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Correlations in metal release profiles following sorption by *Lemna minor*

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ABSTRACT
Following the rapid uptake of contaminants in the first few hours of exposure, plants typically attempt to cope with the toxic burden by releasing part of the sorbed material back into the environment. The present study investigates the general trends in the release profiles of different metal(loid)s in the aquatic macrophyte *Lemna minor* and details the correlations that exist between the release of metal(loid) species. Water samples with distinct contamination profiles were taken from Nilüfer River (Bursa, Turkey), Yeniçağa Lake (Bolu, Turkey), and Beyşehir Lake (Konya, Turkey) and used for release studies; 36 samples were tested in total. Accumulation and release profiles were monitored over five days for 11 metals and a metalloid (^{208}Pb, ^{111}Cd, ^{52}Cr, ^{53}Cr, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{75}As, ^{55}Mn, ^{137}Ba, ^{27}Al, ^{57}Fe, ^{66}Zn, ^{68}Zn) and correlation, cluster and principal component analyses were employed to determine the factors that affect the release of these elements. Release profiles of the tested metal(loid)s were largely observed to be distinct; however, strong correlations have been observed between certain metal pairs (Cr/Ni, Cr/Cu, Zn/Ni) and principal component analysis was able to separate the metal(loid)s into three well-resolved groups based on their release.

KEYWORDS
bioremediation; CA; correlation; duckweed; PCA; pyhtoremediation; release; removal

Introduction

The monitoring and remediation of environmental pollutants is of great importance in the modern world due to the risks posed by many contaminant types on human and animal health. As such, a great number of methods have been developed to reduce the impact of pollutants in soil and freshwater ecosystems, and biological remediation techniques are especially popular for their low costs and ease of application (Xie *et al.* 2013; Li *et al.* 2014). Macrophytes and algae are often used for this purpose (Üçüncü *et al.* 2014a; Harguinteguy *et al.* 2015; Jha *et al.* 2016), and a variety of plant species have been demonstrated to accumulate metals or metalloids at high concentrations (Di Luca *et al.* 2014; Tripathi *et al.* 2014). Plants may retain these elements in their roots, stems or leaves, and their high growth rates and ease of harvest and culture make them ideal for bioremediation efforts under both laboratory and real-life conditions.

Metals and metalloids are important environmental pollutants and may exhibit severe acute or chronic effects on plant, animal and human life (Ullah *et al.* 2015). Nonetheless, macrophytes are able to survive in metal(loid)-contaminated environments by preventing their influx into cells, depositing them in metabolically inactive regions or eliminating their reactivity (Zitka *et al.* 2013). As nonessential metals are able to "leak into" cells through transporter proteins that ordinarily carry essential metals of similar sizes and charges (Üçüncü *et al.* 2014b), the elimination of metal(loid) toxicity also requires their selective transport outside cells or potentially the entire organism. Consequently, plants exposed to metal(loid) burden may accumulate metal(loid)s for some time, only to reverse their accumulation trends and release metal(loid)s back into the environment once their coping mechanisms are activated.

As natural freshwater sources are often contaminated with multiple metal(loid) species, correlations between metal(loid) accumulation and release patterns may allow the design of more effective remediation methods, and we and other groups have previously shown that the accumulation of one metal (loid) may alter the influx of others by competitive and cooperative interactions (Üçüncü *et al.* 2013; Demim *et al.* 2014; Tiwari *et al.* 2014). However, while the transport mechanisms involved in the accumulation of metal(loid)s is a topic of interest, the process of metal(loid) efflux is investigated only to a lesser degree. In this manuscript, we propose that synergistic and antagonistic interactions are also vital for the release of metal(loid)s from the plant into the environment, and present our results on metal(loid) release by *L. minor* in freshwater samples exhibiting distinct contamination profiles from three natural freshwater sources in Turkey.

Materials and methods

Sample collection

The field arm of the study was performed in Nilüfer River (Bursa, Turkey), Yeniçağa Lake (Bolu, Turkey) and Beyşehir Lake (Konya, Turkey). Each region was sampled once

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Table 1. Metal(loid)s profiles of sampling stations used in bioremediation experiments.

Station (μg/L)	Al	^{52}Cr	^{53}Cr	Mn	Fe	Ni	^{63}Cu	^{65}Cu	^{66}Zn	^{68}Zn	As	Cd	Ba	Pb
Y-1	2.19 ± 0.39	0.76 ± 0.34	1.15 ± 0.25	0.69 ± 0.39	823.49 ± 15.52	3.90 ± 0.34	1.65 ± 0.47	1.91 ± 0.44	56.05 ± 25.13	55.12 ± 23.42	1.68 ± 0.36	0.38 ± 0.35	43.68 ± 3.16	1.74 ± 0.84
Y-2	117.4 ± 116	N.D	N.D	177.92 ± 25	1360.50 ± 118	7.39 ± 5.93	N.D	11.07 ± 6.50	2805.17 ± 1.886	2610 ± 1.753	4.86 ± 2.4	1.32 ± 0.8	114.79 ± 13.1	42.50 ± 15.6
Y-3	14.29 ± 6.68	N.D	N.D	N.D	1488.53 ± 484.06	6.21 ± 0.82	N.D	7.52 ± 2.99	2038 ± 1.726	1900 ± 1601	115.76 ± 8.75	N.D	93.72 ± 13.66	9.28 ± 11.05
N-1	11.25 ± 1.15	1032± 6.50	766.50 ± 4.25	119.61 ± 19.71	660.13 ± 6.88	21.97 ± 0.13	9.07 ± 0.39	9.35 ± 0.01	110.48 ± 0.85	104.26 ± 0.91	1.81 ± 0.02	N.D	17.91 ± 0.03	3.96 ± 0.06
N-2	8.44 ± 0.48	302.79 ± 62.46	229.71 ± 48.04	130.85 ± 2.80	564.63 ± 94.63	38.40 ± 6.48	13.46 ± 2.69	11.80 ± 2.09	125.70 ± 25.50	117.80 ± 23.83	2.42 ± 0.34	N.D	13.88 ± 0.02	1.07 ± 0.43
N-3	14.21 ± 3.09	564.06 ± 15.99	422.17 ± 11.96	244.53 ± 7.93	696.01 ± 16.98	70.26 ± 1.93	24.78 ± 4.55	12.21 ± 0.52	278.75 ± 26.29	259.99 ± 24.71	2.61 ± 0.04	0.03 ± 0.01	18.05 ± 0.59	1.75 ± 0.75
N-4	55.23 ± 16.73	55.08 ± 8.24	80.62 ± 3.08	87.37 ± 7.98	1396.94 ± 176.60	17.67 ± 0.53	132.09 ± 52.79	4.17 ± 0.75	2047.56 ± 1.150	1905 ± 1.068	N.D	N.D	18.44 ± 1.11	3.22 ± 0.82
N-5	51.26 ± 19.40	0.89 ± 1.53	4.05 ± 3.17	N.D	819.30 ± 183.99	5.33 ± 0.65	109.32 ± 48.32	3.37 ± 1.15	2377.17 ± 1.045	2205 ± 963	N.D	N.D	13.07 ± 0.75	14.34 ± 8.28
N-6	112.6 ± 31.18	5.42 ± 0.72	N.D	N.D	1168.78 ± 311	0.43 ± 0.43	167.18 ± 74.86	4.72 ± 2.52	5742.11 ± 3.678	5318 ± 3.391	N.D	N.D	12.58 ± 1.48	9.04 ± 11.00
B-1	5.35 ± 0.55	1.21 ± 0.41	N.D	1.06 ± 0.50	412.03 ± 103.77	2.98 ± 0.34	3.32 ± 0.74	1.95 ± 0.84	86.38 ± 22.50	83.51 ± 20.67	2.14 ± 0.74	1.11 ± 0.06	40.68 ± 1.36	2.59 ± 0.73
B-2	11.06 ± 0.19	1.11 ± 0.15	N.D	1.01 ± 0.21	632.83 ± 22.16	2.41 ± 0.20	1.42 ± 0.46	2.19 ± 0.15	96.80 ± 30.52	91.38 ± 28.30	1.35 ± 0.24	0.57 ± 0.18	16.16 ± 0.85	4.27 ± 1.01
B-3	6.48 ± 0.81	0.40 ± 0.14	N.D	0.46 ± 0.08	908.94 ± 23.53	3.61 ± 0.14	0.74 ± 0.21	1.80 ± 0.11	83.96 ± 21.56	82.12 ± 19.88	2.44 ± 0.04	0.10 ± 0.06	53.12 ± 0.52	2.40 ± 1.08

N.D: Not detected, Y:Yeniçağa Lake, N: Nilüfer River, B: Beyşehir Lake

(February 2013) in sampling stations chosen to represent distinct contamination profiles, with the aim of covering a broad range of metal(loid) concentrations present in natural freshwater environments. Water parameters (temperature, dissolved oxygen, pH and electrical conductivity (EC)) were also noted in sampled regions. Three stations each were sampled in Yeniçağa and Beyşehir Lakes, while six stations were sampled in Nilüfer River. All stations were sampled in triplicate and analyzed for metal and metalloid concentrations by ICP-MS. Sample aliquots were acidified in 2% nitric acid prior to ICP measurements and analyzed at regular intervals to monitor the stabilization of metal(loid) concentrations following sampling; experiments were begun only when no changes were noted in sample metal(loid) concentration profiles.

Release experiments

Experimental setup

Lemna plants were provided from Ankara University greenhouse cultures and acclimated to experimental conditions under constant fluorescent light exposure (24 h light/0 h cycles) (Megateli *et al.* 2009; Sekomo *et al.* 2012). Plants bearing two or three fronds were transferred from the main culture for accumulation and release experiments; a total of 30 fronds were used for each replicate (OECD 2002). Phytoremediation experiments were performed directly on freshwater samples with no additional metal(loid) presence; changes in metal(loid) concentrations were measured daily for five days. All tests were repeated in triplicate.

Metal and metalloid analysis

An X-Series II ICP-MS equipped with Cetac Asx-260 autosampler accessories was utilized for water and sample measurements; ^{208}Pb, ^{111}Cd, ^{52}Cr, ^{53}Cr, ^{60}Ni, ^{63}Cu, ^{65}Cu, ^{75}As, ^{55}Mn, ^{137}Ba, ^{27}Al, ^{57}Fe, ^{66}Zn, and ^{68}Zn were the isotopes tested. A 2% nitric acid matrix in ultrapure water was used for all measurements. QCS-27 series of elements were used for the construction of calibration curves. Sample concentrations of metal(loid) were taken into account for the concentration ranges used in calibration; r2 values were > 0.99 for each curve. 10 μg/L ^{209}Bi was used as internal standard. Three runs were performed in total; sampling and washing steps were chosen as 60 s each.

Statistical analyses

Correlation analysis

Correlation analysis was used to investigate whether trends in the accumulation and release of individual metal(loid)s matched the fluctuations of other metal(loid)s. Shapiro-Wilk test was used to monitor the normality of data sets prior to analysis. Pearson test was used on data exhibiting normal distribution; Spearman test was used otherwise (Tunca *et al.* 2013).

Regression analysis

Regression analysis is commonly applied for the predictive modeling of sorption data (Dirilgen 2011; Demim *et al.* 2013a; Ghiani *et al.* 2014) and was used in the present study to determine whether the release of an individual metal(loid) was reliant that of another metal(loid). Data from all sampling stations were pooled for regression analysis.

CA and PCA

Cluster analysis (CA) is an analysis method used to separate data into groups depending on their shared properties. In this work, CA was used to determine the relationships between the accumulation and release trends of metal(loid)s. Euclidean distances and Ward method were used for CA analysis; Z-score correction was also applied to the data (Lopez *et al.* 2004). Principal component analysis (PCA) is another method for investigating relationships between groups of data and determines whether two sets of data are derived from a common background. PCA calculations were performed following Varmuza and Filzmoser and used to group metal(loid)s according to their release profiles (Varmuza and Filzmoser 2009). PCA groups were observed to account for 83.64% of the data (the Kaiser-Meyer-Olkin (KMO) coefficient was found to be 0.85).

Results and discussion

Water parameters and metal(loid) concentrations in freshwater samples

Metal(loid) profiles and water quality parameters associated with Nilüfer River, Yeniçağa Lake and Beyşehir Lake are provided in Tables 1 and 2. While water parameters were altogether similar, the greatly differing metal(loid) concentrations observed across the sampling sites suggest that the regions sampled indeed had distinct contamination profiles.

Accumulation and release profiles of metal(loid)s in Lemna minor

While high metal(loid) concentrations rapidly facilitate metal (loid) entry into aquatic macrophytes, this process is sometimes followed by the release of the sorbed elements back to the environment (Megateli *et al.* 2009). In the present study, statistical methods have been employed to determine whether the release profile of a metal(loid) is affected by others. Correlation analyses between metal(loid) concentrations have been performed on day 2 – day 5 samples (day 1 samples were ignored as the first 24 h typically involves the initial accumulation of all

Table 2. Water parameters of sampling stations used in bioremediation experiments.

Stations	pH	TDS (mg/L)	EC(SPC) (μS/cm)	Salinity (ppt)	NO_3	T (°C)
Nilüfer	7.03 – 7.61	160 – 1449	246 – 2227	0.12 – 1.14	1.02 – 5.89	9.1 – 9.6
Yeniçağa	6.62 – 7.59	255 – 1443	392 – 2218	0.19 – 1.14	1.87 – 6.26	4.0 – 5.0
Beyşehir	7.86 – 8.12	253 – 364	389 – 565	0.19 – 0.27	0.65 – 1.73	11.4 – 12.0

elements tested) and graded as moderate (0.50–0.70), strong (0.70–0.90) or very strong (0.90–1.0) (Table 3).

The strongest correlations observed were between ^{66}Zn and ^{68}Zn (0.96) and ^{53}Cr and Ni (0.94), while other strong correlations were present between ^{52}Cr and ^{53}Cr (0.88), Ni and ^{65}Cu (0.87), ^{52}Cr and ^{65}Cu (0.87), ^{68}Zn and Ni (0.86), ^{52}Cr and Ni (0.86), ^{53}Cr and ^{68}Zn (0.84), ^{65}Cu and ^{66}Zn (0.82), ^{52}Cr and ^{68}Zn (0.82), ^{52}Cr and ^{66}Zn (0.82), ^{53}Cr and Mn (0.81), ^{65}Cu and Mn (0.81) and Al and ^{65}Cu (0.81). As such, strong correlations were observed between Cu, Zn, Mn, Ni, Cr and Al in general. Many of these metals are essential for the function of plants and utilize a large number of shared metabolic pathways: NRAMP metal transporters for Mn, Zn, Cu and Ni (Nevo and Nelson 2006; Manara 2012); type 2 metallothionein, HSP90, GST (Hildebrandt *et al.* 2007), EDTA (Evangelou *et al.* 2007) and ACC deaminase (Grichko *et al.* 2000) for Cu and Zn; anthocyanins for Mn, Zn, and Ni (Pilon-Smits and Pilon 2002); phytosiderophores for Mn, Zn, and Cu (Yang *et al.* 2005); Zn transporters (Hildebrandt *et al.* 2007), OsNramp 1-2-3 (Belouchi *et al.* 1997) and IRT1 (Korshunova *et al.* 1999; Guerinot 2000) for Mn and Zn and TgMTP1, COT1, ZRC1 for Ni and Zn (Persans *et al.* 2001). As such, these strongly correlating metals can share a single transporter, such as NRAMP, or share transporters between two or three-metal groups. In addition, particularly strong Cr/Cu correlations have previously been reported in the literature (Demim *et al.* 2013b). Consequently, shared transport pathways may be a major cause of the correlations observed, although it should be noted that high numbers of potential transporters may also decrease the correlations observed.

The highest correlation observed for Fe is with As, which has previously been observed in rice (Azizur Rahman *et al.* 2011; Tiwari *et al.* 2014). Although As is a metalloid and Fe is a metal, their common oxidation value of +3 may have allowed both elements to be transported through similar mechanisms. While we have observed a weak correlation between these metals, a previous study on *Pistia stratiotes* L. has found a strong (0.89) correlation between the acculumations of Al (another trivalent element) and Fe (Veselý *et al.* 2012). Transporters such as phytosiderophores (Yang *et al.* 2005) and the ZIP family (Ali *et al.* 2013) are responsible for iron transport in plants; in addition, specific transporters for the divalent and trivalent forms of iron have also been reported (Guerinot 2000). As such, the ability of Fe to use multiple, highly specific transporters may account for its lack of strong correlations.

It is known that Cr may compete with Fe, S, and P for binding sites (Wallace *et al.* 1976). However; Cr is a variable element and exists in valence states from −2 to +6 in nature (Ergul-Ulger *et al.* 2014). Cr(III) and Cr(VI) are the most stable forms of Cr, and both forms are uptaken under different mechanisms by plants (Shanker *et al.* 2005). Consequently, Cr is able to share transport mechanisms with a large variety of other ions, which may have resulted in the large number of correlations observed for this metal.

It is worth noting that Ba has displayed negative correlations with every element except Fe and As. These correlations were moderately negative for Al and weakly negative for ^{52}Cr, Mn, ^{63}Cu, ^{65}Cu, and ^{66}Zn. Ba has been reported to be negatively correlated with various elements (Suwa *et al.* 2008), although the mechanisms involved in its accumulation and transport are still largely unknown (Kamachi *et al.* 2015).

As could be expected, very strong correlations were present between isotopes. The strongest isotope-isotope correlation was observed in Zn, while the weakest was in Cu. This effect may be an artifact of the isotope ratios present across the sampling stations, as the isotopic ratio of Zn was very consistent across samples, while the isotopic ratio of Cu isotopes varied greatly. As biological reactions are known to exhibit isotopic preference, differences in isotope ratios may have created these minor changes.

While specialized metabolic pathways are usually lacking for reactions involving non-essential metals, these elements can use transport pathways of essential metals through their physical or chemical similarity to a particular metal ion (Bridges and Zalups 2005; Rodriguez-Hernandez *et al.* 2015). Consequently, competitive or cooperative effects may be observed in the accumulations of essential and non-essential metals. (Liu *et al.* 2003; Degryse *et al.* 2012). However, the nonessential elements As, Pb, and Cd have been observed to show few correlations in the present study. The fact that these metal(loid)s share transport pathways with multiple different metals may have prevented them from correlating with the accumulation of any single essential metal species: Cd for example may use proteins such as IRT1, (Meagher and Heaton 2005), metallothioneins,

Table 3. Release correlations between metal(loid)s in water during 5 days.

	^{27}Al	^{52}Cr	^{53}Cr	^{55}Mn	^{57}Fe	^{60}Ni	^{63}Cu	^{65}Cu	^{66}Zn	^{68}Zn	^{75}As	^{111}Cd	^{137}Ba	^{208}Pb
^{27}Al	1.0													
^{52}Cr	.70^{b}	1.0												
^{53}Cr	.60^{b}	.88^{b}	1. 0											
^{55}Mn	.71^{b}	.73^{b}	.81^{b}	1.0										
^{57}Fe	−.33^{b}	.08	.28^{b}	.03	1.0									
^{60}Ni	.62^{b}	.86^{b}	.94^{b}	.78^{b}	.30^{b}	1.0								
^{63}Cu	.75^{b}	.71^{b}	.74^{b}	.64^{b}	.06	.75^{b}	1.0							
^{65}Cu	.81^{b}	.87^{b}	.81^{b}	.81^{b}	.02	.87^{b}	.78^{b}	1.0						
^{66}Zn	.73^{b}	.82^{b}	.76^{b}	.72^{b}	−.04	.79^{b}	.66^{b}	.82^{b}	1.0					
^{68}Zn	.63^{b}	.82^{b}	.84^{b}	.74^{b}	.14	.86^{b}	.68^{b}	.80^{b}	.96^{b}	1.0				
^{75}As	−.21^{b}	.15^{a}	.44^{b}	.28^{b}	.64^{b}	.43^{b}	.11	.16^{a}	.07	.28^{b}	1.0			
^{111}Cd	.09	−.08	−.08	.18^{a}	−.17^{a}	−.03	−.01	.14	−.07	−.08	.09	1.0		
^{137}Ba	−.62^{b}	−.29^{b}	−.05	−.16^{a}	.61^{b}	−.04	−.21^{b}	−.31^{b}	−.33^{b}	−.12	.70^{b}	.10	1.0	
^{208}Pb	.23^{b}	.14	.14	.29^{b}	−.01	.14	.16^{a}	.30^{b}	.12	.11	.21^{b}	.36^{b}	.14	1.0

$^{a}p < 0.05$ ^{b} $p < 0.01$

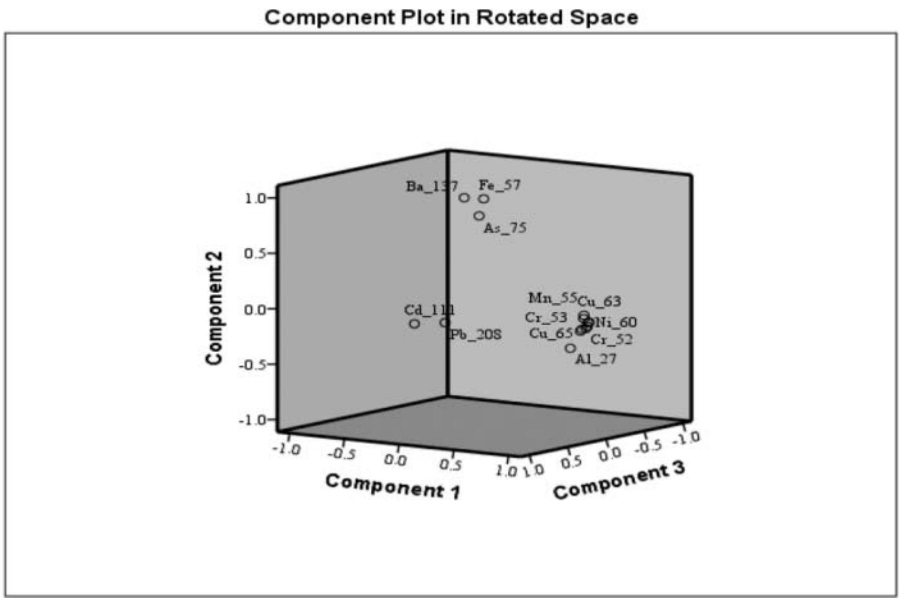

Figure 1. Results of Principal Component Analysis (PCA).

ABC-typetransporter proteins (Lu *et al.* 1997) and glomalin (González-Chávez *et al.* 2004) for its entry, and has shown very few correlations with the elements tested. As plants possess advanced mechanisms for the rapid efflux of essential metals, the selective activation of metal transport genes may also have allowed the removal of essential metals while largely leaving toxic metal(loid)s untouched, leading to a lack of correlation between non-essential metal(loid)s.

PCA results were also in support with correlation analyses and have yielded three major groups, the first containing Al, ^{52}Cr, ^{53}Cr, Mn, Ni, ^{63}Cu, ^{65}Cu, ^{66}Zn, and ^{68}Zn, the second containing Fe, As and Ba and the third group containing Cd and Pb (Fig 1). As such, the main group of strongly correlating elements, the low-correlating Fe/As and Cd/Pb secondary groups were all recovered using PCA. CA dendogram also yielded broadly similar results, although the Al/Cr/Mn/Ni/Cu/Zn group was divided into two subgroups under this method (Fig. 2).

Regression analysis was also performed to observe general trends about metal(loid) release. Although high r^2 values have been obtained for regression results, all metal(loid)s had distinct release profiles throughout the 120-hour

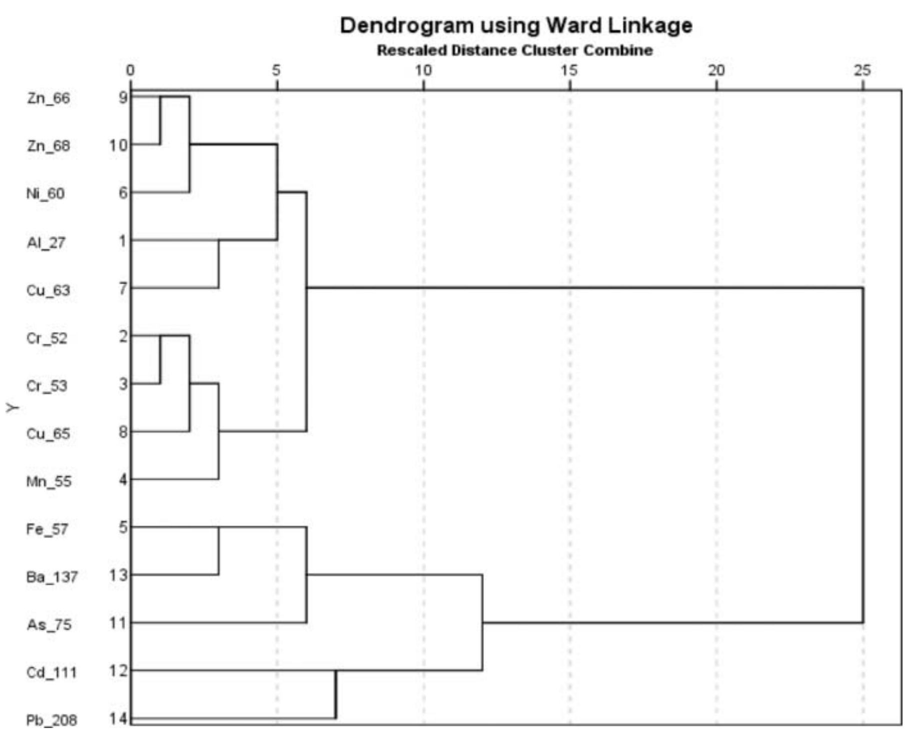

Figure 2. Dendogram of Cluster Analysis (CA).

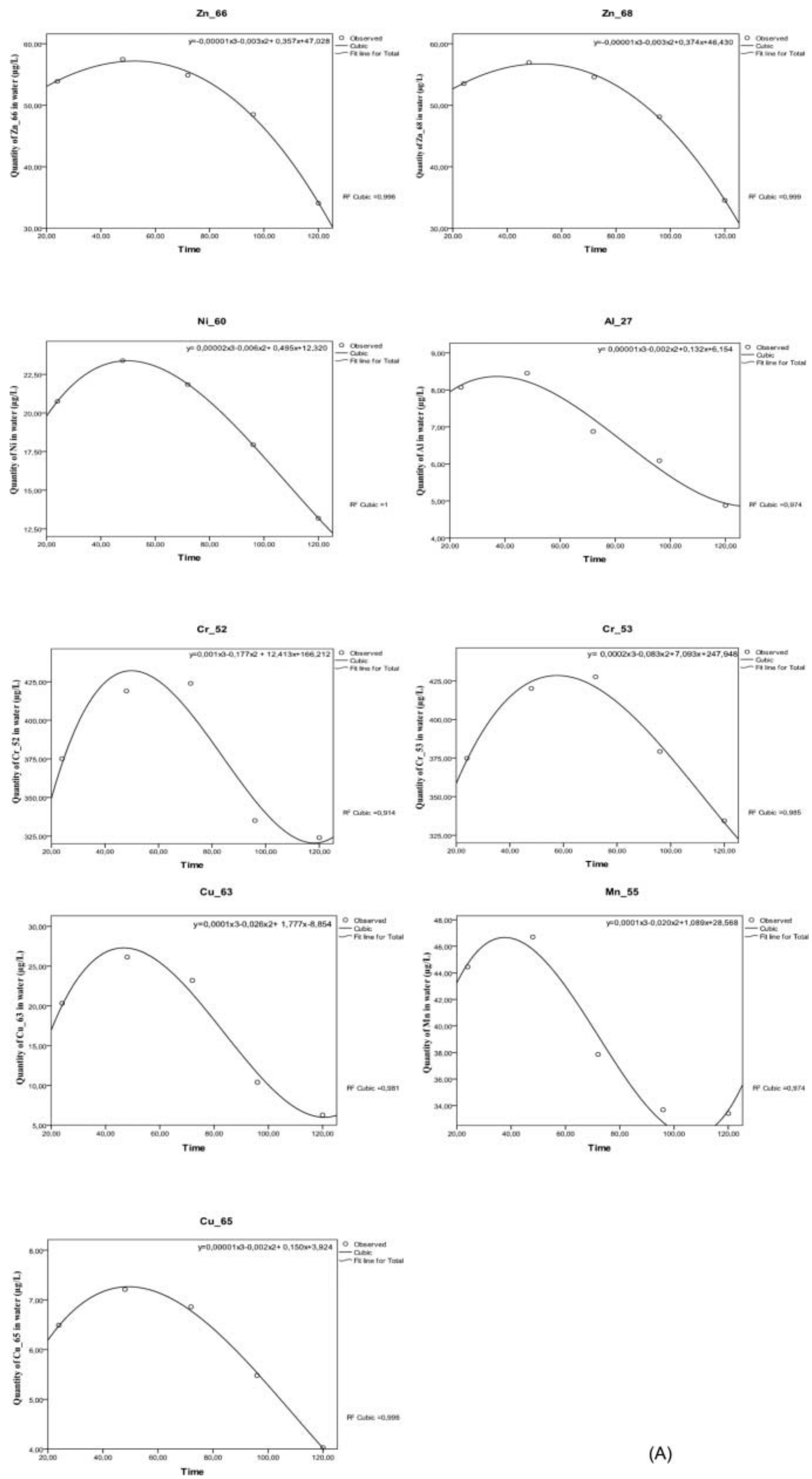

Figure 3.(a-c) Regression analysis of release profiles for 5 days (*Continued*).

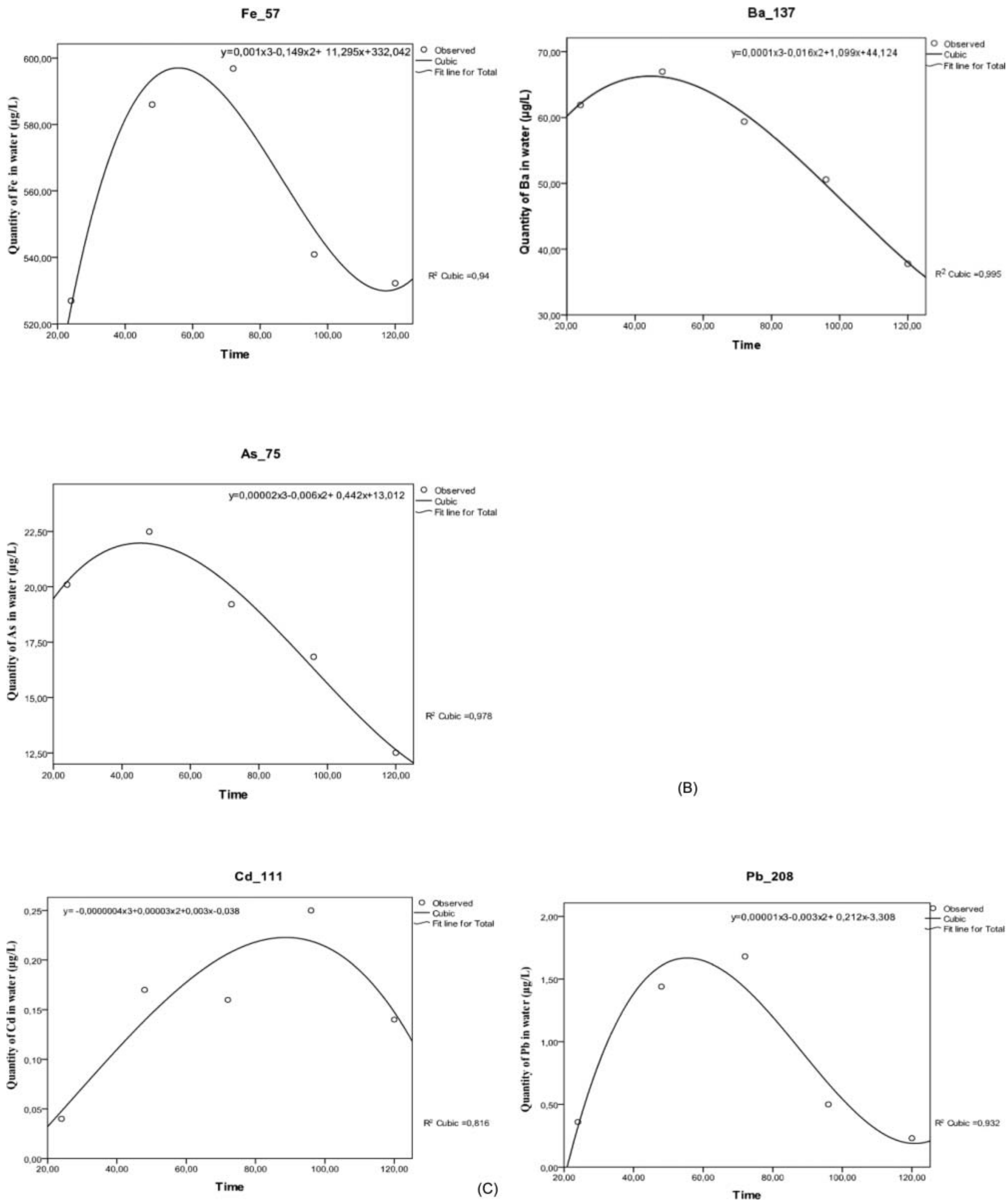

Figure 3. (*Continued*).

experiment period, suggesting that specific mechanisms are responsible for the removal of excess metal(loid)s from plant tissues (Fig 3a-1, Fig 3a-2, Fig 3b, Fig 3c).

Conclusion

Following the first 24 hours of exposure, all metal(loid)s were observed to be alternately released and reaccumulated in an oscillating pattern. However, these oscillation profiles were also demonstrated to be metal(loid)-specific rather than a general physiological response, and correlation trends were outlined between the metal(loid) groups tested based on their release profiles. Consequently, specific efflux mechanisms are likely responsible for reducing the metal(loid) burden of the affected plant; these may involve the metal(loid)-dependent activation of certain transport proteins. The elements tested were recovered under three main groups in cluster analysis and principal component analysis. In addition, correlations between release profiles were observed to agree with correlations reported between accumulation profiles in the literature, suggesting that accumulation and release pathways may use similar transporters. Consequently, the accumulation and release of metal species by *Lemna* appears to be dependent on not just environmental factors such as temperature and water parameters, but also on the presence of other metal(loid) (and potentially non-metal) pollutants in the environment, and this phenomenon may have significant consequences on the remediation of natural freshwater sources that are contaminated by multiple metal(loid)s. However, the ideal means of ensuring maximum remediation efficiency in these environments is unclear, and further studies are necessary to establish the exact mechanisms responsible for these effects.

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References

Ali H, Khan E, Sajad MA. 2013. Phytoremediation of heavy metals-concepts and applications. Chemosphere 91:869–881.

Azizur Rahman M, Mamunur Rahman M, Kadohashi K, Maki T, Hasegawa H. 2011. Effect of external iron and arsenic species on chelant-enhanced iron bioavailability and arsenic uptake in rice (*Oryza sativa* L.). Chemosphere 84:439–445.

Belouchi A, Kwan T, Gros P. 1997. Cloning and characterization of the OsNramp family from *Oryza sativa*, a new family of membrane proteins possibly implicated in the transport of metal ions. Plant Mol Biol 33:1085–1092.

Bridges CC, Zalups RK. 2005. Molecular and ionic mimicry and the transport of toxic metals. Toxicol Appl Pharmacol 204:274–308.

Degryse F, Shahbazi A, Verheyen L, Smolders E. 2012. Diffusion limitations in root uptake of cadmium and zinc, but not nickel, and resulting bias in the Michaelis constant. Plant Physiology 160:1097–1109.

Demim S, Drouiche N, Aouabed A, Benayad T, Dendene-Badache O, Semsari S. 2013a. Cadmium and nickel: Assessment of the physiological effects and heavy metal removal using a response surface approach by *L. gibba*. Ecological Engineering 61:426–435.

Demim S, Drouiche N, Aouabed A, Semsari S. 2013b. CCD study on the ecophysiological effects of heavy metals on *Lemna gibba*. Ecological Engineering 57:302–313.

Demim S, Drouiche N, Aouabed A, Benayad T, Couderchet M, Semsari S. 2014. Study of heavy metal removal from heavy metal mixture using the CCD method. J Ind Eng Chem 20:512–520.

Dirilgen N. 2011. Mercury and lead: Assessing the toxic effects on growth and metal accumulation by Lemna minor. Ecotoxicol Environ Saf 74:48–54.

Di Luca GA, Hadad HR, Mufarrege MM, Maine MA, Sánchez GC. 2014. Improvement of Cr phytoremediation by Pistia stratiotes in presence of nutrients. Int J Phytorem 16(2):167–178.

Ergul-Ulger Z, Ozkan AD, Tunca E, Atasagun S, Tekinay T. 2014. Chromium(VI) biosorption and bioaccumulation by live and acid-modified biomass of a novel *Morganella morganii* isolate. Separation Sci Technol (Philadelphia) 49:907–914.

Evangelou MWH, Ebel M, Schaeffer A. 2007. Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. Chemosphere 68: 989–1003.

Ghiani A, Fumagalli P, Nguyen Van T, Gentili R, Citterio S. 2014. The combined toxic and genotoxic effects of Cd and As to plant bioindicator *Trifolium repens* L. PLoS One DOI: 10.1371/journal.pone.0099239.

González-Chávez MC, Carrillo-González R, Wright SF, Nichols KA. 2004. The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollution 130:317–323.

Grichko VP, Filby B, Glick BR. 2000. Increased ability of transgenic plants expressing the bacterial enzyme ACC deaminase to accumulate Cd, Co, Cu, Ni, Pb, and Zn. J Biotechnol 81:45–53.

Guerinot ML. 2000. The ZIP family of metal transporters. Biochim Biophys Acta-Biomembranes 1465:190–198.

Harguinteguy CA, Pignata ML, Fernández-Cirelli A. 2015. Nickel, lead and zinc accumulation and performance in relation to their use in phytoremediation of macrophytes Myriophyllum aquaticum and Egeria densa. Ecological Engineering 82:512–516.

Hildebrandt U, Regvar M, Bothe H. 2007. Arbuscular mycorrhiza and heavy metal tolerance. Phytochemistry 68:139–146.

Jha VN, Tripathi RM, Sethy NK, Sahoo SK. 2016. Uptake of uranium by aquatic plants growing in fresh water ecosystem around uranium mill tailings pond at Jaduguda, India. Sci Total Environ 539:175–184.

Kamachi H, Kitamura N, Sakatoku1 A, Tanaka1 D, Nakamura S. 2015. Barium accumulation in the metalliferous fern *Athyrium yokoscense*. Theor Exp Plant Physiol DOI: 10.1007/s40626-015-0036-4.

Korshunova YO, Eide D, Clark WG, Guerinot ML, Pakrasi HB. 1999. The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. Plant Mol Biol 40:37–44.

Li Z, Xiao H, Cheng S, Zhang L, Xie X, Wu Z. 2014. A comparison on the phytoremediation ability of triazophos by different macrophytes. J Environ Sci 26(2):315–322.

Liu JG, Liang JS, Li KQ, Zhang ZJ, Yu BY, Lu XL, Yang JC, Zhu QS. 2003. Correlations between cadmium and mineral nutrients in absorption and accumulation in various genotypes of rice under cadmium stress. Chemosphere 52:1467–1473.

Lopez FJS, Garcia MDG, Vidal JLM, Aguilera PA, Frenich AG. 2004. Assessment of metal contamination in Donana National Park (Spain) using crayfish (*Procamburus clarkii*). Environ Monit Assess 93:17–29.

Lu YP, Li ZS, Rea PA. 1997. AtMRP1 gene of Arabidopsis encodes a glutathione S-conjugate pump: Isolation and functional definition of a plant ATP-binding cassette transporter gene. Proceedings of the National Academy of Sciences of the United States of America 94:8243–8248.

Manara A. 2012. Plants and heavy metals. In: Plant responses to heavy metal toxicity. Springer. Dalcorso p. 27–53.

Meagher RB, Heaton ACP. 2005. Strategies for the engineered phytoremediation of toxic element pollution: Mercury and arsenic. J Ind Microbiol Biotechnol 32:502–513.

Megateli S, Semsari S, Couderchet M. 2009. Toxicity and removal of heavy metals (cadmium, copper, and zinc) by *Lemna gibba*. Ecotoxicol Environ Saf 72:1774–1780.

Nevo Y, Nelson N. 2006. The NRAMP family of metal-ion transporters. Biochim Biophys Acta – Mol Cell Res 1763:609–620.

Organisation for Economic Co-operation and Development. 2002. Guidelines for the testing of chemicals Lemna sp. Growth inhibition test. Draft guideline OECD 221.

Persans M, Nieman K, Salt D. 2001. Functional activity and role of cation-efflux family members in Ni hyperaccumulation in *Thlaspi goesingense*. Proceedings of the National Academy of Sciences of the United States of America 98(17):9995–10000.

Pilon-Smits E, Pilon M. 2002. Phytoremediation of metals using transgenic plants. Crit Rev Plant Sci 21(5):439–456.

Rodriguez-Hernandez MC, Bonifas I, Alfaro-De la Torre MC, Flores-Flores JL, Bañuelos-Hernández B, Patiño-Rodríguez O. 2015. Increased accumulation of cadmium and lead under Ca and Fe deficiency in Typha latifolia: A study of two pore channel (TPC1) gene responses. Environ Exp Bot 115:38–48.

Sekomo CB, Rousseau DPL, Saleh SA, Lens PNL. 2012. Heavy metal removal in duckweed and algae ponds as a polishing step for textile wastewater treatment. Ecol Engineering 44:102–110.

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S. 2005. Chromium toxicity in plants. Environ Int 31:739–753.

Suwa R, Jayachandran K, Nguyen NT, Boulenouar A, Fujita K, Saneoka H. 2008. Barium toxicity effects in soybean plants. Arch Environ Contam Toxicol 55:397–403.

Tripathi RD, Singh R, Tripathi P, Dwivedi S, Chauhan R, Adhikari B, Trivedi PK. 2014. Arsenic accumulation and tolerance in rootless macrophyte *Najas indica* are mediated through antioxidants, amino acids and phytochelatins. Aquat Toxicol 157:70–80.

Tiwari M, Sharma D, Dwivedi S, Singh M, Tripathi RD, Trivedi PK. 2014. Expression in Arabidopsis and cellular localization reveal involvement of rice NRAMP, OsNRAMP1, in arsenic transport and tolerance. Plant, Cell Environ 37:140–152.

Tunca E, Ucuncu E, Ozkan AD, Ulger ZE, Tekinay T. 2013. Tissue distribution and correlation profiles of heavy-metal accumulation in the freshwater crayfish *Astacus leptodactylus*. Arch Environ Contam Toxicol 64:676–691.

Üçüncü E, Tunca E, Fikirdesici S, Altindag A. 2013. Decrease and increase profile of Cu, Cr and Pb during stable phase of removal by Duckweed (*Lemna minor* L.). Int J Phytorem 15:376–384.

Üçüncü E, Özkan AD, Kurşungöz C, Ulger ZE, Tekinay T, Ortaç B, Tunca E. 2014a. Interaction modeling of laser ablated silver nanoparticle phytoremediation by *Lemna minor*. Chemosphere 108:251–257.

Üçüncü E, Özkan AD,Ölmez TT, Tunca E. 2014b. Phytoremediation of multiply metal contaminated environments: Synergistic and competitive effects between heavy metals during uptake and transport. In: Heavy metal remediation transport and accumulation in plants. New York (NY): Nova Publishers. p. 179–200.

Ullah A, Heng S, Munis MFH, Fahad S, Yang X. 2015. Phytoremediation of heavy metals assisted by plant growth promoting (PGP) bacteria: A review. Environ Exp Bot 117:28–40.

Varmuza K, Filzmoser P. 2009. Introduction to multivariate statistical analysis in chemometrics. Boca Raton (FL): CRC Press.

Veselý T, Trakal L, Neuberg M, Száková J, Drábek O, Tejnecký V, Balíková M, Tlustoš P. 2012. Removal of Al, Fe and Mn by *Pistia stratiotes* L. and its stress response. Cent. Eur J Biol 7:1037–1045.

Wallace A, Soufi SM, Cha JW, Romney EM. 1976. Some effects of chromium toxicity on bush bean plants grown in soil. Plant Soil 44: 471–473.

Xie WY, Huang Q, Li G, Rensing C, Zhu YG. 2013. Cadmium accumulation in the rootless macrophyte *Wolffia globosa* and its potential for phytoremediation. Int J Phytorem 15(4):385–397.

Yang X, Feng Y, He Z, Stoffella PJ. 2005. Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elements in Medicine Biol 18:339–353.

Zitka O, Krystofova O, Hynek D, Sobrova P, Kaiser J, Sochor J, Zehnalek J, Babula P, Ferrol N, Kizek R and Adam V. 2013. Metal transporters in plants. In: Heavy metal stress in plants. Berlin Heidelberg (Germany): Springer-Verlag. p.19–41.