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The content of microelements (Cu and Zn) in reeds (*Phragmites australis* (Cav.) Trin. ex Steud.) of a constructed wetland system

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ABSTRACT

The method for purification of municipal waste waters with the use of constructed wetland system (CWS) was put in operation in Serbia for the first time in 2004, in the Gložan village near Novi Sad. Biological factors in this anthropogenic ecosystem are microorganisms and reeds (*Phragmites communis* (Cav.) Trin. ex Steud.). In the process of bioaccumulation, among other substances, emergent plants often accumulate large quantities of microelements. Although microelements are present in plants in relatively small quantities, in emergent plants that grow on the surface where wastewater (which contains various substances, including microelements) flows, their quantity in plant dry weight can be higher. This paper presents the results of the three-year study (2004–2006) of Cu and Zn concentration in different organs of reed plants (leaf, stem, rhizome with root and inflorescence) grown in the “Gložan” CWS. The study was carried out on three fields. The first year of the study was taken as control, as that’s when the “Gložan” CWS was established, while 2005 and 2006 were the first and the second years of the system exploitation. In 2004 Cu concentration varied from 3.20 ppm in reed stems to 5.30 ppm in inflorescences. In 2005 Cu concentrations were lower in comparison to the first year, while in the third year the trend of reduction of Cu concentration was present in all analyzed organs in the third field. Concentration of Cu had diminishing tendency in all three fields over time, with exception of inflorescences where it was mostly constant.

In the first year of the study the highest concentrations of Zn were recorded, ranging from 13.97 ppm in rhizomes with roots to 34.60 ppm in inflorescences. In the second year, concentration of Zn was the lowest in all three fields, and in the third year overall concentration of Zn (except for inflorescences) was lower in comparison to the first year of the study (control).

Key words: reed, bioaccumulation, essential microelements, Cu, Zn, constructed wetland system.

Aquatic and wetland plants inhabit a variety of aquatic and wetland ecosystems. Due to their morphological and physiological specificities they can have very positive impact on the functioning of an ecosystem in which they develop. Thus, these plants represent the basis of the primary production of organic matter in shallow aquatic and wetland ecosystems, the development of which depends mainly on the climatic conditions and trophic state of a given ecosystem [Westlake, 1975]. The number and composition of these plants can serve as good bio-indicators of the condition and quality of a given ecosystem [Ghetti, Ravera, 1993; Eglin et al., 1997]. Furthermore, emergent plants have significant anti-thermal effect due to their tall stalks [Krotkevič, 1982] and allelopathic effect as well [Gopal, Goel, 1993]. Therefore these plants form very pure stands in aquatic and wetland ecosystems. However, in terms of modern ecological approaches to environmental protection, the most important feature of these plants is their high potential for uptake, accumulation and transformation of various substances from water, which allows them to be very successful in phytoremediation of aquatic ecosystems [Manny et al., 1991; Weise et al., 1992; Brix, 1994a, b, c; Yurukova, Kochev, 1994; Lewis, 1995; Nikolić et al., 2003, 2014; Ivanova et al., 2012]. Thanks to these capacities, aquatic and wetland plants are nowadays successfully used in artificial systems, defined as constructed wetland systems (CWS), where specific plant species are used for the treatment of wastewaters of different origins [Reddy, De Busk, 1987; Nuttall et al., 1997; Lakatoš, 1998; Cortes-Esquivel et al., 2012; Bragato et al., 2009; Maddison et al., 2009; Bonanno, Giudice, 2010; Zhao et al., 2013]. Phytoaccumulation of metals depends on a number of factors, such as temperature, pH, concentration of ions dissolved in the water, seasonal weather conditions, the nature of a metal and the plant species and the plant's specific morphological and anatomical features [Kadlec, Reddy, 2001; Duman et al., 2007; Drzewiecka et al., 2010; Cortes-Esquivel et al., 2012].

In this paper, the uptake of micronutrients, Cu and Zn, from municipal wastewater at the "Gložan" CWS was studied, as well as the accumulation and distribution of these two elements in different parts of reeds (*Phragmites australis* (Cav.) Trin ex Steud.). The results were expected to point out the level of effectiveness of the "Gložan" CWS and to analyze reed as the main bioaccumulator species of Cu and Zn.

MATERIALS AND METHODS

The "Gložan" CWS comprises three fields with a total area of 9400 m^2. The CWS is the horizontal sub-surface flow type. The substrate consists of gravel strips 0.6 m wide and 0.6 m thick, which alternate with strips of natural soil 1.0 m wide. The surface layer is gravel mixed with soil and planted with reeds. The lining is the impermeable clay layer that underlies the field. Wastewater purification proceeds through all three fields in a row. In Field 1 the wastewater remains for 24 hours, in Field 2 – for 48 hours and in Field 3 – for 33 hours. The technological process comprises collection and transportation of wastewater, purification in the CWS and discharge of the purified water into the channel which empties into the Danube [Josimov-Dunđerski et al., 2011].

The plant material was collected from 2004 to 2006 in September, at the end of the plants' physiological activity. Five specimens of plants were collected from each of the fields; roots, stems and inflorescences were separated. Concentrations of Cu and Zn were determined by flame atomic absorption spectrophotometry (AAS SHIMADZU AA-6300), after ashing the plant material at t = 500 °C and dissolving it in deionized hot water in the presence of 0.25 M HCl.

In order to determine whether the differences in the concentration of Cu and Zn between the analyzed plant parts, CWS fields and examined years are statistically significant, an analysis of variance (ANOVA) and least significant difference test at significance levels of 0.05 and 0.01 were performed, using STATISTICA 12.0 software.

RESULTS AND DISCUSSION

Plants are characterized by specific chemical composition and ratios of macro- and micronutrients. Likewise, reed, one of emergent macrophytes, has specific chemical composition. Moreover, due to its particular morphoanatomical characteristics, it can accumulate higher concentrations of certain elements in its tissues, if exposed to excessive concentrations of mineral elements in available forms, dissolved in water. Reed is the species suitable for phytoremediation because it grows rapidly, produces large biomass and can accumulate significant amounts of pollutants from the ecosystem [Nikolić 2007; Bragato et al., 2009; Maddison et al., 2009; Bonanno, Giudice, 2010; Zhao et al., 2013]. A large number of microe-

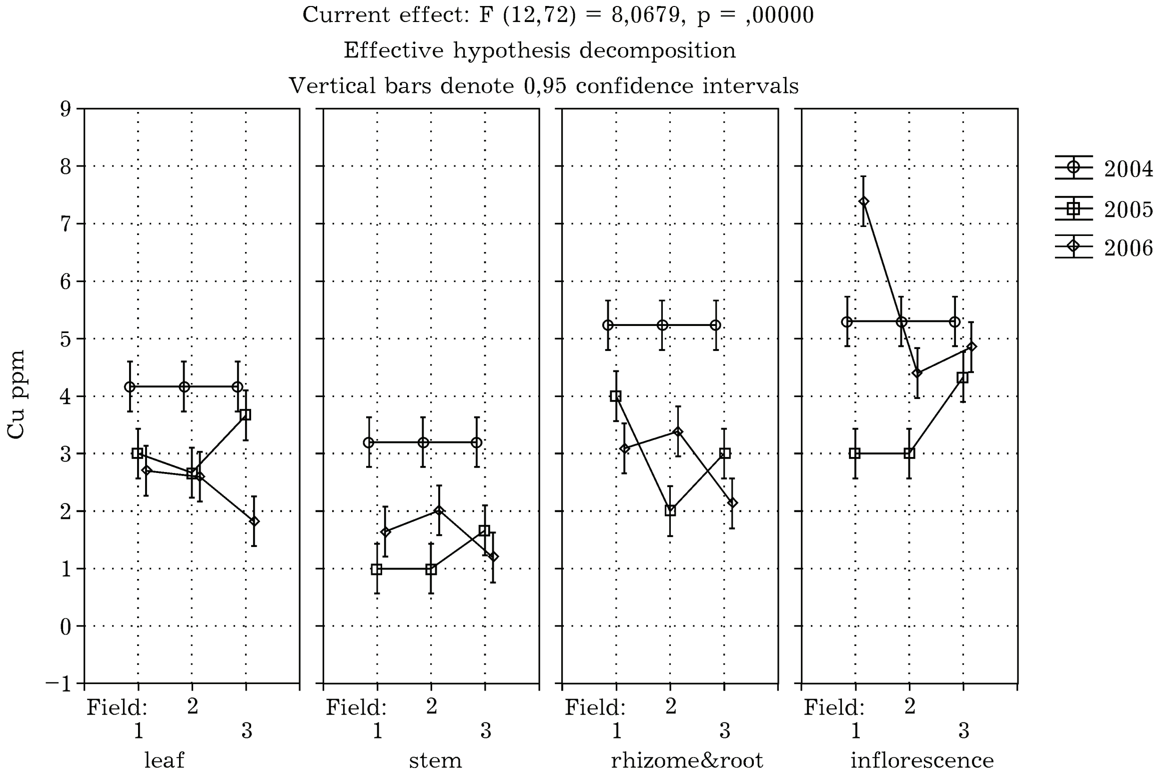

Fig. 1. Average concentrations of Cu in *P. australis* of the "Gložan" CWS (2004–2006)

lements, which belong to the group of heavy metals, are essential for the plants. Nonetheless, when they are present in excessive concentrations in plant tissues, particularly in the form of free ions, they may produce a toxic effect on the plant itself and also on animals and people [Kabata-Pendias, 2010]. It is therefore very important to monitor and identify contents of trace elements in plants and their circulation in the food chain in the soil-plant-animal system, both from the environmental and economic points of view [Kastori, Maksimović, 2008].

The content and distribution of essential microelements Cu and Zn in various organs of reeds, grown on the constructed wetland system "Gložan", which was designed for treatment of municipal wastewaters of the Gložan settlement, was analyzed in this paper.

In dry plant tissues average concentration of Cu is about 6 ppm [Epstein, 1972, 1999], and in reed of the "Gložan" CWS it was somewhat lower (Fig. 1). The lowest concentration of Cu was recorded in reed stalks in all three analyzed years (1.00–3.20 ppm), whereas the highest concentrations were recorded in inflorescences (3.00–7.39 ppm) (Table 1, Fig. 1). Similar results with respect to Cu concentration in reed were published by L. Yurukova and K. Ko-

Table 1

Concentration of Cu in *P. australis* of the CWS "Gložan" (2004–2006)

Cu (ppm)	2004	2005			2006		
		Fields					
		1	2	3	1	2	3
Leaf	4.17	3.00	2.67	3.67	2.70	2.60	1.82
Stem	3.20	1.00	1.00	1.67	1.65	2.01	1.19
Root & rhizome	5.23	4.00	2.00	3.00	3.09	3.39	2.13
Inflorescence	5.30	3.00	3.00	4.33	7.39	4.40	4.85

Table 2

Concentration of Zn in *P. australis* of the CWS "Gložan" (2004–2006)

Zn (ppm)	2004	2005			2006		
		Fields					
		1	2	3	1	2	3
Leaf	15.50	7.67	5.00	5.33	13.45	9.28	8.17
Stem	17.57	1.33	0.00	0.00	4.30	6.37	4.61
Root & rhizome	13.97	4.67	2.33	1.67	10.04	9.13	6.88
Inflorescence	34.60	3.00	10.00	16.00	33.86	35.33	22.53

chev [1994] and Lj. Radak [1995], who found slightly higher concentration of Cu (17.50 mg/kg) in rhizomes and roots of reed from a fishpond. C. Bragato et al. [2009] and G. Bonanno and R. Lo Giudice [2010], however, found much higher concentrations of Cu in roots, rhizomes, leaves and stalks of reed. Unfortunately, none of the aforementioned authors studied concentration of Cu in inflorescences, which in this experiment (except for the plants from the first field in the first year of exploitation of the CWS) was higher than in other tissues. Differences in the concentration of Cu in different organs of reed were statistically significant between Fields 1 and 2 and between Fields 1 and 3, but not between Fields 2 and 3. Such relationship and concentration of Cu in particular organs of reed suggests that municipal waste water that flows into the system is not burdened with excessive Cu. But, nevertheless, the declining trend of Cu concentration in particular organs (except for inflorescences) during the years of investigation, going from the first to the third field, indicates that the "Gložan" CWS effectively retains Cu. However, it has to be stressed that high concentration of Cu in inflorescences is probably the consequence of air pollution. This assumption is supported by the fact that plants, in certain climatic and soil conditions, can indeed uptake and accumulate Cu by their aerial organs [Kastori, Petrović, 1993]. Lj. Radak [1995] found that the concentration of Cu in inflorescences was 33 % lower than in the leaves and stems, whereas in the present experiment it was in average 56 % higher than in the leaves and as much as 2.5 times higher than in the stems. It should be noted that the results of other authors most often refer to the concentration of Cu in the entire shoot, and to phenophases prior to flowering [Bonanno, Lo Giudice, 2010; Bragato et al., 2009]. If, however, concentrations of Cu detected in particular above-ground parts of other plant species are compared to the concentrations of Cu found in inflorescences of reed grown at the "Gložan" CWS, than the number won't be that high. For example, fruit of pepper may contain over 22 mg Cu/kg; fruit of pear and seeds of cumin – about 13.8 mg Cu/kg; apricot and fig fruits – 5 and 2.3 mg Cu/kg, respectively [Kastori et al., 2012].

In dry plant tissues average concentration of Zn is about 20 ppm [Epstein, 1972, 1999], and in the reeds of the "Gložan" CWS it was, except for inflorescences, somewhat lower (Table 2, Fig. 2). The highest concentrations of Zn were found in the first year of the experiment and they ranged from 15.5 in leaves to 34.6 ppm in inflorescences. Similar values were recorded by L. Yurukova and K. Kochev [1994] and somewhat higher values by C. Bragato et al. [2009] and G. Bonanno and R. Lo Giudice [2010]. In the next two years of exploitation of the "Gložan" CWS, the decline in the concentration of Zn in dry matter was noted, though it was less pronounced in inflorescences. Similar relationship between concentrations of Zn was found by Lj. Radak [1995] in the reeds from a fishpond. R. Kastori et al. [2012] reported that in the above-ground organs of other plant species concentration of Zn may be quite high. For example, cucumber fruit contained 23.5 mg Zn/kg, pear and apricot fruits – 13.5 and 11.5 mg Zn/kg respectively, and cumin – as much as 44 mg Zn/kg of dry matter. Moreover, statistically significant differences were found with respect to the concentration of Zn between all three years of

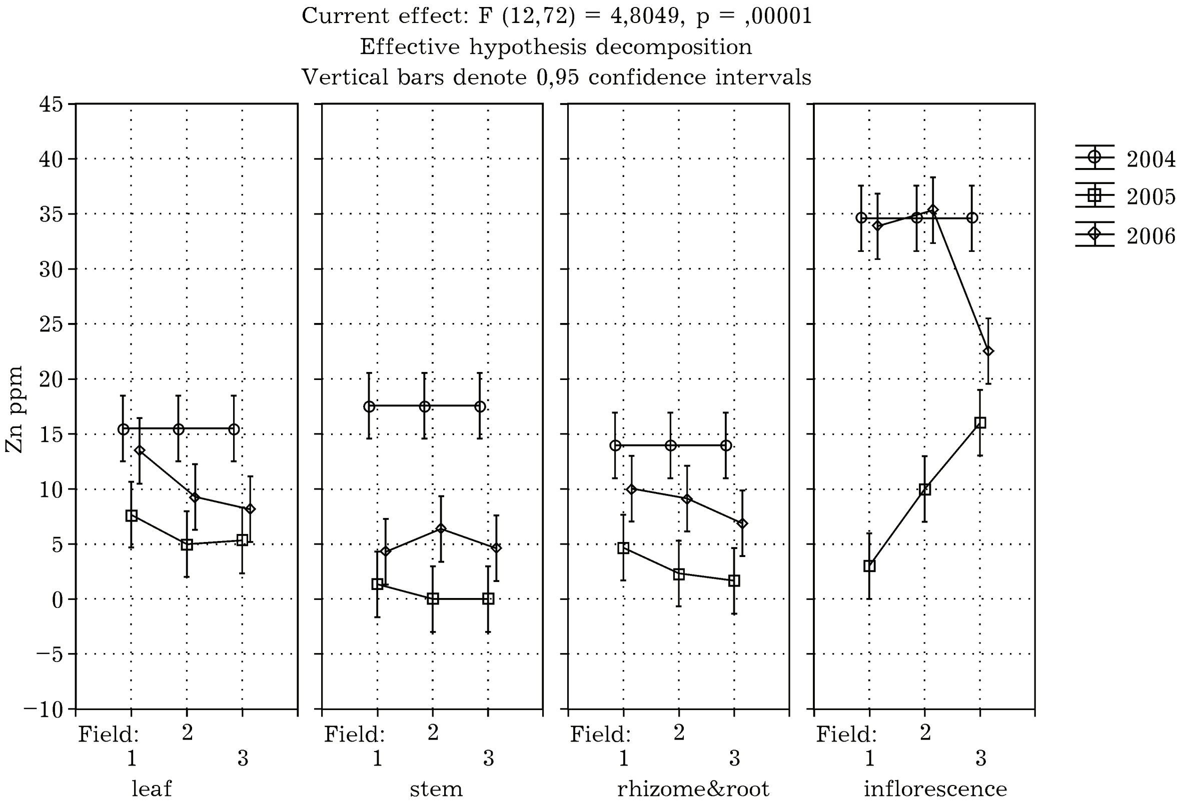

Fig. 2. Average concentrations of Zn in *P. australis* of the "Gložan" CWS (2004–2006)

the experiment, as well as between particular organs, except stems and rhizomes, among which concentrations of Zn were not statistically different. Similarly, there were no statistically significant differences in Zn concentration between the reed organs from different fields of the "Gložan" CWS.

Even thought Cu and Zn are the essential elements for plants, for they are components of many enzymes which take part in vital processes, in excessive concentrations they may exhibit toxic effect on plants. Through plants, these elements may directly enter the food chain or, by casting process of urban waste waters into larger recipients, accumulate in aquatic organisms and enter the food chain indirectly. Our results, based on monitoring of Cu and Zn concentration in reeds on different fields of the "Gložan" CWS over three years, showed that concentrations of these two elements are relatively low. This suggests that municipal wastewaters of Gložan settlement are probably not heavily burdened with Cu and Zn. However, the "Gložan" CWS is efficient in removal of these pollutants, since Cu and Zn concentrations showed a significant declining trend during our study, particularly in vegetative parts of the plants. Somewhat higher content of these metals in inflorescences of reeds which are at a height of 3–4 m can be explained by accidental displacement of air filled with these pollutants from the surrounding roads and agricultural areas. In such areas byproducts of petrol combustion and components of various preparations of pesticides are present in the air; they often contain a variety of metals, including Cu, which has the fungicidal effect [Kastori, Petrović, 1993]. Reeds from the "Gložan" CWS, however, do not contain toxic levels of Cu and Zn, so with respect to the concentration of these two elements, further exploitation of these reeds remains unlimited.

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