Oceanological and Hydrobiological Studies
International Journal of Oceanography and Hydrobiology
Vol. XXXVIII, No.3

Institute of Oceanography (3-14) University of Gdańsk
ISSN 1730-413X 2009 eISSN 1897-3191

DOI 10.2478/v10009-009-0027-3
Original research paper

Received: *November 17, 2008*
Accepted: *July 21, 2009*

The effects of copper and zinc on biomass and taxonomic composition of algal periphyton communities from the River Gharasou, Western Iran

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Key words: artificial stream, algae, biomass, taxonomic composition, heavy metals

Abstract

Indoor artificial streams were used to evaluate the short-term (24 day) influence of copper and zinc, individually and in combination, on the biomass and taxonomic composition of algal periphyton communities. The effects of Cu (50 μg l^{-1}) and Zn (1 mg l^{-1}) were assessed using measurements of biomass, ash-free dry mass (AFDM), chlorophyll-*a* concentration, taxonomic composition and biovolume. In artificial streams with Cu, Zn and Cu+Zn, there were significant ($p < 0.05$) reductions of chlorophyll-*a* concentrations, dry mass and AFDM, and also changes in

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the species diversity and relative abundances of periphyton species. In artificial streams containing both Cu and Zn the taxonomic composition shifted from a Cyanophyceaen dominated community to one consisting mainly of Chlorophyceae, whilst the relative contribution of Bacillariophyceae was not significantly altered.

INTRODUCTION

The Gharasou River in Western Iran suffers from heavy pollution by organic and inorganic toxicants due to the unregulated release of industrial and municipal wastes directly into the river (Atazadeh et al. 2007). Effects of zinc on microbenthic communities have been observed at concentrations ranging from 0.05 mg l^{-1} to 2.5 mg l^{-1} (Niederlehner and Cairns 1992, Loez et al. 1995) and effects of copper have been observed across a range of concentrations from 5 µg l^{-1} to 120 µg l^{-1} (Leland 1984, Real et al. 2003). It is therefore likely that the concentrations of these metals observed in the Gharasou River impact on the periphyton community.

Freshwater microalgae have been widely used for toxicity testing (e.g. Smrchek and Zeeman 1998); however, information obtained from such tests is based on the results for a few freshwater green algae that are easy to manipulate in culture (Mohan and Hosetti 1999). Although toxicity tests with isolated species can provide useful indications for environmental risk assessments of test compounds, they cannot predict changes at different organizational levels in natural communities (Berard et al. 2002). Assessment of impacts of chemical contamination on the environment should take into account the natural variability of biological systems in space and time, and it is particularly noteworthy that any endpoint used to evaluate toxicity may be expected to vary in magnitude under different environmental and biological factors (Schindler 1987).

Using laboratory streams to study the impacts of pollutants on community attributes can provide opportunities to isolate effects of environmental variables on algal communities (e.g. Sabater et al. 2002). For this reason, we decided to use artificial streams to examine the potential effects of metal contamination on periphyton in the Gharasou River.

MATERIALS AND METHODS

This study was conducted in 12 U-shaped fibreglass channels, each 2 m long, 0.5 m wide and 0.4 m deep. Water from the stream head of the Gharasou River (Kermanshah Province, western Iran) was introduced into the experimental channels to a depth of approximately 8 cm. Constant water circulation was provided using water pumps. Water velocity in the channels averaged 17 cm s^{-1}. Artificial light was supplied by 72 metal halide lamps

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(six for each channel, mounted 0.5 m above water level) which provided a broad spectrum of photosynthetically available irradiance. The photon flux density measured at the water's surface in the artificial streams was 360 μmol photon m^{-2} s^{-1}. The photoperiod of the system was set at 12-h light: 12-h dark.

All artificial streams were inoculated with a mixture of algae on the first day of the experiment. The inoculum was prepared by scraping periphyton from rocks collected from the headwaters of the Gharasou River. The scrapings were homogenized and brought to a volume of 120 l with stream water, filtered through a mesh (pore size 1 mm), and then divided so that an equal volume of the periphyton mixture was added to each stream. Each artificial stream contained 200 silica tiles (2.5 × 2.5 cm) as artificial substrata, which were then left for two weeks to allow colonization by the periphyton. Temperature, conductivity, pH, nitrate, phosphate and dissolved oxygen (DO) were measured in each channel. During the course of the experiment the water temperature was 22 ±0.5°C, pH ranged from 7.9–8.2, and conductivity ranged from 245–310 μS cm^{-1}. Average values for NO_3-N, PO_4-P and DO were 0.5 mg l^{-1}, 0.1 mg l^{-1}, and 7 mg l^{-1} respectively.

After the 2-week colonization period, Cu and Zn were introduced to the streams, solutions being injected at the head of each channel by peristaltic pump, and the metal exposure then continued for 24 days. CuCl and $ZnCl_2$ (Pure, Merck) were used to increase the metal concentrations to 50 μg l^{-1} Cu and 1 mg l^{-1} Zn in the streams. These concentrations were similar to mean values observed in polluted sites on the Gharasou River. Four treatments (with three channels of each as replicates) were assigned at random to the 12 channels used in the experiment. After four days of exposure algal periphyton were sampled in order to determine the biomass and taxonomic composition.

In order to determine dry mass, ten tiles were selected at random every four days, dried for 24 hours at 60°C and the attached periphyton scraped from the tiles with a razor blade and weighed. For measurements of AFDM (ash-free dry mass), ten tiles were randomly selected and dried for 24 h at 60°C. Dry mass was estimated as above, then the periphyton was combusted at 525°C in a furnace for four hours and reweighed. AFDM was the difference in the mass before and after incineration.

The periphyton was also scraped from ten randomly-selected tiles into tubes containing 10 ml of 95% ethanol. The samples were then stored overnight in a freezer after which the absorbance of the supernatant at 665 nm was determined using a spectrophotometer (Shimadzu, UV-1201) before and after addition of two drops of 0.1 N HCl. The chlorophyll-*a* concentration was determined from the absorbance using Nusch's equation (1980).

In order to identify algal species present, cells from the slides which had been allowed to become colonized with periphyton were resuspended in water

and then fixed with Lugol's solution. In order to identify diatoms the suspension was boiled in a 2:1 mixture of concentrated nitric and sulphuric acids for 2–4 h. Permanent slides were prepared using the mountant Naphrax. Algae were identified to the lowest possible level (usually species). Species were assigned to three classes: Cyanophyceae, Chlorophyceae, and Bacillariophyceae. The relative abundance of each taxon was estimated by identifying and counting at least 300 cells with a microscope (Olympus, BX51) at ×100-1000 magnification. Algae were identified according to Tiffany and Britton (1971), Pentecost (1984), Entwisle et al. (1997) and Peerapornpisal (2005).

The biovolume for each observed taxon (see Table 1) was estimated following the approach of Hillebrand et al. (1999). Cell counts were first converted to cell densities (cells cm^{-2}) and subsequently diatom cell densities (cells cm^{-2}) were converted to biovolumes (μm^3 cm^{-2}), by multiplying the density of each diatom taxon by the estimated volume per cell. Mean cell dimensions of the diatoms were measured for up to 20 cells using an ocular micrometer at ×1000 (Hill and Knight 1987). The individual sets of measurements were used to calculate a biovolume for each taxon, and these biovolumes were then averaged. Biovolumes for each taxon were calculated using the formula for the geometric shape roughly appropriate for the taxon (Hillebrand et al. 1999), although it should be noted that this is a somewhat inaccurate indication of biovolume since more than one geometric shape can approximate to a cell shape.

Statistical analysis

The significance of differences in dry mass, AFDM, chlorophyll *a* concentration, relative taxon abundance and biovolume data were analyzed using two-way analyses of variance (ANOVA). Differences in biomass and relative abundances were assessed throughout the experiment using two-way ANOVA on repeated measures (Winer 1971). ANOVA of repeated-measures accounts for the dependence of the observations that characterize a temporal sequence. Multiple comparisons between means were analyzed in all cases with a Tukey Honest significant difference (HSD) test (Winer 1971). All statistical analyses were computed with the software package SPSS.

RESULTS

A total of 20 taxa were identified in the control channel. The number was reduced to 19 in channels exposed to copper (T1) and zinc (T2) and to 14 for Cu and Zn in combination (T3) (Table 1). Colonization of algae on the glazed tiles was rapid and after just one week of exposure their surfaces were covered with

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Table 1

Relative abundance (%) of algal taxa in the periphyton communities after 24 day exposure to Cu and Cu+Zn. Relative abundances are calculated on the basis of estimations made on the densities of various taxa in the slides. C: Control, T1: Copper (50 µg l^{-1}), T2: Zinc (1 mg l^{-1}), T3: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}).

	C	T1	T2	T3
Cyanophyceae				
Anabaena sp.	7±2	1±0.5	2±1	0
Microcystis sp.	24±8	12±4	8±4	4±2
Oscillatoria sp.	9±3	4±2	3±1	0
Pseudoanabaena limnetica	2±1	3±1	4±2	0
Lyngbya major	5±2	1±1	0	1±0.5
Schizothrix sp.	3±1	0	1±0.5	0
Chlorophyceae				
Scenedesmus sp.	6±2	11±3	21±1	24±8
Oocystis gigas	3±2	19±5	5±2	7±2
Oocystis crassa	3±1	8±3	23±2	32±9
Stigeoclonium tennue	2±1	17±5	6±6	9±3
Pediastrum tatras	5±2	3±1	3±1	3±1
Geminella minor	4±2	1±1	1±1	2±1
Bacillariophyceae				
Meridion circulare	2±1	3±1	3±2	0
Fragilaria vaucheriae	5±2	2±1	3±1	3±1
Cocconeis placentula	3±1	2±1	3±1	0
Achnanthes sp.	1±0.5	2±1	4±2	1±1
Navicula lanceolata	4±2	3±1	3±1	4±2
Amphora ovalis	4±1	2±1	4±2	3±1
Encyonema minutum	6±2	4±1	1±1	4±1
Gomphonema truncatum	2±1	2±1	2±1	3±1

periphyton. After two weeks this layer was 1-2 mm thick. Visual inspections of communities in the artificial streams showed that periphyton populations exposed to heavy metals tend to become structurally loose and unstable. Chlorophyll-*a* concentrations in both treatment and control channels increased for approximately one week and then declined, until reaching apparent steady state concentrations. However, the magnitude of differences between controls and treated channels was considerable (Fig. 1a). The concentration of chlorophyll-*a* in the control increased up to day 8 then decreased, reaching a final concentration of 35 mg m^{-2}. Equivalent values for the treatment channels at the end of the experiment were 19.5 mg m^{-2}, 14 mg cm^{-2} and 7.1 mg cm^{-2} (Fig. 1a); single factor analysis of variance showed that these differences were significant (F = 12.2, P<0.002) (Table 2).

Biovolumes increased significantly with time (F=18.6, P<0.0003) (Table 2) in all treatments throughout the study (Fig. 1b). They reached 170×10^8 µm^3 cm^{-2} in the control channels, with lower concentrations observed in the two single-metal treatments (99×10^8 µm^3 cm^{-2} for Cu and 111×10^8 µm^3 cm^{-2} for Zn), and the lowest values in the combined metal treatment (56×10^8 µm^3 cm^{-2}) (Fig. 1b).

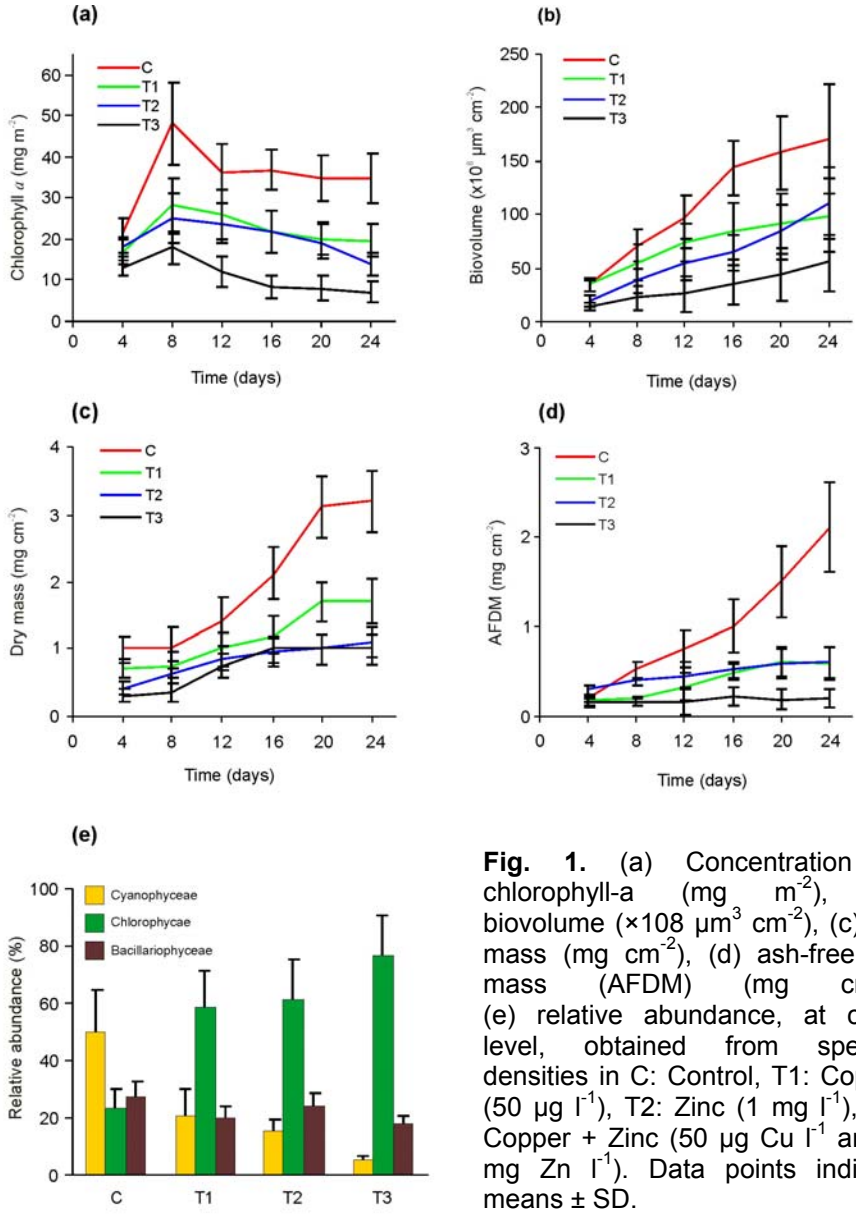


Fig. 1. (a) Concentration of chlorophyll-a (mg m^{-2}), (b) biovolume (×108 μm^3 cm^{-2}), (c) dry mass (mg cm^{-2}), (d) ash-free dry mass (AFDM) (mg cm^{-2}), (e) relative abundance, at class level, obtained from species densities in C: Control, T1: Copper (50 µg l^{-1}), T2: Zinc (1 mg l^{-1}), T3: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). Data points indicate means ± SD.

A general increase in dry mass occurred in both treatment and control channels over the course of the experiment (Fig. 1c). However, growth suppression was observed in all three treatments compared to the controls,

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causing a significant decrease in the amount of dry matter accumulated (F=10.9, P<0.009). The amount of dry mass per unit of surface in the control and treatment channels (Cu, Zn, Cu+Zn) was 3.2 mg cm^{-2}, 1.7 mg cm^{-2}, 1.1 mg cm^{-2} and 1 mg cm^{-2} respectively.

AFDM (ash-free dry mass) increased throughout the experimental period in the control channels, reaching 2.1 mg cm^{-2} by day 24. In the Cu treatment (50 µg l^{-1}) AFDM increased until 20 day and then leveled off at 0.58 mg cm^{-2}, whilst in the Zn treatment (1 mg l^{-1}) it was 0.6 mg cm^{-2} by day 24 and in the Cu+Zn treatment (50 µg Cu l^{-1} and 1 mg Zn l^{-1}) AFDM increased only until 16 day and then leveled off, attaining a final concentration of 0.19 mg cm^{-2} (Fig. 1d). Again, there were significant differences between control and treatments (F=11.4; P<0.003) (Table 2).

Quantitative characterization of the periphyton community is shown in Fig. 1e where algal relative abundances (mean value for all species) are shown for all species belonging to the Cyanophyceae, Chlorophyceae and Bacillariophyceae found in the samples. After 24 days, the Cu, Zn and Cu+Zn treatments all caused shifts from assemblages dominated by Cyanophyceae to assemblages dominated by Chlorophyceae, while the Bacillariophyceae (diatom) populations were not significantly different between channels (F=4.2; P<0.05). In the Cu+Zn channels, the Cyanophyceae were significantly less abundant than in the control communities (Tukey test, P<0.05).

There were significant positive relationships between AFDM, dry mass, biovolume and chlorophyll-*a* concentrations (Fig. 2a-f). The strongest relationship was between AFDM and dry mass (r^2=0.85; Fig. 2a); however, scatter plots suggest that relationships between AFDM and biovolume and chlorophyll are actually curvilinear, as AFDM and biovolume approached plateaus as AFDM increased (Figs 2b, 2c).

Table 2

Results of ANOVA of various characteristics on 24th day experimental periods.

Variable	Cu	Zn	Interaction (Cu:Zn)
Dry mass	51.74 ***	157.61 ***	39.68 ***
AFDM	191.7 ***	184.1 ***	54.94 ***
Chlorophyll *a*	91.79 ***	204.2 ***	13.61 **
Biovolume	100.67 ***	65.97 ***	1.62

Significance: 0 '***' 0.001 '**' 0.01 '*'

DISCUSSION

A number of studies have already demonstrated both short-term and long-term effects of heavy metals on freshwater algae (e.g. Whitton 1970, Soldo and Behra 2000, Sabater et al. 2002) and have identified a number of taxa that are tolerant to copper and zinc (Foster 1982). In this study we have demonstrated that short-term exposure to Cu and Zn in artificial channels leads to algal periphyton communities dominated (45%) by *Oocystis crassa,* a species common in the Gharasou River. Other Oocystaceae have been previously documented as being abundant in Cu- and Zn-polluted sites, and to have a general resistance to metals (Foster 1982, Takamura et al. 1990). *Stigeoclonium tenue*, another taxon that is often recorded as metal-tolerant (Harding and Whitton 1976), is also common in the Gharasou River but did not develop similarly high biomass in this short-term study. Variations were observed in biomass responses of the periphyton communities to the metal additions. Exposure to Cu (50 µg l^{-1}), Zn (1 mg l^{-1}) and combined Cu+Zn (50 µg Cu l^{-1} and 1 mg Zn l^{-1}) caused significant reductions in chlorophyll-*a* concentrations, biovolume, dry mass and AFDM. Chlorophyll concentrations were reduced, relative to biomass, at high metal concentrations (contrast Fig. 1a with Fig. 1b-1d; see also Fig. 2). Observations of reduced algal biomass after exposure to Cu and Zn have previously been described by a number of authors (e.g. Pratt et al. 1987, Paulsson et al. 2002).

As well as general reductions in community biomass, several reports have also demonstrated associated structural changes to algal communities with metal pollution. It has been suggested that changes occurring in species composition of periphyton communities experiencing heavy metal contamination are due to selection for those species that are tolerant to the pollution (Niederlener and Cairns 1992). However, there are few studies that provide adequate evidence indicating that the structural changes observed in metal-exposed communities have actually resulted from direct selection against sensitive species (Admiraal et al. 1999). Several authors have shown that the strong interactions between different components of biofilms, such as bacteria, microalgae and ciliates, mean that establishment of causal relationships is difficult. Part of the resilience of microbenthic communities to metal stress may be due to the input of organic waste providing material for the sorption and chelation of metal ions (Admiraal et al. 1999).

Results obtained from the current study cannot provide direct evidence demonstrating the mechanisms underlying the inhibitory effects of Cu or Zn on the community biomass or structure. However, it has been shown that biofilms are the main sites of metal accumulation in aquatic systems (Feriss et al. 1989). This accumulation has been exploited to monitor the metal contamination of

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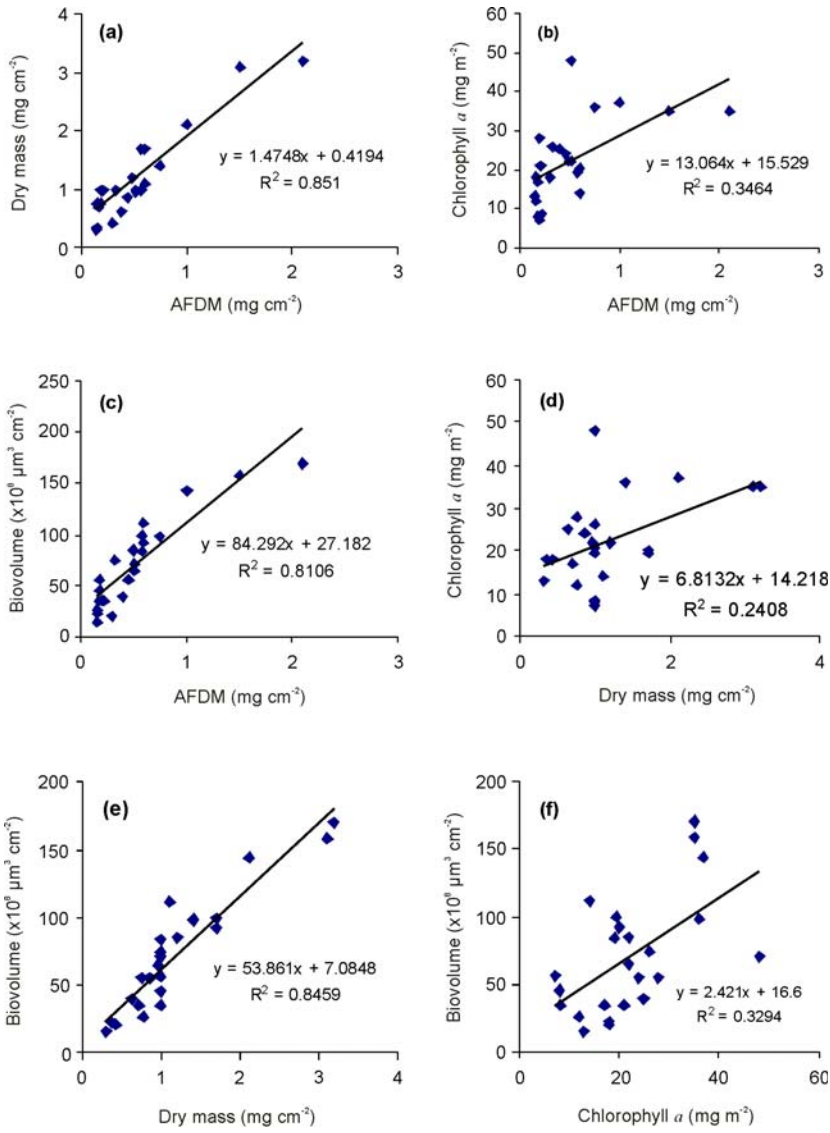


Fig. 2 (a) Relationship between ash-free dry mass (AFDM) and dry mass, (b) relationship between AFDM and chlorophyll-*a* concentration, (c) relationship between AFDM and biovolume, (d) relationship between dry mass and chlorophyll-*a* concentration, (e) relationship between dry mass and biovolume, (f) relationship between chlorophyll-*a* concentration and biovolume.

water columns (Newman and McIntosh 1989, Newman et al. 1985). Various organic compounds produced by periphyton communities, such as mucus and biomass, may prevent the diffusion of Cu and Zn to deeper layers of the biofilm, as has been demonstrated by studies based on autoradiography (Rose and Cushing 1970). In fact the inhibitory effects of a toxicant may be influenced by the sorptive capacities of biofilms (Newman et al. 1985).

It has been shown that the limited penetration of Zn into thick biofilms is a major factor controlling Zn toxicity (Rose and Cushing 1970). The binding of Cu and Zn to various organic matter components produced by the periphyton community can probably explain the insensitivity of some algal species to Cu and Zn pollution. The high resistance of periphyton to metals may be independent of species composition and related more to the physical structure of the biofilm (Guasch et al. 2003).

This study has shown that laboratory exposure of a pristine headwater in western Iran to Cu and Zn pollution caused a marked reduction in biomass along with changes in community taxonomic composition. The presence of high pH, alkalinity and phosphorus concentrations in Gharasou River could cause some protection against Cu and Zn toxicity through reduction of their availability in the water column.

ACKNOWLEDGEMENTS

Thanks to Dr Lydia King (University of Lancaster) for constructive comments on a draft of this paper.

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