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Changes in Periphyton Community in Response to Variable Copper Exposure

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Abstract. This study examined the changes in periphyton communities on exposure to copper under variable conditions. Stock inoculant for the periphyton communities was obtained from the Swan River estuary, Western Australia. Six periphyton communities were colonized onto glass plates for three weeks in copper concentrations between 0.05 – 0.3 mg/L, buffered by ethylenediaminetetraacetic (EDTA). Community development was followed by weekly examining the species composition colonizing on the glass plate substrate. After three weeks, the developed communities were exposed for 24 hours to a series of copper concentrations from 0.16 – 4 mg/L, without EDTA added. Copper has the effect of delaying development of the periphyton communities initially. However, the development picked up subsequently to match that of the non-exposed control community. Copper also has the effect of increasing the species richness and photosynthetic efficiency of the communities. Photosynthesis efficiency (measured as PSII quantum yield) correlated positively with the number of total individuals and species in the communities (Pearson's coefficients of 0.9 at sig < 0.01). The 24-hour exposure to high copper concentrations affect by damaging cellular structures of the community organisms. Pre-exposed communities were more tolerant to the copper effect than the control community.

Keywords: community structure, copper effect, photosynthetic efficiency, periphyton, PSII quantum yield

1. Introduction

Risk assessment aims at protecting ecological communities in addition to human health. Chemical contaminant can alter the structural or functional properties of biological communities. Community structure is commonly studied using biological indices based mainly on taxonomic analysis. On the other hand, community functions such as primary productivity, nutrient cycling, and energy flow have ecological relevance. Furthermore, changes in physiological processes almost always precede community structural changes. Therefore, functional measures that integrate the collective activities of individuals in a community would allow for more effective protection.

This study examined the changes in a periphyton community on exposure to variable copper concentration under laboratory conditions. Periphyton community is important to the productivity of aquatic systems. It is well documented that periphyton are good biological indicators of chemical and physical stress [1]. The periphyton community as a highly diverse community in taxonomy and genetics can provide an integrated source of information for impact studies in nearly every situation [2]. If the response of periphyton community is predictable, it can provide an early warning of impact on the ecosystem. Copper is a common contaminant in urban storm runoff [3]. Although copper is an essential micronutrient to periphyton, it becomes toxic when in excess [4]. Excessive copper can inhibit algal photosynthesis and cause structural changes to periphyton community, thereby degrading aquatic ecosystems [5]. Community changes in both

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function and structure were studied and correlated.

2. Materials and Methods

This study was conducted in the laboratory using a community of periphytic organisms collected from the Swan River Estuary in Western Australia. Conceptually, a community refers to an assemblage of organisms living together interactively as a unit. However, biological entities in natural environments form a continuum and, as such, a natural community cannot be readily defined precisely and truly. Hence, the word “community” is used here in an operational sense. Our experimental design mimicked the typical exposure conditions in aquatic ecosystems receiving contaminated runoff, i.e. prolonged exposure of several months, to some baseline level low in concentration, interrupted by exposure to elevated concentration, for very short duration of within an hour, during a storm event. To simulate this sequence of chronic and acute exposure condition, the periphyton community was exposed for 21 days to low copper concentrations, buffered with ethylenediaminetetraacetic (EDTA), followed by 24-hour exposure to higher copper concentrations, without EDTA buffering. The concentration range (0.05 - 0.3 mg/L Cu) for the long-term chronic exposure was selected to include the ambient concentration range 0.0013 - 0.05 mg/L Cu at a selected site on the Swan River Estuary, Western Australia, Australia [6]. Similarly for the short-term acute exposure, the concentration range of 0.16 - 4 mg/L Cu was selected to cover the concentration of stormwater discharge at the site. As this was an initial study, no attempt was made to simulate runoff flow rates, and therefore static test systems were used.

2.1. Culture Media

The experimental periphyton was maintained in the laboratory using f/2 medium [7], modified by reducing the concentration of copper and zinc to 10 % of the original recipe. The copper concentration was reduced in order to minimise effects from blank culture medium. The zinc concentration was reduced so as to minimise any probable effects of co-tolerance [8]. The medium was prepared in 0.45 μm-filtered seawater with the salinity adjusted to 10 ‰ using deionised water. The culture medium contained 3.82 mM of EDTA (as the disodium salt $C_{10}H_{14}N_2O_8Na_2.2H_2O$, AJAX analytical grade), added to reduce the effective toxicity of copper. The culture medium for the 24-hour exposure was without EDTA.

2.2. Development of Periphyton Communities

Six communities of the periphyton were developed in culture medium spiked to 0.05, 0.1, 0.15, 0.2, 0.25, and 0.3 mg/L Cu for 21 days from a stock culture. The stock periphyton was collected from the Swan-Canning estuary, Western Australia. The communities were allowed to develop on a rack of five glass plates (250 mm x 75 mm x 2 mm clear glass), immersed in a 5-L tank. Incubation was in a cycle of 12 hours in the dark and 12 hours in light (3000 lux, 60 W OSRAM daylight fluorescent lamp) at 20 ℃. The tank solution was agitated by aeration. The tanks were positioned randomly, with weekly re-positioning to offset variability in spatial conditions. Tank solution was renewed weekly. Each set was done in triplicate, including a control.

2.3. Analysis of Community Changes

The periphyton communities were analysed for total number of individuals (N), total number of species (S), species richness (d) and species evenness (J), and the common biodiversity indices of Shannon index (H) and Simpson index ($1-\lambda$). The parameters N and S were determined by direct sample enumeration. Parameters d and S were adjusted to allow for differing number of individuals. The community function determined was photosynthetic efficiency that is related to primary productivity. Photosynthetic efficiency was measured as the effective PSII quantum yield, determined under steady-light condition using a PAM fluorometer (Walz Diving PAM). For the 24-hour exposure to copper, communities were analysed for species presence or absence, and examined for signs of cell damage. The endpoint of no-effect concentration (NEC) was determined by iterating at where the response curve began to decline below the PSII yield of the control test solution. The analysis was performed on the communities developed under treatments of 0.1 mg Cu/L and 0.3 mg Cu/L.

The community parameters were correlated to identify probable link between structural and functional changes, and hence to establish a link with copper exposure. Analysis for differences between community responses and correlation between changes in community structure and function were performed using Primer V 5 (PIMER-E Ltd).

3. Results and Discussion

3.1. Community Development in Copper

In all treatments, the glass substrate was colonized by the first week. The total number of individuals was about 1000 and below (Fig. 1). The early settlers were the pennate diatoms *Nitzschia* and *Naviculi* species, followed by centric diatoms. Others organisms found included protozoa and picoorganisms. The *Nitzschia sp.* was dominant, constituting more than 80% of the community population. The *Naviculoid sp.* varied highly across the communities, from 2.5% to 16% in the 3-week old communities. Protozoa constituted a significant proportion of the communities, varying from 4.5% to 13% in the 3-week old communities.

The features of the *Nitzschia sp*. are particularly advantageous for colonizing onto the smooth glass plates. The flexible, long, distal appendages provide mobility for the organism to navigate. Its rather soft elongated body allows the organism to readily attach onto the smooth surface of the glass plates. The *Nitzschia sp*. joins to one another along the length of their bodies forming a mat that wraps over the glass surface. The mat provided structural support to other organisms of the community such as the circular diatoms, which colonised after the *Nitzschia sp*.

The number of individuals (N) in the communities increased with time. For the control community, N steadied off soon after the second week while for the treated communities, N continued to increase (Fig. 1). Treatment at 0.05 mg Cu/L was consistently higher in N than the control. For treatments ≥ 0.25 mg Cu/L, N was low initially but picked up subsequently so that by the third week, N exceeded that of the control community. Overall, this trend of decreased differences between treatment and control is indicated when all parameters of community structure (N, S, d, J, H, and 1-λ) were taken into consideration (Fig. 2, a non-metric multidimensional scaling (MDS) plot).

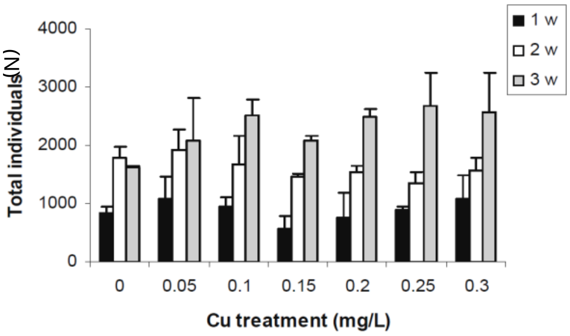

Fig. 1: Weekly counts of total individuals (N) in developing periphyton communities under different copper treatments over three (3) weeks.

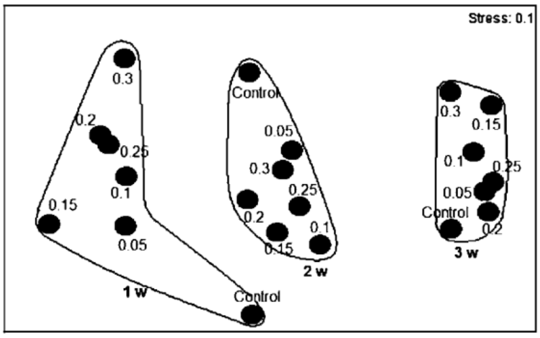

Fig. 2: MDS plot of community structure after 1, 2 and 3 week development in 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 mg Cu/L; between community differences are indicated by the spatial position and distance apart: points that are closer together represent samples that are very similar in their community structures, points that are far apart correspond to very different community structure.

Copper exposure also enhanced photosynthesis performance. Treated communities have higher PSII quantum yield than the control community, 0.66 – 0.72 quantum yield versus 0.63 quantum yield respectively. In general, PSII quantum yield increased with copper concentration. The PSII quantum yield correlated strongly with N and S (Pearson's coefficients of 0.9 at sig. < 0.01).

3.2. Community Response to 24-hour Copper Exposure

High copper concentration impaired the periphyton communities by causing cellular damage in organisms. The *Nitzschia sp*. appeared bloated with disintegrated or fragmented cell structure. The *Naviculoid sp.* also suffered from cellular disintegration even though they retained their form of siliceous cell casing. On the other hand, short-form pennate diatoms inhabiting in tubes tended to stay intact in high exposure concentrations. Cellular damage, observed as disintegrated cell contents or fragmented cell structures, was more apparent with increased concentration of the short-term exposure.

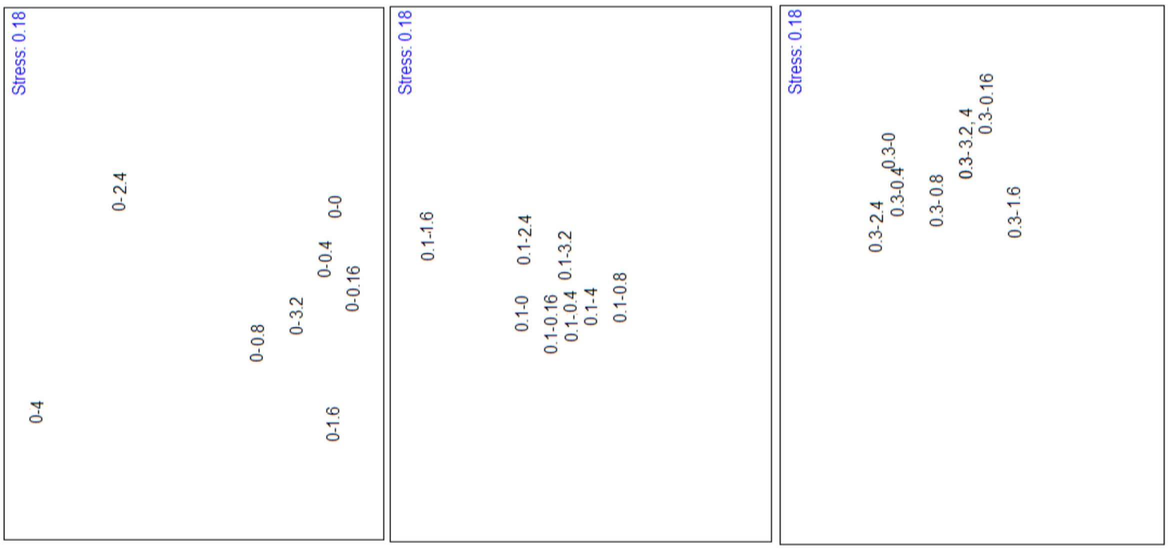

Fig. 3: MDS plots showing differences in community species composition after 24-hour acute copper exposure: the greater the distance apart, the greater the difference; point 'x-y' refers to the test sample where x denotes mg Cu/L of the 21-day treatment and y denotes mg Cu/L of the 24-hour exposure, '0' denotes the control.

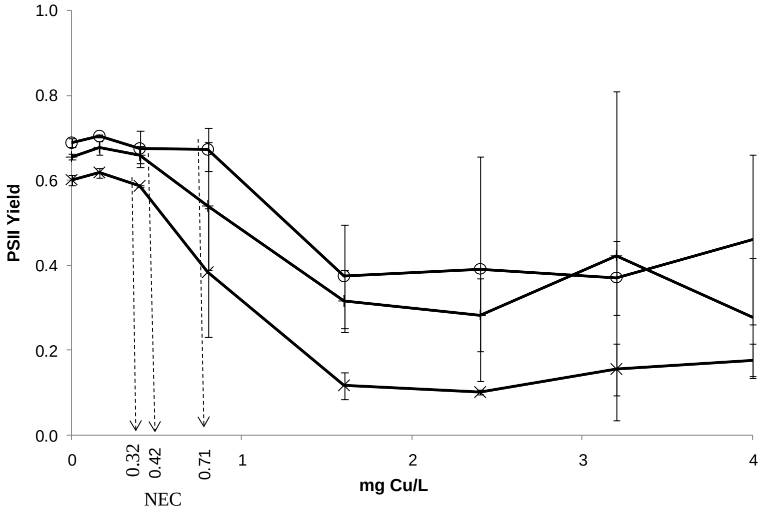

Fig. 4: Community response curve to 24-hour acute copper exposure after 21-day development in 0.1 mg Cu/L (+) and 0.3 mg Cu/L (o) and in control medium (×), respectively; error bar = 1 sd, n = 3; NEC denotes the no effect concentration in mg Cu/L.

In general, the control community responded to the 24-hour exposure with greater changes in both community structure (Fig. 3) and function (Fig. 4) than the treated communities. The no-effect concentration (NEC) of PSII quantum yield was higher for the communities which were developed in copper than for the control community. This indicates that pre-exposure has the effect of enhancing the community tolerance to subsequent copper toxicity. This effect could be due to the beneficial effect of copper as an essential trace nutrient that promoted the fitness of the community during its development stage that subsequently enhanced the tolerance of the developed community to toxicity effect of subsequent acute exposure. Another reason could be due to copper selectivity during pre-exposure whereby more tolerant species, strains or individuals are preferred. However, this latter reason should also lead to lower species richness which was not observed. But again, as the taxonomic analysis in this study was limited to genus level, the latter reason could not be ruled out completely.

4. Conclusion

The periphyton community of the Swan River estuary has a rich diversity of species, predominated by diatoms. Copper exposure induced changes in the community. Photosynthetic performance of communities developed in low copper concentration was enhanced in efficiency and in tolerance to copper toxicity. High copper concentration impaired by causing cellular damages to the community organisms.

The current work focused on algae and ignored other components of the community such as protozoa and pico-organisms. While the ecological relevance of algae is in primary production, the other community components are important in the ecological function of decomposition and should be included in future studies. Identification of community organisms was limited to the genus level. Further work should identify to a level sufficient for determining changes with statistical significance.

5. Acknowledgements

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