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A Multidisciplinary Approach to Evaluate the Efficiency of a Clean-Up Technology to Remove Mercury from Water

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Abstract A microporous material denoted ETS-4 was used as the decontaminant agent to treat water with a low level of Hg contamination. The effectiveness of the treatment was evaluated by assessment of the efficiency of Hg removal and ecotoxicological responses. The results showed that under highly competitive conditions the removal of Hg ranged between 58 % and 73 % depending upon the initial Hg concentration, and that Hg removal was reflected in decreased toxicity to some organisms. The ecotoxicological data indicated that the bacterium *Vibrio fischeri* was the least sensitive organism tested, as no toxicity was observed in either pre- or post-treatment waters. *Daphnia magna* was highly sensitive to Hg. Mercury removal by ETS-4 was not sufficient to completely remove the toxicity of Hg to *D. magna*. However, it was effective in the complete reduction of toxicity for the green alga, *Pseudokirchneriella subcapitata*.

Keywords Mercury · Water remediation · Chemical efficiency · Ecotoxicological effects · Titanosilicate

Mercury (Hg) is an element that occurs naturally in the environment essentially from the weathering of rocks and volcanoes. However, due to its chemical and physical properties, it has been widely used in many fields. The enormous applicability of Hg has resulted in increased amounts of metal released from anthropogenic sources. This Hg flows between the several compartments of the biosphere. Although, Hg is toxic in all forms and in all compartments, it is in the hydrosphere that this metal gives rise to a high level of concern, since it is easily converted into methylmercury, a strong neurotoxin that bioaccumulates and bioamplifies along the food chain (Coelho et al. 2008).

Natural inputs combined with the global anthropogenic sources make Hg contamination a planetary-scale problem, and strict environmental policies on metal discharges have been enforced. Consequently, in the last decade a large number of studies about mercury removal from water by several technologies and/or materials have been reported (Imani et al. 2011; Ghasemi et al. 2012; Figueira et al. 2011; Lo et al. 2012; Lopes et al. 2011; Lv et al. 2012). However, chemical analysis alone is not suitable to explain the effects of contaminants to biota. Consequently, it is crucial to establish a multidisciplinary approach to determine cause-and-effect relationships between concentration of chemicals and consequent environmental damage. Thus, for a proper evaluation of the feasibility of a water treatment, an approach combining both chemical and ecotoxicological assays should be designed, and to the best of our knowledge, the studies that follow this approach are scarce (Mishra and Tripathi 2008).

The microporous titanosilicate ETS-4 [$Na_9Ti_5Si_{12}O_{38}(OH)\cdot 4H_2O$], a synthetic analogue of the mineral zorite, is one material that has been used successfully as a cation exchanger to remove metals from water (Otero et al. 2009; Popa and Pavel 2012; Popa et al. 2006). Moreover in spiked (50 μg/L Hg^{2+}) ultra-pure water, the ion-exchange efficiency of this material can be superior to 99 %, achieving Hg^{2+} concentrations in solution lower than the guideline value for drinking water quality (1 μg/L,

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Directive 98/83/EC) (Lopes et al. 2009). Nonetheless, until now only the chemical efficiency of the ion-exchange process, under the influence of the main operating parameters, has been investigated (Lopes et al. 2010), and a significant gap exists in our knowledge of the ecotoxicological consequences of the process.

Accordingly, the main goal of this study was to evaluate the ecotoxicological consequences of an ion-exchange process for Hg removal by assessing the water toxicity to organisms from different taxonomic groups that exhibit different key functions at the ecosystem level. To accomplish the proposed goal, we carried out the ecotoxicological assays in the water before and after the clean-up technology and the results obtained were related with the chemical efficiency of the process.

Materials and Methods

To assess both chemical efficiency and ecotoxicological effects of the water treatment, stirred batch experiments (i.e. fixed volume in a closed vessel) were carried out at 20 ± 2°C using American Society for Testing and Materials (ASTM) hard water medium (ASTM 1992) spiked with an initial concentration of Hg^{2+} ($C_{Hg,0}$) (100 and 50 μg/L), and 75 mg/L of ETS-4 particles. ETS-4 particles were synthesized as previously described by Lopes et al. (2009).

All Hg^{2+} solutions were prepared by dilution from a stock solution of $Hg(NO_3)_2$ at 1,000 ± mg/L. Before the beginning of each experiment, an aliquot of the Hg^{2+} solution (10 mL) was collected to check the initial Hg^{2+} concentration. The ion-exchange experiments began when the accurately known amount of ETS-4 was added to the Hg^{2+} solution. After equilibration time, one-half of the volume of each solution was filtered through a 0.45-μm Millipore membrane to separate ETS-4 particles from solution. Afterward, both solutions, with and without ETS-4 particles, were transferred to Schott Duran bottles (500 mL) and kept at 4°C until Hg^{2+} quantification and completion of bioassays. Mercury analyses were performed by cold vapor atomic fluorescence spectroscopy, using $SnCl_2$ (10 % m/v) as a reducing agent. The Hg^{2+} concentration was quantified through a calibration curve (0.0–0.5 μg/L). In this range, the limit of detection of the method was 0.02 μg/L and the precision and accuracy (expressed, respectively, as relative standard deviation and relative error) were <5 %. A blank experiment (without Hg^{2+}) and a control experiment (without ETS-4) were run under the same experimental conditions.

The ecotoxicological effects of the treatment were evaluated by carrying out bioassays with organisms representative of different trophic levels: the bacterium *Vibrio fischeri* (decomposer); the green microalga *Pseudokirchneriella subcapitata* (producer) and the cladoceran *Daphnia magna* (primary consumer). All bioassays were conducted using the following samples: ASTM medium containing ETS-4 (ASTM/ETS4); water before treatment with $C_{Hg,0}$ of 50 and 100 μg/L (WBT_{50} and WBT_{100}); water after treatment, without (WAT_{50} and WAT_{100}) and with ETS-4 particles ($WAT/ETS4_{50}$ and $WAT/ETS4_{100}$).

The ecotoxicity of the different water samples was assessed for the bacterium *V. fischeri* using the Microtox® basic bioluminescence inhibition assay (AZUR 1998). The bioluminescence measurements were monitored after 5, 15 and 30 min of exposure (AZUR 1998).

The microalga *P. subcapitata* was selected to perform the bioassays, since it is a species that is readily available for culture collections, easily maintained in the laboratory under reproducible culture conditions, and has been widely used and recommended for toxicity testing (OECD 2006; USEPA 1994). Individual cultures of this species were maintained in nonaxenic batch cultures, in 5 L flasks, with 4 L of sterilized Woods Hole nutritive culture medium [MBL, (Stein 1973)], with continuous aeration and under controlled temperature (20 ± 1°C) and continuous light. The bioassays were carried out in sterile 24-well microplates (with 1 mL of medium/well), following the EC (1992) standard guidelines. The test species was exposed for a 72-h period to a range of dilutions of the water samples (0 %, 19.8 %, 29.6 %, 44.4 %, 66.7 % and 100 %) using the synthetic culture medium of algae—MBL as dilution water (Stein 1973), at 23 ± 1°C and with a constant luminous intensity (60–120 $\mu E/m^2/s$, equivalent to 6,000–10,000 lx). For this, each well in the microplates was filled with 900 μL of test water and inoculated with 100 μL of the correspondent algal-inoculum solution (10^5 cells/mL), so that the nominal initial cell concentration in the test was 10^4 cells/mL (the absorbance of this solution was measured in a spectrophotometer at 440 nm, Jenway, UV–VIS 6505, Chelmsford, Essex, England). Three replicates were set up randomly for each treatment and a control (MBL medium) per microplate. The peripheral wells were filled with 500 μL of distilled water to minimize evaporation in the test wells. During the exposure period, each well was shaken manually twice per day. After 72 h of exposure the concentration of algae was computed at each replicate by measuring absorbance at 440 nm and using the equation: $C = 17{,}107.5 + ABS \times 7{,}925{,}350$ ($R^2 = 0.99$; $p < 10^{-8}$) (fitted after making measurements in a set of ten serial dilutions of *P. subcapitata* suspensions in the spectrophotometer at 440 nm and in parallel counting cells at the optical microscope), where C is the algae concentration (cells per milliliter) and ABS is the absorbance obtained at 440 nm. For each concentration, the average specific growth rate (μ) (for exponentially growing cultures) and the percentage reduction in average growth rate compared to the control value were calculated, after a

period of 72 h (OECD 2006), using the following equations: $\mu = (lnC_{72h} - lnC_{0h})/T$ and $reduction = (\mu_{control} - \mu) \times 100/\mu_{control}$, where T is the time of exposure expressed in days. The validity of this test was assessed by by assuring that algal growth in the controls was ≥16-fold that for the initial concentration at time 0, and that the coefficient of variation in the controls was <7 %.

The test organism *D. magna* was chosen to perform the laboratory assays because it is a standard test species commonly used and recommended for lethal and sublethal toxicity assays. This species was provided by the Department of Biology of the University of Aveiro (Aveiro, Portugal). It was continuously reared in the laboratory, under semi-static conditions and controlled photoperiod (16:8 h light:dark) and temperature (20 ± 1°C) in ASTM hard water medium. This ASTM medium was supplemented with vitamins and a standard organic extract, Marinure 25 (Glenside, Stirling, UK) (7.5 mL/L of a suspension, absorbance of 620 units at 400 nm), to provide essential microelements to daphnids. Cultures were renewed every 2 days and the organisms were fed daily with *P. subcapitata* at a rate of 3.0×10^5 cells/mL/day. Neonates (>6 and <24 h old), from the third to the fifth generation, were used to perform toxicity assays. Five neonates were placed in 12 mL test flasks containing 10 mL of the test solution (OECD 2004). Each set of tests was comprised of five dilutions and an ASTM control. A range of dilutions of water samples (0 %, 3.1 %, 6.3 %, 12.5 %, 25 %, 50 % and 100 %) was obtained using ASTM culture medium as dilution water. The survival was checked after 24, 48 and 72 h of exposure. The validity of this test was assessed using the criteria that the % of mortality in the controls should be lower than 10 % and the dissolved O_2 should be higher than 3 mg/L.

Ecotoxicity testing results were evaluated through the calculation of the effective (EC) or lethal (LC) concentration that causes 20 % (toxic effects threshold) and 50 % of effect ($EC_{20\ and\ 50}$ and $LC_{20\ and\ 50}$). The EC and LC values were calculated in percentage of dilution of the starting full-strength test solutions (*v/v*). The Microtox-Omni software was used to collect the data for the Microtox® toxicity test and it was also used to calculate both EC_{20} and EC_{50} (after 5, 15 and 30 min of exposure). The Probit Program version 1.63, a parametric statistical method, for the analysis of inhibition/mortality data (Finney 2009), was used to calculate the EC_{20} and EC_{50} for microalga *P. subcapitata* and *D. magna*, with the respective 95 % confidence limit. One-way analysis of variance was used to test statistical differences in the growth of algae exposed to the different dilutions of the non- and remediated samples. When significant differences were found, the Dunnett's test was performed (STATISTICA version 10, Tulsa, OK, USA) to determine the no-observed effect dilution (NOEC) and the lowest-observed effect dilution (LOEC; inhibition relatively to the control).

Results and Discussion

Chemical analysis of water solutions revealed that there was no Hg contribution from ETS-4 particles, and that a significant decrease of the Hg concentration in solution occurred in their presence (Fig. 1a). The chemical efficiency for removal of Hg from water by the ETS-4 particles was 73 % when the $C_{Hg,0}$ was 100 μg/L and 58 % when the $C_{Hg,0}$ was halved. A slightly lower efficiency was recorded for the water samples containing the ETS-4 particles (Fig. 1b), attributed to a small release from the ETS-4 particles back into solution (see proposed mechanism ahead). Although for the higher initial contamination level the water cleaning process was sufficient to reduce Hg levels to values lower than the guideline value (50 μg/L) for Hg discharge from industrial sectors (Directive 84/156/EEC), the cleaning process was not as efficient as that obtained by Lopes et al. (2009) in ultra-pure water (99 %).

The framework of microporous titanosilicate ETS-4 comprises corner-sharing SiO_4 tetrahedra, TiO_5 pentahedra and TiO_6 octahedra. Since each Ti^{4+} ion has an associated −2 charge, the global neutrality is achieved by the presence of extra-framework cations in the channels, usually Na^+. The latter may be ion-exchanged by other cations, such as Hg^{2+}. Therefore, in an ideal theoretical ion exchange mechanism, 1 mol of Hg^{2+} will replace 2 mol of Na^+, according to the following mechanism (M_1.):

$$M_1.\quad ETS\text{–}Na_2\,(solid) + Hg^{2+}\,(aq) \leftrightarrow ETS\text{–}Hg\,(solid) + 2Na^+\,(aq)$$

where ETS refers to the titanosilicate structure. However, in more complex systems such as ASTM hard water, other phenomena can occur, including competition between Hg^{2+} and other cations, such as Ca^{2+}, Mg^{2+}, K^+ and H^+, for the ion exchange sites of the ETS-4 particles (see M_2.), the partial hydrolysis of the titanosilicate (see M_3.), or even the ion-exchange of the Hg^{2+} previously ion-exchanged by other cations present in water (see M_4.). Hence, more than one mechanism can be proposed for this system, as shown below:

$$M_2.\quad ETS\text{–}Na_2\,(solid) + 2/nX^{n+}\,(aq) \leftrightarrow ETS\text{–}X_{2/n}\,(solid) + 2Na^+\,(aq)$$

$$M_3.\quad ETS\text{–}Na_2\ (solid) + H_2O(aq) \leftrightarrow ETS\text{–}H_2\ (solid) + 2Na^+\,(aq) + 2OH^-\,(aq)$$

$$M_4.\quad ETS\text{–}Hg\,(solid) + 2/nX^{n+}\,(aq) \leftrightarrow ETS\text{–}X_{2/n}\,(solid) + Hg^{2+}\,(aq)$$

where X represents possible cations (Ca^{2+}, Mg^{2+}, K^+, Na^+ or H^+) and n is the valence of the cation.

In ASTM hard water, the huge amount of ions are an important interference to the ion-exchange of Hg^{2+} ions and are responsible for the lower removal efficiency when

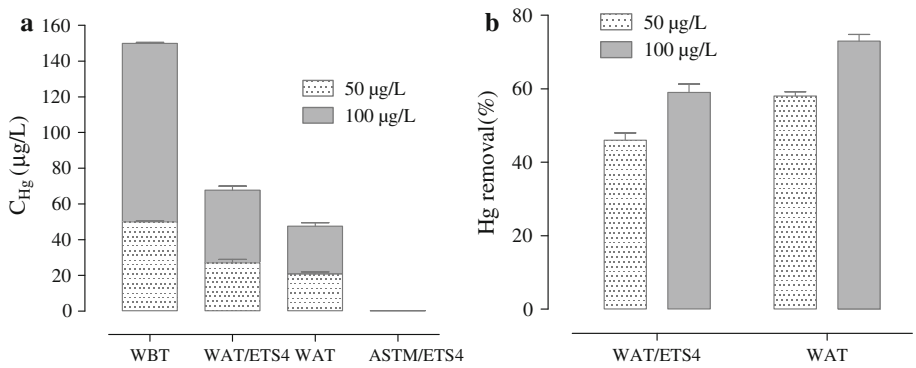

Fig. 1 Mercury concentrations in solution (**a**) and removal efficiency, **b** for the tested solutions

Table 1 Ecotoxicity results expressed as the percentage of effect (%), the effective or lethal concentration values inhibiting by 20 % (EC_{20} and LC_{20}) or 50 % (EC_{50} and LC_{50})

		Treatment[a]						
		ASTM/ETS4	WBT_{50}	WAT_{50}	$WAT/ETS4_{50}$	WBT_{100}	WAT_{100}	$WAT/ETS4_{100}$
V. fisheri	Effect 30 min	NTEO[b]	NTEO[b]	NTEO[b]	NTEO[b]	NTEO[b]	NTEO[b]	NTEO[b]
P. subcapitata	Effect (%) 72-h	NTEO[b]	7.3	0	0	36.2	0	0
	EC_{20} (%) 72-h	NTEO[b]	–	NTEO[b]	NTEO[b]	62.1 (53.6–71.5)	NTEO[b]	NTEO[b]
	EC_{50} (%) 72-h	NTEO[b]	–	NTEO[b]	NTEO[b]	–	NTEO[b]	NTEO[b]
	NOEC (%)	NTEO[b]	66.6	NTEO[b]	NTEO[b]	29.6	NTEO[b]	NTEO[b]
	LOEC (%)	NTEO[b]	100	NTEO[b]	NTEO[b]	44.4	NTEO[b]	NTEO[b]
D. magna	Effect (%) 72-h	0	100	100	100	100	100	100
	LC_{50} (%) 24-h	NTEO[b]	41.7 (34.5–50.6)	58.5 (47.0–74.8)	36.9 (30.9–44.6)	32.7 (28.2–38.4)	53.6 (50.4–57.1)	28.7 (24.2–34.1)
	LC_{50} (%) 48-h	NTEO[b]	29.7 (25.1–35.2)	37.7 (–;–)	26.4 (24.9–28.2)	8.8 (6.5–11.5)	42.1 (35.5–49.8)	17.7 (15.2–20.5)
	LC_{50} (%) 72-h	NTEO[b]	14.4 (11.7–17.8)	33.9 (29.3–39.6)	21.4 (17.4–26.5)	4.7 (3.4–6.0)	17.1 (13.4–21.6)	7.4 (5.9–9.2)
	LC_{20} (%) 24-h	NTEO[b]	29.1 (21.4–35.0)	36.4 (25.1–45.4)	35.4 (29.6–42.7)	26.3 (21.1–30.3)	49.1 (46.2–52.3)	21.9 (16.3–25.8)
	LC_{20} (%) 48-h	NTEO[b]	22.9 (17.2–26.8)	28 (–;–)	24.2 (22.7–25.7)	4.2 (2.5–5.5)	32.4 (24.2–38.0)	14.8 (12.1–17.0)
	LC_{20} (%) 72-h	NTEO[b]	9.2 (6.6–11.4)	27.7 (22.6–31.9)	13.7 (9.9–16.9)	2.7 (1.4–3.7)	9.8 (6.5–12.7)	4.5 (3.1–5.8)

The endpoints are presented as dilute of the starting full-strength test solutions (*v/v*) with 95 % confidence limits in parentheses for the EC and LC percentages

[a] Water before (WBT_{50} and WBT_{100}) and after treatment, without (WAT_{50} and WAT_{100}) and with ETS-4 particles ($WAT/ETS4_{50}$ and $WAT/ETS4_{100}$)

[b] No toxic effect observed

compared to that achieved in soft water, while the increase of efficiency for higher initial Hg^{2+} concentrations is due to an increase of the relative amount of Hg^{2+} ions regarding the total ionic force. This reinforces the need to perform this type of study in matrices as similar as possible to real scenarios.

Under experimental conditions, the concentrations of Hg^{2+} ions in the solid phase (i.e. q_{Hg}), calculated from the mass balance, $q_{Hg} = (C_{Hg,0} - C_{Hg}) \times (V/m)$, where subscript 0 denotes the initial condition, C is Hg concentration in solution, V is the volume of solution and m is the mass of ETS-4, ranged between 0.39 mg/g for a $C_{Hg,0}$ of 50 µg/L and 0.98 mg/g for a $C_{Hg,0}$ of 100 µg/L. Under the same initial Hg concentration (50 µg/L) and similar mass of sorbent, the ETS-4 particles perform better than some biosorbents like rice husk (q_{Hg} of 0.16 mg/g) or cork powder from used stoppers (q_{Hg} of 0.12 mg/g) (Rocha et al. 2013; Lopes et al. 2013) but are less effective than functional materials like magnetite coated with siliceous hybrid shells, properly designed for Hg uptake (Tavares et al. 2013).

The results of the bioassay with bacteria *V. ficheri* indicated that none of the solutions (pre- and post-treatment and clean-up agent) caused bioluminescence inhibition, i.e. no toxic effects were observed with this species (Table 1). The lack of response observed for this species can be associated to the low Hg levels tested in this study, including the water before treatment.

The *P. subcapitata* growth rate was not affected upon exposure to the ASTM medium with ETS-4 particles, indicating that the clean-up agent did not cause any adverse effects to this species. Significant differences in *P. subcapitata* growth were observed between the control MBL medium and the WBT_{50} (one way ANOVA: $F_{5,15} = 97.2$, $p < 0.05$); however, this level of contamination caused only slight toxicity, inhibiting the algal growth by 7 %. The

values found for the NOEC and LOEC were 66.7 % and 100 %, respectively (Table 1). After the treatment with ETS-4 particles, no toxic effects were observed during the exposure of *P. subcapitata* to the samples with (WAT/ETS4$_{50}$) and without (WAT$_{50}$) particles. Algal growth was sensitive to the pre-treated sample with a higher concentration of Hg^{2+} and its exposure to WBT$_{100}$, with approximately 36 % inhibition occurring after 72 h of exposure. The value obtained for EC$_{20}$ after a period of exposure of 72 h exposure was 62.1 %, and the NOEC and LOEC values (one way ANOVA: $F_{5,15} = 38.8$, $p < 0.05$) were 44.4 % and 29.6 %, respectively (Table 1). The algal growth rates in the presence of WAT$_{100}$ and WAT/ETS4$_{100}$ samples were close to that for the MBL control, with no differences being observed between the control and WAT$_{100}$ (one way ANOVA: $F_{6,17} = 15.8$, $p < 0.05$) or the control and WAT/ETS4$_{100}$ (way ANOVA: $F_{6,17} = 64.8$, $p < 0.05$).

No mortalities were observed during the exposure of *D. magna* to the ASTM medium containing ETS-4 particles. The exposure of *D. magna* to both WBT$_{50}$ and WBT$_{100}$ samples, i.e. to the full strength solutions and higher dilutions (50 %), caused 100 % mortality, and the 72-h LC$_{50}$ values were 14.4 % and 4.7 %, respectively (Table 1). The post-treatment samples, for both WAT$_{50}$ and WAT$_{100}$ concentrations also caused 100 % of mortality to *D. magna* after a period of exposure of 72 h. Still, the toxicity was reduced, in particular for 50 μg/L, resulting in LC$_{50}$ values of 33.9 % for WAT$_{50}$, 21.4 % for WAT/ETS4$_{50}$, 17.2 % for WAT$_{100}$ and 7.4 % for WAT/ETS4$_{100}$ (Table 1).

The data from the bioassays clearly show that the three species selected have different responses to the different levels of contamination and have different sensibilities toward Hg. The order of sensitivity was cladoceran *D. magna* > microalga *P. subcapitata* > bacterium *V. ficheri*.

ETS-4 was found to be an innocuous material to the selected test organisms in the present study. It decreased the levels of Hg in the water from 50 to 21 μg/L and from 100 to 28 μg/L, with an application of 75 mg/L of particles. These reductions in Hg concentrations were sufficient to completely protect *P. subcapitata* from acute effects, but not for complete protection of *D. magna*. This organism was highly sensitive to Hg, which allowed for recognition of the fact that waters containing ETS-4 particles after treatment were less efficient in the detoxification process than waters without the particles still present. This fact may be due to the previously mentioned mechanism M_4, since the Hg in the WAT/ETS4 samples is distributed between the liquid (solution) and solid (ETS-4 particles) phases. Some of the Hg^{2+} associated with ETS-4 may be exchangeable with Ca^{2+} and/or other ions present in ASTM hard water, thereby releasing Hg^{2+} back into the water.

In conclusion, this study showed that treatment of Hg-containing water with ETS-4 particles partially reduced the Hg concentration in water, did not cause toxic effects to the aquatic organisms tested, and effectively protected the microalga *P. subcapitata* from acute effects. However, the water treatments were not sufficient to completely removing the toxicity to *D. magna*, which was found to be highly sensitive to Hg. Additionally, the bacterium *V. ficheri* was found to be an unsuitable organism to evaluate the efficiency of this water treatment, due to its low sensitivity to Hg.

Chemical analysis alone, as usually performed, is not recommended as being sufficient for an evaluation of the efficiency of water treatment. Rather, a multidisciplinary approach combining both chemical and ecotoxicological tools affords more reliable conclusions about the real effectiveness of the clean-up technologies proposed for contaminated waters.

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