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Research Paper

Bioaccumulation of alkaline soil metals (Ca, Mg) and heavy metals (Cd, Ni, Pb) patterns expressed by freshwater species of *Ulva* (Wielkopolska, Poland)

key words: Ulva, *Enteromorpha*, heavy metals, freshwater, macroalgae

Abstract

The paper includes results of the first study on the accumulation of selected metals in freshwater populations of *Ulva* taxa at 16 sites in Poland. The thalli examined contained very high concentrations of Ca and Mg, owing to well-developed surface incrustations of carbonate. Among the heavy metals investigated, the most significant concentration in the thalli was Ni, whereas the lowest was Pb. The median concentrations of Ni and Cd in the freshwater *Ulva* thalli were significantly higher than those reported in the available literature for marine taxa of the same genus. Several statistically significant correlations were found when the concentrations of different metals in the thalli and the water were analysed.

1. Introduction

The examination of marine water contamination by heavy metals may be difficult if classical methods of analysis are used to determine concentrations of the elements in water. The problem results primarily from the low concentrations of heavy metals found in sea water. This situation demands that multiple samples must be collected repeatedly over a large area. Only in this way reliable measurements can be obtained (Villares *et al.*, 2001). In addition, measurements of heavy metal concentrations only in water or in the sediment supply no information on the bioavailability of these metals to organisms (Bryan *et al.*, 1985). Another factor that hampers the correct evaluation of the concentration of metals in sediments is the influence of environmental factors, such as the sediment's accumulation rate and chemical changes occurring in the sediment (Phillips, 1977).

One of the methods that provides a solution to the above mentioned problems is the use of organisms that are able to accumulate metals, and thereby indicate the level of water contamination. Organisms used as bioindicators need to have many characteristics. They should exhibit considerable tolerance to high concentrations of the contaminant(s), and they should be common species within a particular taxonomic group that may be easily collected and processed.

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Most importantly, the concentrations of heavy metals found in these organisms should exhibit a simple correlation with the concentration of the metals in the habitat examined (PHILLIPS, 1990).

Macroalgae, especially those belonging to the genus *Ulva* (including taxa from the former *Enteromorpha* genus) are among the best-described and examined bioindicators for heavy metal contamination in marine water. Species of this genus are believed to serve as good indicators of contamination in an aquatic environment. Their cosmopolitan nature and their ability to develop and grow in extremely contaminated areas make them exceptionally valuable as bioindicators (HO, 1990). Several algae belonging to the genus *Ulva* have been examined to determine their suitability as bioindicators of water contamination with heavy metals. The species examined for this purpose include *Ulva lactuca* (TALBOT and CHEGWIDDEN, 1982), *U. rigida* (BOUBONARI *et al.*, 2008; USTUNADA *et al.*, 2011), *U. linza* (SEELIGER and EDWARDS, 1977), and *U. flexuosa* (TABUDRAVU *et al.*, 2002). *Ulva* species characterised by distromatic frondose thalli (*e.g.*, *U. lactuca*) are often used for biomonitoring marine waters contaminated with heavy metals, whereas the species with monostromatic tubular thalli (*e.g.*, *U. intestinalis*) are used less frequently.

Previously, no study has been conducted to investigate the ability of *Ulva* species occurring in freshwater ecosystems, such as lakes, rivers, ponds or streams (with water salinity < 0.5‰), to accumulate heavy metals and serve as bioindicators. This situation is the result of the species' scarcity in inland ecosystems and of their evanescence. Since 1850, freshwater populations of *Ulva* have been observed at approximately 170 sites worldwide (MESSYASZ and RYBAK, 2011). Most sites at which freshwater *Ulva* taxa commonly occur have been found in Great Britain (WHITTON and DALPRA, 1968), the United States (TAFT, 1964; REINKE, 1981), the Czech Republic (MAREŠ, 2009), Japan (ICHIHARA *et al.*, 2009) and Poland (MESSYASZ and RYBAK, 2011). Only the taxa having a monostromatic tubular thallus have been found in inland ecosystems. To date, the following species have been reported in freshwater habitats: *Ulva compressa* L., *U. flexuosa* Wulfen, *U. intestinalis* L., *U. limnetica* Ichihara et Shimada, *U. paradoxa* C. Agardh, and *U. prolifera* J. Agardh.

The goal of this research was to measure the concentration of selected alkaline soil and heavy metals in the thalli of *Ulva* macroalgae. This genus is commonly used as a bioindicator of the level of environmental contamination by heavy metals in marine ecosystems. This study examined the possibility of using freshwater populations of *Ulva* as a bioindicator of metal contamination in ecosystems, such as lakes, rivers or ponds. Furthermore, relationships between the observed concentrations of metals in the thallus and in water were analysed. The concentrations of metals in algae as obtained in the study were then compared with the results of other studies of metal-accumulating ability of *Ulva* species, especially those species having monostromatic tubular thalli.

2. Materials and Methods

2.1. Sampling

The research was conducted during the summers of 2009 and 2010, when freshwater populations of *Ulva* were in their optimal phase of development. Samples of thalli of *Ulva* spp. were taken from the Wielkopolska region (Central Europe, Poland), where the macroalgae were found at 16 sites (Fig. 1). The sites with freshwater *Ulva* were located in streams (the Dworski Rów and the Świątnica), lakes (Licheńskie, Pątnowskie, Gosławskie, Malta), one river (the Nielba), ponds (Śródka and Tulce) and unnamed reservoirs along highways (Table 1 and 2). Most studies on the bioaccumulation and bioindication of heavy metals by *Ulva* have neglected freshwater species growing in rivers, ponds, lakes and canals. This reflects that fact that only a few hundred inland–water locations exists around the world, where *Ulva* has been reported, and only a few of these sites are documented to be permanent habitats for these macroalgae. For this reason, macroalgae thalli were collected from sites, where *Ulva* was observed before the period of the study. The choice of research sites also influenced the type of catchment area

Figure 1. The study area in the Wielkopolska Region (Poland), showing the locations of the sampling stations.

Table 1. The types and the locations of the freshwater ecosystems examined in the study.

Station code	Station name	Ecosystem	Date	Coordinates	*BLHM (mg/kg)
D1	Dworski Rów	stream	04.07.2010	N 52°20′40.04″ E 17°2′32.74″	Ni: <2 Cd: <1 Pb: 5–7
D2	Dworski Rów	stream	04.07.2010	N 52°20′38.84″ E 17°2′31.86″	
D3	Dworski Rów	stream	14.06.2010	N 52°20′38.40″ E 17°2′31.72″	
L	Licheńskie Lake	lake	08.07.2010	N 52°18′47.5″ E 18°20′56.5″	no data
P1	Pątnowskie Lake	lake	09.07.2010	N 52°18′05.6″ E 18°16′34.9″	no data
P2	Pątnowskie Lake	lake	09.07.2010	N 52°18′05.8″ E 18°17′04.8″	
G1	Gosławskie Lake	lake	07.07.2010	N 52°17′17.9″ E 18°12′45.2″	no data
G2	Gosławskie Lake	lake	07.07.2010	N 52°18′12.1″ E 18°14′49.5″	
M	Malta Lake	lake	01.07.2010	N 52°24′01.9″ E 16°58′46.3″	Ni: <2 Cd: <1 Pb: <24
N	Nielba River	river	01.08.2010	N 52°48′7,41″ E 17°12′31.43″	no data
S1	Świątnica	stream	28.08.2010	N 52°21′37″ E 17°02′40″	Ni: 3–5 Cd: <1 Pb: <24
S2	Śródka	pond	16.09.2009	N 52°17′45.9″ E 17°07′06.1″	Ni: 9–13 Cd: <1 Pb: >49
T	Tulce	pond	17.06.2010	N 52°20′35″ E 17°04′40″	Ni: <2 Cd: <1 Pb: 5–7
W1	non name	water reservoir	17.09.2010	N 52°18′47.8″ E 17°32′28.4″	no data
W2	non name	water reservoir	16.09.2010	N 52°18′25.8″ E 17°10′11.2″	no data
W3	non name	water reservoir	16.09.2010	N 52°18′34.4″ E 17°14′07.3″	no data

* Background levels of heavy metals

development. Streams, such as Dworski Rów (D1–D3), Świątnica (S1) and the Śródka pond (S2), are located in agricultural areas. Small water bodies (W1–3) are the receivers of water flowing from the highway. On the other hand, the Tulce pond (T) and the Nielba river (N) are located in suburban areas of large cities. Also, the lake Malta (M) is located in the center of a large metropolis. Licheńskie (L), Pątnowskie (P1–P2) and Gosławickie (G1–G2) lakes are part of a power plant cooling system. With such diverse types of aquatic ecosystems we wanted to capture the potential impact of the catchment area development on the listed concentrations of metals in freshwater *Ulva* thalli.

The thalli that were sampled from the examined sites were identified as *Ulva flexuosa* and *U. compressa*. However, many specimens could not be assigned to any species. This problem stemmed from the complex taxonomy of the genus and the high morphological resemblance among the specimens. Similar problems with the identification of *Ulva* species were encountered during the research on *Ulva*'s bioindicative capability in marine ecosystems (Villares *et al.*, 2001). Given the very similar physiology of different *Ulva* species, one may assume that metal accumulation in different species occurs at a

Table 2. The physicochemical properties of the water at the sampling stations.

Property Units	Depth cm	SD* m	Temperature °C	Conductivity μS cm^{-1}	Oxygen mg l^{-1}	Oxygen %	pH –	Cl^{-} mg l^{-1}
Stations								
D1	45	0.45	17.7	934.01	1.96	20.6	7.75	95.5
D2	45	0.45	18.4	889.01	3.07	32.5	7.66	104.25
D3	150	1.50	14.8	1430.8	6.75	52.0	7.61	94.25
L	51	0.51	27.6	619.0	6.06	77.0	8.59	62.4
P1	95	0.95	24.2	565.0	7.96	95.1	8.77	70.85
P2	90	0.90	24.7	577.0	6.83	82.3	8.55	72.5
G1	72	0.70	22.0	485.5	9.33	106.9	8.76	64.56
G2	112	0.70	23.8	551.0	11.12	131.9	8.91	60.79
M	150	0.50	25.2	644.0	10.1	122.8	8.71	165.0
N	70	0.70	18.6	720.0	8.43	81.0	8.16	60.5
S1	52	0.52	20.3	1421.3	7.3	76.0	8.83	120.0
S2	250	2.0	13.0	1261.0	6.6	70.2	8.4	53.0
T	125	1.25	12.2	1229.2	8.3	71.4	10.01	90.0
W1	15	0.50	13.3	1547.0	7.3	85.0	8.2	225.0
W2	50	0.50	12.0	2599.0	9.2	106.0	7.6	732.5
W3	50	0.50	12.4	983.0	2.6	29.0	7.95	39.0

* SD Secchi disk.

very comparable level (LITTLER and LITTLER, 1980; VILLARES *et al.*, 2001). Additionally, no statistically significant differences in the accumulation of metals by diverse *Ulva* species were found in a laboratory study (SEELIGER and WALLNER, 1988).

The water samples were collected at the same time as the thalli samples. The samples were placed in a plastic container, cooled down to 4 °C, and transported to the laboratory. The thalli were rinsed a few times with distilled water to remove any adhering algae, vascular plants (lemnids), and snails. Next, the thalli were dried for 2 hours at 105 °C and put into 100 ml plastic containers. The collected samples were first treated with 15% nitric acid (HNO_3) and then placed in a freezer at –20 °C. All glassware used in the following procedures was acid-washed (with 15% nitric acid).

The basic physiochemical parameters of the water (temperature, conductivity, concentrations of oxygen and Cl^- and pH) at the study sites were measured with a *YSI Professional Plus* meter.

2.2. Sediment, macroalgae and water mineralisation

The labile fraction in sediment samples was extracted using 1 M HCl. Samples were left overnight to remove the effervescence produced by carbonates. They were then kept at room temperature under mechanical shaking for 1 h. The extract was centrifuged at 5000 rpm for 2 min.

Metal extraction in each case was performed by digesting 0.5 g of algae, 25 ml of water and 0.4 g of the total fraction of sediment in a mixture of 15 ml of 65% HNO_3 and 5 ml of 30% H_2O_2 in Teflon tubes in a MarsX5 microwave oven. All samples were mineralised in two steps: I – 300 s and power 400 W; II – 300 s and power 800 W. The samples were analysed qualitatively and quantitatively for Ca, Mg, Ni, Cd and Pb by inductively coupled plasma optical emission spectrometry (ICP-OES). The concentrations of metals were determined by ICP-OES using a Varian spectrometer. Calibration was performed with aqueous standard solutions.

2.3. Statistical analyses

STATISTICA 9.0 software was used for the statistical analysis of the data. The correlation between the metals' concentration in the algae and in the water was analysed using the Pearson correlation coefficient.

3. Results

The sites at which *Ulva* was found were located in the central and western parts of Greater Poland Voivodeship (also known as the Wielkopolska Region). The samples of thalli were collected from ecosystems that included diverse habitats. All *Ulva* sites were located within the littoral zone of lakes and ponds, or along the banks of rivers and streams. The values of conductivity, oxygen concentration and pH differed among the sites. Freshwater *Ulva* species were not observed in ecosystems characterised by a pH lower than 7.6 and Cl^- concentrations less than 39.0 mg l^{-1} (Table 2).

Of all the metals examined, Ca (± 162836.5 µg g^{-1}) and Mg (± 6952.6 µg g^{-1}) had the highest concentrations in the *Ulva* thalli. The lowest concentration in the thalli was that of Pb (± 4.5 µg g^{-1} d.m.) (Table 3). In ranked order from highest to lowest, the concentrations of heavy metals and alkaline earth metals observed in the macroalgal thalli were as follows: Ca > Mg > Ni > Cd > Pb. The highest concentrations in the water samples studied were Ca (± 87.5 µg l^{-1}) and Mg (± 19.4 µg l^{-1}). The lowest concentration was found for Cd as (± 0.00057 µg l^{-1}). In ranked order, the concentrations of the heavy metals and alkaline earth metals observed in the water from the studied sites were: Ca > Mg > Ni > Pb > Cd.

The highest concentration of Ca was in thalli collected in Licheń Lake (338051.39 µg g^{-1} d.m.) (Fig. 2a). In contrast, the Ca concentration in the water of Licheń Lake was one of the lowest values for this element (Fig. 2b). The highest concentrations of Mg occurred in *Ulva* thalli from the Świątnica stream (14271.46 mg g^{-1} d.m.) and in water from Śródka pond (114.67 µg l^{-1}) (Fig. 2c and d). The concentration of Mg in the water sample from the latter site was twice that at the remaining 15 *Ulva* sites. Moreover, the thalli from Świątnica stream exhibited the highest observed concentration of Pb (13.47 µg g^{-1} d.m.) (Fig. 3a). Relative to the values at other sites, the highest concentration of Pb (0.019 µg l^{-1}) was at one site on the Nielba River (Fig. 3b). The highest level of Ni in the *Ulva* was in samples collected in Malta and Gosławice Lake, and in Śródka Pond. These values ranged from 99.31 to 225.77 µg g^{-1} d.m. (Fig. 3c). The differences among the concentrations of Ni in waters from *Ulva* sites allowed the classification of the sites into 2 groups. The first group included 9 sites at which the Ni concentration ranged from 0.14 – 0.16 µg l^{-1}, and the second group exhibited high concentrations of Ni ranging from 2.05 to 2.68 µg l^{-1} (Fig. 3d). The

Table 3. The metal concentrations observed in freshwater *Ulva* thalli (µg g^{-1}) and water (µg l^{-1}) from the sampling stations.

Metal	Place	Min.	Max.	Mean	SD[c]	SE[d]
Ca	t[a]	29996.84	338051.4	162836.5	88169.94	22042.49
	w[b]	37.14	147.6	87.5	30.57	7.64
Mg	t	2598.96	14271.5	6952.6	2867.67	716.92
	w	7.12	40.1	19.4	6.62	1.66
Cd	t	0.0	179.6	23.6	52.66	13.17
	w	0.0	0.001	0.0005	0.00051	0.00012
Ni	t	23.59	225.8	102.8	71.03	17.76
	w	0.14	2.7	1.2	1.22	0.30
Pb	t	0.0	13.5	4.5	4.03	1.01
	w	0.001	0.0186	0.0069	0.00567	0.00141

n = 16.
[a] thalli.
[b] water.
[c] standard divisions.
[d] standard error.

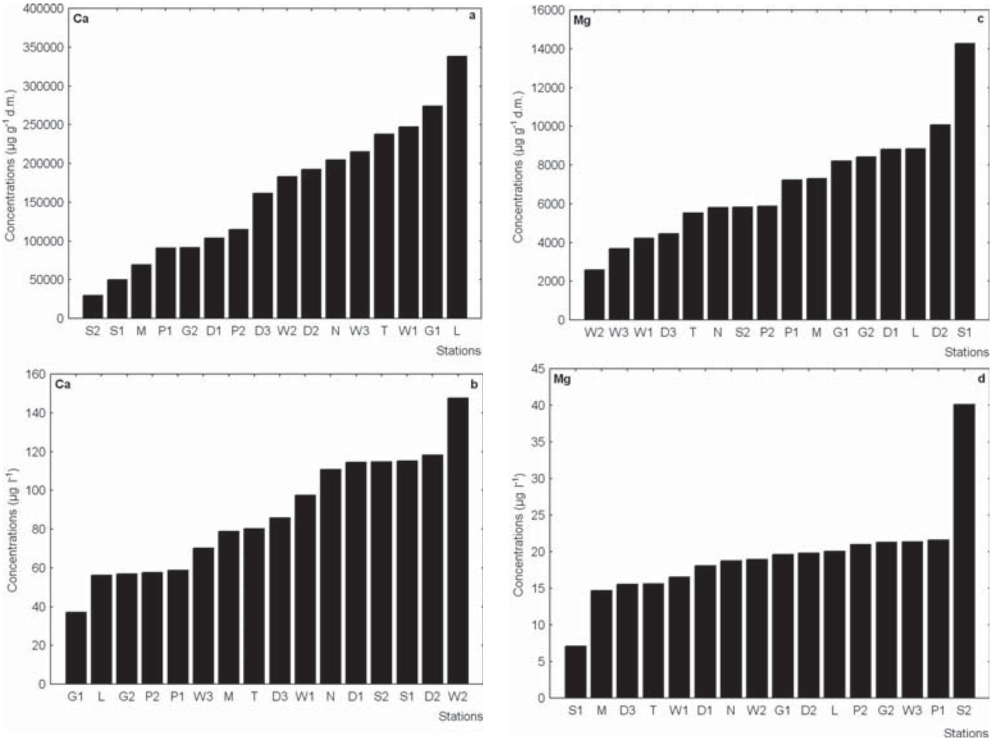

Figure 2. The Ca content of freshwater *Ulva* thalli (a), water (b) and the Mg content of freshwater *Ulva* thalli (c) and water (d) at different sampling stations.

accumulation of Cd in the thalli also appeared roughly bimodal. Half of the samples had substantially higher concentrations of this metal (from 15.01 to 179.64 µg g^{-1} d.m.), whereas the Cd concentration in the remaining thalli samples was much lower and ranged from 0.09 to 5.0 µg g^{-1} d.m. (Fig. 4a). The Cd concentration in the water from the study sites was at detection limit. The highest concentrations were approximately 0.001 µg l^{-1} (at stations D3, S1, S2, T, P1, W1, W2 and W3) (Fig. 4b).

The concentrations of heavy metals and alkaline earth metals in *Ulva* thalli were from a few hundred to several thousand times higher than the corresponding concentrations in water in which the *Ulva* developed. The concentration of Ca in the thalli was 1860 times higher than in the water. The concentration differences of the other metals between the thalli and the water expressed as accumulation factors (number of times higher in thalli than in water), were as follows; Mg, 358; Cd, 41403; Ni, 85; and Pb, 652. In ranked order, the ability of freshwater *Ulva* to accumulate the metals examined can be represented as Cd > Ca > Pb > Mg > Ni.

No statistically significant correlations between the concentration of any metal in water and in thalli (*e.g.*, between the concentration of Pb in the water and in *Ulva*) were found. However, a few significant correlations were present between concentrations of different metals in water, in thalli, and in water *vs*. thalli. In water samples from the *Ulva* sites, the Pb and Ni concentrations correlated negatively ($r = -0.63$; $P = 0.009$). Likewise, a positive correlation between the Ni and Cd concentrations was observed ($r = 0.61$; $P = 0.012$). The correlation between Ni concentration in water and Cd concentration in thalli ($r = 0.52$;

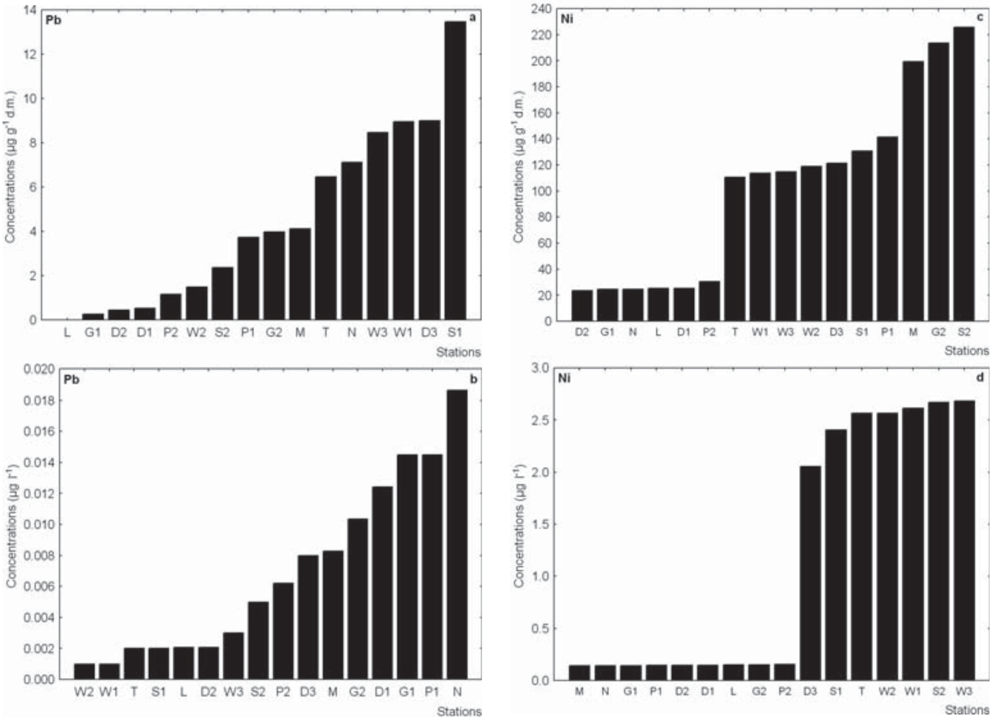

Figure 3. The Pb content of freshwater *Ulva* thalli (a), water (b) and the Ni content of freshwater *Ulva* thalli (c) and water (d) at different sampling stations.

$P = 0.04$) was positive, as was the correlation between Ni in water and Pb in thalli ($r = 0.57$; $P = 0.019$). In thalli, a positive correlation between the Pb and Cd concentrations ($r = 0.50$; $P = 0.04$) and a negative correlation between the Ni and Ca concentrations ($r = –0.60$; $P = 0.013$) were observed (Table 4).

4. Discussion

This study found very high concentrations of alkaline earth metals, in particular Ca, in the thalli of freshwater *Ulva.* The high concentration of Ca resulted from the presence of a rich $CaCO_3$ incrustation on the surface of the thalli. The $CaCO_3$ incrustations often covered over 90% of the thallus area. Accordingly, carbonates were dominant in the biomass of freshwater *Ulva* (MESSYASZ *et al.*, 2010). This rich incrustation can also affect the development of thalli by preventing light and nutrients from reaching the cells directly. These constraints are further enhanced by a diatom periphyton that occurs massively on the surface of *Ulva* (KAMERMANS *et al.*, 2002). In marine ecosystems, one observes complex trophic relationships in the genus *Ulva.* For example, snails (*e.g.*, *Hydrobia ulvae*) and crustaceans (*e.g.*, *Gammarus locusta*) eat away the periphyton along with the incrustation adhering to the thalli (JENSEN and SIEGISMUND, 1980; KAMERMANS *et al.*, 2002). By eating the epiphytes, shellfish and snails stimulate the alga's development. This phenomenon will influence on the growth and biomass of the thalli (KAMERMANS *et al.*, 2002).

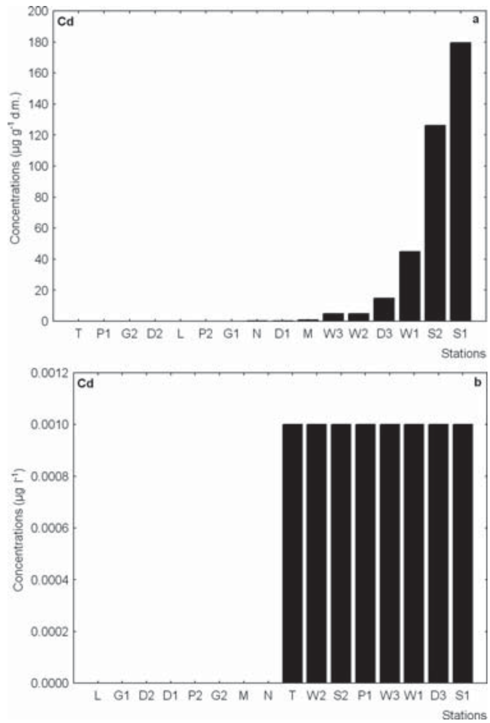

Figure 4. The Cd content of freshwater *Ulva* thalli (a) and water (b) at different sampling stations.

The high concentrations of Ca observed in the thalli examined in this study can therefore be attributed to an intensive process of calcification occurring in the thalli. This process occurs especially in fresh water of slightly alkaline pH.

During the study at freshwater *Ulva* sites with pH values ranging from 7.6 to 10.01 were observed. The pH is the most import parameter affecting the bioaccumulation process. A study of MICHALAK and CHOJNACKA (2009) showed that the uptake of Cr from aqueous solution by *Ulva prolifera* is pH-dependent, and is most efficient at higer pH (> 5). For species *U. prolifera* sorption of metals from solution proceeds efficiently at pH 5–7, while in alkaline medium (pH 8–10.5), the process occurs at a constant rate (CHOJNACKA, 2008). In our study there was no statistically significant correlation between the pH of water from *Ulva* sites and the concentration of metals in the macroalga thallus.

Trace metals ions, which we studied (Ni, Cd and Pb), are toxic to plant cells and can cause many problems in the physiology and the cell structure. Such disturbances are the result of three basic mechanisms (i) the generation of reactive oxygen species (ROS) and free radicals (FR), (ii) the blocking of functional groups, or (iii) changing the essential metals building biomolecules to another's (SAMARDAKIEWICZ and WOŹNY, 2000). Metals such as Cd, Ni and Pb can be incorporated into the molecule during the synthesis of chlorophyll instead of Mg, which results in a male-function of light harvesting (PHETSOMBAT *et al.*, 2006). In studies on green alga *Haematococcus lacustris* conducted by XYLÄNDER and BRAUNE (1994) high concentrations of Ni reduced the germination rate as well as the protein and chlorophyll contents in cells. However, in *Chlamydomonas reinhardtii* it was observed that nickel and lead had stimulated the photosynthetic efficiency (PE) (DANILOV and EKELUND, 2001). It was also found that Cd, Ni and Pb ions may cause an inhibi-

Table 4. The Pearson correlation coefficients (r) between the metal concentrations in the water and the metal concentrations in the thalli.

	Ca	Mg	Cd	Ni	Pb
Freshwater *Ulva*					
Ca	1.000	–0.25 [ns]	–0.47 [ns]	**–0.60***	–0.15 [ns]
Mg		1.000	0.43 [ns]	–0.12 [ns]	0.04 [ns]
Cd			1.000	0.38 [ns]	**0.50***
Ni				1.000	0.31 [ns]
Pb					1.000
Water					
Ca	1.000	–0.01 [ns]	0.13 [ns]	0.42 [ns]	–0.29 [ns]
Mg		1.000	0.06 [ns]	0.04 [ns]	0.08 [ns]
Cd			1.000	**0.61***	–0.20 [ns]
Ni				1.000	**–0.63****
Pb					1.000
Freshwater *Ulva*			Water		
Ca	–0.26 [ns]	–0.17 [ns]	–0.19 [ns]	–0.01 [ns]	–0.16 [ns]
Mg	–0.05 [ns]	–0.28 [ns]	–0.27 [ns]	–0.38 [ns]	0.06 [ns]
Cd	0.37 [ns]	0.04 [ns]	0.31 [ns]	**0.52***	–0.31 [ns]
Ni	0.05 [ns]	0.26 [ns]	0.43 [ns]	0.40 [ns]	–0.15 [ns]
Pb	0.15 [ns]	–0.47 [ns]	0.44 [ns]	**0.57***	–0.16 [ns]

$n = 16$.
* significant at $P < 0.05$.
** significant at $P < 0.01$.
[ns] not significant at significance level 0.05.

tion of electron transport and oxidative phosphorylation as well as changes in the mitochondrial membrane permeability (OLESZCZUK, 2008). Of particular importance in avoiding the toxicity of heavy metals by plants and algae cells is the accumulation of metals in cell walls, which is an important protective barrier for protoplast (IRMER *et al.*, 1986). In cell walls of *Chlamydomonas reinhardtii* almost twice higher metal concentrations were recorded than in the protoplasts of the cells (MACFIE *et al.*, 1994). Moreover, metal deposits in cell structures and a significant thickening of cell walls in all parts of the thallus were observed in freshwater species of the *Ulva* genus (MESSYASZ *et al.* – in press). Therefore, it is probable that the main mechanism to avoid toxicity of trace metals by freshwater *Ulva* is the binding of metal ions through the cell wall components – mainly pectic substances.

Previously, the accumulation of heavy metals by *Ulva* has only been examined in species that occur in marine and estuarine ecosystems. Particular *Ulva* species serve as indicators for specific metals. HO (1990) has stated that *Ulva lactuca* can be a good indicator of water contamination for Cd, Fe, Mn and Pb, but is not a good indicator for Cu and Zn. Haritonidis and MALEA (1999) have shown that *U. rigida* is a good indicator of water contamination by Pb, Zn and Cd. Research on the *Ulva* species characterised by monostromatic tubular thalli has previously been conducted only in saline estuarine waters (SAY *et al.*, 1990). An experimental study of 2 species having tubular thalli, *U. clathrata* and *U. flexuosa* demonstrated a linear relationship between the concentrations of Cd, Cu, Pb and Zn in the water and the corresponding concentrations in the thalli (SEELIGER and WALLNER, 1988). The results of the study related the observed concentration of heavy metals in the thalli to the concentrations of these metals in water. A similar relationship by SEELIEGER and EDWARDS (1977) demonstrated relevant correlations between the concentrations of Cu and Pb in water and thalli of *U. linza*.

The present study shows that compared to the geochemical background of the study areas (available only for sites: D1–3, M, S1, S2 and T), the identified concentrations of heavy metals for Cd and Pb were within an acceptable limit. However, in the case of nickel concentrations the geochemical background level was exceeded by an average of 0.34 mg l^{-1} at D3, S1 and T sites (Table 1). Very high concentrations of Ni (from 23.59 to 225.8 μg g^{-1}) were in the thalli of freshwater *Ulva*. The available literature supplies no information on the concentrations of this metal exceeding 70 μg g^{-1} in species of *Ulva* having tubular thalli (HÄGERHÄLL, 1973). In freshwater *Ulva* thalli from most sites, the Ni concentration always exceeded approximately 23 μg g^{-1}. This value seems to represent the largest known concentration of this metal in thalli of marine *Ulva*.

The Pb concentration in thalli of freshwater *Ulva* ranged between 0–13.5 μg g^{-1}. In the thalli of *U. flexuosa* species from the Fiji Islands, the concentrations of Pb were reported to range between < 5 and 62 μg g^{-1} (TABUDRAVU *et al.*, 2002). High concentrations of Pb (± 28.4 μg g^{-1}) have also been discovered by CAPONE *et al.* (1983) in the tubular *Ulva* thalli collected from lagoons in Sardinia. HORNUNG *et al.* (1992) have found concentrations of Pb reaching 12.6 μg g^{-1} in the thalli of *U. compressa* from the Mediterranean Sea coast in Israel. It may therefore be assumed that freshwater thalli are characterised by much lower concentrations of Pb than those found in *U. flexuosa* or *U. compressa* occurring in estuarine zones. Yet in some cases, for example in the Piran-Rovinj region on the Northern Adriatic, the Pb concentrations in tubular thalli of *Ulva* species fluctuated between 2 and 9 μg g^{-1} (MUNDA and HUNDNIK 1991). Additionally, GOSAVI *et al.* (2004) reported a Pb concentration of ±6.0 μg g^{-1} in the tubular thalli of species described as *Enteromorpha* sp. from Moreton Bay in Australia. This value resembles the average Pb concentration (4.5 μg g^{-1}) measured in freshwater *Ulva* thalli from the Wielkopolska region.

Cadmium concentrations ranging from 0.1 to 0.9 μg g^{-1} have been reported in marine populations of tubular species of *Ulva* from the northern Adriatic Sea. Moreover, low concentrations (0–0.8 μg g^{-1}) of the same metal have been confirmed by HORNUNG *et al.* (1992) in thalli of *U. compressa*. In the tubular thalli of *Ulva* from 6 estuaries located along the British North Sea coast, SAY *et al.* (1990) recorded Cd concentrations ranging from 0.07 to 4.8 μg g^{-1}. Evidently, the tubular thalli of *Ulva* from estuarine regions exhibit lower concentrations of Cd than those in *Ulva* occurring in freshwater ecosystems. The available literature does not report Cd concentrations higher than the values of up to 179.6 μg g^{-1} d.m. in the current study.

SAY *et al.* (1990) have stressed the fact that the estuarine water, in which *Ulva* thalli exhibited Cd values above 1.5 μg g^{-1} and Pb values above 60 μg g^{-1}, should be defined as highly contaminated. However, the authors point out that the correct and thorough monitoring with *Ulva* species requires repeated collections in a particular region. Moreover, other researchers have discussed cases in which the concentrations of accumulated metals vary across *Ulva* species. For example, the ranked order of metal concentrations in the thalli of *U. linza* was reported as Fe > Zn > Pb > Cu > Cd (MALEA and HARITONIDIS, 1999). The ranked for thalli of *U. lactuca* was similar from Navachiste Bay (ORDUÑA-ROJAS and LANGORIA-ESPINOZA, 2006). However, *U. rigida* from the North Aegean Sea and from sites located near the Dardanelles had the ranked order of Zn > Cu > Pb > Cd (BOUBONARI *et al.*, 2008; USTANADA *et al.*, 2010). In *U. linza*, *U. lactuca* or *U. rigida* thalli, Cd exhibited the lowest concentrations of all metals examined. The present research obtained a similar result for Cd. A relatively low level of Cd accumulation therefore appears to be characteristic of species with monostromatic tubular and distromatic frondose thalli. This result does not seem to depend on the habitat occupied by the species (*e.g.*, saline, brackish or freshwater).

5. Conclusions

Our research showed that freshwater *Ulva* occurring in inland ecosystems having no contact with saline water are able to accumulate Ni and Cd to a higher degree than marine taxa. The *Ulva* thalli were characterised by very high concentrations of alkaline earth metals (Ca and Mg). These high levels were related to the well-developed carbonate incrustation on the surface of these algae. We found in our study the lack of statistically significant correlations between the concentration of metals in water samples of a given study site and the corresponding concentration in the thallus. The freshwater *Ulva* thalli contained concentrations of heavy metals and alkaline earth metals that were from several hundred to several thousand times higher than the corresponding concentrations of these metals in water from the *Ulva* sites.

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