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Multi-metal interactions between Cd, Cu, Ni, Pb and Zn in water flea *Daphnia magna*, a stable isotope experiment

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ABSTRACT

Metal interaction effects were investigated in *Daphnia magna* during a simultaneous exposure to essential (Cu, Ni and Zn) and non-essential (Cd and Pb) metals at environmentally relevant concentrations using a stable isotope technique. The metals were applied in the following concentration ranges: 0.0125–0.2 μM for ^{106}Cd, 0.025–0.25 μM for ^{65}Cu and ^{204}Pb, 0.1–1.25 μM for ^{62}Ni and ^{67}Zn. Cadmium and copper exhibited a suppressing effect on the uptake rates of all other metals present in the mixture with the exception to lead at all studied concentrations. The effect was already pronounced at low Cd and Cu concentrations and reached a maximum at the higher concentrations. Nickel and zinc showed weaker interactions with cadmium and between each other, while having no effect on copper and lead uptake. There was a high degree of correlation between Cd, Ni and Zn uptake rates indicating that these metals share in part common uptake or interaction pathways. Moreover, a significant correlation between Zn and Cu uptake processes suggests that more than one mechanism is involved in Zn accumulation since Cu is known to interact with Na uptake sites. The uptake of lead was marked by a high initial rate, but the uptake process reached saturation within 24 h. Cd applied at a concentration of 0.2 μM was the only metal which affected the lead uptake process by stimulation of the Pb uptake. Added to the medium at a concentration of 0.25 μM, lead in turn, increased copper uptake. Current work illustrates that metal interactions are significant and occur at low environmentally realistic concentrations affecting bioavailability of both toxic and essential metals.

1. Introduction

Natural waters are frequently contaminated by trace metals as a result of human activities. In such conditions aquatic organisms are often exposed to a mixture of metals rather than a single element (More and Ramamoorthy, 1984). Some of these trace metals, such as Cu and Zn, play an important role in cellular metabolism and their body concentrations can be regulated by the organisms. Others, such as Ag, Cd and Pb, are toxic even at low concentrations and tend to accumulate in the body (Rainbow, 1997, 2002). Essential and non-essential metals may, however, share common uptake routes and interact with each other affecting uptake, bioaccumulation and toxicity. The results of such interactions are highly variable ranging from antagonism to synergism depending on the metal, its external concentration and exposure scenario, length of exposure, studied species and examined organs (Amiard-Triquet and Amiard, 1998; Norwood et al., 2003). Evidence exists that in crustaceans Cd and Zn are taken up by the same transport pathway. However, the type of interaction varies showing both positive, negative or no interaction and is species-dependent (Rainbow et al., 2000). For example, zinc additions reduced cadmium toxicity in the amphipod *Corophoum volutator* (Bat et al., 1998), while increased toxicity was observed in the water flea *Daphnia magna* and the shrimp *Callianassa australiensis* during exposure to Cd–Zn mixtures compared to individual metals (Biesinger et al., 1986; Negilski et al., 1981). Synergetic effects of metals interactions in Cu–Cd and Cu–Pb mixtures have also been reported for fish (Pelgrom et al., 1995; Tao et al., 1999).

At present, the BLM models, which aim to predict dissolved metal toxicity to aquatic organisms as a function of external metal concentration and water chemistry characteristics, are used for setting site-specific water quality criteria. However, these models have been developed for single metals and do not take into account possible metal interactions. Nevertheless, numerous studies indicate the necessity of such consideration and incorporation of metal interactions is an important future task (Paquin et al., 2002).

The present study is aimed to investigate the interactive effects of Cd, Cu, Ni, Pb and Zn in *D. magna*, a standard organism for toxicity testing, during simultaneous exposure to these metals at low concentrations. The number of studies investigating the

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effect of metal interactions on trace metal uptake and toxicity in *D. magna* is limited. Contradictory results on toxicity of Cd–Zn mixtures have been reported in several studies. In particular, Shaw et al. (2006) found no interactions between Cd and Zn in *D. magna*, although increasing cadmium concentrations decreased the toxicity of Cd–Zn mixtures in other *Daphnia* species (*D. ambigua*, *D. pulex* and *C. dubia*). Similarly, Jak et al. (1996) examined the effects of simultaneous exposure to As, Cd, Cr, Cu, Hg, Ni, Pb and Zn on population density of *D. magna* and observed no interactions among metals. In contrast, Canizares-Villanueva et al. (2000) observed an antagonistic effect of zinc on cadmium toxicity in *D. magna*. Another study performed by Barata et al. (2002) showed increased cadmium tolerance of three *D. magna* clones induced by exposure to elevated zinc concentrations. In this work we have studied the effects of metal interactions during a multi-metal exposure at environmentally relevant concentrations on metal uptake rates using a stable isotope technique that allows direct measurement of newly accumulated metal by ICP-MS. The choice of exposure concentrations was justified by current Flemish water quality criteria set to 1 $\mu g\,L^{-1}$ Cd, 50 $\mu g\,L^{-1}$ Cu, 30 $\mu g\,L^{-1}$ Ni, 50 $\mu g\,L^{-1}$ Pb and 200 $\mu g\,L^{-1}$ Zn (Flemish Government, 2000) and concentrations of these metals in natural water systems in Flanders (Belgium), which vary between 0.1–12.2 $\mu g\,L^{-1}$ Cd, 1–30.1 $\mu g\,L^{-1}$ Cu, 2.7–19.8 $\mu g\,L^{-1}$ Ni, 0.1–1 $\mu g\,L^{-1}$ Pb and 47–2168 $\mu g\,L^{-1}$ Zn (Bervoets and Blust, 2003; Reynders et al., 2008).

2. Materials and methods

2.1. Culturing conditions

The water flea *D. magna* was cultured under controlled laboratory conditions in 1 L polypropylene aquaria. The soft aerated freshwater was used as a rearing medium and had the following composition: 0.5 mM $CaCl_2{\cdot}2H_2O$, 0.5 mM $MgSO_4{\cdot}7H_2O$, 2 mM NaCl and 0.077 mM KCl, pH 7. The pH of the medium was controlled by adding a non-complexing Good's biological buffer (1 mM MOPS brought to pH 7 with addition of NaOH) (de Schamphelaere et al., 2004). During a rearing period *Daphnia* were fed a mixture of the green algae *Pseudokirchneriella subcapitata* and *Chlamidomonas reinhardtii* in a 3:1 ratio. Three times a week the medium was renewed and daphnids were fed 400×10^6 algal cells L^{-1}. The culture was maintained at $21 \pm 1\,^{\circ}C$ under a 14 h light:10 h dark cycle. Daphnids, 15–16 days old, from fourth generation were used for the experiments.

2.2. Experimental procedure

All chemicals were purchased from VWR Int. (Leuven, Belgium) and were reagent grade. Stable isotopes were obtained from STB Isotope GmbH, Germany. Ultra pure water (MilliQ, $R > 18.2\,M\Omega$) was used for the preparation of the medium. All uptake experiments were carried out in 750 mL polypropylene containers. The exposure medium was spiked with increasing amounts of ^{106}Cd, ^{65}Cu, ^{62}Ni, ^{204}Pb, and ^{67}Zn according to Table 1. No food was provided to the organisms during a test period. At 0, 24, 48, 72 and 96 h both *Daphnia* and water samples were collected to be analysed for metals. Ten to fifteen animals were collected from each of three replicates and rinsed with MQ. The daphnids were dried at 60 °C to a constant weight and digested with 69% HNO_3 in a microwave. The 20 mL water sample was filtered through a 0.45 μm membrane (NC45, Schleicher & Schüll), acidified to 2% HNO_3 with concentrated (69%) nitric acid and kept in a freezer until the analysis.

Table 1
Exposure concentrations of Cd, Cu, Ni, Pb and Zn used in the study

Condition	Metal
All metals at minimum level	0.0125 μM Cd, 0.025 μM Cu, 0.1 μM Ni, 0.025 μM Pb and 0.1 μM Zn
Cd 0.05	0.05 μM Cd, 0.025 μM Cu, 0.1 μM Ni, 0.025 μM Pb and 0.1 μM Zn
Cd 0.2	0.02 μM Cd, 0.025 μM Cu, 0.1 μM Ni, 0.025 μM Pb and 0.1 μM Zn
Cu 0.05	0.0125 μM Cd, 0.05 μM Cu, 0.1 μM Ni, 0.025 μM Pb and 0.1 μM Zn
Cu 0.25	0.0125 μM Cd, 0.25 μM Cu, 0.1 μM Ni, 0.025 μM Pb and 0.1 μM Zn
Ni 0.25	0.0125 μM Cd, 0.025 μM Cu, 0.25 μM Ni, 0.025 μM Pb and 0.1 μM
Ni 1.25	0.0125 μM Cd, 0.025 μM Cu, 1.25 μM Ni, 0.025 μM Pb and 0.1 μM
Pb 0.1	0.0125 μM Cd, 0.025 μM Cu, 0.1 μM Ni, 0.1 μM Pb and 0.1 μM Zn
Pb 0.25	0.0125 μM Cd, 0.025 μM Cu, 0.1 μM Ni, 0.25 μM Pb and 0.1 μM Zn
Zn 0.25	0.0125 μM Cd, 0.025 μM Cu, 0.1 μM Ni, 0.025 μM Pb and 0.25 μM Zn
Zn 1.25	0.0125 μM Cd, 0.025 μM Cu, 0.1 μM Ni, 0.025 μM Pb and 1.25 μM Zn

Concentrations of major cations were kept constant at all conditions (2 mM Na^+; 0.078 mM K^+; 0.5 mM Ca^{2+}; 0.5 mM Mg^{2+}; pH 7, ionic strength 0.0084 M).

2.3. Analytical and computational procedures

All samples were analysed for metals by inductively coupled plasma mass spectrometry (Varian Expert 700 quadrupole ICP-MS, Mulgrave, Australia). The calibration standards were prepared from a 1000 mg L^{-1} ICP multi-element standard solution IV (Merck) by dilution with 1% (v/v) nitric acid. Yttrium was added to all standards and samples as an internal standard. The machine was recalibrated every 15–20 samples to account for any instrumental drift. At least two isotopes were measured for each metal. Non-tracer isotopes, which were not added to the exposure medium (i.e. ^{111}Cd, ^{63}Cu, ^{60}Ni, ^{64}Zn, ^{208}Pb), were used for background concentration corrections or determination of the concentrations of metals already present in the organism based on known natural abundances of isotopes. For example, to calculate the background concentration of ^{106}Cd, ^{111}Cd was also monitored and the following equation was applied:

$$[^{106}Cd]_{background} = [^{111}Cd]_{sample} \times \left[\frac{\text{abundance } ^{106}Cd}{\text{abundance } ^{111}Cd}\right] \quad (1)$$

$$[^{106}Cd]_{background} = [^{111}Cd]_{sample} \times \left[\frac{1.21}{12.75}\right] \quad (2)$$

Calculated background isotope concentrations of spiked isotopes were subtracted from measured values.

The experimental data were fitted to either linear or hyperbolic function using GraphPad Prism 4 software to estimate the uptake rates. If a simple linear function was fitting the data the best, then the uptake rate was equal to the slope of the corresponding line. In cases where experimental data could be better described by a hyperbolic function of the form

$$C_{org} = \frac{at}{b + t}$$

where C_{org} is a metal concentration inside the organism ($\mu M\,kgdw^{-1}$) and t is time (h), then the initial uptake rate was calculated as a/b. An ANOVA test was used to determine differences in the uptake rates. Since we used three separate incubations for each condition as replicates representing repeated measurements, it was possible to construct individual uptake curves for each repli-