Microchim Acta 154, 261–268 (2006)
DOI 10.1007/s00604-006-0519-8

Microchimica Acta
Printed in Austria

Original Paper

Trace Metals in Microcrustaceans and Brazilian Waterweed from a Contaminated Chilean Wetland Using Total Reflection X-Ray Fluorescence Spectrometry

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Received September 30, 2005; accepted January 9, 2006; published online May 11, 2006

Abstract. The trace element content of individual copepod specimens and of the Brazilian water weed (*Egeria densa*) from a metal-contaminated wetland in Southern Chile were determined using total reflection X-ray fluorescence spectrometry. Sampling of the water and the organisms was carried out at three sampling sites during 2004. Enhanced concentrations of dissolved Fe and Mn were found in the column water and in the pore water. The Fe content in the benthic copepods was significantly elevated compared to other aquatic organisms from different Chilean lakes. Regarding *E. densa*, healthy (green coloured) plants showed mass fractions of Fe, Mn, Ni, Cu and Zn which were typical for uncontaminated systems. In contrast, damaged (brownish coloured) plants exhibited very high Fe and Mn concentrations indicative of contamination or processes which changed the element load from the environment to the plant.

Key words: Bioaccumulation; cyclopods; TXRF; *Egeria densa*; heavy metal uptake.

Elemental analysis of freshwater biota is important to investigate the uptake, the bioaccumulation and the transfer of pollutants along the trophic chain [1–3]. Heavy metals as toxic substances are important parameters to understand main processes in aquatic systems [4]. They are released into the environment from a wide range of natural and anthropogenic sources. Because the rate of influx of these heavy metals into the environment exceeds their removal by natural processes, there is a tendency of heavy metals to accumulate in the aquatic environment, especially in sediments and biota [4–6]. Important bioindicators for the characterisation of the water quality and the metal toxicity are microcrustaceans and macrophytes [7]. In this way element determination in microcrustaceans and macrophytes give first information on the bio-availability of elements and their bioaccumulation in biota [8, 9].

Here, we present first results on the trace element content of individual copepod specimens and of the Brazilian waterweed (*Egeria densa*) from the wetland Carlos Anwandter Sanctuary (River Cruces watershed) near the southern Chilean city of Valdivia. This wetland suffered a dramatic change during 2004. At least 130 swans were found dead since October 2004 and most of the survivors migrated from the wetland, which was home to more

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than 6,000 swans. The swans' disappearance was related to a massive die-off of their prime source of food, the predominanting Brazilian water weed (*Egeria densa*, Hydrocharitaceae). It was argued that the chemistry in the wetlands changed during 2004, just some months after the operative start of a huge pulp mill factory (Kraft Paper; Valdivia Project of Celulosa Arauco and Constitución S.A.). This uses water from the river Cruces, 25 km up stream, and where it also dumps its industrial effluent after tertiary treatment containing also heavy metals.

The aim of this study was to determine the element mass fraction in single specimens of the very small benthic copepod *Eucyclops serrulatus* and of the water weeds in order to detect enhanced concentrations of trace metals which may indicate pollution.

Experimental

Sampling Sites and Sampling Procedure

The wetland Carlos Anwandter Sanctuary is located mid-way along the Pacific coast in the Los Lagos Region immediately north of the city of Valdivia in Southern Chile (39°41′S 073°11′W) (Fig. 1). The entire area is part of an estuarine complex, lowing to this variation in the water level (less than 1 m). Most parts of the wetland are about 0.5–1.0 m deep, only the central part of the wetland with the River Cruces is 9 to 16 m deep. Some 40 % of the total area (4,877 ha) comprises flooded areas. Amongst the submerged and flooded plant species the Brazilian waterweed (*Egeria densa*) was the dominant species. The wetland is eutrophic with low conductivity (100–500 $\mu S\,cm^{-1}$), except the southern part which receives saline water during tides. The catchment is largely used as agricultural land, but since February 2004 the wetland receives also waste water from a pulp mill factory which is located about 25 km above the wetland.

Sampling of the water, of the benthic copepod *E. serrulatus* and of the pore water was carried out at three sampling sites during November (site 3) and December (sites 1 and 2) in 2004 (Fig. 1). The water samples and the copepods were taken near the

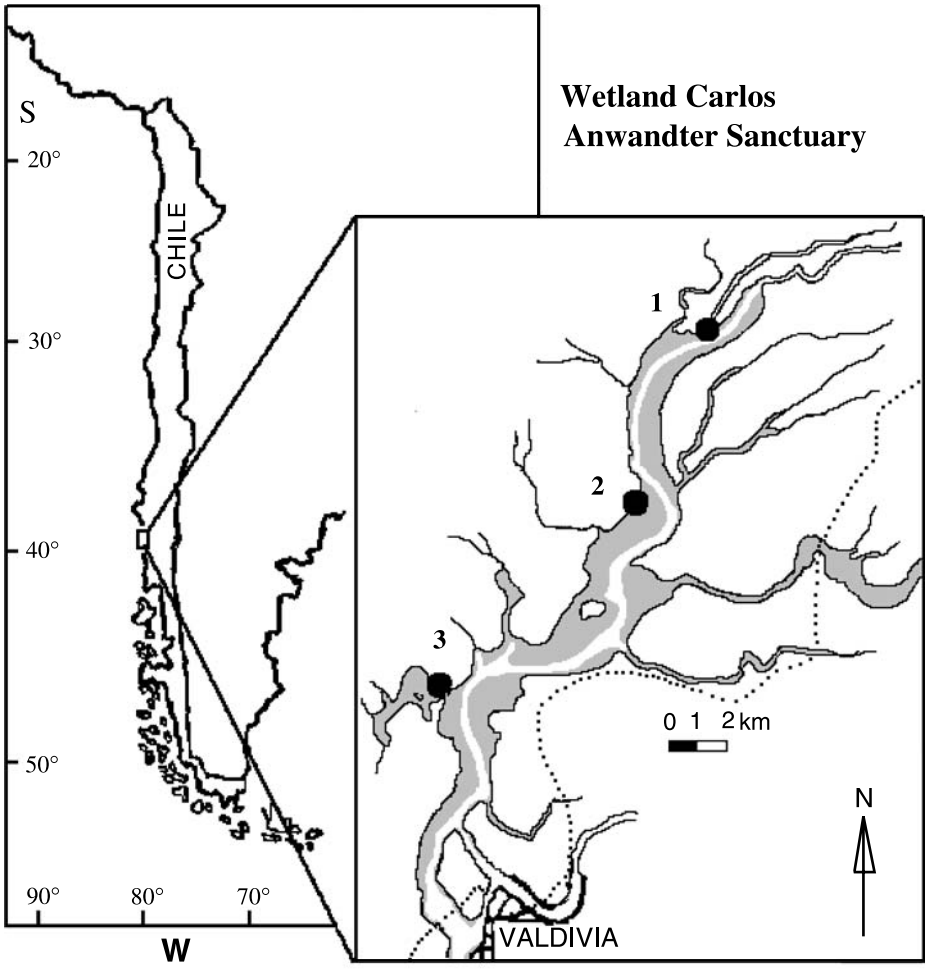

Fig. 1. Sampling sites of the wetland Carlos Anwandter Sanctuary (Chile)

bottom in 0.5 to 1.0 m depth using a plastic bottle (volume 1 L) mounted to a 3 m large wooden stick. To collect pore water, sediment samples were taken at every sampling site and filtered (volume 50 mL) using 0.2 µm cellulose ester filter. Afterwards, both the 0.2 µm-filtered water samples and the pore water were filled into 1.5 mL polypropylene bottles and preserved using 2 µL 65% HNO_3 (Suprapure grade, Merck, Darmstadt, Germany). Finally, the element concentration in the water was analyzed by TXRF on 10 µL subsamples [10].

After collection the copepods were transported to the laboratory and prepared according to the *dry method* [10] including in cold plasma ashing techniques to improve the homogeneity of the sample on the glass carrier [11].

The waterweed *Egeria densa* was sampled only at site 2, because it had already disappeared at the other sampling sites. We found healthy and polluted plants: (1) green coloured, obviously young plants in relatively healthy conditions and (2) brownish coloured, plants in moribund conditions. In order to test, whether the heavy metal content varied within these two kinds of plants we collected ten green and ten brownish coloured plants.

Preparation of Copepods and Egeria densa for TXRF Analysis

After sampling the copepods were washed three times in 0.2 µm prefiltered sample water and finally washed in high purity water (double deionised water) put into small teflon bottles (3 mL volume) and frozen in liquid nitrogen. After freeze drying individual specimens (mostly adults) were selected, their dry weight was determined using a microbalance (Sartorius Ultramicro S4, sensitivity 0.1 µg) and corrected for relative humidity (RH) [10]. RH varied between 31.1% and 53.3%. After weighing, single specimens were put onto quartz glass carriers, fixed with 2 µL high purity water and air dried. Finally, 5 ng Gallium (suspended in nitric acid 2%) as internal standard was added and the individual specimens were digested with 5 µL HNO_3 while the quartz glass carriers were put onto a hot plate and dried at 100 °C once more. In a second step, the specimens were ashed in a cold plasma asher (CPA) (Plasma-System 100, Technics Plasma GmbH, Kirchheim, Germany) in order to remove the organic matrix which improves the detection of trace elements [11]. Specific operation parameters were: oxygen pressure 100 Pa; oxygen purity factor 4.5 (99.995% oxygen), microwave power 300 W, reaction time two hours.

Prior to lyophilisation, *E. densa samples* were thoroughly washed three times with 0.2 µm prefiltered sample water and once with pure water. After that, small parts of leaves and stems (dry weight 0.5–5 mg) of the plants were cut and put into teflon bottles (volume 3 mL), frozen in liquid nitrogene and lyophilised. The dry leaves and stems were weighed and put into PTFE vials (7 mL, Savillex, Canada), and with addition of 500 µL HNO_3 and 10 µL H_2O_2 (Suprapur grade, Merck, Germany). Ga as an internal standard (100 ng) was also added directly into the PTFE vials. The vials were then tightly closed with screw caps. The digestion took 3 h at 120 °C on a hot plate. Finally, the element mass fractions were determined by TXRF on 10 µL subsamples.

Bioaccumulation of Trace Metals

The bioaccumulation (BA) of the trace metals in the three micrustaceans was calculated as follows:

$$BA = C_{org}/C_{water} \quad (1)$$

where C_{org} is the element mass fraction in the organism (µg g^{-1} dry weight) and C_{water} is the element concentration of the water (µg mL^{-1}). The bioaccumulation was calculated only for the water phase, not for the pore water.

Instrumentation for TXRF Analyses

Total reflection X-ray fluorescence spectrometer 8030 C (FEI Company Munich, Germany) was used. The spectrometer was equipped with an 80-mm^2 Si(Li) detector having a resolution of 158 eV (at 5.9 keV, Mn-Kα line), Mo fine focus tube operated at 50 kV and 55 mA and a computer controlled multichannel analyser system combined with a spectrum deconvolution program. Measurement time was 1000 s.

Quality Assurance

In order to test accuracy of the TXRF measurements, 0.372 mg of reference material CRM414 plankton [12], which consists >98% by the freshwater cladoceran *Daphnia magna*, were weighted and put into PFA microvials (volume 7 mL), and also addition of 500 µL HNO_3 and 10 µL H_2O_2. Ga as an internal standard (100 ng) was also added directly into the PTFE vials. The vials were than tightly closed with screw caps. The digestion took 3 h at 120 °C on a hot plate. TXRF measurements were done on twelve sub samples of 10 µL of the digested reference material.

Statistical Analysis

The significance or non-significance of differences between the median element concentrations of the copepods and of *Egeria densa* and between sampling sites was tested using the Student t-test on the assumption of a normal distribution and equal variances [13]. The normality of the data was verified by Chi square test and the variance check was tested with Bartlett's test. In most cases, the data were not normally distributed and did not show equal variances, thus non-parametric Kruskal-Wallis 1 way ANOVA test (K-W test) was used [14]. The significance of the difference between the two series is verified if the P value was <0.05, otherwise both series originate from the same population and the difference between the series (concentration and sampling sites in our case) is not significant. All statistical analysis were done using Statgraphics Plus 5.0 for Windows and XL-Stat 7.2.

Results and Discussion

Quality Assurance

The TXRF measurements of certified reference material of plankton CRM 414, which consisted >98% by the freshwater microcrustacean *Daphnia magna*, were largely in acceptable agreement with the certified values considering the small sample size of only 372 µg (see Table 1). For Mn, Fe, Bi, Cu and Zn the recovery rates and the relative standard deviation (RSD) varied between 87 and 117% and between 5.6 and 13.0%, respectively (exception Ni: 41.6%). This is about 1.4–4.1 times higher (only Mn, Fe, Cu, Zn) than the RSD reported for the reference material CRM

Table 1. TXRF analysis of reference material CRM 414 (European Community Bureau reference material 414, trace elements in plankton with >98% of the cladoceran *Daphnia magna*; sample dry weight: 300–400 µg; $n = 12$)

Element	Reference values of CRM 414			TXRF: samples			R_r (%)
	Mean mass fraction (µg g^{-1} DW)	SD (µg g^{-1} DW)	RSD (%)	Mean mass fraction (µg g^{-1} DW)	SD (µg g^{-1} DW)	RSD (%)	
Ca	65000[c]	2000	3.1	63909	2278	3.6	98
Mn	299[a]	12	4.0	259.8	14.5	5.6	87
Fe	1850[b]	190	10.3	1808	160	8.8	98
Ni	18.8[a]	0.8	4.3	17.7	7.4	41.6	94
Cu	29.5[a]	1.3	4.4	34.6	4.5	13.0	117
Zn	112[a]	3	2.7	108.1	12.3	11.3	97
Sr	261[a]	25	9.6	222	7.7	3.5	83

SD standard deviation; *RSD* relative standard deviation; R_r Recovery rate = mass ratio of measured and referenced material, [a] certified, [b] indicative, [c] informative value.

Table 2. TXRF analysis of dissolved metal concentrations of the water-sediment interphase and the pore water at three sampling sites of the wetland Carlos Anwandter Sanctuary (mean ±1 SD). All data are given in µg L^{-1}. The data for Lake Riñihue and Lake Rapel are taken from [8, 10]

Element	Wetland Carlos Anwandter Sanctuary					Lake Riñihue	Lake Rapel
	Site 1		Site 2		Site 3		
	River water ($n = 5$)	Pore water ($n = 1$)	River water ($n = 5$)	Pore water ($n = 1$)	River water ($n = 5$)	Lake water ($n = 5$)	Lake water ($n = 6$)
Mn	1541 ± 27.4	1298	2340 ± 31.1	2623	2232 ± 52.6	<4.96	9.0 ± 8.5
Fe	353 ± 9.4	5695	473 ± 10.9	9725	331 ± 8.0	9.17 ± 1.19	18.3 ± 4.0
Ni	1.88 ± 0.40	5.10	1.99 ± 0.63	<2	2.92 ± 0.68	<1.78	2.7 ± 0.9
Cu	3.55 ± 0.52	11.23	3.48 ± 0.46	<1.5	3.36 ± 0.71	<2.3	9.6 ± 6.0
Zn	2.41 ± 0.26	9.67	2.95 ± 0.30	1.61	6.74 ± 0.52	5.65 ± 1.21	2.8 ± 1.6

n number of replicates.

414, but falls in the same range like measurements made by others [8, 10, 15].

Element Concentration of the Water Phase and of the Pore Water

Table 2 shows the concentrations for Mn, Fe, Ni, Cu and Zn of the wetland compared to the lake water of the oligotrophic Andean Lake Riñihue and the eutrophic Rapel reservoir in Central Chile as reported by [8, 10]. The data revealed high element concentrations for Mn and Fe from the wetland compared to lake water, but similar values for Ni, Cu and Zn for all sites.

Mn was about five times higher than Fe in both the water phase and the pore water of the wetland. These values are one to two magnitudes higher than those from Lake Riñihue and Rapel reservoir. These relatively high concentrations of Mn and Fe probably are the result of the currents and wave action which affect the deposition of sediment by continually resuspending the sediment [6, 16]. Furthermore, probably the sediment collection and the modifications to conditions, such as aeration of overlying waters caused the elevated metal concentrations we found in the water phase, but not in the pore water [16]. Finally, Fe and Mn naturally show high concentrations in the soil, which may enhance the load of these metals from the catchment area, especially during heavy rainfalls.

Element Mass Fraction of Copepods

Figure 2 and Table 3 display the element mass fraction for Mn, Fe, Ni, Cu and Zn for *E. serrulatus* from the three sites of the wetland Carlos Anwandter Sanctuary. The number of samples from the three sites was quite different between the sites. Most specimens were found at site 1 ($n = 62$) followed by site 3 ($n = 32$) and site 2 ($n = 5$). In general, the individual dry weight (DW) of the copepods varied from 3 to 15 µg with slightly higher values found at site 1. If all values of every element from the three sampling sites

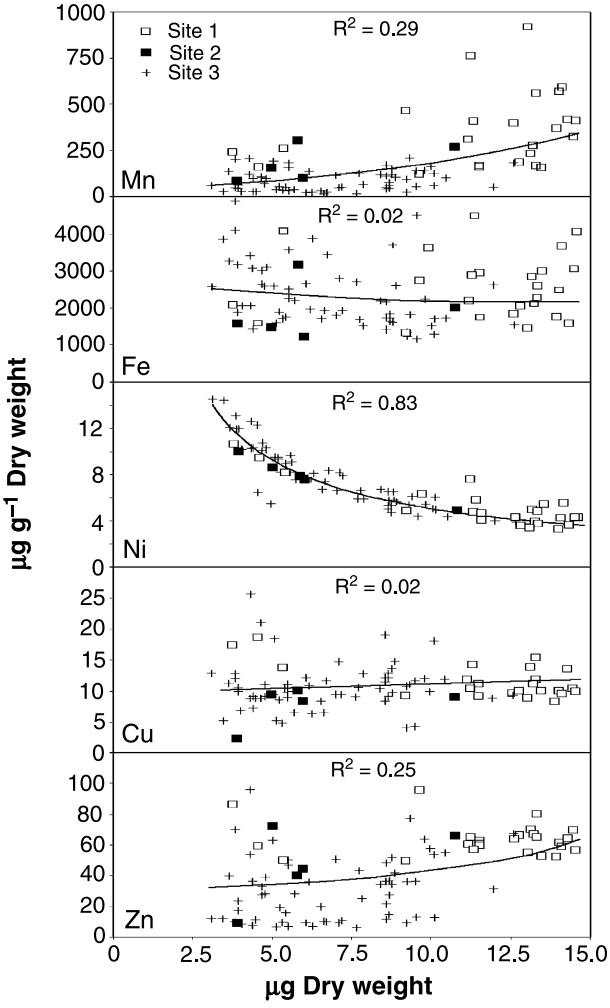

Fig. 2. Trace element concentrations of Mn, Fe, Ni, Cu and Zn in *E. serrulatus* from three sampling sites of the wetland Carlos Anwandter Sanctuary (Chile)

are considered as originated from the same copepod population, only Ni revealed a significant relationship between the element mass fraction and the DW. All other metals showed a much weaker (Mn, Zn) or no such relationship. Unfortunately, comparable literature data for the relationship between DW and mass fraction in freshwater copepods completely lack, but there exist some information for cladocera [8, 10]. According to this, there is a general trend of an inverse relationship between the element mass fraction and the DW, at least for Ni, Fe and Mn. We suggested that the remarkable differences between cladocera and copepods might be related to the ability of the copepods to discriminate between different food particles and to regulate actively their internal metal concentrations [17]. This would allow to maintain their internal metal concentration stable to a certain degree and to be independent from the trace element concentration in the water phase and in the food.

Regarding the element mass fraction of the copepods from the wetland, no significant differences between the sampling sites were found for Fe and Cu and between sites 2 and 3 for Ni and Zn. The most important differences between the trace element content and the sampling sites was found for Mn, which was two to four times more highly concentrated at site 1 than it was at sites 2 and 3. This means, that the copepods at site 1 accumulated Zn and Mn about twice to four times higher compared to sites 2 and 3 despite the more or less similar concentration of both metals in the water phase and in the pore water.

There are only few comparable measurements on trace element analysis in freshwater microcrustaceans from South America or on a worldwide scale in the literature [1, 8–10]. According to that, our data on Zn, Cu and Ni were generally consistent with unspecific zooplankton values from uncontaminated systems from Hungary and twenty North American lakes [1, 9]. Comparable results on Fe and Mn are not available. Because these metals are of special interest in our study, we included here unpublished results on copepods from two Chilean lakes where already trace element analysis on cladocera have been published [8, 10]. According to these values, Fe was significantly 6 to 12 times higher concentrated in copepods from the wetland than in copepods from the two lakes. The other metals (except Cu) were in the same range, but showed still significant differences. Only Cu was much higher concentrated in copepods from Lake Rapel, because this lake received drainage water from a copper mine [8].

The calculation of the bioaccumulation of the trace metals under consideration revealed that copepods from the wetland accumulated – despite the much higher concentrations of Mn and Fe in the water phase – much less Mn and Fe than copepods from the two lakes (Fig. 3). For Ni, Cu and Zn the bioaccumulation in the wetland was similar or lower than in the two lakes. We suggest that our data may reflect the different metal content of the copepod's food sources rather than the different metal levels (especially Fe and Mn) in the aqueous phase, because copepods assimilate metals principally vía ingestion of food particles and not directly from the water phase [1, 17]. Therefore, we conclude that the element mass

Table 3. Trace element concentrations of Mn, Fe, Ni, Cu and Zn in copepods from three sampling sites of the wetland Carlos Anwandter Sanctuary

Element		*E. serrulatus*			Significant differences between species	P-value	
		Site 1 ($n=62$)	Site 2 ($n=6$)	Site 3 ($n=32$)			
Mn	mean ± SE	346 ± 39.9	185 ± 44.4	84.7 ± 7.2	site 1 – site 2	0.041	significant
	median	294	158	64.7	site 1 – site 3	0.000	significant
					site 2 – site 3	0.000	significant
Fe	mean ± SE	2533 ± 166	1903 ± 338	2346 ± 111	site 1 – site 2	0.086	non-significant
	median	2383	1610	2064	site 1 – site 3	0.285	non-significant
					site 2 – site 3	0.220	non-significant
Ni	mean ± SE	5.10 ± 0.38	7.97 ± 0.84	7.80 ± 0.35	site 1 – site 2	0.006	significant
	median	4.49	7.97	7.40	site 1 – site 3	0.000	significant
					site 2 – site 3	0.800	non-significant
Cu	mean ± SE	11.89 ± 0.55	8.05 ± 1.39	10.74 ± 0.49	site 1 – site 2	0.072	non-significant
	median	11.28	9.22	10.50	site 1 – site 3	0.057	non-significant
					site 2 – site 3	0.134	non-significant
Zn	mean ± SE	63.8 ± 2.11	46.5 ± 11.2	30.6 ± 2.69	site 1 – site 2	0.004	significant
	median	46.4	44.2	27.7	site 1 – site 3	0.000	significant
					site 2 – site 3	0.192	non-significant

Mean, standard error of the mean (SE) and median are given. All concentration data are given in $\mu g\, g^{-1}$ dry weight. The significance of the differences between the medians of the three copepod species were tested applying the Kruskal-Wallis test. Significance level $P < 0.05$.

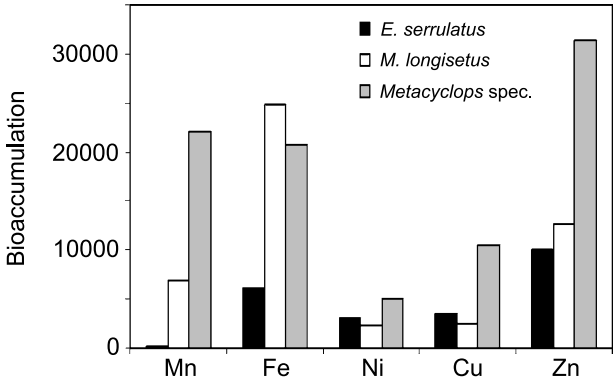

Fig. 3. Bioaccumulation of Mn, Fe, Ni, Cu and Zn in *E. serrulatus* ($n=92$) from wetland Carlos Anwandter Sanctuary, *M. longisetus* ($n=32$) from Lake Riñihue and of *Metacyclops* sp. ($n=32$) from Rapel reservoir (Chile)

fraction in copepods only in part can be considered as indicative of enhanced metal concentration or metal load in the aqueous phase. Compared to literature data on unspecified zooplankton fractions from 20 lakes in the northeastern United States, our results on the bioaccumulation factor are one to three magnitudes lower [1]. This probably can be explained by the much lower trace element concentration of the lake water and the zooplankton composition in these lakes, which are dominated by highly efficient filter feeder.

Element Mass Fraction of the Brazilian Water Weed

Table 5 displays the mass fraction of Mn, Fe, Ni, Cu and Zn in leaves and stems of green and brownish specimens of *E. densa*. Whereas Ni, Cu and Zn were in some cases significantly different in green and brownish plants, their mass fraction varied only by the factor two between the different plants. The most important differences were found for Fe and Mn. These trace elements were significantly different in leaves and stems of both green and brownish plants and between green and brownish plants. The mass fraction of Fe and Mn from leaves and stems were 7 to 30 times and 3 to 9 times higher concentrated in brownish plants than in green plants. In comparison with literature data on *E. densa* and *Elodea canadensis* (Table 6), our data on Fe and Mn from brownish plants were much higher than reported values for uncontaminated and even contaminated systems. Only green plants revealed Fe and Mn concentrations similar to those from uncontaminated site.

The high Fe concentrations in brownish plants in part could be explained by precipitates of probably iron-hydroxid on the leaves and on the stems of *E. densa*. Moreover, we could also observe by an optical microscope that the cell walls of the outer cell layers were brownish coloured. This may indicate a penetration of Fe- or Mn-precipitates in the plant itself. Such

Table 4. Trace element concentrations of Mn, Fe, Ni, Cu and Zn in copepods from the wetland Carlos Anwandter Sanctuary (*E. serrulatus*) compared to Lake Riñihue (*Mesocyclops longisetus*) and Rapel reservoir (*Metacyclops* sp.)

Element		*E. serrulatus* ($n = 92$)	*M. longisetus* ($n = 32$)	*Metacyclops* sp. ($n = 32$)	Significant differences between species	P-value
Mn	mean ± SE	166 ± 17.5	34.2 ± 1.01	195 ± 13.7	*E. serrulatus* – *M. longisetus*	0.000
	median	116	35.5	194	*E. serrulatus* – *Metacyclops* sp.	0.003
					M. longisetus – *Metacyclops* sp.	0.000
Fe	mean ± SE	2374 ± 89.5	228 ± 9.2	379 ± 31.8	*E. serrulatus* – *M. longisetus*	0.000
	median	2172	236	345	*E. serrulatus* – *Metacyclops* sp.	0.000
					M. longisetus – *Metacyclops* sp.	0.000
Ni	mean ± SE	7.04 ± 0.3	4.1 ± 0.95	13.7 ± 0.7	*E. serrulatus* – *M. longisetus*	0.000
	median	6.31	2.6	13.4	*E. serrulatus* – *Metacyclops* sp.	0.000
					M. longisetus – *Metacyclops* sp.	0.000
Cu	mean ± SE	12.0 ± 1.1	5.6 ± 0.3	101.4 ± 6.7	*E. serrulatus* – *M. longisetus*	0.000
	median	10.4	5.7	95.7	*E. serrulatus* – *Metacyclops* sp.	0.000
					M. longisetus – *Metacyclops* sp.	0.000
Zn	mean ± SE	40.8 ± 2.5	71.6 ± 2.3	87.9 ± 5.5	*E. serrulatus* – *M. longisetus*	0.000
	median	40.8	73.0	82.9	*E. serrulatus* – *Metacyclops* sp.	0.000
					M. longisetus – *Metacyclops* sp.	0.007

Mean, standard error of the mean (SE) and median are given. All concentration data are given in $\mu g\, g^{-1}$ dry weight. The significance of the differences between the medians of the three copepod species were tested applying the Kruskal-Wallis test. Significance level $P < 0.05$.

Table 5. Trace element concentrations of Mn, Fe, Ni, Cu and Zn in *Egeri densa* from the wetland Carlos Anwandter Sanctuary

Element		Green plants		Brownish plants		Significant differences between	P-value	Differences
		Leaves ($n = 10$) (a)	Stem ($n = 10$) (b)	Leaves ($n = 10$) (c)	Stem ($n = 10$) (d)			
Mn	mean ± SE	4295 ± 967	739 ± 71.0	27338 ± 2734	6761 ± 1509	(a) – (b)	0.013	significant
	median	4237	716	28115	5561	(a) – (c)	0.000	significant
						(c) – (d)	0.000	significant
						(b) – (d)	0.000	significant
Fe	mean ± SE	2224 ± 326	542 ± 50.4	15438 ± 3187	15091 ± 6259	(a) – (b)	0.000	significant
	median	1992	490	11957	3712	(a) – (c)	0.000	significant
						(c) – (d)	0.149	non-significant
						(b) – (d)	0.000	significant
Ni	mean ± SE	39.8 ± 4.79	20.4 ± 3.93	40.7 ± 4.54	19.6 ± 2.24	(a) – (b)	0.005	significant
	median	35.5	16.8	37.7	19.9	(a) – (c)	0.890	non-significant
						(c) – (d)	0.000	significant
						(b) – (d)	0.705	non-significant
Cu	mean ± SE	22.3 ± 2.83	15.8 ± 2.80	23.2 ± 2.00	11.7 ± 1.35	(a) – (b)	0.041	significant
	median	19.8	14.2	21.9	12.2	(a) – (c)	0.496	non-significant
						(c) – (d)	0.000	significant
						(b) – (d)	0.406	non-significant
Zn	mean ± SE	94.4 ± 7.08	96.0 ± 2.37	94.2 ± 13.4	65.2 ± 9.72	(a) – (b)	0.540	non-significant
	median	93.4	95.0	90.2	64.8	(a) – (c)	0.705	non-significant
						(c) – (d)	0.257	non-significant
						(b) – (d)	0.034	significant

Mean, standard error of the mean (SE) and median are given. All concentration data are given in $\mu g\, g^{-1}$ dry weight. The significance of the differences between the medians were tested applying the Kruskal-Wallis test. Significance level $P < 0.05$.

a penetration seems to be possible, because *E. densa* neither possesses an epidermis nor a cuticula which act as protective layer. Because it is known from the literature that high iron concentrations can cause the destruction of plant cells or inhibit the plant growth [21]. It should be studied the input of Fe and Mn from the catchment area into the wetland and the metal exchange near the sediment-water interphase.

Table 6. Literature data on the trace element concentrations of Mn, Fe, Ni, Cu and Zn in different aquatic plants. All concentration data are given in $\mu g\, g^{-1}$ dry weight

Species	Site description	Sample description	Mn	Fe	Ni	Cu	Zn	Reference
Egeria densa	Chile, wetland	green plants	800–4300	540–2220	20–40	16–22	94–96	this study
		brownish plants	6700–27340	15100–15400	20–41	12–23	65–94	
Egeria densa	Brazil, Uncontaminated river	entire plants		2155–2540		5.9–20.7	21–103	[18]
Egeria najas		entire plants		2670–2960		5.7–18.0	20–131	
Elodea canadensis	Rivers in Pennsylvania	entire plants	1000–4000	990–1100		18–54		[20]
Elodea canadensis	Finland Eutrophic lake	leaves	1200–9800	2500–4400	13–32	13–20	35–49	[19]
		shoots	690–3900	610–1450	4–6	6–11	34–69	
Elodea canadensis	Three polish rivers Contaminated/ uncontaminated	entire plants	38–770	18–287	3–313	3–217	24–770	[22]

Conclusions

In this study we present first data on the element mass fraction of benthic copepods and of the macrophyte *E. densa* from a contaminated wetland in Southern Chile using total reflection X-ray fluorescence.

We found considerably enhanced concentrations of dissolved Fe and Mn in the column water and in the pore water at three sampling sites within the wetland. Also, the mass fraction of Fe in the benthic copepods was significantly elevated compared to other aquatic systems. In case of *E. densa*, green and brownish coloured plants were analysed. Green (= more healthy) plants showed mass fractions of Fe, Mn, Ni, Cu and Zn which are typical for uncontaminated systems. In contrast, the brownish coloured plants exhibited very high Fe and Mn concentrations indicative of contamination or processes which changed the Fe load from the environment to the plant. It is probably that the high Fe and Mn concentrations had a negative impact on the growth and/or survival of the water weed.

Acknowledgements. A part of this study was financed by the *Convenio Complementario Específico No. 1210-1203/2004-12-14* CONAMA X[a] Región de los Lagos – Universidad Asutral de Chile.

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