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# Use of algae for monitoring of heavy metals in the River Vardar, Macedonia

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## Abstract

*Aiming to resolve some of the problems regarding monitoring of heavy metals in rivers using Cladophora glomerata and epilithic algal communities, a year's survey of Co, Cd, Cu, Fe, Mn, Pb and Zn has been conducted on the river Vardar, FY Republic of Macedonia. Obtained results and statistical analysis clearly point out the well documented possibility of using epilithon (basically diatom communities) as a monitoring tool, since correlation patterns for epilithon are either better or the same as those for Cladophora, while at the same time epilithon is much more reliable for monitoring, especially in cases when no other plant material can be obtained.*

**Keywords:** Biomonitoring, Heave metals, *Gladophora,* Epilithon, Vardar (Axios).

## Introduction

A large number of investigations into the effect of heavy metals on water environments and their accumulation in various hydrobionts can be found in literature data (KELLY & WHITTON, 1989a; GENTER 1996; WANG & LEWIS, 1997). Submerged and emerged photosynthetic organisms are very important components of river ecosystems, but their application to monitoring systems has not reached level of their significance (WHITTON & KELLY, 1995). Organisms show an integrating response to their environment, as well as to fluctuations in water quality, which may be missed by intermittent chemical analysis of water (COX, 1991). Besides direct chemical analysis of river water which is spread out in large amounts very frequently, the content of components and chemical substances in tissues, organs and the whole body, enable the obtaining of valid data for monitoring purposes. Taking into account that concentration factors for heavy metals have a range $10^2$-$10^5$ (KEENEY *et al.,* 1976; OERTEL, 1991), this kind of analysis has great importance in the detection of the incident or discontinued inputs of toxic substances, especially chemicals with a low content in river water (concentrations at detection limit of the instruments). In many cases, contents of heavy metals in algal thali are much better indicators of the pollution load of the ecosystem. This is especially pointed for lead (FORSTER, 1982; LEVKOV, 2001). The idea of using organisms, or communities of organisms, to register and evaluate certain characteristics of the environment is based on the ecological theorem of congruence between the prominence of environmental factors and the requirements of the species (MARKERT *et al.,* 1997).

*Ecological investigations on the River Vardar*

Interest in heavy metal pollution of River Vardar increased after the building of a metal industry in the city of Veles in the late seventies. Cadmium, one of most toxic heavy metals, was determined in the River Vardar in high concentrations (VELJANOVSKI, 1982). The concentrations have maximal values in the region of Veles, but it could be determined downstream from Veles to the border with Greece. GRIZO (1995) gave a review of heavy metals pollution during 1981-1995 and noticed that the values of concentration of particular heavy metal are decreasing. But this situation was a result mainly of decreased production and functioning of metal industry, but not from the measurement that was taken from industry (VELJANOVSKI, 1987).

More complex saprobiological investigations took place in the mid-nineties as a basis for establishing the biomonitoring based on diatoms (KRSTIC & MELOVSKI, 1994; KRSTIC, 1995; KRSTIC *et al.,* 1994, 1996, 1997). Presented data reveal physic-chemical parameters (concentration of main cations, and anions, oxygen regime, pH, etc) and biological features (qualitative composition of diatom flora, abundance of particular species and their saprobiological characteristics).

The data concerning the heavy metal accumulation in sediments are very few (MELOVSKI *et al.,* 1997) and incidental, but useful in determining the hot spots in the River Vardar and the ability of sediment to concentrate heavy metals.

Taking into account all data on the River Vardar, as well as characteristics of the river net, industry and lack of waste water treatment plants in FYR Macedonia, the hydrobiology team at the Institute of Biology started a project entitled *"Biomonitoring of heavy metals in the River Vardar"* to determine the level of contamination of algae with heavy metals and their impact on the algal community in the River Vardar as well as to determine the most valid algal object for biomonitoring of heavy metals in the River Vardar.

## Materials and Methods

The River Vardar is the main watercourse in FYR Macedonia, having a catchment area of 28.410 km$^2$, more than 15 large tributaries and is 388 km long, up to its mouth in the Aegean sea. The concentration of settlements and industry in the River Vardar valley (cities Skopje and Veles, chemical industry, smelters, and irrigation catchment, Fig.1) makes it a complex ecosystem with strong pollution pressure, primarily due to lack of any waste water treatment facility (KRSTIC *et al.,* 1997).

Field investigations on the River Vardar were realized in a period of one year (1998-1999) on ten sampling sites (Fig. 1). Water temperature, conductivity, pH, and concentration of oxygen were measured monthly in the field with appropriate instruments. Water for analysis of concentration of filtrable heavy metals was collected in 1 ½ L polyethylene bottles. After filtration of 500 mL water, it was concentrated 10 times with evaporation and the addition of 1 mL "$H_2SO_4$" and measured on an atomic spectrometer Varian 10BQ.

The sediment was also collected monthly from five different positions at sampling point. Interstitial water was removed with filtration of sediment. After 24 h drying on T=105 °C, 0.5 g of dried material was digested (wet digestion) using the nitric-perchloric-sulfuric acid method (10:2:1) for preparing the probes for AAS according to MOORE & CHAPMAN (1985). Analysis of concentration of heavy metals in total sediment was performed due to


***Fig. 1:*** River Vardar water course in FYR Macedonia and 10 sampling sites.

obtaining the initial data of heavy metal load in the river bed on the sampling sites.

Samples of *Cladophora*, collected monthly from five different positions at sampling site (5 replicates), were vigorously washed in river water, and stored in plastic bottles in a refrigerator (WHITTON *et al.,* 1989). Samples were then transported to the laboratory and washed approximately 10 times in distilled water remove all attached algal cells (checked under the microscope). After 24 h drying on T=105 °C, 0.5 g of dried material was digested (wet digestion) using nitric-perchloric-sulfuric acid method (10:2:1) for preparing the probes for AAS according to MOORE & CHAPMAN (1985). Samples of epilithon were scraped from the surface of five bigger stones with a plastic knife, dried out and checked for presence of *Cladophora glomerata*. The procedure followed for digestion was the same for sediment as well. After dissolving of the digested material in distilled water, the samples were measured on atomic spectrometer Varian 10BQ.

The statistical analysis were done with STATISTICA for Windows (StatSoft, Inc. 1995). Two multiple regressions were performed. In the first, the concentration of heavy metals in water as an independent variable, and the content of heavy metals in sediment, *Cladophora* and epilithon as dependent variable was taken, to predict the linkage of these two variables. The second correlation analysis was performed as an attempt to predict the influence of sediment on the accumulation of heavy metals in algae.

## Results and Discussion

The most variable value for pH was obtained on sampling site $T_8$ (Table 1), due to the great impact of waste waters of the chemical industry of Veles (CIV). The waste waters show great variability of pH value from 12.9 to 1.1 (KRSTIC, 1995). Low pH values have an the impact on the dissolving or sedimentation of heavy metals (TACK *et al.,* 1996), as well as on the accumulation processes in algae (VYMAZL, 1995). Beside pH, other physico-chemical parameters have an impact on heavy metal concentrations in natural waters: suspended particles (BOTELHO *et al.,* 1994a; PELLETIER, 1996), redox potential (VERLOO & COTTENIE, 1985) sulfides and phosphates (RECZYNSKA-DUTKA, 1991), chemical interactions (RULE & ALDEN, 1996), organic substances (WHITTON & SAY, 1975; BOTELHO *et al.,* 1994b). The analysis of the concentration of heavy metals in water often gave only an approximate view of heavy metal load. The relativity of obtained data could be established in short-term or incidental (discontinued) load of heavy metals (VOGEL & CHOVANEC, 1992).

The communal waste waters from the capital Skopje have a great impact on the River Vardar (KRSTIC, 1995), but the concentration of filterable heavy metals is not as high as on the next sampling sites. This could be as a result of the huge load of organic substances, phosphates and sulfides with communal origin. The maximal values for the concentration of Cd, Pb and Zn are determined on sampling site $T_7$ due to the load of waste waters from the metal industry in Veles. The other three analysed elements, Cu, Mn and Fe, show maximal values on the next sampling site ($T_8$ CIV) as a result of the direct input of waste waters with extremely low pH.

Comparing the obtained results (Table 1) with analogous results from other authors, (WHITTON *et al.,* 1989; RECZYNSKA-DUTKA, 1991), it can be found that for some ions (Cd, Pb and Zn) values are much higher. But, compared with analogous data from Vardar in the past several decades the tendency towards decreasing of values could be noticed. This situation is a result of the decreased level of functioning and production of the metal industry in Veles and definitely not as a result

**Table 1**
**Average, maximal and minimal values of measured parameters along the River Vardar (T1-T10) water course in FYR Macedonia.**

| Parametrs | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
|---|---|---|---|---|---|---|---|---|---|---|
| T water (°C) | 12.25<br>(19.1-9.6) | 11.12<br>(17.2-6.7) | 13.16<br>(20.5-6.1) | 14.33<br>(19.6-7.9) | 13.51<br>(20.5-6.9) | 14.81<br>(29.1-5.4) | 15.87<br>(25.9-5.7) | 17.94<br>(28.7-9.5) | 16.36<br>(28.8-5.5) | 16.23<br>(28.8-6.1) |
| pH | 7.61<br>(8.63-6.75) | 7.62<br>(7.97-7.05) | 7.72<br>(8.73-7.29) | 7.78<br>(8.71-7.42) | 7.65<br>(7.92-7.15) | 7.57<br>(7.87-7.25) | 7.51<br>(7.97-7.25) | 6.13<br>(11.3-2.61) | 7.51<br>(7.95-7.06) | 7.80<br>(8.54-7.32) |
| Conductivity (µS/cm) | 102.40<br>(200-84.7) | 200.00<br>(300-100) | 254.55<br>(500-200) | 340.00<br>(400-40) | 345.45<br>(400-200) | 354.55<br>(400-300) | 427.27<br>(600-300) | 854.55<br>(3700-300) | 427.27<br>(700-300) | 418.18<br>(600-300) |
| Cd (µg/L) | 0.02<br>(0.05-0.01) | 0.11<br>(0.6-0.01) | 0.38<br>(1.2-0.01) | 0.33<br>(1.9-0.01) | 0.61<br>(2.9-0.01) | 1.07<br>(4.9-0.01) | 14.40<br>(36-0.5) | 9.32<br>(14.4-0.1) | 1.54<br>(4.7-0.01) | 1.14<br>(3.3-0.01) |
| Cu (µg/L) | 5.93<br>(13-2.9) | 12.58<br>(56-4.4) | 13.27<br>(40-7.8) | 23.03<br>(90-12.3) | 18.87<br>(84-8.2) | 17.15<br>(59-7.5) | 20.27<br>(70-9.6) | 26.67<br>(86-9.9) | 18.62<br>(98-6.9) | 18.26<br>(86-6.9) |
| Fe (µg/L) | 19.25<br>(40-2) | 38.50<br>(96-5) | 50.75<br>(79-22) | 72.42<br>(262-21) | 110.08<br>(311-26) | 120.91<br>(265-26) | 130.50<br>(377-32) | 495.25<br>(1904-43) | 74.17<br>(140-22) | 66.92<br>(142-14) |
| Mn (µg/L) | 1.81<br>(8.5-0.1) | 3.55<br>(6.4-0.1) | 5.13<br>(10.3-0.2) | 19.13<br>(56.2-2.3) | 10.38<br>(29.8-1.1) | 19.58<br>(69.8-2.8) | 47.91<br>(249-10.4) | 61.33<br>(236-8.6) | 12.47<br>(27-5.8) | 9.69<br>(20-0.01) |
| Pb (µg/L) | 12.31<br>(37-1) | 26.25<br>(90-4) | 27.58<br>(103-4) | 37.75<br>(104-4) | 40.58<br>(104-11) | 39.99<br>(104-9) | 62.83<br>(138-16) | 41.83<br>(130-14) | 40.08<br>(101-6) | 40.17<br>(104-4) |
| Zn (µg/L) | 6.92<br>(17.9-3.8) | 30.58<br>(220-4.8) | 15.61<br>(44.6-6) | 15.05<br>(26.7-7.4) | 16.29<br>(24.5-7.4) | 14.96<br>(25-5) | 168.47<br>(397.2-39.7) | 96.98<br>(472.5-12.6) | 16.39<br>(20.6-11) | 30.13<br>(213.5-5.3) |

of measures undertaken for the treatment of waste waters.

The sediment is a depot where a large amount of heavy metals is accumulated (JOHNSON, 1998), but the high heavy metal content in sediment could be the result of high background concentration. In addition the content of heavy metals in sediment could not be correlated with concentration in river water (VERLOO & COTENIE, 1985) due to the their binding in undissolved sulfides and phosphates.

According to VOGEL & CHOVANEC (1992), there are several advantages in using sediment to monitor heavy metals: (i) emission sources can be located and characterized by sediment analyses; (ii) data from single sediment sample represent the pollution of the river over a longer time period; (iii) due to the low concentration in water, some substances can be hardly detected, and substances which accumulate in sediments can be easily detected by sediment analyses.

When the content of heavy metals in sediment is analysed, two different approaches could be applied: (i) heavy metal in the whole sediment and (ii) heavy metals in different fractions of sediment. The fine sediment fraction (<0.2 mm) is particularly useful for determination of heavy metal load in rivers and to distinguish between natural (background, geogenic) and anthropogenic sources (KRALIK, 1999). The size fraction of sediment could be divided into two groups: (1) coarse particles less than 0.2 mm and (2) fine particles less 0.02 mm. Nevertheless, there is experimental uncertainty associated with all presently available methods of metal speciations. In particular, solid phase fractionation schemes suffer from serious limitations: sample handling, reagent selectivity and specificity, interference, etc. (TACK & VERLOO, 1995). Some elements, such as Cd, for example, in natural sediments are strongly bound with organic substances (sulfides), but heavy metals with anthropogenic origin are bound very weakly (RULE & ALDEN, 1992). Nevertheless, the content of heavy metals in sediment depends on different factors such as pH (TACK *et al.,* 1996), type of sediment, ionic strength, conductivity, (ZHOU & KOTT, 1995), algal biofilms (WOODROOFF *et al.,* 1999), redoxpotential (VERLOO & COTTENIE, 1985), size fractions (KRALIK, 1999), etc.

Analyses of sediment in the River Vardar (Table 2) show that on sampling sites located in the upper part of the River Vardar (from source to Skopje) the anthropogenic pressure is low. Decreasing of values on sampling sites located after the communal channels of the towns Tetovo and Gostivar ($T_2$ and $T_3$), is a

**Table 2**
**Average, maximal and minimal values of content of heavy metals in the River Vardar sediment during investigated period 1998-1999.**

| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cd (µg/L) | 0.92 (0.20-3.80) | 0.90 (0.2-2.0) | 1.08 (0.1-2.5) | 2.32 (0.1-3.8) | 2.09 (0.1-5.1) | 2.23 (0.4-3.2) | 133.98 (18.3-212.9) | 35.40 (4.95-27.4) | 8.79 (1.7-25.0) | 4.85 (0.5-14.0) |
| Co (µg/L) | 13.99 (10.5-16.6) | 13.99 (9.4-20.4) | 11.36 (7.3-17.5) | 13.15 (9.7-17.1) | 14.62 (12.5-17.8) | 13.91 (10.3-17.0) | 13.13 (10.3-18.4) | 14.39 (8.6-22.1) | 12.97 (9.1-15.5) | 11.73 (6.7-17.1) |
| Cu (µg/L) | 46.83 (24.8-66.4) | 45.29 (23.5-65.0) | 38.37 (24.3-54.4) | 52.52 (23.5-104.5) | 53.21 (27.9-104.5) | 49.29 (22.6-84.3) | 146.11 (44.8-260.0) | 74.14 (31.1-112.2) | 37.55 (27.2-49.9) | 39.22 (14.1-129.3) |
| Fe (µg/L) | 3234.88 (2509-4925) | 3619.39 (3050-4913) | 3404.92 (2645-4490) | 2889.99 (2256-4106) | 2975.52 (2687-4131) | 3127.94 (2282-4627) | 2851.80 (1975-4095) | 3255.65 (2794-4898) | 3004.00 (2041-4795) | 3127.73 (2058-4361) |
| Mn (µg/L) | 790.95 (600-1625) | 1068.20 (543-1446) | 858.09 (453-938) | 638.78 (440.2-879) | 929.74 (765-1184) | 864.93 (561-1516) | 872.60 (532.5-1141) | 956.14 (590-1270) | 775.60 (494-1492) | 602.86 (413-1368) |
| Pb (µg/L) | 84.81 (15.0-157) | 122.93 (70.0-185.0) | 116.08 (66.0-210.0) | 163.03 (86.3-271.5) | 136.13 (30.0-271.5) | 117.43 (27-207.5) | 630.41 (96.7-1257) | 170.28 (101.0-242) | 139.63 (68.0-201.5) | 121.39 (75.5-214.5) |
| Zn (µg/L) | 102.40 (83.8-143.3) | 87.13 (53.8-106.5) | 76.35 (47.5-120.0) | 173.98 (85.3-257.3) | 214.66 (96.3-454.4) | 196.77 (96.8-369.5) | 1905.31 (524-5573) | 420.83 (286.8-676) | 196.24 (121.8-263) | 134.24 (72.5-177.1) |

result of physico-chemical characteristics of sediment on those locations. Nevertheless, the sediment is composed mainly of inorganic silica particles which have very a weak ability to adsorp heavy metals (ZHOU & KOTT, 1995; BIJLSMA *et al.,* 1994). Starting from sampling site T4 (located in Skopje), the content of heavy metals starts to increase. The highest values for all investigated heavy metals are determined at T7 (Veles). When obtained results from this sampling site are compared with reference dates (GONÇALVES *et al.,* 1992) enormous difference can be noticed. The values for Cd are about 670 times greater than background concentration. This remarkable increase in values also could be noticed for Pb and Zn (VOGEL & CHOVANEC, 1992; DAUVALTER, 1992; FACETTI *et al.,* 1998). According to these, characteristics sediment at this sampling site should be classified and treated as extremely dangerous (KELDERMAN & DROSSAERT, 1999).

WHITTON *et al.,* (1981) suggested 10 plant species as appropriate for biomonitoring of heavy metals in river ecosystems, and four of them belong to the group of algae: *Cladophora glomerata, Enteromorpha sp., Lemanea fluviatilis and Nitella flexilis.*

WHITTON (1970) had pointed out *Cladophora glomerata* as potential species for biomonitoring of heavy metals as a result of wide distribution in water ecosystems. This feature of *Cladophora* was referred to by several authors (BLUM, 1956; ROSEMARIN, 1985; WHITTON *et al.*1989; DODDS, 1991; DODDS & GUDER, 1992; SHEATH & COLE, 1992; WHITTON & KELLY, 1995). The Natural population of *Cladophora* in rivers (Table 3) shown great ability to accumulate of heavy metals (KEENEY *et al.*1976; ADO-RADY, 1980; WHITTON *et al.,* 1981, 1989; KELY & WHITTON, 1989, JACKSON *et al.,* 1990; OERTEL, 1991, 1993). Obtained high significant statistical correlation between concentration of heavy metals in water and *Cladophora* suggest that this species is a very good object for the monitoring of heavy metals (WHITTON *et al.,* 1989; LEVKOV, 2001). In addition, *Cladophora* could be used in the monitoring of eutrophication, e.g. long-term biomonitoring of concentration of nitrogen and phosphorus (WIESE *et al.,* 1986; FREEMAN, 1986).

KELLY & WHITTON (1989) had compared the accumulation ability of aquatlic mosses *(Rhynostegium riparoides, Amblistegium riparium and Fontinalis antypiretica)* and macrophytic algae *(Cladophora glomerata, Lemanea fluviatilis and Stigeoclonium tenue)* and concluded that the background concentrations for algae are lower than mosses. Accumulation processes increase with the

**Table 3**
**Average, maximal and minimal values for content of heavy metals in Cladophora on nine sampling sites during investigated period.**

| | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
|---|---|---|---|---|---|---|---|---|---|
| Cd (μg/L) | 0.50 (0.01-1.30) | 0.75 (0.01-3.12) | 1.38 (0.1-3.97) | 1.55 (0.1-7.39) | 1.12 (0.1-2.79) | 166.36 (12.99-237.9) | 31.31 (0.560-67.37) | 8.78 (0.1-31.95) | 5.11 (0.1-15.83) |
| Co (μg/L) | 1.54 (0.30-2.48) | 3.91 (0.1-4.55) | 4.27 (0.1-8.75) | 5.31 (0.1-11.17) | 4.45 (0.1-10.15) | 6.19 (0.25-18.32) | 8.33 (2.50-22.6) | 3.83 (1.9-12.1) | 2.78 (0.2-7.3) |
| Cu (μg/L) | 18.50 (9.52-28-.5) | 20.83 (14.43-33.05) | 46.58 (12.9-153.3) | 29.48 (18.98-45.4) | 29.74 (14.95-37.19) | 68.42 (16.55-206.5) | 52.22 (15.5-143.8) | 26.08 (11.90-52.6) | 31.69 (11.37-52.6) |
| Fe (μg/L) | 7.78 (3.58-12.8) | 7.67 (2.56-11.78) | 7.46 (4.06-12.91) | 6.96 (3.58-11.57) | 6.31 (4.20-9.13) | 10.65 (4.20-17.7) | 12.77 (4.91-20.42) | 9.27 (3.72-12.06) | 9.76 (3.84-16.85) |
| Mn (μg/L) | 699.2 (272-1258.8) | 1035.5 (230.3-3244) | 887.9 (226.4-1773) | 866.4 (206.6-1936) | 1141.0 (336.3-2112) | 2094.2 (411.3-6214) | 1332.2 (273-2241) | 1079.5 (285-2773) | 625.4 (191.25-1668) |
| Pb (μg/L) | 31.70 (9.50) | 33.81 (6.0-76.5) | 53.53 (18.0-213.5) | 60.49 (11.1-151.1) | 41.67 (27.49-92.0) | 259.97 (71.5-891.8) | 195.28 (55.5-591.7) | 57.16 (31.0-102.0) | 46.60 (7.0-86.0) |
| Zn (μg/L) | 36.47 (20.3-72.1) | 62.60 (28.27-140.4) | 138.18 (33.07-239.6) | 194.36 (51.3-322.2) | 115.43 28.10-197.5 | 1385.41 (265.3-5049) | 372.23 (88.6-807.4) | 163.75 (92.0-382.9) | 145.18 (35.86-264.0) |

increase of the concentration of particular heavy metals (WEHR & WHITTON 1983a, 1983b; WEHR *et al.,* 1987). But the intensity of heavy metals accumulation in algae is higher than in aquatic mosses. This feature makes algae a better and more sensitive object for the monitoring of heavy metals (LEVKOV 2001).

Many studies for detection of heavy metal uptake and accumulation in *Cladophora* are based on a single collection (or several field investigations) of material in the summer period when thre population of alga is dominant in rivers. Data for seasonal changes in the content of heavy metals in *Cladophora* are scarce (RANG & STIKES, 1987). This situation is mainly the result of the decrease in *Cladophora's* population especially in winter months and it is not most the representative (dominant) alga in the river ecosystem. In this period, basal parts of the alga only can be found, but they are not appropriate for chemical analysis due to calcification (WHITTON, 1981). Additionally, large biomass of epiphytic and grazer species makes cleaning of the material more difficult and increase the possibility of contamination (LEVKOV, 2001). Nevertheless, enough material for chemical analysis can be found with a detailed search of the river bottom.

Intensity and kinetics of the bioaccumulation processes of heavy metals were also tested in laboratory conditions (SCHANZ & THOMAS, 1981; VYMAZAL 1987, 1990a, 1990b, 1995; SOBHAN & STENBERG 1999). The kinetics of accumulation of heavy metals consists of two phases; (i) the initial phase is very rapid, occurring immediately after exposure to heavy metal; usually lasting for less than 30 minutes (VYMAZAL, 1990; GENTER, 1996; WANG & DEI, 2001a). This phase is probably passive, involving physical sorption of heavy metals. According to PICKERING & PUA (1969), this phase can be divided in two separate phases: a) intensive accumulation in the first 20 minutes and b) linear increasing in a period of 90 minutes. (ii) second phase, named as metabolism-depend uptake (GENTER, 1996), is extended and slow with duration of more than one month. The accumulation trend could be linear in laboratory conditions (SAKAGUCHI *et al.,* 1979) or hyperbolic in natural conditions (CONWAY & WILLIAMS 1979). Also, nutrients such as N, P and Si have a great influence on heavy metal uptake and kinetics (WANG, 1987, WANG & DEI, 2001b, 2001c).

Comparing the literature data for heavy metal content it could be noticed that for all investigated metals obtained values for the River Vardar are much higher (Table 4). Nevertheless, VYMAZAL (1987, 1990, 1995) investigated the processes under laboratory conditions with exposure of *Cladophora* to concentrations of 200 mg/L of a particular heavy metal over period of six hours. This concentration is much higher than values detected in the River Vardar (and by other researchers), and it is very possible that it results in inhibition of metabolic processes in *Cladophora* and accumulation is due to passive (osmotic) transport of heavy metals and existing high gradient of concentration. These data are very important for explaining the kinetics of heavy metal uptake in *Cladophora.* Beside, algal populations in the River Vardar have permanent heavy metal pressure throughout the year and accumulation processes depend both on the metabolic condition of algae and environmental

**Table 4**
**Maximal detected values for heavy metal content in *Cladophora glomerata* (μg/g dry weight).**

| | Cd | Cu | Pb | Zn |
|---|---|---|---|---|
| Vyzamal (1995)* | 109.6 | 115.2 | 157 | 117.6 |
| Vyzamal (1987)* | / | / | / | 643.0 |
| Vyzamal (1990)* | 119.1 | / | 159.1 | |
| Whitton *et al.* (1989)** | 6.97 | 33 | 330 | 1130.0 |
| Oertel (1991)** | 6.09 | 28.2 | 128 | 270.0 |
| Keeny *et al.* (1979)** | 3.9 | 7.2 | 12.5 | 23.7 |
| Abo-Rady (1980)** | 0.94 | 9.1 | 5.2 | 62.0 |
| Levkov (2001)** | 799.5 | 206.5 | 891.5 | 5048 |

* Data from culture exposed to high concentrations of heavy metal under laboratory conditions
** Natural populations of *Cladophora*

conditions. This is especially expressed at sampling site T8 (CIV) where due to the low pH value of river water (Table 1) accumulation processes are decreased. The obtained values from the River Vardar, compared with WHITTON *et al.* (1989) and OERTEL (1991), show the real heavy metal load in the river. Nevertheless, of cadmium and zinc content is more than 110 and 20 times higher respectively, than most polluted sites in Great Britain and the river Danube.

Beside numerous advantages that *Cladophora sp.* has as a potential biomonitoring object, it also has several limitations and disadvantages. Namely, the growth of *Cladophora* is mainly limited to mesotrophic to eutrophic waters, and depends on concentration of nitrogen and phosphorus (NEIL & JACKSON 1982; MANTAI *et al.,* 1982; MILLNER *et al.,* 1982), temperature, (WHITTON, 1970), light (GRAHAM *et al.,* 1982), water velocity and composition of the bottom (DODDS, 1991). The growth is seasonal and representative material for chemical analysis can be found only in seven to eight months during the year.

Several macrophytic algal species, beside *Cladophora*, were used to determine the effect of high heavy metal concentrations and the possibility of using those species as biomonitoring objects. Due to several features, such as easy recognition and collection of the talus and good ability to accumulate heavy metals, *Lemanea fluviatilis* is one of the potential algal species for biomonitoring (HARDING & WHITTON, 1981; KELLY & WHITTON 1989a) which shows special resistance to zinc and lead (WHITTON & SAY, 1975).

Very similar accumulation characteristics to *Cladophora*, have *Ulotrix zonata* and *Stigeoclonium tenue*, but as a result of limited ecological valence and distribution, application of these species for biomonitoring of heavy metals in river ecosystems is very limited (JACKSON *et al.,* 1990; KELLY & WHITTON, 1989a, 1989b).

The limited ecological valence of *Cladophora glomerata* to pollution parameters and the seasonal pattern of the life cycle are the main factors that make the biomonitoring of heavy metals based on this species uncertain and difficult. This is characteristic mainly of the winter months when *Cladophora* populations decrease. To avoid these problems, an alternative solution is the method based on epilithic communities (biofilms). According to Round (1991) epilithic communities have several advantages as biomonitoring tool, as (i) there is significant difference in content of heavy metal in epilithon for natural (unpolluted) and polluted sites (Table 5); (ii) the ecological valence of epilithon is much wider than filamentous species such as *Cladophora* (iii) the biomass of epilithon, in general, is increases with the increase of the pollution level.

Effects of increased levels of heavy metals on epilithic algal communities is studied under laboratory and natural conditions. Generally in natural communities effects are investigated at chronic (long term) exposures, what is “more realistic, what algae will experience in nature” (GENTER, 1996). They react more completely than filamentous algae or macrophytes (IVORRA *et al.,* 1999). On the other hand, there are very few literature data concerning accumulation of heavy metals in natural diatom communities (LEVKOV, 2001). IVORRA (2000) shows that there is a large difference in the content of heavy metals in algal communities from unpolluted and polluted sites, and mainly shows linear correlation with concentration of adequate heavy metal in ambient water (ABSIL & van SCHEPPINGEN, 1996). This difference is due to the different species composition in diatom communities (ADMIRAAL *et al.* 1997; GENTER, 1987). Some diatom species have developed tolerance mechanisms against cytotoxic effects of heavy metals (TORRES *et al.,* 1995, 1997) to reduce heavy metal toxicity by producing intracellular and extracellular binding components (AHNER *et al.,* 1995; AHNER & MOREL, 1995).

**Table 5**
**Average, maximal and minimal values for content of heavy metals in epilithon on 10 sampling sites during investigated period .**

| | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Cd (μg/L) | 0.35 (0.15-3.5) | 0.24 (0.05-0.55) | 0.17 (0.1-0.25) | 10.05 (3.60-17.85) | 5.00 (0.60-7.85) | 3.14 (1.35-8.05) | 62.93 (41.32-83.9) | 21.05 (5.25-29.0) | 2.98 (1.60-4.30) | 1.96 (1.80-2.15) |
| Co (μg/L) | 5.81 (4.88-6.65) | 8.46 (7.60-9.75) | 9.33 (8.55-10.30) | 11.18 (8.85-12.95) | 11.11 (9.05-15.80) | 11.90 (10.7-17.6) | 13.35 (10.7-17.6) | 11.98 (9.80-13.95) | 11.76 (10.95-13.8) | 12.28 (11.7-12.95) |
| Cu (μg/L) | 42.74 (26.20-72.3) | 31.22 (25.65-41.8) | 34.43 (30.5-40.1) | 49.11 (32.20-61.3) | 20.95 (17.30-25.6) | 24.89 (21.2-32.15) | 102.01 (94.8-119.5) | 68.55 (44.5-115.7) | 32.34 (28.0-41.0) | 25.76 (24.05-27.5) |
| Fe (μg/L) | 6.73 (5.37-8.95) | 12.42 (11.9-13.96) | 16.28 (11.8-21.40) | 20.82 (17.99-25.4) | 14.45 (11.55-19.3) | 18.03 (10.9-25.41) | 21.06 (19.85-25.5) | 20.57 (19.25-21.7) | 23.24 (20.5-26.27) | 20.34 (19.26-21.1) |
| Mn (μg/L) | 438.1 (338.8-66.3) | 771.5 (659-914) | 1667.9 (1145-2399) | 1343.7 (943.8-2114) | 1095.3 (981-1202) | 1046.3 (965-1150) | 1255.9 (1093-1337) | 971.7 (601-1325) | 1381.7 (1273-1496) | 923.4 (636-1113) |
| Pb (μg/L) | 7.26 (4.50-12.0) | 15.29 (9.15-20.00) | 13.13 (9.50-16.50) | 20.94 (17.75-25.5) | 19.94 (18.50-23.0) | 24.30 (20.50-31.5) | 247.63 (107.5-595) | 50.50 (46.0-56.60) | 28.31 (26.25-29) | 22.74 (19.75-28.0) |
| Zn (μg/L) | 113.10 (67.5-17.9) | 65.74 (54.2-72.5) | 64.33 (53.7-75.63) | 160.48 (126.9-200) | 107.19 (88.5-148.8) | 128.94 (82.5-165.0) | 1619.53 (320.5-2743) | 299.18 (255-316.3) | 204.64 (166.3-261) | 129.13 (90.0-230.0) |

## Statistical Analysis

The heavy metal content in Cladophora was correlated with concentrations of heavy metals in water and sediment. WHITTON (1971) noticed that content of heavy metals in sediment does not have an effect on *Cladophora glomerata*, but the author noticed that populations of *Cladophora* could be found at sites with high levels (content) of zinc in sediment. WHITTON *et al.* (1989) show that the content of heavy metals in river water has great impact on the content of heavy metals in *Cladophora*. There are positive linear correlations with high statistical significance between the concentration of a particular ion in water and algae. Besides these types of the correlation, the authors show that other ions have an impact on heavy metal accumulation, too. Obtained results on the River Vardar show the great impact of Zn on the accumulation of other elements such as Cd, Fe, and Pb (Table 6).

Statistical analysis of the River Vardar data shows that much higher correlations are establish between data sets obtained for sediment and *Cladophora*, than to the data sets for water and *Cladophora*.

Conducted statistical analysis represents the final evidence for the validity of epilithic communities as biomonitoring objects. Comparing the obtained correlation coefficients (r) for epilithon and *Cladophora* it could be noticed that both of them have very similar values and patterns of behavior at pollution stress caused by heavy metals. This fact could be noticed when data sets for *Cladophora* and epilithon were compared for each heavy metal (Figs 2,3).

Obtained higher values for correlation coefficients for epilithon suggest that epilithic communities are better biomonitoring objects for heavy metal pollution than algae such as *Cladophora glomerata, Lemanea fluviatilis* and *Fritschiella tuberosa* or aquatic mosses *Fontinalis antypiretica* and *Rhynostegium riparoides.*

## Conclusions

Bearing in mind all the problems that a proper monitoring system faces today in relation to the heavy metal pollution of river ecosystems, according to presented results, we would like to recommend epilithon communities as a monitoring tool due to following conclusions based on investigations conducted on the River Vardar:

- The River Vardar in FYR Macedonia represents an ecosystem of severe human impact, originating both from industrial and communal waste water systems. Con-

**Table 6**
**Correlation coefficient (r) between metal concentrations in water and sediment and the corresponding metals in *Cladophora* and epilithon.**

| | $Cd_{epi}$ | $Co_{epi}$ | $Cu_{epi}$ | $Fe_{epi}$ | $Mn_{epi}$ | $Pb_{epi}$ | $Zn_{epi}$ | $Cd_{clad}$ | $Co_{clad}$ | $Cu_{clad}$ | $Fe_{clad}$ | $Mn_{clad}$ | $Pb_{clad}$ | $Zn_{clad}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $Cd_{aq}$ | 0.69 | 0.51 | 0.47 | 0.26 | -0.26 | 0.73 | 0.41 | 0.55 | 0.43 | 0.38 | 0.43 | 0.17 | 0.32 | 0.25 |
| $Co_{aq}$ | -0.07 | -0.27 | 0.03 | -0.32 | -0.20 | -0.09 | -0.13 | -0.10 | -0.13 | 0.03 | -0.19 | -0.11 | -0.10 | -0.10 |
| $Cu_{aq}$ | 0.12 | 0.22 | 0.08 | 0.28 | -0.22 | 0.04 | 0.12 | 0.13 | 0.34 | 0.27 | 0.06 | 0.22 | 0.17 | 0.19 |
| $Fe_{aq}$ | -0.02 | 0.05 | 0.43 | 0.09 | -0.24 | 0.06 | -0.06 | 0.00 | 0.20 | 0.25 | 0.11 | -0.03 | 0.10 | -0.06 |
| $Mn_{aq}$ | 0.56 | 0.55 | 0.58 | 0.40 | -0.10 | 0.86 | 0.48 | 0.30 | 0.44 | 0.52 | 0.28 | 0.29 | 0.35 | 0.31 |
| $Pb_{aq}$ | 0.27 | 0.02 | 0.35 | -0.09 | -0.05 | 0.65 | 0.556 | 0.53 | 0.23 | 0.37 | 0.18 | 0.50 | 0.53 | 0.63 |
| $Zn_{aq}$ | 0.47 | 0.17 | 0.54 | 0.14 | -0.28 | 0.45 | 0.78 | 0.69 | 0.55 | 0.51 | 0.37 | 0.55 | 0.69 | 0.75 |
| $Cd_{sed}$ | 0.80 | 0.45 | 0.88 | 0.31 | -0.03 | 0.87 | 0.76 | 0.71 | 0.48 | 0.83 | 0.28 | 0.35 | 0.57 | 0.60 |
| $Co_{sed}$ | -0.03 | -0.13 | 0.13 | -0.17 | 0.14 | -0.01 | -0.09 | -0.08 | -0.05 | 0.06 | -0.10 | -0.15 | -0.05 | -0.13 |
| $Cu_{sed}$ | 0.62 | 0.22 | 0.82 | 0.13 | -0.17 | 0.69 | 0.78 | 0.74 | 0.55 | 0.80 | 0.24 | 0.67 | 0.77 | 0.78 |
| $Fe_{sed}$ | -0.24 | -0.30 | 0.18 | -0.20 | -0.32 | -0.11 | -0.18 | -0.15 | -0.10 | -0.01 | -0.24 | -0.17 | 0.01 | -0.11 |
| $Mn_{sed}$ | 0.11 | -0.08 | 0.12 | -0.34 | -0.23 | 0.10 | 0.07 | 0.10 | 0.22 | -0.04 | -0.12 | 0.17 | 0.12 | 0.08 |
| $Pb_{sed}$ | 0.65 | 0.21 | 0.71 | 0.18 | 0.03 | 0.69 | 0.95 | 0.88 | 0.50 | 0.76 | 0.20 | 0.75 | 0.82 | 0.98 |
| $Zn_{sed}$ | 0.80 | 0.52 | 0.73 | 0.28 | 0.02 | 0.98 | 0.75 | 0.61 | 0.45 | 0.73 | 0.26 | 0.44 | 0.51 | 0.56 |

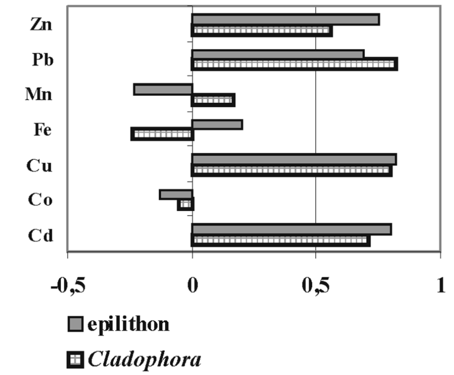


***Fig. 2:*** Correlation coefficients (r) for content of heavy metals in epilithon and *Cladophora* versus river water.

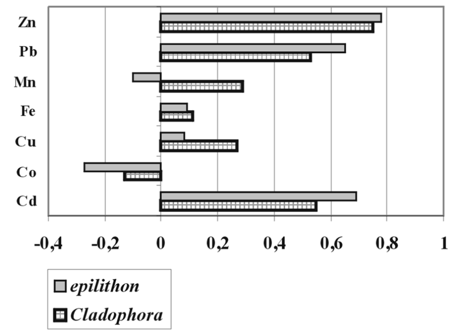


***Fig. 3:*** Correlation coefficients (r) for content of heavy metals in epilithon and *Cladophora* (dependent variable) versus sediment (independent variable).

centrations of heavy metals in water and especially in sediment are probably the highest recorded recently, thus putting the river biota under enormous survival stress or maximal accumulation rates. Due to a wide range of influences (pH, phosphates, sulfates, organic compounds, etc.) there are frequent records of complete extinction of river bottom life, which is another reason to monitor epilithon since it is the first to develop after devastating human influence;

- River sediment has a great ability to accumulation of heavy metals and could be used for the monitoring of heavy metal pollution;
- *Cladophora glomerata* is a precise biomonitoring tool for determination and quantification of heavy metal pollution in the River Vardar. Concentrations of particular heavy metals in *Cladophora* reflect the heavy metal load in the River Vardar;
- Statistical correlation matrices reflect significant correlation coefficient regarding heavy metal concentration both in *Cladophora* and epilithon, versus concentrations of heavy metals in waters or sediment (except for Mn which are not statistically relevant after all). These findings suggest that epilithon can be relevantly

chosen for a biomonitoring tool of heavy metal pollution in rivers;

- Concentrations of heavy metals in epilithon were usually very much related to those recorded for the sediment, obviously pointing to the concentration of the particular metal in the sampling site and thus representing a better monitoring tool for a prolonged period of time;
- Epilithon (predominantly diatoms) is present in all river systems, although sometimes difficult to locate in large rivers, but can easily be introduced onto natural stones, and does not depend on most factors that influence filamentous algae, bryophytes or higher plants. It is also present throughout the year, but left alone tends to predominate in winter, when all the other possible monitoring organisms disappear.

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