

UPTAKE OF SELECTED HEAVY METALS AND THEIR EFFECTS ON SOME PHYSIOLOGIC PARAMETERS AND MINERAL NUTRITION IN *PHRAGMITES AUSTRALIS* IN KARASU RIVER-TURKEY

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ABSTRACT

Water contamination by heavy metals is a serious global problem due to increased environmental and health-related issues. In this study, we have comparatively investigated the heavy metal concentrations and mineral nutrient levels in Karasu river sediments and in plants samples of *Phragmites australis* (Cav.) in order to assess the biomonitoring capacity. Plant and sediment samples were collected from four different localities along Karasu River, and those samples were analyzed in terms of heavy metals such as Fe, Zn, Mn, Ni, Cr, Cu, Pb, Co and Cd, and in terms of mineral elements such as K, Ca, Mg, Na, Al, and B using ICP-OES. In addition, lipid peroxidation, chlorophyll and carotenoid levels in plant samples were also checked. Sediments in Karasu River contained high concentrations of Ni and Cr metals. Moreover, *P. australis* showed hyper-accumulation for Cd and Zn in root and leaf samples, respectively. Although there was no significant difference in chlorophyll amounts among locations, lipid peroxidation was found to be high in samples taken from İliç.

Keywords: Chlorophyll; Lipid peroxidation; Heavy Metals; Sediment; Phragmites australis

1. Introduction

In recent years, water contamination by heavy metals has become a serious global problem due to increased environmental and health-related issues. The amount of heavy metals in aquatic ecosystems has significantly increased related to anthropogenic and industrial activities (Akguc *et al.*, 2010). Heavy metals do not decrease autogenously, but to accumulate in aquatic micro and macrophytes, and enter into the food chain (Dirilgen, 2011). Many sensitive aquatic vascular plants accumulate metal ions, therefore, they are recognized as biomonitors for metals (Fargasova, 2001; Dhir *et al.*, 2009; Dirilgen, 2011). High concentrations of pollutants have direct and indirect effects on aquatic ecosystems. Heavy metals are one of the main pollutants and moreover, they are both accumulative and highly mobile (Monferrán, 2012). Metals contaminate ecosystems through domestic, industrial and agricultural activities (Demirezen *et al.*, 2007; Yasar *et al.*, 2010; Monferrán, 2012). Using biological materials in environmental cleaning is a quite cheap and reliable method of decontamination. Biomonitors are organisms that provide quantitative information on environmental pollution levels (Markert *et al.*, 2003; Akguc *et al.*, 2008; Aksoy *et al.*, 2012).

P. australis is a rather common macrophyte in aquatic ecosystems. Biomonitoring studies have demonstrated that *P. australis* has a bioaccumulation capacity for trace elements (Lesage *et al.*, 2007;

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Vymazal *et al.*, 2007; Fawzy *et al.*, 2012; Bonanno, 2012). Heavy metals such as Zn, Cu and Ni are trace elements and they play important roles in plant growth and development. However, high concentrations are toxic to the plants (Rengel, 2004; Bragato *et al.*, 2009). On the other hand, dispensable elements such as Cd, Cr, and Pb, could have toxic effects on plants even at low concentrations (Kabata-Pendias and H. Pendias, 1992; Zayed and Terry, 2003). Decontamination of areas with medium levels of contamination is effective and reasonable (Bose, 2008). Effective improvement methods have also been developed for the less contaminated areas (Weis and Weis, 2004; Bose, 2008). Contrary to organic pollutants, decontamination of heavy metals requires extremely expensive methods. Therefore, using plants that have phytoremediation properties stand as potential means to reduce the decontamination costs (Lasat, 2002; Ghosh and Singh, 2005; Bragato, 2009). Macrophytes are common plant species that serve as biomonitors (Demirezen and Aksoy, 2006; Vymazal *et al.*, 2007; Bonanno 2012). Phragmites australis (common reed) is one of the most common macrophytes in aquatic ecosystems, and also several studies have proved its capacity of trace element bioaccumulation (Duman *et al.*, 2007; Bragato *et al.*, 2009; Maddison *et al.*, 2009)

In this study, we have comparatively investigated the heavy metal concentrations and mineral nutrient levels in Karasu river sediments and in *P. australis* plant samples to assess the biomonitoring capacity of *P. australis*. In addition, some physiological features such as lipid peroxidation and chlorophyll amounts of *P. australis* collected from different locations were comparatively analyzed.

2. Materials and methods

2.1 Plant ecology and morphology

P. australis is common in lakes, rivers, swamps, ditches and on the coasts. It can be found approximately at maximum height of 2400 meters. It is a perennial plant with one stem of about 3 meters. The plumes are like hair shape and thick on the ligula. The height of a plume is 0.5-1 mm. The leaf is knife-shaped on the base and approximately 3 cm wide, and reaches up to 60 cm long. Panikul is almost 40 cm and there are more feathers on the base. Each spike includes 3–6 flowers (Davis, 1965).

P. australis, known as common reed, is a kind of perennial grass living in the lakes and rivers or brackish wetlands such as marshes across the temperate and tropical regions all over the world. It belongs to Poaceae family and is included among the most common species of the Phragmites genus (Bonanno, 2011). These species prefer eutrophic and stagnating waters and can tolerate to a moderate level of salinity It is a rhizomatous hemicryptophyte/geophyte and creates large areas known as reed beds that provide microhabitats for many birds and mammals (Bonanno, 2011)

2.2 Study location

The River Euphrates has the highest water potential in Turkey. It originates from Eastern Anatolia of Turkey and joins into Karasu and Murat Rivers. The River Murat joins in Euphrates at 720 km away from its source, whereas River Karasu joins at 460 km away from its source. The total length of Euphrates is 2.800 km, of which 1.263 km are within Turkey. After leaving the borders of Turkey and joining with River Dicle, Euphrates merges into the Basra Gulf (Anonymous, 2012). The River Karasu goes through Tercan, Erzincan, Kemah and İliç, which are located in Eastern Anatolian of Turkey.

2.3 Sample collection and analysis

Plant and sediment samples have been collected from these places where River Karasu flows during July-August 2012 (Fig. 1). Plant samples were divided into root, stem and leaf. Sediment samples were air-dried on floor. Plant materials were oven dried at 80 °C for 24 h, milled in a micro-hammer cutter and then sieved. After each milling, to protect plant sample from microbial decomposition, mortar was cleaned with ethyl alcohol and distilled water. To ensure the uniform distribution of metals in sample, materials were milled in a micro-hammer cutter, sieved through a 1.5-mm sieve and kept in clean polyethylene bags (Markert, 1993; Osma *et al.*, 2012). Lipid peroxidation level was measured using

Heath and Packer method (1968) with slight modifications (Ananieva *et al.*, 2002). 0.5 g leaf sample was homogenized in 3 ml 0.1% TCA and centrifuged at 15000 × g for 30 min at 4 °C. To 0.5 ml aliquot of the supernatant, 1 ml reagent (0.5% thiobarbituric acid (TBA) in 20% TCA, w/v) was added. For a negative control, 0.5 mL 0.1% TCA and 1 ml reagent were added. The test-tubes were heated at 95 °C for 30 min and then quickly cooled in an ice bath. After cooling and centrifugation to give a clear supernatant, absorbance of supernatant was read at 532 nm and value for the non-specific absorption at 600 nm was subtracted. The level of malondialdehyde (MDA) was estimated by using the mmol/L extinction coefficient of 155 mmol/L⁻¹ cm⁻¹ (Mutlu *et al.*, 2011, 2012).



Figure 1. Location of study area

Total chlorophyll and carotenoid content in fresh leaves were estimated using Lichtenthaler and Buschmann method (2001). 0.5 g fresh leaf tissue was ground in a mortar containing 5 ml acetone (80%). The optical density of solution was read at 662 and 645 nm (chlorophyll) and 470 nm (carotenoids). Photosynthetic pigments were expressed as mg/g⁻¹ FW (Erdal2012).

Plant samples were then added to 8 ml 65% HNO₃ (Merck), soil samples were added to 5 ml 65% HNO₃, 3 ml 37% HCI and 2 ml 48% HF. Teflon plates were closed, then placed in a microwave oven (Berghof - MWS2). The heat of the microwave was gradually increased to 175 °C and held for 20 minutes. Then, the Teflon plates were filtered with Whattman filters into 50-ml sterile tubes and were added up to 50 ml by using ultra-pure water. Finally, these samples were made ready for element analysis with ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy – Perkin Elmer-Optima 7000 DV). Before analyzing, standards were prepared using 1000 ppm multi-element solution (Merck). Elemental analysis was conducted with ICP-OES (Aksoy and Ozturk, 1996; Osma *et al.*, 2012).

2.4. Data analysis

Statistical comparisons were significant at \leq 0.05. To determine the standard deviation, ANOVA test were used. Tukey HSD tests were used to determine differences in multiple comparison test. In addition, correlations were examined between lipid peroxidation in plant samples (Osma *et al.*, 2012).

3. Results and discussion

In the present study, we have analyzed the concentrations of heavy metals and mineral elements in sediment and in *P. australis* samples collected from along the Karasu River. In addition, we have analyzed the lipid peroxidation, chlorophyll and carotenoid levels in plant samples. In this study, nine heavy metals and six mineral elements, in total, 15 metals were analyzed. The heavy metal concentrations in sediment and plant samples showed following sequence:

In sediment: Fe > Mn > Ni > Cr > Zn > Co > Cu > Pb > Cd;

In root: Zn > Fe > Mn > Ni > Cr > Cu > Pb > Co > Cd;

In stem and leaf: Fe > Zn > Mn > Ni > Cr > Cu > Pb > Co > Cd (Figure 2).

The concentrations of mineral elements in sediments and plants samples were ranked as follows:

In sediment: Al > Mg > Ca > K > Na > B;

In root, stem and leaf: K > Ca > Mg > Na > Al > B (Figure 2).

Statistical analysis showed the differences between element concentrations (Figure 2). While Al showed high concentration in sediment, its uptake by *P. australis* was quite low. Concentration of K in sediment was lower than that of plant parts, especially compared to the concentration in the leaf samples. Among mineral elements, Ca was the second element in plant samples after K.

There were differences in heavy metal concentrations between upper and lower sides of the river. In particular, sediment samples showed higher concentrations of Ni and Cr. Concentrations of Cd and Zn were higher in sediments than in the plant samples.

Varol (2011) reported higher Cd (1.2-4.9 μ gg⁻¹ dw), Mn (282-1,657 μ gg⁻¹ dw), Pb (62-566 μ gg⁻¹ dw) and Zn (123-2,396 μ gg⁻¹ dw) levels in sediment, but Ni, Cr and Co were parallel with our results. Bonanno (2011) found higher Al (21,600 μ gg⁻¹ dw) and B (<0.5 μ gg⁻¹ dw) concentrations in plant material but lower values in sediment; the concentration of Co was similar to the present study, whereas Fe was higher in sediment but similar in plant material. Vymazal (2009) reported higher values for Al (188 μ gg⁻¹ dw), Fe (109 μ gg⁻¹ dw), B (5.2 μ gg⁻¹ dw), and Mn (175 μ gg⁻¹ dw) but lower values for metals such as Cr (0.18 μ gg⁻¹ dw), Cd (0.01 μ gg⁻¹ dw), Ni (1.63 μ gg⁻¹ dw), Pb (0.23 μ gg⁻¹ dw), Co (0.08 μ gg⁻¹ dw) and Zn (27.1 μ gg⁻¹ dw). Bonanno (2012) studied *Arundo donax*, and reported lower concentrations of Mn (815 μ gg⁻¹ dw), Cr (19.2 μ gg⁻¹ dw) and Ni (17.5 μ gg⁻¹ dw); similar levels of Cd; and Pb and Zn in sediment but similar levels to the present study in the plant material. When (Bonanno and Giudice, 2010)'s research results were examined, in sediment, Cd (0.66 μ gg⁻¹ dw), Mn (181.8 μ gg⁻¹ dw) were lower than our data;

Cu and Pb were parallel with ours; Zn (9.69 μ gg⁻¹ dw), Ni (29.14 μ gg⁻¹ dw), Cr (40.1 μ gg⁻¹ dw) were lower than our findings, however, in the parts of *P. australis* Cd (0.68-1.13 µgg⁻¹ dw) and Pb (9.87-16.54 μgg⁻¹ dw) were higher than our results, Cu and Zn was parallel with our values again; Cr (0.40-6.97 μgg⁻¹ ¹ dw) were lower than ours. Bragato (2006) studied *P. australis*, and reported similar Cr and Cu levels but lower Ni and Zn. A study by Demirezen and Aksoy (2004) was compared with our results, in this study, it was pointed out that Cd was higher; Cu, Pb and Zn were parallel with our values but Cr (1-12 μgg⁻¹ dw) and Ni (2-12 μgg⁻¹ dw) were lower. Singha (2003) reported higher Cr, Mn, Fe, Cu, Zn and Pb levels; Co and Cd were parallel, whereas Ni was lower than our findings. Samecka-Cymermana and Kempers (2004) reported higher concentrations of Zn (246-515 µgg⁻¹ dw), Ca (12.900-19.200 µgg⁻¹ dw), Fe (14.200-25.200 µgg⁻¹ dw), Al (147-354 µgg⁻¹ dw), Cd (1.1-8.8 µgg⁻¹ dw), Co (33-98 µgg⁻¹ dw), Cu (720-1.040 μgg⁻¹ dw), Mn (3,990-6,660 μgg⁻¹ dw), Ni (18-59 μgg⁻¹ dw) and Pb (151-850 μgg⁻¹ dw) heavy metals in plants than those in the resent study, whereas Mg (1,730-5,260 µgg⁻¹ dw) and Cr (1.3-2.7 µgg⁻¹ ¹ dw) similar to our findings. Obolewski (2011) reported higher levels of Cd (1.3-2.1 µgg⁻¹ dw), Pb (14.4-26 μgg⁻¹ dw), Fe (97-333 μgg⁻¹ dw), Na (501-12,454 μgg⁻¹ dw) and Ca (5,686-10,285 μgg⁻¹ dw) than the present study; Zn (8.6-44.4 μ gg⁻¹ dw), Cu (1.5-11.1 μ gg⁻¹ dw) and Co (3-5 μ gg⁻¹ dw) were lower than ours, whereas Cr, Ni, Mn, K and Mg were parallel with our findings.







Figure 2. The concentration of Mn, Cu, Cd, Pb, Zn, Co, Ni, Cr, Fe, Mg, K, Ca, Al, Na and B. Significance of differences between sediment, root, stem and leaf from paired t-test, are indicated above the columns. (Keys: *p<0.05; **p<0.01; ***p<0.001 significant; I, Tercan, II, Erzincan, III, Kemah, IV, İliç and S, sediment, R, root, St, Stem, L, leaf)

The concentrations of metals obtained from the plant measurements in this study were parellel reported by Ross (1994), and Kacar and Inal (2008), but there are important differences between localities.

The result of our physiological experiments revealed that the rate of MDA which is a lipid peroxidation at İliç location was slightly higher than the other locations. The higher amounts of some heavy metals found at İliç location compared with the other locations were considered to be effective on MDA rate. Related with chlorophyll size, chlorophyll concentration was also lower at Erzincan location.

Many studies have also examined lipid peroxidation and chlorophyll level of certain plants by administering metals in laboratory environments alongside control groups. These studies reported significant differences in lipid peroxidation and the chlorophyll levels. As a result of these research,

they have found significant differences at the lipid perodixation and the chlorophyll levels (Vecchia *et al.*, 2005; Nouairi *et al.*, 2006; Vollenweider *et al.*, 2006; Popova *et al.*, 2009).

5. Conclusion

In addition to element analyses in sediment and plant samples, some physiological features of plant samples such as lipid peroxidation, chlorophyll a, chlorophyll b and chlorophyll a+b amounts were investigated. Although there were not large differences in chlorophyll amounts for all locations, lipid peroxidation activity was found to be slightly higher at İliç (Figure 3). Sediments in Karasu River had high concentrations of Ni and Cr metals. *P. australis* showed hyper-accumulation for Cd and Zn, therefore, it could be defined as a good biomonitoring plant. Zn level was particularly high in roots while Cd was higher in leaves. However, K was present in all parts of the plant. Lipid peroxidation was high in samples taken from İliç, and these surplus were caused by high level of lipid peroxidation.



Figure 3. The lipid peroxidation (mmol/L⁻¹ cm⁻¹) and concentration of chlorophyll (mg/g⁻¹) in *P. australis*

Elements	Leaf-MDA
Zn	0,50
Cd	0,90
Cu	0,89
Cr	0,90
Ca	0,65
Na	0,57
В	0,91

Table 1. The correlation between lipid peroxidation and plants.

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