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Bioindication of Heavy Metals by *Mimulus guttatus* from the Czeska Struga Stream in the Karkonosze Mountains, Poland

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The study of the aquatic vegetation of running streams provides information on the quality of their environment (Grasmuck et al. 1995). Among the principal characteristics of aquatic macrophytes is their ability to accumulate nutrients and accelerate nutrient cycling in the environment (Nogueira et al. 1996). Aquatic macrophytes concentrate elements and integrate temporal fluctuations in water, therefore making them more useful for monitoring purposes compared to chemical analyses of water and sediments (Jones 1985). Therefore, these taxa can be used as indicators of the trophic level of the ambient water (Carbiener et al. 1990). The evaluation of the role of aquatic macrophytes in nutrient cycling requires studies of the chemical composition of plant tissues as well as of the water and sediments in which the plant community develops (Nogueira et al. 1996). Among the aquatic macrophytes, emergent and free floating plants show a high productivity and have been recently utilized for waste water treatment (Tsuchiya 1991).

Mimulus guttatus (common yellow monkey flower) is an emergent perennial macrophyte that has a North American origin. This introduced species has an expanded range among other native macrophytes in the Karkonosze Mountains (Mróz et al. 1994). *Mimulus guttatus* grows on calcareous soils on the banks of cold, well oxygenated, running waters and has a blooming period from June to September. Within the genus *Mimulus*, a copper tolerant endemic (*Mimulus cupriphilous*) is known from copper mines in California (Shaw 1990; Searcy and Macnair 1993). Mróz et al. (1994) concluded that *Mimulus guttatus* exhibits a high tolerance towards ammonium and has also a maximum frost resistance to -6 °C. Therefore, it can be expected that this plant could be used in biological purification and wastewater treatment in mountaineous areas. The aim of this study was to determine the chemical characteristics of *Mimulus guttatus*, water, and sediments from the Czeska Struga stream in the Karkonosze Mts.

MATERIALS AND METHODS

Investigations were conducted in the Czeska Struga stream (Fig. 1), a northern tributary of the Kamienna River (belonging to the stream system of the Bobr and Oder) near Szklarska Porêba (SW of Jelenia Gora) in the Karkonosze Mountains (Poland). The stream is embedded in granites, covered by muddy sediments and bordered by alternately built up areas, meadows, pastures and forest.

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Figure 1. Location of the four sampling sites in the Czeska Struga.

Any system of water purification is absent in the catchment area of the stream and the wastewater from some small factories and numerous houses in the stream area is transported without treatment by means of various drains to the Czeska Struga. In addition solid waste deposits and ashes from coal and cokes pollute the stream banks. In this stream 4 sampling sites were selected, distributed over the entire 3 km length of the stream. At each site, water, sediments and samples of *Mimulus guttatus* were collected in five replications. Although the Czeska Struga receives untreated municipal sewages, *Mimulus guttatus* grew abundantly.

Prior to analysis, the water samples were filtered using a glass microfibre filter (pore size 0.5 µm). The following parameters were measured within two days after collection of the water samples: pH (potentiometrically), ammonium, nitrate, phosphate and chlorides (calorimetric) and sulphates (nephelometric) with a Technicon Autoanalyzer System II TRAAC 800), Ca, Mg, K, Al, Fe, Cr, Co, Ni and Zn with a Simultaneous Sequential ICPES (Spectra Analytical Instruments), Cd, Cu and Pb with AAS (graphite furnace Philips PU 9200X).

Fresh sediments were used for measurement of the pH (potentiometrically).

After collection, plants were washed thoroughly with streamwater and dried (together with the sediments) at 60 °C and homogenized.

Sediments were extracted for: PO_4 by means of a solution of 0.3 *M* sodium citrate and 1 *M* sodium bicarbonate (Olsen and Sommers 1982) and for Ca, Mg and K by means of an 1 *N* ammonium acetate solution (Knudsen and Peterson 1982).

Plant and soil materials (200 mg) were digested in duplicate with 1.5 ml nitric acid (Merck, suprapur quality, 65%) and hydrogen peroxide, during which temperatures were raised to about 95 °C until evolution of nitrous gas stopped and the digest became clear. After dilution to a total of 10 ml, the digests were analysed for total N and P (only plant material), Fe, Cr, Co, Ni, Zn, Cd, Cu and Pb.

All elements were measured using the same instrumentation as described for the water samples and were measured against standards with the following maximum concentrations in mg/L: 0.1 NH_4^+, 10 NO_3^-, 0.5 PO_4^{---}, 5 Cl^-, 50 SO_4^{--}, 10 Ca^{++}, 10 Mg^{++}, 10 K^+, and in µg/L: 500 Al^{+++}, 1000 Fe^{+++}, 100 Cr^{++}, 200 Co^{++}, 500 Ni^{++}, 1000 Zn^{++}, 5 Cd^{++}, 25 Cu^{++}, 50 Pb^{++} (BDH Chemicals Ltd, reagent grade) and blanks. All analyses were done in duplicate and all results for plants and soil were calculated on a dry weight basis.

At each sampling site, all plants (69 plants in site 1, 128 in site 2, 140 in site 3 and

187 in site 4) were measured for the following biometrical features: length of plants, number of leaves, length and width of the longest leaf, number of flowers, length of inflorescence and number of lateral branches. In addition, the number of plants per m^2 was established.

Differentiation between sampling sites with respect to the mean concentrations of elements in plants, water and sediments and biometrical features of plants were evaluated by the least significant difference (LSD) test (Parker 1983). Frequency distribution of the length of plants was evaluated with the chi square test (Parker 1983). Linear regressions, multiple regressions and correlation coefficients (Zuk 1989) were calculated to examine the relationships between biometrical features and concentrations of elements in water, sediments and plants. The selected variables formed the regression model on the probability level of error lower than 0.05. All variables in the model were significant at this probability level. Selection of independent variables was conducted by rejection of those, which from ecological (biological) point of view could not influence the dependent variable. The statistical selection of data, which was the next step of data analysis, consisted of a stepwise regression analysis. Calculations were made of: regression weights (B), standardized regression weights (BETA), coefficients of multiple correlation (R); and R-square coefficients of multiple determination (Zuk 1989).

All calculations were done with the program CSS Statistica 3.1 (StatSoft 1993).

RESULTS AND DISCUSSION

The mean concentrations of elements in water and sediments of the examined sampling sites as well as in plants and the biometrical features of plants are presented in tables l-4. The LSD test indicated that all plants, water and sediments from their sampling sites differ significantly with respect to the contents of the examined elements. Plants of all populations differ significantly with respect to the biometrical features.

According to the values established for polluting elements in unpolluted surface water by the Dutch Government (Ministerie van VROM 1992), the investigated streams surpassed the upper limits of 1 µg/L in the water for Cd (all sites) and 20 µg/L for Pb (all sites). Also, according to Dojlido (1987), the concentrations of Ni, Cr and Zn surpassed background levels which are 3, 2 and 10 µg/L respectively.

The Ca:Mg ratio in natural water is usually 2-4 (Dojlido 1987). Higher values are usually caused either by an increase of mineralization of natural origin or by pollution. Only at site 4 was the ratio above this range, caused probably by pollution as indicated by a significant higher concentration of chlorides (one of the indicators for water pollution) in site 4.

According to the values established for polluting elements in unpolluted sediments by the Dutch Government (Ministerie van VROM 1992) the investigated sediments surpassed the upper limits of 1 mg/L for Cd (sites 2, 3 and 4) and 50 mg/L for Pb (sites 3 and 4). According to Kabata-Pendias and Pendias (1984) the concentration of Pb in all sites surpassed the background level of 20 mg/kg sediment.

Table 1. Concentration of elements in water ± the standard deviation. S=site.

S	NO_3	SO_4	K	Ca	Mg	Cl	Fe	NH_4	PO_4	Cu	Cr	Ni	Cd	Al	Co	Pb	Zn
				mg/L							µg/L						
1	2.4±0.1	32±3	2.7±0.1	48±1	13.4±0.3	10.5±0.3	110±10	1±0.3	60±10	8.1±0.3	4.0±0.3	6.1±0.3	3.9±0.2	11.2±0.4	14.1±0.2	34±0.4	41±0.4
2	2.2±0.1	47±4	2.4±0.1	49±1	15.4±0.5	12.7±0.2	80±3	58±8	200±20	9.9±0.3	5.6±0.4	7.8±0.3	4.1±0.3	15.0±0.6	15.0±0.4	49±0.6	44±0.5
3	3.2±0.1	53±4	2.8±0.1	45±1	21.5±0.6	20.8±0.1	120±20	101±11	230±20	11.3±0.2	7.5±0.6	9.3±0.4	3.5±0.3	16.1±0.6	16.6±0.5	112±1.6	49±0.5
4	3.2±0.2	54±5	2.8±0.1	52±1	10.1±0.3	80.7±1.2	130±30	110±5	280±40	12.7±0.3	11.3±0.8	15.0±0.6	4.1±0.2	18.0±0.5	17.3±0.5	55±0.7	47±0.6

Table 2. Concentration of elements (mg/kg) in sediments ± standard deviation. S=site.

S	pH	PO_4	K	Ca	Mg	Fe	Cu	Cr	Ni	Cd	Al	Co	Pb	Zn
1	5.8±0.1	43±2	100±2	7400±130	1500±110	300±19	8.4±0.2	5.7±0.1	5.9±0.2	0.88±0.06	3700±56	2.7±0.03	36±1	47±1
2	6.3±0.2	48±2	90±2	6200±130	770±47	260±16	9.7±0.3	8.7±0.2	11.4±0.3	1.15±0.07	4900±62	3.1±0.04	41±1	85±1
3	6.5±0.2	46±1	94±2	6000±140	660±36	193±12	11.2±0.2	6.4±0.1	9.9±0.2	1.49±0.08	5400±37	4.9±0.04	57±1	58±1
4	7.0±0.2	54±3	62±2	1800±160	980±41	182±13	11.7±0.1	12.4±0.2	10.0±0.2	1.27±0.06	5100±31	5.9±0.03	64±1	122±1

Table 3. Concentration of elements (mg/kg) in *Mimulus guttatus* ± standard deviation. S=site.

S	N	P	K	Ca	Mg	Fe	Cu	Cr	Ni	Cd	Al	Co	Pb	Zn
1	35000±159	1050±31	27500±137	1110±67	380±16	167±10	8.9±0.1	1.01±0.02	0.91±0.02	0.77±0.02	98±5	2.32±0.1	4.6±0.1	72±3
2	21500±132	1070±32	30000±174	870±24	510±18	222±10	9.3±0.1	1.11±0.02	1.24±0.04	1.06±0.02	240±13	2.66±0.1	8.1±0.2	96±3
3	33000±178	1050±41	26900±156	930±36	470±21	193±7	10.4±0.2	1.42±0.04	1.36±0.03	1.21±0.03	248±13	2.86±0.2	8.1±0.3	255±6
4	34000±194	1060±21	30700±167	800±41	510±13	900±12	11.3±0.2	1.99±0.05	1.83±0.04	1.07±0.04	242±13	3.25±0.3	10.5±0.4	209±5

Table 4. Biometrical features of *Mimulus guttatus* ± standard deviation. S=site.

S	Length of plant (cm)	Number of leaves	Length of longest leaf (cm)	Width of longest leaf (cm)	Number of flowers	Length of in-florescence (cm)	Number of lateral branches	Number of plants/m^2
1	81.3±1.5	17.6±0.4	6.4±0.5	4.8±0.2	12.6±0.6	20.3±1.1	2.9±0.1	96±3
2	65.7±1.3	17.5±0.5	5.4±0.3	4.1±0.2	12.5±0.5	20.1±0.9	3.1±0.1	100±4
3	63.2±1.1	12.1±0.3	4.7±0.1	3.3±0.1	9.5±0.3	14.1±0.6	3.1±0.1	101±3
4	60.2±0.9	11.7±0.3	4.6±0.1	3.2±0.1	7.3±0.2	11.7±0.5	2.9±0.07	102±4

Table 5. Statistically significant relations (Pearson correlations) between chemical characteristics of water, sediments and plants.

Relations between	*R*	*Significance level*
Cr in water and Cr in plants	0.98	0.017
Ni in water and Ni in plants	0.98	0.022
Co in water and Co in plants	0.97	0.031
Zn in water and Zn in plants	0.97	0.030
Cu in water and Cu in plants	0.97	0.030
PO_4 in water and P in plants	0.98	0.019
Cu in sediments and Cu in plants	0.96	0.040
Cd in sediments and Cd in plants	0.97	0.028
Co in sediments and Co in plants	0.95	0.040
Al in sediments and Al in plants	0.96	0.036
PO_4 in sediments and P in plants	0.97	0.029
Al in water and length of plants	-0.98	0.024
Al in water and length of longest leaf	-0.97	0.028
Al in plants and number of plants/m^2	-0.97	0.027
Pb in plants and length of plants	-0.96	0.039
Zn in plants and number of leaves	-0.96	0.038
SO_4 in water and length of plants	-0.98	0.011
SO_4 in water and length of longest leaf	-0.99	0.007
SO_4 in water and width of longest leaf	-0.96	0.037
NH_4 in water and length of plants	-0.97	0.034
NH_4 in water and length of longest leaf	-0.99	0.001
NH_4 in water and width of longest leaf	-0.99	0.009
NH_4 in water and number of plants/m^2	-0.98	0.020
Co in water and length of plants	-0.97	0.029
Co in water and length of longest leaf	-0.99	0.002
Co in water and width of longest leaf	-0.98	0.018
Zn in water and width of longest leaf	-0.95	0.048
Cu in water and number of plants/m^2	0.95	0.047
Cu in sediments and number of plants/m*	0.95	0.049
Ca in sediments and Fe in plants	-0.98	0.019
Ca in sediments and Ni in plants	-0.95	0.044
Mg in sediments and Cd in plants	-0.96	0.039
Co in sediments and number of leaves	-0.95	0.043
Co in sediments and number of flowers	-0.98	0.013
Co in sediments and length of inflorescence	-0.98	0.019
Al in sediments and length of longest leaf	-0.96	0.037
Pb in sediments and number of leaves	-0.97	0.032
Pb in sediments and width of longest leaf	-0.97	0.033
Pb in sediments and number of flowers	-0.98	0.015
Pb in sediments and length of inflorescence	-0.98	0.017

The concentrations of Zn, Cd, Pb, Cu, Co and Ni in all plants (except for Ni in plants of site 1) were higher than the background values as established by Markert (1992) and Kabata-Pendias and Pendias (1984) and generally increased (together with Cr) downstream in all plants (table 3).

The analyses of water and sediments indicated potential of pollution with Cd and

Pb and analyses of *Mimulus guttatus* indicated elevated levels of Cd, Pb, Cu, Ni, Zn and Co.

The concentrations of nutrients in the examined plants were within the ranges described by (Markert 1992). The lower limits of N, P and K in plants, critical for growth of aquatic macrophytes, are respectively: 13000, 1000 and 8000 mg/kg (Gerloff 1975). The concentrations of these elements in the investigated *Mimulus guttatus* were higher than the abovementioned limits and were therefore not limiting the growth. In this investigation, only P gave a significant positive correlation between the concentrations in plants and in the environment (table 5). Lehtonen (1989) and Maury-Brachet et al. (1990) state that some helophytes take up nutrients, like N and P, from the sediments through their roots. K however appears to originate from the water column despite the abundance of this element in sediments.

The correlations between concentrations of elements in water, soil and *Mimulus guttatus* (table 5) suggest that this plant may accumulate nutrients from both water and soil. Robach et al. (1995) proved that the P content in several rooted aquatic macrophytes was strongly correlated with the annual mean concentration of P in water and that there was no correlation between P in plant tissue and in sediments. In this investigation, P in water and in sediments both appear to have an influence on the P concentration in *Mimulus guttatus.*

A negative influence of the ammonium content in water on the increase in length of *Mimulus guttatus* was found (table 5), which is in contradiction to Mroz et al. (1994) who state that this species is characterized by a high tolerance towards ammonium. This discrepancy could not be explained by the fact that ammonia could be more toxic in alkaline water than in acidic water where it is present in a non-toxic ionized form (Robach et al. 1995), because the pH of the stream (6.4-6.6) examined in this investigation was even slightly lower than that one examined by Mroz et al. (6.6-7.6). Additionally, it was found that ammonium had a negative influence on the length and width of the longest leaf (table 5).

Franzin and McFarlane (1980) found a significant negative correlation between plant metal accumulation and dissolved Ca, suggesting that Ca in solution might modify (reduce) the uptake of metals by plants. Plants may counteract heavy metal toxicity by maintaining high Ca levels (Angelone et al. 1993), since this element is known to reduce the toxic influence of heavy metals. Mg serves in a similar, protective role (Samecka-Cymerman and Kempers 1994). According to Brooks (1972), the capacity of a plant to absorb mineral salts obviously has finite limits. If the element is accumulated to an excessive degree, this will have to be compensated for by reduced uptake of another element. In the present study, negative correlations between concentrations of Ca in soil and Fe and Ni in plants and between concentrations of Mg in soil and Cd in plants were found (table 5). These results are in agreement with Markert and Wtorova (1992), who stated that heavy metal accumulation seems to be directly associated with a reduced uptake of Mg and Ca by plants.

Positive correlations between concentration of Cu in water, sediment and plants, Cr in water and plants, Ni in water and plants, Co in water, sediments and plants, Cd in soil and plants and Al in soil and plants indicated that *Mimulus guttatus* may be a useful organism to identify potential pollution from metals. This is in accordance with Jones et al. (1985), who states that strong positive associations between metal concentrations in plants and their environment suggest that this plant has a potential for pollution monitoring in general and for the examined metals in particular. Searcy and Macnair (1993) are of the opinion that *Mimulus guttatus* is a Cu tolerant plant. Other heavy metals revealed a negative correlation with the growth of this species (table 5).

The frequency distribution of the length of *Mimulus guttatus* was normal only in the upstream populations of sampling sites 1 and 2. In all other sites, environmental conditions are believed to be disturbing the proper development of the populations of this species, expressed by a downstream decrease of the length of the plant, number of leaves and flowers, length and width of the longest leaf and length of inflorescence (table 4). This downstream decrease of biological features was probably caused by a downstream increasing pollution (site 1 characterized the lowest concentrations of heavy metals in water and sediments), accompanied by a downstream increase of Zn, Cr, Cd, Pb, Cu, Co and Ni in all plants (table 3). A possible negative influence on the growth of *Mimulus guttatus* was also indicated by negative correlations between contents of Al, SO_4, NH_4 Co, Zn and Pb in water or sediments and some biometrical features (table 5).

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