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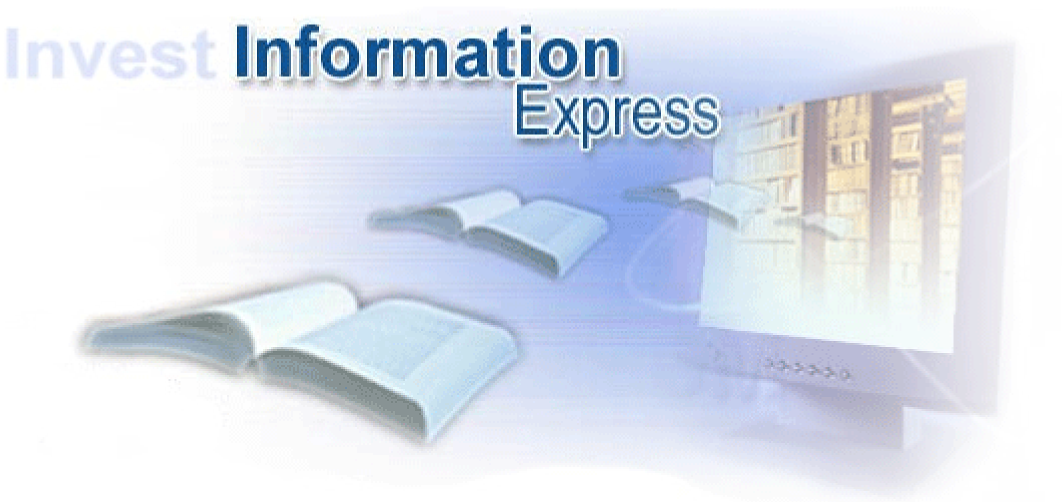

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BIOMONITORING IN RUNNING RIVER WATER WITH AQUATIC BRYOPHYTES

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Abstract:

Heavy metals Ni, Co, Zn, Pb, Cd, Cu and toxic element As as well as the macronutrients N, K, Ca, Mg, Fe, P, Mn, S, Al and Na were measured in water and in the 17 aquatic bryomonitors sampled from 23 locations in the Maritsa River (East Aegean Sea Area, Bulgarian territory). These species are used to evaluate the distribution of elements along the river water, contained elevated levels of all studied elements. According to the specific accumulation capacity and bioconcentration factors two specimens - Fontinalis antipyretica and Amblystegium riparium should be used as suitable biomonitors in the different parts of the river. It was established that the moss Fontinalis antipyretica has higher accumulation capacity for analyzed heavy metals compared with Amblystegium riparium at the sites they occurred naturally together.

Key words: *biomonitoring, aquatic bryophytes, Maritsa River*

INTRODUCTION

For years the possibility of observing the element status of our environment using living organisms instead of direct measurements of the emission into the ecosystems has been intensively discussed. Passive biomonitoring or method '*in situ'* is a popular method of using plant species grown into the survey area as diagnostic means for environmental pollution. Bryophytes play an important role in the freshwater ecosystems providing food and habitat for a host of invertebrates (Gerson, 1982; Varga, 1992) and vertebrates. For a variety of reasons these organisms are preferred in researches of environmental contamination: the absence of roots which excludes substrate influence (Smith, 1980), bryophytes could survive at high levels of pollutants (Empain, 1977); some species display high accumulation factors in relation to the ambient water medium, etc. There is quite a large body of literature about the response of bryophytes species to water pollution. According to Dierssen (2001) *Fontinalis antipyretica* indicates oligo-mesosaprobic zone in freshwater ecosystems. There are diverse data on the response of that bryophyte species to water pollution (Say & Whitton, 1983; Lopez & Carballeira, 1989, 1993; Yurukova et al., 1996, 1997; Bruns et al., 1997). Its resistance to elevated pollutant levels combined with its wide distribution suggests that it could represent promising indicator for water metal content. Utilizing aquatic bryophytes to assess water pollution is not widely distributed in Bulgaria. *Fontinalis antipyretica* was used as bioindicators in an investigation of the Arda River (Kirin et al., 2002). The international river Maritsa runs through an area of approximately 321 km in the Bulgarian territory and flows into the Aegean Sea (Fig. 1). We have been investigating 17 aquatic bryophyte species in the Maritsa River and their specific accumulation capacity and bioconcentration factors. The aim of the current paper is to summarize some important results of 3 years observations. Some of our previous papers are focused on the attempt for the active biomonitoring with *Fontinalis antipyretica* in the Maritsa River (Yurukova & Gecheva, 2003) and characteristic of the river sediments (Filcheva et al., 2003).

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Fig. 1. Map of Bulgaria and Maritsa River.

MATERIAL AND METHODS

Samples of aquatic bryophyte species and river water have been collected in summer and autumn seasons from 2001 till 2003. Twenty-three sites were selected along the main bed of the river (Fig. 2). The names of the taxa followed current nomenclatures for the mosses (Corley et al., 1981; Cortini Pedrotti, 2001) and for the liverworts (Grolle & Long, 2000). The categories of threat are according to the European Red data Book of Bryophytes (ECCB, 1995). The background site is situated at the beginning of the Maritsa River, Central Rila Reserve, 1668 m a.s.l. The samples were dried at 40^o C and then wet ashed. About 1-2 g powdered material was treated with 15 ml nitric acid overnight. The wet ashed procedure was continued with heating on a water bath, following by addition of 2 ml hydrogen peroxide. This treatment was repeated till full digestion. The filtrate was diluted with double distilled water to 50 ml. All solutions were stored in plastic flasks. Macro-elements (N, P, K, Ca, S, Mg, Mn, Fe, Al, Na) and micro-elements (Zn, Cu, Pb, Cd, Co, Ni, As) have been determined by atomic emission spectrometry with inductively coupled plasma (ICP – AES) using SPECTROFLAME instrument (Germany). Analytical precision was checked with replicating, blanks and stock standard solutions. The quality control was assured with moss-reference samples prepared during the European moss survey in 1995 (Yurukova, 2000). The results of ICP-AES analysis are in good agreement with certified values (RSD < 5%). The value for each period is the average of 3 samples and for separate sample is the mean of 5 analytical determinations. Nitrogen was analyzed by the method of Kjeldahl (H_2SO_4+H_2O_2). The concentrations are presented in mg/kg dry weight. Chemical analysis of river water was performed within two weeks after sampling. 300 ml from natural water samples were evaporated to dryness and then diluted to 10 ml volume with double-distilled water and a drop of nitric acid, and then filtered and analyzed with ICP-AES. The bioconcentration factor as a ratio of the concentration of element X in moss tissues and concentration of element X in river water was calculated for the species from each location.

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Fig. 2. Sampling sites along Maritsa River.

RESULTS AND DISCUSSION

Bryophyte flora of the Maritsa River includes 66 mosses and 4 liverworts (Gecheva, 2003; Yurukova & Gecheva, 2004). Three species are rare (*Schistidium agassizii* Sull. & Lesq., *Pohlia obstusifolia* (Brid.) L.F.Koch and *Bryum bicolor* Dicks), *Orthotrichum pumilum* Sw. is endangered, *Brachythecium plumosum* (Hewd.) Bruch, Schimp. & W.Gümbel is not sufficiently studied as well as the first species is included in the European Red Data Book of Bryophytes (Gecheva, 2003; Yurukova & Gecheva, 2004). Two species *Amblystegium fluviatile* (Ganeva & Gecheva, 2002) and *Bryum supapiculatum* were found for the first time in Bulgaria. The prevailing part of the macro- and microelements have expected minimum content in the upper stream river water and insignificant alterations through the research period. The river water from the middle stream has improved quantitative indices at end of the investigation. Twelve bryophyte species applied as biomonitors in the upper stream are Plagiochila porelloides, *Scapania undulata*, *Atrichum undulatum*, *Bryum pseudotriquetrum*, *Rhizomnium punctatum*, *Fontinalis antipyretica*, *Amblystegium riparium*, *Sanionia uncinata*, *Warnstorfia exannulata*, *Brachythecium velutinum*, *B. plumosum* and *Rhynchostegium riparioides*. The inorganic element content of 17 bryophytes analyzed as biomonitors of elements in different seasons and locations of the Maritsa River could be summarized as follows. The obtained data from 38 moss samples show in the prevailing part nitrogen has maximum content. The mean elements concentrations in the tested bryophytes from unpolluted river regions have the following diminishing order: Ca, K, Fe, Al, S, P, Mg, Mn, Na, Zn, Cu, Pb, Co, Ni, Cd. The decreasing order of the same elements in bryophyte species from polluted areas located along the middle river stream is: Ca, K, Fe, Mn, Al, P, Mg, S, Na, Zn, Pb,Cu, Ni, Cd, Co, As. *Amblystegium riparium* accumulates the highest content of P (up to 6117 mg/kg), Ca (up to 38524 mg/kg), S (up to 6080 mg/kg), Mg (up to 5740 mg/kg), Fe (up to 11469 mg/kg), Zn (up to 2096 mg/kg), Cu (up to 174 mg/kg), Pb (up to 240 mg/kg), Cd (up to 48 mg/kg), Co (up to 11 mg/kg), Ni (up to 26 mg/kg) and As (up to 8.0 mg/kg), *Fontinalis antipyretica* (upper 10 cm fragments) – of Mn (up to 35650 mg/kg) and Al (up to 8144 mg/kg), *Rhizomnium punctatum* – of K (up to 12227 mg/kg), and *Amblystegium fluviatile* – of Na (up to 3180 mg/kg). The variation of the values found is very high for Mn (463-fold) and Cd (159-fold), followed by Zn (89-fold), Pb and Co (35-fold). Minimum variation is observed for S (4-fold), P and K (6-fold) concentrations. *Atrichum undulatum* accumulates in its tissues the lowest content of Ca, Mn, Zn, Co and Ni. Minimum concentrations of P, Na and Mg are found in *Scapania undulata*, of S and Cu are in *Sanionia uncinata*, and of Fe and Al in *Rhizomnium punctatum* and of K in *Warnstorfia exannulata*. The concentrations of As with the detection limit of 0.6 mg/kg have only maximum

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values (up to 7 mg/kg d.w.). Changes in the concentration of heavy metals Cd, Co, Ni, Cu, Pb and Zn in the upper segments of *Fontinalis antipyretica* from the beginning of the river to the last site where the species is distributed is shown at Fig. 3. The metals, especially Zn show high levels at the village of Momina Klisura probably due to the periodically discharge of water after the lock before the village. Second expected increasing of the heavy metals concentration occurs before the town of Plovdiv. Heavy metals content in *Amblystegium riparium* found in 13 locations from the middle part of the river are shown at Fig. 4. Accumulation moss capacity confirmed elevated water pollution at the region of the Plovdiv city (Zn, Pb, Cu, Ni and Cd) and the highest values of Zn, Cd, Ni and close to the maximum content for Cu and Pb at the sampling site between the towns of Parvomai and Dimitrovgrad. *Fontinalis antipyretica* clearly shows higher (especially for lead) sensibility in the accumulation of the heavy metals compared with *Amblystegium riparium* at the sites they inhabit together.

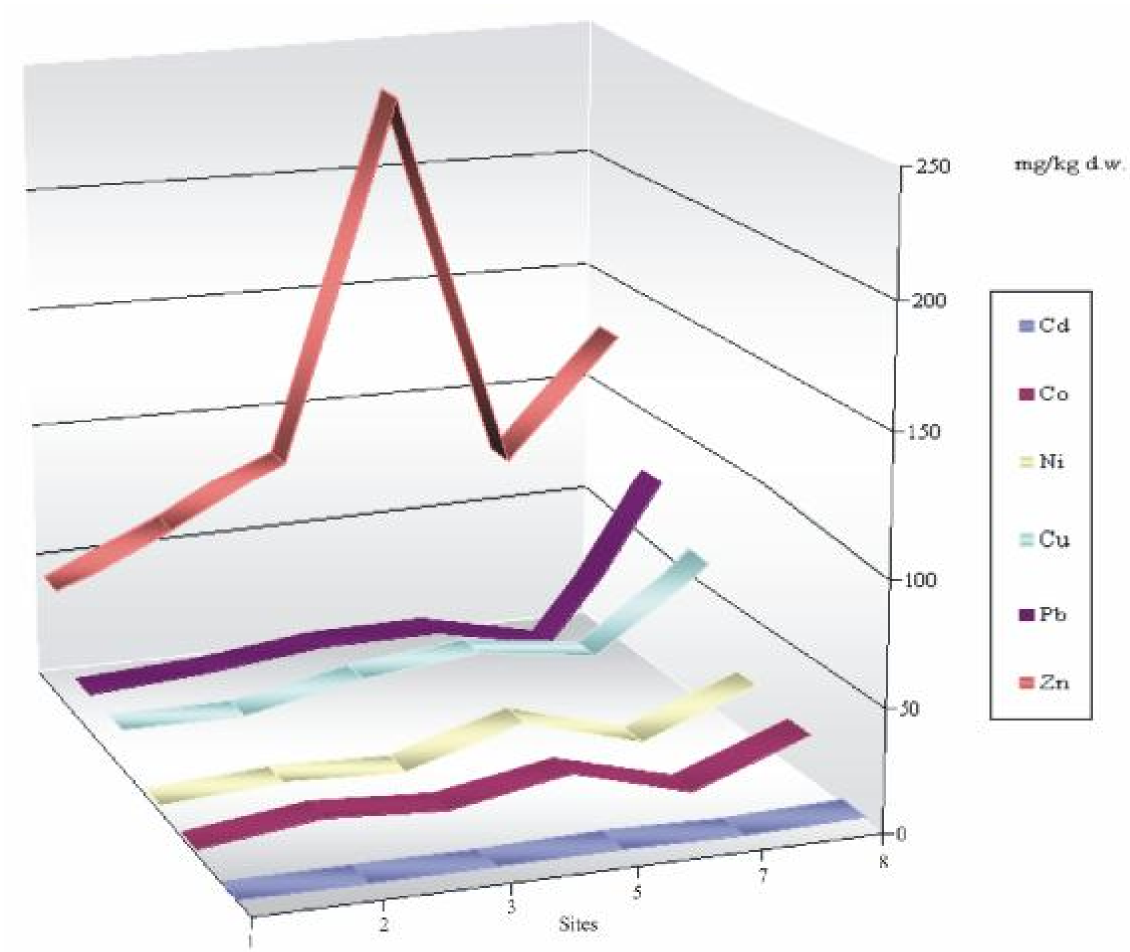

Fig. 3. Changes in Cd, Co, Ni, Cu, Pb and Zn concentrations in *Fontinalis antipyretica* from natural locations of Maritsa River, mg/kg d.w., site 1 – Maritsa chalet, Rila Mountain; site 2 – village of Raduil; site 3 – town of Dolna Bania; site 5 – village of Momina Klisura; site 7 – NW from the town of Septemvri; site 8 – before the town of Plovdiv.

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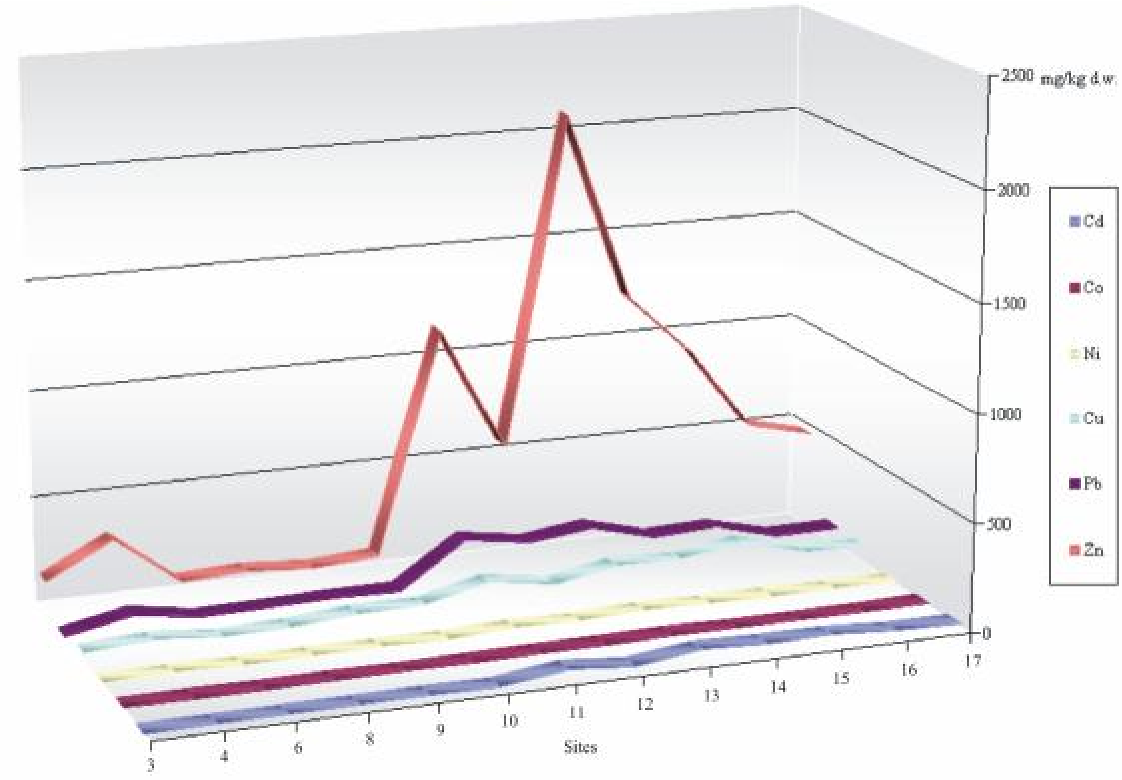

Fig. 4. Changes in Cd, Co, Ni, Cu, Pb and Zn concentrations in *Amblystegium riparium* from different locations of Maritsa River, mg/kg d.w.; site 3 – town of Dolna Bania; site 4 – town of Kostenets; site 6 – NE from the village of Zlokuchene; site 8 – before the town of Plodiv; site 9 – town of Plovdiv; site 10 – after the town of Plovdiv; site 11 – before the village of Hristo Milevo; site 12 – after the village of Hristo Milevo; site 13 – town of Parvomai; site 14 – town of Dimitrovgrad; site 15 – village of Brod; site 16 – town of Liubimets; site 17 – town of Svilengrad.

The bioconcentration factors for all 12 species in the upper stream are as follow: 10^2 for Na, 10^2 – 10^3 for S, Mg, Zn, 10^3 for Pb, 10^2 – 10^4 for K, Ca, Ni, 10^3 – 10^4 for Cu, 10^3 – 10^5 for P, 10^4 – 10^5 for Al, 10^4 – 10^6 for Mn and 10^5 – 10^6 for Fe. The orders for 7 mosses found in the middle stream remain the same only for Mg and Fe. A greater variation of orders values for this area is established: 10^1 – 10^4 for Zn, Cu, Pb, Ni, 10^2 – 10^6 for Mn, 10^3 – 10^6 for Al. The factors of K, Ca, S and Na diminished: 10^2 - 10^3 for K and Ca, and 10^1 - 10^2 for S and Na. The value for P is 10^4 – 10^5. As a result of the passive biomonitoring could be noted that bioconcentration factors for *Fontinalis antipyretica* (upper fragments) are 10^5 for P, Mn, Fe, Al, 10^4 for K, Pb, Cd, Co, 10^3 for Ca, S, Mg, Zn, Cu, Ni in the background sites in the upper stream of the river. The factors for this moss species are one order of magnitude less for P, K, Ca, S, Mg, Al, Cd, with the same order for Fe, Zn, Pb, Co and one order of magnitude up for Cu, Mn, Ni in the Plovdiv district. *Amblystegium riparium* showed bioconcentration factors after town of Belovo as follows: 10^5 for Fe, 10^4 for P, Mn, Al, 10^3 for Zn, Cu, Ni, 10^2 for K, Ca, S, Mg. The factors of Mn, Fe, Al, Zn, Cu, Ni for this species increased to the border (village of Generalevo), whereas those for P, K, Ca, S, Mg were retained. Although the biomonitoring process has been carried out in a large number of localities with different level of pollution and with diverse bryophyte species, the bioconcentration factors have varied in close limits. It was made an attempt at confirming the heavy metals distribution values in *Fontinalis antipyretica* and *Amblystegium riparium* from all investigated sites mosses were distributed and different periods (Fig. 5). Carballeira & López (1997) used *Fontinalis*

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antipyretica (from 36 rivers in NW Spain) to identify background level of metals, their results are close to results of this study for Fe, Pb and Cd from the Central Rila Reserve. The concentrations of Co, Ni, Mn, Cu and Zn given in the quoted paper are higher than in the upper part of Maritsa River. Say & Whitton (1983) investigated the accumulation of heavy metals in *Fontinalis antipyretica* from 52 sites in North England and Belgium. In comparison our results for the sample of the Plovdiv region were similar to their maximum concentration for Ca, to mean values for Mn, Cu, Cd and Pb, and to minimum concentration for Zn. Comparing the obtained data with those of Lopez et al. (1993) from 32 rivers in NW Spain showed that some of the values in this study were higher. Lead in the species at Plovdiv district exceeded more than 75% the mean value given in the quoted paper. The other element with higher concentration was Fe – 8776 mg/kg at Plovdiv region and 6254 µg/g mean value at the polluted rivers from NW Spain respectively.

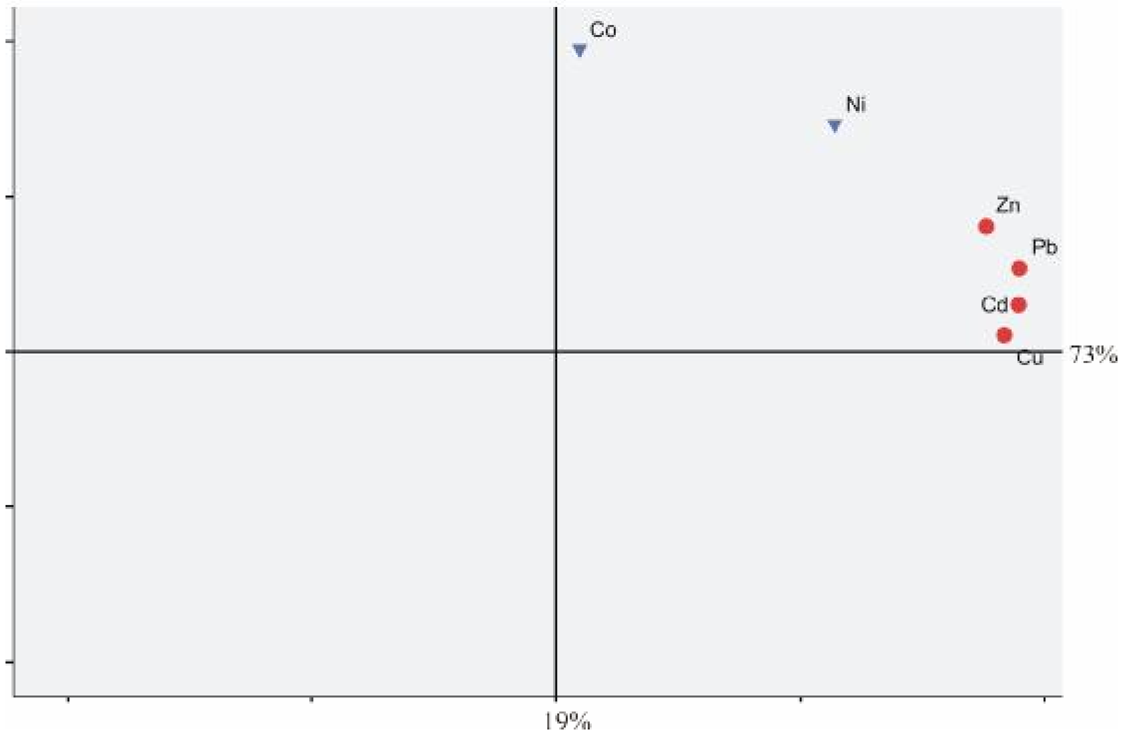

Fig. 5. Principal Component Analysis.

CONCLUSION

The Maritsa River suffer from urban and industrial pollution but the observed decreasing of heavy metals and toxic element As concentrations in water samples from studied sites at the end of the research period represents promising alteration. Bryophyte species tested as monitors have the following bioconcentration factors: 10^1-10^3 for Na and S, 10^2-10^3 for Ca and Mg, 10^2-10^4 for K and Ni, 10^3 for As, 10^2-10^5 for Zn, 10^3-10^4 for Cu, Pb and Cd, 10^3-10^5 for Co:, 10^4-10^5 for Al, 10^4-10^6 for P, Fe and Mn. The results show that the mosses *Fontinalis antipyretica* and *Amblystegium riparium* have high accumulation ability and should be used as suitable biomonitors in the different parts of the river. One of the method advantages for aquatic bryophytes is the possibility to be implemented throughout the year. Comparison between the two main bryomonitors tested at their common sites illustrated *Fontinalis antipyretica* has higher accumulation capacity for Cd, Co, Ni, Cu, Pb and Zn.

ISBN 954-9368-16-5

REFERENCES

1. Gerson, U. 1982. Bryophytes and invertebrates. – In: Bryophyte ecology, A.J.E. Smith (Eds.), Chapman and Hall, New York, 291-332.

2. Varga, J. 1992. Analysis of the fauna of protected moss species. Biological Conservation. Vol. 59, no. 2-3, 171-173.

3. Smith, A.J.E. 1980. The moss flora of Britain and Ireland. Cambridge University Press, Cambridge, 706.

4. Empain, A.M. 1977. Ecologie des populations bryophytes aquatiques de la Meuse de la Sambre et de la Somme. Doctoral Dissertation, University of Liege, Belgium.

5. Dierssen, K. 2001. Distribution, Ecological Amplitude and Phytosociological Characterization of European Bryophytes. J. Cramer, Berlin-Stuttgard, 289.

6. Say, P.J., B.A. Whitton. 1983. Accumulation of Heavy Metals by Aquatic Mosses. 1: *Fontinalis antipyretica* Hedw. Hydrobiologia, 100, 245-260.

7. Lopez, J., A. Carballeira. 1989. A Comparative Study of Pigment Contents and Response to Stress in Five Aquatic Bryophytes. - Lindbergia, 15, 188-194.

8. Lopez, J., A. Carballeira. 1993. Interspecific Differences in Metal Bioaccumulation and Plant-Water Concentration Ratios in Five Aquatic Bryophytes. – Hydrobiologia, 263, 95-107.

9. Yurukova, L.D., A.S. Ganeva, A.A. Damyanova. 1996. Aquatic Bryophytes as Bioconcentrators of Macro- and Microelements. -In: Observatorie de montagne de Moussala, OM2, J.P. Calbonnel, J. Stamenov (Eds.), Sofia, 4, 127-136.

10. Yurukova, L., A. Damyanova, K. Ivanov, V. Janminchev. 1997. Bioaccumulation of *Fontinalis antipyretica* from Musalenska Bistrica River, Rila Mountain. - In: Observatoire de montagne de Moussala - OM2, J.P. Calbonnel, J. Stamenov (Eds.), Sofia, 6: 77-86.

11. Bruns, I., K. Friese, B. Markert, G.-J. Krauss. 1997. The Use of *Fontinalis antipyretica* L. ex Hedw. as a Bioindicator of Heavy Metals. 2. Heavy Metal Accumulation and Physiological Reaction of *Fontinalis antipyretica* L. ex Hedw. in Active Biomonitoring in the River Elbe. – Sci. Total Environ., 204, 161-176.

12. Kirin, D., G. Buchvarov, N. Kuzmanov. 2002. Biological diversity and ecological evaluation of the fresh water ecosystems of the Arda River. - Journal of Environmental Protection and Ecology, v. 3, 2: 449-456.

13. Yurukova, L., G. Gecheva. 2003. Active and passive biomonitoring using *Fontinalis antipyretica* in Maritsa River, Bulgaria. – J. Balkan Ecology, v. 6, 4: 390-397.

14. Filcheva, E., L. Yurukova, G. Gecheva. 2003. Some characteristics of sediments from Maritsa River, Bulgaria. In: - Ecology and Future, v. II, 3-4: 30-33.

15. Corley, M.F.V., A.C. Crundwell, R. Düll, M.O. Hill, A.J.E. Smit. 1981. Mosses of Europe and the Azores: an annotated list of species, with synonyms from the recent literature. - Journal of Bryology, 11: 608.

16. Cortini Pedrotti, C. 2001. New check-list of the mosses of Italy. - Fl. Medit., 11: 23-107.

17. Grolle, R., D.G. Long. 2000. An annotated check-list of the Hepaticae and Anthocerotae of Europe and Macaronesia. - Journal of Bryology, 22: 103.

ISBN 954-9368-16-5

18. ECCB. 1995. Red Data Book of European Bryophytes. - European Committee for Conservation of Bryophytes, Trondheim.

19. Yurukova, L. 2000. First Bulgarian data in the European bryomonitoring of heavy metals – ISBN 954-9746-03-8. Sofia, 30.

20. Yurukova, L, G. Gecheva. 2004. Biomonitoring in Maritsa River using aquatic bryophytes. – J. Environmental Protection and Ecology.

21. Gecheva, G. 2003. Aquatic bryophytes from the upper stream of the Maritsa River – part of the biodiversity of the Rila Mountain. – In: International Scientific Conference, Forest Research Institute, 1-5 October, Sofia, 277-279.

22. Ganeva, A., G. Gecheva. 2002. *Amblystegium fluviatile* (Hedw.) Bruch, Schimp.& W.Gümbel (*Amblystegiaceae*, Bryopsida): a new species for the Bulgarian bryoflora. - Phytologia Balkanica, v. 8, 3: 311-314.

23. Carballeira, A., J. López. 1997. Physiological and statistical methods to identify background levels of metals in aquatic bryophytes: Dependence on Lithology. – J. Environmental Quality, 26: 980-988.