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Long-term effects of copper on the structure of freshwater periphyton communities and their tolerance to copper, zinc, nickel and silver

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Abstract

A community adapted to elevated ambient levels of a particular pollutant is expected, compared to a non-exposed community, to display an increased tolerance to that pollutant. The potential of tolerance measurements as a method to detect metal-induced structural impacts at the community level is poorly known. Particularly, the determination of increased tolerance to various metals may confound conclusions related to the causes of the impact. In this study the effects of long-term copper exposure on the community structure of freshwater periphyton, and the short-term community tolerance of photosynthesis to copper, zinc, nickel and silver were determined. Using an outdoor flow-through aquaria system, we carried out long-term exposure of freshwater periphyton communities to copper (0, 0.05, 0.1, 0.5, 1 and 5 μM copper). After 12 weeks we examined how the copper exposure affected the taxonomic composition, photosynthesis rate and tolerance thereof to copper, zinc, nickel and silver. Effects included changes in the distribution of algal classes from a community dominated by Cyanophyceae to one dominated by Chlorophyta. The relative abundance of *Oocystis nephrocytioides* increased from less than 1% in the control aquaria to 56% in the 5 μM copper treatments. Except at the highest copper exposure, communities did not significantly differ in their photosynthesis rate, although the short-term tolerance of photosynthesis to metals was affected by the copper treatments. Significant increases in tolerance to copper were found in communities previously exposed to ≥ 0.1 μM copper concentrations. Communities exposed to copper also displayed an increased co-tolerance to zinc, nickel and silver. These observations suggest that copper-induced structural impacts on periphyton communities can be evidenced as an increased tolerance to copper. However, because of the occurrence of co-tolerance, the identification of the metals that have induced the structural and tolerance changes may require metal determinations in organisms.

Keywords: Periphyton; Species composition; Copper tolerance; Co-tolerance; Nickel; Silver; Zinc

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1. Introduction

Assessment of long-term impacts of chemical contamination of an environment should account for the natural variability of biological systems in space and time. In fact, any endpoint used to evaluate toxicity may be expected to vary in magnitude because of changes in environmental and biological variables (Schindler, 1987), even in the absence of chemical contamination. This points to the difficulty of interpreting biological changes, in terms of causation, from between-site comparisons of biological data alone. Approaches are needed that are suitable for indicating the presence of stress and for identifying the stress-causing pollutant.

Blanck et al. (1988) have proposed that assessment using functional tolerance measurements permits chemical-induced structural impacts to be inferred at the community level and the pollutant actually affecting the community to be identified. The replacement of sensitive individuals or species by tolerant ones is an expected outcome of chronic chemical exposure. Thus, the tolerance of a community previously exposed to a toxicant is anticipated to be greater than that of a community that has never been exposed. Pollution-induced community tolerance (PICT) has been validated with controlled microcosm experiments, and has been detected in phytoplankton and periphyton communities in contaminated environments (e.g. Blanck and Dahl, 1996; Knauer et al., 1999).

However, with metals, cause-and-effect relationships between concentration and community response, such as shifts in the tolerance to metals, is confounded by the influence of chemical speciation on bioavailability. In laboratory experiments on algae, uptake and toxicity of metals by algae was shown to depend on the free metal concentration (e.g. Campbell, 1995, and references cited therein). Consideration of metal speciation is therefore important in the design of toxicity tests and in the evaluation of metal impact studies. This is not a trivial task, however, considering that species formation is influenced by locally prevailing physicochemical conditions, such as acidity, salinity, inorganic and organic ligands, and the presence of particles (Sigg and Xue, 1994). The uptake and toxicity of metals also depends on the intrinsic tolerance of an organism and its ability to trigger defense mechanisms in response to metal exposure. Moreover, co-tolerance can occur when defense mechanisms are effective against other metals. In such cases, identifying which metal has actually had an effect on the community is difficult.

In this study, we investigated the effects of long-term copper exposure on the community structure of freshwater periphyton and the short-term community tolerance of photosynthesis to copper, zinc, nickel and silver. With this approach, including controlled copper exposure of natural communities, we tested whether short-term tolerance measurements were predictive of structural changes. Moreover, we examined co-tolerance patterns to zinc, nickel and silver to understand co-tolerance patterns in terms of the mechanisms conferring metal tolerance. The results are discussed in relation to the potential application of tolerance comparisons in the assessment of metal pollution.

2. Materials and methods

2.1. Experimental design

An outdoor flow-through glass aquaria system, installed on the site of the sewage plant in Fällanden (canton Zürich, Switzerland) and with access to the river Glatt, was used from July to October 1995 for periphyton colonization and copper exposure experiments (Fig. 1). The experimental site is located near to the outlet of lake Greifen. The Glatt river runs through a catchment dominated by carbonate rocks, its alkalinity ranges from 1.6 to 2.4 mM, and pH from 8.0 to 8.3 (Swiss National Hydrological and Geological Survey, 1996). Using a submersible pump, river water containing microorganisms was pumped into a tank and distributed by gravity into each of 18 aquaria, at a flow rate of 0.2 l min^{-1}. Three PVC racks, each holding eight microscope slides (76×26 mm), were placed in each aquarium to allow periphyton colonization.

Copper exposure was started after a 4-week colonization period and was continued for 16 weeks. A peristaltic pump (Ismatech 726) delivered concentrated copper solutions (as $CuSO_4$) so as to yield nominal copper concentrations in each of three aquaria of 0 (control communities), 0.05, 0.1, 0.5, 1 and 5 μM copper. Overflow water from the aquaria was collected in a tank and pumped into the basin of the sewage plant. Periphyton was sampled for taxonomic analyses and for short-term photosynthesis tests after 12 weeks of exposure to copper.

2.2. Photosynthesis and metal tolerance

Photosynthesis rate and sensitivity thereof to copper, zinc, nickel and silver were measured in short-term tests by the ^{14}C-technique. Algae from each aquarium were scraped from the slides, washed three times with an incubation medium (0.1 mM $MgCl2_6 \cdot H_2O$, 1 mM $CaCO_3$, 30 μM $NaNO_3$ and 20 nM $NaH_2PO_4 \cdot H_2O$) at pH 6.5, and then resuspended in incubation medium so as to obtain an optical density at 680 nm of about 0.2. Aliquots of 2 ml were placed in glass scintillation vials and preincubated for 2 h at 10°C in the dark at five different metal concentrations (0, 10, 50, 100 and 150 μM; $n = 3$). The incubation time was chosen after preliminary experiments indicated that inhibitory effects of copper on periphyton photosynthesis occur in less than 30 min (unpublished observations). Metals were added as $CuSO_4$, $ZnSO_4$, $NiSO_4$ and $AgSO_4$. Sensitivity of photosynthesis to copper was determined in all long-term copper treatments, and that to zinc, nickel and silver in the 0, 0.5 and 5 μM long-term copper treatments. After preincubation, 37 kBq $NaH^{14}CO_3$ (2035 MBq $mmol^{-1}$; Amersham) were added to each vial and the vials were incubated for another 15 min in a water bath (10°C) at a light intensity of 125 μE m^{-2} s^{-1}. The reaction was stopped by adding 50 μl of concentrated HNO_3, and samples were bubbled with air for 50 min to remove excess $^{14}CO_2$. After adding 3 ml of Lumagel (Lumac), the incorporated radioactivity was detected by liquid scintillation counting (BETAmatic I, Kontron). Photosynthesis was normalized to the chlorophyll (Chl) *a* content of the algal suspension. The inhibition of photosynthesis by metals was calculated by normalizing the mean of three treatment replicates to the mean of three control replicates.

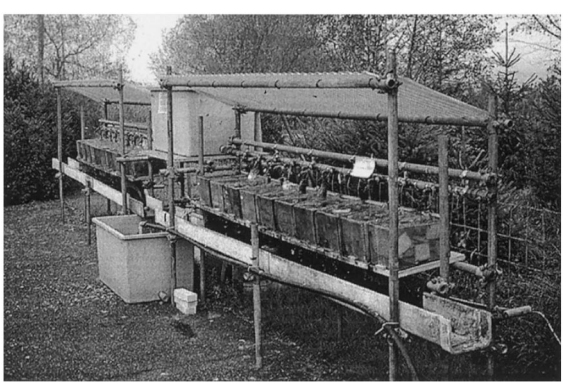

Fig. 1. The flow-through aquaria system used for periphyton colonization and long-term copper exposure.

2.3. Taxonomic analyses

The periphyton on the glass slides was suspended in water and fixed with Lugol's solution for identification and enumeration of algae. Identification was done to the species or, when this was not possible, to the lowest possible taxonomic level. The relative abundance of each taxon was estimated by identifying at least 300 cells (Neubauer cell) with an inverted microscope (Zeiss) at a 100–1000 × magnification.

Chl *a* content of periphyton samples, used to normalize photosynthesis rate, was determined spectrophotometrically after extraction in 90% ethanol (Meyns et al., 1994).

2.4. Copper analyses

Water samples for measurements of copper concentrations in the Glatt river and in the aquaria were filtered (pore size 0.45 μm) and acidified to 0.01 M HNO_3. Analyses of dissolved copper were done by graphite furnace atomic absorption spectrometry (AAS; Varian). The background concentration of copper in the river Glatt was 25 nM. Concentrations of dissolved copper in the aquaria were determined once, after

12 weeks of long-term exposure. Maximal deviations from nominal concentrations ranged from −32% to +40% in 1 μM and 0.5 μM copper exposures, respectively (not shown).

The concentrations of the free copper ions in the incubation medium were computed with the chemical speciation program ChemEQL V2.0 (Westall, 1979; Müller, 1996). Calculations indicated that, at pH 6.5 and in the range of total copper concentrations applied in the photosynthesis measurements, about 95% occurred as free Cu^{2+}.

2.5. Data analyses

For each copper exposure concentration the mean and the 95% confidence interval were calculated from the data of three aquaria replicates. Photosynthesis rate and relative abundance data were analysed using one-way analysis of variance (ANOVA). Differences in metal tolerance data between the control and the copper-exposed aquaria were analysed using two-way ANOVA. Comparison of photosynthesis inhibition in the short-term tests within each aquaria treatment and between each copper-exposed aquaria with the control were made using Tukey's Honest Significant Difference Test (Zar, 1984). The tests were performed on $\log_{10}$ transformed data after having tested for normality and homogeneity of variance with Kolmogorov's and Cochran's test, respectively ($P > 0.5$). Unless otherwise stated, a significance level of 0.05 was applied. All statistical analyses were computed with the software package Statistica for Macintosh 4.1 (Statsoft, 1994).

3. Results

The 12-week long-term exposure of periphyton communities to copper caused a dominance shift from Cyanophyceae to Chlorophyta (Fig. 2). In the highest copper treatment, the Cyanophyceae were significantly less abundant than in control communities ($P < 0.05$). The relative abundance of Bacillariophyceae was similar in all copper treatments. Each class showed broad interspecific

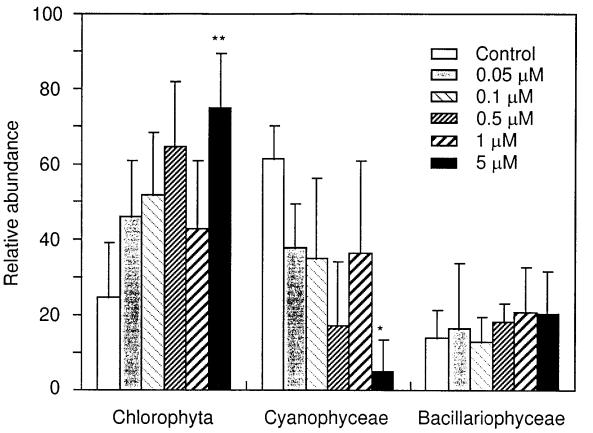

Fig. 2. Relative abundance (%) of algal classes in periphyton communities after 12 weeks of exposure to different concentrations of copper. Bars represent mean ± 1 S.D. ($n = 3$). Significantly different from control treatment: $*P < 0.05$, $**P < 0.01$.

differences in sensitivity to copper. *Microcystis* sp., *Gomphospheria* sp. and *Oscillatoria* sp. were virtually absent in the 1 μM copper treatments (Table 1). At this concentration, *Pseudoanabaena catenata* was the dominant Cyanophycea, although it was absent in 5 μM copper treatments. Most chlorophytes tolerated exposure concentrations up to 1 μM copper, but were sensitive to the 5 μM copper exposure (Table 1). There was a particularly remarkable change in the relative abundance of the unicellular green alga *Oocystis nephrocytioides*, from less than 1% in the control aquaria to 56% in the 5 μM copper treatments. Among diatoms, *Achnanthes* was the dominant genus at the highest copper exposure. These structural shifts were observable after 4 weeks of exposure (not shown).

Algal biomass in the aquaria was not quantified but it was visually abundant in all but the highest copper treatment, where biomass was very sparse (not shown).

The photosynthesis rate of the periphyton communities was not significantly influenced by the long-term exposure to copper, except at the highest copper treatment (Fig. 3). Specific ^{14}C uptake of communities exposed to ≤ 1 μM copper ranged from 182.2 ± 39.9 to 255.5 ± 18.7 μg C μg^{-1} Chl *a* h^{-1}, whereas at 5 μM copper the value was 395.1 ± 89.1 μg C μg^{-1} Chl *a* h^{-1}.

Table 1
Relative abundance (%) of algal taxa in periphyton communities after 12 weeks of exposure to copper[a]

	Control	Copper concentration (μM)				
		0.05	0.1	0.5	1	5
Chlorophyta						
Cosmarium sp.	<1	<1	1	1	4	0
Geminella interrupta	7	3	18	11	3	0
Mougeotia sp.	3	6	7	11	1	0
Oedogonium sp.	<1	14	2	4	0	0
Oocystis nephrocytioides	<1	14	8	22	20	56
Phacotus lenticularis	1	<1	1	1	1	5
Scenedesmus spp.	4	5	9	2	0	0
Others	9	3	7	13	13	13
Bacillariophyceae						
Achnanthes sp.	6	6	2	4	0	14
Fragilaria sp.	2	1	2	1	3	2
Tabellaria fenestrata	3	2	4	3	3	1
Others	3	8	5	11	15	3
Cyanophyceae						
Gomphospheria sp.	10	7	24	3	2	0
Microcystis sp.	27	6	3	6	0	0
Oscillatoria sp.	25	21	0	0	0	5
Pseudoanabaena catenata	<1	3	8	8	34	0

[a] Values represent means of three measurements done with composite samples taken from each of three treatment replicates.

After 12 weeks, the short-term tolerance of photosynthesis to copper of communities exposed to copper was higher compared to that of the control communities (Fig. 4). Significant tolerance increases ($P < 0.05$) were found in communities exposed to 0.1, 1 and 5 μM copper when tested at short-term concentrations of 50, 100 and 150 μM copper. While the photosynthesis rate of control communities and of communities exposed to 0.05, 0.1 and 0.5 μM copper was significantly affected by 50 μM copper ($P < 0.05$), the photosynthesis of communities established at the highest copper concentration (5 μM) was not affected by the highest copper concentration applied in the short-term test (150 μM).

Long-term exposure to copper also resulted in an increase in community tolerance to zinc, nickel and silver (Fig. 5). While photosynthesis rate of control communities was inhibited by about 40% at the lowest tested zinc concentration (10 μM), about 100 μM zinc was required to inhibit photosynthesis rate of communities exposed to 0.5 μM copper to this same level. Communities exposed to 0.5 and 5 μM copper were significantly more tolerant to zinc than control communities at all the tested short-term zinc concentrations ($P <$

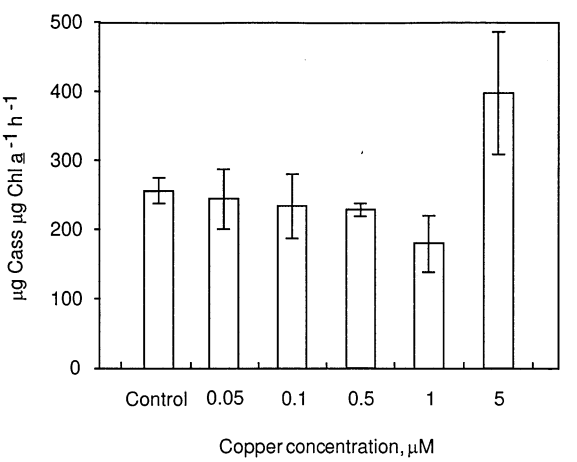

Fig. 3. Photosynthesis rate of periphyton communities after 12 weeks of exposure to different concentrations of copper. Bars represent mean ± 1 S.D. ($n = 3$).

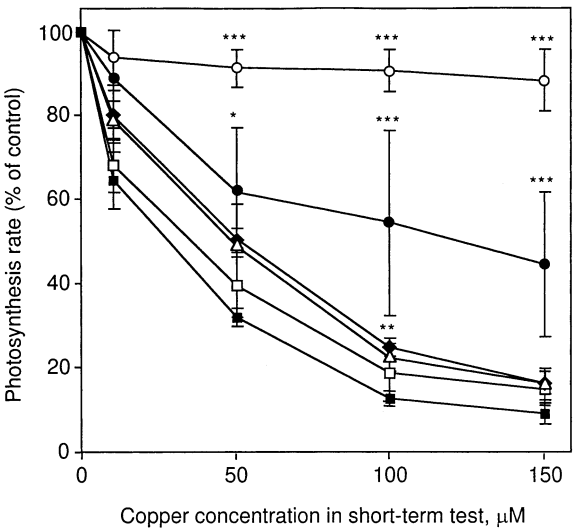

Fig. 4. Inhibition of photosynthesis rate by copper in periphyton communities after 12 weeks of exposure to different concentrations of copper. Bars show mean ± 1 S.D. ($n = 3$). Copper treatment in aquaria: (■) control, (□) 0.05 μM, (◆) 0.1 μM, (△) 0.5 μM, (●) 1 μM, (○) 5 μM. Significantly different from control treatment: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

0.01). The control communities and those exposed to 0.5 μM copper showed the same tolerance to nickel. Still, communities exposed to 5 μM copper displayed a significantly increased tolerance to nickel ($P < 0.05$) at 100 and 200 μM nickel. Tolerance to silver increased with the increase in copper exposure concentration. Concentrations of 10 μM silver in the short-term test almost completely inhibited the photosynthesis in the control communities. At the same silver concentration, photosynthesis of the communities established at 0.5 μM and 5 μM copper was significantly more tolerant ($P < 0.001$). At concentrations of 100 μM silver, the photosynthesis was completely inhibited in all communities.

4. Discussion

Long-term exposure to copper changed the taxonomic composition of periphyton communities. Major changes included a decline of Cyanophyceae and an increase in Chlorophyta. These results confirm the high sensitivity of Cyanophyceae to copper (Takamura et al., 1989), which are generally scarce in copper-polluted en-

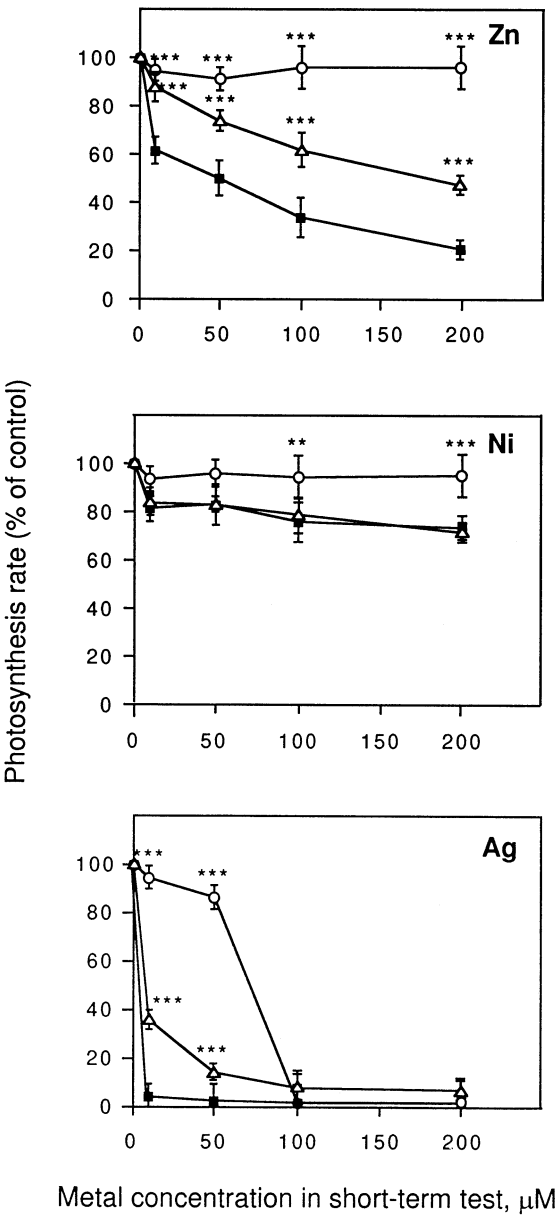

Fig. 5. Inhibition of photosynthesis rate by zinc, nickel and silver in periphyton communities after 12 weeks of exposure to different concentrations of copper. Bars show mean ± 1 S.D. ($n = 3$). Copper treatment in aquaria: (■) control, (△) 0.5 μM, (○) 5 μM. Significantly different from control treatment: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

vironments (Horne and Goldman, 1974; Leland and Carter, 1984; Cattaneo, 1992). In contrast, various taxa belonging to the Chlorophyta were insensitive up to 1 μM copper, which reflects the high tolerance of green algae to copper and other metals (Foster, 1982). At the highest copper concentration, periphyton communities were dominated (56%) by the unicellular green alga *Oocystis nephrocytioides*. Other Oocystaceae have been previously documented as being abundant in copper-polluted sites and to have a general resistance to metals (Foster, 1982; Takamura et al., 1990). As with *Oocystis nephrocytioides*, the increased density of other taxa in the presence of copper, particularly *Geminella interrupta* and *Pseudoanabaena catenata*, reflects a reduced competition by more copper-sensitive algae.

Periphyton communities subjected to long-term copper 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 on Chl *a* synthesis (Rai et al., 1990). The response in terms of photosynthesis of periphyton communities in metal-polluted environments has been shown to be quite complex, usually following a pattern of decline and recovery (Crossey and La Point, 1988; Takamura et al., 1990; Balczon and Pratt, 1994). The high variability in photosynthesis rate further suggests the limited utility of this parameter as an indicator of metal induced impacts.

Unlike photosynthesis rate, the tolerance of photosynthesis to copper increased in response to long-term copper exposure (Fig. 4). While photosynthesis of control communities was affected by 10 μM copper in the short-term test, photosynthesis of communities exposed at the highest copper concentration (5 μM) was less affected by 150 μM copper. Significant increases in copper tolerance were detectable at exposure concentrations of only 0.1 μM copper ($P < 0.01$). That a pollutant-impacted community is more tolerant to the same pollutant relative to a non-impacted community is to be expected, considering, as suggested by Blanck et al. (1988), that tolerance will increase as the most sensitive components of the community are lost. Accordingly, the taxonomic composition of the algal communities changed with increasing copper exposure concentration (Fig. 2). The shift in community structure with change in tolerance, and the increase in tolerance with the strength of copper exposure reflects the usefulness of short-term tolerance measurements for the assessment of copper-induced impacts on periphyton communities.

The sensitivity of individual species in the communities was found to span a range from 0.1 to 5 μM copper. The lowest copper concentration found to change the taxonomic composition and the copper tolerance of periphyton communities is comparable to the water quality criteria for copper established in different countries (Behra et al., 1994). Similar copper concentrations were reported to affect the structure and tolerance of phytoplankton but not periphyton communities (Gustavson and Wängberg, 1995). In terms of free copper concentrations the sensitivity range might be considerably larger. At different sites along the Glatt river, total dissolved copper varies between 20 and 35 nM and the free copper ion concentration is in the range of 10^{-13} to 10^{-15} M (Xue et al., 1996). In the control aquaria the copper ion concentration was 10^{-15} M and in the 5 μM copper exposures around 10^{-11} M (Xue, unpublished data). In comparison, the copper concentration required to inhibit photosynthesis rate upon short-term incubation with copper was in the micromolar range. Upon long-term exposure, the most sensitive species in the periphyton communities were thus eliminated at free copper concentrations of about 10^{-14} M, whereas other species tolerated about three orders of magnitude higher concentrations. Similar free copper concentrations have been found to be tolerated by various algal species (Knauer et al., 1997).

Communities subjected to long-term copper exposure also displayed an increased tolerance to zinc, nickel and silver. The occurrence of co-tolerance is not surprising, considering that the exposed communities comprised various species presumably differing in their mechanisms for providing tolerance to metals (Reed and Gadd, 1990). Our results suggest the involvement of cellular ligands in confering tolerance to the tested metals. Consideration of the relative ability of

copper, zinc, nickel and silver to bind to organic ligands provides insight into potential functional groups that may be important in the immobilization of these metals. Although copper, like zinc and nickel, shows no general preference for O- or S-donating ligands, it displays similar binding preferences to sulfhydryl groups as silver (Mason and Jenkins, 1995). The co-tolerance pattern observed in our study may be partly explained by the involvement of phytochelatin in the regulation of cellular metal levels. Phytochelatins are metal-binding peptides with a high cysteine content (Grill et al., 1987) that bind various heavy metals by thiolate coordination (Gekeler et al., 1988). Additional mechanisms that may be involved in tolerance to copper detected in communities exposed to the highest copper concentration and dominated by *Oocystis nephrocytioides* include cellular exclusion and extracellular immobilisation of copper. However, this last mechanism seems to be less important, because in this case, other species would have been protected from the copper effects. Such a view is consistent with earlier studies showing that several Oocystaceae species excreted none or only weak copper ligands (Swallow et al., 1978; McKnight and Morel, 1979).

The occurrence of metal co-tolerance in communities and algal populations has been reported before (Reed and Gadd, 1990; Gustavson and Wängberg, 1995). Detected co-tolerance patterns are various, which most probably reflects the variety of mechanisms through which algae are metal-tolerant. Nevertheless, detecting co-tolerance implies the difficulty of conclusively assigning tolerance shifts to the presence of a particular metal in the environment. It is also questionable whether parameters other than photosynthesis will allow more precise conclusions, particularly in cases where co-tolerance results from intrinsic physiological properties of the organisms (e.g. production of mucilage). The combination of tolerance measurements with analyses of metals in the organisms may provide a better approach.

Before using tolerance measurements as an ecotoxicological tool for the assessment of metal-induced impacts, a few additional points need examination. The evaluation of results that do not indicate increases in tolerance seems to be particularly problematic. In our study, tolerance increases were clearly detectable after 12 weeks of copper exposure. In the study reported by Gustavson and Wängberg (1995), exposure to copper resulted in a gradual increase of tolerance to copper in phytoplankton but not in periphyton. The time required before increased tolerance can be detected will depend on the tolerance mechanisms potentially operating in the community. These, in turn, will depend on the species present in the community at the onset of exposure. Previously, tolerance has been expressed as the response in photosynthesis (Blanck and Wängberg, 1988) or growth (Foster, 1982) to metals. The choice of alternative biochemical parameters based on the mechanisms of metal tolerance may allow an earlier detection of tolerance increases.

In conclusion, our results support the notion of pollution induced community tolerance, which conceives the increased tolerance to a pollutant as evidence for species selection and structural shifts of the community due to the presence of that pollutant (Blanck and Wängberg, 1988). Metal-induced community impacts may be better assessed by improving the sensitivity of tolerance detection and by the combination of tolerance measurements with metal analyses.

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