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Selecting a sensitive battery of bioassays to detect toxic effects of metals in effluents

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ABSTRACT

The use of bioassay batteries is necessary to evaluate toxic effects at various biological levels. The selection of bioassays without prior testing and determination of the most sensitive/suitable groups for each impact may allow the discharge of effluents that pose a threat to the environment. The present study tested and selected a battery of sensitive ecotoxicological bioassays for detecting toxic effects of metals. The sensitivities of six organisms were evaluated (algae *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*, Cladocera *Daphnia similis* and *Ceriodaphnia dubia*, and fish *Poecilia reticulata* and *Danio rerio*) after exposure to 10 individual metal species deemed toxic to the aquatic environment (Ag^{+}, Cd^{2+}, Cu^{+}, Cu^{2+}, Cr^{3+}, Cr^{6+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, and Hg^{2+}) and to real (steel-mill) and laboratory simulated effluents. In the bioassays, fish were the least sensitive; *D. rerio* showed no sensitivity to any of the effluents tested. *P. subcapitata* was a good bioindicator of Cr^{3+} toxicity, and *D. similis* was the most sensitive organism to Hg^{2+}; but the toxic effect of effluents with higher levels of Hg^{2+} was better detected by *C. dubia*. The most sensitive battery of bioassays to detect low concentrations of dissolved metals in effluents was the 72-h chronic test with *C. vulgaris* and the 48-h acute test with *C. dubia*.

1. Introduction

Ecotoxicological bioassays are valuable tools for assessing the combined toxic effects of pollutants in the aquatic environment. The use of a battery including different trophic levels is recommended for evaluating the toxicity of industrial effluents (Tigini et al., 2011). This approach allows to estimate the potential effects on producers, primary consumers, predators, and decomposers of one location. It is assumed that by protecting the most sensitive trophic level, all other group of organisms are protected as well, and that protecting the structure of an ecosystem also protects ecosystem functions (Backhaus and Faust, 2012). Many countries have adopted the use of batteries using at least three organisms, usually a vertebrate, an invertebrate, and a plant species (Australian and New Zealand Environment and Conservation Council (ANZECC) and Agriculture and Resource Management Council of Australia and New Zealand (ARMCANZ), 2000; Canada—Environment Canada, 1999; United Kingdom—UK Water Industry Research Limited (UKWIR), 2001; USA—United States Environmental Protection Agency (US EPA), 1991).

Until recently in Brazil, as in most Latin American countries, the quality assessment of effluent was based only on its physicochemical and chemical characteristics. Priority substances lists were developed to restrict or eliminate discharge of toxic chemicals into the environment (Brazil, 2011). However, industrial effluents are generally a complex and poorly characterized mixtures of a large number of chemicals. In 2011, the Brazilian legislation (CONAMA Resolution no. 430; Brazil, 2011) was aligned with international recommendations to use standardized bioassays with species representing at least two trophic levels for the ecotoxicological assessment of effluents. There are now 12 species standardized by the Brazilian National Standards Organization (ABNT): 1 bacterium, 3 algae, 5 microcrustaceans, and 3 fish. However, the mere standardization of bioassays does not guarantee sensitivity to all stressors because organisms do not have all relevant target sites for all contaminants (Johnson et al., 2004).

To determine the best battery of bioassays, two approaches have been employed: one is the evaluation of the sensitivity of bioassays

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to individual contaminants from a variety of classes with different toxicokinetic and toxicodynamic mechanisms (Hoekzema et al., 2006; Martins et al., 2007), and another is the test of bioassays for wastewater with a series of aqueous mixtures and effluents containing contaminants belonging to different classes and industrial categories (Johnson et al., 2004; Manusadžianas et al., 2003; Pablos et al., 2009; Pandard et al., 2006; Ren and Frymier, 2003). Both approaches have limitations. The sensitivity of bioindicators depends on the toxic substance evaluated and its interaction with the organism (uptake, transport, internal bioavailability, and elimination). Therefore, it is necessary to have a battery of bioassays that are more sensitive to certain classes of contaminants (Carballeira et al., 2012; Tigini et al., 2011), and individual assays in the battery should complement rather than duplicate the information obtained from other assays in the battery.

Brazil is the largest producer of steel in Latin America with 29 plants including the ninth largest producer and the fifth largest exporter of steel in the world (34.5 million tons) (Brazil Steel Institute, 2012). Steel-mill effluent is characterized mainly by its high concentrations and varieties of metals in addition to auxiliary substances, such as acids, alkalis, and phenols. The toxicity of metals depends on their chemical species, and is correlated with the concentration of free ions in the environment and their interaction with organism ligands (Balistrieri and Mebane, 2014). This interaction assumes a binding pattern with cell membranes where monovalent metals typically affect Na^+ transport, and divalent metals disrupt Ca metabolism due to increased competition for binding sites (Niyogi and Wood, 2004). Meanwhile, the entry of mixed metals into an organism is not the same as the entry of individual metals; there is competition among the various metals for binding sites, and the entry of some metals can be favoured in relation to others (Franklin et al., 2002). Thus, it is difficult to extrapolate the toxicity of mixtures from individual toxic effects and highlights the importance of using bioassays. In addition, this “favouring” varies from one organism to another, resulting in some species being more sensitive to some metals and less sensitive to others (Komjarova and Blust, 2009a).

The objective of this study is to select a battery of bioassays that is sensitive to metal toxicity. Bioassays with metals were performed individually – 10 metal species deemed toxic to the aquatic environment (Ag^+, Cd^{2+}, Cu^+, Cu^{2+}, Cr^{3+}, Cr^{6+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, and Hg^{2+}) – and in mixtures, taking as an example real (steel-mill effluents) and laboratory simulated effluents. Six organisms belonging to three trophic levels were used for chronic or acute tests (algae *Pseudokirchneriella subcapitata* and *Chlorella vulgaris*, Cladocera *Daphnia similis* and *Ceriodaphnia dubia*, and fish *Poecilia reticulata* and *Danio rerio*). Although many studies determined the toxicity of metals for most of these organisms, test conditions were varied (such as the pH, hardness, culture media, dilution water, etc.), and it is known that these factors affect metal bioavailability. Thus, in the present study we used water dilutions and culture media with the smallest possible number of interfering factors. In addition, our results contribute with knowledge on ecotoxicological data for the alga *C. vulgaris* and fish *P. reticulata*, data that is missing in published literature. With the recent development of prediction models for the toxicity of mixtures, the identification of a battery of tests that is sensitive to individual metals and mixtures help create models based on these organisms.

2. Materials and methods

Our test strategy was divided in two steps. First we assessed the individual effects of toxic metals. Second, toxic effects of metals in mixtures were evaluated (four steel-mill effluents and one laboratory simulated effluent). All steps were performed with acute and chronic toxicity standard bioassays.

2.1. Individual metals

Assays with individual metals were performed with analytical grade compounds. Cadmium sulphate ($CdSO_4/8:3H_2O$—98%), lead nitrate ($Pb(NO_3)_2$—99%), potassium dichromate ($K_2Cr_2O_7$—99.9%), basic chromium III sulphate ($Cr(OH)SO_4$—25%), copper chloride (CuCl—97%), cupric sulfate ($CuSO_4/5H_2O$—98%), zinc chloride (ZnCl—98%), mercury sulphate ($HgSO_4$—98%), nickel sulfate ($NiSO_4/6H_2O$—98%), silver nitrate ($AgNO_3$—99%) were chosen as model cations. Stock solutions of metals were prepared by dissolving in ultrapure water (Milli-Q®, conductivity values less than 0.1 μS/cm) which was subsequently diluted in test medium (according to each organism maintenance medium as described below) to obtain test concentration. The reaction with each test medium generated solutions with different pH. It is well known that pH and hardness of water interfere on metal toxicity. In order to standardize all bioassays, each concentration test had the pH adjusted for 7.0 (6.75–7.32) and hardness of the dilution water used was between 20 and 50 mg/L $CaCO_3$, being characterized as a soft water.

2.2. Effluents containing mixtures of metals

2.2.1. Steel-mill effluents

Four effluent samples of a large steel industry were collected. Effluent 1 (EF1) was taken after the process of continuous casting lines, electrolytic tinning and chromium plating, characterized by high concentrations of Cr^{6+}, Sn^{2+}, Fe and low pH. Afterwards it passes for a chromic sewage basin where the effluent receives $FeCl_2$ solution and $Na_2S_2O_5$ to reduce Cr^{6+} to Cr^{3+}, at this point effluent 2 (EF2) was collected. Effluent 3 (EF3) was sampled from acid/alkali washing process, galvanization (zinc and lead-tin plating) and tinning process, characterized by high concentration of metals and alkaline pH. Effluent 4 (EF4) was sampled after EF2 and EF3 were mixed in the treatment plant and followed for neutralization with lime and compressed air injection for precipitation of metals, coagulation, flocculation and decantation. EF4 is thus the treated effluent that was ready to be discharged.

Effluents EF1, EF2 and EF3 were sampled before treatment. EF1 and EF2 were acid (pH 3–4) and EF3 was alkaline (pH 8.5–9.5). These pH values are toxic to our test organisms (pH tolerance range of 5–9), and our objective was to compare the sensitivity of bioassays to detect toxicity of metals in mixtures. Also, one of the steps of the treatment is the correction of pH (as described above). Considering that pH interferes on metal solubility (bioavailability) and that each bioassay is performed in a different medium, pH was adjusted to 7.0 (6.75–7.32) for each concentration tested to guarantee all species are exposed to the same metal concentration.

Four collections were made over two years. Chemical composition and biological responses varied very little between the four collections because it is a standardized production process. Thus, the mean numbers was used for analyzes (Table 1). Steel-mill effluent samples were preserved in polyethylene bottles and kept under refrigeration (4 °C) in the dark. Samples were tested within 48 h of collection. In a few cases where this was not possible, the sample was frozen at −20 °C. Concentrations of effluent were expressed as a percentage following a dilution factor of two, considering the raw sample as 100%.

2.2.2. Laboratory simulated effluent

The simulated effluent was prepared in the laboratory using the dilution water indicated for each test organism and by adding metals (Table 2) in the maximum allowed concentration (MAC) for effluent discharge in surface water bodies of classes 3 and 4, according to the Brazilian legislation (Brazil, 2011). Metal concentrations were: 0.1 mg/L Ag^+; 0.2 mg/L Cd^{2+}; 0.5 mg/L Pb^{2+}; 0.5 mg/L Cu^+; 0.5 mg/L Cu^{2+}; 1.0 mg/L Cr^{3+}; 0.1 mg/L Cr^{6+}; 2.0 mg/L Ni^{2+}; 5.0 mg/L Zn^{2+} and 0.01 mg/L Hg^{2+}. The simulated effluent was tested at pH 6.0, 7.0 and 9.0 (SE6, SE7 and SE9, respectively—Table 2). These pH are within the limit of tolerance of all test organisms and are within the range of permitted pH for effluent release in surface waterbodies (pH between 5 and 9). This approach enabled us to identify the most sensitive organisms even in situations where the bioavailability of metals was low. This approach was used only with the simulated effluent because it contained no other interfering substances. Algae bioassays were performed only with initial pH of 6.0 because algal activity increased the final pH to 8.2–8.5. Concentrations of effluents were expressed as a percentage following a dilution factor of two, considering the raw sample as 100%. Adjustments to pH were made using NaOH 1 mol/L and HCl 1 mol/L.

2.3. Characterization of samples

For all effluents, the parameters dissolved oxygen, pH, salinity, temperature, conductivity, and TDS were measured using a multi-parameter (HACH *Senslon* 378); total hardness, calcium and magnesium hardness, Ca and Mg concentration were measured by EDTA titration method following the procedure USEPA 2340C (Tables 1 and 2). For steel-mill effluents, concentrations of Ag, Al, Cd, Cr, Pb, Fe, Sn, Ni and Zn were measured. These metals are described as characteristic of this industrial process (Ahmadi et al., 2014; Neto et al., 2008). The acid-soluble metal fractions were determined for all effluent types (Tables 1 and 2) after acid