ORIGINAL ARTICLE

A comparison of metal accumulation by the cnidarian *Hydra vulgaris* directly from water or through contaminated prey and effects upon reproduction and regeneration

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Abstract

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The cnidarian *Hydra* has been widely used to assess the acute toxicity of freshwater pollutants, but very little is known about pollutant accumulation by this animal. The purpose of this study was to measure the accumulation of the three metals, i.e., copper, cadmium and zinc directly from water and also via its prey and to relate the recorded tissue concentrations to any change in biological activities. It was found that copper, cadmium and zinc all were accumulated in the tissues of *Hydra* exposed directly to the metals in water and also those exposed indirectly through feeding on contaminated prey. The bioconcentration factor (BCF) recorded at 48 hours following direct uptake from water was greatest for copper (773), followed by cadmium (409) and zinc (125), although the greatest increase in body burden occurred with cadmium, Metal

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body burdens of *Hydra* fed on contaminated prey (*Artemia*) increased in the same metal sequence as for direct uptake from water and the increase was highest (250 times that of control *Hydra*) for cadmium; however, biomagnification factors (BMFs) were all < 1.0 indicating that there was little potential for increasing accumulation via the food chain. There was significant inhibition of regeneration and bud production in polyps which had fed on cadmium-containing *Artemia* but not on copper or zinc- containing *Artemia*.

Key words : Hydrozoa, pollutant, bioconcentration, bioaccumulation, biomagnification

บทคัดย่อ

วรรณชไม การถนัด[1] และ เดวิด พาสโค[2]

การเปรียบเทียบการสะสมของโลหะในไฮดราจากน้ำโดยตรงหรือจากอาหารที่ปนเปื้อน และผลของโลหะต่อการสืบพันธุ์และงอกใหม่ของไฮดรา

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ไฮดราเป็นสัตว์น้ำจืดขนาดเล็กที่ใช้กันทั่วไปในการทดสอบความเป็นพิษเฉียบพลันที่เกิดขึ้นจากมลพิษในแหล่งน้ำ แต่มีการศึกษาน้อยมากเกี่ยวกับการสะสมทางชีวภาพของสารพิษในไฮดรา การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อตรวจหาการสะสมทางชีวภาพของโลหะ 3 ชนิด ได้แก่ ทองแดง แคดเมียมและสังกะสีที่ได้รับโดยตรงจากน้ำหรือจากอาหารที่ปนเปื้อน อีกทั้งหาความสัมพันธ์ของปริมาณโลหะหนักที่ตรวจพบได้ในเนื้อเยื่อกับการเปลี่ยนแปลงทางชีววิทยาที่เกิดขึ้นในไฮดรา ผลการศึกษาพบทองแดง แคดเมียม และสังกะสี สะสมในเนื้อเยื่อของไฮดราที่ได้รับโลหะโดยตรงจากน้ำและโดยการกินอาหารที่ปนเปื้อนโลหะ ค่าการสะสมทางชีวภาพซึ่งเปรียบเทียบจากความเข้มข้นของโลหะในน้ำและในเนื้อเยื่อ ที่เวลา 48 ชั่วโมงของทองแดง มากกว่าแคดเมียมและสังกะสี โดยมีค่า 773, 409 และ 125 ตามลำดับ อย่างไรก็ตามอัตราการเพิ่มขึ้นของแคดเมียมในเนื้อเยื่อของไฮดราเกิดขึ้นมากที่สุด นอกจากนี้การสะสมโลหะในเนื้อเยื่อของไฮดราหลังจากกินอาหาร (อาร์ทีเมีย) ที่ปนเปื้อนโลหะ มีลำดับจากมากไปน้อยเช่นเดียวกันกับการได้รับจากน้ำโดยตรง โดยเฉพาะแคดเมียมเพิ่มขึ้นมากที่สุด (250 เท่า) เมื่อเปรียบเทียบกับกลุ่มควบคุม อย่างไรก็ตามค่าการสะสมทางชีวภาพที่เปรียบเทียบความเข้มข้นของโลหะในเนื้อเยื่อกับความเข้มข้นในอาหารที่กินเข้าไปจะมีสัดส่วนน้อยกว่า 1 ในโลหะทุกชนิด ซึ่งค่าดังกล่าวบ่งชี้ว่ามีความเป็นไปได้น้อยมากที่โลหะหนักจะสะสมเพิ่มขึ้นตามการถ่ายทอดในห่วงโซ่อาหารที่ซับซ้อนขึ้น นอกจากนี้ไฮดราที่กินอาหารปนเปื้อนแคดเมี่ยมมีผลยับยั้งการสร้างหน่อและการงอกใหม่อย่างมีนัยสำคัญทางสถิติ โดยผลดังกล่าวจะไม่เกิดขึ้นในกรณีที่ปนเปื้อนทองแดงและสังกะสี

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The sedentary cnidarian *Hydra* occurs in many freshwaters (Campbell and Bode, 1983) and is widely used for studying various aspects of animal development, including patterning and cell differentiation (Bosch and Khalturin, 2002), regeneration (Galliot and Schmid, 2002), as well as morphogenesis (Martin *et al*., 1997) and symbiotic relationships (Kessler *et al*., 1988; Karntanut and Pascoe, 2005). It is also employed in assessing the toxicity of freshwater pollutants (Benson and Boush, 1983; Hyne *et al*., 1993; Beach and Pascoe, 1998; Pollino and Holdway, 1999). Traditionally, toxicity has been recorded using mortality as a response criterion, however, a fuller understanding of a chemical's action and potential effects can be gained using sub-lethal indicators such as the ability to regenerate lost tissue (Wilby, 1988; Wilby and Tesh, 1990; Pascoe *et al*., 2003) or to undergo sexual (Pascoe *et al*., 2002) or asexual development (Fukuhori *et al*.,

2005). For some species, investigations of pollutant accumulation into an organism have proved valuable in helping to explain toxicity. For example, phenol toxicity can be correlated to a bioaccumulation threshold in the isopod *Asellus aquaticus* (McCahon *et al*., 1990) and zinc toxicity related to accumulation and depuration rates by the amphipod *Gammarus pulex* (Xu and Pascoe, 1993). Although very small (approximately 4 µg dry weight per animal) *Hydra vulgaris* Pallas 1776 is an important predator in freshwater ecosystems (Taylor *et al*., 1995) and could play a role in the trophic transfer of pollutants, but unfortunately, mainly due to this small size, very little is known about its ability to accumulate pollutants (Santiago-Fandino, 1983; Hyne *et al*., 1992).

There is a vast literature on metal bioaccumulation by fish and invertebrates, but it is generally recognised that bioconcentration factors (BCFs) and biomagnification factors (BMFs) for metals, unlike those for lipophilic organic chemicals, typically decrease with exposure concentration and are of little value in hazard assessment (McGeer *et al*., 2003). Nevertheless, for comparison between metals under the same experimental conditions and at the same exposure time, they can be useful indicators of potential effect.

The aims of this investigation were to compare the accumulation of three important metal pollutants (copper, cadmium and zinc) by *H. vulgaris* directly from water and also via its prey and to relate the recorded tissue concentrations to important biological activities. As well contributing to our understanding of pollutant effects, such information could help determine if transfer of toxicants via this freshwater predator could occur.

Materials and Methods

Source of animals

Hydra vulgaris (Zurich strain, male clone) were cultured in *Hydra* medium (Lenhoff, 1983) within glass aquaria (33×24×21 cm) and fed daily with *Artemia nauplii*. Cultures were kept at a temperature of 20±1°C with a light regime of 16 hours light and 8 hours dark as described previously (Beach and Pascoe, 1998; Karntanut and Pascoe, 2000; 2002).

Test chemicals

Concentrations of each metal used were based on 1/10 of the 48 h LC_{50} determined in a previous acute toxicity study (Karntanut and Pascoe, 2000). This concentration range was selected to ensure that many animals would survive the exposure, and so sublethal toxic effects could be examined yet it was high enough for detectable tissue levels to be attained. The nominal range of toxicant concentrations (copper 5.4 µg/l, cadmium 84 µg/l and zinc 3000 µg/l) was prepared from 100 mg/l stock solutions of copper sulphate ($CuSO_4$. 5 H_2O), cadmium chloride ($CdCl_2$. 2½ H_2O) and zinc sulphate ($ZnSO_4$. 7 H_2O) and made up using *Hydra* medium as diluent.

Direct metal accumulation from water

Approximately 4000 *Hydra* were required for this study because in order to achieve a biomass >1000 µg dry weight 250 animals were needed for each exposure time at each metal concentration. The three metal solutions listed above and a control (*Hydra* medium) were prepared, and toxicity exposures were carried out in 15 ml in each of two 5 cm petri dishes for each metal and the control. Solutions were added to the petri dishes for 24 hours before the start of the test in order to equilibrate and minimise the loss of the test chemical. Approximately 500 *Hydra* polyps were then placed into each of eight dishes. The experiments were performed at 20±1°C in a temperature controlled room with a photoperiod of 16 h light : 8 h dark and continued for 72 hours.

After specific exposure times (10, 48 and 72 hours), 250 animals were collected from the petri dishes for each metal and control, and rinsed with *Hydra* medium to remove any surface test solution which could have confused the results. The samples were dried at 95°C for at least 48 hours before being weighed, digested in Aristar® nitric acid and analysed, as described later, so that metal bioconcentration over 72 hours could be determined. At each exposure time, water samples were

taken to measure the actual metal concentrations and water quality (pH, total hardness, DO and conductivity).

Indirect metal accumulation from food.

Preparation of contaminated prey as a food source

Newly hatched brine shrimp, *Artemia* were used as a prey organism for *Hydra*. They were contaminated by 24 hours exposure to nominal metal concentrations of 10 mg/l copper, cadmium or zinc in artificial seawater. This high concentration was used to be sure that the *Artemia* would acquire sufficient metal to act as contaminated prey. A further batch of *Artemia* was maintained in seawater as a control food source. Exposure solutions were analysed for actual metal concentrations as were samples of *Artemia* after 24 hour metal exposure and the *Artemia* maintained as controls in sea water. Data were used to calculate the metal accumulation by *Artemia*.

Feeding of Hydra on contaminated food

Approximately 1600 *Hydra* were required for three separate experiments. Four hundred *Hydra* used for each copper, cadmium, zinc and control feeding exposure were placed in petri dishes containing *Hydra* medium. They were fed daily for five days with excess contaminated (Cu, Cd or Zn) *Artemia* previously rinsed thoroughly five times with *Hydra* medium before use to avoid the additional transfer of metal solution with the food. The control group was fed with uncontaminated *Artemia*. *Hydra* medium was renewed daily with fresh solution after removal of the uningested *Artemia* in order to reduce the likelihood of metal leaching from contaminated food to the water.

Metal biomagnification by Hydra from contaminated Artemia

After five days of feeding on contaminated prey, about 250 *Hydra* were randomly selected for each metal and rinsed with *Hydra* medium.The *Hydra* were then weighed and oven dried and the metal concentrations were determined as described later. Water used to rinse the *Artemia* before they were fed to *Hydra* was also analysed for metal, to confirm that this was not the source of contamination, as was the *Hydra* medium in the petri dishes. Data were used to calculate the concentration of metal after five days exposure compared to control *Hydra*. Concentrations in *Hydra* were also related to metal concentrations in the food (*Artemia*) to determine the extent of biomagnification.

Effect of metal accumulation via contaminated Artemia on polyp regeneration.

During the five days feeding regime described above, 10 *Hydra* were taken on five consecutive days for each metal and transferred to another petri dish containing *Hydra* medium. They were rinsed thoroughly with *Hydra* medium and allowed to relax before the digestive region was excised (Wilby, 1988; Pascoe *et al*., 2002) giving a total of 50 isolated digestive regions (10 per day) for each metal (Cu, Cd and Zn) and control. Individual digestive regions (i.e. polyps lacking a hypostome, tentacles and foot) were then placed into a glass vial containing 3 ml of *Hydra* medium in order to observe the effect of metal accumulation from contaminated food on regeneration. Regeneration was assessed as described by Wilby (1988) at 24, 48 and 72 hours with a score of 0-10 assigned depending on the degree of regeneration. This experiment was conducted at 20±1°C in a temperature controlled room with 16 h light: 8 h dark.

Effects of metal accumulation via contaminated food on Hydra bud production.

At the end of the five day feeding regime described above, a further ten *Hydra* were taken for each metal and individually placed in a glass vial containing 3 ml of *Hydra* medium. Five Cu, Cd or Zn-contaminated *Artemia* were used daily to feed the *Hydra*. The controls were fed on non-contaminated *Artemia*. The study continued for one month, at 20±1°C with 16 h light: 8 h dark, and solutions were renewed daily after feeding. New *Hydra* buds were recorded throughout the test and morphological changes were also noted.

Water quality and toxicant analysis

Water quality (pH, hardness, dissolved oxygen and conductivity) of *Hydra* medium was monitored throughout the experiment. To determine the *Hydra* and *Artemia* metal concentrations, dried tissue in a glass vial was digested in 0.1 ml of Aristar® nitric acid in a thermostat block (60°C) over night until the solution was clear. The final volume was made up to 1 ml with double distilled water in a volumetric flask and analysed for actual metal concentration by Inductively Coupled Plasma Mass Spectrometry (ICPMS). All samples of test solution and the *Hydra* medium used for rinsing were analysed in the same way. In view of the small size of the animals (approximately 4 ug each), a large number were needed to provide enough tissue for accurate metal analysis, and so to maximise the potential to detect differences between metals at different exposure times as many animals as possible were used in single analyses for metal content rather than using replicates. The accuracy of metal analysis was confirmed using certified reference tissue (reference mussel GBW 08571).

Data analysis and evaluation

Data which were normally distributed (Shapiro-Wilk test) and homogenous (Bartlett's χ^2 test) were analysed by one way ANOVA. Data which were not normally distributed were analysed by the non-parametric Kruskal-Wallis and Mann-Whitney tests. All tests were carried out using Minitab software (version 13).

The bioconcentration factor (BCF) was calculated as:

$$\frac{\text{(metal conc. of exposed } \textit{Hydra} \text{ µg/ g dw) - (metal conc. of control } \textit{Hydra} \text{ µg/ g dw)}}{\text{(metal concentration in water µg/ ml)}}$$

and the biomagnification factor (BMF) as:

$$\frac{\text{(metal conc. of fed } \textit{Hydra} \text{ µg/ g dw) - (metal conc. of control } \textit{Hydra} \text{ µg/ g dw)}}{\text{(metal concentration in } \textit{Artemia} \text{ µg/ g dw)}}$$

Results

Water quality parameters measured through-out the investigation were pH (7.5±0.24), total hardness as $CaCO_3$ (151.6±53.4 mg/l), DO (9.3± 0.14 mg/l) and conductivity (449±43.8 µS/cm). For quality assurance purposes, measured and certified values of reference standard tissue for copper, cadmium and zinc (GBW 08571) were compared. The measured and certified (mean±SD) values, respectively, were for copper (10.5 and 7.7±0.9) µg/g dry weight, for cadmium (4.0 and 4.5±0.5) µg/g dry weight and for zinc (121.1 and 138±0.9) µg/g dry weight.

Direct metal accumulation from water

All data analyses were based on the actual toxicant concentration. The mean (±SD) concentrations of copper, cadmium and zinc to which 1000 *Hydra* were exposed were 110 (±0.1), 77 (±0.1) and 2500 ± (0.3) µg/l, respectively, i.e., higher than intended for copper but close to nominal for cadmium and zinc. The mean concentrations (±SD) of copper, cadmium and zinc recorded in non-exposed control *Hydra* were 23.7 (±0.1), 0.8 (±0.1) and 338.2 (±0.1) µg/g dry weight, respectively. Accumulation of metals by *Hydra* was observed to increase over 72 hours, (48 hours for copper) (Figure 1) and bioconcentration factors of 773 for copper, 409 for cadmium and 125 for zinc were recorded at 48 hours (Figure 2). At this time the body burdens had increased by factors of 3.6 (Cu), 38.9 (Cd) and 0.9 (Zn) compared to control *Hydra*.

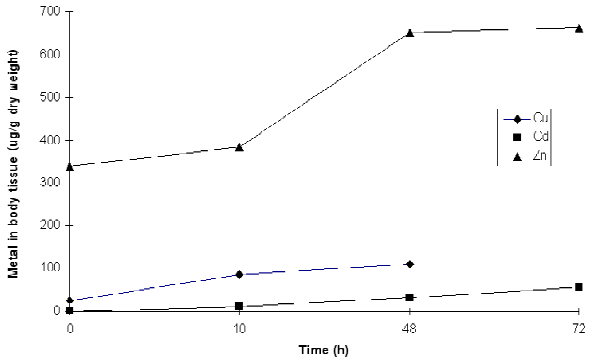

Figure 1. Bioconcentration of copper, cadmium and zinc by *H. vulgaris* (Zurich) during 72 hours exposure. Time zero shows the metal concentration in control *Hydra*.

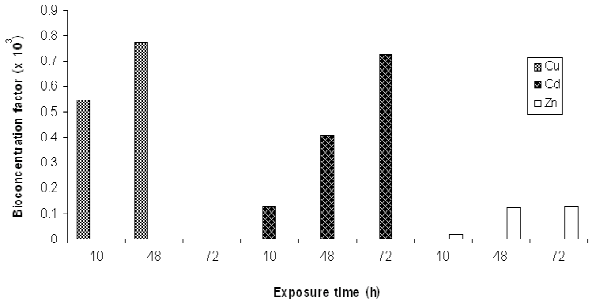

Figure 2. A comparison of bioconcentration factors for copper, cadmium and zinc at different exposure times.

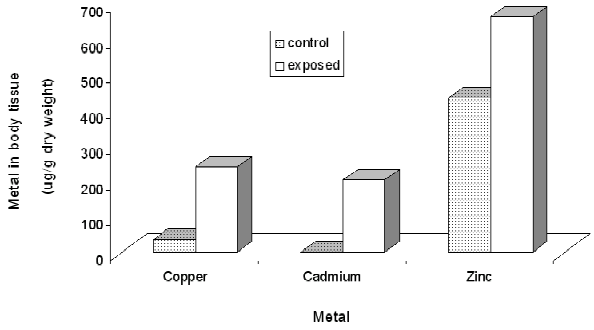

Figure 3. Concentrations of copper, cadmium and zinc in *H. vulgaris* after feeding on metal-contaminated or control *Artemia* for five days.

Indirect metal accumulation from food.

Preparation of contaminated prey as a food source.

The concentrations of copper (11.07 mg/l), cadmium (11.28 mg/l) and zinc (10.30 mg/l) to which the *Artemia* were exposed for 24 hours were close to the nominal concentration (10 mg/l). Metal concentrations in *Artemia* maintained under control conditions were 11.2 (Cu), 0.2 (Cd) and 248.1 (Zn) µg/g dry weight, while the metal concentrations of Cu, Cd and Zn-contaminated *Artemia* after 24 h exposure were 756, 302 and 2385 µg/g dry weight reflecting BCFs of 0.07, 0.027 and 0.21, respectively.

Metal biomagnification by Hydra from contaminated Artemia

The accumulation of metals in *Hydra* via contaminated food (*Artemia*) was measured after five days. Copper, cadmium and zinc concentrations in the *Hydra* medium throughout the feeding experiment were relatively low at 1.8, 0.1 and 376 µg/l compared to those in contaminated *Artemia*. Water used to rinse the *Artemia* before they were fed to *Hyd*ra was also low in copper, cadmium and zinc, with concentrations of 2.5±0.7; 0.3±0.4 and 39.4±0.2 µg/l, respectively, in the fifth rinsing solution. Feeding on Cu, Cd and Zn-contaminated *Artemia* resulted in elevated metal concentrations in *Hydra* compared to control *Hydra* feeding on non-contaminated *Artemia* (Figure 3), with body burdens increased by factors of 5.6 (Cu), 250 (Cd) and 0.5 (Zn). Biomagnification factors relative to the metal concentrations in the contaminated prey (Figure 4) were < 1.0 for all metals.

Effect of metal accumulation via contaminated Artemia on polyp regeneration.

All control isolated digestive regions, from polyps which had been fed on non-contaminated food for 1-5 days, achieved full development with a hypostome, tentacles and foot (score 10) over a 72-h period of regeneration (Figure 5). Digestive regions from *Hydra* feeding on Cu or Zn-contaminated *Artemia* for up to five consecutive days also completely regenerated. In contrast,

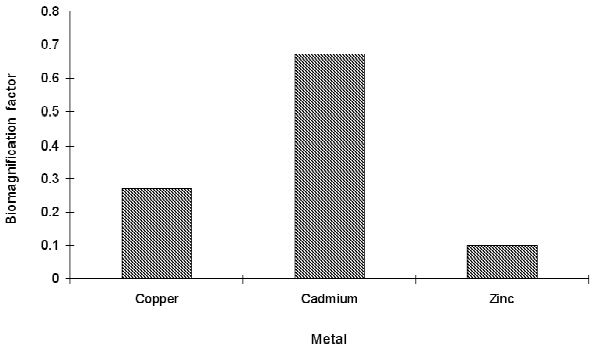

Figure 4. Biomagnification factor for copper, cadmium and zinc in *H. vulgaris* feeding for five days on metal-contaminated *Artemia*.

regeneration was inhibited in the group feeding on Cd-contaminated *Artemia,* with only those digestive regions from polyps which had fed for one day achieving full regeneration. Those isolated following two days feeding on contaminated *Artemia* reached median score 9 after 72 hours, but for those isolated following 3-5 days feeding there was no regeneration (non parametric Kruskal-Wallis $p<0.05$).

Effects of metal accumulation via contaminated food on Hydra bud production.

All control *Hydra* survived over the 1-month period of this experiment. The *Hydra* feeding daily on Cu or Zn-contaminated *Artemia* also survived, in contrast to those feeding on Cd-contaminated *Artemia* for which increasing mortality was observed until at the end of the one month study, 60% remained alive. Even taking this mortality into account the mean number of buds produced within one month for *Hydra* feeding on Cd-contaminated *Artemia* was significantly less $p<0.001$ (one-way ANOVA) than for *Hydra* feeding on Cu or Zn-contaminated *Artemia* and those feeding on non-contaminated *Artemia* (Figure 6).

Morphological changes in *Hydra* over one month were also observed. *Hydra* polyps feeding on Cu or Zn-contaminated *Artemia* showed slight changes compared to the control, for example tentacles were partially contracted and reactions were slow, whereas in control *Hydra* the tentacles and body remain extended and active (Figure 7a). The effect was greater on *Hydra* fed on Cu-contaminated *Artemia* than zinc. However, major damage was observed in *Hydra* feeding on Cd-contaminated *Artemia*. (Figure 7 b-d), with greater effects seen in those polyps fed on contaminated prey for longer periods. Effects were mainly seen in the body column with constrictions, splitting between the constricted areas and eventual degeneration below the hypostome leaving an isolated tentacle ring (Figure 7 b-d).

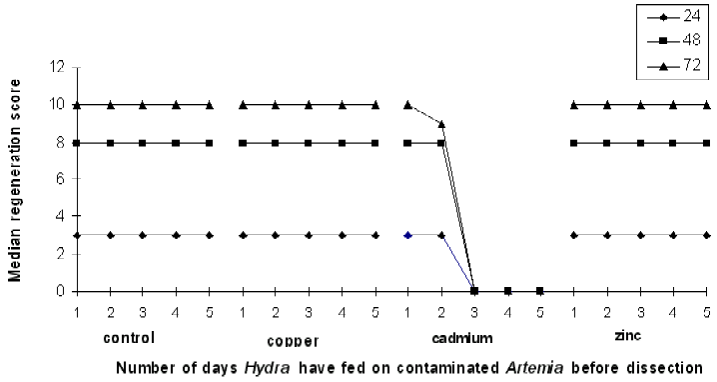

Figure 5. A comparison of median regeneration scores at 24, 48 and 72 hours for *H. vulgaris* digestive regions following 1-5 days feeding on copper, cadmium and zinc contaminated *Artemia* or on non-contaminated *Artemia*.

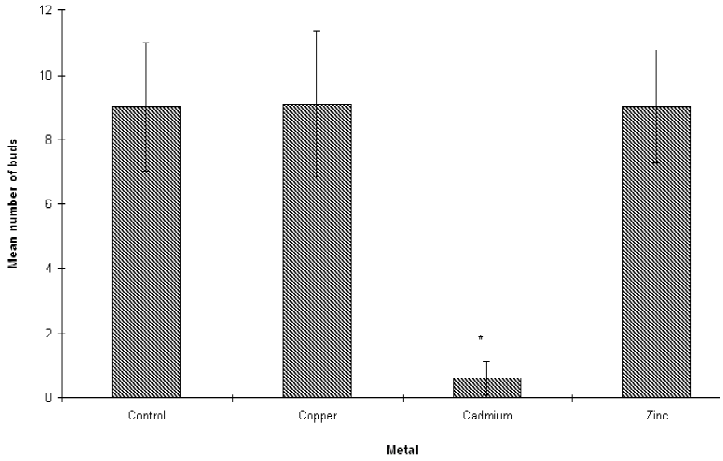

Figure 6. Mean (SD) number of buds produced by *H. vulgaris* polyps during 1 month while feeding on metal-contaminated *Artemia* (* indicates significant difference p<0.001 oneway ANOVA from control polyps feeding on non-contaminated *Artemia*).

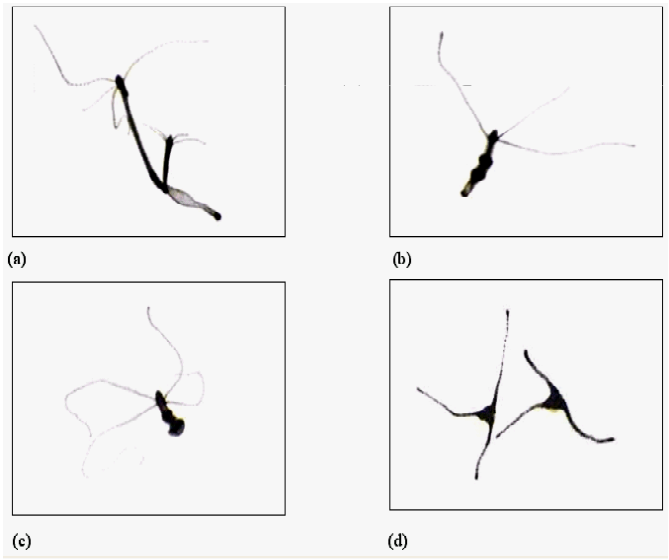

Figure 7. Morphological damage seen in *Hydra* polyps feeding on cadmium contaminated *Artemia*. Control animals show extended tentacles and an active body (a) Cadmium exposed animals have the tentacles and body slightly contracted and body constricted (b), the body become more contracted and constricted (c), then splits and degenerates but the tentacle ring is still intact (d).

Discussion

Several workers have reported on the acute toxicity of metals to a variety of *Hydra* species (Pyatt and Dodd, 1986; Pollino and Holdaway, 1999; Holdaway *et al*., 2001; Karntanut and Pascoe, 2000; 2005), and in a study with four different *Hydra*, relative metal toxicity decreased from copper to cadmium and with zinc the least toxic for all species (Karntanut and Pascoe, 2002). Metal uptake by freshwater invertebrates may occur at the body surface and via the gut. However, we

are not aware of any detailed investigations of metal accumulation by *Hydra* although Santiago-Fandino (1983), using radioisotope tracers, attempted to relate nickel accumulation by *H. littoralis* to growth rate. Hyne *et al.* (1992) detected uranium in the discharged nematocysts of *H. viridissima* following exposure to water from a uranium mine and noted a reduced ability of these animals to capture live prey.

In the current study copper, cadmium and zinc were all found to be present in the tissues of *Hydra* exposed directly to the metals in water and indirectly through feeding on contaminated prey. Direct accumulation from water increased with time, but within the relatively short exposure period (72 hours) there was no indication of a plateau concentration which would have suggested a balance between uptake and depuration rates (Xu and Pascoe, 1993, 1994; Shuhaimi-Othman and Pascoe, 2003) as seen with many organic substances. The bioconcentration factor (BCF) recorded at 48 hours was greatest for copper (773), followed by cadmium (409) and zinc (125) although the greatest increase in body burden occurred with cadmium. Regulation of metals within animals is an integration of the processes of absorption, metabolism, excretion and storage, and in some aquatic invertebrates has been reported to be associated with metallothioneins. However, there was no evidence for regulation in this investigation and metal binding proteins or metallothioneins (Andersen *et al*., 1988) are reported to be absent from *Hydra*. Santiago-Fandino (1983) demonstrated that *Hydra* was unable to control the accumulation of nickel and no release of the metal occurred.

When metal-contaminated *Artemia* were used as an indirect source of pollutant by feeding them to *Hydra* for five days, metal concentrations increased in test *Hydra* compared to control animals fed on non-contaminated prey. Body burdens increased in the same metal sequence as for direct accumulation from water, with the greatest increase (250 times that of control *Hydra*) for cadmium. The relative difference in the accumulation of heavy metals from water and from food in freshwater invertebrates has been studied in several aquatic species including *Asellus aquaticus* (Van Hattum *et al*., 1989), *Daphnia magna* (Carney *et al*., 1986) and *Chironomus riparius* (Timmermans *et al*., 1992). Xu and Pascoe (1994) reported that the amphipod *Gammarus pulex* was able to regulate its total body zinc level and obtained most of the metal directly from water rather than from food.

Species that contain bioaccumulated chemicals could be another source of pollutants for predators, thus making pollutants available for higher trophic levels. *Hydra* plays an important part as a predator in the freshwater food chain feeding on *Daphnia*, copepods and other small organisms. Although previous investigators have demonstrated that some aquatic organisms accumulate metals through the food, as well as from ambient water, the study of trace metal effects on invertebrate predator species are limited (Brown and Pascoe, 1988). Hence, it is important to investigate heavy metal accumulation in a predator such as *Hydra*; however, this study actually revealed that biomagnification factors (BMFs) were all < 1.0, indicating that there was no potential, in these circumstances, for increasing accumulation via the food chain.

The ability of dissected polyps to regenerate missing hypostome, tentacles and foot after feeding for up to five days on prey containing high levels of copper and zinc was not inhibited and occurred at the same rate as seen in control animals fed on non-contaminated prey. However, there was significant inhibition of regeneration in polyps which had fed on cadmium-containing *Artemia*. It is well documented that substantial amounts of cadmium have a toxic effect on many aquatic organisms. For example, in *Hydra littoralis*, it has been reported by Santiago-Fandino (1983) that nickel and cadmium affected the mean specific growth rate. The insect *Chironomus*, after long term exposure to a sublethal concentration of cadmium, showed significantly impaired development of first and second instars (Timmermans *et al*., 1992) and adult emergence (Pascoe *et al*., 1989). In *Daphnia* exposed to cadmium, re-

production and growth were impaired (Allen *et al*., 1995).

In a longer term assessment of asexual reproduction, cadmium was again seen to have a damaging effect with polyps fed for > 1 month on contaminated prey producing significantly less buds than control polyps or those feeding on copper or zinc contaminated *Artemia*. These polyps also suffered major structural damage to the main body column. *Hydra* species have been shown to be sensitive to both metal and organic pollutants regarding bud production (Stebbing and Pomroy, 1978; Pollino and Holdway, 1999). The number of buds produced within a month in the present study was markedly affected and may be attributed to high cadmium accumulation, whereas no significant difference ($p>0.05$) was seen for copper, zinc and control groups. Additionally, tests with a long exposure (one-month) indicated that cadmium accumulation via contaminated food was very damaging to *Hydra*. Some mortality occurred after six days feeding on Cd-contaminated *Artemia*, while those feeding on Cu and Zn-contaminated *Artemia* survived for the entire test period. The pattern of morphological changes indicated considerable damage to the body column and may be attributed to the penetration of cadmium through the gastrovascular cavity. Non-essential uranium accumulation in *Hydra* has been reported to cause reduced population growth and a feeding dysfunction (Hyne *et al*., 1993). In contrast, when *Hydra* were exposed for a short time to an inorganic lead compound at various concentrations stimulation of growth as measured by the rate of bud production actually occurred (Brown and Davis, 1977).

Conclusions

Copper, cadmium and zinc all accumulated in the tissues of *Hydra* exposed directly to the metals in water and indirectly through feeding on contaminated prey. The BCF recorded at 48 hours following direct uptake from water was greatest for copper, followed by cadmium and zinc, but the greatest increase in body burden was seen to occur with cadmium. When *Hydra* were fed on contaminated prey, the body burdens increased in the same metal sequence as for direct uptake from water and again the increase was greatest for cadmium. BMFs were all < 1.0 so that there would seem to be no potential for increasing accumulation via the food chain. There was significant inhibition of regeneration and bud production in polyps which had fed on cadmium-containing *Artemia* but not on copper or zinc- containing *Artemia*. This demonstration that three important metal pollutants (copper, cadmium and zinc) accumulate in the tissues of *H. vulgaris* following uptake from water and food contributes to our understanding of toxic effects on biological function and the potential for toxicant transfer via the food chain.

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References

Allen, Y., Calow, P. and Baird D.J. 1995. A mechanistic model of contaminant induced feeding inhibition in *Daphnia magna*. Env. Tox. Chem. 14(9): 1625-1630

Andersen, R.L., Wiger, W., Daae, H.L. and Eriksen, K.D.H. 1988. Is the metal binding protein metallothionein present in the coelenterate *Hydra attenuata*? J. Comp. Physiol. 91C(2): 555-557.

Beach, M.J. and Pascoe, D. 1998. The role of *Hydra vulgaris* (Pallas) in assessing the toxicity of freshwater pollutants. Wat. Res. 32: 101-106.

Benson, B. and Boush, G.M. 1983. Effect of pesticides and PCBs on budding rates of green *Hydra*. Bull. Environ. Contam. Toxicol. 30: 344-350.

Bosch, T.C.M. and Khalturin, K. 2002. Patterning and cell differentiation in *Hydra*: novel genes and the limits to conservation. Can. J. Zool. 80: 1670-1677.

Brown, C.L. and Davis, L.E .1977. Cellular mechanisms of stimulation of bud production in *Hydra*

by low levels of inorganic lead compounds. Cell. Tiss. Res. 177: 555-570.

Brown, A.F. and Pascoe, D. 1988. Studies on the acute toxicity of pollutants to freshwater macroinvertebrates: 5. The acute toxicity of cadmium to twelve species of predatory macroinvertebrates. Archiv. Fur. Hydrobiol. 114: 311-319.

Campbell, R.D. and Bode, H.R. 1983. Terminology for Morphology and cell types **In**: Lenhoff HM (Ed.), *Hydra* Research Methods. Plenum Press, New York, pp. 5-14.

Carney, G.C. Shore, P. and Chandra, H. 1986. The uptake of cadmium from a dietary and soluble source by the crustacean *Daphnia magna*. Environ. Res. 39: 290-298.

Fukuhori, N., Kitano, M. and Kimura, H. 2005. Toxic effects of Bisphenol A on sexual and asexual reproduction in *Hydra vulgaris*. Arch. Environ. Contam. Toxicol. 48: 495-500.

Galliot, B. and Schmid, V. 2002. Cnidarians as a model system for understanding evolution and regeneration. Int. J. Dev. Biol. 46: 39-48.

Holdaway, D., Lok, K. and Semann, M. 2001. The acute and chronic toxicity of cadmium and zinc to two *Hydra* species. Environ. Toxicol. 16: 557-565.

Hyne, R.V., Rippon, G.D., White, J. and Ellender, G. 1992. Accumulation of uranium by freshwater hydra into discharged nematocysts. Aquatic Toxicol. 23: 231-246.

Hyne, R., Rippon, G.D. and Ellender, G. 1993. Investigation of uranium - induced toxicity in freshwater *Hydra*. **In**: Dallinger R and Rainbow P (Eds.), Ecotoxicology of Metals in Invertebrates. CRC press, Florida.

Karntanut, W. and Pascoe, D. 2000. A comparison of methods for measuring acute toxicity to *Hydra vulgaris*. Chemosphere. 41:1543-1548.

Karntanut, W. and Pascoe, D. 2002. The toxicity of copper, cadmium and zinc to four different *Hydra*. Chemosphere. 47: 1059-1064

Karntanut, W. and Pascoe, D. 2005. Effects of removing symbiotic green algae on the response of *Hydra viridissima* (Pallas 1776) to metals. Ecotox. Environ. Saf. 60: 301-305.

Kessler, E., Huss, V.A.R. and Rahat, M. 1988. Species-specific ability of *Chlorella* strains (Chlorophyceae) to form stable symbioses with *Hydra* viridis. Plant. Syst. Evol. 160: 241-246.

Lenhoff, H.M. 1983. Culturing large numbers of *Hydra*. **In**: Lenhoff HM (Ed.), *Hydra* Research Methods. Plenum Press, New York, pp 53-62

Martin, V.J., Littlefield, C.L., Archer, W.E. and Bode, H.R. 1997. Embryogenesis in *Hydra*. Biol. Bull. 192:345-363.

McCahon, C.P., Barton, S.F. and Pascoe, D. 1990. The toxicity of phenol to the freshwater crustacean *Asellus aquaticus* (L) during episodic exposure - Relationship between sub-lethal responses and body phenol concentrations. Arch. Env. Cont. Tox. 19: 926-929.

McGeer, J.C., Brix, K.V., Skeaff, J.M., DeForest, D.K., Brigham, S.I., Adams, W.J. and Green, A. 2003. Inverse relationship between bioconcentration factor and exposure concentration for metals: Implications for hazard assessment of metals in the aquatic environment. Environ. Toxicol. Chem. 22: 1017-1037.

Pascoe, D., Karntanut, W. and Muller, C.T. 2003. Do pharmaceuticals affect freshwater invertebrates? A study with the cnidarian *Hydra vulgaris*. Chemosphere 51: 521-528.

Pascoe, D., Williams, K.A. and Green, D.W.J. 1989. Chronic toxicity of cadmium to *Chironomus riparius* (Meigen) - effects upon larval development and adult emergence. Hydrobiologia 175: 109-115.

Pascoe, D., Carroll, K., Karntanut, W. and Watts, M.M. 2002. Toxicity of 17α-ethinylestradiol and Bisphenol A to the freshwater cnidarian *Hydra vulgaris*. Arch. Environ. Contam. Toxicol. 43: 56-63

Pollino, C.A. and Holdway, D.A. 1999. Potential of two *Hydra* species as standard toxicity test animals. Ecotox. Environ. Saf. 43:309-316.

Pyatt, F.B. and Dodd, N.M. 1986. Some effects of metal ions on the freshwater organisms, *Hydra oligactis* and *Chlorohydra viridissima*. Indian J Exp Bio. 24: 169-173.

Santiago-Fandini, V.J.R. 1983. The effects of nickle and cadmium on the growth rate of *Hydra littoralis* and an assessment of the rate of uptake of ^{63}Ni and ^{14}C by the same organism. Wat. Res. 8: 917-923.

Shuhaimi-Othman, M. and Pascoe, D. 2003. Bioconcentration of the heavy metals copper, cadmium and zinc by the freshwater amphipod *Hyalella azteca*. Malays. Appl. Biol. 32: 1-8.

Stebbing, A.R.D. and Pomroy, A.J. 1978. A sublethal technique for assessing the effects of contaminants using *Hydra littoralis*. Wat. Res. 12: 631-635.

Taylor, E.J., Morrison, J.E., Blockwell, S.J., Pascoe, D. and Tarr, A. 1995. Effects of lindane on the predator-prey interaction between *Hydra oligactis* Pallas and *Daphnia magna* Strauss. Arch. Environ. Contam. Toxicol. 29: 291-296.

Timmermans, K.R., Peeters, W. and Tonkes, M. 1992. Cadmium, zinc, lead and copper in *Chironomus riparius* (Meigen) larvae (Diptera, Chironomidae): uptake and effects. Hydrobiol. 242: 119-134.

Van Hattum, B., De Voogt, P. and Van Den Bosch, L. 1989. Bioaccumulation of cadmium by the freshwater isopod *Asellus aquaticus* (L.) from aqueous and dietary sources. Environ. Pollut. 62: 129-151.

Wilby, O.K. 1988. The *Hydra* regeneration assay. Proceedings of workshop organised by Association Francaise de Teratologie. pp. 108-124.

Wilby, O.K. and Tesh, J.M. 1990. The *Hydra* assay as an early screen for teratogenic potential.Toxicol. In Vitro. 4/5: 582-583.

Xu, Q. and Pascoe, D. 1993. The bioconcentration of zinc by *Gammarus pulex* (L.) and the application of a kinetic model to determine bioconcentration factors. Water Research 27: 1683-1688.

Xu, Q. and Pascoe, D. 1994. The importance of food and water as sources of zinc during exposure of *Gammarus pulex* (Amphipoda). Arch. Env. Cont. Toxicol. 26: 459-465