

Efficiency of a cleanup technology to remove mercury from natural waters by means of rice husk biowaste: ecotoxicological and chemical approach

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Abstract In the present work, the efficiency of rice husk to remove Hg(II) from river waters spiked with realistic environmental concentrations of this metal ($\mu\text{g L}^{-1}$ range) was evaluated. The residual levels of Hg(II) obtained after the remediation process were compared with the guideline values for effluents discharges and water for human consumption, and the ecotoxicological effects using organisms of different trophic levels were assessed. The rice husk sorbent proved to be useful in decreasing Hg(II) contamination in river waters, by reducing the levels of Hg(II) to values of *ca.* 8.0 and 34 $\mu\text{g L}^{-1}$, for an Hg(II) initial concentration of 50 and 500 $\mu\text{g L}^{-1}$, respectively. The remediation process with rice husk biowaste was extremely efficient in river waters spiked with lower levels of Hg(II), being able to eliminate the toxicity to the exposed organisms algae *Pseudokirchneriella subcapitata* and rotifer *Brachionus calyciflorus* and ensure the total survival of *Daphnia magna* species. For concentrations of Hg(II) tenfold higher (500 $\mu\text{g L}^{-1}$), the remediation process was not adequate in the detoxification process, still, the rice husk material was able to reduce considerably the toxicity to the bacteria *Vibrio fischeri*, algae *P. subcapitata* and rotifer *B. calyciflorus*, whose responses were fully inhibited during its exposure to the non-remediated river water. The use

of a battery of bioassays with organisms from different trophic levels and whose sensitivity revealed to be different and dependent on the levels of Hg(II) contamination proved to be much more accurate in predicting the ecotoxicological hazard assessment of the detoxification process by means of rice husk biowaste.

Keywords Metals · Natural waters · Ecotoxicity · Rice husk · Biosorption · Remediation

Introduction

Metals are persistent in nature and become toxic when they are not metabolised by the body and accumulate in the soft tissue. Mercury (Hg) is considered by the Environmental Protection Agency (EPA) as a highly dangerous metal because of its toxicity and accumulative and persistent character in the environment and biota (Uría and Sanz-Medel 1998). With the ecological and human health risks that arise from the excessive release (due to industrialization and urbanisation) and proliferation of Hg and other metals into aquatic environments, there is an increasing need to remediate waters that receive these contaminants and as a result, new, safer and economical remediation approaches are continually being developed to supplement traditional methods (Srivastava and Goyal 2010; Naja et al. 2010).

In the last two decades, the sorption of contaminants by sorbents of natural origin (biosorption) gained a particularly importance, as these materials represent unused resources that are widely available and environmentally friendly, and as result sorption methods present economic, ecological and

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technologic advantages (Demirbas 2008; Lesmana et al. 2009; Naja et al. 2010; Pacheco et al. 2011; Sud et al. 2008). Among the variety of biowastes used to remove Hg from water and wastewater, one of the materials that has been used is rice husk (Tiwari et al. 1995; Khalid et al. 1999; Feng et al. 2004; Krishnani et al. 2008; El-Said et al. 2010; El-Shafey 2010), a lignocellulosic agricultural waste generated abundantly in rice milling industries. Recently, Rocha et al. (2013a, b) explored the sorption efficiency of the natural form of rice husk in the removal of realistic concentrations of Hg, using minimal amounts of this biowaste material. These studies, like most of the approaches driven in the field of water treatment, aim only the quantification of the residual levels of metals and the amount of the metal effectively sorbed by the material applied (Tiwari et al. 1995; Khalid et al. 1999; Feng et al. 2004; Krishnani et al. 2008; El-Said et al. 2010; El-Shafey 2010). However, chemical analysis alone is not adequate to explain the effects of contaminants to the biota and, therefore, it is important to establish a cause-effect relationship between the concentration of chemicals and consequent environmental damage. Thus, for a correct valuation of the efficiency and environmental hazard of the method developed, a multidisciplinary approach, combining chemical and ecotoxicological essays should be designed (Manusadzianas et al. 2003). As far as we know, there is little information concerning the efficacy of water treatment technologies with biological wastes in reducing or eliminating the toxicity of waters contaminated with metals (Mishra and Tripathi 2008; Gupta and Torres (1998)).

In this study, the real efficiency of natural rice husk on Hg(II) decontamination was evaluated, by assessing the residual levels of Hg(II) in river water and comparing the water toxicity to biota, before and after the biosorption process. The biosorption process was conducted in river water spiked with concentrations of Hg(II) reflecting contamination problems that can possibly be found in the environment. A battery of bioassays, with organisms from different taxonomic groups and exhibiting different key functions at the ecosystem levels, the bacterium *Vibrio fisheri* (decomposer), the green algae *Pseudokirchneriella subcapitata* (formerly known as *Selenastrum capricornutum* Printz) (producer), the rotifer *Brachionus calyciflorus* (primary consumer) and the cladoceran *Daphnia magna* (primary consumer), was carried out to evaluate the sub-lethal effects posed by natural waters spiked with Hg(II) in the pre and posttreatment phase, and also to evaluate possible toxic effects caused by the rice husk biowaste applied in the decontamination process. The use of a natural water instead of a synthetic one allowed us to achieve conditions close to the ones found in aquatic systems and to evaluate the influence posed by this natural matrix (chemically very complex) on the speciation and dynamics of Hg, that can eventually hinder or enhance the sorption process and as well the toxicity to biota.

Materials and methods

Material and chemicals

All chemicals were of analytical reagent grade and solutions were prepared with ultra-pure water (18.2 M Ω cm, Milli-Q system). The standard stock solution of mercury nitrate (1,001 \pm 2 mg L⁻¹) and the nitric acid 65 % (suprapur) were purchased from Merck. All glassware used in the experiments was acid-washed prior to use.

Rice husk material

The rice husk was kindly supplied by the industries of Álvaro Alves Borges Lda. from Figueira da Foz, Portugal. The rice husk material was washed with distilled water and dried at 60 °C. Afterwards, the material was triturated using a coffee mill (model Taurus aromatic). Different fractions of the rice husk samples were obtained using a mechanical sieve shaker, but only the fraction that comprises particle sizes between 200 and 500 μ m was studied. The material was stored at room temperature in plastic containers until further use. Other information regarding the morphological, chemical and physical description of the rice husk material used in this work can be found in previous studies (Rocha et al. 2013a, Rocha et al. 2013b).

Sampling and characterization of the river water

The natural river water was collected in the Vouga River, located in central Portugal and that empties into the Atlantic Ocean through a delta called "Ria de Aveiro". The water was collected near the abstraction of water of Carvoeiro (40° 40' 42" N, 08° 22' 18" W), from a depth of about 10 cm and using polyethylene bottles that were rinsed with surface water, before filling with the sample. The water was fully characterised: pH, conductivity, suspended particulate matter (SPM), organic matter (dissolved organic carbon DOC), Hg concentration (dissolved), major (total Ca, K, Mg, Na, Si) and minor (total Al, As, B, Ba, Be, Cd, Co, Cr, Cu, Fe, Li, Mn, Ni, P, Pb, Sr, Ti, V, Zn) elements.

The pH and conductivity of the river water were recorded on a WTW series 720 pH and WTW LF530 meters, respectively. For SPM determination, water samples were filtered through a pre-weighted 0.45 μ m pore size cellulose acetate membrane filter (Millipore). The filters were dried at 60 °C until constant weight and then reweighed to estimate the dry mass of SPM. For DOC determination, water samples were filtered through a precombusted 0.47 μ m pore size glass fibre filter (Whatman GF/F). The filtrated water was analysed on a Total Organic Carbon Analyzer (Shimadzu Corporation). For Hg determination, water sample was filtered through a 0.45 μ m pore size cellulose acetate membrane filter

(Millipore), acidified to pH below 2 (with 65 % HNO₃) and analysed by CV-AFS as described afterwards ([Hg quantification and QA/QC procedures](#)). For the determination of major and minor elements, non-filtrated water samples were analysed by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in a Jobin-Yvon JY70 Plus Spectrometer.

Batch sorption experiments

Kinetic studies

Batch sorption kinetic experiments were performed at room temperature (21±1 °C) by contact of a certain amount of rice husk with river water (RW) spiked with a known concentration of Hg(II). The experimental trials were carried out in volumetric flasks (2 L) under constant stirring conditions (1,400 rpm). Two initial Hg(II) concentrations were selected: 50 µg L⁻¹ (RW₅₀) representing the maximum value for Hg discharges from industrial sectors, and a value ten times higher, i.e. 500 µg L⁻¹ (RW₅₀₀). No pH adjustments were applied to the water samples. The dose of rice husk used in all experiments, 0.50 g L⁻¹, was selected according to the data from a previous study (Rocha et al. 2013a). In that study, the authors verified that using a dose of 0.50 g L⁻¹, the efficiency of the removal process is assured (>90 %) in consecutive treatments, minimising the amount of sorbent, and therefore issues concerning with secondary waste disposal problems are reduced.

The river water was spiked with the standard stock solution (Hg(NO₃)₂) (1,000±1 mg L⁻¹) or with an intermediate solution (10 mg L⁻¹) to obtain the desired initial concentrations, 500 and 50 µg L⁻¹, respectively. An aliquot of the Hg(II) solution (10 mL) was collected before the beginning of each experiment to check the initial Hg(II) concentration (C_{Hg,0}). Experiments started when a known mass of the biosorbent was added to Hg(II) solutions and stirring was initiated. Aliquots (10 ml) were collected at increasing times, filtered through an acid-washed 0.45 µm Millipore membrane and adjusted to pH<2 with 65 % HNO₃. Each experiment was maintained until the Hg(II) concentration in the solution remained constant (situation of equilibrium). After that, half volume of each solution was filtered through a 0.45-µm Millipore and the solutions were transferred to Schott Duran bottles (500 mL) and kept at 4 °C until Hg(II) quantification and bioassays completion. All experiments were performed in duplicate (R₁ and R₂), and the results were always expressed as the mean value obtained. Additionally, experiments in river water spiked with both Hg(II) concentrations but without rice husk were performed, in order to assess the Hg(II) losses during the experimental trials. Also, an experiment with rice husk and without Hg(II) was carried out (RW_{RH}), in order to assess Hg contribution from the biosorbent.

Hg quantification and QA/QC procedures

The concentration of Hg(II) in all samples was evaluated by cold vapour atomic fluorescence spectroscopy (CV-AFS) on a PSA cold vapour generator, model 10.003, associated with a Merlin PSA detector, model 10.023, using SnCl₂ as reducing agent. The Hg(II) concentration was quantified through a calibration curve of five standards prepared in a HNO₃ solution (2 %v/v) by dilution from the certified standard solution of Hg(NO₃)₂, and which concentration ranged from 0.0 to 0.5 µg L⁻¹. In this range, the limit of detection of the method is 0.02 µg L⁻¹ and the precision and accuracy are <5 %.

Ecotoxicity assays

In the present work, a battery of ecotoxicological assays was performed in spiked water before (RW₅₀ and RW₅₀₀) and after remediation with rice husk (RW₅₀-rem and RW₅₀₀-rem) and in non-spiked water (RW). An experiment with rice husk but without the addition of Hg(II) (RW_{RH}) was also performed to verify the toxic effects that might arise from the use of this agricultural waste. In order to obtain an accurate ecotoxicological hazard assessment of the detoxification process, a battery of bioassays with organisms representative of different trophic and functional levels were conducted. The following organisms were used: the bacterium *V. fischeri* (decomposer), the green algae *P. subcapitata* (producer), the rotifer *B. calyciflorus* (primary consumer) and the cladoceran *D. magna* (primary consumer).

Bacterium V. fischeri

The Microtox acute toxicity test measures the decrease in bioluminescence output by the test organism (Microtox® basic test; AZUR 1998). The ecotoxicity of all water samples (RW, RW_{RH}, RW₅₀, RW₅₀₀, RW₅₀-rem and RW₅₀₀-rem) were assessed for the bacterium *V. fischeri*. The Microtox® test reagent and test solutions were purchased from Quincomer Inc. (Lisboa, Portugal). The Microtox® test was performed in a Microtox Model 500 Analyser (Microbics Corporation 1992), according to the Microbics Corporation detailed protocol for the basic test, and bioluminescence measurements were monitored after 5, 15 and 30 min of exposure (AZUR 1998).

Green algae P. subcapitata

The microalga *P. subcapitata* has been widely used and recommended as a model species for toxicity testing and the purpose of this assay is to evaluate algal growth inhibition in the presence of contaminants (OECD 2006; USEPA 1994; EC 1992). Individual cultures of microalga *P. subcapitata* were maintained in nonaxenic batch cultures, in 5 L flasks, with 4 L

of sterilised Woods Hole nutritive culture MBL medium (Stein 1973), with continuous aeration and under controlled temperature (20 ± 1 °C) and continuous light. The assays were carried out in 24-well microplates (with 1 mL of medium/well), following the EC (1992) standard guidelines. Prior to its use and in order to avoid the Hg(II) losses during the experimental trial, the microplates wells were filled with 65 % HNO₃ and then washed with Milli-Q water. The microalga was exposed to all water samples for a 72 h period at 23 ± 1 °C and with a constant luminous intensity (60–120 $\mu\text{E}/\text{m}^2/\text{s}$, equivalent to 6,000–10,000 lx). For RW, RW_{RH} no dilutions were applied, as no effect on algal growth was observed. A range of dilutions using the culture medium of algae–MBL as dilution water (Stein 1973) were made to the remaining samples. For RW_{50-rem}, the dilutions made were 29.6, 44.4, 66.7 and 100 %, for RW_{500-rem} and RW₅₀ were 19.8, 29.6, 44.4, 66.7 and 100 %, and for RW₅₀₀ were 3.9, 5.8, 8.8, 13.2, 19.8, 29.6, 44.4, 66.7 and 100 %. Each well in the microplates was filled with 900 μL of the sample being tested 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. Three replicate were set up randomly for each treatment and a control (MBL medium) per microplate. The peripheral wells were filled with 1,000 μL of distilled water, to minimise 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 (Jenway, 6505 uv/vis spectrophotometer) and using the equation: $C = 17107.5 + ABS \times 7925350 (R^2 = 0.99; p < 10^{-8})$, where C is the algae concentration (cells per millilitre) and ABS is the absorbance at 440 nm. For each sample, the average specific growth rate, given by the $\mu = (\ln C_{72h} - \ln C_{0h})/T$ (for exponentially growing cultures), where T is the period of exposure (expressed in hours). Additionally, the values for the percentage of inhibition in average growth rate, compared to the control value, were calculated after a period of 72 h (OECD 2006).

Rotifer *B. calyciflorus*

The aim of the toxicity test with freshwater rotifer *B. calyciflorus* is to measure the decrease in reproduction of the rotifers under toxic stress after 48 h exposure (Rotokit FTM, short chronic, Microbiotest). Test animals were obtained by hatching cysts of *B. calyciflorus* in synthetic freshwater. Hatching was performed at 25 °C under a light intensity of 3,000 lx. After 18 h of incubation, cysts were checked to ensure collecting the test animals within 2 h. After 16–18 h of incubation and prior starting the short-chronic toxicity test, freshly hatched rotifers were pre-feed with a specific inert food for a period of 2 h, in order to increase the reproduction

rate of the test organisms during the test (ROTOXKIT FTM, short chronic, Microbiotest). A range of dilutions using the synthetic freshwater as dilution water were made to the following samples: 29.6, 44.4, 66.7 and 100.0 % for RW_{500-rem}; 19.8, 29.6, 44.4, 66.7 and 100 % for RW₅₀; and 3.9, 5.8, 8.8, 13.2, 19.8, 29.6, 44.4, 66.7 and 100 % for RW₅₀₀. For the remaining samples that include RW, RW_{RH}, RW_{50-rem}, no dilutions were applied (100 %), as no effects were observed at the non-diluted sample.

The assays were carried out in multiwell plates and each well was filled with 1,000 μL of the sample being tested. To allow the growth of rotifers during the exposure period, 100 μL of algal concentrated food stock (corresponding to a final concentration of 2×10^6 algal cells/mL) was added to each well. For a statistical acceptable evaluation of the effects, each sample and its corresponding dilutions, as well as the control (synthetic freshwater) was assayed in eight replicates ($n=8$). The test was initiated by transferring one (N_{initial}) newly hatched rotifer into each well which was incubated at 25 °C in darkness. After 48 h of incubation, the total number of live (N_{final}) and dead rotifers were counted. The population reproduction rates (r) were calculated by means of the following expression $r = (\ln N_{\text{final}} - \ln N_{\text{initial}})/T$, where T is the period of exposure (expressed in hours). Additionally, the percentages of reproduction inhibition, compared to the control value, were calculated after a period of 48 h.

Cladoceran *D. magna*

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 sub-lethal toxicity assays. The sub-lethal feeding test with *D. magna* is an ecologically relevant test and it aims to measure the inhibition in feeding responses due to the presence of contaminants (McWilliam and Baird 2002; Rosa et al. 2010). *D. magna* was provided by the Department of Biology of the University of Aveiro (Aveiro, Portugal). Neonates (4 days old) from the third to the fifth generation were used to perform toxicity assays. Five neonates were placed in a 12-mL test flask containing 10 mL of the test solution. Each set of tests was comprised of five dilutions and a hardwater medium (ASTM–American Society for Testing and Materials 2002) control. A range of dilutions using ASTM culture medium as dilution water were made to the following samples: 6.25, 12.5, 25, 50 and 100 % for RW_{RH}, RW₅₀, RW_{50-rem} and RW_{500-rem}. For RW₅₀₀, a different set of dilutions were made: 0.39, 0.78, 1.56, 3.12, 6.25, 12.5, 25, 50 and 100.0 %, as a high mortality was observed with the first serial dilutions. For RW samples, no dilutions were made, as no effect was observed. Four replicates of each sample and its corresponding dilutions were made. The tests were carried out at 20 ± 1 °C under darkness and static conditions in the presence of food, corresponding to

a *P. subcapitata* concentration of 3×10^5 cells/mL. After a period of exposure of 24 h, the concentration of algae for each set of dilutions and at each replicate ($n=4$) was computed by measuring absorbance by UV/VIS as described previous in the experimental section for green algae *P. subcapitata*. The feeding rates were calculated by means of the following expression: $F = (\ln C_{0h} - \ln C_{24h})/nT$, where n is the number of organisms for each replicate and T is the period of exposure (expressed in hours). Furthermore, the survival was checked in the same samples after the period of exposure of 24 h and in the presence of food.

Data analysis

Sorption kinetic data

The removal efficiency of the biosorption process was evaluated by calculating the removal percentage (R), which at time t is defined by:

$$R_{\text{Hg(II)}} (\%) = \frac{(C_{0,\text{Hg}} - C_{t,\text{Hg}})}{C_0} \times 100 \quad (1)$$

where $C_{0,\text{Hg}}$ ($\mu\text{g L}^{-1}$) is the initial concentration of Hg(II) in the solution and $C_{t,\text{Hg}}$ ($\mu\text{g L}^{-1}$) is the concentration after a particular period of time t (h).

Speciation modelling

Visual MINTEQ, a programme supported by the Swedish Research Council (VR) and by the Foundation for Strategic Environmental Research (MISTRA), was used to simulate the speciation of Hg in natural river water. Due to the complexity of the matrix used in this study, the NICA-Donnan model was used for realistic assessment of Hg to dissolved organic carbon (DOC).

Ecotoxicological data

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 EC_{50} and LC_{20} and LC_{50}). The EC and LC values were calculated in percentage of dilution of the contaminated river water (volume/volume, v/v). The MicrotoxOmni 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 1971), was used to calculate the EC_{20} and EC_{50} for microalga *P. subcapitata*, *B. calyciflorus* and

D. magna (both sub-lethal and lethal tests), with the respective 95 % confidence limit.

One-way analysis of variance (ANOVA) was used to test statistical differences in the growth of algae and reproduction of rotifers 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) to determine the no-observed effect dilution (NOEC) and the lowest-observed effect dilution (LOEC; inhibition relatively to the control).

Results and discussion

Physico-chemical characteristics of non- and spiked river water before and after treatment with rice husk

The physical and chemical characterization of non-spiked water from Rio Vouga indicates that the pH value of the river sample was 7.02, its conductivity was $58.7 \mu\text{S cm}^{-1}$ and some elements like K, Mg, Ca, Na and Si were detected at mg L^{-1} concentrations, while Al, Ba, B, Cu, Fe, Li, Mn, P, Sr and Zn were detected at $\mu\text{g L}^{-1}$ concentrations (Table 1). The amount of suspended particulate matter (SPM) was 7.89 mg L^{-1} and the total carbon (TC) and organic carbon (OC) in the dissolved fraction found were 3.48 and 1.15 mg L^{-1} , respectively. The amount of Hg(II) in the natural water was found to be very low (3.75 ng L^{-1}), typical of non-contaminated waters.

Spiked river water before (RW_{50} and RW_{500}) and after ($\text{RW}_{50\text{-rem}}$ and $\text{RW}_{500\text{-rem}}$) the remediation process with rice husk, was also characterised (Table 1). The spiked river water show a slight decrease in pH values, in particular for the highest Hg(II) concentration due to the acid character of the standard solution added. Most of the levels found in river water for the elements initial detected were maintained in pre- and posttreatment waters. However, there were some exceptions, specifically the levels detected for P, K and Fe. The samples containing rice husk sorbent ($\text{RW}_{50\text{-rem}}$, $\text{RW}_{500\text{-rem}}$ and RW_{RH}) showed a substantially increment in the concentration of K of *ca.* 3 to 4 times and of *ca.* 2 to 4 times for P, which is attributed to the nature of the rice husk. The EDS analysis of this material (Rocha et al. 2013a) identifies a high content of C, O and Si, but also the presence of Ca and K. Together with the biosorbent pore size (48 \AA), these results suggest the occurrence of an ion-exchange mechanism involved in the sorption process with rice husk (Rocha et al. 2013b). Additionally, the levels detected for Fe decrease *ca.* 56 to 72 % in the posttreatment waters suggesting the sorption of Fe onto the rice husk. Eventually, the $\text{Fe}^{2+}/\text{Fe}^{3+}$ in solution may compete with Hg(II) for the available binding sites in the rice husk surface and as a result, the efficiency of Hg(II) removal may be affected by the presence of Fe co-ion. The increase in the conductivity of posttreatment waters

Table 1 Physical (pH and conductivity) and chemical characterization (dissolved organic carbon (DOC), minor and major elements) of: river water (RW), river water treated with rice husk (RW_{RH}), non-remediated (RW₅₀ and RW₅₀₀) and remediated river waters (RW_{50-rem} and RW_{500-rem}) spiked with two Hg(II) concentrations (50 and 500 µg L⁻¹). The rice husk dose used in these experiments was 0.50 g L⁻¹

	C _{0,Hg} µg L ⁻¹	Dose _{RH} g L ⁻¹	pH	Conductivity µS cm ⁻¹	DOC mg L ⁻¹	Major elements (mg L ⁻¹)						Minor elements (µg L ⁻¹)								
						Ca	Na	Si	Mg	K	P	Al	B	Ba	Cu	Fe	Li	Mn	Sr	Zn
RW	0	–	7.02	57.8	1.15	2.83	7.47	4.33	1.12	1.52	16.4	18.69	5.91	4.96	4.33	125.3	3.06	37.49	14.47	8.29
RW _{RH}	0	0.50	7.38	65.0	2.86	2.56	7.59	4.76	1.14	5.26	28.2	11.81	23.51	<3	<3	34.74	3.25	<3	12.74	8.61
RW ₅₀	50	–	6.56	53.4	1.73	2.75	12.7	4.52	1.13	1.50	<15	24.69	20.62	4.57	4.33	94.70	<3	33.62	13.87	7.61
RW _{50-remR1}	50	0.50	6.53	67.4	3.33	2.83	15.2	5.15	1.34	5.67	59.3	15.28	27.20	3.92	3.33	37.25	3.4	90.77	14.60	11.91
RW _{50-remR2}	50	0.50	6.61	81.0	–	2.77	14.3	5.21	1.20	5.20	63.6	14.9	37.93	3.80	3.12	29.81	3.15	87.52	13.27	11.57
RW ₅₀₀	500	–	5.75	84.8	1.63	2.68	8.65	4.40	1.18	1.96	<15	22.27	7.94	4.38	<3	76.21	3.54	21.46	16.85	7.25
RW _{500-remR1}	500	0.50	6.34	94.7	3.60	2.73	8.36	4.92	1.24	5.78	51.0	12.30	13.59	3.56	<3	33.35	3.35	81.38	14.31	10.3
RW _{500-remR2}	500	0.50	6.46	96.3	–	2.77	20.3	5.00	2.80	6.00	63.7	<10	14.95	3.35	<3	27.06	3.45	77.52	20.18	9.44

corroborates the hypothesis of an ion-exchange mechanism, and the release of ions from the rice husk surface to the solution.

The levels of dissolved organic carbon (DOC) increased in the samples treated with rice husk, mainly composed by cellulose (29 to 34 %), hemicellulose (21 to 29 %) and lignin (19 to 30 %) (Ahmaruzzaman and Gupta, 2011), probably due to the dissolution of some of the soluble components (eg. waxes, proteins, non-cellulosic polysaccharides and some lignin surrounding the fibre bundles) during the sorption process that was carried out under vigorous stirring conditions.

Chemical efficiency of Hg(II) removal by rice husk from spiked river water

The feasibility of the natural form of rice husk in the treatment of spiked river waters contaminated was evaluated for two initial Hg(II) concentrations (50 and 500 µg L⁻¹). Figure 1 shows the kinetic curves (*R*_{Hg(II)} vs *t*) obtained for the removal of Hg(II) by rice husk, expressed as mean value of two replicate experiments performed under the same conditions.

For an initial Hg(II) concentration (*C*_{0,Hg}) of 50 µg L⁻¹ (Fig. 1a), the removal of Hg(II) was slow at the beginning of the sorption process (between 0 and 6 h), confirmed by the low values of Hg(II) removal percentage (*R*_{Hg(II)}<15 %). After 24 h, the removal of this metal increased considerably, achieving values *ca.* 56 %, reaching the maximum value, 81.0 ±2.4 %, at equilibrium (*t*=168 h). By increasing the initial concentration of Hg(II) to 500 µg L⁻¹ (Fig. 1b), the removal efficiency at the beginning of the sorption process (first 6 h) was faster, reaching 59 %. After that, the removal efficiency increases continually and reaches its maximum value (93.0±0.2 %) at the equilibrium, obtained after a period of contact of 168 h.

Comparing the Hg removal percentages obtained in spiked river water with the ones obtained in spiked ultra-pure water, under identical experimental conditions (*C*_{0,Hg} and rice husk dose) (Rocha et al. 2013a), it was observed that there is no relevant difference between the obtained values, respectively 81.0 vs. 84 % for a *C*_{0,Hg} of 50 µg L⁻¹ and 92 vs. 93.0 % for a *C*_{0,Hg} of 500 µg L⁻¹. Although the removal percentages are similar in both matrixes, it must be highlighted that there are different processes contributing to the Hg(II) removal process: one is the rice husk material and the other are the water components, mainly DOM. The speciation of Hg in the river water for both Hg(II) initial concentrations was modelled by the NICA-Donnan model of Visual MINTEQ. The speciation data obtained indicate that for *C*_{0,Hg} of 50 µg L⁻¹, 31.5 and 16.5 % of Hg is organically complex to the carboxylic and phenolic functional groups of fulvic substances, respectively, and 52.0 % is available in solution as Hg(OH)₂. For *C*_{0,Hg} of 500 µg L⁻¹, the dominant Hg species in the solution are

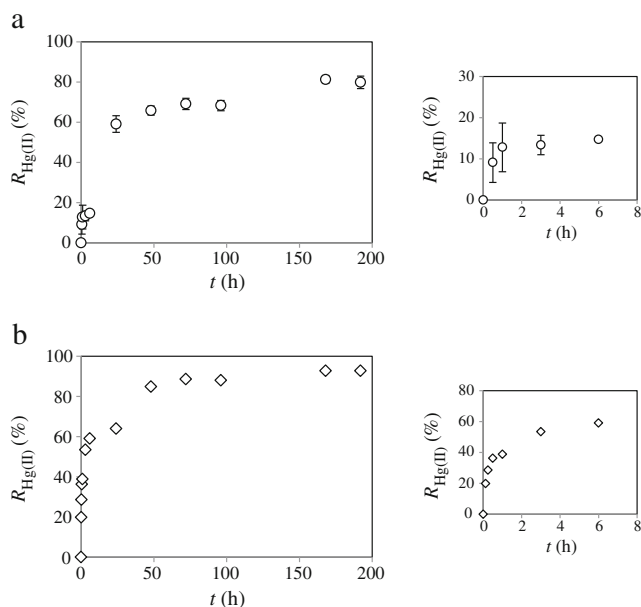


Fig. 1 Variation of $R_{\text{Hg(II)}}$ with time (t , h) for the sorption process of Hg(II) onto rice husk using spiked river water and for initial Hg(II) concentrations of $50 \mu\text{g L}^{-1}$ (a) and $500 \mu\text{g L}^{-1}$ (b). Other experimental conditions: pH ~ 6.0 and RH dose of 0.50 g L^{-1} . $C_{\text{Hg(II)}}$ was expressed as the mean value of two replicates

Hg(OH)_2 (89.6 %), a very small fraction is available as HgOH^+ (0.1 %) and the remaining fraction of Hg is organically complex to the carboxylic (7.3 %) and phenolic groups (3.1 %) of fulvic substances. Therefore, the overall removal of Hg in natural river waters results from: (1) the sorption of Hg onto the rice husk and (2) the presence of the DOC content (matrix), which influences greatly the speciation of Hg(II).

Even in very complex matrices like natural river water, the rice husk sorbent proved to be useful in decreasing Hg(II) contamination in river waters. The residual Hg(II) concentration in $\text{RW}_{50\text{-rem}}$ samples was $7.95 \pm 0.82 \mu\text{g L}^{-1}$ ($n=2$) and as a result, it was not possible to achieve residual concentrations of Hg(II) as low as the guideline value for drinking water quality ($C_{\text{Hg(II)}} < 1 \mu\text{g L}^{-1}$, Council Directive 84/156/EEC). Nevertheless, values lower than the value for Hg discharges from industrial sectors, i.e. $50 \mu\text{g L}^{-1}$ (Council Directive 98/83/EC), were obtained for $\text{RW}_{50\text{-rem}}$.

Ecotoxicity testing

A battery of ecotoxicological tests was applied to the non-remediated samples (RW, RW_{50} and RW_{500}) and remediated samples (RW_{RH} , $\text{RW}_{50\text{-rem}}$ and $\text{RW}_{500\text{-rem}}$). Table 2 presents the effects observed due to exposure of test organisms to non-diluted samples (nominal concentration of 100 %) and Table 3 the toxicity results expressed as the percentage of dilution of the contaminated river water, that produces 20 and 50 % of inhibition/mortality (EC_{20} and EC_{50}).

Bacterium V. fischeri

The results show that the river water (RW) used to perform the sorption experiments did not exert any adverse effect to the bacteria *V. fischeri*. The effect of the river water containing rice husk in RW_{RH} showed that there was no significant bioluminescent bacterial inhibition, indicating that this agricultural waste do not cause toxicity to this specie. No toxic effects were observed by exposing the bacteria *V. fischeri* to non-remediated (RW_{50}) and remediated ($\text{RW}_{50\text{-rem}}$) river waters spiked with $50 \mu\text{g L}^{-1}$ of Hg(II), while for river water samples spiked with a $C_{0 \text{ Hg(II)}}$ of $500 \mu\text{g L}^{-1}$, the effects observed due to the exposure of test organisms to non-diluted untreated sample (RW_{500}) increased with exposure time: 76.1, 99.0 and 99.8 %, after a period of bacteria exposure of 5, 15 and 30 min, respectively (Table 2). The percentage of dilution of the contaminated river water, that caused 50 % inhibition of luminescence (EC_{50}), was calculated and the values obtained were 0.68 % after a period of bacteria exposure of 5 and 15 min and of 0.86 % after 30 min. After the remediation with rice husk, these effects were eliminated, as no toxicity was observed (0 % effect) in the non-diluted $\text{RW}_{500\text{-rem}}$ treated samples. These results show the effectiveness of the treatment procedure by means of rice husk biowaste under these experimental conditions.

Green algae P. subcapitata

Algal growth inhibition tests were performed, and the results were expressed as the percentage of inhibition in growth rate relative to the control (Table 2). The values found for *P. subcapitata* growth rate were not affected during the exposure to the river water (RW) and the river water treated with rice husk (RW_{RH}), indicating that neither the river water nor the material used in the decontamination treatment exert an adverse effect on this species. The results applying a paired t test showed that there are no significant differences ($n=3$, $P \leq 0.05$) between the growth rate of the control and that from the RW and RW_{RH} . The river water spiked with an initial concentration of Hg(II) of $50 \mu\text{g L}^{-1}$ (RW_{50}) inhibited algae growth rate in 39 % (when compared to the control), before the remediation with rice husk. The value obtained for EC_{20} after a period of exposure of 72 h was 63.8 %. Significant differences in *P. subcapitata* growth were observed between the control and the RW_{50} dilutions (one-way ANOVA: $F_{4,23} = 26.1$, $P < 0.05$), and the values found for NOEC and LOEC were 44.4 and 66.7 %, respectively. The use of rice husk material to remediate the river water with these levels of Hg(II) contamination reduced the toxicity, and the inhibition in algae growth rate was just 7.4 and 9.8 % for $\text{RW}_{50\text{-rem}}$ R1 (replicate 1) and $\text{RW}_{50\text{-rem}}$ R2 (replicate 2), respectively, when compared with the control medium. It was not possible to calculate an EC_{20} , still significant differences were found

Table 2 Percentage of effects (bioluminescence, growth and feeding inhibition, and mortality) relative to the respective control observed due to exposure of test organisms to non-diluted (100 %) river water samples

Test	Sample C Hg(II) ($\mu\text{g L}^{-1}$) Duration	RW 3.75×10^{-3} Effect (% v/v)	RW _{RH} 9.53×10^{-3}	RW ₅₀ 50.0	RW _{50-remR1} 8.53	RW _{50-remR2} 7.37	RW ₅₀₀ 500	RW _{500-remR1} 32.60	RW _{500-remR2} 34.85
<i>V. fischeri</i>	5 min	NT	NT	NT	NT	NT	76.1	NT	NT
	15 min	NT	NT	NT	NT	NT	99.0	NT	NT
	30 min	NT	NT	NT	NT	NT	99.8	NT	NT
<i>P. subcapitata</i>	72 h	NT	1.5	39.3	7.4	9.8	100	22.3	21.1
<i>B. calyciflorus</i>	48 h	NT	6.5	46.6	NT	NT	100	32.7	30.4
<i>D. magna</i> (survival)	24 h	NT	NT	0	NT	NT	0	0	0
<i>D. magna</i> (feeding)	24 h	NT	36.3	96.4	60.8	69.2	100.0	97.7	98.2

NT no toxicity

between RW_{50-rem} dilutions and the control ($F_{5,22}=10.2$ and $F_{4,23}=27.8$ for replicate R1 and R2, respectively, $P<0.05$), showing a NOEC of 66.7 % and a LOEC of 100 %.

Algal growth was very sensitive to the sample with a higher level of Hg(II) contamination RW₅₀₀, inhibiting its growth in 100 %. The EC₅₀ value for the RW₅₀₀ sample was 31.0 % and the values obtained for NOEC and LOEC were 3.9 and 8.5 %, respectively. Under these circumstances, the treatment with rice husk was extremely efficient in the decontamination process and was able to reduce the toxicity in ca. 80 %. In this case, the remediated samples showed an inhibition in algal growth of 22.3 and 21.1 % for RW_{500-rem} R1 (replicate 1) and RW_{500-rem} R2, (replicate 2), respectively. For RW_{500-rem} R1, it was possible to calculate an EC₂₀ and the value found was 96.3 %. The results from one-way ANOVA indicate that the RW_{500-rem} R1 and RW_{500-rem} R2 tested dilutions were significantly different from the control ($F_{4,22}=82.3$ and $F_{4,23}=75.4$ for replicate R1 and R2, respectively, $P<0.05$), and the NOEC and LOEC values obtained were 44.4 % and 66.7 %, respectively for RW_{500-rem} R1 and 66.7 and 100 %, respectively, for RW_{500-rem} R2.

Rotifer B. calyciflorus

The river water used in the present study did not cause toxicity to rotifer *B. calyciflorus* and a paired *t* test showed that the reproduction rate of RW was not statistically different from the control ($n=8$, $P\leq 0.05$). The toxicity of the river water treated with rice husk was evaluated and the results indicate that the inhibition in reproduction is affected in less than 7 %, and according to a significant test, no differences were observed in the reproduction rate between the control and RW_{RH} (one-way ANOVA: $F_{5,60}=0.32$, $P<0.05$). This shows that the sorbent used in the decontamination process of Hg(II) in natural waters do not pose toxicity to this species. The results from the exposure of *B. calyciflorus* to the untreated sample

with different degrees of Hg(II) contamination (0, 50 and 500 $\mu\text{g L}^{-1}$) before (RW, RW₅₀ and RW₅₀₀) and after being treated with rice husk material (RW_{RH}, RW_{50-rem} and RW_{500-rem})

RW₅₀ indicate that reproduction is affected, with an inhibition of ca. 47 % in the non-diluted sample. The EC₅₀ value found for RW₅₀ after 48 h was 94.2 % and the NOEC and LOEC were 66.7 and 100 %, respectively. After the treatment procedure with rice husk sorbent, the reproduction rate of RW_{50-rem} R1 and RW_{50-rem} R2 were close to that from the control and no significant differences were found between them ($n=8$, $P\leq 0.05$), showing an efficacy in the remediation procedure.

The effects from the exposure of *B. calyciflorus* are much more drastic in the untreated sample RW₅₀₀ than those from RW₅₀, the former one with an Hg(II) initial concentration ten times higher. In this case, the levels found in the non-diluted RW₅₀₀ are lethal, causing absolute mortality to the organisms (0 % survival rate). The RW₅₀₀ untreated sample presents an LC₅₀ of 15.9 (11.8–20.1 %) after a period of exposure of 48 h. The EC₅₀ value for RW₅₀₀ was 9.9 %, and the value found for NOEC was 5.8 %, for LOEC 8.7 %. The levels of Hg(II) found after the treatment with rice husk, indicate that RW_{500-rem} R1 and RW_{500-rem} R2 do not cause mortality to the organisms, revealing once more the efficacy of the treatment with rice husk sorbent. However, the toxicity is not completely eliminated after treatment and the results indicate an inhibition in the reproduction of rotifers of 32.7 and 30.4 % for RW_{500-rem} R1 and RW_{500-rem} R2, respectively, when compared with the control. In this case, it was not possible to calculate the EC₅₀ and the values obtained for EC₂₀ were 72.6 % for RW_{500-rem} R1 and 64.1 % for RW_{500-rem} R2. The results from the analysis of variance indicate that there are no significant differences between the control and the test dilutions of RW₅₀₀ R1 ($F_{6,67}=0.70$, $P=0.65$) and RW₅₀₀ R2 ($F_{5,59}=1.77$, $P=0.13$).

Cladoceran D. magna

No mortality was observed during the exposure of *D. magna* to the river water and to the river water treated with rice husk.

Table 3 Ecotoxicity of river waters with and without Hg(II) before and after being treated with rice husk material expressed as effective or lethal concentration values inhibiting by 20 % (EC₂₀ and LC₂₀) or 50 % (EC₅₀ and LC₅₀) the endpoint measured given in percentage of the water samples in test medium (v/v) and respective 95 % confidence intervals

Test	Duration	Effect	Samples									
			RW	RW _{RH}	RW ₅₀	RW ₅₀ -rem R1	RW ₅₀ -rem R2	RW ₅₀₀	RW ₅₀₀ -rem R1	RW ₅₀₀ -rem R2		
<i>V.fischeri</i> (luminescence inhibition)	5 min	EC ₂₀ (% v/v)	NT	NT	NT	NT	NT	0.42 (0.12–1.53)	NT	NT	NT	
	15 min	EC ₂₀ (% v/v)	NT	NT	NT	NT	—(NC)	NT	NT	NT		
	30 min	EC ₂₀ (% v/v)	NT	NT	NT	NT	—(NC)	NT	NT	NT		
<i>P.subcapitata</i> (growth inhibition)	5 min	EC ₅₀ (% v/v)	NT	NT	NT	NT	0.68 (0.26–1.77)	NT	NT	NT		
	15 min	EC ₅₀ (% v/v)	NT	NT	NT	NT	0.68 (0.03–16.04)	NT	NT	NT		
	30 min	EC ₅₀ (% v/v)	NT	NT	NT	NT	0.86 (0.04–11.29)	NT	NT	NT		
	72 h	EC ₂₀ (% v/v)	NT	NT	63.79 (56.21–72.85)	NT	13.69 (8.22–18.84)	*(1)	96.31 (86.03–114.4)	NT		
	72 h	EC ₅₀ (% v/v)	NT	NT	NT	NT	31.04 (23.05–43.07)	NT	NT	NT		
	72 h	NOEC (% v/v)	NT	NT	44.4	66.7	3.9	44.4	44.4	44.4		
<i>B.calyflorus</i> (reproduction inhibition)	72 h	LOEC (% v/v)	NT	NT	66.7	100	66.7	66.7	66.7	66.7		
	48 h	EC ₂₀ (% v/v)	NT	NT	41.68 (34.15–48.21)	NT	6.24 (5.64–6.80)	72.62 (64.87–81.83)	64.11 (52.05–81.19)			
	48 h	EC ₅₀ (% v/v)	NT	NT	94.17 (80.456–118.3)	NT	9.87 (9.20–10.6)	—(NC)	—(NC)			
	48 h	NOEC (% v/v)	NT	NT	66.7	NT	5.8	—(NC)	—(NC)			
<i>D.magna</i> (survival)	48 h	LOEC (% v/v)	NT	NT	100	NT	8.7	—(NC)	—(NC)			
	24 h	EC ₂₀ (% v/v)	NT	NT	22.17 (21.22–23.17)	NT	1.41 (1.00–1.68)	23.88 (21.95–26.06)	22.90 (21.31–24.64)			
	24 h	EC ₅₀ (% v/v)	NT	NT	23.54 (22.53–24.61)	NT	1.87 (1.56–2.28)	25.5 (23.41–27.86)	25.24 (23.47–27.21)			
<i>D.magna</i> (feeding)	24 h	EC ₂₀ (% v/v)	NT	61.42 (52.07–72.39)	6.22 (2.27–10.04)	34.15 (28.81–39.52)	30.71 (26.11–35.20)	1.90 (1.16–2.55)	5.92 (1.77–10.04)	9.24 (3.82–14.23)		
	24 h	EC ₅₀ (% v/v)	NT	—(NC)	16.51 (10.29–24.20)	77.86 (66.36–95.24)	64.86 (56.64–76.11)	3.35 (2.50–4.48)	16.85 (9.91–25.59)	21.78 (14.13–32.60)		
	24 h	NOEC (% v/v)	NT	25	—(NC)	12.5	25	0.8	—(NC)	6.2		
24 h	LOEC (% v/v)	NT	50	6.2	25	50	1.6	6.2	12.5			

NT no toxicity, —(NC) Not Computed

The exposure of *D. magna* to both the untreated samples (RW₅₀ and RW₅₀₀) and to the samples treated with rice husk and for initial Hg(II) concentration of 500 µg L⁻¹ (RW₅₀₀-rem R1 and RW₅₀₀-rem R1) caused 100 % of mortality. However, no lethal effects (0 % mortality) were observed for the treated samples with lower Hg(II) levels (RW₅₀-rem R1 and RW₅₀-rem R2). These results reveal the effectiveness of rice husk to treat samples with lower levels of contamination (C₀ Hg(II) of 50 µg L⁻¹), but not to treat samples with a C₀ Hg(II) ten times higher. The *D. magna* lethality test showed LC₅₀ values of 23.5 and 1.9 % for untreated RW₅₀ and RW₅₀₀ samples, respectively. The LC₅₀ values calculated for the treated samples were: 25.5 % for RW₅₀₀-rem R1 and 25.2 % for RW₅₀₀-rem R2.

The sub-lethal effects on *D. magna* were evaluated by measuring the feeding rates with respect to the ASTM control. The results indicate that *D. magna* feeding responses were not affected during its exposure to river water and that there were no significant differences between this sample and the control ($n=4$, $P\leq 0.05$). However, the river water treated with rice husk, inhibited the feeding rate by 36 % and showed an EC₂₀ of 61.4 %. Significant differences were observed between the feeding rate of the control and the test dilutions of RW_{RH} ($F_{5,21}=4.26$, $P<0.05$), and the values obtained for the NOEC and LOEC were 25 and 50 %, correspondingly. A possible explanation for this behaviour can be attributed to the higher content of dissolved organic carbon (DOC) (ca. 2.5 times) on the RW_{RH} sample when compared with RW, resultant from the dissolution of some soluble components of rice husk vegetable biomass during the sorption process (Kuan and Yuen 2012). According to some studies, the DOC content represents an additional source of energy for *Daphnia* and the amount ingested increases with DOC concentration (Speas and Duffy 1998). As a result, the higher levels of DOC found in RW_{RH} represents an additional feeding supply for *D. magna*, decreasing the feeding rate resultant from the ingestion of algae.

Additionally, the results obtained showed that the feeding rates were inhibited by ca. 100 % on both the untreated samples (RW₅₀ and RW₅₀₀) and on the treated samples with higher Hg(II) levels (RW₅₀₀-rem R1 and RW₅₀₀-rem R2). The EC₅₀ values calculated for the untreated and treated sample with higher Hg(II) levels of contamination were 3.4 % for RW₅₀₀, 16.8 for RW₅₀₀-rem R1 and 21.8 % for RW₅₀₀-rem R2. Significant differences were observed between the control and the tested dilutions of RW₅₀₀ ($F_{6,22}=15.8$, $P<0.05$), RW₅₀₀-rem R1 ($F_{5,17}=12.5$, $P<0.05$) and RW₅₀₀-rem R2 ($F_{5,20}=13.2$, $P<0.05$). For the untreated sample RW₅₀₀, the value found for NOEC was 0.8 % and for LOEC was 1.6 % and for the treated sample RW₅₀₀-rem R2 a NOEC and LOEC of 6.2 and 12.5 % was obtained, respectively. For sample RW₅₀₀-rem R2, the value of LOEC was 6.2 % and NOEC could not be computed (<6.2 %). For the samples with lower

Hg(II) levels, the values obtained for EC₅₀ were 16.5 % of C₀ Hg(II) for RW₅₀ untreated sample, and 77.8 and 64.9 % for RW₅₀-rem R1 and RW₅₀-rem R2 treated samples, correspondingly. The results from the analysis of variance indicate the existence of significant differences between the control and the test dilutions of RW₅₀ ($F_{5,20}=14.3$, $P<0.05$), RW₅₀-rem R1 ($F_{5,20}=6.75$, $P<0.05$) and RW₅₀-rem R2 ($F_{5,19}=4.11$, $P<0.05$). Additionally, the values obtained for NOEC and LOEC were correspondingly 12.5 and 25 % for RW₅₀-rem R1, 25 and 50 % for RW₅₀-rem R2. For the untreated sample RW₅₀ and according to the dilution used, the value for NOEC could not be detected (<6.2 %) and the value of LOEC was 6.2 %.

Depending on the degree of Hg(II) contamination, different responses can be obtained and this will be reflected on the calculated EC₅₀ and EC₂₀ values. Based on the values obtained, it is possible to see that in general, the lower EC values, which reflect a higher toxicity, were obtained for the samples that contained higher Hg(II) concentrations.

From the bioassays performed in this study, *D. magna* proved to be more sensitive to the levels of contamination by Hg(II) than the other tested organisms. In this particular case, the efficacy in the detoxification process was not fully achieved and some inhibitory responses could be observed after the remediation process.

Conclusions

In the present study, the efficacy of rice husk to remove Hg(II) from river waters spiked with realistic environmental concentrations of this metal (µg L⁻¹ range) was evaluated, by determining the levels of this pollutant and assessing the ecotoxicological effects. The sorption process with rice husk proved to be effective and was able to reduce the levels of Hg(II) in solution to 7.95±0.82 µg L⁻¹, for an initial Hg(II) concentration of 50 µg L⁻¹, and to 33.7±1.6 µg L⁻¹, for an initial concentration ten times higher. The results from the ecotoxicity tests indicate that the remediation process with rice husk, for river waters spiked with lower Hg(II) concentrations (50 µg L⁻¹), was able to eliminate the toxicity to the exposed organism's algae *P. subcapitata* and rotifer *B. calyciflorus* and to ensure the total survival of *D. magna* species, and only some inhibitory effects were observed on *D. magna* at sub-lethal levels. Increasing ten times the concentrations of Hg(II) (500 µg L⁻¹) in river water, the results from the remediation with rice husk were not suitable in the detoxification process. Still, the toxicity of bacteria *V. fischeri*, algae *P. subcapitata*, rotifer *B. calyciflorus* and *D. magna*, whose responses were fully inhibited during its exposure to the non-remediate sample was considerably reduced after treatment with rice husk. This work highlights the importance of using a battery of bioassays with organisms from different

trophic levels, to obtain an accurate ecotoxicological hazard assessment of the detoxification process using rice husk biowaste.

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