Ecotoxicology and Environmental Safety 142 (2017) 322–329

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Prediction of toxicity of zinc and nickel mixtures to *Artemia* sp. at various salinities: From additivity to antagonism

Évila Pinheiro Damasceno[a], Lívia Pitombeira de Figuerêdo[a,1], Marcionília Fernandes Pimentel[a], Susana Loureiro[b,*], Letícia Veras Costa-Lotufo[a,*,2]

[a] Instituto de Ciências do Mar, LABOMAR/UFC, 60165-081 Fortaleza, Ceará, Brazil
[b] Department of Biology and CESAM, University of Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

Keywords:
Acute toxicity test
Artemia sp.
Zinc
Nickel
Mixtures
Salinity range

ABSTRACT

Few studies have examined the toxicity of metal mixtures to marine organisms exposed to different salinities. The aim of the present study was to investigate the acute toxicity of zinc and nickel exposures singly and in combination to *Artemia* sp. under salinities of 10, 17, and 35 psu. The mixture concentrations were determined according to individual toxic units (TUs) to follow a fixed ratio design. Zinc was more toxic than nickel, and both their individual toxicities were higher at lower salinities. These changes in toxicity can be attributed to the Biotic Ligand Model (BLM) rather than to metal speciation. To analyze the mixture effect, the observed data were compared with the expected mixture effects predicted by the concentration addition (CA) model and by deviations for synergistic/antagonistic interactions and dose-level and dose-ratio dependencies. For a salinity of 35 psu, the mixture had no deviations; therefore, the effects were additive. After decreasing the salinity to 17 psu, the toxicity pattern changed to antagonism at low concentrations and synergism at higher equivalent LC_{50} levels. For the lowest salinity tested (10 psu), antagonism was observed. The speciations of both metals were similar when in a mixture and when isolated, and changes in toxicity patterns are more related to the organism's physiology than metal speciation. Therefore, besides considering chemical interactions in real-world scenarios, where several chemicals can be present, the influence of abiotic factors, such as salinity, should also be considered.

1. Introduction

Human activities are responsible for the introduction to the marine environment of an increasing number of chemicals that are found to be complex mixtures (Cedergreen et al., 2008). A regulatory risk assessment of the aquatic environment is mainly based on the exposure and effects of single chemicals. However, aquatic organisms are invariably vulnerable to chemical mixtures, and the joint effects can be based on the sum of the individual effects or even higher or lower than the sum, increasing or decreasing the overall mixture toxicity (Barata et al., 2006).

At the beginning of the 20th century, two models were developed to predict and analyze the effects of possible mixtures in pharmacological studies: the concentration addition (CA) model, applied to chemicals with similar modes of action (Loewe and Muischnek, 1926), and the independent action (IA) model, for chemicals with different modes of action (Bliss, 1939). These two models were considered the starting point of the notion that chemicals do not interact with each other and their effects are additive; the CA model also predicted the toxicities of chemicals in mixtures, as they were dilutions of each other. However, other patterns were often observed, where increases (synergism) or decreases (antagonism) in toxicities were depicted mainly due to chemical interactions under toxicodynamic processes. In 2005, Jonker and co-authors described the Mixtox approach/tool, adding other possible and combinatory deviations, mainly changing from synergism to antagonism (or vice versa) when concentration levels changed (low to high or high to low concentrations) or when the chemical ratio changed (high levels of one chemical with low of other[*s*]). This approach using conceptual models (CA and IA) and deviations for synergism or antagonism has been applied to freshwater organisms (Holmstrup et al., 2010; Pavlaki et al., 2011) or edaphic organisms (Santos et al., 2011; Svendsen et al., 2010), and few were devoted to marine or estuarine environments (Otitoloju, 2002; Figuerêdo et al., 2015).

* Corresponding authors.
E-mail addresses: sloureiro@ua.pt (S. Loureiro), costalotufo@usp.br (L.V. Costa-Lotufo).
[1] São Carlos School of Engineering (EESC), University of São Paulo (USP). 13.560–970 São Carlos - São Paulo – Brazil.
[2] Instituto de Ciências Biomédicas, University of São Paulo (USP), 05508-900 São Paulo, São Paulo Brazil

http://dx.doi.org/10.1016/j.ecoenv.2017.04.020
Received 22 November 2016; Received in revised form 7 April 2017; Accepted 10 April 2017

Because estuarine and marine environments are prone to receiving xenobiotics from different sources (e.g., point or diffuse), the aim of the present study was to predict the toxicity of metallic compound mixtures in a test organism under different salinities, mimicking natural variations that can be found in estuaries. For that, experiments were carried out with the microcrustacean *Artemia* sp. exposed to zinc and nickel as model chemicals, as well as to their binary mixtures. *Artemia* sp. was chosen as a test species, as it plays an important role in marine and estuarine food chains, but also because it can handle wide ranges of salinity. Furthermore, organisms from this genus are easily maintained in the laboratory, and toxicity tests are well developed and cost effective (Nunes et al., 2006). The main pollution sources for zinc are related to the manufacturing of galvanized steel, in alloy with other metals in many objects, in the manufacturing of rubber to neutralize acidity, and in agriculture as a crop nutrient (John et al., 2007). Nickel enters into the environment via the production, processing, and recycling of various nickel products, including stainless steel, electroplating, pigments, and ceramics (Chowdhury et al., 2008). These two metals originate from different sources, but they can be simultaneously found in the environment at high concentrations. Zinc is an essential metal for marine organisms and nickel has also been described as essential for phytoplankton, but increased levels might result in toxic effects (Hogstrand, 2011; Pyle and Couture, 2011). To study potential changes in toxicity patterns due to changes in salinity, acute toxicity tests were carried out at different salinity levels (35, 17, and 10 psu), and the patterns of response for zinc and nickel isolated and in mixture exposures were assessed and related to both metal speciation and the organism's physiology.

2. Materials and methods

2.1. Chemicals analysis

Zinc sulfate heptahydrate (CAS 7446-20-0) and nickel (II) chloride hexahydrate (CAS 7791-20-0) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

The concentrations of zinc (500 and 1,000 mg L^{-1}) and nickel (1000, 2,500, and 6000 mg L^{-1}) of stock solutions were confirmed by flame atomic absorption spectrometry (FAAS) measurements following Standard Methods for the Examination of Water and Wastewater, 21st Edition (APHA, AWWR, 2005).

2.2. Speciation analysis

The chemical equilibrium model Visual MINTEQ ver. 3.0/3.1 (developed by J.P. Gustafsson) was used to calculate the speciation of nickel and zinc in seawater. Seawater constituents included in the model input data were based on Millero (Millero, 2006), with some adaptations regarding salinity and SO_4^{2-}. From nickel (Ni) and zinc (Zn) spiking, extra SO_4^{2-} was added to the test solutions and therefore corrected in the model accordingly.

2.3. Test organism

The brine shrimp (*Artemia* sp.) cysts were purchased commercially (Bio Artemia) and were kept at 4 °C in a refrigerator before being used for hatching. To obtain the nauplii, the cysts were initially hydrated in distilled water for one hour and then moved into a beaker with sodium hypochlorite in vigorous shaking until the cysts' colors changed from brown to orange. After, the cysts were washed and maintained in a 1-L flask containing filtered natural seawater (0.8 µm, salinity 35 psu) under constant aeration at 24 ± 2 °C and 12 h light: 12 h dark until hatched. The nauplii were then used in bioassays 48 h after hatching as nauplii stage II. Nauplii stage was confirmed under the stereo microscope, where the yolk sac and the external opening of the gut were observed.

2.4. Test medium

The natural seawater used in the cyst maintenance and toxicity tests was collected 3 km from the coastline of Fortaleza, Ceará, Brazil (3°41'S 38°30′W). The dissolved organic carbon reported for seawater collected from this area ranges from 3.3 mg L^{-1} in the rainy season to 4.2 mg L^{-1} in the dry season (non-published data). Before use, natural seawater was filtered to 0.8 µm membranes to remove suspended particles. Then, filtered seawater was diluted in distilled water to obtain different salinities.

Test solutions for zinc (50 to 1200 mg Zn L^{-1}) and nickel (25–600 mg Ni L^{-1}) were obtained by the dilution of stock solutions with seawater at the proper salinity (10, 17, or 35 psu). The salinity values were confirmed using a refractometer (Biobrix, model 211). The tested concentrations were selected based on preliminary range-finding tests (data not shown).

2.5. Artemia toxicity test

Experiments were performed according to Pimentel et al. (2009), with some adaptations. Toxicity tests were conducted in non-treated sterile polystyrene 24-multi-well plates by adding 10 nauplii to 0.5 mL of seawater using a fixed volume micropipette per replicate/well, which was then filled with 2 mL of the test concentration (total final volume of 2.5 mL). Tested concentrations ranged from 50 to 1200 mg Zn L^{-1} and 25–600 mg Ni L^{-1}. Five experiments were conducted in triplicate for each metal individually under three different salinities (35, 17, and 10 psu). These data were afterwards pooled for statistical analysis (see details below). Plates were kept at 24 ± 2 °C and under a 12 h light: 12 h dark photoperiod. After 24 h, plates were observed under stereo microscope (20x), and the number of dead animals was assigned using the criterion of immobilization for 10 s.

2.6. Binary-mixture exposures

The exposure concentrations (C*i*) for each of the metals in the binary mixtures were derived by converting concentrations into toxic unit (TU) equivalents, considering the 24 h LC_{50} as a unit of strength:

$$TU = Ci/LC50$$

For the three salinities, the TUs calculated for each individual metal were multiplied by decimal numbers to achieve a dilution series to define the concentrations used in the mixture experiments. Thus, the combination of zinc and nickel had seven dilution ratios (4:1, 3:2, 2:1, 1:1, 1:2, 2:3, and 1:4), representing zinc and nickel, respectively, and each number being a multiple of the respective TU (Fig. 1). Each ray in Fig. 1 represents the concentrations of two experiments for each dilution ratio.

The experimental setup included separate experiments, where each dilution ratio was run independently from the others. The five independent experiments carried out for the single toxicity tests bridge the variability on the responses that may exist regarding both metals' exposure. This allowed a wider exposure range testing, where data could be analyzed as single dilution ratios or pooled together (see below Section 2.5).

The methodology for exposures in the mixture trials was similar to that described above for the single exposures.

2.7. Statistical analysis

Toxicity data were expressed as LC_{50} with a respective standard error (SE), which was calculated using a sigmoidal (logistic, 3 parameter) equation (Sigma Plot 10.0) for both total metal concentration and the bioavailable free ionic forms (Ni^{2+} and Zn^{2+}) previously obtained from the Visual MINTEQ. For each metal and for each salinity value, data from approximately five experiments were pooled and