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Monitoring of toxicity of As(V) solutions by AMPHITOX test without and with treatment with zerovalent iron nanoparticles

Cristina S. Pérez Coll[a,b,c,*], Carolina Pabón-Reyes[a,b,d], Jorge M. Meichtry[a,d], Marta I. Litter[a,b,d]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[b] Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de Gral. San Martín, Campus Miguelete, Av. 25 de Mayo y Francia, 1650, San Martín, Provincia de Buenos Aires, Argentina
[c] Escuela de Ciencia y Tecnología, Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de San Martín, Campus Miguelete, Av.25 de Mayo y Francia, 1650, San Martín, Provincia de Buenos Aires, Argentina
[d] Gerencia Química, Centro Atómico Constituyentes, Comisión Nacional de Energía Atómica, Av. Gral. Paz 1499, 1650, San Martín, Provincia de Buenos Aires, Argentina

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ABSTRACT

Changes in toxicity of As(V) solutions from acute to chronic exposure have been evaluated by the AMPHITOX test. This test employs *Rhinella arenarum*, a widely distributed toad in Argentine areas. LOEC values were 6.37 and 1.88 mg L^{-1} for embryos and larvae, respectively, and serious sublethal effects have been observed. Toxicity of As(V) solutions has been also evaluated after treatment with zerovalent iron nanoparticles (nZVI). After 60 min of treatment with nZVI, As(V) removal was 77%, and neither lethal nor sublethal effects were observed. However, nZVI had to be eliminated before the bioassay because they caused adverse effects in both embryos and larvae. This work highlights the high sensitivity of *R. arenarum* to As(V), the relevance to assess toxicity on different periods of the lifecycle, and the need to expand exposure to As(V) to chronic times. The utility of the test for monitoring toxicity changes in As(V) solutions after nZVI treatment has been also shown.

1. Introduction

Regions with high natural arsenic (As) levels in groundwater are well-known in different countries of the world such as China, Hungary, India, Bangladesh and Vietnam. In Latin America, the problem affects at least 14 countries (Argentina, Bolivia, Brazil, Chile, Colombia, Cuba, Ecuador, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Peru and Uruguay), and the number of exposed people can be calculated to be around 14 million. The most critical areas are in Argentina, Chile and Mexico (Figueiredo et al., 2010). It is currently estimated that the population living in areas with As contaminated water in Argentina rises to about 4,000,000 people (Bardach et al., 2015).

Although minor sources of As in water come from anthropogenic activities, As pollution is mainly natural (Litter et al. 2010). Concerns on human health due to consumption of waters containing high As concentrations have prompted numerous research studies worldwide (Bardach et al., 2015; Hughes et al., 2011; Mandal and Suzuki, 2002). The phenomenon, known as Chronic Endemic Regional Hydroarsenicism (*hidroarsenicismo crónico regional endémico*, HACRE, in Spanish) in Argentina and in some other Latin American countries, affects largely human health. In many provinces of Argentina such as Santiago del Estero and Santa Fe, elevated levels of As in water and food as well as elevated excretion of As in urine of the population have been reported (Swiecky et al., 2006). Moreover, the International Agency for Research on Cancer (IARC) classifies As in Group 1, as there is sufficient evidence of a relationship between exposure to As and human cancer (IARC, 2018), and the World Health Organization recommends a limit of 0.01 mg As L^{-1} in drinking water (WHO, 2011). Although the consequences in humans have been very much studied (Vahter and Concha, 2001; Bardach et al. 2015), the possible impacts of As-contaminated water on the ecosystems and wildlife have been less investigated. Particularly, amphibians play a key role in the food webs, living near or in water reservoirs affected by the presence of As. The decline and extinction of amphibians is a major concern for biodiversity protection worldwide, alerted since the 1960's (Blaustein et al., 2003; Pérez Coll et al., 2017), because these organisms are extremely sensitive as they have permeable skin and eggs that readily absorb chemicals from the environment. Because of their high sensitivity, mainly during the

Abbreviations: nZVI, zerovalent nanoparticles; nZVIR, nZVI removed; TOP, Toxicity Profile; AMPHITOX, Amphibian Toxicity Test; ASol, AMPHITOX Solution; LC, Lethal Concentration; EC, Effective Concentration; LOEC, Low Observed Effect Concentration; TI, Teratogenic Index

* Corresponding author at: Instituto de Investigación e Ingeniería Ambiental, Universidad Nacional de Gral. San Martín, Campus Miguelete, Av. 25 de Mayo y Francia, 1650, San Martín, Provincia de Buenos Aires, Argentina.
E-mail address: perezcoll@unsam.edu.ar (C.S. Pérez Coll).

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developmental period of their life cycle, they are increasingly used for toxicity screening purposes. Standardized tests employing amphibian embryos and larvae are successfully used to evaluate the toxicity of hazardous substances and environmental samples (Herkovits and Pérez-Coll, 2003; Hoke and Ankley, 2005; Pérez Coll et al., 2017). The assessment of toxicity in both embryos and larvae allows to search an eventual differential susceptibility between them. The finding of the most sensitive period of the life cycle of a species is critical for conservative and ecological purposes, and these studies are important to make available the toxicity profile of a chemical as completely as possible (Pérez Coll et al., 2017).

Embryos and larvae of *Rhinella arenarum*, the common South American toad (Fam. Bufonidae), are used in AMPHITOX as a valuable biological material to perform toxicity tests (Herkovits et al., 2002; Pérez Coll et al., 2017). This toad exhibits an extensive neotropical distribution, including countries such as Argentina, Uruguay, Paraguay, Brazil and Bolivia. Contrary to other bioassays that only assess the acute toxicity of chemicals or the toxicity on a unique stage of the life cycle, AMPHITOX evaluates toxicity by using different endpoints (exposure times and developmental stages), offering a more complete information about the toxicity of a chemical species. Examination of Toxicity Profile (TOP) curves, based on the plot of the Lethal Concentration 10 (LC_{10}, considered as the Low Observed Effect Concentration value, LOEC, from a statistical approach), LC_{50} and LC_{90} (or LC_{100}), from acute to chronic exposure, allows the visualization of concentration- and time-exposure thresholds, as well as the range of concentrations that exert adverse effects in each case. These TOP curves provide, within a systemic toxicity approach, a more complete and appropriate set of data than the classical LC_{50}-48 h (Herkovits et al., 1997; Pérez-Coll and Herkovits, 2004).

It is well known that there is a differential susceptibility of living organisms to both As chemical species, As(III) and As(V), with the first form shown to be more toxic than the latest one (Gardner et al., 2017; Ventura-Lima et al., 2011). While As(III) dominates in reducing environments such as groundwater, As(V) is dominant in oxidizing environments such as surface waters (Chen et al., 2009), where amphibians live and breed. However, the toxicity of As(III) to several amphibians have been mainly reported, with LC_{50} values ranging from 0.04 mg L^{-1} for the eastern narrow-mouthed toad embryos (*Gastrophryne carolinensis*) (Birge et al., 1979), 0.25 mg L^{-1} for the green pond frog larvae (*Rana hexadactyla*) (Khangarot et al., 1985) to 4.45 mg L^{-1} for the marbled salamander (*Ambystoma opacum*) (Birge, 1978). For embryos of *Xenopus laevis*, the South African clawed frog, a 96-h LC_{50} of 470.5 mg L^{-1} for As(V) was estimated (Gornati et al., 2002), indicating a low sensitivity of *X. laevis* to As(V). Mortality values for As (V), for other freshwater organisms (microinvertebrates) have been reported, such as 48-h LC_{50} of 2.44 mg L^{-1} for *Daphnia carinata* (He et al., 2009) and 3.9 mg L^{-1} for *Daphnia pulex* (Shaw et al., 2007).

In recent years, several studies on As removal from water were performed based on the use of zerovalent iron nanoparticles (nZVI) as innovative treatment. In comparison with other methods, nZVI can simultaneously remove As(V) and As(III) without previous oxidative treatment and does not require the use of additional chemicals, attaining very good removal efficiencies in very short times (Levy, 2013; Litter et al., 2010; Morgada et al., 2009; Mosaferi et al., 2014; Rahmani et al., 2010; Rahmani et al., 2011; Ramos et al., 2009).

Previously, we have demonstrated the usefulness of the AMPHITOX test for monitoring the toxicity changes after the application of highly efficient technologies for the removal of difficult contaminants. In that case, TiO_2-heterogeneous photocatalysis was used to drastically decrease Cr(VI) concentrations from aqueous systems, and AMPHITOX represented an optimal test to detect the abatement of the heavy metal during the application of this innovative methodology (Hojman et al., 2015).

The aims of the present work have been: i) to evaluate the toxic effects of As(V) on *R. arenarum* embryos and larvae from acute to chronic exposure, and ii) to assess the usefulness of AMPHITOX for monitoring changes in the toxicity of As(V) solutions submitted to nZVI treatment.

2. Materials and methods

2.1. Reagents

The zerovalent iron nanoparticles (nZVI) were provided by NANO IRON S.R.O. (Czech Republic) as NANOFER 25 in aqueous suspension (C_{Fe} = 242 mg L^{-1}). Sodium arsenate dibasic 7-hydrate ($Na_2HAsO_4.7H_2O$, Baker) and all other chemicals were of the highest purity. In all experiments, Milli-Q water was used (resistivity = 18 MΩ cm).

2.2. As removal with nZVI

To 200 mL of a 10 mg L^{-1} As(V) aqueous solution (pH 7) in a thermostatted (25 °C) glass cylindrical cell open to air, drops of nZVI suspension were added to reach 745 mg L^{-1} nZVI (1:100 As:Fe molar ratio), and the system was stirred with a paddle stirrer, open to the air. Samples were taken at 5, 15, 30, 45 and 60 min, nZVI were removed by centrifugation using an Eppendorf AG 5810 centrifuge, and the supernatants were used in toxicity bioassays. The removal experiment was performed by duplicate and the experimental error was never higher than 2% of the initial value, as calculated by standard deviation among the duplicate experiments; error bars for the averaged experiments are shown in the corresponding figure. These samples will be called nZVIR (nZVI removed) 5–60 min. As(V) concentration was determined in each sample by spectrophotometry, using the arsenomolybdate complex, measuring at 868 nm (Lenoble et al., 2003).

2.3. Obtaining Rhinella arenarum embryos and larvae

Rhinella arenarum adults weighing approximately 200–250 g were acquired in Lobos, Buenos Aires province, Argentina (35°11′ S; 59°05′ W). Toad care, breeding, embryo acquisition and analysis were conducted according AMPHITOX protocols (Herkovits and Pérez-Coll, 2003; Pérez Coll et al., 2017). Oocytes were fertilized in vitro using fresh sperm suspended in AMPHITOX solution, ASol (36.0 mg L^{-1} NaCl, 0.5 mg L^{-1} KCl, 1.0 mg L^{-1} $CaCl_2$ and 2.0 mg L^{-1} $NaHCO_3$). For bioassays with larvae, embryos were kept in ASol and maintained at 20 ± 2 °C, until organisms reached the complete operculum stage, S.25 (Del Conte and Sirlin et al., 1951).

2.4. Toxicity bioassays

Three independent experiments were performed in which 2 groups of ten embryos (S.2) and early larvae (S.25) were randomly placed by triplicate in covered 10 cm-diameter glass Petri dishes containing 40 mL of solution under different conditions. To evaluate the lethal and sublethal effects of As(V) on *R. arenarum* embryos and larvae and to construct the survival and TOP curves, the following conditions were set: 1) absolute control: ASol, 2) 2–10 mg L^{-1} As(V) solution in ASol, assaying the following five As(V) concentrations: 2, 4, 6, 8 and 10 mg L^{-1}. To assess the usefulness of AMPHITOX for monitoring changes in the toxicity of As(V) solutions submitted to treatment with nZVI, the following conditions were used: 1) absolute control: ASol, 2) 10 mg L^{-1} As (V) solution in ASol, 3) 10 mg L^{-1} As(V) solution treated with 745 mg L^{-1} (13.34 mM) nZVI for the indicated reaction time and then centrifuged (nZVIR 5–60 min), 4) supernatant of a pure (no As) 745 mg L^{-1} nZVI aqueous suspension centrifuged after 60 min (nZVIR), and 5) pure (no As) 745 mg L^{-1} nZVI aqueous suspension without centrifugation, 60 min after its preparation (nZVI). In conditions 3), 4) and 5), AMPHITOX salts were added to the solutions before exposure to achieve the concentration of the absolute control. Prior to testing and renewal of

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