Environmental Pollution 215 (2016) 203–212

Contents lists available at ScienceDirect

Environmental Pollution

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

Ecotoxicity and genotoxicity of cadmium in different marine trophic levels☆

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ARTICLE INFO

Article history:
Received 16 January 2016
Received in revised form
2 May 2016
Accepted 4 May 2016

Keywords:
Acartia tonsa
Palaemon varians
Solea senagalensis
DNA damage
Comet assay
Ionic cadmium

ABSTRACT

Cadmium ecotoxicity and genotoxicity was assessed in three representative species of different trophic levels of marine ecosystems – the calanoid copepod *Acartia tonsa*, the decapod shrimp, *Palaemon varians* and the pleuronectiform fish *Solea senegalensis*. Ecotoxicity endpoints assessed in this study were adult survival, hatching success and larval development ratio (LDR) for *A. tonsa*, survival of the first larval stage (zoea I) and post-larvae of *P. varians*, egg and larvae survival, as well as the presence of malformations in the larval stage of *S. senegalensis*. In vivo genotoxicity was assessed on adult *A. tonsa*, the larval and postlarval stage of *P. varians* and newly hatched larvae of *S. senegalensis* using the comet assay. Results showed that the highest sensitivity to cadmium is displayed by *A. tonsa*, with the most sensitive endpoint being the LDR of nauplii to copepodites. Sole eggs displayed the highest tolerance to cadmium compared to the other endpoints evaluated for all tested species. Recorded cadmium toxicity was (by increasing order): *S. senegalensis* eggs < *P. varians* post-larvae < *P. varians* zoea I < *S. senegalensis* larvae < *A. tonsa* eggs < *A. tonsa* LDR. DNA damage to all species exposed to cadmium increased with increasing concentrations. Overall, understanding cadmium chemical speciation is paramount to reliably evaluate the effects of this metal in marine ecosystems. Cadmium is genotoxic to all three species tested and therefore may differentially impact individuals and populations of marine taxa. As *A. tonsa* was the most sensitive species and occupies a lower trophic level, it is likely that cadmium contamination may trigger bottom-up cascading effects in marine trophic interactions.

1. Introduction

During the last 50 years, the environmental hazard of cadmium has been assessed in aquatic and soil ecosystems (Burger, 2008; Nordberg, 2009). This naturally occurring metal, is found both in water and soil/sediments at low concentrations due to natural processes, such as volcanic eruptions, natural crust erosion and also anthropogenic activities, such as mining and smelting (World Health Organization, 2003). Being a common by-product of zinc mining, cadmium often runoffs into aquatic systems (Environment Programme, 2008) and subsequently ends up in brackish and marine environments (Chiodi-Boudet et al., 2013). This non-essential metal to life forms, is commonly toxic even at relatively low concentrations and can cause adverse effects due to its high bioaccumulation tendency (Chandurvelan et al., 2013a, 2012). Reported concentrations of cadmium in the marine environment ranging from less than 5 ng/L (World Health Organization, 2003) to an average of 40 ng/L in unpolluted surface waters (Ray, 1984), while up to 250 ng/L can be recorded in coastal areas of northern Europe (World Health Organization, 2003). This feature has been associated with riverine inputs and/or due to direct human impact (Elinder, 1985; World Health Organization, 2003).

The International Agency for Research on Cancer (1993) classifies cadmium as a human carcinogen and teratogen with probable mutagenic properties. Indeed, this metal can induce genotoxicity in organisms, such as DNA strand breaks, chromosomal aberrations and micronuclei formations (MN) (Sarkar et al., 2015). Cadmium contamination can trigger the production of reactive oxygen species (ROS) (Amirthalingam et al., 2013), which have been suggested to be the promoters of genotoxicity (Bertin and Averbeck, 2006). Nocuous effects of chemicals are usually foreshadowed by cellular

☆ This paper has been recommended for acceptance by Harmon Sarah Michele.

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http://dx.doi.org/10.1016/j.envpol.2016.05.010
0269-7491/

and/or molecular shifts that sometimes may only be perceptible over time through the shaping of adult populations (Migliarini et al., 2005). Therefore, under these scenarios it may be too late to take any suitable measures to mitigate the negative impacts associated with chemical contamination. Recent research has pointed out the need of assessing the genotoxic effects of chemicals in aquatic organisms (Chang et al., 2009; Sarkar et al., 2015). The single cell gel electrophoresis assay, also known as comet assay, has proven to be a good assessment tool, as it is considered a simple, sensitive and fast way to assess DNA damage and repair (Frenzilli et al., 2009; Migliarini et al., 2005; Nigro et al., 2002; Sarkar et al., 2015; Thompson and Bannigan, 2008).

Cadmium's toxicity to aquatic organisms varies significantly and depends mainly on the concentration of its free ionic form, rather than the concentration of total dissolved cadmium (Engel and Fowler, 1979; Sunda et al., 1978). Marine toxicology, in comparison to freshwater toxicology, remains "poorly" studied, mostly due to the complexity and constraints associated with the calculations of chemical speciation of contaminants in seawater (Hayes and Kruger, 2014; Leung et al., 2001). Cadmium contamination is no exception to this rule. One possible way to overcome the bottlenecks associated with speciation is to assess cadmium concentration through the use of chemical equilibrium speciation models. Various chemical equilibrium models, such as MINEQL+, Visual MINTEQ or MINTEQA2, allow the quantification and speciation of metals, in order to better evaluate ecotoxic effects and facilitate comparisons between studies.

In this way, in order to gain further knowledge on the effects of cadmium contamination in different marine trophic levels, the present study tested the following null hypotheses: a) cadmium toxicity is not life stage or species specific; and b) cadmium does cause genotoxicity to tested species. Three different species, representing different marine trophic levels, were used to assess lethal and sublethal effects of free ionic cadmium, including the comet assay to evaluate genotoxicity. In addition, different life stages were also used in order to infer any differences in their sensitivity to cadmium exposure. *Acartia tonsa*, a primary consumer, is a marine/estuarine copepod that is part of the diet of several macro invertebrates and fish. It is easily maintained in large cultures under laboratory conditions and it is widely used and accepted as an alternative to *Artemia* in aquacultures due to its higher nutritional value and fatty acids profile (Ajiboye et al., 2010; Shields et al., 1999; Støttrup, 2003, 2000; Støttrup et al., 1986, 1998). *Palaemon varians*, the Atlantic ditch shrimp or grass shrimp, a secondary consumer, is an edible estuarine shrimp. It plays a key role in these ecosystems, as it feeds on decaying matter and promotes nutrient recycling; it is also an important prey for juvenile and larval fish stages of commercial importance (Sykes et al., 2006). *Solea senegalensis* is a benthic flatfish often considered a top predator commonly found in abundance around European, Atlantic and Mediterranean coasts. It is of high economic value for fisheries and aquaculture (Bejarano-Escobar et al., 2010; Dinis, 1992; Dinis et al., 1999).

2. Materials and methods

2.1. Test organisms

2.1.1. Copepod culture

Full life-cycle cultures of the marine calanoid copepod, *Acartia tonsa*, were kept in artificial seawater (ASW) prepared by mixing a commercially available salt mixture (Tropic Marin® Pro Reef salt; Tropic Marine, Wartenberg, Germany) with freshwater purified by a four-stage reverse osmosis unit (Aqua-win RO-6080). Cultures were started from eggs (kindly provided by the Escola Superior de Tecnologia do Mar, IPL, Peniche, Portugal) using 15-L poly(methyl methacrylate) (PMMA) cylindrical-conical tanks provided with constant aeration (3 bubbles/sec), at a density of ≈130 adults/L. Salinity was kept at 20 ± 1, temperature at 20 ± 1 °C and photoperiod at 16 h light: 8 h dark. Newly hatched copepod nauplii were separated into 15 L PMMA tanks by filtering them using a 125 μm mesh and fed daily ad libitum with the cryptophyte, *Rhodomonas lens* CCMP 739 (minimum 2×10^7 cells/mL). Tanks were cleaned daily to collect resting eggs, remove dead organisms, fecal pellets and excess of food. Total water renewal was performed once a week. Eggs of *A. tonsa* were stored at 4 °C for starting new cultures whenever necessary.

2.1.2. Shrimp culture

Ovigerous female ditch shrimp *Palaemon varians* were collected from the end of April till middle of October from a non-polluted salt marsh at Troncalhada, Aveiro, Portugal (40°38′40.1″N, 8°39′52.0″W) (Rodrigues et al., 2011). All specimens were stocked at a temperature of 20 °C, salinity 35 ± 1 and a photoperiod of 16 h light: 8 h dark in a recirculated maturation system described by Calado et al. (2007) until larval hatching. Newly hatched larvae (zoea I) were either used to run ecotoxicity trials (see below) or raised in a recirculated rearing system described by Calado et al. (2008) until they had reached their first post-larval stage (≈12 days after hatching). Larvae were fed daily ad libitum with newly hatched *Artemia* nauplii and decapsulated *Artemia* cysts until used for testing.

2.1.3. Sole stock

Fertilized eggs of *Solea senegalensis* (less than 12 h) were kindly provided by Safiestela, S.A. (group Sea8), a commercial sole hatchery in Póvoa de Varzim, Portugal. Fertilized eggs were transferred to the laboratory using sealed plastic bags (1/3 seawater and 2/3 oxygen saturated atmosphere) under constant temperature (18 °C). Upon arrival they were stocked under continuous aeration in 2-L flat bottom conical glass vials at salinity 35 ± 1 and a temperature of 18 ± 1 °C for 2–3 h prior to testing. Eggs were chosen according to their developmental stage, being selected for testing when shifting from the blastula to the gastrula stage.

2.1.4. Test chemicals

The chemical compound used in the present study was cadmium chloride anhydrous (CAS No. 10108-64-2, Sigma-Aldrich, Germany). Stock solutions of 100 mg of Cd/L and 10 g of Cd/L were prepared with ultrapure water using a Millipore® Academic Milli-Q system. Tested concentrations were then accomplished through dilutions in artificial seawater (ASW) (see above for details) adjusting the salinity to each organism's culture conditions. To confirm the actual concentrations in the spiking process, dilutions were made in ultrapure water and acidified samples of the dilutions as well as acidified samples from the stock solutions used were sent for analysis to LCA (Central Laboratory of Analysis, University of Aveiro, Portugal). Chemical analysis was performed by Inductively Coupled Plasma Mass Spectrophotometry (ICP – MS) using a calibration curve made with seven standards obtained by successive dilutions of multi-element standard ICPMS-71A, from Inorganic Ventures, Virginia, USA. The method was verified using a Certified Reference Material (CRM), 1643e available from NIST. Quantification limits were 0.1 μg/L and detection limits were 0.33 μg/L. As mentioned before, free ionic cadmium is considered to be the bioavailable chemical species, responsible for causing toxicity to the organisms. Therefore, the chemical equilibrium model Visual MINTEQ ver. 3.0/3.1 (Gustafsson, 2013) was used to estimate the metal speciation of cadmium in ASW medium using the concentration of all salt constituents (information supplied by the manufacturer) to obtain the concentration (in form of