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The Effects of Cu and Cu+Zn on Biomass and Taxonomic Composition in Algal Periphyton Communities

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Abstract: In order to evaluate the long-term (24-day) influence of copper alone (Cu) and with combination of Cu+Zn on biomass and taxonomic composition in algal periphyton communities, indoor artificial streams were used with copper (50 µg.l^{-1}) and copper and zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). The effects of copper and zinc on community productivity were assessed by measuring biomass, ash-free dry mass (AFDM), chlorophyll-*a*, taxonomic composition, relative abundance and biovolume. In artificial streams with Cu and Cu + Zn, there was a significant ($p < 0.05$) reduction of chlorophyll-*a*, dry mass and AFDM. Species diversity and relative abundance of periphyton species changed drastically under the influence of copper and zinc. In an artificial streams containing copper and zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}), the taxonomic composition of the population shifted from a cyanophyceaen dominated community to a community mainly consisting of chlorophyceae, while changes among species within the bacillariophyceae did not show a marked difference under the influence of copper and zinc. In artificial streams with copper alone *Oocystis gigas* and *Stigeoclonium tennue* became dominant species while in artificial streams containing copper and zinc *Oocystis crassa* and *Scenedesmus* sp. dominated the periphyton community. This study showed that copper alone or with combination of zinc not only influences community productivity but also causes changes in species composition.

Key words: Artificial stream, biomass, biovolume, chlorophyll-*a*, periphyton, relative abundance, taxonomic composition, copper, zinc.

Introduction

There is, to date, no information available regarding copper and zinc pollution in inland freshwater wetlands in Iran. However, extensive pollution caused by various domestic and industrial wastewaters has turned many small rivers into unhealthy watercourses, which may have high doses of heavy metals. Copper and zinc are present in most European rivers and its widespread use has generated much research on its effect in aquatic ecosystems. During 1997-1998, mean annual copper concentration in Catalan Streams (NE Spain) ranged from 1.3 to 17.5 µg.l^{-1}; peak values (> 40 µg.l^{-1}) were detected in highly polluted stream sites in agriculture and industrial catchments and in certain period, values of up to 100 µg.l^{-1} have been occasionally detected in an extremely polluted stream in the same region (Real et al., 2003). The concentration of zinc in polluted systems has been reported to range from nmol.l^{-1} to nearly 100 µmol.l^{-1} (Whitton et al., 1982). Long-term effects of zinc on microbenthic communities have generally been reported at contamination ranges from 0.05 mg.l^{-1} (0.8 µM) to 2.5 mg.l^{-1} (38 µM) (Williams & Mount, 1965; Genter et al., 1987; Colwell et al., 1989; Dean-Ross,

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1990; Niederlehner & Cairns, 1992, 1993; Loez et al., 1995). Several authors (e.g. Foster, 1982; Leland & Carter, 1984; Soldo & Behra, 2000) report changes in periphyton community composition after long-term exposure to copper and zinc.

Biofilms are layer of polysaccharide-rich materials with active microbial components on the surface of inert materials and organic matter that modulate biological availability, hence the potential toxicity of heavy metals in the environment (Real et al., 2003). There are indications that heavy metals and phosphorus interact to decrease biomass production (Bates et al., 1985; Kuwabara, 1985; Pratt et al., 1987).

There have been some studies, under both laboratory and natural conditions, on the relationship between the concentration of copper and zinc in water and the algal periphyton community. It has been shown that an increase in copper and zinc concentration will, in the long term, cause a shift in composition to more tolerant species (Soldo & Behra, 2000). In laboratory experiments with algae, the uptake and toxicity of zinc have been shown to be related to the free metal concentration (Campbell, 1995). For this reason freshwater microalgae have been subjected to many phytotoxicity tests. However, most phytotoxicity information obtained from these tests is based on the results for a few freshwater green algal species (Mohan & Hosetti, 1999). Although phytotoxicity tests with isolated species can provide useful indications for environmental risk assessment of the test compound, they cannot predict changes in a natural community at different organizational levels. Assessment of long-term impacts of chemical contamination on the environment should account for the natural variability of biological systems in space and time. In fact, as has been shown in many toxicological studies, any endpoint used to evaluate toxicity may be expected to vary in magnitude under various environmental and biological factors (Schindler, 1987).

Using laboratory streams for studying the impacts of pollutants on community attributes can provide the opportunity to isolate the effect of environmental variables on algal communities (e.g. Lamberti & Steinman, 1993; Sabater et al., 2002). In contrast to the difficulties associated with the "laboratory to natural environment generalization", several laboratory studies have shown that algal periphyton are sensitive to changes in water quality. This has caused several authors to use algal periphyton as an indicator of water quality in aquatic environments (Lewis et al., 2002; Nayar et al., 2003).

The main aim of present study was to address the paucity of information regarding the toxicity effects of Cu and Cu + Zn at the community level by evaluating the toxic effect of zinc on various aspects of the productivity of a periphyton community. We also aimed to identify changes that occur, in response to copper and zinc, in the species composition of this community.

Materials and Methods

This study was conducted in 15 U-shaped fiberglass channels, each 2 m in length, 0.5 m in width and 0.4 m in depth. Each fiberglass channel was divided into two equal sections by placing fiberglass sheets down the middle of the channels. Water from the head stream of the Gharasou river (Kermanshah Province, western Iran) was used in the experimental channels at the depth of approximately 0.08 m. A constant water circulation was provided using water pumps. Water velocity in the channels averaged 17 $cm.s^{-1}$. Artificial light was supplied by 90 metal halide lamps (six for each channel), which provided a broad spectrum of photosynthetically available irradiance. Metal lamps were mounted 0.5 m above the channels. Photon flux density measured at the water surface in the artificial streams was 90 $\mu E.m^{-2}.s^{-1}$. 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 Gharasou River. The scrapings were homogenized and brought to a volume of 120 litres with water, filtered through a mesh (pore size 1 mm), and then an equal volume of the filtrated mixture of periphyton algae was added to the streams. Each artificial stream contained 200 tiles (2.5 × 2.5 cm), which were allowed to become colonized by periphyton algae for two weeks. Temperature, conductivity, pH, nitrate, phosphate and dissolved oxygen (DO) were measured in each channel. During the course of the experiment, water temperature was 22 ± 0.5°C, pH ranged from 7.9-8.4, and conductivity ranged from 245-310 $\mu S.cm^{-1}$. Average values for NO_3-N, PO_4-P and DO were 0.5 $mg.l^{-1}$, 0.005 $mg.l^{-1}$, and 7 $mg.l^{-1}$ respectively.

Cu and Cu + Zn exposure was started after a 2-week colonization period and was continued for 24 days. We used CuCl and $ZnCl_2$ (Pure, Merck) to increase copper and zinc concentration in the artificial streams, three treatment (with five channels as replicates) were assigned at random to the 15 channels used in the experiments: (c) control; (T1) copper (50 $\mu g.l^{-1}$); (T2) copper + zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). After four days of exposure algal periphyton was sampled in order to determine biomass and taxonomic composition.

Samples for determination of dry mass were randomly selected every four days, dried for 24 hours at 60°C and the attached algal periphyton scraped from the tiles with a razor blade and weighed. For measuring AFDM (ash-free dry mass), ten tiles were randomly selected and dried for 24 h at 60°C. Algal periphyton organisms were scraped off the tiles as for dry mass estimates, weighed, combusted at 525°C in a furnace for four hours, and reweighed. Representing all organic matter in the algal periphyton, AFDM was the difference in the mass before and after incineration. AFDM was calculated as $mg.cm^{-2}$ of the original substrate.

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

In order to identify periphyton species, specimens gathered on glass slides were suspended in water and fixed with Lugol's solution. Identification was done to the species or, when this was not possible, to the lowest possible taxonomic level. Species were assigned to three classes: Cyanophyceae, Chlorophyceae, and Bacillariophyceae. The relative abundance of each taxon was estimated by identifying at least 300 cells with a microscope (Olympus, BX51) at 100-1000 × magnification. Using species density as a measure of species abundance, the general diversity in each periphyton community was determined using the Shannon Weiner, index of general diversity (Shannon & Weiner, 1949). The dominance for each taxon in the artificial streams was calculated using the Simpson index (Simpson, 1949) of dominance.

The biovolume for each taxon was estimated employing the methods used by Litteral (1995). In estimating periphyton biovolume the cell counts were first converted to cell density ($cells.cm^{-2}$) and subsequently diatom cell densities ($cells.cm^{-2}$) were converted to diatom biovolume. This was then expressed as $\mu^3.cm^{-2}$ by multiplying the diatom density of each taxon by the estimated volume per cell. Cell volumes of diatoms were measured for up to 20 cells using an ocular micrometer at 1000× to obtain mean cell dimensions (Hill & Knight, 1987). Each individual set of measurements was used to calculate a biovolume for each taxon and then these biovolumes were averaged. Biovolumes for each taxon were calculated using the formula for the geometric shape roughly appropriate for the taxon. This does not compromise accuracy of biovolume calculations because more than one geometric shape can approximate a cell shape.

Statistical Analyses

The quantitative analysis of algal community composition was carried out on days 4, 8, 12, 16, 20 and 24. We randomly selected ten tiles from each channel and these were preserved in a 4% formalin solution. At least 300 cells were examined under 100-1000× magnification. For each Cu and Cu+Zn exposure concentrations, the mean and the 95% confidence interval were calculated from the data from the experimental channels. Dry mass, AFDM, chlorophyll-*a*, relative abundance and biovolume data were analyzed using one-way analyses of variance (ANOVA). Differences in treatment concentrations between the control and the copper and zinc-exposed channels were also analyzed using one-way analysis of variance (ANOVA).

Results

Changes in concentration of chlorophyll-*a* in treatments and in control channels demonstrated a general early increase and subsequent decrease until reaching an apparent steady state concentration. However, the difference in the magnitude of changes between controls and treated channels was considerable (Figure 1). The concentration of chlorophyll-*a* in the control increased up to day 8 and then from day 12 a rapid decrease began. At the end of the experiment the concentration of

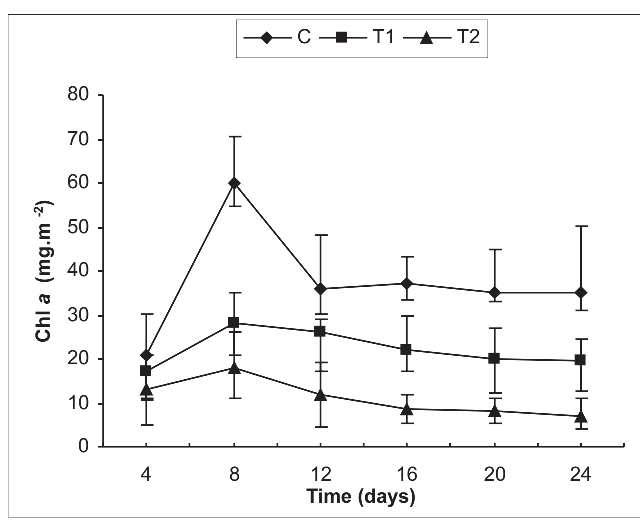

Figure 1: Concentration of chlorophyll-*a* ($mg.m^{-2}$) in C: Control, T1: Copper ($50\ \mu g.l^{-1}$), T2: Copper + Zinc ($50\ \mu g$ $Cu\ l^{-1}$ and $1\ mg\ Zn\ l^{-1}$). Data points indicate means ± SD.

chlorophyll-*a* reached a final concentration of 35 $mg.m^{-2}$ in the control channels. Equivalent values for treatment channels at the end of experiment were 19.5 $mg.m^{-2}$ and 7.1 $mg.cm^{-2}$ respectively (Figure 1). Using single factor analysis of variance between concentration of chlorophyll-*a* (Table 1) in control and two treatment channels demonstrates a significant difference ($p < 0.05$).

The effects of Cu and Cu+Zn on the biovolume of the periphyton community are shown in Figure 2. Biovolume in the controls increased and by the end of the experimental period reached 170×10^8 $µm^3.cm^{-2}$. In Cu treatment (50 $µg.l^{-1}$), biovolume increased to 99×10^8 $µm^3.cm^{-2}$. In Cu+Zn treatment (50 µg Cu l^{-1} and 1 mg Zn l^{-1}) led to a final biovolume of 56×10^8 $µm^3.cm^{-2}$ (Figure 2).

A general increase in biomass in both treatment and control artificial streams can be seen (Figure 3). However, the suppression effect of the two concentrations of Cu and Cu+Zn caused a significant decrease in the amount of dry matter accumulated (Table 1). The amount of dry mass per unit of surface in the control and treatment channels was 3.2 $mg.cm^{-2}$, 1.7 $mg.cm^{-2}$ and 1 $mg.cm^{-2}$ respectively.

AFDM (ash-free dry mass) in controls increased upto day 24 and at the end of experimental period reached to 2.1 $mg.cm^{-2}$. In the Cu treatment (50 $µg.l^{-1}$) AFDM increased until day 20 and then leveled off at 0.58 $mg.cm^{-2}$, whereas in the Cu+Zn treatment (50 µg Cu l^{-1} and 1 mg Zn l^{-1}) AFDM increased only until day 16 when it attained 0.19 $mg.cm^{-2}$ (Figure 4).

Table 1: Results of ANOVA of various characteristics during 24 days experimental periods

	C vs T1		*C vs T2*	
	F-value	P-value	F-value	P-value
Chlorophyll-*a*	18.2	0.002	51.2	0.000
Biovolume	9.5	0.03	14	0.01
Dry mass	15.1	0.009	16.45	0.004
AFDM	13.4	0.003	25.5	0.001

C: Control, T1: Copper (50 $µg.l^{-1}$), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}).

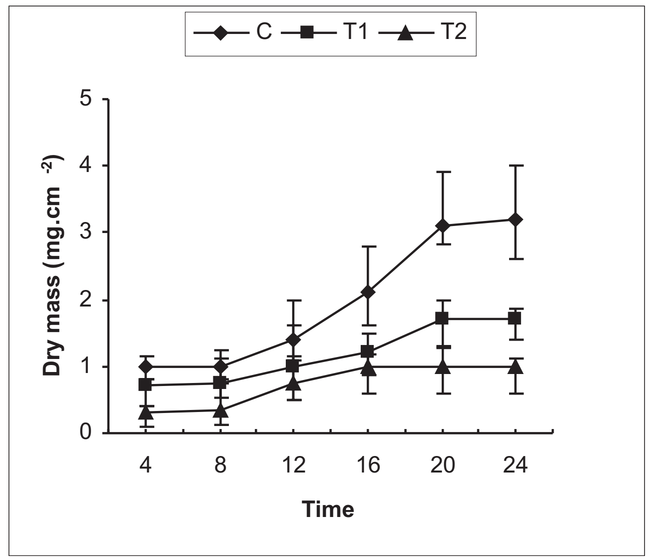

Figure 3: Dry mass ($mg.cm^{-2}$) in C: Control, T1: Copper (50 $µg.l^{-1}$), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). Data points indicate means ± SD.

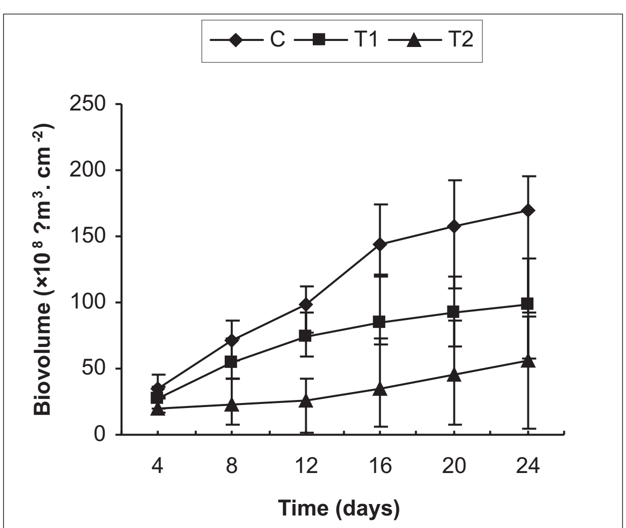

Figure 2: Biovolume (×10^8 $µm^3.cm^{-2}$) in C: Control, T1: Copper (50 $µg.l^{-1}$), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). Data points indicate means ± SD.

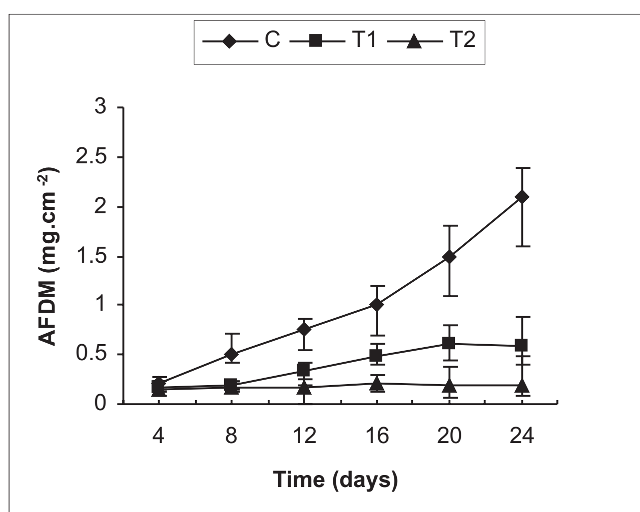

Figure 4: AFDM ($mg.cm^{-2}$) in C: Control, T1: Copper (50 $µg.l^{-1}$), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). Data points indicate means ± SD.

Colonization of algae on the glazed tiles took place fairly rapidly. After only one week of exposure, the surface of tiles were covered with the periphyton and after two weeks a 1-2 mm thick layer of periphyton gradually developed. Quantitative characterization of the periphyton community is shown in Figure 5 where algal relative abundances (mean value for all species) are shown for all species belonging to the three classes—Cyanophyceae, Chlorophyceae, and Bacillariophyceae. The day 24 exposure of periphyton communities to Cu and Cu+Zn caused a dominance shift from Cyanophyceae to Chlorophyceae while the Bacillariophyceae (diatoms) did not show significant differences ($p < 0.05$) between channels. In Cu+Zn channels, the Cyanophyceae were significantly less abundant than in control communities ($p < 0.05$).

Two components of species diversity, species number and species dominance, in the artificial streams were affected following 24 days of exposure to Cu and Cu+Zn. A total number of 20 taxa were identified in the control channel (Table 2). Exposure to copper and zinc caused the periphyton community, which was mainly comprised of Cyanophyceae (50%), Chlorophyceae (23 %) and Bacillariophyceae (27 %), to shift to a community dominated by Chlorophyceae (77 %) in Cu+Zn channels (Table 2).

Although the general decrease in species diversity is evident from the number of taxa, the abundance of each taxa was also affected by this exposure. Using the Shannon & Weiner index of diversity, which combines species number with relative abundance for each species, demonstrates a clear reduction in species diversity following exposure to copper and zinc (Figure 6). The pattern of species dominance as indicated by the Simpson index of dominance (Figure 7) in the periphyton communities was also affected by exposure to copper and zinc. Low values obtained for this index (as found in

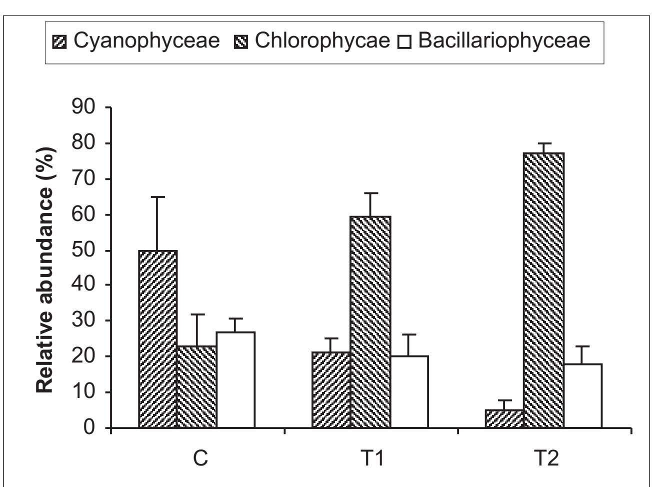

Figure 5: Relative abundance, at class level, obtained from species densities in C: Control, T1: Copper (50 µg.l^{-1}), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). Data points indicate means ± SD.

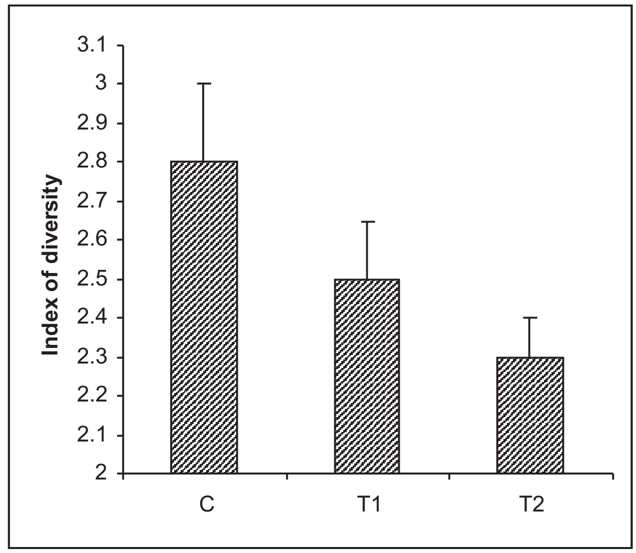

Figure 6: The effect of zinc on the periphyton diversity index (Shannon Weiner index of general diversity). C: Control, T1: Copper (50 µg.l^{-1}), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). Data points indicate means ± SD.

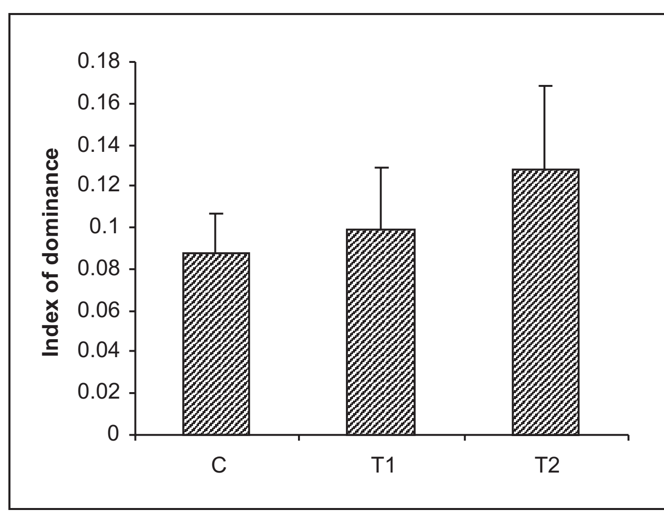

Figure 7: Simpson index of dominance calculated for the artificial streams on the basis of the density of species. In the polluted streams, dominance is restricted to fewer species. C: Control, T1: Copper (50 µg.l^{-1}), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}). Data points indicate means ± SD.

Table 2: 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	*T1*	*T2*
Cyanophyceae			
Anabaena sp.	7	1	0
Microcystis sp.	24	12	4
Ocillatoria sp.	9	4	0
Pseudoanabaena limnetica 2	3	0	
Lyngbya major	5	1	1
Schizothrix sp.	3	0	0
Chlorophyceae			
Scenedesmus sp.	6	11	24
Oocystis gigas	3	19	7
Oocystis crassa	3	8	32
Stigeoclonium tennue	2	17	9
Pediastrum tatras	5	3	3
Geminella minor	4	1	2
Bacillariophyceae			
Meridion circulare	2	3	0
Fragilaria vaucheria	5	2	3
Cocconeis placentura	3	2	0
Achnanthes sp.	1	2	0
Navicula lanceolata	4	3	4
Amphora ovalis	4	2	3
Cymbell minuta	6	4	4
Gomphonema truncatum	2	2	3

C: Control, T1: Copper (50 µg.l^{-1}), T2: Copper + Zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}).

the control) demonstrate that the importance (abundance) values for species in the control community are more evenly distributed amongst the species while higher values indicate that the community exposed to copper and zinc has gradually changed to one in which abundance of species is less evenly distributed within the periphyton population.

Discussion

Although toxicity tests have shown that heavy metals can cause lethal effects on various terrestrial and aquatic plants and animals, there is still insufficient information regarding the influence of these metals on groups of species at the community level. Of those studies that have been carried out, investigations by various authors demonstrate contrasting results. For example Whitton (1970) and Sabater (2002) have been able to identify species of chlorophyta sensitive to heavy metals. However, several investigations have shown that changes in species composition exposed to heavy metal can be observed readily (e.g. Soldo & Behra, 2000). In the present study it has been shown that long-term exposure of periphyton communities to Cu and Cu+Zn can cause a dominance shift from cyanophyceae to chlorophyta. At the same time, the bacillariophyceae in the artificial streams did not show a significant change under copper and zinc treatments. In artificial streams with copper alone *Oocystis gigas* and *Stigeoclonium tennue* became dominant species while in artificial streams containing copper and zinc *Oocystis crassa* and *Scenedesmus* sp. dominated the periphyton community.

In contrast various taxa belonging to the cholophyta were insensitive to copper and zinc, which reflects the high tolerance of green algae to copper and zinc (Foster, 1982). At Cu+Zn channels, algal periphyton communities were dominated (45%) by the *Oocystis crassa*, other oocystaceae have been previously documented as being abundant in copper and zinc polluted sites and to have a general resistance to metals (Foster, 1982; Takamura et al., 1990; Soldo & Behra, 2000).

Periphyton communities subjected to long-term copper and zinc exposure at different concentrations did not differ significantly in their photosynthesis rate. The higher rates measured in the 5 µM treatments may be due to inhibitory effects of copper and zinc on chlorophyll-*a* synthesis (Rai et al., 1990). The response in terms of photosynthesis of periphyton communities in metal-polluted environment has been shown to be quite complex, usually following a pattern of decline and recovery (Takamura et al., 1990; Balezon & Pratt, 1994). The high variability in photosynthesis rate further suggests the limited utility of this parameter as an indicator of metal induced impacts (Soldo & Behra, 2000).

In the present study, similar changes were shown in various aspects of the community productivity. Thus, exposure to copper (50 µg.l^{-1}) and copper and zinc (50 µg Cu l^{-1} and 1 mg Zn l^{-1}) caused significant reductions in chlorophyll-*a*, biovolume, dry mass and AFDM. The reduction in algal biomass by exposure to copper and zinc has been described by a number of authors (Bates et al., 1985; Kuwabara, 1985; Pratt et al., 1987; Paulsson et al., 2002).

Along with a general reduction in community productivity, there are several reports that also demonstrate some associated structural changes to the communities. 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 & Cairns, 1992). However, there are few studies that provide adequate evidence indicating that the structural changes observed in metal-exposed community 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, provide such a complex situation in which establishment of any causal relationship is not easily possible. In the present study gross visual inspections of communities in the control and treatment of artificial streams showed that when periphyton populations are exposed to the heavy metal they became structurally loose and unstable.

Results obtained from the current study cannot provide direct evidence demonstrating the mechanisms underlying the inhibitory effects of copper and zinc on the community productivity or the community 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 metal contamination of the water column (Newman & McIntosh, 1989; Newman et al., 1985). Various organic compounds produced by the periphyton community such as mucus and biomass may prevent the diffusion of copper and zinc to deeper layers of the biofilm, as has been demonstrated by studies based on autoradiography (Rose & Cushing, 1970). In fact the inhibitory effects of a toxicant may be under the influence of the sorptive capacities of biofilms (Newman et al., 1985). All these factors are likely to interact in the present study. The mucus layer, at least of the epilithon, was well developed and capable of trapping particles (Atazadeh, unpublished observations).

It has been shown that the limited penetration of zinc into thick biofilms is a major factor controlling zinc toxicity (Rose & Cushing, 1970). The binding of copper and zinc to various organic matter components produced by the periphyton community can probably explain the insensitivity of various algal species to copper and zinc concentrations. It seems that the high resistance of periphyton is independent of species composition and related more to the physical structure of the biofilm (Guasch et al., 2003). Investigations carried out by Guasch et al. (2003) showed that the penetration of zinc into thick biofilms is limited. Copper and zinc cannot penetrate well into the periphyton layer. The inability of heavy metals to penetrate into a biofilm has also been documented by Barranguet et al. (2000) using copper.

This study has shown that laboratory exposure of a pristine headwater in western Iran to copper and zinc pollution caused a marked reduction in productivity along with changes in species composition. It also demonstrates that the periphyton community can significantly reduce the concentrations of copper and zinc in water column. Potentially, the high pH and high alkalinity in this river, similar to conditions in most arid rivers, may have had some contribution to an elevated response level of the periphyton community to heavy metal exposure. The presence of high phosphate concentrations in River Gharasou could cause some protection against copper and zinc toxicity through reduction of the availability of copper and zinc in the water column. However, it is not clear that the phosphate present in the laboratory streams actually led to reduced copper and zinc availability in the water.

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