Ecotoxicology (2013) 22:558–573
DOI 10.1007/s10646-013-1048-y

The accumulation of metal (Co, Cr, Cu, Mn and Zn) in freshwater *Ulva* (Chlorophyta) and its habitat

Andrzej Rybak · Beata Messyasz · Bogusława Łęska

Accepted: 3 February 2013 / Published online: 12 February 2013

Abstract The possibility of using freshwater *Ulva* (Chlorophyta) as a bioaccumulator of metals (Co, Cr, Cu, Mn and Zn) in lake and river water was examined weekly in the summer of 2010 in three types of samples: the water, the sediment and the thalli of *Ulva.* Samples of freshwater *Ulva* were collected from two aqueous ecosystems lie 250 km away from the basin of the Baltic Sea and 53 km from each other. A flow lake located in the centre of the big city was the first water reservoir (ten sites) and second, the suburban river (six sites). The mean metal concentrations in the *Ulva* tissue from the river and the lake decreased in the following order: Mn > Zn > Cr > Cu > Co and Mn > Cr > Zn > Cu > Co, respectively. Moreover, a negative and statistically significant correlation between Mn concentrations in the *Ulva* thalli and the river water was observed. Additionally, numerous correlations were noted between the different concentrations of metals within the *Ulva* thalli, in the water and in the sediment. The great concentrations of Mn and Zn and the smallest of Co were found in thalli of *Ulva*, irrespective of the type of the ecosystem from which samples of algal thalli originated. Freshwater *Ulva* populations examined in this study were clearly characterized a dozen or so times by the higher Mn and Cr accumulation than taxa from that genera coming from sea ecosystems. The calculated bioconcentration factor confirm the high potential for freshwater *Ulva* to be a bioaccumulator of trace metals in freshwater ecosystems.

Keywords Chlorophyta · Bioconcentration factor · Bioaccumulator · Freshwater *Ulva* · Heavy metals

A. Rybak (✉) · B. Messyasz
Department of Hydrobiology, Institute of Environmental Biology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland
e-mail: rybakandrzej@interia.eu

B. Łęska
Department of Supramolecular Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Grunwaldzka 6, 60-780 Poznan, Poland

Introduction

Trace metals in the sea and inland waters are found mainly in low concentrations. However, as a result of the modern civilization, high concentrations of heavy metals are discharged into the water from industrial and municipal pollutants. This metal-containing water supplies lakes and marine waters through rivers and channels, causing many trophic problems and influencing the stability of the aquatic ecosystem.

Using organisms as bioindicators for heavy metal contamination is widespread. Both plants and animals are capable to accumulate metals. The repeated accumulation is very efficient, resulting in concentrations of metals in tissues that are a few thousand times larger than the concentrations of the same metals in the environment (Bryan and Langston 1992).

Macroalgae, such as red algae (Muse et al. 1995), brown algae (Bryan and Langston 1992) and green algae (Villares et al. 2002; Gąbka et al. 2010), are also being used to monitor the pollution of water with heavy metals (Lobban and Harrision 1997). The ability of marine macroalgae to accumulate heavy metals leads to the following order: Chlorophyta > Phaeophyta > Rhodophyta (Al-Shwafi and Rushdi 2008). Therefore, green algae are most often used to monitor the pollution. Species of the genera *Ulva* (including species of the genera *Enteromorpha*) were examined to determine which were the best within the

Chlorophyta to be used for monitoring the bioaccumulation of heavy metals (Haritonidis and Malea 1995).

For experiments concerning the bioaccumulation of heavy metals by species of the genera *Ulva*, the freshwater populations of these taxa, which develop in such ecosystems as rivers, ponds, lakes or streams, have not been reported in the literature. Freshwater *Ulva* have previously been observed starting around 1895 in Poland (Rybak in press), in the United States (Reinke 1981) and in the Czech Republic (Mareš 2009). At these sites, only taxa with monostromatic tubular thalli (e.g., *U. flexuosa*) were noted. Taxa of *Ulva* with distromatic frondose thalli (e.g., *U. lactuca*) are not found in freshwater ecosystems (Messyasz and Rybak 2009). The aquatic ecosystems that were selected for the present examinations of the freshwater *Ulva* populations were observed every year since the mid-nineties of the last century. Additionally, the location of Lake Malta (in the centre of a large city) and the Nielba River (in an urban area) likely determined the amounts of the metals that were reaching the water.

The aim of the present study is to provide new data on the accumulation capacity of freshwater *Ulva* for selected heavy metals, with a particular focus on using freshwater *Ulva* as a bioaccumulator for heavy metal contamination in water. We present a comprehensive study by analysing changes in the concentration of heavy metals in freshwater *Ulva* during the growing season and compare these data with reference samples from water and sediments.

In the present study, the change in Co, Cr, Cu, Mn and Zn concentrations were analyzed. The set of these metals was chosen purposeful in consideration of planned future work in the laboratory, which will be connected with an artificial enrichment the biomass of freshwater *Ulva* thalli in such elements. *Ulva* thalli enriched in the above-mentioned metals as feed supplements for chicken will be tested on laying hens (Michalak and Chojnacka 2008).

Materials and methods

Study area

The studies were conducted from June to August 2010 during the optimum growth period for the freshwater populations of *Ulva*. Thalli samples of the genus *Ulva* were collected from two aquatic ecosystems in the western part of Poland, from Malta Lake (ten sites) and from Nielba River (six sites) (Fig. 1).

The Nielba River, which is located in the city of Wągrowiec, has a length of approximately 27 km and reaches a width of 2.0–2.5 m in the locations where the samples were collected (N1–N6) (Fig. 1a). The Malta Lake, which is located in the centre of Poznan city, has a surface of 0.64 km^2, with a length of 2.2 km, a width of 0.46 km and an average depth of 3 m. Freshwater *Ulva* were found in the northern part of the lake (sites M1–M4) and on the southern shore (M5–M10) (Fig. 1b).

Sampling

Samples of water, sediment and thalli were collected in parallel at each site. Water was sampled by hand to the 1.0 L sterile plastic bottles (Roth) directly from under freshwater *Ulva* mats. Veterinary grade gloves (with long sleeves) were used for the purpose of sampling to avoid any pollution. River samples were taken by means of telescopic extension arm (Geomor-Technik). On the lake, water samples were taken from a rowing boat in order to approach cautiously the freshwater *Ulva* mat on the pelagic zone side because of the need to avoid potential deposit displacement (stagnant water). Each sample was filtered through a coarse plastic sieve in order to separate water from the vascular plants (such as e.g. Lemna) and filament algae (such as e.g. Cladophora). Next water samples were placed in a 0.5 L sterile plastic container (Roth), preserved using 15 % HNO_3 and stored at 4 °C. In the laboratory, the samples were filtered through a nitrocellulose microbiological filter with a pore size of 0.45 microns and deposited in a freezer at −20 °C.

Sediment samples (1 cm of the surface layer) were collected from directly underneath the *Ulva* mat using a tube sediment sampler (with a diameter of 5 cm and a length of 1 m). The sediment was sieved through a nylon sieve (with a mesh diameter of 1 mm–500 µm) to remove plant debris, sand, stones and other impurities. The resulting sediment fraction was dried for 2 h at 105 °C (Kamala-Kannan et al. 2008).

Thalli samples were collected from the centre of the macroalgae mats that were formed by *Ulva*. Thalli were sampled by hand using veterinary-grade gloves (similarly as in the case of water samples). Given the mass development of *Ulva* mats in the Nielba River at a very low water level in the streamway, thalli were sampled directly from the river bank. In the case of the Malta Lake, a rowing boat was used for manual thalli sampling into 1 L sterile plastic containers (Roth). Approximately 500 g of thalli was collected and transported at 4 °C. In the laboratory, the *Ulva* thalli were rinsed five times with distilled water to remove the attached filamentous algae, vascular plants and sand. The thalli were then dried for 30 min at room temperature and later for 2 h at 105 °C to the dry weight (AOAC 1997).

Physico and chemical analysis of water

The physicochemical parameters of waters from all sites containing freshwater *Ulva* were analyzed. Water temperature,

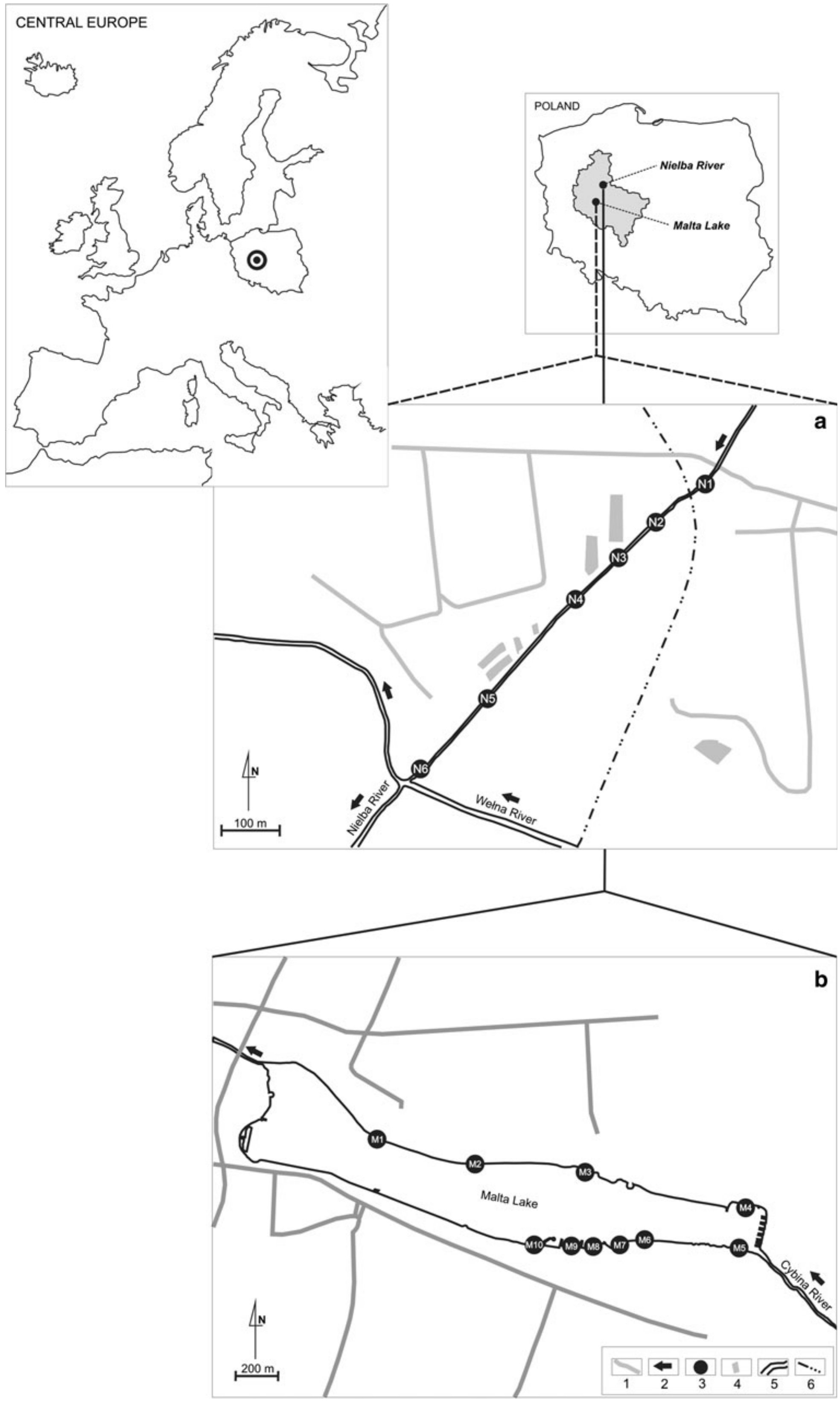


Fig. 1 The locations of the sampling sites on the **a** Nielba River and **b** Malta Lake. *1* main road, *2* water flow direction, *3* research sites, *4* ponds, *5* river, *6* railway

pH, TDS, conductivity and oxygen levels were measured using YSI Professional Plus with this analysis. Additionally, water samples (500 mL) were collected for chemical analyses and preserved with 0.5 mL of chloroform. These samples were then stored in refrigerators at 4 °C, with chemical analyses performed using standard methods for a HACH DR 2800 spectrophotometer (Hermanowicz et al. 1999). Concentrations were determined for the following variables: ammonium, nitrate, phosphate, sulfate, sodium chloride and iron.

Sample mineralisation

The extraction of the metal from the *Ulva* thalli was carried out by digesting 0.5 g of algae with a mixture of 15 mL of 65 % HNO_3 and 5 mL of 30 % H_2O_2 in Teflon containers in a Mars X5 microwave oven. All samples were mineralised in two steps: I—300 s at 400 W; II—300 s at 800 W (AOAC 1997). After mineralisation, the samples were quantitatively transferred into 10 ml flasks and filled with redistilled water (Ho 1987). Subsequently, the content ions was determined. All glass wares, plastic devices and Teflon devices were thoroughly acid washed (Moody and Lindstrom 1977).The labile fraction of the sediment was extracted with 1 M HCl. The samples were stored overnight to allow for the complete removal of generated CO_2. Subsequently, the samples were shaken mechanically at room temperature for 1 h. The extract was centrifuged at 5,000 rpm for 2 min. The extraction of the metal was carried out by digesting 0.4 g of the sediment and following the procedure for macroalgae mineralisation (see above).

The extraction of the metal from the water was carried out by digesting 25 mL and following the same protocol as described for thalli and sediment.

Analytical procedures

The content of the Co, Cr, Cu, Mn and Zn ions in the samples were determined after mineralisation in a Mars Xpress microwave oven, using an inductively coupled plasma emission spectrometer VISTA-MPX (VARIAN ICP). The calibration was performed using aqueous standard solutions. The detection limits values for the studied elements were as follows (μg g^{-1} or μg mL^{-1}): Co −1, Cr −1, Cu −2, Mn −1 and Zn −1.

Bioconcentration factor

The bioconcentration factor (BCF) provides an index of the ability of the plant to accumulate metal with respect to the metal concentration in the substrate. The BCFs were calculated as described by Zayed et al. (1998) based on the concentration of a given element in water, sediment and plant tissues. The BCF was calculated as follows: BCF = the mean concentration of the metal in the plant tissue (μg g^{-1} dry weight)/the mean concentration of the metal in the water (μg mL^{-1}) or the sediment (μg g^{-1} dry weight).

Statistical analysis

The STATISTICA 10.0 software was used for the statistical analysis. The correlation between the metal concentration in the algae, the sediment and the water was defined using a Spearman rank correlation coefficient. The differences in the average concentrations of the metals between the sites were estimated using the *F* test (ANOVA). The differences in average concentration of the physico-chemical properties of water between the Nielba River and the Malta Lake were estimated using the Kruskal–Wallis test.

Results

Physical and chemical parameters of water

During the study the values of analyzed physico-chemical parameters of water at sampling sites in the littoral of Lake Malta looked very congruent (sites M1–M10). A similar situation occurred in case of the Nielba River, where chemical parameters from different sites were homogenous as a result of constant flow and fast current of waters in the river as well as the proximity of sampling positions (sites N1–N6) (Fig. 1). When compared, the water in the Nielba River was characterized by a lower temperature, pH, oxygen and ammonium ions concentrations in relation to the waters of Lake Malta (Table 1). Despite the differences in the measurements, there were no significant statistical differences between the concentrations of DO, P–PO_4, Cl^-, Fe_{total} and N–NH_3 found in the waters collected from the river and the lake. For the above parameters the values of Kruskal–Wallis test were successively: 0.65 ($P = 0.4203$), 0.0024 ($P = 0.9607$), 0.02 ($P = 0.8874$), 0.4254 ($P = 0.5143$) and 0.1254 ($P = 0.7233$). However, statistically significant differences between the values of parameters in the waters of the river and the lake were noted in case of: TDS (KW–H = 36.15; $P < 0.05$), conductivity (KW–H = 35.8; $P < 0.05$), SO_4^{2-} (KW–H = 33.92; $P < 0.05$), N–NO_3 (KW–H = 29.7; $P < 0.05$), pH (KW–H = 25.73; $P < 0.05$), and water temperature (KW–H = 23.3; $P < 0.05$). Due to its current the water of the Nielba River was cooler than at the sites of the shallow littoral of Lake Malta. During the study period the water of the river was also more mineralized (TDS > 520 mg L^{-1}; conductivity > 780 μS cm^{-1}) than the water of the lake and contained higher concentrations

Table 1 Physico-chemical properties of the Nielba River and the Malta Lake

	Temp (°C)	pH (−)	DO ($mg\ L^{-1}$)	Cond. (µS cm)	TDS ($mg\ L^{-1}$)	Cl^- ($mg\ L^{-1}$)	$N\text{-}NO_3^-$ ($mg\ L^{-1}$)	$N\text{-}NH_3^+$ ($mg\ L^{-1}$)	$P\text{-}PO_4^{3-}$ ($mg\ L^{-1}$)	SO_4^{2-} ($mg\ L^{-1}$)	Fe_{total} ($mg\ L^{-1}$)
Nielba River n = *21*											
Mean (SE)	20.92 (0.39)	8.15 (0.11)	8.51 (0.25)	784.19 (13.67)	530.90 (2.62)	64.74 (2.37)	1.10 (0.11)	0.41 (0.07)	0.17 (0.03)	143.10 (6.12)	0.05 (0.02)
Min–max	18.60–24.46	7.40–9.97	6.11–10.74	719–860	519–563	55.50–110	0.40–1.90	0.17–1.40	0.03–0.69	90–200	0–0.29
Malta Lake n = *29*											
Mean (SE)	24.57 (0.38)	8.67 (0.03)	8.74 (0.03)	648.90 (3.76)	426.51 (1.94)	62.62 (0.31)	0.24 (0.04)	0.50 (0.08)	0.16 (0.02)	81.04 (1.94)	0.02 (0.01)
Min–max	21–27.90	8.24–11.85	4.44–8.99	607.01–681	416–448.5	59.25–65.50	0–0.60	0.02–1.34	0.03–0.41	62–102	0.01–0.05

DO dissolved oxygen, *Cond* conductivity, *TDS* total dissolved solids, *SE* standard error

of nitrate (>1.0 mg L^{-1}) and sulfate ions (>140.0 mg L^{-1}) (Table 1).

The metal concentrations in the water and the sediment

Higher concentrations of metals were found in the river sediment under the *Ulva* mats as compared to water. The average concentrations for Co, Cr, Cu, Mn and Zn in the sediment amounted to 2.80, 13.85, 25.21, 160.66 and 111.70 µg g^{-1}, respectively. However, in the river water, the Co concentration was below the range of detectability. The remaining metal concentrations were 0.16 µg mL^{-1} for Cr, 0.01 µg mL^{-1} for Cu, 0.06 µg mL^{-1} for Mn and 0.03 µg mL^{-1} for Zn. The mean metal concentrations from the river sediment and water decreased in the following order: Mn > Zn > Cu > Cr > Co and Cr > Mn > Zn > Cu > Co, respectively (Table 2). In the river sediment, the highest Co (5.63 µg g^{-1}) and Cu (58.57 µg g^{-1}) concentrations were noted at site N3 and of Cr at site N4 (45.53 µg g^{-1}), while the lowest for these metals was found at site N5 (0.75, 2.45, 3.88 µg g^{-1}, accordingly). In the river water, the highest (0.23 µg mL^{-1}) and the lowest (0.07 µg mL^{-1}) Cr concentrations were simultaneously observed at site N4, where also the highest concentration of Cu appeared (0.04 µg mL^{-1}).

Interestingly, Mn was one of two elements that had high concentrations in the sediment at a majority of the sites. The highest Mn concentration in the sediment was found at site N3 (512.18 µg g^{-1}) and the lowest at the last site, N6 (43.26 µg g^{-1}). In the water the lowest detectable Mn concentration (0.02 µg mL^{-1}) appeared once during the period of examinations at all river sites. The concentration of Zn in the river sediment was the highest at site N3 (263.20 µg g^{-1}), while in the river water it ranged from 0.01 µg mL^{-1} (N5, N6) to 0.08 µg mL^{-1} at site N4 (Table 2).

The average concentrations of Co, Cr, Cu, Mn and Zn in the sediment samples from the lake were 1.90, 7.15, 43.53, 268.81 and 40.32 µg g^{-1}, respectively. As in the river water, the Co concentration in the lake water was below the threshold of detectability, but the values for Cr, Cu, Mn and Zn were 0.13, 0.02, 0.10 and 0.03 µg mL^{-1}, appropriate. The mean metal concentrations in the lake sediment decreased in the following order: Mn > Cu > Zn > Cr > Co and in water decreased in the same way as the river water: Cr > Mn > Zn > Cu > Co (Table 3).

In lacustrine sediments, the highest concentration of Co (9.20 µg g^{-1}) and Cr (36.74 µg g^{-1}) was found at site M1, while the lowest concentration was found at sites M6 (Co—0.48 µg g^{-1}) and M5 (Cr—1.97 µg g^{-1}). For Cu, the highest concentration in the sediment was found at site M4 (306.65 µg g^{-1}) and the lowest at site M5 (0.14 µg g^{-1}). At sites M6, M7 and M8, a high concentration of Cu was noted

Table 2 The mean value (x) ± the standard error, the range of metal concentrations in freshwater *Ulva*, the sediment and the water from all of the stations in the Nielba River (μg g^{-1} dry weight for freshwater *Ulva* and the sediment, μg mL^{-1} for water)

	Freshwater *Ulva*		Sediment		Water	
	x ± SE	Range	x ± SE	Range	x ± SE	Range
Co	1.35 ± 0.12 (21)	0.63–2.52	2.80 ± 0.31 (22)	0.75–5.63	BDL	BDL
Cr	45.49 ± 4.18 (21)	36.57–115.72	13.85 ± 1.98 (22)	3.88–45.53	0.16 ± 0.01 (22)	0.07–0.23
Cu	11.37 ± 1.42 (21)	2.26–26.29	25.21 ± 3.69 (22)	2.45–58.57	0.01 ± 0.01 (22)	BDL–0.04
Mn	323.85 ± 39.62 (21)	99.94–911.59	160.66 ± 20.69 (22)	43.26–512.18	0.06 ± 0.01 (22)	0.02–0.27
Zn	157.98 ± 44.03 (21)	7.60–738.07	111.70 ± 15.40 (22)	9.31–263.20	0.03 ± 0.01 (22)	0.01–0.08

The number of samples is given in brackets

BDL below detection limit

Table 3 The mean value (x) ± standard error, the range of metal concentrations in freshwater *Ulva*, the sediment and the water from all of the stations in Malta Lake (μg g^{-1} dry weight for freshwater *Ulva* and the sediment, μg mL^{-1} for water)

	Freshwater *Ulva*		Sediment		Water	
	x ± SE	Range	x ± SE	Range	x ± SE	Range
Co	3.46 ± 0.44 (29)	0.63–8.83	1.90 ± 0.42 (30)	0.48–9.20	BDL	BDL
Cr	253.25 ± 34.89 (29)	43.06–676.06	7.15 ± 1.24 (30)	1.97–36.74	0.13 ± 0.01 (30)	0.06–0.16
Cu	18.47 ± 2.80 (29)	5.89–70.63	43.53 ± 12.38 (30)	0.14–306.65	0.02 ± 0.01 (30)	0.01–0.03
Mn	549.90 ± 42.02 (29)	140.51–1120.73	268.81 ± 70.80 (30)	21.72–1637.40	0.10 ± 0.01 (30)	0.03–0.23
Zn	36.34 ± 10.61 (29)	6.80–322.72	40.32 ± 7.09 (30)	6.88–173.10	0.03 ± 0.01 (30)	0.01–0.06

The number of samples is given in brackets

BDL below detection limit

in water (0.03 μg mL^{-1}). The concentration of Mn in the lacustrine sediment was the highest of all elements investigated (max. 1,637.4, min. 21.72 μg g^{-1}). The Mn concentration in water was on average a few hundred to a few thousand times lower than that in the sediment. For Zn, concentrations in the sediment were the highest at site M4 (173.10 μg g^{-1}) and the lowest at site M5 (6.88 μg g^{-1}). In lake water, the Zn concentrations were low and reached values of only 0.01 μg mL^{-1} at site M7 to 0.06 μg mL^{-1} at site M5.

The metal concentrations in freshwater Ulva from the Nielba River

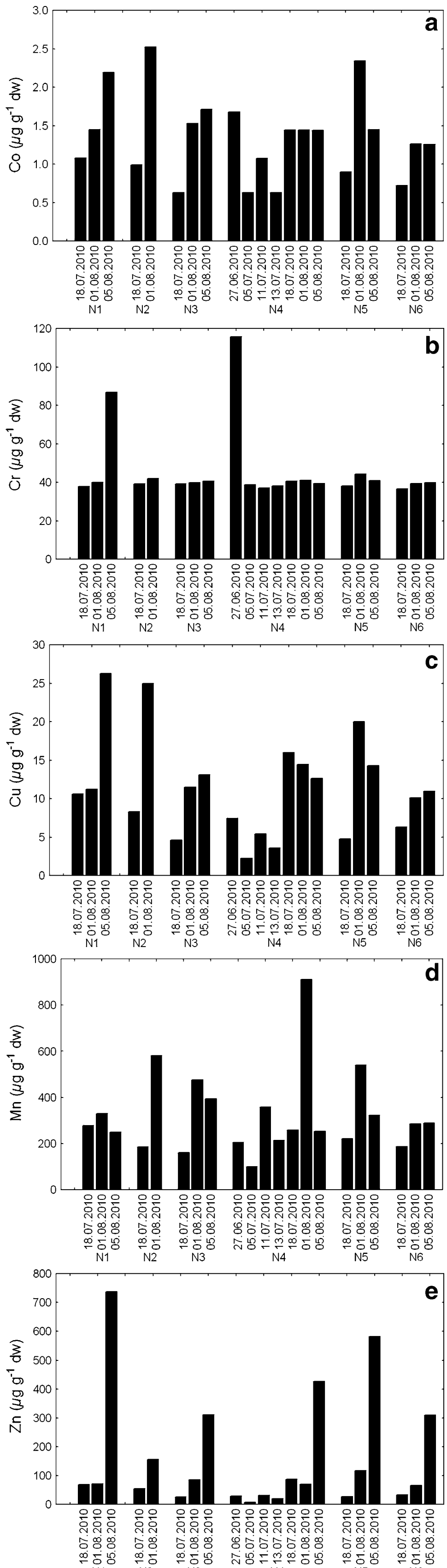
The average concentrations of Co, Cr, Cu, Mn and Zn in the *Ulva* thalli from the river were 1.35 μg g^{-1} dry weight (d.w.), 45.49, 11.37, 323.85 and 157.98 μg g^{-1} d.w., respectively (Table 2). The mean metal concentrations in the *Ulva* thalli from the river decreased in the following order: Mn > Zn > Cr > Cu > Co. This series was quite different compared to data of the lacustrine sediments or of the lake water. The highest concentration of Co in the algal thalli was noted at site N2 (2.52 μg g^{-1} d.w.) in August, while the lowest concentration was found in *Ulva* from site N4 (0.63 μg g^{-1} d.w.), which were collected in July. In the thalli from sites N1, N2 and N6, a regular increase in the Co concentration was observed from June or July to August (Fig. 2a).

The highest concentration of Cr in the thalli was noted in June at site N4 (115.72 μg g^{-1} d.w.), and the lowest in July at site N6 (36.57 μg g^{-1} d.w.) but usually stayed on comparatively level (~40 μg g^{-1} d.w.) over time and were not subject to distinct changes (Fig. 2b).

The concentrations of Cu in the *Ulva* cells were the highest at sites N1 (26.29 μg g^{-1} d.w.) and N2 (24.97 μg g^{-1} d.w.) in August, while the lowest value was observed at site N4 (2.45 μg g^{-1} d.w.) at the beginning of July. An increase in the concentration of Cu was noted at sites N1, N2, N3, and N6, while a decrease was noted at sites N4 and N5 (Fig. 2c). A similar situation was found with respect to changes in the Co concentrations in thalli.

The maximum Mn level (August—911.59 μg g^{-1} d.w.) and the lowest (July—99.94 μg g^{-1} d.w.) for *Ulva* was observed at site N4. Fluctuations in the Mn level in thalli were found at the majority of river sites except, for sites N2 and N6, where increases in the level of this metal were recorded (Fig. 2d).

The highest level of Zn (738.07 μg g^{-1} d.w.) was found in the *Ulva* thalli that were collected at site N1, and the lowest at site N4 (7.60 μg g^{-1} d.w.). At the beginning of



◀ **Fig. 2** The variation in metal levels in *Ulva* for the period from June to August 2010 for each sampling site in the Nielba River. *N1–N6* sampling sites in the river

the vegetative season, in June and July, slight fluctuations in the Zn level of the *Ulva* thalli appeared at site N4 (Fig. 2e).

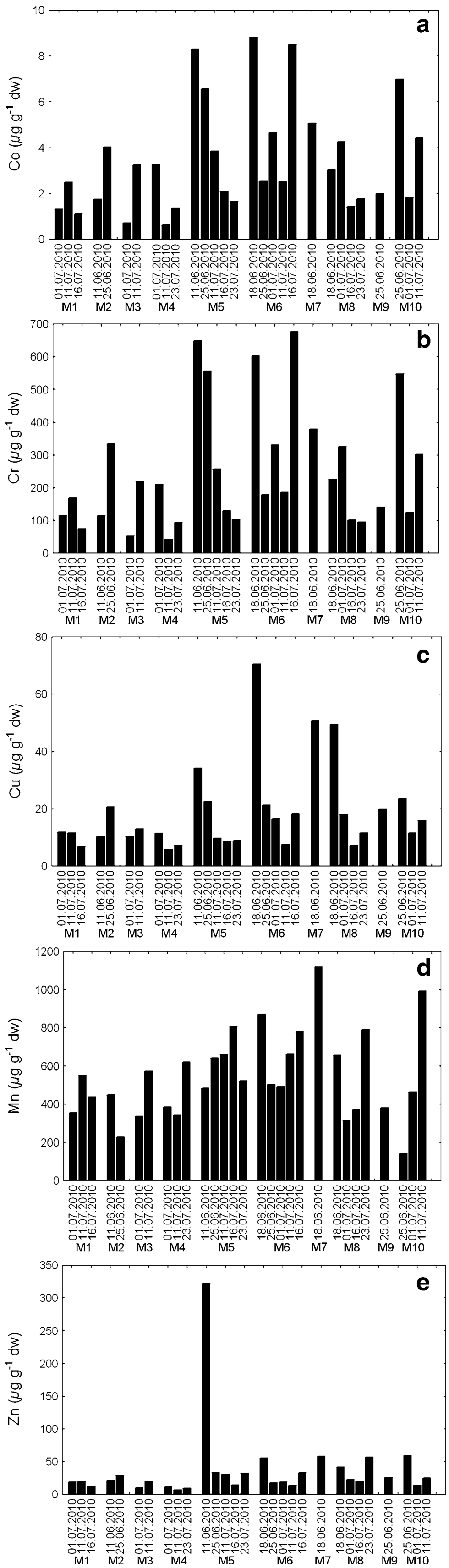
The metal concentrations in freshwater Ulva from Malta Lake

The mean metal concentrations in the *Ulva* thalli decreased in the following order: Mn > Cr > Zn > Cu > Co. The average concentrations of Co, Cr, Cu, Mn and Zn in the *Ulva* thalli were 3.46, 253.25, 18.47, 549.90 and 36.34 µg g^{-1} d.w., respectively (Table 3). The highest concentration of Co in the *Ulva* thalli that were developing in the lake was noted at site M6 (June—8.83 µg g^{-1} d.w.) and the lowest concentration was registered at site M4 (July—0.63 µg g^{-1} d.w.). Fluctuations in the concentration of Co in thalli were found at sites M1, M4, M6, M8 and M10, while at site M5, there was a steady Co decrease from 8.30 µg g^{-1} d.w. in June to only 1.66 µg g^{-1} d.w. at the end of July (Fig. 3a).

When analysing the concentrations of Cr, the highest concentration in the thalli was observed in July at site M6 (676.06 µg g^{-1} d.w.), and the lowest (43.06 µg g^{-1} d.w.) at site M4. During the *Ulva* development, the concentration of Cr in its thalli almost tripled during the consecutive periods of sample collection at the M2 and M3 sites. A fluctuation in the Cr concentrations from thalli coming from the M4, M6 and M7 sites, with a distinct decrease in concentration from 648.55 µg g^{-1} d.w. in June to 103.77 µg g^{-1} d.w. in July, was also observed (Fig. 3b).

The concentration of Cu in the *Ulva* thalli was the highest at site M6 (70.63 µg g^{-1} d.w.), and the lowest at the beginning of July at site M4 (5.89 µg g^{-1} d.w.). A small increase in the Cu concentration was found at the M2 and M3 sites, while a slight fluctuations were observed at the remaining sites (Fig. 3c).

The highest Mn concentration (1,120.73 µg g^{-1} d.w.) was observed in the macroalgae from site M7, and the lowest (140.51 µg g^{-1} d.w.) was found in the thalli from site M10. In the algal cells at sites M3 and M10, there was an increase in the concentration of Mn during this study. Moreover, a decrease was observed for the Mn level only in the thalli from site M2, from 449.71 to 226.44 µg g^{-1} d.w. (Fig. 3d). Fluctuations in the concentration of Zn were recorded at all of the sites, except for M2 and M3, where a slight increase of this metal was found over time (Fig. 3e).



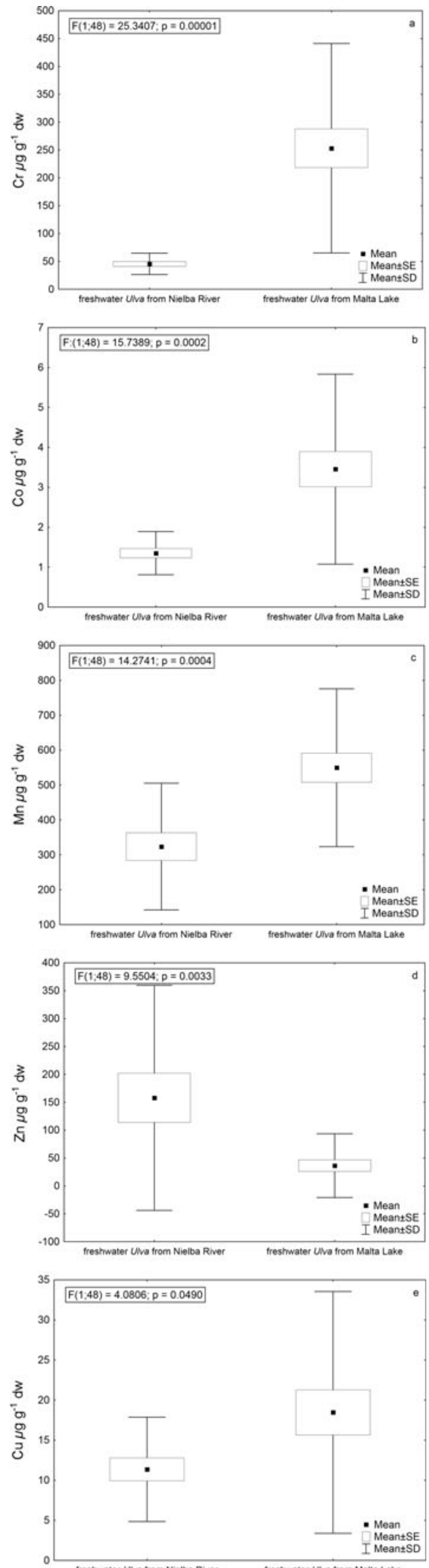
◀ **Fig. 3** The variation in metal levels in *Ulva* for the period from June to July 2010 for each sampling site in Malta Lake. *M1–M10* sampling sites in the lake

The comparison of the metal concentrations in Ulva from two freshwater ecosystems

Statistically significant differences were noted between the mean concentrations of the heavy metals that were examined in the thalli of the *Ulva* that were collected from the river and from the lake. The greatest difference appeared in the mean concentrations of Cr, where $F_{(1;48)} = 25.34$; ($P < 0.05$) (Fig. 4a). Similarly, the average concentrations of Co and Mn in the *Ulva* thalli differed between the samples from the different water ecosystems (Fig. 4b, c). In the case of Zn and Cu, the differences between the concentrations in the thalli from the river and lake were $F_{(1;48)} = 9.55$ ($P < 0.05$) and $F_{(1;48)} = 4.08$ ($P < 0.05$), respectively (Fig. 4d, e). In the *Ulva* thalli that were collected from Lake Malta, the mean concentration of Cr was on average 5.5 times higher than that in from the Nielba River.

The correlation coefficients of metals in the water, the sediment and in the freshwater Ulva

As a result of the Spearman rank correlations between concentrations of the same metals, which were found in the investigated fractions (*Ulva* thalli, the sediment, and the water), statistically significant relationships were found in the fluvial and lake populations. In the *Ulva* river population, a statistically significant negative correlation was found between the concentration of Mn in the *Ulva* thalli and in the water ($P < 0.05$; $rs = -0.59$) (Table 4). The concentration of Mn in the lake water demonstrated a positive correlation ($P < 0.05$; $rs = 0.45$) only with the sediment (Table 5). Numerous statistically significant correlations, however, were found between the concentrations of individual metals in the *Ulva* thalli, the sediment, and the water. For the river-macroalgae, the most statistically significant positive correlation between the metal concentrations within the *Ulva* thalli was found between Zn and Cu ($P < 0.001$; $rs = 0.88$) (Table 6). However, the strongest correlation was found between Cr and Co levels ($P < 0.001$; $rs = 0.98$) (Table 7) for the lake-originating *Ulva* thalli. Correlations between the concentrations of Zn and Cu ($P < 0.001$; $rs = 0.79$) and Zn and Mn ($P < 0.01$; $rs = 0.57$) were found in the fluvial water, whereas a negative correlation between the levels of Zn and Cr ($P < 0.001$; $rs = -0.75$) was found in the lake water. Only statistically significant correlations between the concentrations of metals in the fluvial sediment was found. The



◀**Fig. 4** The comparison of the mean concentrations of metals in freshwater *Ulva* thalli (µg g^{-1}dry weight) between populations from Malta Lake and the Nielba River. *SD* standard deviation, *SE* standard error

strongest positive correlation was found between Zn and Cu ($P < 0.001$; $rs = 0.97$). Statistically significant results were not found between all metal levels in the lake sediment. No correlation was found between Cr and Cu, Cr and Mn, or Cr and Zn. However, for the lake sediment, the strongest correlation was found between Zn and Cu ($P < 0.001$; $rs = 0.76$) (Tables 6, 7).

The correlation coefficients of metals in the freshwater Ulva and physico-chemical properties of water

The Spearman rank correlation analysis between physico-chemical parameters of the river and the lake water which contained freshwater *Ulva* and the concentration of metals in macroalgae thalli showed the series of statistically significant results. Changes in the concentration of all tested metals in freshwater *Ulva* thallus originating from the river were correlated with temperature, conductivity, oxygen concentration, and nitrate ions. In case of the *Ulva* population from the river the strongest correlation was observed between the concentration of zinc and nitrate ions ($P < 0.001$; $rs = 0.82$). Similarly, cobalt concentrations were correlated with changes in the concentration of nitrate ions in the water ($P < 0.001$; $rs = 0.75$). For other metals, the strongest correlations were found for temperature and they amounted to: for Cr $rs = 0.69$ ($P < 0.001$), for Cu $rs = -0.78$ ($P < 0.001$) and for Mn $rs = -0.66$ ($P < 0.01$) (Table 8). Changes in the concentrations of individual metals in the thalli of river *Ulva* populations were correlated with a number of physico-chemical parameters of waters. Therefore, changes in the concentration of metal levels correlated with water parameters in the following way: zinc with six, Mn, Cr and Co with four, and in Cu levels with five water parameters.

In case of freshwater *Ulva* populations found in the lake there was only a single statistically significant correlation between the concentrations of metals in algal thalli and physico-chemical parameters of the water. For, while the changes in the concentration of Cu in thalli were negatively correlated with the water temperature ($P < 0.001$; $rs = -0.67$) and conductivity ($P < 0.01$; $rs = 0.56$), they were positively correlated with the changes of total dissolved solid ($P < 0.05$; $rs = 0.46$). On the other hand, changes in the concentration of zinc in freshwater *Ulva* thalli were affected by temperature ($P < 0.05$; $rs = -0.38$) and TDS ($P < 0.05$; $rs = 0.56$). There was also a negative correlation between manganese in algal thalli and the concentration of nitrate ions in the lake water ($P < 0.05$; $rs = -0.41$) (Table 8).

Table 4 The Spearman rank correlation coefficients between metal concentrations in freshwater *Ulva*, the sediment and the river water

	Co	Cr	Cu	Mn	Zn
Freshwater *Ulva*–river water	0.139^{ns}	−0.159^{ns}	−0.04^{ns}	**−0.597***	0.287^{ns}
Freshwater *Ulva*–sediment	0.054^{ns}	0.187^{ns}	0.106^{ns}	0.003^{ns}	0.01^{ns}
River water–sediment	0.083^{ns}	−0.06^{ns}	−0.08^{ns}	0.116^{ns}	−0.08^{ns}

* $P < 0.05$, $n = 21$ for water and sediment, $n = 22$ for *Ulva*

ns not significant at significance level 0.05

Bold value is statistically significant correlation

Table 5 The Spearman rank correlation coefficients between metal concentrations in freshwater *Ulva*, the sediment and the lake water

	Co	Cr	Cu	Mn	Zn
Freshwater *Ulva*–lake water	−0.153^{ns}	0.227^{ns}	0.185^{ns}	0.083^{ns}	−0.514^{ns}
Freshwater *Ulva*–sediment	−0.159^{ns}	−0.259^{ns}	−0.106^{ns}	0.027^{ns}	0.081^{ns}
Lake water–sediment	−0.019^{ns}	−0.206^{ns}	0.295^{ns}	**0.451***	0.080^{ns}

ns not significant at significance level 0.05

* $P < 0.05$, $n = 30$ for water and sediment, $n = 29$ for *Ulva*

Bold value is statistically significant correlation

Table 6 The Spearman rank correlation coefficients between the concentrations of different metals in freshwater *Ulva*, the river water and the sediment

	Co	Cr	Cu	Mn	Zn
Freshwater Ulva					
Co	1.000	**0.865*****	**0.857*****	**0.663****	**0.740*****
Cr		1.000	**0.761*****	**0.442***	**0.589****
Cu			1.000	**0.668*****	**0.885*****
Mn				1.000	**0.562****
Zn					1.000
River water					
Co	1.000	0.333^{ns}	0.230^{ns}	0.136^{ns}	0.364^{ns}
Cr		1.000	0.413^{ns}	0.224^{ns}	0.339^{ns}
Cu			1.000	0.279^{ns}	**0.799*****
Mn				1.000	**0.573****
Zn					1.000
Sediment					
Co	1.000	**0.924*****	**0.926*****	**0.706*****	**0.953*****
Cr		1.000	**0.817*****	**0.578****	**0.840*****
Cu			1.000	**0.678*****	**0.977*****
Mn				1.000	**0.705*****
Zn					1.000

ns not significant at significance level 0.05

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $n = 21$ for water and sediment, $n = 22$ for *Ulva*

Bold values are statistically significant correlations

Table 7 The Spearman rank correlation coefficients between the concentrations of different metals in freshwater *Ulva*, the lake water and the sediment

	Co	Cr	Cu	Mn	Zn
Freshwater Ulva					
Co	1.000	**0.983*****	**0.768*****	0.336^{ns}	**0.662*****
Cr		1.000	**0.787*****	0.271^{ns}	**0.666*****
Cu			1.000	0.154^{ns}	**0.729*****
Mn				1.000	0.293^{ns}
Zn					1.000
Lake water					
Co	1.000	0.099^{ns}	0.072^{ns}	0.081^{ns}	0.164^{ns}
Cr		1.000	1.131^{ns}	0.302^{ns}	**-0.757*****
Cu			1.000	0.157^{ns}	**-0.05**^{ns}
Mn				1.000	**-0.150**^{ns}
Zn					1.000
Sediment					
Co	1.000	**0.628*****	**0.563****	**0.424***	**0.727*****
Cr		1.000	0.354^{ns}	0.051^{ns}	0.360^{ns}
Cu			1.000	**0.562****	**0.761*****
Mn				1.000	**0.640*****
Zn					1.000

ns not significant at significance level 0.05

* $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$, $n = 30$ for water and sediment, $n = 29$ for *Ulva*

Bold values are statistically significant correlations

Table 8 The Spearman rank correlation coefficients between the concentrations of different metals in freshwater *Ulva* and the physico-chemical properties of water

	Co	Cr	Cu	Mn	Zn
River water					
Temperature	$\mathbf{-0.74}^{***}$	$\mathbf{0.69}^{***}$	$\mathbf{-0.78}^{***}$	$\mathbf{-0.66}^{**}$	$\mathbf{-0.76}^{***}$
pH	0.32^{ns}	0.24^{ns}	0.35^{ns}	$\mathbf{0.57}^{**}$	0.39^{ns}
DO	$\mathbf{0.66}^{**}$	$\mathbf{0.68}^{***}$	$\mathbf{0.73}^{***}$	$\mathbf{0.52}^{*}$	$\mathbf{0.75}^{***}$
Conductivity	$\mathbf{-0.66}^{**}$	$\mathbf{-0.51}^{*}$	$\mathbf{-0.70}^{***}$	$\mathbf{-0.65}^{**}$	$\mathbf{-0.76}^{***}$
Cl^-	-0.04^{ns}	-0.05^{ns}	-0.20^{ns}	-0.15^{ns}	-0.24^{ns}
$N–NO_3^-$	$\mathbf{0.75}^{***}$	$\mathbf{0.62}^{**}$	$\mathbf{0.72}^{***}$	$\mathbf{0.59}^{**}$	$\mathbf{0.82}^{***}$
$N–NH_3^+$	-0.05^{ns}	0.07^{ns}	0.11^{ns}	-0.27^{ns}	0.31^{ns}
$P–PO_4^{3-}$	-0.23^{ns}	-0.24^{ns}	0.02^{ns}	-0.27^{ns}	0.22^{ns}
SO_4^{2-}	-0.17^{ns}	-0.09^{ns}	-0.29^{ns}	-0.28^{ns}	$\mathbf{0.46}^{*}$
Fe_{total}	0.35^{ns}	0.26^{ns}	0.37^{ns}	0.24^{ns}	$\mathbf{0.52}^{*}$
Lake water					
Temperature	-0.29^{ns}	-0.35^{ns}	$\mathbf{-0.67}^{***}$	0.14^{ns}	$\mathbf{-0.38}^{*}$
pH	-0.30^{ns}	-0.36^{ns}	-0.31^{ns}	-0.07^{ns}	-0.37^{ns}
DO	0.16^{ns}	0.16^{ns}	0.34^{ns}	0.02^{ns}	0.03^{ns}
Conductivity	-0.19^{ns}	-0.23^{ns}	$\mathbf{-0.56}^{**}$	0.10^{ns}	-0.17^{ns}
TDS	0.33^{ns}	0.34^{ns}	$\mathbf{0.46}^{*}$	0.10^{ns}	$\mathbf{0.56}^{**}$
Cl^-	-0.30^{ns}	-0.28^{ns}	-0.19^{ns}	0.01^{ns}	0.07^{ns}
$N–NO_3^-$	-0.19^{ns}	-0.16^{ns}	-0.12^{ns}	$\mathbf{-0.41}^{*}$	-0.29^{ns}
$N–NH_3^+$	-0.23^{ns}	-0.20^{ns}	-0.08^{ns}	0.07^{ns}	0.02^{ns}
Fe_{total}	0.08^{ns}	0.07^{ns}	-0.02^{ns}	0.24^{ns}	-0.12^{ns}

ns not significant at significance level 0.05

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Bold values are statistically significant correlations

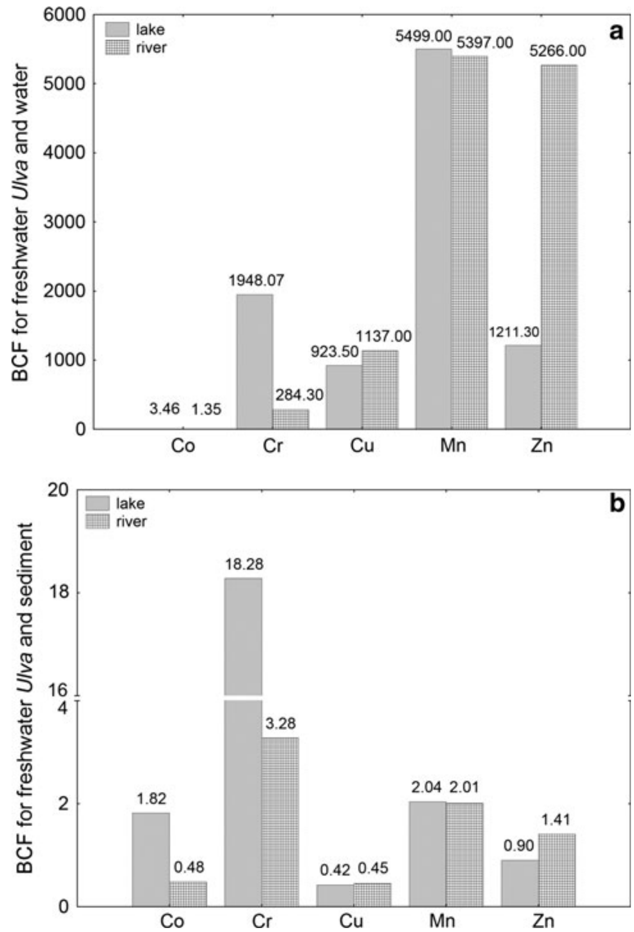


Fig. 5 The bioconcentration factors (BCF) for freshwater *Ulva*. **a** BCF for *Ulva* and water **b** BCF for *Ulva* and sediment

Bioconcentration factor

The maximum BCF values (for lake freshwater *Ulva*) for Mn, Cr, Zn, Cu and Co were, respectively: 5,499; ~1,948; ~1,211; ~923, and ~3.4. The BCF values (for fluvial freshwater *Ulva*) for Mn, Zn, Cu, Cr, and Co were 5,397; 5,266; 1,137; ~284; and 1.35 (Fig. 5a). The BCF values that were calculated for thalli and sediment fractions were very low. However, the largest BCF value for this system was calculated for Cr, which was analysed in the freshwater *Ulva* thalli that were sampled from the lake (BCF = 18.28) (Fig. 5b).

Discussion

Macroalgae are generally known for their ability to accumulate heavy metals in their cells; consequently, many brown and green algae (mostly of the *Ulva* genus) are commonly recognised as good biomonitors and bioindicators (Haritonidis and Malea 1995; Villares et al. 2002). However, the legitimacy of using these organisms for monitoring contaminated waters will require the availability of complete information on metal concentrations in the macroalgae habitat. Such information is obtained through the analysis of sediment and water samples as well as samples of different organisms that grow together with the macroalgae species (Phillips 1977). The detailed analysis of metal concentration fluctuations in freshwater *Ulva* thalli, in the water environment in which it develops and in the sediment that lies under the macroalgae mat, was a part of our study. The metal concentrations from the fluvial and the lake sediment were higher than those in the water samples that were taken from those aquatic ecosystems. In the fluvial sediment, the Co, Cr, and Zn concentrations were higher than those in the same lake-originating fraction. However, the Cu and Mn concentrations in the sediment from the lake were definitely higher than those in the samples from the river. Very small Co concentrations, usually under the detection limit, were found in water from the two ecosystems. Concentrations of the remaining tested elements in the water from the ecosystems that were specific to the freshwater *Ulva* development were very similar, specifically with regard to

Table 9 The comparison of metal concentrations (μg g^{-1} dry weight) in *Ulva* species that were observed by different authors

Species	Description	Metals					References
		Co	Cr	Cu	Mn	Zn	
Ulva compressa	Hong Kong (China)	–	–	8.0–9.4	18.0–25.0	17.0	Ho (1987)
Ulva compressa	Bosphorus (Turkey)	–	–	26.79	31.69	68.45	Güven et al. (1993)
Ulva clathrata	Agaba (Jordan)	–	–	38.6	22.4	41.8	Wahbeh (1984)
Ulva fasciata	Goa (India)	6.1–11.0	–	6.7–7.3	111.0–1075	2.8–24.0	Agadi et al. (1978)
Ulva fasciata	Rio de Janeiro (Brazil)	3.2	3.3	4.5	–	22.0	Lacerda et al. (1992)
Ulva flexuosa	Hong Kong (China)	–	–	7.9–10.2	74.0–98.0	28.0–63.0	Ho (1987)
Ulva lactuca	Oresund (Denmark)	–	–	9.5–22.0	–	78.0–91.0	Hägerhäll 1973
Ulva lactuca	Hong Kong (China)	–	–	14.03–134.19	–	1.40–8.61	Wong et al. (1982)
Ulva lactuca	Hong Kong (China)	–	–	16.0–18.0	53.0–211.0	27.0–66.0	Ho (1990)
Ulva lactuca	Bosphorus (Turkey)	2.6–3.9	2.9–7.8	13.0–25.0	11.0–24.0	31.0–67.0	Güven et al. (1993)
Ulva lactuca	Pulicat Lake (India)	–	24.68	–	–	–	Kamala-Kannan et al. (2008)
Ulva linza	Thermaikos Gulf (Greece)	–	–	1.9–4.3	–	49.7–141.3	Malea and Haritonidis (1999)
Ulva reticulata	Goa (India)	–	–	13.85	1721.04	8.89	Agadi et al. (1978)
Ulva rigida	Sinop (Turkey)	2.3–2.6	5.3–5.9	4.5–5.7	26.0–28.0	6.0–12.0	Güven et al. (1992)
Ulva rigida	Thermaikos Gulf (Greece)	–	0.18–10.7	–	–	–	Haritonidis and Malea (1995)
Ulva rigida	Lagoon Venice (Italy)	0.2–4.0	0.1–14.0	2.1–21.0	14.0–480.0	8.0–110.0	Favero et al. (1996)
Ulva rigida	Thermaikos Gulf (Greece)	0.14–2.36	0.53–28.4	2.5–8.2	27.0–783.0	6.3–54.7	Malea and Haritonidis (2000)
Enteromorpha sp.	North Sea (England)	–	–	–	–	19.0–437.0	Say et al. (1990)
Enteromorpha sp.	Oporto (Portugal)	–	–	6.4–16.9	–	–	Leal et al. (1997)
Freshwater *Ulva*	Nielba River (Poland)	0.63–2.52	36.57–115.72	2.26–26.29	99.94–911.59	7.60–738.07	Present study
Freshwater *Ulva*	Malta Lake (Poland)	0.63–8.83	43.06–676.06	5.89–70.63	140.51–1120.73	6.80–322.72	Present study

Enteromorpha sp. (syn. *Ulva* sp.)—only species with monostromatic tubular thalli

Cu and Zn. Likewise, Kamala-Kannan et al. (2008) found lower levels of heavy metals (Cr—five times, Cd—a hundred times) sampled from the *Ulva lactuca* habitat in the waters of North Chennai (India) when compared to their concentrations in the sediment. Alternatively, in the study conducted on the eastern Aegean coast by Akcali and Kucuksezgin (2011) the Cr concentration in the sediment was up to 150 times higher than that in the water and 30 times higher for Cu and Zn. Villares et al. (2001) and Puente (1992) highlight that the level of sediment contamination by heavy metals in estuaries is usually higher than that in the open sea due to their location in proximity to rivers, which continually supply impurities to the estuary.

The average concentrations of the heavy metals Co, Cr, Cu, and Mn were higher in freshwater lake-originating *Ulva* thalli than those in the thalli of the river *Ulva*. The only metal that had a higher concentration in the river thalli was Zn, which was more than four times higher than that in the thalli that were sampled from the lake. In the lake-originating *Ulva*, the concentration of Mn ranged from 140 to 1.120 μg g^{-1} d.w., compared to a range of 99–911 μg g^{-1} d.w. for the river thalli. Very high concentrations of Mn were also found in the thalli of marine *Ulva* species (Table 9). In the thalli of the two *Ulva* freshwater populations, the Co concentration was found to be the lowest out of all the tested elements. Our values for Co were very similar to those obtained by Lacerda et al. (1992), Güven et al. (1993) and Malea and Haritonidis (2000) in *U. fasciata*, *U. lactuca* and *U. rigida*. Cobalt is the least efficiently accumulated heavy metal by opportunistic taxa from *Ulva* genus. The two test populations of freshwater *Ulva* featured higher levels of Cr, Mn, and Zn when compared with the marine species of this genus, which were sampled at other geographical regions (Table 9).

Internal physiological parameters, such as macroalgae metabolic activity, cell wall thickness, growth dynamics, and chelator species, have an impact on the metal concentrations that are found in the macroalgae thalli. A similar influence can be attributed to the external conditions which prevail in algae habitats such as pH, temperature, the organic matter volume, the dissolved organic carbon, and natural ligands as well as the chemical parameters of the water, specifically the chloride levels (Ansari et al. 2004). Metal concentrations listed in algal thalli of *Ulva* genus is therefore associated with a number of biological, physical and chemical properties of water and sediment (Wangersky 1986). In laboratory studies it was frequently indicated that macroalgae accumulate only the available dissolved metal ions from the medium (Luoma 1983). As was shown by Lee and Wang (2001), the absorption rate of metals such as Fe, Mn, Zn, Cd and Pb by *Ulva fasciata* is also closely related to light intensity and nutrient availability. Another important factor influencing the bioavailability of heavy metals is salinity. Concentrations of physico-chemical parameters recorded in the Nielba River and the Malta Lake when conducting investigations classify them to rich in nutrients, eutrophic ecosystems (Table 1).

In coastal ecosystems such as estuaries, whose waters feature low salinity, the thalli of the local *Ulva* species feature higher heavy metal concentrations when compared to the same species that are sampled from more saline waters (Wang and Dei 1999). Furthermore, the results from an experimental study indicated that Cu accumulation by *U. reticulata* at salinity of 20 psu was three times higher than that at 35 psu (Mamboya et al. 2009). Similar results were published for two cosmopolitan *Ulva* species, where the accumulation of Zn and Cd in *U. rigida* and Cr and Zn in *U. lactuca* increased along with the decreasing salinity (Favero et al. 1996). In the experimental studies it was observed that reducing the salinity of the medium increases the amount of Cu ions accumulated by *Ulva reticulata* thalli (Mamboya et al. 2009). In our study, the freshwater *Ulva* populations lived in water, where the average Cl^- concentrations were below 110 mg L^{-1} in the river and below 65.50 mg L^{-1} in the lake (Messyasz 2009; Messyasz and Rybak 2011; Rybak and Messyasz 2011). Similar Cl^- concentrations were found in many estuaries in which studies focused on heavy metal bioavailability to species from the *Ulva* genus were conducted (Say et al. 1990). Hence, we can reasonably state that compared to sea species, the higher bioaccumulation of metals by freshwater *Ulva* (specifically with regards to Cr, Mn, and Zn) is most likely assisted by the low chloride levels in water at sites of these macroalgae. However, in case of examined freshwater *Ulva* populations there was no statistically significant correlation between the concentrations of Cl^- in the water and changes in the concentration of metals in the thallus. For marine species *U. lactuca,* along with increasing salinity of the water the decrease of metals accumulation such as Cu, Fe and Mn was observed while the levels of Cd and Zn rose (Kaimoussi et al. 2004).

The important factor influencing the bioavailability of metals is also the abundance of nutrients in surrounding waters. High concentrations of nutrients, such as phosphates and nitrates, have been reported to reduce the toxicity of heavy metals by several authors (Haglund et al. 1996). In our studies on freshwater *Ulva*, there were no correlations between the accumulation of metals and changes in the concentration of phosphate and ammonium ions in the water observed. However, in case of marine *Ulva fasciata* was demonstrated that Cd uptake increases with the rise in the concentration of nitrate in the medium. Besides, for freshwater *Ulva* we also indicated a strong correlation between the increase in the level of Co, Cr, Cu, Mn, and Zn accumulation and the rise of nitrates concentration in the water. It was observed, however, that in an environment with very high concentrations of nutrients in the water the inhibition of metals absorb due to the complexes formation between nutrients and ions of metals was observed (Göthberg et al. 2004). The knowledge about the chemical properties concentration and intensity of physical factors can broaden our attainments of the efficiency in the metals bioaccumulation by macroalgae. However, because of the comprehensive and synergistic impacts in these parameters between each other and between the metals, a full understanding of these processes is possible only in the case of experimental studies (Stokes 1983). The bioavailability of metals in water and sediments is also influenced by the impact of biological factors (Sunda and Huntsman 1998; Langston 1990). Likewise, the chemical conversions following in the sediment (changes in redox potential and pH), strongly determines the bioavailability of metals in the water. In our study limited to the freshwater *Ulva* population from the river, we pinpointed that with increasing pH value there was a rise in manganese accumulation. Michalak and Chojnacka (2009) in experimental studies on metal accumulation by *Ulva prolifera* (2009) observed that the species most efficiently collected chromium at pH values ranging from 5 to 7. However, the fixed level of accumulation of chromium in alga *U. prolifera* occurred at pH > 8 (Chojnacka 2008).

Absorption of metals by lifeless organic matter results in a decrease of metal toxicity by complexing ion fractions (Peterson et al. 1984). In low pH conditions heavy metals are predominant as cations in the water column, whereas at alkaline pH they precipitate as insoluble hydroxides, carbonates and phosphates (Florence et al. 1984). In consideration of this interaction to determine the concentration of metals in the water it is many times not correlated with the toxicity of metals in macroalgae thalli (Rai et al. 1981).

Still very little is known about the influence of temperature on the metals uptake by macroalgae. The study of Rai et al. (1981) demonstrated as well an increase as a decrease in the metal concentration in macroalgal thallus together with increasing temperature in the experimental system. In fact, for freshwater *Ulva,* temperature was one of the most important environmental factors affecting the level of metals accumulation. While the increase in the water temperature negatively influenced the accumulation of cobalt, copper, manganese and zinc, it also triggered the accumulation of chromium.

The temperature has a significant effect on the rate of chemical and biological processes in water and sediments that influence directly on the metal toxicity and their bioavailability to organisms (Fritioff et al. 2005).

There is also no available information on to what extent the changes in mineralization (TDS, conductivity) and the supply of iron, sulfates, and oxygen in the water affect the rate of accumulation of metals by algal taxa from *Ulva* genus. However, with the increase of water conductivity the decrease of Co, Cr, Cu, Mn and Zn accumulation in freshwater *Ulva* thalli was observed. This situation was particularly evident in the example of *Ulva* from the Nielba River. In addition, we observed that an increased concentration of oxygen in the water had a positive effect on the accumulation level of all examined metals for this population. Kaimoussi et al. (2004) in environmental studies carried out on *U. lactuca* noted a decrease in the accumulation of Cu, Mn and Zn together with the increase of oxygen concentration in the water. In contrast, the rise of the cadmium accumulation level in the thalli of this marine species coincided with increasing oxygen concentration in the environment. The relationship between the heavy metal concentrations in the thalli of the *Ulva* genus and its habitat has been studied by many authors (Villares et al. 2001; Malea and Haritonidis 1999). In the case of the freshwater *Ulva* river population in our study, a statistically significant yet negative correlation was found between Mn levels in the thalli and in water. Villares et al. (2001) also found a significant negative correlation between Mn levels in the sediment and the thalli of the *Ulva rigida* sea species. For Mn, our study found a significant correlation between the concentration in the lake water and that in the sediment. For investigated freshwater *Ulva*, the correlations between Cr levels in alga and in both the sediment and water was not found in any tested population. Alternatively, Kamala-Kannan et al. (2008) reported that Cr in the bay water demonstrated a positive correlation with the sediments from the *U. lactuca* habitat in the Pulicat Gulf. For Cu and Zn, Akcali and Kucuksezgin (2011) did not find any statistically significant correlations between the concentrations of these elements in the thalli, water, or sediment. The absence of such relationships was also confirmed in our *Ulva* test populations.

Heavy metal bioaccumulation power fluctuates considerably across various macroalgae species (Akcali and Kucuksezgin 2011). The BCF is mostly used for the purpose of evaluating the bioaccumulation power in macroalgae. The BCF allows for the estimation of the status of an aquatic ecosystem's maintenance stability or for monitoring the contamination thereof (Conti and Cecchetti 2001). In our study, the BCF was used to determine as the difference between the bioaccumulation of metals in two freshwater *Ulva* populations. Moreover, the BCF informs about the bioavailability rate of specific metals as early as at the first trophic level (producers). These BCF results indicate that the potential for Mn accumulation by freshwater *Ulva* was slightly higher than that for other metals. The results also confirm the use of freshwater *Ulva* as a good hyperaccumulator of Mn, Zn, Cu, and Cr. However, the BCF values were very low for Co (1.35 and 3.46). Based on the criteria given by Zayed et al. (1998), the BCF value over 1.000 is generally considered to be proof that the plant will be useful for phytoremediation. This study resulted in BCF values above 10^3 for Mn, Zn, Cr (only for *Ulva* from the lake), and Cu (only for *Ulva* from the river), indicating that freshwater *Ulva* are good hyperaccumulators of Mn and Zn, irrespective of the type of aquatic ecosystem for thalli development. In a study on the accumulation of metals by vascular aquatic species, the highest BCF (tissue-metal-to-water level ratio) was also found for Mn. For *Elodea canadensis*, the BCF was 743, whereas for *E. nuttallii*, it was 547. Conversely, the lowest BCF values were found for Cu, Cr, and Pb in the two investigated macrophytes (Thiébaut et al. 2010). In a study of metal phytoaccumulation in *Lemna gibba*, Khellaf and Zerdaoui (2010) found that the BCF values ranged from 300 to 966 for Cu and from 33 to 100 for Ni. These results confirm that the bioaccumulation of metals by macrophytes is less productive than algae. Experimental studies have determined that *Pithophora varia* (Chlorophyta) macroalgae have as much as ten times greater accumulation of Zn and Cu than the vascular aquatic plants (Michalak and Chojnacka 2008). Furthermore, the simple thalli and cell structure will also translate to higher BCF values in algae than in more developed plants. Macroalgae are able to accumulate higher concentrations of metals than seawater. We note that marine macroalgae from the *Ulva* genera are also a source of food for invertebrates and fish (Gerking 1994; Kamermans et al. 2002). Thus far, the findings have confirmed the emergence of a considerable biomass of freshwater *Ulva* in a water basin, which may become a source of food for pond snails, as it is often more attractive than the macrophytes (Rybak and Messyasz in press). Accordingly, a freshwater *Ulva* biomass containing high concentrations of Mn, Zn, and Cr becomes a way to introduce these elements into the trophic chain of native aquatic organisms. In this context, studies on the impact of the toxicity of these metals on the organisms that feed on freshwater *Ulva* thalli and on their predators seem reasonable.

Conclusions

The results of this study indicated that heavy metals such us Co, Cr, Cu, Mn and Zn were accumulated by freshwater *Ulva* growing both in the river and lake waters. An elevated ability to accumulate Mn and Cr in freshwater *Ulva* thalli compared with marine species from this genera suggested that in inland aquatic ecosystems its may play an important role as a bioaccumulator for heavy metals contamination in water. This conclusion was supported by the few hundred times higher levels of Mn and Zn concentrations observed in the freshwater *Ulva* thallus than in water from the same sites and statistically significant correlations between concentrations of Mn in the fluvial algal population and the water. Increasing water temperature and conductivity were the most important environmental factors affecting negatively the level accumulation of metals (except Cr for temperature) by macroalgae. In contrast, especially in the river, increase in the concentration of nitrates in water and the pH was present that appeared to have developed strong positive relationship with the accumulation of tested metals by *Ulva*. Additionally, significant differences in the level of all metals accumulation recorded between the river and lake *Ulva* populations suggested that locally derived *Ulva* spread may periodically contribute significantly to the high trace/heavy metals accumulation in its biomass.

Acknowledgments This project was supported by funds from the Polish Ministry of Science and Higher Education; Grant Number N N304 013 437. The field research was funded partially by the Project GDWB-07/2011. Andrzej Rybak has received a PhD fellowship from the Adam Mickiewicz University Foundation. We also thank the anonymous reviewers of this paper for the valuable, critical and helpful comments on the manuscript.

References

Agadi VV, Bhosle NB, Untawale AG (1978) Metal concentration in some seaweeds of Goa. Ind Bot Mar 21:247–250

Akcali I, Kucuksezgin F (2011) A biomonitoring study: heavy metals in macroalgae from eastern Aegean coastal areas. Mar Pollut Bull 62(3):637–645

Al-Shwafi NA, Rushdi AI (2008) Heavy metal concentrations in marine green, brown, and red seaweeds from coastal waters of Yemen, the Gulf of Aden. Environ Geol 55(3):653–660

Ansari TM, Marr IL, Tariq N (2004) Heavy metals in marine pollution perspective: a mini review. J Appl Sci 4:1–20

AOAC (1997) Official methods of analysis, 16th edn. AOAC International, Arlington

Bryan GW, Langston WJ (1992) Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries—a review. Environ Pollut 76:89–131

Chojnacka K (2008) Using biosorption to enrich the biomass of seaweeds from the Baltic sea with microelements to produce mineral feed supplement for livestock. Biochem Eng J 39(2): 246–257

Conti ME, Cecchetti G (2001) Biological monitoring: lichens as bioindicators of air pollution assessment—a review. Environ Pollut 114:471–492

Favero N, Cattalini F, Bertaggia D, Albergoni V (1996) Metal accumulation in a biological indicator (*Ulva rigida*) from the Lagoon of Venice (Italy). Arch Environ Contam Toxicol 31(1): 9–18

Florence TM, Lumsden BG, Fardy JJ (1984) Algae as indicators of copper speciation. In: Kramer CJM, Duinker JC (eds) Complexion of trace metals in natural waters. Dr. W. Junk Publishers, Netherlands, pp 411–418

Fritioff Å, Kautsky L, Greger M (2005) Influence of temperature and salinity on heavy metal uptake by submersed plants. Environ Poll 133:265–274

Gąbka M, Owsianny PM, Burchardt L (2010) The influence of co-occurring vegetation and habitat variables on distribution of rare charophyte species *Lychnothamnus barbatus* (Meyen) in lakes of western Poland. Pol J Ecol 58(1):15–25

Gerking SD (1994) Feeding ecology of fish. Academic Press, San Diego, pp 57–59

Göthberg A, Greger M, Holm K, Bengtsson B (2004) Influence of nutrients on uptake and effects of Hg, Cd and Pb in *Ipomoea aquatica*. J Environ Qual 33:1247–1255

Güven KC, Topcuoğlu S, Kut D, Esen N, Erentürk N, Saygi N, Cevher E, Güvener B, Öztürk B (1992) Metal uptake by Black Sea algae. Bot Mar 35:337–340

Güven KC, Saygi N, Öztürk B (1993) Survey of metal contents of Bosphorus algae, *Zostera marina* and sediments. Bot Mar 36: 175–178

Hägerhäll B (1973) Marine botanical-hydrographical trace element studies in the Öresund area. Bot Mar 16:53–64

Haglund K, Björklund M, Gunnare S, Sandberg A, Olander U, Pedersén M (1996) New method for toxicity assessment in marine and brackish environments using the macroalga *Gracilaria tenuistipitata* (Gracilariales, Rhodophyta). Hydrobiologia 326–327(1):317–325

Haritonidis S, Malea P (1995) Seasonal and local variation of Cr, Ni and Co concentrations in *Ulva rigida* C. Agardh and *Enteromorpha linza* (Linnaeus) from Thermaikos Gulf, Greece. Environ Pollut 89(3):319–327

Hermanowicz W, Dożańska W, Dojlido J, Koziorowski B (1999) Fizyczno-chemiczne badania wody i ścieków. Arkady, Warszawa, pp 1–50

Ho YB (1987) Metals in 19 international macroalgae in Hong Kong waters. Mar Pollut 18:564–566

Ho YB (1990) *Ulva lactuca* as bioindicator of metal contamination in intertidal waters in Hong Kong. Hydrobiologia 203:73–81

Kaimoussi A, Mouzdahir A, Saih A (2004) Seasonal Variations of Metal Contents (Cd, Cu, Fe, Mn and Zn) in Seaweed Ulva lactuca from the Coast of El Jadida City (Morocco). Comptes Rendus Biol 327(4):361–369

Kamala-Kannan S, Prabhu Dass Batvari B, Lee KJ, Kannan N, Krishnamoorthy R, Shanthi K (2008) Assessment of heavy metals (Cd, Cr and Pb) in water, sediment and seaweed (*Ulva lactuca*) in the Pulicat Lake, south east India. Chemosphere 71(7):1233–1240

Kamermans P, Malta EJ, Verschuure JM, Schrijvers L, Lentz LF, Lien ATA (2002) Effect of grazing by isopods and amphipods on growth of *Ulva* spp. (Chlorophyta). Aquat Ecol 36:425–433

Khellaf N, Zerdaoui M (2010) Growth response of the duckweed *Lemna gibba* L. to copper and nickel phytoaccumulation. Ecotoxicology 19(8):1363–1368

Lacerda LD, Fernandez MA, Calazans CF, Tanizaki KF (1992) Bioavailability of heavy metals in sediments of two coastal lagoons in Rio de Janeiro, Brazil. Hydrobiologia 228:65–70

Langston WJ (1990) Toxic effect of metals and the incidence of metal pollution in marine ecosystems. In: Furness RW, Rainbow PS (eds) Heavy metals in the marine environment. CRC Press, Boca Raton, pp 101–122

Leal MCF, Vasconcelos MT, Sousa-Pinto I, Cabral JPS (1997) Biomonitoring with benthic macroalgae and direct assay of heavy metals in seawater of the Oporto coast (Northwest Portugal). Mar Pollut Bull 34(12):1006–1015

Lee WY, Wang WX (2001) Metal accumulation in the green macroalgae *Ulva fasciata*: effect of nitrate, ammonium and phosphate. Sci Total Environ 213:273–277

Lobban CS, Harrision PJ (1997) Seaweed ecology and physiology. Cambridge University Press, United Kingdom

Luoma SN (1983) Bioavailability of trace metals to aquatic organisms—a review. Sci Total Environ 28:1–22

Malea P, Haritonidis S (1999) Metal content in *Enteromorpha linza* (Linnaeus) in Thermaikos Gulf (Greece). Hydrobiologia 394:103–112

Malea P, Haritonidis S (2000) Use of the green alga *Ulva rigida* C. Agardh as an indicator species to reassess metal pollution in the Thermaikos Gulf, Greece, after 13 years. J Appl Phycol 12(2): 169–176

Mamboya F, Lyimo TJ, Landberg T, Björk M (2009) Influence of combined changes in salinity and copper modulation on growth and copper uptake in the tropical green macroalga *Ulva reticulata*. Estuar Coast Shelf Sci 84:326–330

Mareš J (2009) Combined morphological and molecular approach to the assessment of *Ulva* (Chlorophyta, Ulvophyceae) in the Czech Republic. Master thesis. University of South Bohemia

Messyasz B (2009) *Enteromorpha* (Chlorophyta) populations in River Nielba and Lake Laskownickie. Hydrobiol Oceanol Stud 38:1–9

Messyasz B, Rybak A (2009) The distribution of green algae species from the *Ulva* genera (syn. Enteromorpha: Chlorophyta) in Polish inland waters. Ocean Hydro Stud 38(1):121–138

Messyasz B, Rybak A (2011) Abiotic factors affecting the development of *Ulva* sp. (Ulvophyceae: Chlorophyta) in freshwater ecosystems. Aqua Ecol 45(1):75–87

Michalak I, Chojnacka K (2008) The application of macroalga *Pithophora varia* Wille enriched with microelements by biosorption as biological feed supplement for livestock. J Sci Food Agric 88(7):1178–1186

Michalak I, Chojnacka K (2009) Edible macroalga *Ulva prolifera* as microelemental feed supplement for livestock: the fundamental assumptions of the production method. World J Microbiol Biot 25(6):997–1005

Moody JR, Lindstrom PM (1977) Selection and cleaning of plastic containers for storage of trace element samples. Anal Chem 49:2264–2267

Muse JO, Tudino MB, d'Huicque L, Troccoli OE, Carducci CN (1995) A survey of some trace elements in seaweeds from Patagonia, Argentina. Environ Pollut 87:249–253

Peterson HG, Healey FP, Wagemann R (1984) Metal toxicity to algae: a highly pH dependent phenomenon. Can J Fish Aquat Sci 41:974–979

Phillips DJH (1977) The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments. Environ Pollut 13:281–317

Puente X (1992) Metais pesados en organismos bentónicos dos esteiros de Galicia. Tesis de Licenciatura. University of Santiago de Compostela, pp. 58

Rai L, Gaur JP, Kumar HD (1981) Physiology and heavy metal pollution. Biol Rev 56:99–151

Reinke DC (1981) *Enteromorpha*, a marine alga in Kansas. Trans Kans Acad Sci 84(4):228–230

Rybak A, Messyasz B (2011) *Ulva flexuosa* subsp. *pilifera* (Chlorophyta, Ulvophyceae) on the new freshwater locality in Poznań. Chrońmy Przyr Ojcz 67(2):182–188

Say PJ, Burrows JG, Whitton BA (1990) *Enteromorpha* as a monitor of heavy metals in estuaries. Hydrobiologia 195:119–126

Stokes PM (1983) Responses of freshwater algae to metals. Prog Phycol Res 2:87–112

Sunda WG, Huntsman SA (1998) Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. Sci Total Environ 219:165–181

Thiébaut G, Gross Y, Gierlinski P, Boiché A (2010) Accumulation of metals in *Elodea canadensis* and *Elodea nuttallii*: implications for plant-macroinvertebrate interactions. Sci Total Environ 408(22):5499–5505

Villares R, Puente X, Carballeira A (2001) *Ulva* and *Enteromorpha* as indicators of heavy metal pollution. Hydrobiologia 462(1–3):221–232

Villares R, Puente X, Carballeira A (2002) Seasonal variation and background levels of heavy metals in two green seaweeds. Environ Pollut 119(1):79–90

Wahbeh M (1984) Levels of Zn, Mn, Mg, Fe and Cd, in three species of seagrasses from Agaba (Jordan). Aquat Bot 20:179–183

Wang WX, Dei RCH (1999) Kinetic measurements of metal accumulation in two marine macroalgae. Mar Biol 135:11–23

Wangersky J (1986) Biological control of trace metal residence time and speciation: a review and synthesis. Mar Chem 18:269–297

Wong MH, Kwok TT, Ho KC (1982) Heavy metals in *Ulva lactuca* collected within Tolo Harbour, an almost landlocked sea. Hydrobiol Bull 16(2–3):223–230

Zayed A, Gowthaman S, Terry N (1998) Phytoaccumulation of trace elements by wetland plants: I. Duckweed. J Environ Qual 27(3):715–721