Ecotoxicology and Environmental Safety 118 (2015) 37–46

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Effects of the essential metals copper and zinc in two freshwater detritivores species: Biochemical approach

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ARTICLE INFO

Article history:
Received 6 February 2015
Received in revised form
7 April 2015
Accepted 8 April 2015

Keywords:
Copper
Zinc
Oxidative stress
Integrated biomarker response
Freshwater invertebrates

ABSTRACT

The input of metals into freshwater ecosystems from natural and anthropogenic sources impairs water quality and can lead to biological alterations in organisms and plants, compromising the structure and the function of these ecosystems. Biochemical biomarkers may provide early detection of exposure to contaminants and indicate potential effects at higher levels of biological organisation. The effects of 48 h exposures to copper and zinc on *Atyaephyra desmarestii* and *Echinogammarus meridionalis* were evaluated with a battery of biomarkers of oxidative stress and the determination of ingestion rates. The results showed different responses of biomarkers between species and each metal. Copper inhibited the enzymatic defence system of both species without signs of oxidative damage. Zinc induced the defence system in *E. meriodionalis* with no evidence of oxidative damage. However, in *A. desmarestii* exposed to zinc was observed oxidative damage. In addition, only zinc had significantly reduced the ingestion rate and just for *E. meridionalis*. The value of the integrated biomarkers response increased with concentration of both metals, which indicates that might be a valuable tool to interpretation of data as a whole, as different parameters have different weight according to type of exposure.

1. Introduction

In freshwater ecosystems, the level of trace metals can be enhanced as a consequence of natural and/or industrial activities. In addition to water quality degradation, the increase of metal levels can lead to biological alterations in organisms and plants. Trace metals can be transferred along food chains resulting in health consequences for humans (Rainbow, 1997). Metal exposure may produce sub-lethal effects that can compromise organism biochemistry, physiology, and reproductive success and eventually affect the long-term survival of populations (MacFarlane et al., 2006). In contaminated environments, the levels of copper and zinc can reach more than 40 and 100 mg l^{-1}, respectively (Macedo-Sousa et al., 2008).

Biochemical biomarkers are considered to be one of the most promising tools for ecotoxicological applications (Huggett et al., 1992; Peakall, 1992) as they may provide early detection of contaminant exposure, and an early indication of potential effects at higher levels of biological organisation, e.g., population and the ecosystem effects (McLoughlin et al., 2000). Metals are known inducers of oxidative stress in organisms with the subsequent production of reactive oxygen species (ROS) (Stohs and Bagchi, 1995). Hydrogen peroxide (H_2O_2), superoxide ($O2^{\bullet-}$) and hydroxyl (OH-) radicals, are among the most reactive compounds produced during metal stress (Dazy et al., 2009). These are generally associated with alterations at the level of DNA, proteins and membranes, and can result in cellular damage (Huggett et al., 1992; Vieira et al., 2011). Organisms are able to cope with ROS, through an antioxidant defence system, and only its failure results in oxidative stress and subsequent cellular damage (Livingstone, 2001; Lushchak, 2011).

Copper (Cu) and zinc (Zn) are essential metals required in small doses by all living organisms for metabolic functions and oxygen transport, however they can produce deleterious effects when internal concentrations exceed the requirements of the organism and its detoxification capability (Correia et al., 2002; Viarengo et al., 1990). Copper deficiency decreases the enzymatic activity of several enzymes implicated in oxidative defence systems and changes the cellular content of ROS scavengers (Uriu-Adams et al., 2005). On the other hand, the excess of Cu ions induces the formation of ROS via the generation of oxidising radicals, and ultimately leads to cellular toxicity (Gaetke and Chow, 2003; Livingstone, 2001; Viarengo et al., 1990). Waterborne and dietary exposure to high concentrations of Cu induced oxidative stress (in Lushchak, 2011). Zinc has several physiological/biochemical roles in organisms, and is involved in the functioning of more than 200

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http://dx.doi.org/10.1016/j.ecoenv.2015.04.006
0147-6513/

enzymes (Geret and Bebianno, 2004; Muyssen and Janssen, 2002) and maintenance of the membrane structure and function (Stohs and Bagchi, 1995). Several authors have proposed zinc as an antioxidant (Powell, 2000; Valko et al., 2005) and a deficiency of Zn levels could result in lipid peroxidation (Stohs and Bagchi, 1995). However, others suggest that an excess of Zn (0.1 and 1.0 mg l^{-1}) is related to ROS generation or the decline of antioxidant enzymes in several organisms including invertebrates (Geret and Bebianno, 2004).

The major mechanism through which ROS can cause tissue injury is lipid peroxidation (LPO) that can result in impaired cellular function, alterations of membrane properties and consequently disrupt vital functions (Rikans and Hornbrook, 1997). To reduce the negative effects of ROS, organisms have a protective and effective antioxidant defence system (Novais et al., 2011; Timofeyev, 2006; van der Oost et al., 2003; Vieira et al., 2011; Woo et al., 2009). This system is comprised of three principal groups of several enzymes that scavenge: ROS, such as catalase (CAT); internal lipid peroxidation products, namely glutathione peroxidase (GPx); and secondary oxidation radical products, like glutathione-S-transferase (GST). Catalase is the major enzyme involved on the scavenging of H_2O_2, converting it to water and oxygen. Several recent works observed alterations to the activity of this enzyme after metal exposure (Barata et al., 2005; Gravato et al., 2006; Liu et al., 2006; Oliva et al., 2012; Vieira et al., 2011). Glutathione peroxidase (GPx) also detoxifies H_2O_2 and organic hydroperoxides produced, for example by lipid peroxidation (LPO) (Correia et al., 2003). Several authors found alterations on GPx activity in different organisms associated with or after metal exposure (Ahmad et al., 2005; Ahmad et al., 2006; Gravato et al., 2006; Liu et al., 2006). GST catalyses the conjugation of glutathione (GSH) with xenobiotics including metals, playing an important role on their detoxification (Huggett et al., 1992; Jemec et al., 2010). Some authors reported alterations on GST activity by the presence of metals and products of oxidative stress (Hayes and Pulford, 1995; van der Oost et al., 2003). Liu et al. (2006) and Gravato et al. (2006) observed an induction of GST activity in liver of the goldfish and eels, respectively, as a result of copper exposures. Glutathione (GSH), one of the major thiol containing molecules in the cell, protects cells from the effects of metals and other electrophilic compounds (Oliveira et al., 2009) by being a substrate for GPx, a co-factor for glutathione-S-transferase (GST) and directly linked to pro-oxidants, such as transition metals (Lushchak, 2011; Meister, 1995a; 1995b; Saint-Denis et al., 1998). The glutathione reductase (GR) also protects cell by catalysing the reduction of GSSG (oxidised glutathione form) into GSH (reduced and active form) (Huggett et al., 1992; Novais et al., 2011; Saint-Denis et al., 1998). Gravato et al. (2006) found a slight decrease of GR activity, in the liver of European eel after exposure to copper.

The results of a battery of biomarkers can be combined into a general "stress index", the integrated biomarker response (IBR), which is used to easily describe the toxically induced stress level of populations (Beliaeff and Burgeot, 2002). Generally, this index is used in field studies (Broeg and Lehtonen, 2006; Oliveira et al., 2009; Wang et al., 2010), although it seems to be a promising tool to integrate and interpret responses at different organisation levels even in laboratory assays that evaluate the "health condition" of the organisms. This index also allows the selection of several parameters that will be more discriminatory for the type of contamination concerned.

The objective of this study was to evaluate the effects of two essential metals, copper and zinc, at biochemical and organism level on two detritivores species, the shrimp *Atyaephyra desmarestii* (Millet) and the amphipod *Echinogammarus meridionalis* (Pinkster). We also determined the capability of the IBR index to integrate the results and give an overview of the effects at different organisation levels for the selected metals for each species. And, finally, we assessed the value of this battery of biomarkers for metal exposure.

2. Material and methods

2.1. Sampling and acclimation of organisms

Organisms of both species were collected with a kick-sampling or an handling net at Rio Ceira near Coimbra, Portugal (40°10′13.21″N 8°23′26.28″W, *A. desmarestii*) and at Rio Lena near Leiria, Portugal (38°35′28.3″N 8°40′30.2″W, *E. meridionalis*), and transported to the laboratory in local water. Rio Lena sampling site is less that 200 m of river spring and with no evident contaminant sources. Rio Ceira sampling site is near the end of the river and have some agricultural and urban impacts. Organisms were maintained for at least one week in aerated artificial pond water (APW) (ASTM, 1980), at 20 °C with a photoperiod of 16:8 h (light: dark) for acclimation purposes and dry alder leaves were given ad libitum during this period (Macedo-Sousa et al., 2007; Pestana et al., 2007).

2.2. Exposure assays

In order to evaluate the effects of copper and zinc on feeding and biochemical biomarkers of the shrimp *A. desmarestii* and the amphipod *E. meridionalis*, the organisms were exposed during 48 h in plastic beakers filled with 300 ml of testing solutions with increasing concentrations of each metal. The test solutions were prepared by dissolving copper ($CuCl_2 \cdot {}_2H_2O$) and zinc ($ZnCl_2$) stock solutions in artificial pond water (APW). Pre-weight leaf discs were soaked in APW or the corresponding metal solutions for 48 h prior to use, in order to reduce adsorption of metal ions to the leaf material during the exposure period. Both species were exposed to sub-lethal concentrations of zinc and copper bellow the LC50 previously determined, for *E. meridionalis* 11.86 and 0.05 mg l^{-1}, respectively and for *A. desmarestii* 7.95 and 0.13 mg l^{-1}, respectively (unpublished data). Briefly, ten replicates were used with three shrimps or ten amphipods per beaker with 250 ml of treatment solution. Copper nominal concentrations for assays with shrimps were 0.0–0.2 mg l^{-1} and for assays with amphipods were 0.0–0.05 mg l^{-1}. Zinc nominal concentrations for both species were 0.0–3.0 mg l^{-1}. 0.05 and 3.0 mg l^{-1} of copper and zinc, respectively, are the maximum permissible values for human consume water in national legislation (DL. 236/98). The duration of exposure was 48 h, in a 20 °C temperature controlled room, with a photoperiod of 16:8 h (light:dark), with aeration and food supply (pre-weight leaf discs). Conductivity, dissolved oxygen, pH and temperature were measured daily. At the end of the experiment, organisms were used for enzymatic assays. The leaf discs of each treatment were dried until no variation of their weight was observed and the ingestion rates were estimated as follows:

$$Ir = \frac{\Delta Lw}{Lwi} \times \frac{1}{d \times Ow}$$

where ΔLw, is the variation of leaves weight (initial minus final weight); Lwi, is the leaves initial weight; d, is the number of days; and Ow, is the organism weight. Ir is given in μg mg^{-1} day^{-1} (adapted from (Pestana et al., 2007).

2.3. Enzymatic assays

The abdomen of shrimp and the whole individual amphipods of each replicate were sonicated separately in 0.1 M K-phosphate buffer (pH 7.4) at the proportion of 1:15 (100 mg of tissue in