Ecotoxicology and Environmental Safety 122 (2015) 545–550

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Abatement of toxicity of effluents containing Cr(VI) by heterogeneous photocatalysis. Toxicity assessment by AMPHITOX assay

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ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form
21 September 2015
Accepted 22 September 2015

Keywords:
Heterogeneous photocatalysis
Hexavalent chromium
AMPHITOX test

ABSTRACT

Toxicity of a Cr(VI) solution before and after treatment by TiO_2 heterogeneous photocatalysis (HP) was performed with AMPHITOX bioassay. Changes in toxicity on *Rhinella arenarum* larvae for 10-d were monitored after exposure to an untreated Cr(VI) solution and to the same solution after HP treatment. The HP treatment of a 41.60 mg L^{-1} Cr(VI) solution reduced to 37.5% the concentration of the metal ion. A 10-fold reduction in toxicity at acute exposure (72 h) and 150-fold reduction in toxicity after 240 h was found. Further, the LOEC value increased from 0.001% for the untreated solution to 0.153% after HP treatment. Moreover, the safe concentration in untreated solution corresponded to 0.0001% sample, and it was 0.01% after the treatment, i.e., 100 times higher. A saving of water of about 100,000 L per L of effluent would be possible through dilution to allow safer concentrations for discharge; the saving would reach the highest value (1,000,000 L per L) at 240 h. Sub-lethal effects were completely absent in larvae exposed to the treated solution. The AMPHITOX test allowed to detect chronic effects at low Cr concentrations, i.e. at environmentally relevant levels.

1. Introduction

The presence of chromium in wastewater is a source of constant concern as the metal is widely used in several industries, e.g. steel, electronics, metal finishing, paints, wood preservatives (the chromated copper arsenate (CCA) process), leather tanning agents, etc (Downing et al., 2002; Mosquera-Corral et al., 2007; Assem and Zhu, 2007). Its presence in water bodies causes high environmental and social impact (WHO, 1993), and recently, several incidents related to pollution of water by Cr(VI) have been reported (Parliament of New South Wales, 2001; Vasilatos et al., 2008). Among the stable chromium species, hexavalent chromium (Cr (VI)) is the most toxic one, and has been classified by IARC in Group I, due to sufficient evidence of carcinogenicity and mutagenic effects on humans (Katz and Salem, 1993). A high mobility in water favors its occurrence in water bodies. The maximum level of Cr(VI) in drinking water recommended by the WHO is 0.05 mg L^{-1} (WHO, 1993). In contrast, Cr(III) is considered non-toxic or of very low toxicity, presenting a lower mobility in water (Katz and Salem, 1993; U.S. Environmental Protection Agency, 1999; IARC Chromium (VI) Compounds, 2012).

The discharge of industrial wastes into water bodies without appropriate treatment causes a decrease in the water quality for living organisms and human health. Large volumes of water are used to dilute effluents to reach the threshold levels required for the discharge of dangerous pollutants. Although the CCA-treated wood process consumes small amounts of water (U.S. EPA, 2001), other processes related to chromium need huge quantities. For example, the chromium metal finishing process consumes between 2 and 20 L water per m^2 of treated surface, generating a chromium rich effluent. Tanneries consume from 20 to 100 m^3 of water per ton of raw leather, generating about 60 m^3 of wastewater (Mosquera-Corral et al., 2007). Although tanneries mainly use Cr(III), Cr(VI) has been detected after heating wastes in the presence of oxygen or other oxidants as such as MnO_2 (Apte et al., 2005).

Conventional treatments for Cr(VI) removal from water involve reduction to Cr(III) with different chemicals, with the subsequent economical costs and generation of hazardous wastes (Lan et al., 2006; Barrera-Díaz et al., 2012). Advanced Oxidation Technologies (AOTs) for treatment and disposal of industrial wastes have the potential to drastically reduce water consumption (Litter, 2005;

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http://dx.doi.org/10.1016/j.ecoenv.2015.09.036
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Wang and Xu, 2012). One of these AOTs, heterogeneous photocatalysis with TiO_2 (HP), has been thoroughly studied for Cr(VI) reduction, with rapid complete Cr(VI) transformation to Cr(III) (see e.g. Refs. Litter, 1999; Wang et al., 2008; Litter and Quici, 2014; Litter, 2015; Meichtry et al., 2014). However, generated oxidative radicals, intermediate species, or TiO_2 itself might produce adverse effects on the environment. The analytical determinations to separate and characterize the products and to gain information about the toxicity of the treatment can be very complex and expensive. In contrast, simple and inexpensive bioassays represent a very useful tool in the safe implementation of the treatment technologies (Konstantinou and Albanis, 2003), a scarcely studied aspect in the case of photocatalytic systems (Bozzi et al., 2004). The eventual detoxification of different matrices by the use of AOTs (Konstantinou and Albanis, 2003; Bozzi et al., 2004) and the acute toxicity of TiO_2 nanoparticles to freshwater aquatic organisms (Hall et al., 2009) have been assessed by different techniques, e.g., luminescent bacteria (Bozzi et al., 2004; Hall et al., 2009; Oturan et al., 2008; Plahuta et al., 2014), algae (Hall et al., 2009; Oturan et al., 2008; Andreozzi et al., 2004), microinvertebrates (Bozzi et al., 2004; Oturan et al., 2008; Plahuta et al., 2014; Andreozzi et al., 2004; Nagel-Hassemer et al., 2011) and fishes (Hall et al., 2009; Plahuta et al., 2014; Nagel-Hassemer et al., 2011). However, chronic toxicity studies during application of AOTs have scarce records.

Standardized toxicity bioassays are very useful to evaluate the toxicity of a variety of matrices and processes. Amphibians are very convenient for toxicity tests because they are ecosystem key species, with biphasic life cycle and high permeability of epithelia, making them highly susceptible to xenobiotics (Herkovits et al., 2002; Ferrari et al., 2005; Herkovits and Pérez-Coll, 2003; Mann, 2005). The AMPHITOX test (Herkovits and Pérez-Coll, 2003), which employs developmental stages of *Rhinella arenarum*, the South American toad, was developed to obtain ecotoxicological reports of chemical impacts on Latin American species, providing information of the exposure to a wide spectrum of matrices, and exploring effects in both acute and chronic exposures. Chronic toxicity results are obtained by expanding the evaluation time, which is a great advantage since the majority of toxicity studies focus only on acute conditions. Chronic toxicity assessment is environmentally mandatory because the most frequent contamination events occur at low concentration (Herkovits et al., 2002; Herkovits and Pérez-Coll, 2003). On the other hand, although the evaluation of lethality endpoint just requires quick and easy analyses, with little observer expertize, sub-lethal effects, which are subtle in many cases, would remain hidden. The evaluation of sub-lethal effects is highly valuable because the indirect effects could hamper the continuity of the affected populations. The analysis of all these parameters renders the toxicity tests more realistic for management and conservation decisions.

Lethal concentration (LC), i.e., the effluent concentration that causes death in a given percent of the exposed population (e.g., LC10 and LC50 for 10% and 50% of the test population, respectively) and lowest-observed-effect-concentration (LOEC), i.e., the lowest concentration of effluent that causes adverse effects on the test organisms (US EPA, 2002), are the most used toxicological parameters. From a statistical approach, the LC10 can be considered the LOEC value. LC50 and LOEC values allow to construct toxicity profiles to estimate the changes in toxicity of an effluent during an AOT treatment (EPA, 2002; Herkovits and Helguero, 1998; Pérez-Coll and Herkovits, 2004). However, the LOEC is not environmentally conservative because it implies the possibility that 10% organisms die. A better parameter, established by the US EPA (2002), is the "safe concentration" (SC), i.e., the highest concentration of toxicant at which there is no significant death of exposed organisms, and which allows the normal propagation of aquatic life in receiving waters. SC is a biological concept, whereas LOEC is a statistically estimated concentration.

In this work, the toxicity evolution by AMPHITOX of a Cr(VI) solution during a HP treatment, and the efficiency of HP as treatment for a wastewater containing Cr(VI), have been evaluated, with focus on the reduction of the water volume necessary to discharge the effluents.

2. Material and methods

2.1. Chemicals

Cr(VI) ($K_2Cr_2O_7$, 99.9% Merck), and all other chemicals were reagent grade. For pH adjustments, 1 M HCl (70%) or $NaHCO_3$ (c) were used. TiO_2 Degussa P25 was provided by Degussa AG Germany (now Evonik) and used as received. In all experiments, Milli-Q water was used (resistivity = 18 MΩ cm).

2.2. Irradiation procedure

The TiO_2 suspension (1 g L^{-1}) was prepared by mixing 0.25 g of the photocatalyst with 250 mL of a 41.60 mg L^{-1} Cr(VI) solution; then, the suspension was adjusted at pH 3 and ultrasonicated for 2 min. The irradiation setup was the same as used previously (Kleiman et al., 2011). Briefly, four 10 cm diameter borosilicate glass Petri dishes, whose caps acted as filters for wavelengths lower than 300 nm, were irradiated simultaneously; two Petri dishes contained 30 mL of the Cr(VI)/TiO_2 suspension, and the other two contained 30 mL of the same Cr(VI) solution without TiO_2. Irradiation time was 2 h and magnetic stirring was used. A Phillips HPA 400 S UV lamp ($\lambda > 310$ nm, maximum emission at 365 nm) was employed. The mean UV irradiance was 4800 μW cm^{-2}, measured with a Spectroline model DM-365 XA radiometer.

Samples of 1 mL were periodically taken and diluted 1/10 with water for analysis; the suspensions were previously filtered through 0.22 μm cellulose acetate Sartorius membranes. Changes in Cr(VI) concentration were monitored by the absorbance at 352 nm with a UV–vis HP 8453 A spectrophotometer (Wei et al., 1993). Experiments were done by quadruplicate, with a relative standard deviation lower than 5%.

2.3. Acquisition of Rhinella arenarum larvae

Adults of *R. arenarum*, weighing approximately 200–250 g, were obtained in Lobos (Buenos Aires province, Argentina, 35°11′ S; 59°05′ W). Toad care, breeding, embryo acquisition and analysis were conducting according to AMPHITOX protocols (Herkovits et al., 2002; Herkovits and Pérez-Coll, 2003). Briefly, ovulation of a female toad was induced by an intraperitoneal injection of homologous hypophysis suspended in 1 mL of AMPHITOX solution (AS), which contained 36 mg L^{-1} NaCl, 0.5 mg L^{-1} KCl, 1 mg L^{-1} $CaCl_2$ and 2 mg L^{-1} $NaHCO_3$ in distilled water. Oocytes were fertilized *in vitro* using fresh sperm suspended in AS. Embryos were kept in AS at 20 ± 2 °C until they reached the complete operculum stage, S.25 (Del Conte and Sirlin, 1951).

2.4. Solutions used in the bioassays

Sample A was a 41.60 mg L^{-1} Cr(VI) solution in distilled water at pH 3. Sample B was sample A after irradiation in the presence of 1 g L^{-1} TiO_2 for 2 h ([Cr(VI)] = 15.60 mg L^{-1}). Sample C was sample A stirred for 2 h under UV light ([Cr(VI)] = 41.60 mg L^{-1}). Sample D was a 1 g L^{-1} TiO_2 suspension in water at pH 3 stirred under UV irradiation for 2 h. Sample E was the same as sample D

Table 1
Solutions for the bioassays.

Sample	A (untreated)	B (HP treated)	C (UV irradiated, no TiO_2)	D (UV irradiated TiO_2 suspension)	E (dark TiO_2 suspension)
Cr(VI) (mg/L)	41.60	15.60	41.60	0	0
TiO_2 (g/L)	0	1	0	1	1
UV irradiation	No	Yes	Yes	Yes	No

but in the dark. Table 1 summarizes the composition of the solutions used in the bioassays.

After 2 h stirring, all suspensions (Samples B, D and E) were filtered to remove TiO_2 particles. The obtained solutions and samples A and C were neutralized at pH 7–8 with $NaHCO_3$ (c). A control AS solution, as maintaining medium for *R. arenarum* larvae, was also tested.

2.5. Toxicity bioassays

The toxicity bioassays were performed with larvae at complete operculum stage (S.25) (Del Conte and Sirlin, 1951) following the AMPHITOX conditions (Herkovits et al., 2002; Herkovits and Pérez-Coll, 2003). For each condition, triplicate batches of ten larvae were placed in covered 10 cm diameter glass Petri dishes containing 30 mL of each sample. A preliminary 24-h toxicity test, by exposing larvae to samples without dilution, was performed. Due to the high acute toxicity registered, eight dilutions of samples A and B between 100% (41.60 and 15.60 mg L^{-1} for A and B, respectively) and 0.0001% (0.00004 and 0.00001 mg L^{-1} for A and B, respectively) were prepared. The lethal and sub-lethal data at different times were obtained from the same dilution series. Prior to testing and renewal of solutions, pH, conductivity and dissolved oxygen were measured to ensure acceptable levels. Larvae were acutely and chronically exposed by extending the observations up to 240 h. Bioassays were semistatic: test solutions were entirely replaced every 48 h, and temperature was maintained at 20 ± 2 °C throughout the experiments. Dead larvae were removed and survival was daily evaluated. Larvae were fed with Tetra Color Fin Sinking Granules for Goldfish *ad libitum* every other day, coincident with the changes of the solutions.

The care and use of animals were conducted in accordance with the guidelines of the international standards on animal welfare (Canadian Council on Animal Care in Science, 1993).

2.6. Data analysis and examination of sub-lethal effects

Survival, anomalies and behavioral disturbances were assessed and recorded every 24 h, discarding dead individuals.

The SC, i.e. the highest concentration of toxicant for which there was no significant death of exposed organisms with respect to controls (EPA, 2002), was empirically established for samples A and B. Sub-lethal effects were studied with a Zeiss StemiDV4 stereoscopic microscope and identified according to the Atlas of Abnormalities (Bantle et al., 1998), analyzing all surviving individuals.

Behavioral alterations such as abnormal fast rotations which are a sign of neurotoxic stress, lying on the lateral or dorsal side, and abnormal swimming patterns were evaluated. Smooth movements of the Petri-dishes, followed by stimulation with a light source were done. In the case of no response, soft mechanic stimulation with a glass rod was made and finally heartbeat was checked with the stereoscopic microscope. The incidence of sub-lethal effects was calculated from the empirical data obtained of the bioassays according to the following expression:

$$\%\ \text{sub-lethal effects} = (\text{number of abnormal larvae/total number of larvae}) \times 100 \quad (1)$$

2.7. Statistical analyses

Lethality data were statistically analyzed according to common regression analysis by the USEPA Probit Program (US EPA, 1988). Toxicity profiles, as isotoxicity curves, were plotted based on LC10 and LC50 with their confidence limits at different times obtained by probits (Herkovits and Helguero, 1998; Pérez-Coll and Herkovits, 2004). The LC10 was considered the LOEC value; therefore, the lowest concentration of sample A and sample B in which there was an observable effect on the survival of the exposed organisms was statistically estimated. To establish statistical differences among the different LC10 and different LC50 values obtained, a comparison was made, considering the statistically significant difference when the higher LC50/lower LC50 ratio exceeded the critical value (95% confidence interval) established by APHA (American Public Health Association, 2005). Comparisons were made among samples A, B and controls.

3. Results and discussion

3.1. Photocatalytic experiments

The irradiation for 120 min of a Cr(VI) solution (41.60 mg L^{-1} = 0.8 mM) with addition of TiO_2 (1 g L^{-1}) at pH 3 allowed a diminution of 62.5% of the initial Cr(VI) concentration with transformation to Cr(III) (Fig. 1). Experimental conditions were the same as used in previous works of the group (Litter, 1999; Litter and Quici, 2014; Litter, 2015; Meichtry et al., 2014), the

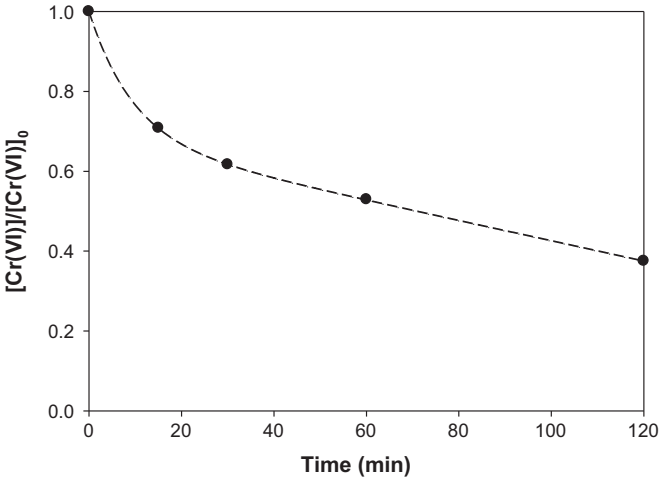

Fig. 1. Temporal evolution of normalized Cr(VI) concentration during a heterogeneous photocatalytic experiment. Conditions: $[Cr(VI)]_0 = 41.60$ mg L^{-1}, $[TiO_2] =$ 1 g L^{-1}, pH 3, $\lambda > 310$ nm, $\lambda_{max} = 365$ nm, $E = 4800$ μW cm^{-2}. The dashed line is only for a better visualization of the experimental points and does not obey to any fitting equation.

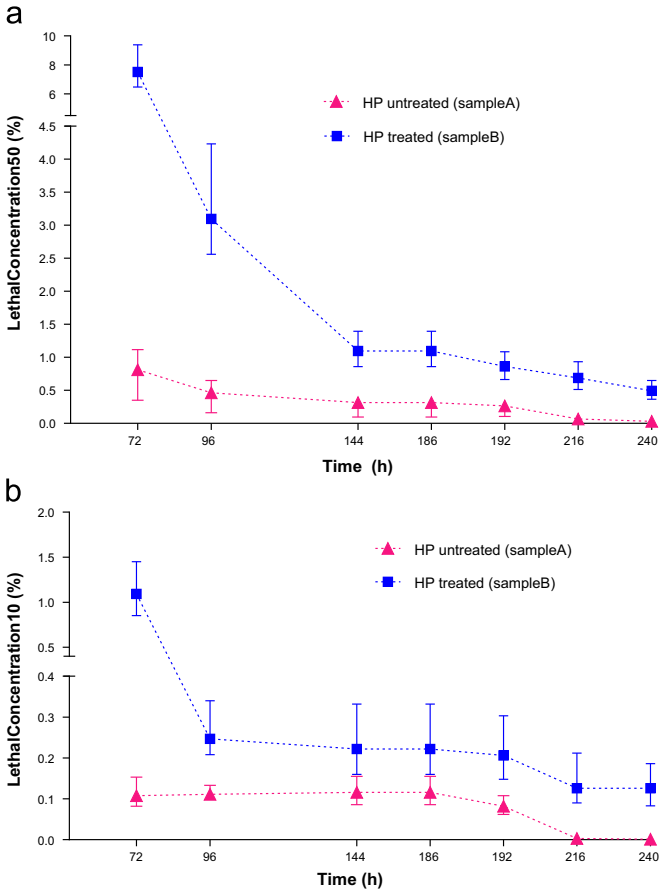

Fig. 2. Toxicity profile curves based on LC50 (a) and LC10 (b) and their confidence limits of *R. arenarum* larvae exposed to samples A and B for 240 h.

Cr(VI) concentration being that typically found in wastewaters (Owlad et al., 2009).

The temporal profile indicates a rapid reaction during the first 30 min followed by a decrease of the reaction rate. For this reason, the irradiation was stopped at 2 h and not after the total Cr(VI) depletion. As previously observed [Meichtry et al., 2014 and references therein], Cr(VI) removal was observed only in the presence of TiO_2 and under UV irradiation.

3.2. Toxicity bioassays

The toxicity of Cr(VI) on *R. arenarum* larvae was time-dependent. Fig. 2(a) and (b) show the toxicity profile curves (LC50 and LC10, respectively) of *R. arenarum* larvae (S.25) exposed to the untreated sample A and to the HP treated sample B, depicting the beneficial effect of HP reflected on larval survival. Upper curves, corresponding to the HP treated solution, reflect the decrease of Cr (VI) concentration and the consequent toxicity fall.

The figures show a fall in LC data along time, indicating that it is necessary to dilute the samples to achieve the same lethality, i.e., 50% or 10%. Thus, the sharp survival fall between 72 and 96 h exposure (acute period) takes place mainly for the treated solution; however, from this time on, a similar shape of toxicity curves of Cr(VI) for both treated and untreated conditions was observed, reaching relatively constant values during the extended exposure, but maintaining significant differences between the absolute LC values.

Considering Fig. 2(a), i.e., in terms of the classic LC50, the HP treatment yielded a difference for the acute toxicity (72 h) more than 9 times higher for the treated sample (7.51%) than the untreated sample (0.81%). When the exposure was extended to 240 h (chronic period), the difference climbed to nearly 16 times more (0.03% and 0.49%, respectively).

Table 2
LOEC values based on LC10 for *R. arenarum* larvae exposed to samples A and B for 72 h and 240 h and volume of water needed for dumping 1 L of effluent.

Sample	LOEC 72 h (%)	LOEC 240 (%)	V_{H_2O} to dump 1 L of effluent with Cr(VI) at 72 h (L)	V_{H_2O} to dump 1 L of effluent with Cr(VI) at 240 h (L)
A	0.108	0.001	925.9	100,000
B	1.093	0.153	91.5	654

For LC10 (Fig. 2(b)), the difference jumped to one order of magnitude between the toxicity of the untreated sample (0.11%) and the treated sample (1.09%) at 72 h, and more than 150 times when the exposure was extended to 240 h (0.001% and 0.153%, respectively).

These results reflect the fall in the Cr(VI) concentration in the HP treated solution, showing the efficiency of this technology for water detoxification. This suggests, additionally, that TiO_2 does not generate new chemical species that, in contact with Cr(VI) solutions, could cause toxicity after TiO_2 removal. The combination TiO_2/UV is responsible for reducing the initial toxicity of the solution but the elimination of TiO_2 from the treated effluent is mandatory to assure the absence of toxicity (Hall et al., 2009).

Considering our remarkable results and assuming the LOEC values for larvae exposed to samples A and B for 72 and 240 h, respectively, Eq. (2) can be used to estimate the volume to which the sample must be diluted to reach the harmless concentrations for discharge into water bodies (Table 2).

$$V_i \times C_i = V_f \times C_f \quad (2)$$

where V_i and V_f are the initial and final volumes of the effluent, and C_i and C_f are the initial and final (LOEC) Cr(VI) concentrations for each case.

The calculation* indicates that a saving of 834.4 L water at 72 h and of 99,346 L at 240 h per L of effluent can be obtained.

According to that indicated in Section 2.6, the SC of the untreated sample on *R. arenarum* larvae empirically obtained in this study corresponded to the 0.0001% sample, while it was 0.01% after the treatment, i.e., 100 times higher. Thus, taking into account the concept of SC (EPA, 2002), the saving water would reach the highest value of 1 L × 100/0.0001 = 1,000,000 L per L of effluent at 240 h. This is an important result as, according to Nriagu (1988), "it has been estimated that the toxicity of all the metals being released annually, into the environment, far exceeds the combined total toxicity of all the anthropogenic radioactive and organic wastes as measured by the quantity of water needed to dilute such wastes to the drinking water standard".

Fig. 3 shows the survival curves of *R. arenarum* larvae (S.25) exposed to different control samples. By comparing the absolute control (AS) with sample A, it can be seen that the larval survival for sample A falls totally apart at 72 h. Larvae exposed to solutions D and E (both after TiO_2 filtration) did not show any significant differences with respect to AS. This result is very relevant, because it indicates that the treatment does not cause toxicity if TiO_2 is removed from the effluent. There were no differences in toxicity between Cr(VI) samples free of TiO_2 before and after UV exposure

* At 72 h, for 1 L of untreated initial effluent, V_f = 1 L × 100/0.108 = 925.9 L; for the treated effluent, V_f = 1 L × 100/1.093 = 91.5 L; the difference (925.9 L − 91.5 L) gives a saving of water of 834.4 L. A similar calculation gives V_f = 1 L × 100/0.001 = 100,000 at 240 h; V_f = 1 L × 100/0.153 = 654 L; the difference (100,000 L − 654 L) gives a saving of water of 99,346 L.

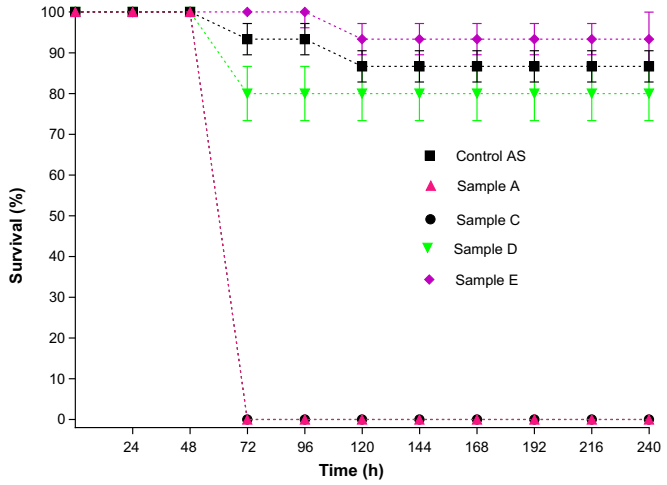

Fig. 3. Survival curves of *R. arenarum* larvae (S.25) exposed to sample A and different control samples.

Table 3
Percentages of sub-lethal effects caused by different conditions on *R. arenarum* larvae registered at the end of a chronic exposure (240 h).

Condition*	Sub-lethal effects (%)
0.0001% A (SC)	3.33
0.001% A (~LC 10)	6.66
0.05% A (~LC 50)	100
0.1% A	Dead
0.01% B (SC)	3.33
0.1% B (~LC 10)	6.66
0.5% B (~LC 50)	80
5% B	Dead
0.001% C	6.66
100% D	3.33
100% E	3.33
AS (absolute control)	3.33

* Larvae were exposed to different diluted solutions of samples A, B and C and to 100% control samples D and E (Section 2.5). Concentrations higher than 0.1% A and 5% B caused lethality of 100% of larvae. Larvae exposed to diluted solutions of control sample C showed the same malformation incidence than larvae exposed to the same diluted solutions of sample A.

(samples A and C, respectively), indicating that UV irradiation alone does not change the toxicity, and that the use of the photocatalyst is essential for the success of the treatment.

3.3. Sub-lethal effects

The stereoscopic studies of *R. arenarum* larvae after exposure to the untreated Cr(VI) solution showed, as expected, generalized cellular desquamation, collapse of the coelomic spaces, edemas and abnormal behavior. Table 3 shows the percentages of sub-lethal effects caused on *R. arenarum* larvae under different conditions at the end of the chronic exposure (240 h) and indicates the wide range between the percentages of dilution that caused a similar incidence of sub-lethal effects. For example, a similar sub-lethal effect was obtained in 100% of larvae exposed to the 0.05% dilution of the untreated solution (A) and in 80% of larvae exposed to the 0.5% of the HP treated solution (B). These alterations have been also reported for other amphibians exposed to Cr(VI) during their embryo-larval development, as the cases of *Rana tigrina* (Abbasi and Soni, 1984) and *Xenopus laevis* (Bosisio et al., 2009). However, these effects were completely absent in larvae exposed to the HP treated solution, reflecting the fall of the Cr(VI) concentration during the treatment. These sub-lethal effects are non Cr-specific, as they were also observed in *R. arenarum* larvae exposed, for example, to Pb (Pérez-Coll and Herkovits, 1990), Cd (Pérez-Coll and Herkovits, 1996), Ni (Sztrum et al., 2011) and Cu (Aronzon et al., 2011).

4. Conclusions

To our knowledge, this is the first work on the evaluation of the toxicity of solutions containing metal ions such as Cr(VI), treated by heterogeneous photocatalysis. The evaluation by AMPHITOX indicated that HP reduced the initial toxicity of a Cr(VI) solution on *R. arenarum* larvae in about 150 times during chronic exposure. This fact means a saving of water resources of around 835 L per each L of effluent for acute exposure, and a water saving of almost 100,000 L/L after chronic exposure. Moreover, by considering the SC criterion, the saving water would reach 1,000,000 L per L of effluent. The importance of saving water is relevant not only from an ecological but also from an industrial point of view. It is pertinent to mention that the presence of organic matter in real waters does not interfere with the HP technology; moreover, most organic compounds exert a synergistic effect on the photocatalytic efficiency for Cr(VI) reduction (Litter, 1999; Litter and Quici, 2014; Litter, 2015). Also, it is very well known that organic matter itself diminishes the toxic effects caused by Cr(VI) in natural waters by means of its reduction to Cr(III) (Fendorf, 1995).

The solution coming from the HP treatment, after TiO_2 removal, did not cause toxicity to the organisms, and sub-lethal effects were completely absent in exposed larvae. The evaluation of sub-lethal effects is highly valuable because the indirect effects could hamper the continuity of affected populations.

Based on these results, it can be concluded that: 1) TiO_2 does not generate new chemical species that in contact with Cr(VI) solutions could cause toxicity after TiO_2 removal, and 2) its combination with UV light (HP) is responsible for reducing the initial toxicity of an effluent. It is important to indicate, however, that TiO_2 must be eliminated from the effluent to assure the lack of toxicity. The safety of the method allows to confirm that HP is an environmentally sustainable technology for a Cr(VI)-containing wastewater treatment, or to remediate focal events of metal contamination in the environment.

The application of AMPHITOX, in contrast to the majority of tests performed under acute conditions, allowed to detect chronic effects at low Cr concentrations, i.e., at environmentally relevant levels. As an additional value, this test allowed to employ a native species that provided relevant information about the local impact of Cr(VI).

Acknowledgments

This work was performed as part of Agencia Nacional de Promoción Científica y Tecnológica PICT-512-2006, PICT-0463-2011 and PICT 0245-2013 projects.

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