DIFFERENT PATTERNS OF UPTAKE AND DEPURATION OF CADMIUM BY PERIPHYTON COMMUNITY AND A GRAZER SPECIES (*PHYSA* SP.): A MESOCOSM EVALUATION

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Introduction

Widespread pollution by heavy metals generated by various industries has serious adverse effects on human health and the environment. Cadmium is a heavy metal recognised as one of the most hazardous environmental pollutants (Lodeiro et al. 2005). It is a non-essential and non-beneficial element to organisms, causing toxicity and other deleterious effects on various components of the aquatic environment. The ability of algal periphyton to concentrate cadmium from fresh water is well known (Hill et al. 2000). Moreover, periphyton communities are able to accumulate large amounts of cadmium despite its low concentration in fresh water. Cadmium bioaccumulation has been studied in bacteria (Trevors et al. 1986; Volesky & Holan 1995), Cyanobacteria (Les & Walker 1984; Inthorn et al. 1996), algae (Sakaguchi et al. 1979; Geisweid & Urbach 1983; Holan & Volesky 1995) and fungi (Fourest & Roux 1992; Holan & Volesky 1995). Periphyton algae, bacteria, and fungi that develop on stream substrata, can accumulate high concentrations of metals and may be an important source of metal exposure to benthic macroinvertebrates in mining-impacted streams (Kiffney & Clements 1993).

There is, to date, no information available regarding cadmium 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. In natural fresh waters, cadmium sometimes occurs at concentrations of less than 0.01 μg L^{-1}, but in environments impacted by man, concentrations can be several micrograms per litre or greater (U.S. EPA 2000). There have been some studies, under both laboratory and natural conditions, on the relationship between the concentration of cadmium in water and the algal periphyton community (Nayar et al. 2003). 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 organisational

levels. Assessment of long-term impacts of chemical contamination on the environment should also take account of the natural variability of biological systems in space and time. The use of laboratory streams to study the impacts of pollutants on community attributes can provide the opportunity to isolate the effect of environmental variables on algal communities (Lamberti & Steinman 1993). This has led many authors to use algal periphyton as an indicator of water quality in aquatic environments (Nayar et al. 2003). In the present study, we asked two basic questions: Does cadmium accumulate along a food chain consisting of the periphyton community and a grazer species (*Physa* sp.) under semi-natural conditions provided by artificial streams? If not, which one can better indicate the water quality?

The artificial streams

This study was conducted in three replicate fibreglass channels, each 2 m in length, 0.5 m in width and 0.4 m in depth. Each fibreglass channel was divided into two equal sections along its length, by placing a fibreglass sheet along the middle of the channel, with water circulating up one side and down the other side of the divide. Water from the head stream of the Sarab Chehr (Kermanshah Province, western Iran) was used to fill the experimental channels to a depth of approximately 0.08 m. A constant water circulation was provided using a water pump. Water velocity in the channels averaged 17 cm s^{-1}. Artificial light was supplied by 18 metal halide lamps (6 for each channel), which provided a broad spectrum of photosynthetically available irradiance. Photon flux density was 90 µE m^{-2} s^{-1} as measured at the water surface in the artificial streams. The photo period was set at 16L:8D. During the experiment, which lasted for 49 days, water temperature ranged between 18 °C and 21 °C, pH was in the range 7.8–8.2, and conductivity ranged between 245 µS cm^{-1} and 310 µS cm^{-1}. Average values for NO_3-N, PO_4-P and dissolved oxygen were 0.6 mg L^{-1}, 0.075 mg L^{-1} and 7 mg L^{-1} respectively.

Experimental treatments

All artificial streams were inoculated with a mixture of benthic algae on the first day of the experiment, 24th December 2005. The inoculum was prepared by scraping periphyton from rock collected from Sarab Chehr. The scrapings were homogenised and made up to a volume of 120 L with stream water, filtered through a mesh (pore size 1 mm), and then an equal volume of the filtrated mixture of periphyton algae was added to each stream. Each artificial stream contained 200 tiles (2.5 × 2.5 cm), which were allowed to become colonised by periphyton algae for 2 weeks prior to the introduction of snails. This period was sufficient for visible

colonisation of algae. Snails (*Physa* sp.) were allowed to depurate over three days, rinsed in de-ionised water to remove attached algae, and then introduced to the streams on the 14th day.

On the 15th day of the experiment, the three artificial streams were smeared with a stock solution of $CdCl_2$ to provide a nominal Cd concentration of 20 µg L^{-1}. To compensate for absorbtion of cadmium by the biotic and abiotic community/environment, and to keep the dissolved cadmium in the water column at a relatively stable concentration above the detection limit for atomic absorption spectrometry, a similar 'contamination' with $CdCl_2$ was introduced on the 30th day of the experiment.

Experimental analysis

The cadmium content of a sample of snails and of periphyton from each channel was measured every 5 days. At each sampling, randomly selected snails were placed in a Petri dish and dried in an oven for a period of 24 h to 48 h at 100 °C until their weights became constant. This procedure (Heng et al. 2004) was followed by acid digestion using a mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) at a ratio of 5:1 (w/w) in a microwave oven on 0.5 g of each sample. After filtration of samples by filter paper, the cadmium concentration was measured using an atomic absorption spectrometer (AA-680/G; Shimadzu).

Samples of periphyton scraped from the tiles were dried for 24 h at 60 °C and then combusted in a 525 °C furnace for 4 h. Acid decomposition of samples of approximately 0.5 dry weight was performed in a mixture of 10 mL 65 % HNO_3 and 10 mL distilled H_2O for 50 minutes, using a microwave oven. Cadmium concentration of samples was measured after filtration by filter paper, using the same atomic absorption spectrometer (AA-680/G; Shimadzu).

Periphyton samples for determination of dry mass were randomly selected every 5 days, dried for 24 hours at 60 °C and the attached algal periphyton scraped from the tiles with a razor blade and weighed. In order to measure chlorophyll *a* in the periphyton community, 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).

During the experiment, differences in results between the three replicate channels were determined using analysis of variance (ANOVA) with 95 % confidence (probability limit of $p < 0.05$) and found to be not significant.

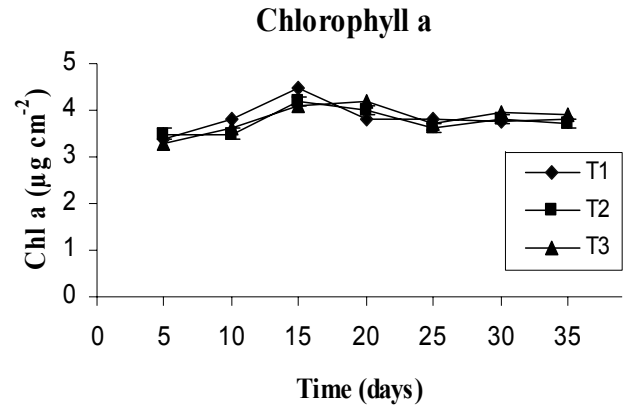
The effect of cadmium on the periphyton

Assuming chlorophyll *a* and dry mass as an indicator of well-being of the periphyton community, the periphyton appeared to suffer no adverse effects from the cadmium contamination (Fig. 1). Cadmium concentrations in the periphyton community slowly increased to a steady state concentration and there were no signs of depuration (Fig. 2b). The amount of cadmium measured was comparable to several similar experiments. Maximum intracellular accumulation under low cadmium concentration in *Chlorella* sp. was estimated to be 2.4 mg Cd /g dry cells (Matsunaga et al. 1999). Geisweid & Urbach (1983) have found cadmium concentration in several species of algae such as *C. vulgaris* to be 5 mg g^{-1}. Similarly, Sakaguchi et al. (1979) have indicated cadmium concentration in *C. regularis* to be 7.9 mg g^{-1}.

The pattern of accumulation of cadmium in the periphyton community in the current experiment demonstrates a slow increment that gradually approaches an apparent upper asymptote. This pattern of metal accumulation is in contrast with metal sorption reported by several authors in microalgae which is often characterised by an initial phase of rapid sorption followed by a second phase of slower uptake (Genter et al. 1988; Reinfelder & Chang 1999). In such a biphasic model of metal uptake, metal ions are believed to bind to and saturate negatively charged sites (e.g. carboxylic groups) on the exterior of cells in the first phase and are then transported to the interior of the cell via biologically active processes in the second phase (Xue et al. 1988). The relatively long exposure time and possibly fewer colloidal particles in our indoor artificial streams imply that internal uptake was the most important component of cadmium accumulation and external adsorption of the heavy metal would be negligible. It is also possible that the essentially linear pattern of uptake by the periphyton community in this experiment is to some extent influenced by the comparatively low cadmium concentration in the artificial streams that allows active transport to keep pace with adsorption. Such an assumption can be substantiated by reports indicating that the biphasic sorption of cadmium by phytoplanktonic diatoms becomes more linear as cadmium concentration decreases (Conway & Williams 1979).

Internal cadmium concentration in the periphyton increased with periphyton biomass (Fig. 3). This could be related simply to the increasing duration of exposure, but could also reflect an increase in exposure surface in the periphyton with increasing biomass. It has been shown that both biomass and water flow can have some influence on the sorption of cadmium by the periphyton community (Hill et al. 2000). According to Hill et al. (2000) increasing cadmium sorption with increasing periphyton biomass implies a significant contribution of subsurface cells to the metal

a)



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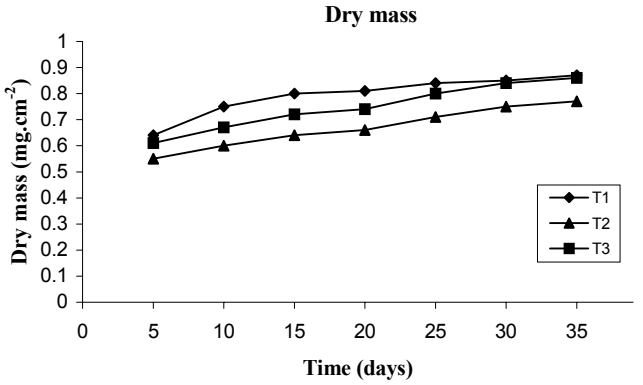
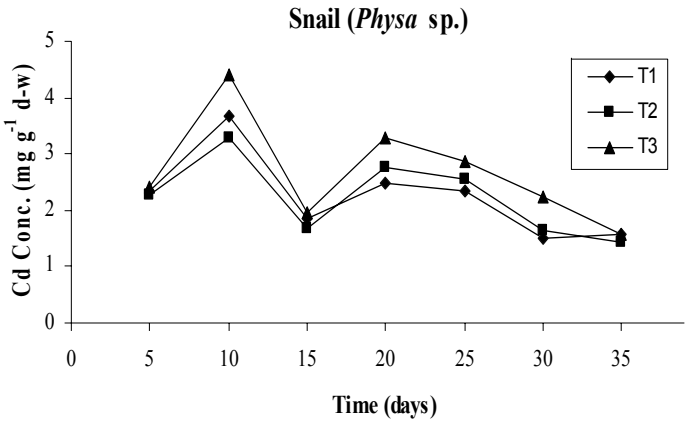


FIG. 1. *(a)* Chlorophyll *a* concentration (µg cm^{-2}) and *(b)* dry mass (mg cm^{-2}) in periphyton algae, over 35 days of exposure to cadmium in three replicate artificial streams (T_1, T_2 and T_3).

a)



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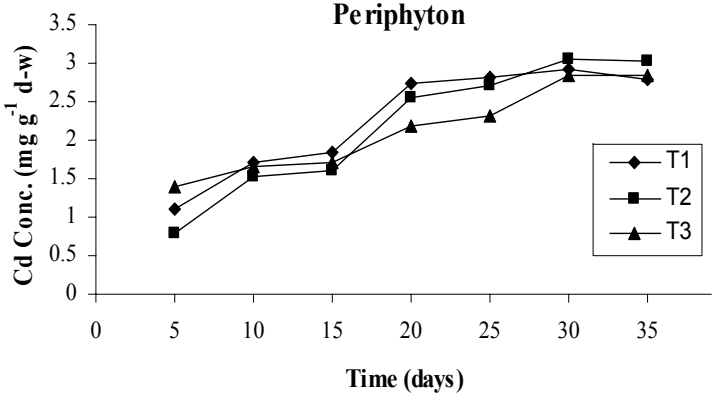

FIG. 2. Concentration of cadmium (mg g^{-1} dry weight) in *(a) Physa* sp. and *(b)* the periphyton, over 35 days of exposure to cadmium in three replicate artificial streams (T_1, T_2 and T_3).

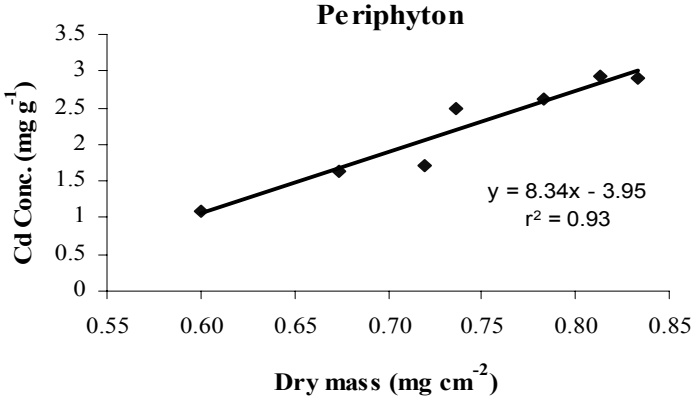

FIG. 3. Relationship between cadmium concentration and dry mass of periphyton over 35 days of exposure to cadmium. Results shown are mean values for three replicate channels.

sorption. These cells may contribute directly to sorption by providing additional sorption sites, or they may contribute indirectly by changing water acidity.

The effect of cadmium on *Physa*

No mortality or other overt signs of toxic effect were noticed in *Physa* sp. during this study. In contrast to the periphyton, there was an early rapid increase in cadmium concentration in the snails and a subsequent decrease until an apparent steady state concentration was reached (Fig. 2a). With regard to efficient grazing behaviour, the snail had the apparent opportunity to ingest high concentrations of cadmium through the food chain. However, the amount of cadmium accumulated at the upper asymptote for the snail is only slightly greater than in the periphyton ($\overline{X}_s = 3.78$, $\overline{X}_p = 2.91$, $t = 3.9$, $n = 7$, $p = 0.003$). Weiss et al. (1981) cite several studies which found low metal concentrations in heterotrophic species. It is important to notice that accumulation of heavy metals by herbivorous organisms in the freshwater environment is under the influence of various factors. A grazer species such as *Physa* sp., in addition to uptake of cadmium through food, could also adsorb some cadmium to external surfaces. The ability of heterotrophic organisms in adsorbing heavy metals has been documented by measuring accumulation of cadmium on dead and living organisms (Dressing et al. 1982).

Modelling of bioaccumulation

Modelling of bioaccumulation of cadmium in the periphyton community and in the snail was carried out using a first-order kinetic equation. For the periphyton community the model can be described with the following differential equation (e.g. Connell 1990):

$$\frac{dC_p}{dt} = k_1 C_w - k_2 C_p$$

where C_p is the metal concentration in periphyton algae (mg g^{-1} tissue dry wt), t is the exposure time, C_w is the bio-available dissolved metal concentration in water (µg L^{-1}), k_1 is the uptake rate constant from water and k_2 is the elimination rate constant. Assuming that C_w is constant and the elimination rate negligible the solution to the differential equation is:

$$C_p = \left[\frac{k_1 C_w - k_2 C_p}{k_2}\right] \times [1 - \exp(k_2 t)]$$

For *Physa* sp. with a clear depuration of cadmium following a rapid increase in cadmium concentration the pattern of uptake and depuration follows the following equation (Connell 1990):

$$C_s = (\frac{k_1 Cw}{k_2})(1 - \exp(-k_2 t)) + C_{se} \exp(-k_2 t)$$

where C_{se} is the cadmium concentration of the snail at equilibrium level. The initial background metal content of the periphyton and *Physa* sp. was assumed to be negligible.

Comparisons between observed and predicted values of cadmium as indicated by the first order kinetic equations (Fig. 4) show that cadmium uptake followed theoretical models of heavy metal bioaccumulation (Connell 1990) with good coefficients of variation for periphyton (r^2 = 0.83) and snails (r^2 = 0.51).

Biological monitoring of cadmium pollution

The present study suggests that detection of cadmium pollution in streams may be facilitated by using periphyton and its grazer (*Physa* sp.) as biological monitors, rather than by attempting to assess directly the metal concentrations of water or sediments. Potentially toxic metals such as cadmium are often not detected or are found only in very low aqueous concentration. However, even if aqueous concentrations are high enough to permit detection, grab samples or 'spot' (discontinuous) water samples may not intercept pulses of pollution which can be considerably greater

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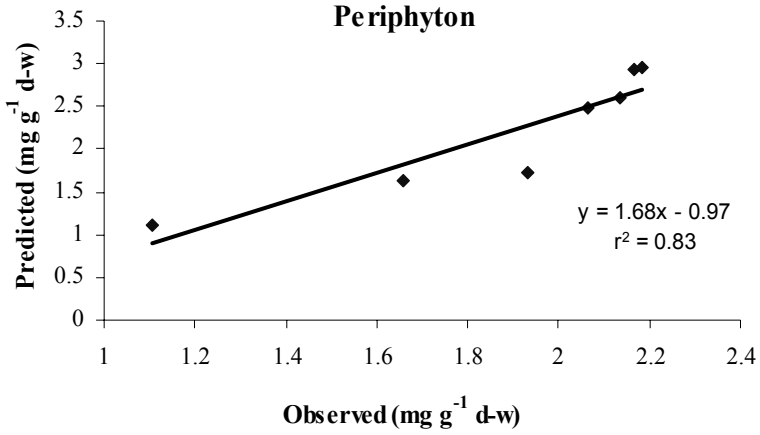

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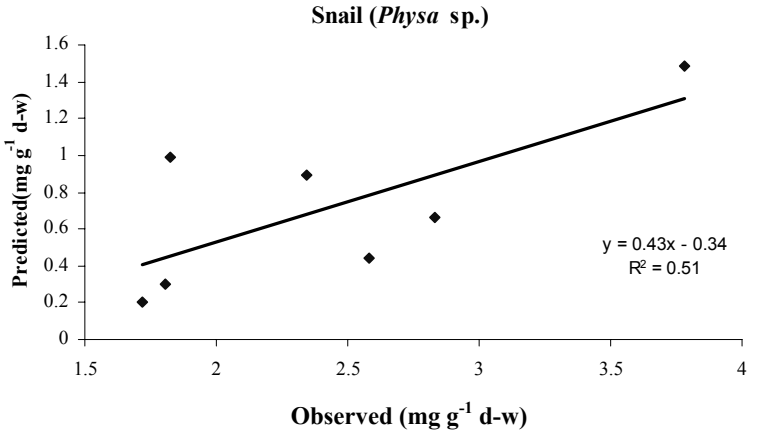

FIG. 4. Relationship between observed and predicted values for the mean cadmium concentration of *(a)* periphyton and *(b)* the snail *Physa* sp. in three replicate channels over 35 days of exposure to cadmium.

than background levels. In constrast, periphyton metal concentrations provide an integrated picture of metal pollution in freshwater environments (Gold et al. 2003).

Concentrations of cadmium in both the periphyton community and in the snail (*Physa* sp.) were more than three orders of magnitude greater than the nominal concentrations in the artificial streams. The average rate of cadmium uptake was 0.05 mg g^{-1} and 0.14 mg g^{-1} per day for periphyton and snail respectively. It is possible that the periphyton community may be able to concentrate metals by active as well as passive uptake, with adsorption by non-biotic segments of the periphyton, while in *Physa* both uptake and depuration are influenced by biological active processes. The apparent lack of depuration in the periphyton, however, may mean that the periphyton community may provide a more realistic representation of spatial and temporal variation in ambient metal concentrations. We believe that the use of artificial substrata to collect periphyton for monitoring metal pollution in streams provides a viable alternative to monitoring protocols which include analysis of metals only in water or in sediments.

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