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Assessment of Pollution with Aquatic Bryophytes in Maritsa River (Bulgaria)

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Abstract Bryophyte species composition and 26 common physico-chemical and inorganic chemical parameters were assessed at 23 selected sites in the Maritsa River (BG) over a 4-year period. Principal components analyses (PCA) of both bryophytes and water variables distinguished different locations in the ecosystem. The data imply that the content of elements measured in bryophytes represents river contamination, while species compositional patterns reflect hydromorphology and general degradation. This study for the first time combined aquatic bryophyte occurrence, the bioaccumulation of 17 macro-and microelements in 17 species, and 26 water factors by principal components analysis (PCA) in an assessment of river pollution.

Keywords Aquatic bryophytes · River pollution · Heavy metals · Toxic elements

River pollution is a major environmental issue affecting freshwater habitats and human health. Lotic ecosystems constantly react to external and internal changes. Their recovery after human alterations depends on a variety of factors, among them living organisms, including bryophyte communities. Aquatic bryophytes influence both river biodiversity and water chemistry. An understanding of the relationship between bryophytes and water chemistry is critical for a comprehensive assessment of a river ecosystem. Studies applying separate aquatic bryophyte species as biomonitors indicate bryophytes are an appropriate tool to rank contaminated sites (Samecka-Cymerman et al. 2002; Cesa et al. 2006, 2010; Vieira et al. 2009).

Water and bryophyte samples were studied for 4 years within the Bulgarian territory (321 km) of the transboundary Maritsa River—the largest river on the Balkan Peninsula. The river belongs to Ecoregion 7 (East Balkans) based on the biogeographic regions (Illies 1978). A small part (10.5 km) of the river upper course runs through a protected territory. Its middle course is one of the largest linear protected Natura 2,000 sites in Bulgaria (BG0000578; 147 km^2), flowing predominantly through agricultural and urban areas. This study was an attempt to illustrate the correlation between aquatic bryophyte species and their element accumulation in relation to river contamination, based on water parameters in corresponding samples from 23 selected river sites.

Materials and Methods

Bryophytes and river water were sampled at 23 sites along affected by human activities and minimally disturbed, along the main bed of the Maritsa River (Fig. 1). The first data resulted from 4 years of study (2002–2005), with additional field observations made during the next three growing seasons (2006–2008). The nomenclature of the identified species followed Hill et al. (2006) for mosses and Grolle and Long (2000) for liverworts. In situ measurements of acidity (pH), electrical conductivity (C, $\mu S\ cm^{-1}$) and redox potential (Eh, mV) of river water were made using a Multiline P3 (WTW, Germany), and

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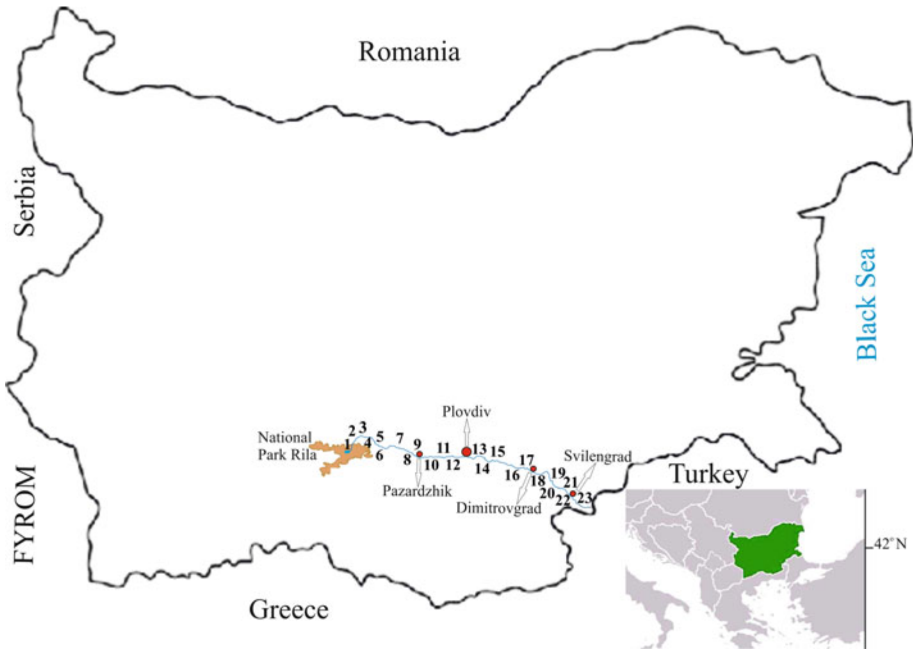

Fig. 1 Map of Bulgaria with location of the 23 sampling sites and major cities along river course (1. Marichini Lakes in Rila Mountain—1,700–2,925 m a.s.l.; 2. Forest near chalet Maritsa; 3. above Raduil village—923 m a.s.l.; 4. before Dolna Banya town; 5. after Kostenets town; 6. village Momina Klisura; 7. NW from Septemvri town; 8. N from Zlokuchene village; 9. before Parzardjik town; 10. N from Sinitovo village; 11. S from Ognyanovo village; 12. 7 km before the town of Plovdiv—160 m a.s.l.; 13. place "Ribarska sreshta"; 14. after Popovitsa village; 15. NW from Hristo Milevo village; 16. before the town of Parvomay; 17. before chemical factory "Vulkan", Dimitrovgrad town; 18. S from Brod village; 19. E near Simeonovgrad town; 20. SE from Harmanli town; 21. NE near Lyubimets town; 22. town of Svilengrad—50 m a.s.l.; 23. SE from Generalevo village—Bulgarian-Turkish border)

anions—sulphates, chlorides and nitrates (mg L^{-1}) with a Lovibond Photometer PC 22 (Germany). Bryophyte samples were dried at 40°C until constant weight and then wet ashed. About 1–2 g powdered material was treated with 15 mL nitric acid (9.67 M) overnight. The wet ash procedure was continued with heating on a water bath after addition of 2 mL hydrogen peroxide. This treatment was repeated until full digestion. The filtrate was diluted with double distilled water (0.06 µS cm^{-1}) to 50 mL. All solutions were stored in plastic flasks. Macroelements (P, K, Ca, S, Mg, Mn, Fe, Al, Na) and microelements (Zn, Cu, Pb, Cd, Co, Cr, Ni, As) were determined by atomic emission spectrometry with inductively coupled plasma (ICP–AES) using a SPECTROFLAME instrument (Germany). The detection limits were 0.002 mg L^{-1} for Mn, 0.004 mg L^{-1} for Cd, Co, Cr, Cu, Ni and Zn, 0.02 mg L^{-1} for As, 0.03 mg L^{-1} for Pb, 0.04 mg L^{-1} for Al and Fe, and 0.5 mg L^{-1} for Ca, K, Mg, Na, P and S. Analytical precision was checked with replication (deviation between the duplicates was below 5% in all cases), blanks and stock standard solutions (1,000 µg L^{-1} Merck) for the preparation of working aqueous solutions. Quality control was assured with 3 moss reference materials for the European moss surveys. The measured concentrations were in agreement with recommended values published by Steinnes et al. (1997). The value for each site/period is the average of 3 samples, and for separate analyses is the mean of 3 analytical determinations. Nitrogen in bryophytes was analyzed by a modification of the method of Kjeldahl ($H_2SO_4 + H_2O_2$). The concentrations are presented in mg kg^{-1} dry weight. Chemical analysis of river water was performed just after sampling. Natural water samples of 300 mL were evaporated to dryness and then diluted to 10 mL volume with double-distilled water, a drop of nitric acid added, and the filtrate was analyzed by the ICP-AES method. Data analyses were conducted using CANOCO ver. 4.5 (Ter Braak and Smilauer 2002). Linear method PCA was carried out on bryophyte and river water data without transformation, based on a correlation matrix and standard deviation as inflation factor.

Results and Discussion

Registered bryophyte composition (67 mosses and 6 liverworts) comprised almost 10% of the total Bulgarian bryoflora (Ganeva and Natcheva 2003; Natcheva and Ganeva 2005) indicating that Maritsa River bryophyte diversity is comparatively high. Along the upper course, the 28 species we found represent about 55% of the aquatic bryoflora in Bulgaria. In communities dominated by *Fontinalis antipyretica*, we found only a limited presence of *Platyhypnidium riparioides*, contrary to the wide distribution and tolerance reported previously (Peñuelas 1984). The predominant species in the middle course was *Leptodictyum riparium* on a wide spectrum of substrates. Bryophytes were absent from many sites, especially those dominated by sandy substrates, and the importance of catchment geology and of substrate suitability is thus confirmed (Scarlett and O'Hare 2006). Bryophytes were uncommon also where the river bed is amenable to erosion. In general, unstable river habitats were characterized by small-sized species, while stable ones were colonized by large mosses (i.e. *Fontinalis*, *Hygrohypnum*).

PCA was carried out on dataset of 1,825 bryophyte and river water data. Basic concentrations of parameters are presented in Table 1.

Seventeen bryophyte species were applied as biomonitors (Fig. 2). Fifty-seven bryophyte samples were analyzed. Element concentrations in bryophytes were submitted to PCA (ordination in Fig. 2) and compared to species distribution. PCA revealed a model with four principal components (PCs) explaining 80.7% of the total variance in the data set. The eigenvalue for axis one was 0.473, whereas eigenvalues for axes 2, 3 and 4 were only 0.142, 0.135 and 0.056 respectively. The latter three axes were considered of lesser importance since the first axis therefore captured 47% of the variation in the environmental data set. First PC (PC1) was positively contributed by the environmental variables Mn, P, Na, S, Co, Mg, Ca, Ni, As, all having positive weights and representing pollution impact. The above group of variables was negatively correlated with the

Table 1 Results from analyses of water and/or bryophyte samples collected from 23 selected sites during 4 annual sessions (from 2002 to 2005)

	Water			Bryophytes		
	Min	Max	Median	Min	Max	Median
pH	6.61	8.5	7.85	n.a.		
C (μS cm^{-1})	25	789	488	n.a.		
Eh (mV)	-88	345	185	n.a.		
Anions (mg L^{-1})						
Cl^{-}	0.8	19.3	9.4	n.a.		
SO_4^{2-}	2	400	89.5	n.a.		
NO_3^{-}	0.44	44	7.48	n.a.		
Micro- and macroelements (mg L^{-1}, mg kg^{-1})						
Al	0.0009	0.1122	0.0406	749	8,144	2,988
As	<0.00001	0.0075	0.0016	<0.03	7.8	4.2
Ba	0.0021	0.0236	0.007	n.a.		
Ca	2.6	56.1	27.5	3,629	38,524	13,292
Cd	<0.000003	0.0005	0.0003	<0.007	48	1.0
Co	<0.000003	0.0006	0.0003	<0.007	22	3.8
Cr	0.0010	0.0024	0.0016	n.a.		
Cu	0.0013	0.0109	0.0055	7.7	174	25
Fe	0.0019	0.0401	0.013	727	11469	5078
K	0.28	11.4	3.7	2050	12,890	6,169
Mg	0.31	23.3	6.7	831	5,740	2,734
Mn	0.0022	0.06	0.0051	77	35,650	1,045
N	n.a.			11,800	31,900	18,200
Na	1.2	45.9	19.1	76	3,180	989
Ni	0.0007	0.0478	0.0017	1.8	32	5.1
P	0.004	1.0	0.11	1,022	6,117	3,194
Pb	<0.00003	0.0042	0.0027	7.1	240	22
S	1.1	41.1	15.5	1,379	6,080	2,459
Se	<0.000027	0.0030	0.0016	n.a.		
Sr	0.064	1.2	0.49	n.a.		
Zn	0.0093	0.3772	0.0294	24	2,096	83

n.a. not analyzed

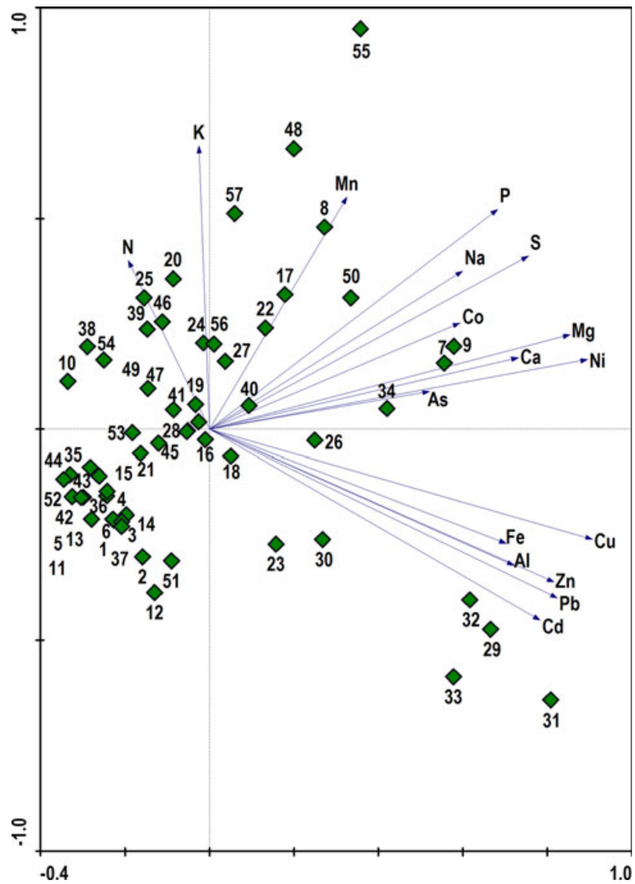

Fig. 2 Ordination of the 57 bryophyte samples towards 17 macro- and microelement concentrations in the tissues. *Arrows* indicate environmental variable effects. *Diamonds*—relevant moss sample (1–3, 16, 25 *Platyhypnidium riparioides*; 4–6 *Scapania undulata*; 7–9, 17, 19, 22, 24, 26–34 *Leptodictyum riparium*; 10 and 39 *Rhizomnium punctatum*; 11 and 13 *Sanionia uncinata*; 12 *Warnstorfia exannulata*; 14 *Bryum pseudotriquetrum*; 15, 21, 23 *Brachythecium rivulare*; 18 *Hygroamblystegium tenax*; 20 *Hygroamblystegium fluviatile*; 35 *Plagiochila porelloides*; 36 *Brachytheciastrum velutinum*; 37 *Sciuro-hypnum plumosum*; 38 *Atrichum undulatum*; 40 *Schistidium agassizii*; 41 *Plagiomnium rostratum*; 42–57 *Fontinalis antipyretica*)

6 variables that dominated PC2 (Cu, Fe, Al, Zn, Pb, Cd). Macroelements N and K contributed to PC4 and were also negatively correlated with the group of variables that exhibited their highest values at PC2.

The 57 bryophyte samples were pinpointed on the biplot. The second component revealed a gradient along which sites located in the upper river course were separated from those in the middle course. In the 1st quadrant were located samples from sites in the beginning of the middle course and sites located close to larger cities, indicated by higher values for macroelements P, Ca, Mg, Mn and microelements Co, Ni, As, and represented by *F. antipyretica*, *Schistidium agassizii* and *L. riparium*. Points located in the 4th quadrant represented samples with high Fe, Al, Zn, Cu, Pb and Cd values, collected from sites (15–18) suffering from relevant anthropogenic disturbance. The species having its centre of distribution close to these sites was again *L. riparium*. The quadrant included also sites 5, 8 and 12 dominated by *Hygroamblystegium tenax*, *L. riparium* and *Brachythecium rivulare*. The finding of *Hygroamblystegium tenax* in these habitats was in disagreement with Dierssen (2001), who stated this species is an indicator of an oligosaprobic zone, and therefore absent from sites with elevated anthropogenic pressure. Points located in the 3rd quadrant represented samples with below average values for all environmental variables, collected from the highest river course and characterized by reference conditions and several taxa: *F. antipyretica*, *Bryum pseudotriquetrum*, *Plagiochila porelloides*, *P. riparioides*, *Sanionia uncinata*, *Scapania undulata*, and *Warnstorfia exannulata*. In the same quadrant was also the third sampling site (3) from the upper course. It should be noted that data from *F. antipyretica* (#s 45 and 53 in Fig. 2) placed the site close to the center of the diagram, i.e. to the mean values of measured elements, compared to *Brachytheciastrum velutinum* and *Sciuro-hypnum plumosum* sampled at the same site which placed it distantly probably due to their lower bioconcentrational capacity. An analogous situation was observed for site 8 which is placed next to the center (# 16) according to data from *P. riparioides*, and at the very third quadrant according to date from *B. rivulare* (#s 15 and 21). Close to the mean values was also sampling site 13 represented by *L. riparium* (# 28). The 2nd quadrant also represented samples collected from the upper river course and site 8, characterized by higher N and K values. The results clearly reflect that elemental content in bryophyte tissue is more representative of water chemistry. The species distribution is probably more dependent on altitude and substrate.

The results for 26 parameters of the river water measured once a year at each monitoring site conformed with the requirements for first (highest) category surface running waters in Bulgaria (Regulation 7 1986), except for SO_4^- and electrical conductivity (Table 1). Values for the 26 measured variables were divided into three groups (physico-chemical, anions and macro- and microelements), and for each of them a separate PCA was performed. Water pH, conductivity and redox potential PCA: four PCs described 82.1% of the variance in the data set. Eigenvalues for the first two axes were 0.297and 0.247 (capturing 54.4% of the total variance). The first component revealed a gradient along which sites with below average values were separated from those (12–13) with higher values, located close to the largest town. Anions PCA: four PCs described 83.2% of the variance. The first and second principal components having eigenvalues 0.374 and 0.205 respectively, accounted for 57.9% of the total variation. The regularity of higher bryophyte diversity in higher pH values at alkaline mountain springs found by Glime and Vitt (1987) was not found to comport our results. Species composition at Rila Mountain where the lowest pH levels (6.61–7.28) were measured was characterized by the highest biodiversity probably due to cumulative ecological factors (altitude, geology, temperature, etc.), as well as the complex of the micro- and macroelements ions in reference conditions. *F. antipyretica*—one of the most common species in the upper course, was also observed at anthropogenically influenced sites along the middle course. The pH interval (6.6–8.4) for sites where *F. antipyretica* was collected was wider and higher than its tolerance range reported by Dierssen (2001): 7.0–7.5. *F. antipyretica*, *P. riparioides*, *B. rivulare* and *S. undulata* were representative mainly of the upper course where conductivity and sulphates had low values. Nevertheless, we registered *B. rivulare* and *F. antipyretica* also in the middle course habitats with elevated levels of the two parameters (up to 359 μS cm^{-1} and 88 mg L^{-1}, respectively). These results suggest the two species above show a greater resistance to high conductivity and sulphate concentrations than reported by López et al. (1997).

Macro- and microelement concentrations in water during each sampling event were submitted to PCA (ordination in Fig. 3). Four PCs described 71.3% of the overall data variation in the data set of 20 elements in river water. Eigenvalues for axes 1 and 2 were 0.258 and 0.213, while those for axes 3 and 4 were only 0.130 and 0.112 respectively, and were not considered further. The first two axes therefore captured 47.1% of the variation in the environmental data set. The variables with positive weight on PC1 were Co and Se (1st event sampling); As (1st and 2nd event sampling), Mn and Cr (2nd event sampling); P, Ca, S, Na and Mg (3rd and 4th event sampling); K, Mn, Fe, Zn, Cu and Ni (4th event sampling). The group of variables formed by Ni (2nd and 3rd), K, Mn, Fe, Zn, Cu and Pb (3rd) and Al (3rd and 4th sampling events) contributed positively to PC2. The following variables contributed positively to PC4: K, Ca, S, Na, Mg, Fe, Al, Zn, Cu, Pb, Ni, Cr, Ba, Sr (1st sampling); P, Mn and Co (2nd sampling) and Cd. PC1, PC2 and PC4 were contributed by the environmental variables measured during the different sampling events. At the bottom-left quadrant of the diagram were sites with lower than average values of measured variables. Among them were sites 15 and 18, characterized as the most anthropogenically disturbed based on bryophyte tissue content. This confirms the importance of measuring the concentrations of elements in bryophyte tissues in describing water quality. Similar results have been observed in a previous study based on *F. antipyretica* collections along the Nysa River and its main tributaries (Vazquez et al. 2004), confirming bryophytes are excellent tools for water quality assessment.

Bryophyte populations were seriously affected by structural changes along the middle course of the Maritsa

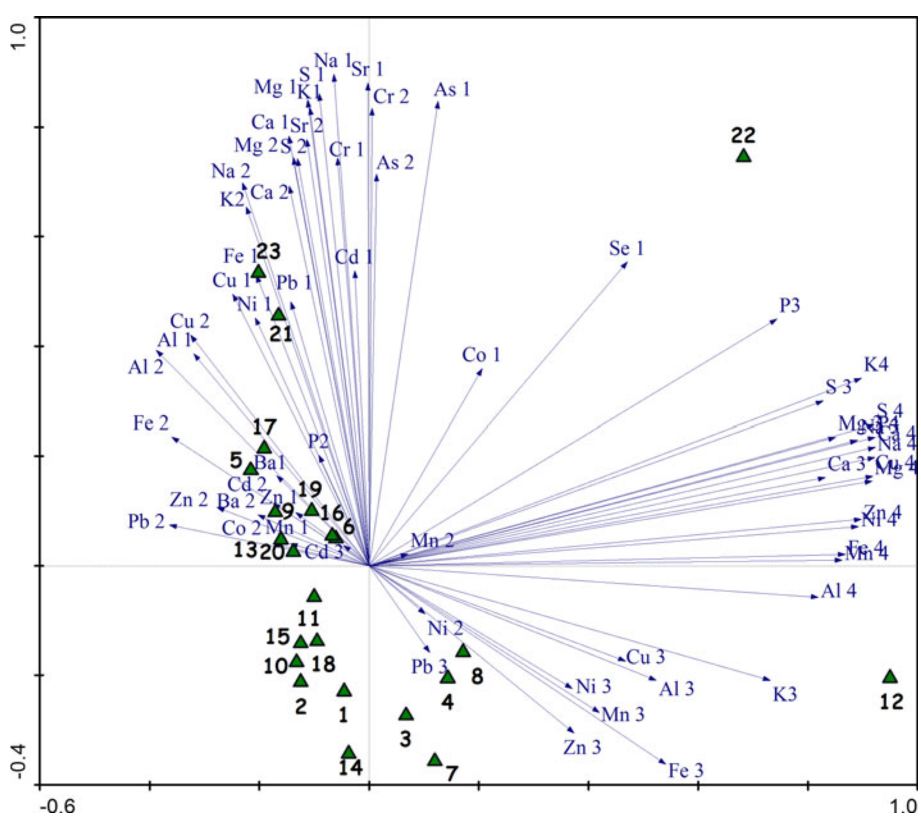

Fig. 3 Ordination of the 23 sample sites towards 20 micro- and macroelement concentrations in the water. *Arrows* indicate environmental variable effects. The number next to each variable symbol refers to the sampling event (from 1 to 4). *Triangles*—relevant sampling sites with corresponding numeration (according Fig. 1)

River as a consequence of some extreme natural disturbances (e.g. floods and droughts). Sites there were also subject to anthropogenic pressure due to agriculture, urbanisation and industrialisation, but also to riverbed morphological modifications. Species compositional patterns suggest that flow regime is the most influent factor for aquatic bryophyte diversity. The flow regime is affected by floods and droughts, which, in turn, determines the availability of substrate for byrophyte colonization.

The elemental content in bryophyte tissue and corresponding water samples was the major component that differentiated sampling sites. PCA also indicated that *F. antipyretica* and *L. riparium* had the ability to accumulate high amounts of trace elements, and possessed a particular tolerance towards strong anthropogenic influence. Therefore, we suggest employing these two moss species to monitor sites disturbed by multiple pollution sources, as in industrial and urban areas. Physico-chemical water variables represent the major component differentiating bryophyte assemblages at sites affected by increasing levels of anthropogenic pressure. But even if the observed overall compositional patterns were significantly concordant, the relative importance of environmental factors underlying their community compositions differed strongly. Thus, in the assessment of surface water quality, bryophyte species composition is representative of river hydromorphology, while the content of elements in bryophyte tissue reflects water chemistry.

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