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Uptake and toxicity of Cd, Cu and Pb mixtures in the isopod *Asellus aquaticus* from waterborne exposure

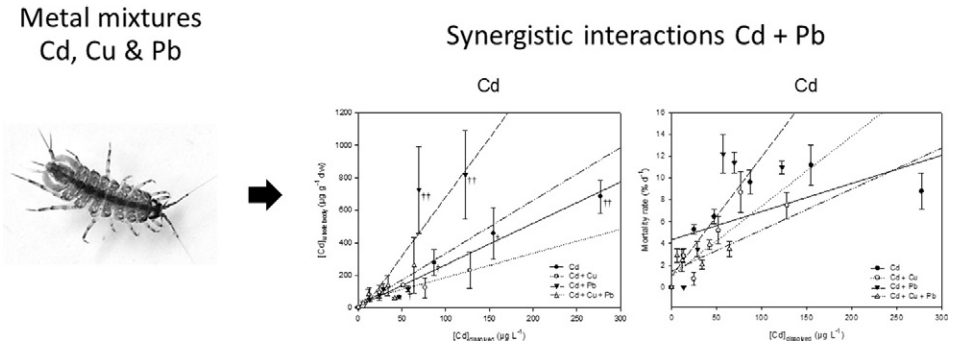
M. Van Ginneken *, M. De Jonge, L. Bervoets, R. Blust

Department of Biology, Systemic Physiological and Ecotoxicological Research (SPHERE), University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

HIGHLIGHTS

- Synergistic interactions of Cd and Pb mixtures on uptake and toxicity were observed.
- Effects of Cd and Pb mixtures on toxicity were related to increased Cd uptake.
- No mixture interactions between Cu and Cd or Pb were observed.

GRAPHICAL ABSTRACT



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ABSTRACT

The present study evaluated interactions of waterborne Cd, Cu and Pb mixtures on metal uptake rates in the isopod *Asellus aquaticus* and related this to mixture effects on toxicity. Secondly, it was assessed whether observed mixture effects were better related to isopod body concentrations compared to exposure concentrations. Isopods were exposed for 10 days to single, binary and tertiary mixtures including five different concentrations of Cd (0.107 to 277 μg L^{-1}), Cu (3.35 to 2117 μg L^{-1}) and Pb (0.782 to 443 μg L^{-1}). Mortality was assessed every day while isopod body concentrations, growth (biomass) and energy reserves (glycogen, lipid and protein reserves) were assessed at the end of the experiment.

Synergistic interactions of combined Cd and Pb exposure on Cd and Pb uptake as well as on growth rates and mortality rates were observed. Mixture effects of combined Cd and Pb exposure on toxicity endpoints were directly related to increased Cd uptake in the Cd + Pb treatment. No mixture interactions of Cu on Cd or Pb uptake (and vice versa), nor on toxicity endpoints were observed. All toxicity endpoints were related to body concentrations. However, mixture effects disappeared when growth and mortality rates were expressed on body concentrations instead of exposure concentrations.

By combining information of mixture effects on metal uptake with mixture toxicity data, the present study provides more insight in the way metal mixtures interfere with aquatic organisms and how they can induce toxic effects.

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1. Introduction

Metal pollution still poses a threat to aquatic ecosystems all over the world and managing ecological risks of metal-contaminated systems

* Corresponding author.
E-mail address: marjolein.vanginneken@uantwerpen.be (M. Van Ginneken).

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remains an important challenge. In the natural environment trace metals most often occur in different mixtures, in which metals can strongly interfere with each other, producing antagonistic, synergistic or additional toxic effects (Norwood et al., 2003; Borgmann et al., 2008).

Besides effects on metal speciation in the medium (Norwood et al., 2013), exposure to metal mixtures encloses different possible biochemical interactions. Firstly, metal mixtures can interact at biotic ligands resulting in competitive, anti-competitive or non-competitive inhibition and/or increase of metal uptake and bioaccumulation (Norwood et al., 2007; Borgmann et al., 2008; Komjarova and Blust, 2008). Secondly, metal mixtures can induce toxicity responses, both directly at the site of toxic action and indirectly by interfering with internal pathways (e.g. detoxification processes) (Norwood et al., 2003; Vijver et al., 2011). Recent reviews on effects of metal mixtures have shown a large variety in combined effects which can differ depending on the metal combinations and their concentrations, the species used and the endpoint measured (Weltje, 1998; Norwood et al., 2003; Vijver et al., 2011). For example, the study of Franklin et al. (2002) observed additive responses for bioaccumulation, but synergistic responses for growth after exposing the freshwater alga *Chlorella* sp. to Cd–Cu mixtures. The latter illustrates the need for metal mixture studies combining interactions at metal uptake and bioaccumulation with toxicity data, in order to reveal at which level interactions of metal mixtures are occurring. To date, only few studies have related metal mixture influences on uptake and accumulation to toxicity effects, especially for aquatic invertebrates (but see Franklin et al., 2002 and Abboud and Wilkinson, 2013 for algae; and Birceanu et al., 2008 for fish). Furthermore, mortality has been most frequently used as an endpoint to study metal mixture interactions, which is rather drastic and requires high exposure concentrations, while sublethal endpoints such as growth remain only poorly documented (Vijver et al., 2011).

The aim of the present study was first to evaluate interactions of waterborne Cd, Cu and Pb mixtures on metal uptake rates in the isopod *Asellus aquaticus* (L.) and relate this to mixture effects on sublethal endpoints as well as mortality. Secondly, it was assessed whether observed mixture effects were better related to exposure concentrations or to measured isopod body concentrations. Recently, Norwood et al. (2013) developed an effects addition model based on body concentrations to predict chronic mortality of metal mixtures in the amphipod *Hyalella azteca*. In fact, invertebrate body concentrations have been shown to be a useful predictor of metal mixture effects since they account for metal interactions that can occur at biotic ligands and they integrate multiple routes of exposures as well as pulse exposure events (Borgmann et al., 2008; Norwood et al., 2013; De Jonge et al., 2013). Therefore, we hypothesize that body concentrations will be a better predictor of mixture effects than exposure concentrations. Cadmium, Cu and Pb were chosen since they generally differ in uptake mechanism for aquatic organisms; i.e. Cd^{2+} is known to be a Ca^{2+} uptake inhibitor (Niyogi and Wood, 2004; Rainbow and Black, 2005), Cu^{2+} is a known Na^{+} uptake inhibitor (De Schamphelaere and Janssen, 2002; Grosell and Wood, 2002) and Pb^{2+} has been observed to inhibit both Ca^{2+} and Na^{+} uptake pathways (Rogers and Wood, 2004; Rogers et al., 2005). The isopod crustacean *A. aquaticus* is an important decomposer of organic material and can be found worldwide in diverse freshwater ecosystems of the temperate region. This species is known to accumulate trace metals both from waterborne and dietary sources (van Hattum et al., 1989; De Jonge et al., 2012) and has been frequently used in ecotoxicological assays (e.g. van Hattum et al., 1989; Bloor and Banks, 2006; De Jonge et al., 2012).

2. Material and methods

2.1. Experimental design and water chemistry

A 10-day lab experiment was executed using the isopod *A. aquaticus*. All exposures occurred in acid-washed (1% HCl) polypropylene containers (125 mL) under controlled temperature (20 ± 1 °C) and light conditions (photoperiod of 16 h light and 8 h dark) in a climate chamber type WT15′/+ 5DU-WB (Weiss Technik, Reiskirchen-Lindenstruth, Germany).

Exposure concentrations were based on single metal toxicity data of *A. aquaticus* or related species from literature data, i.e. for Cd: 10-day LC_{50} of 54 μg L^{-1} (*A. aquaticus*; Ham et al., 1995), for Cu: 4-day LC_{50} of 650 μg L^{-1} (*A. meridianus*; Brown, 1976) and for Pb: 2-day LC_{50} of 280 μg L^{-1} (*A. meridianus*; Brown, 1976). Stock solutions of each metal were freshly prepared with analytical grade salts of cadmium chloride hydrate ($CdCl_2 \cdot H_2O$), copper chloride dihydrate ($CuCl_2 \cdot 2H_2O$) and lead chloride ($PbCl_2$) (all metal salts were purchased from VWR Int., Leuven, Belgium). Ultra-pure water (Milli-Q; Millipore, MA, USA) was used for the preparation of the stock solutions. Starting from the stock solution, for each metal five single metal exposure concentrations were prepared in 100 mL reconstituted freshwater (EPA medium-hard water; US EPA, 2002), resulting in total dissolved concentrations (analytically verified, see below) ranging from 24.9 to 277 μg L^{-1} for Cd, 151 to 2117 μg L^{-1} for Cu and 15.1 to 443 μg L^{-1} for Pb (Table 1). Maximal concentrations used in the present study are relatively high, although they can be encountered in metal-contaminated surface waters (e.g. Bryan and Gibbs, 1983; Bervoets et al., 2005; Stockdale et al., 2010; De Jonge et al., 2010, 2013). Mixture treatments were prepared by taking 50% of the single metal concentrations for the binary mixtures (Cd + Cu; Cd + Pb and Cu + Pb) (ray design with 1:1 concentration ratio) and 25% of the single metal concentrations for the tertiary mixture (Cd + Cu + Pb), resulting in four mixture treatments per metal (one single, two binary and one tertiary), each consisting of five different concentrations and one control containing background metal concentrations of 0.107 ± 0.034 μg L^{-1} Cd; 3.35 ± 0.61 μg L^{-1} Cu; and 0.782 ± 0.264 μg L^{-1} Pb.

Individuals of *A. aquaticus* were purchased from Blades Biological Ltd (Edenbridge, Kent, UK). Prior to exposure, all organisms were acclimatized for four weeks in acid-washed aquaria containing medium-hard EPA water and ad libitum amounts of food (alder leaves, *Alnus glutinosa*). At the start of the experiment three individuals of equal length (9.43 ± 0.17 mm) were introduced in each container filled with 100 mL test medium. Organisms were separated by a three-part polypropylene septum to avoid cannibalism (Bloor and Banks, 2006). No food was provided throughout the experiment. Experiments were executed in duplicate. Mortality and growth were assessed for all six organisms from both replicate chambers per treatment. Metal body concentrations and energy reserves, however, were each assessed for three organisms from different replicate chambers. General water characteristics (dissolved oxygen, pH and electrical conductivity) in all experimental containers were monitored every working day. At the beginning (day 0) and end (day 10) of the experiment water samples were taken from all containers with a syringe (10 mL), filtered through a 0.20 μm cellulose acetate filter (Schleicher & Schuell MicroScience GmbH, Dassel, Germany) and acidified to 1% HNO_3. Trace metals (total dissolved Cd, Cu, Pb and major ions Na, K, Mg and Ca) were analyzed using an inductively coupled plasma optic emission spectrometer (ICP-OES; Thermo scientific, ICAP 6300 Duo, Waltham, MA, USA). Dissolved organic carbon (DOC) was quantified using a TOC-analyzer (TOC-VCPH, Shimadzu Corporation, Kyoto, Japan). Analytical results regarding exposure concentrations and water chemistry are presented in Table 1.

2.2. Determination of body concentration

Metal body concentrations and sublethal toxicity endpoints (growth and energy reserves) were measured at the end of the experiment (day 10). However, due to early mortality in both the Cd single and Cd + Pb treatments, Cd and Pb body concentrations, growth and energy reserves were determined in animals that had died after seven days in the 87.1 and 155 μg L^{-1} single Cd concentration and the 57.2 μg L^{-1}

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