of the pollutant mixture (MTI). The evaluated indices in table 1 allow to estimate another cumulative toxicity index (MTI), using the relation (4). The resulted value is $MTI = 8.05 > 1$. As suggested in the literature [12], the obtained supra-unitary value for MTI re-confirms the synergistic action of the heavy metals present in the investigated environment. The synergistic action of the mixture of pollutants (heavy metals in this case) means that the cumulative toxic effect of the heavy metals present in the environment is greater than the sum of their individual toxicities.

Table 1. Individual and cumulative toxicity indices of the analyzed heavy metals.

Heavy metal Index “i”	Average concentration in water (C_i) ($\mu\text{g/L}$)	$EC_{50,i}$ ($\mu\text{g/L}$)	Individual toxicity index (TU_i)	Cumulative toxicity index (M)
Cd	0.028	18	0.0016	0.071
Cr	0.627	1200	0.0005	
Cu	1.850	5000	0.0004	
Ni	0.984	750	0.0013	
Pb	0.535	29	0.0185	
Zn	0.239	4.9	0.0488	

3.3.3. *The similarity index of the pollutants (λ)*. Another important toxicity index that characterizes a mixture of pollutants is the so-called “the similarity index of the pollutants (λ)” [9]. This cumulative toxicity index can be estimated by solving the non-linear equation (7), using the values from Table 1. The resulted value is $\lambda = 0.06$. According to the above-mentioned literature, such a value fits in the range of ($0 < \lambda < 1$), thus indicating an independent action mechanism of the analyzed heavy metals.

4. Conclusions

The present studies performed in the aquatic environment by using *Phragmites australis* as a test organism, have shown that this plant has the ability to bio-accumulate all the studied heavy metals. Consequently, one can conclude that this plant may be a good candidate for the bio-remediation of contaminated wetlands, and can actively contribute to prevent the bio-accumulation of heavy metals in the aquatic environment [10, 11]. The accumulation potential of the studied metals decreases in the following order: Zn > Cd > Cr > Ni > Pb > Cu. The present analysis of individual and cumulative toxicity of the analyzed heavy metals present in the studied wetland pointed-out the synergistic behavior of these pollutants. That is, the cumulative toxic effect of the heavy metals present in the environment is greater than the sum of their individual toxicities. Moreover, according to the similarity index of pollutants (λ), the analyzed heavy metals present an independent action mechanism on the aquatic receptor.

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