



Removal of mercury by silica-supported ionic liquids: Efficiency and ecotoxicological assessment

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ARTICLE INFO

Keywords:

Ionic liquids-functionalized silica
Mercury removal
Ulva lactuca
Toxicity
Oxidative stress

ABSTRACT

Anthropogenic impacts have affected the coastal environment and contributed to its contamination. Mercury (Hg) is widespread in nature and has been shown to be toxic in even the smallest amounts, negatively affecting not only the marine ecosystem but also the entire trophic chain due to its biomagnification. Mercury ranks third on the Agency for Toxic Substances and Diseases Registry (ATSDR) priority list and it is therefore imperative to develop more effective methods than those currently available to avoid the persistence of this contaminant in aquatic ecosystems. The present study aimed to evaluate the effectiveness of six different silica-supported ionic liquids (SIL) in removing Hg from contaminated saline water, under realistic conditions ([Hg] = 50 µg/L), and to ecotoxicologically evaluate the safety of the SIL-remediated water, using as test model the marine macroalga *Ulva lactuca*. The results revealed that SIL [Si][C3C1im][SCN] (250 mg/L) was the most effective in removing Hg from solution, with an efficiency up to 99 % in just 6 h, that enable to obtain < 1 µg/L Hg (European guideline in drinking water). *U. lactuca* exposed to either the SIL and/or the remediated water showed no significant changes in relative growth rate and chlorophyll a and b levels, compared to the control condition. Biomarker analysis (LPO, GSH, GSSG, SOD, GPx, CAT and GRed) also showed no significant changes in the biochemical performance of *U. lactuca*. Therefore, it could be assumed that water treatment with SIL or its presence in an aqueous environment does not pose toxicity levels that could inhibit the metabolism or cause cell damage to *U. lactuca*.

1. Introduction

Despite the continued implementation of increasingly restrictive regulations and water quality monitoring, discharges of wastewater without any form of treatment or remediation are still a reality today (United Nations, 2021). Coastal ecosystems are the usual recipients of discharged contaminated water, mainly industrial and domestic effluents, which often contain potentially toxic elements (PTEs) such as metals (Deycard et al., 2014; Schweitzer and Noblet, 2018). In aquatic systems, metals can exist as free ions or complexed with inorganic anions and organic ligands (Haripriyan et al., 2022), and the most soluble metallic compounds can be easily absorbed and bioaccumulated by living organisms (Costa et al., 2020).

One of the most toxic metals, even in low concentrations, mercury (Hg), has caused global concern because it is non-degradable and

persists in ecosystems, being continually enriched through the trophic chain (biomagnification) (La Colla et al., 2019). This problem is compounded when Hg is found in the form of methylmercury (MeHg⁺), the most toxic and bioaccumulative form of Hg (Clarkson and Magos, 2006). The third place on the Toxic Substances and Disease Registry Agency (ATSDR) priority list emphasizes the importance of Hg control and elimination (ATSDR, 2022). Several intergovernmental agencies have imposed increasingly stringent limits on the use and release of Hg (Decision No 2455/2001/EC, 2001; Directive 2000/60/EC, 2000; Directive 2008/105/EC, 2008), restrictions which gained a new glow with the signing of the international agreement resulting from the Minamata Convention on Mercury. As example, currently, European Union (EU) regulation restricts the import of Hg into the EU, prohibits the export of Hg and requires that Hg waste in liquid form undergo appropriate conversion/immobilization into a more stable and less soluble form

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<https://doi.org/10.1016/j.aquatox.2023.106611>

Received 27 April 2023; Received in revised form 11 June 2023; Accepted 12 June 2023

Available online 13 June 2023

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(solid form) (European Commission, 2018).

Different conventional methods, such as chemical precipitation, adsorption, or membrane filtration have been used to reduce water contamination, including Hg removal (Qasem et al., 2021). However, most of them share serious disadvantages such as high cost, generation of large amounts of toxic waste, or inefficiency when applied to the treatment of highly diluted effluents, which requires the application of an additional process at the end of the effluent treatment (Henriques et al., 2019). New methods of water remediation have been proposed, such as biosorption/bioaccumulation or the use of chemically engineered materials (Imdad and Dohare, 2022). Nevertheless, despite their potential in metal-contaminated water treatment (Razzak et al., 2022), the lack of studies under realistic conditions, which validate the method, has postponed the transition from the laboratory to the market (Ali-dokht et al., 2021). Most studies still deal with unrealistic high concentrations of contaminant in ultrapure or distilled water, disregarding the complexity of natural waters (Dias et al., 2021).

Composed of an asymmetric organic cation and an inorganic anion, Ionic Liquids (ILs) are considered to be a promising solution for removing metals from water (Goutham et al., 2022). One of the benefits of the ILs is the possibility to adjust some properties such as conductivity, viscosity or hydrophobicity and to select the composition ions to optimize a particular process (Karimi et al., 2018). Ammonia cation-based ILs can form ion pairs or complexes with metal ions in aqueous matrices and remove them with high efficiency (Raj et al., 2022). In many cases, ILs immobilization as with silicas is required (Nguyen et al., 2022). The supported ILs (SILs) maintain the high specific surface area and mechanical properties circumventing the problem of mass transport limitation and the need for high amounts of ILs (Wolny and Chrobok, 2022).

Although ILs and SILs have been identified as green approaches, little is known about their environmental impact and potential ecotoxicity. Toxicological assessment of ILs has been mainly limited to microorganism and simple cells (Chen and Mu, 2021). Still, few studies have shown the toxicological evaluation of ILs to aquatic organisms (Cho et al., 2021; Gonçalves et al., 2021), and until now the main conclusion resides in the fact that the biological response changes according to the change in the ILs structure. Thus, it is of vital importance to continue to conduct studies for higher levels of biological organization, (Fan et al., 2019a; Kumar et al., 2011; Liu et al., 2018; Parajó et al., 2019; Sanches et al., 2023; Santos Klienchen Dalari et al., 2022) to facilitate and ensure their complete utilization in practical water treatment applications. Macroalgae, such as *Ulva lactuca*, have been used extensively for bio-monitoring of environmental pollution and ecotoxicological assessment (Pereira et al., 2009; Yang et al., 2019). Their use is particularly advantageous since these organisms are more complex than microalgae and are as well compose the basis of the trophic chain (Torres et al., 2008).

The present study aimed to assess the performance of silica-supported ionic liquids (SILs) in the removal of Hg from contaminated saline water at realistic levels; and particularly to evaluate its efficiency in terms of the ecotoxicological safety for aquatic organisms (both decontaminated water and SIL). For that, *Ulva lactuca* was exposed to different conditions (presence and absence of Hg and/or SIL) and the effects on organism growth, pigmentation, and biomarkers (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, reduced glutathione, oxidized glutathione, and lipid peroxidation) were analysed.

2. Material and methods

2.1. Materials and reagents

The reagents used in this work were of analytical quality. Nitric acid (65 %, m/m, Suprapur®) and the standard mercury solution (1001 ± 2 mg/L) were purchased from Merck. All solutions used, including

standards for calibration curves, were obtained by diluting the commercial standard solution. All glass material was washed with nitric acid (25 %) for at least 24 h followed by ultrapure water (18 MΩ/cm) before use.

2.2. Collection of seawater and *Ulva lactuca*

The saline water used for macroalgae acclimatization and assays was collected from the Atlantic Ocean, Aveiro, Portugal (40°6441N, 8°7453W) (salinity 35). In the laboratory, the saline water was filtered using Millipore porosity filters (0.45 μm) and subsequently diluted to salinity 15, using ultrapure water (18 MΩ/cm).

U. lactuca collection was carried out in Ria de Aveiro, Portugal (40°3839N, 8°4443W). In laboratory, the macroalgae were washed with saline water to remove possible debris and epibionts. A sample was immediately frozen at −80 °C, for later quantification of the total natural Hg concentration. Macroalgae were kept in aerated aquaria (salinity 15) and exposed to natural light at room temperature (20 ± 2 °C) for acclimation during one week prior to the start of the experiments.

2.3. Evaluation of silica-supported ionic liquids (SILs) efficiency in batch mode

To evaluate the removal efficiency of Hg from saline water, assays in batch mode were conducted for 24 h. Six different ionic liquids, supported on a silica matrix, were studied (Table 1). Each SIL was placed in contact with filtered saline water (salinity 15), which was previously spiked with Hg to obtain the initial concentration of 50 μg/L (maximum value allowed for industrial effluent discharges) (Council Directive 84/156/EEC, 1984). The water pH was adjusted to 7.8 using NaOH (0.1 mol/L). Two different amounts of SIL were analysed (50 mg/L and 250 mg/L) together with control solutions (saline water spiked with Hg in the absence of SIL). After adding the SIL, water samples were collected at pre-defined time intervals (0, 1, 6, 12, and 24 h), filtered (pore size of 0.45 μm), and immediately acidified with 65 % (m/m) HNO₃.

The mercury removal efficiency (R, %) was calculated by Eq. (1):

$$R = 100 \times \frac{(C_0 - C_t)}{C_0} \quad (1)$$

where C_0 (μg/L) is the initial concentration of Hg in the spiked water and C_t (μg/L) the concentration at time t .

2.4. *Ulva lactuca* ecotoxicological assays

To evaluate the potential toxicity associated with SIL use, ecotoxicological assessment assays with *U. lactuca* were conducted. Five conditions were studied (Table 2), in which the SIL analysed was the one that presented the best Hg removal efficiency from saline water. *U. lactuca* (8 discs of $\varnothing = 25 \pm 1$ mm and 6 discs of $\varnothing = 58 \pm 1$ mm) were added to 500 mL of water to achieve approximately 1 g in fresh weight (FW).

The assays were carried out in triplicate for 48 h and water samples (5 – 10 mL) were taken immediately before the addition of the discs (time 0 h) and after 24 and 48 h. Samples were stored in polystyrene

Table 1
Silica-supported ionic liquids evaluated in mercury removal from water.

| | |
|-----------------------------------|-------|
| [Si][C3C1im]Cl | SIL 1 |
| [Si][C3C1im][N(CN) ₂] | SIL 2 |
| [Si][C3C1im][Male] | SIL 3 |
| [Si][C3C1im][NTf ₂] | SIL 4 |
| [Si][C3C1im][Tos] | SIL 5 |
| [Si][C3C1im][SCN] | SIL 6 |

Table 2Conditions to which *U. lactuca* was exposed for 48 h ($n = 3$).

| | |
|---|---|
| A | <i>U. lactuca</i> in clean saline water (salinity 15) – Control. |
| B | <i>U. lactuca</i> in saline water (salinity 15) spiked with Hg (50 µg/L). |
| C | <i>U. lactuca</i> in saline water (salinity 15) formerly spiked with Hg (50 µg/L) and remediated with the SIL (250 mg/L). SIL was separated at the end. |
| D | <i>U. lactuca</i> in saline water formerly contaminated with Hg (50 µg/L) and remediated with SIL. SIL remained in solution. |
| E | <i>U. lactuca</i> in saline water containing SIL (250 mg/L). |

tubes, previously acidified with HNO₃ (65 % m/m) to guarantee a pH < 2 and kept at 4 °C until analysis. At each collection time, the pH of the water was measured. At the end of the experiment, macroalgae discs were collected and immediately frozen at –80 °C for further analysis.

2.5. Mercury quantification

Mercury quantification in water samples was performed by atomic fluorescence spectrometry with a cold steam generator (CV-AFS), on a PSA cold vapor generator with a Merlin PSA detector (model 10.025). Tin chloride (SnCl₂) (2 % w/v in 10 % v/v HCl) was used as the reducing agent (Fabre et al., 2020). Each water sample was analysed at least in triplicate (accepted coefficient of variation < 5 %). The limit of quantification of the method was 0.02 µg/L.

Mercury quantification in the macroalgae biomass was performed by atomic absorption spectrometry with thermal decomposition, on a LECO® AMA-25. The analysis was carried out directly on the lyophilised sample (Fabre et al., 2020). The limit of quantification was 0.03 ng of Hg. Samples were analysed in triplicate with a coefficient of variation < 10 %. To assure the quality of the results a certified reference material (ERM-CD200 Bladderwrack (*Fucus vesiculosus*) (European Reference Materials, 2014)) was analysed in parallel with the samples.

2.6. Impacts evaluation in *U. lactuca*

After *U. lactuca* exposure to the different conditions (Table 2), the following parameters were evaluated: 1) macroalgae growth rate; 2) content of chlorophylls *a* and *b*; and 3) biochemical performance.

Growth rate was analysed by measuring the area of *U. lactuca* discs (placed in transparent plastic sheets and scanned with a resolution of 200 ppi) with Fiji software (Schindelin et al., 2012). The relative growth rate (RGR, %/day) was calculated, according to Eq. (2):

$$RGR \left(\frac{\%}{\text{day}} \right) = \frac{\ln A_t - \ln A_0}{t} \times 100 \quad (2)$$

where, A_t represents the area at time t (t in days) and A_0 the initial area (Henriques et al., 2021).

Quantification of chlorophyll *a* and *b* was performed in 2 discs per condition replicate ($\varnothing = 25 \pm 1$ mm), which were stored in Falcon tubes wrapped in aluminum foil to avoid photodegradation of pigments, at –80 °C, until analysis. The discs were macerated in 6 mL ethanol (90 % v/v) and left overnight at 4 °C to extract the pigments. Samples were then vortexed for 1 min, centrifuged at 4000 rpm, for 5 min, and analysed on a GBC UV/Vis 918 spectrophotometer (400 to 800 nm). Chlorophyll *a* and *b* were determined according to Ritchie (2006), following Eqs. (3) and 4:

$$Chl a \left(\frac{\mu\text{g}}{\text{mL}} \right) = -5.201 (649 \text{ nm} - 750 \text{ nm}) + 13.528 (665 - 750 \text{ nm}) \quad (3)$$

$$Chl b \left(\frac{\mu\text{g}}{\text{mL}} \right) = 22.433 (649 \text{ nm} - 750 \text{ nm}) - 7.074 (665 - 750 \text{ nm}) \quad (4)$$

where, *Chl a* corresponds to the amount of chlorophyll *a* and *Chl b* to the amount of chlorophyll *b*.

Biochemical markers related to the macroalgae oxidative state were evaluated. Antioxidant enzymes activity was assessed by superoxide

dismutase activity (SOD, which converts O²⁻ to H₂O₂), catalase activity (CAT, which converts H₂O₂ into water, quenching the generation of lipid peroxides), glutathione peroxidase (GPx, which convert the SOD product (H₂O₂) into water) and glutathione reductase (GRed, which converts GSSG to GSH). Cellular damage level was assessed by lipid peroxidation, (LPO, which indicates the cellular damage level) and reduced glutathione content (GSH, which represents the redox state of cells). A 0.3 g sample of macroalgae from each of the 3 replicates of each condition was analysed in a microplate reader (Biotek) (in duplicate).

For LPO quantification, samples were sonicated for 1 min and then centrifuged (10,000 g) for 20 min at 4 °C, with trichloroacetic acid (TCA) 20 % (w/v) (Henriques et al., 2021). For GSH and GSSG content determination, samples were extracted by sulfosalicylic acid (0.6 % (w/v)) in potassium phosphate buffer (0.1 mol/L dipotassium phosphate; 0.1 mol/L potassium dihydrogen phosphate; 5 mmol/L EDTA; 0.1 % (v/v) Triton X-100; pH 7.5) and quantification followed the method of Rahman et al. (2007).

For SOD, CAT and GPx quantification, samples were sonicated for 1 min and centrifuged for 20 min (10,000 g) at a temperature of 4 °C with potassium phosphate buffer (50 mmol/L potassium dihydrogen phosphate; 50 mmol/L potassium phosphate dibasic; 1 mmol/L ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA); 1 % (v/v) Triton X-100; 1 % (w/v) polyvinylpyrrolidone (PVP); 1 mmol/L dithiothreitol (DTT); pH 7.0). Superoxide dismutase activity was quantified following the method of Beauchamp and Fridovich (1971), while CAT activity quantification followed the method of Johansson and Håkan Borg (1988) with modifications by Carregosa et al. (2014). Glutathione peroxidase was determined following Paglia and Valentine (1967) modifications.

For GRed the extraction was in sodium phosphate buffer, pH 7.0 (50 mmol/L sodium dihydrogen phosphate monohydrate, 50 mmol/L disodium hydrogen phosphate dihydrate, 0.15 % (v/v) Triton X-100) and determination was based on the method of Carlberg and Mannervik (1985).

Results were submitted to a non-parametric statistical hypothesis testing using Permutational Analysis of Variance and the software PERMANOVA and Primer v6 (Anderson et al., 2008). Significant differences between conditions were evaluated and the null hypothesis established was: for each biomarker, no significant differences between conditions tested existed. Principal coordinates analysis (PCO) was performed, applying a multivariate analysis to the obtained physiological and biochemical parameters. Spearman's correlation vectors (correlation higher than 75 %) of all biochemical descriptors obtained were superimposed on the PCO graph.

3. Results

3.1. Removal of mercury by silica-supported ionic liquids

For the lowest amount of material (50 mg/L), removals varied between 40 and 82 %, the lowest achieved by [Si][C3C1im][Male] and the highest by [Si][C3C1im][SCN] (Fig. 1A). Regardless of the SIL, the mass increase from 50 to 250 mg/L raised the removal of Hg. Still, SIL 6 ([Si][C3C1im][SCN]) was found to be the most effective with a removal efficiency of over 99 %.

The mercury removal by SILs over time was characterized by two phases: the first with rapid sorption, resulting in 60 % of removal in the first hour in the case of SIL 6 (Fig. 1B) and the second slower and approaching a plateau state between 6 and 24 h

3.2. Mercury concentration in water and in the macroalgae

After 48 h, Hg concentration in water was 0.04 µg/L for condition B, 0.03 µg/L for condition C and 0.32 µg/L for condition D. Mercury concentration in condition A and E (absence of Hg spike) remained below the limit of quantification.

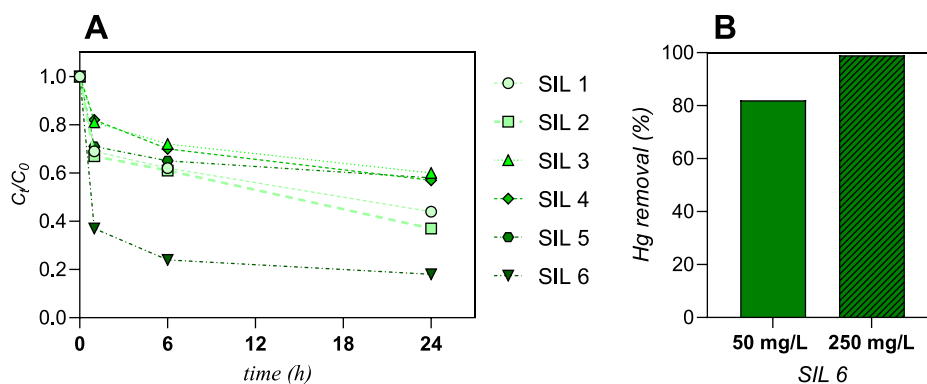


Fig. 1. A - Kinetic profiles of normalized concentrations of Hg in the presence of the SIL (although not shown, C_t/C_0 remained approximately 1 in the absence of SIL); B - Mercury (Hg) removed from saline water by the SIL 6 ([Si][C3C1im][SCN]) after 24 h of contact. Initial concentration of Hg of 50 $\mu\text{g/L}$ and SIL dosage of 50 and 250 mg/L.

Before exposure, Hg concentration in *U. lactuca* was 0.29 $\mu\text{g/g}$ dry weight (DW). After 48 h of exposure, Hg concentration in macroalgae tissue was 0.06 $\mu\text{g/g}$ for conditions A and E, 82.3 $\mu\text{g/g}$ for condition B, 1.1 $\mu\text{g/g}$ in condition C and 47.6 $\mu\text{g/g}$ in condition D, all in DW.

3.3. Evaluation of [Si][C3C1im][SCN] toxicity in *U. lactuca*

3.3.1. Effect on macroalgae growth

The relative growth rate of *U. lactuca* varied between 2.2 %/day and 3.7 %/day ($n = 9$). The lowest RGR was observed for condition E (presence of SIL and absence of Hg) and the highest rate recorded was for condition B, where *U. lactuca* was exposed only to mercury.

The RGR observed (Table 3) in condition A showed no significant differences from condition E ($p > 0.05$). Both conditions, A and E, differed significantly from conditions B, C and D ($p < 0.05$). No color loss or visible changes were observed.

3.3.2. Effect on macroalgae pigmentation

The content in Chlorophyll *a* was always higher than Chlorophyll *b*. Conditions B to E presented lower Chlorophyll *a* (1.04 – 1.10 $\mu\text{g/mg}$) when compared to condition A (1.18 $\mu\text{g/mg}$), yet there were no significant differences between conditions. Chlorophyll *b* values varied between 0.62 – 0.74 $\mu\text{g/mg}$ among conditions. The ratio between chlorophyll *a* and *b* was higher in condition B (1.74 $\mu\text{g/mg}$), while the lowest ratio between chlorophylls was observed in condition C (1.49 $\mu\text{g/mg}$). Mean values of chlorophyll *a* and *b* are presented in Table 4.

3.3.3. Effect on macroalgae oxidative stress

Lipid peroxidation (LPO) levels were significantly lower in the macroalgae exposed to conditions D and E compared to control (A) and conditions B and C. No significant differences were observed between the macroalgae exposed to control (A) and conditions B and C (Fig. 2).

Reduced glutathione content was significantly lower in macroalgae exposed to condition E when compared to conditions A, C and D. No significant differences were observed among conditions A, B, C and D (Fig. 3I).

No significant differences between macroalgae exposure conditions

Table 3

Relative growth rate (RGR, %/day; $n = 9$) of *U. lactuca* exposed to conditions summarized in Table 2.

| Condition | RGR (%/day) mean value | Standard deviation |
|-----------|------------------------|--------------------|
| A | 2.5 | 0.52 |
| B | 3.6 | 0.31 |
| C | 3.4 | 0.25 |
| D | 3.0 | 0.56 |
| E | 2.2 | 0.81 |

Table 4

Chlorophyll *a* and *b* content (mean values; $n = 3$) in *U. lactuca* after 48 h of exposure to conditions summarized in Table 2. Quantification of chlorophyll using UV/Vis spectroscopy, after extraction with ethanol 90 % (v/v) overnight.

| ($\mu\text{g/mg}$) | Condition | | | | |
|----------------------------|-----------|------|------|------|------|
| | A | B | C | D | E |
| Chlorophyll <i>a</i> | 1.18 | 1.08 | 1.10 | 1.09 | 1.04 |
| Chlorophyll <i>b</i> | 0.68 | 0.62 | 0.74 | 0.70 | 0.62 |
| Chl <i>a</i> /Chl <i>b</i> | 1.73 | 1.74 | 1.49 | 1.56 | 1.68 |

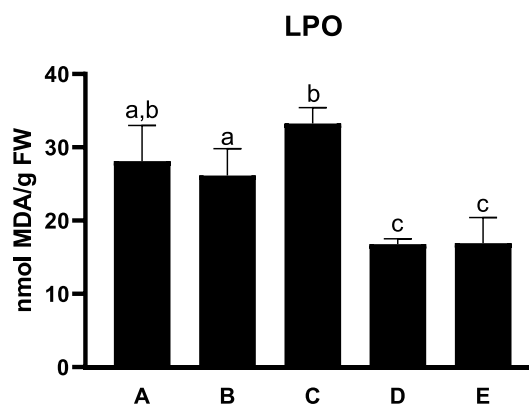


Fig. 2. Lipid peroxidation levels in *U. lactuca* after 48 h of exposure to conditions summarized in Table 2.

were observed for GSSG content when compared with control condition (A) (Fig. 3II).

Superoxide dismutase (SOD) activity was significantly lower in macroalgae exposed to condition B compared to control (A). No significant differences were observed among macroalgae exposed to C, D and E conditions (Fig. 4I).

Catalase (CAT) activity was significantly higher for macroalgae in condition C in when compared with control (A). No significant differences were observed among macroalgae exposed to conditions B, D and E (Fig. 4II).

Glutathione peroxidase (GPx) activity was significantly higher in macroalgae exposed to conditions D and E when compared to control (A). Conditions B and C did not show significant differences compared to control levels (Fig. 4III).

Glutathione reductase (GRed) activity levels were significantly lower in condition D compared to the control and condition B. A significant higher activity was observed for macroalgae exposed to condition E. No

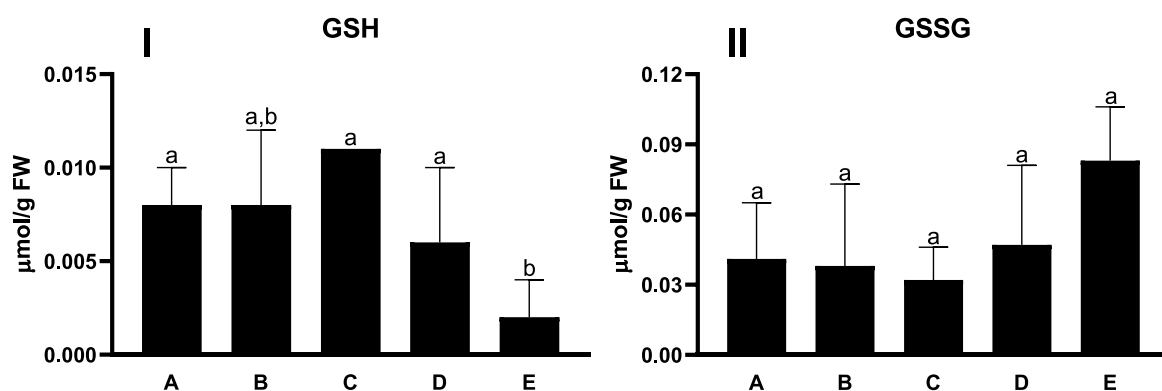


Fig. 3. I - Reduced glutathione (GSH) levels; II - Oxidized glutathione (GSSG) levels in *U. lactuca* after 48 h of exposure to conditions displayed in Table 2.

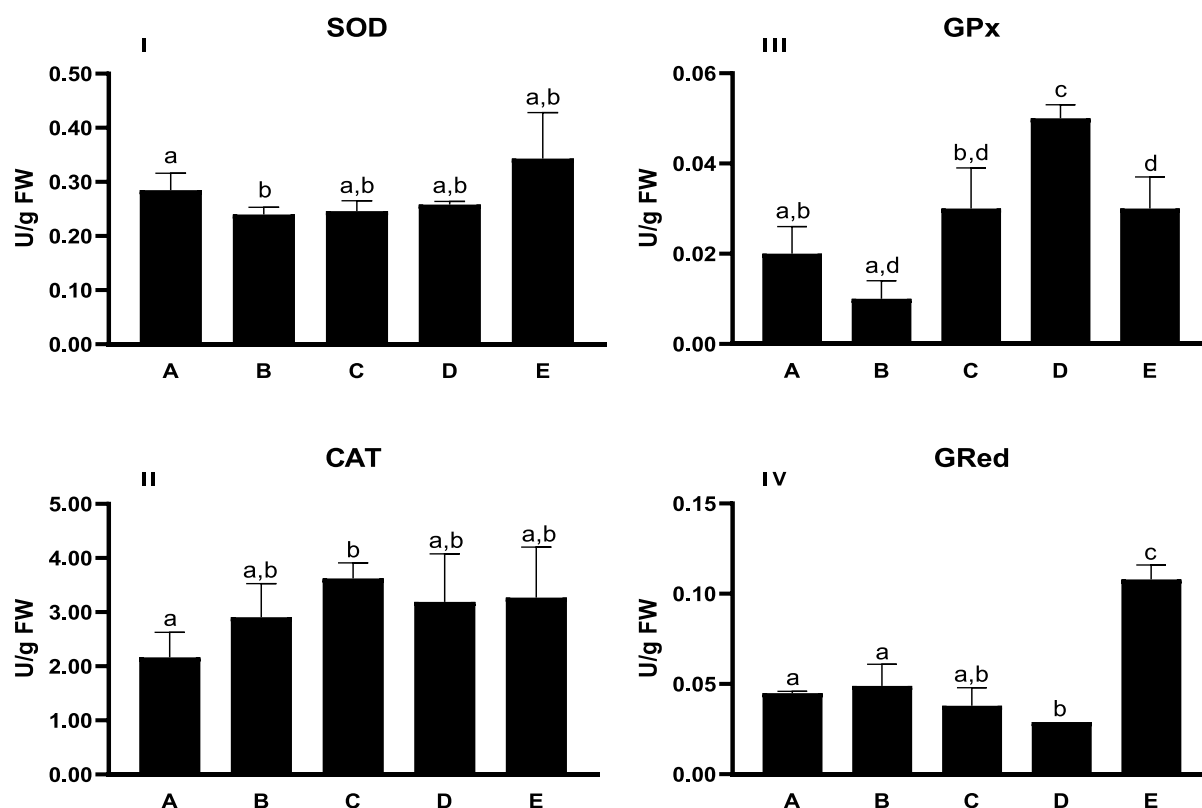


Fig. 4. I - Superoxide dismutase (SOD) activity; II - Catalase (CAT) activity; III - Glutathione peroxidase (GPx) activity; IV - Glutathione reductase (GRed) activity in *U. lactuca* after 48 h of exposure to conditions displayed in Table 2.

significant differences were observed for condition B and C compared to control (A) (Fig. 4IV).

Principle coordinates at horizontal dimension (PCO1) explained 49.1 % of total variation, while PCO vertical dimension (PCO2) explained only 27.4 % of total variation (Fig. 5). PCO1 separated macroalgae exposed to condition E at the positive side. The PCO2 separated macroalgae exposed to conditions A and B in the positive site from condition C and D. GPx showed the highest correlation with the negative axis of PCO2 ($r > -0.75$) (Fig. 5).

4. Discussion

4.1. Mercury removal by SIL

The sorption capacity of SILs has already been demonstrated for non-steroidal anti-inflammatory drugs and for hazardous gas compounds

(Almeida et al., 2020; Kohler et al., 2014). Here, under realistic conditions, the sorption capacity for Hg, currently a top-priority contaminant in the water sector, was evaluated and the results showed that Hg levels as low as the drinking water norm (1 µg/L, European legislation) (Decision No 2455/2001/EC, 2001; Directive 2000/60/EC, 2000) can be achieved, using a small amount of SIL (250 mg/L).

Among the SILs evaluated, SIL [Si][C3C1im][SCN] was the one with the best removal rates for Hg, with nearly 80 % of removal within 6 h of contact, for the lowest sorbent mass analysed (50 mg/L). This superior efficiency can be attributed to the thiocyanate (SCN⁻) group (Jabbari et al., 2001), once mercury has a known stronger affinity and interaction with sulfur (as a class B metal, the order is: $S > N > O$) (Nieboer and Richardson, 1980; Wang et al., 2020). Based on this knowledge, numerous thiol-functionalized sorbent materials have been designed and proposed for Hg capture with enhanced binding affinity and uptake capacity (Wang et al., 2020), such as silica gel, metal-organic

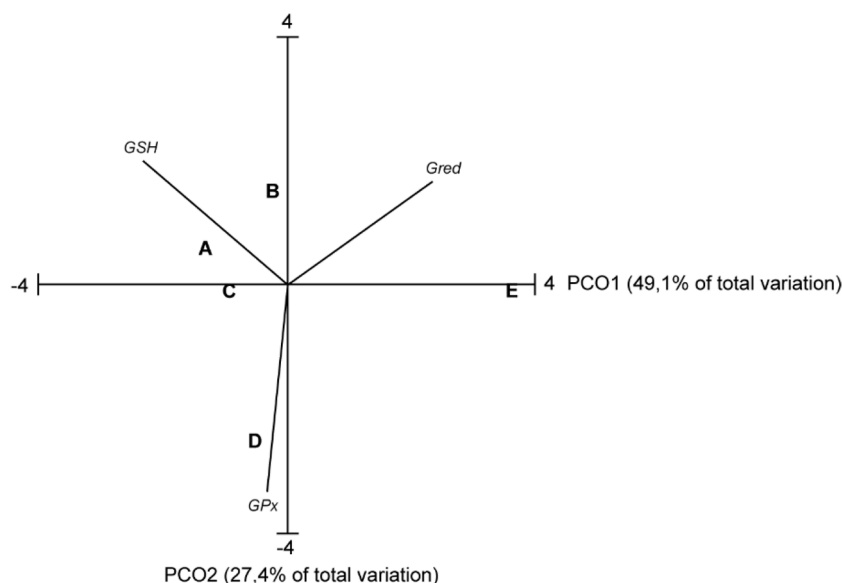


Fig. 5. Principal Component Ordination performed using biochemical markers measured in *U. lactuca* after 48 h of exposure to conditions displayed in Table 2. GSH, reduced glutathione; GPx, glutathione peroxidase activity; GRed, glutathione reductase activity.

frameworks, and carbon-based materials (Ji et al., 2022; Liang and Zou, 2020; Xia et al., 2019). However, unlike the present study, all these studies were conducted solely in deionized or ultrapure water.

4.2. Ecotoxicological safety of SIL 6 and remediated water for *Ulva lactuca*

Marine macroalgae have a known ability to uptake and accumulate metals and other trace elements from water, playing an important role in the global mercury cycle in aquatic systems (La Colla et al., 2019), and are efficient bioindicator organisms for environmental contamination (Areco et al., 2021; Rakib et al., 2021). In the present work, when exposed to 50 $\mu\text{g/L}$ of Hg (condition B), *U. lactuca* incorporated a large amount of Hg from the contaminated water (99 % of the Hg initially in water), corresponding to 82 $\mu\text{g Hg/g}$, DW). This high sorption capability underscores the environmental remediation capacity of this species pointed in various studies for different contaminants (Areco et al., 2021; Henriques et al., 2019; Smii et al., 2023). It also demonstrates the ease of bioaccumulation of Hg in organisms and hence the need to remove this contaminant from water to avoid environmental and human health risks.

The SIL [Si][C3C1im][SCN] showed similar performance in removing Hg from water (98 % in 24 h), even using 10 times less sorbent (250 mg/L) than that of macroalgae. *U. lactuca* exposed to the water remediated by [Si][C3C1im][SCN] for 48 h (condition C), accumulated much less Hg (80-fold) in comparison to untreated water (condition B), although it promoted an additional improvement in water quality by decreasing the Hg concentration from 0.72 $\mu\text{g/L}$ to 0.03 $\mu\text{g/L}$. These results demonstrate not only the viability of [Si][C3C1im][SCN] as a Hg sorbent, but also the possibility of using macroalgae use as a complement to other Hg removal methods (Henriques et al., 2015).

Nevertheless, the macroalga exposed to condition D (with [Si][C3C1im][SCN] remaining in the water after Hg sorption) had a Hg content of 41 $\mu\text{g/g}$, which is 40 times higher than that in condition C. This shows that *U. lactuca* could compete for part of the Hg that was sorbed onto [Si][C3C1im][SCN]. However, in a practical application, the Hg-laden SIL will always be separated from the solution after treatment, and safely stored or regenerated, as commonly done to most sorbents.

Although the results demonstrate the viability of [Si][C3C1im][SCN] in remediation from a chemical perspective, it is important to

understand the toxicological impact that the use of this SIL as a remediation method can have on aquatic organisms.

4.2.1. Relative growth rate (RGR, %/day)

The relative growth rate (RGR) is one of the indicators of the metabolic and welfare effects of *U. lactuca* (Bews et al., 2021). In the present work, the lowest growth rate was verified in the condition in which the macroalgae were exposed to the SIL [Si][C3C1im][SCN], in the absence of Hg (condition E). This could indicate some toxicity of the material, however, the RGR observed for condition E was not significantly different from that observed in condition A (macroalgae in saline water). The highest growth rate (3.6 ± 0.31 %/day) was observed in condition B where the macroalgae were exposed only to Hg, consistent with that reported by Costa et al. (2011) for the same conditions of salinity, Hg concentration and exposure time (salinity 15, 50 $\mu\text{g/L}$, 48 h). This result can be framed in the concept of *hormesis*, a central concept in the biological and biomedical sciences with important implications for environmental risk assessment, that consists in 'low dose stimulation and high dose inhibition' (Bews et al., 2021). Macroalgae from conditions C and D (saline water post-remediation with [Si][C3C1im][SCN]) presented positive RGR, that not statistically differed from that of condition B. This proves that, by it-self, the SIL [Si][C3C1im][SCN] had no impact on *U. lactuca* growth.

4.2.2. Chlorophyll a and b

The amount of chlorophyll in photosynthetic organisms is an important monitoring parameter (Bews et al., 2021), especially chlorophyll a as this is the primary pigment of photosynthesis in most photosynthetic organisms and the presence or absence of other types of chlorophyll is of taxonomic importance (Patel, 2011). In addition, the content of secondary chlorophyll, such as chlorophyll b versus chlorophyll a reflects fluctuations in light intensity and stress due to environmental conditions (Singh et al., 2020).

In the present work, the production of chlorophyll a and b remained statistically the same, regardless of the conditions the macroalgae were exposed to, suggesting that neither the presence of Hg nor SIL affected the photosynthetic capacity of the organism. The observed chlorophyll a and b values observed are consistent with algal values stated in literature, where chlorophyll a is predominant. Control showed a ratio of Chl a/Chl b = 1.7, which is a value found in healthy green algae (Cruces et al., 2019; Malta et al., 2002). Also, the mean value of Chl a/Chl b, 1.62

$\pm 0.078 \mu\text{g}/\text{mg}$, was within the range reported for healthy green algae (molar ratios of 1.5 – 2.2). Similar results were observed in *U. armoricana* exposed to different Cu concentrations (25, 50, 100 and 250 $\mu\text{g}/\text{L}$), where there were no significant changes in the photosynthetic activity until exposure to the highest concentrations (100 and 250 $\mu\text{g}/\text{L}$) (Han et al., 2008). Other studies reported stress effects of PTEs on green algae, namely Chl *a*/Chl *b* (Omar, 2002; Rocchetta et al., 2012; Simon et al., 2001) and also on the protective function of carotenoids (Genç et al., 2020) against photooxidation of light-harvesting pigments such as chlorophyll *a* (protection against chlorophyll-induced photosensitization (Pinto et al., 2003)), reducing SOD activity and the cost of immunity (Tan et al., 2020).

4.2.3. Biomarker analysis

Exposure to stressful conditions can activate an enzymatic action of the macroalgae (Rezayian et al., 2019), hence the biochemical analysis performed in the present study aimed to understand the biochemical state of the macroalgae *U. lactuca* after exposure to Hg, to the sorbent [Si][C3C1im][SCN], and to the remediated water. The results showed that in the presence of Hg or Hg + SIL (B and C conditions) the macroalgae presented levels of oxidative stress higher than those observed in D and E conditions. The higher LPO levels in condition C were promptly remediated by the increased activity of CAT to eliminate excess reactive oxygen species (ROS), because of exposure to stress conditions. A study by Elbaz et al. (2010) with *Chlamydomonas reinhardtii* also indicated that the increased antioxidant activity was effective in preventing cellular damage in Hg-exposed organisms. Oxidative stress appears to be due to the presence of Hg and not of SILs. Although no cellular damage was identified in the macroalgae exposed to Hg on the remediated water (Hg + SIL), *U. lactuca* was found to be under oxidative stress in conditions B and C, evidenced by the increased activity of antioxidant enzymes.

Regarding condition D (remediated water, where SIL remained in solution), and E (saline water + SIL) the activities of CAT and SOD enzymes were similar to that measured in the control. Condition E showed significantly lower LPO levels than control condition (condition A) and indicated that the oxidative stress was resolved by increased GPx and GRed activity, thereby eliminating ROS and preventing oxidative stress and cell damage. Given these results, it could be assumed that the presence of [Si][C3C1im][SCN] in an aquatic environment does not exhibit toxicity levels capable to inhibit metabolism or cause cellular damage in *U. lactuca*, which was not observed to other IL, such as the 1-decylpyridinium bromide ([DPy]Br) (Liu et al., 2018). Decreased chlorophyll content, cell membrane permeability and increased SOD and CAT activities were measured in *Scenedesmus obliquus* by Liu et al. (2018), suggesting that [DPy]Br could damage photosystem II (PSII). Other IL also induced oxidative damage on *S. obliquus*, and in high concentration treatments, ROS could not be effectively removed by the antioxidant system, leading to oxidative damage and ultimately resulting in growth inhibition and cell death (Fan et al., 2019b).

Regarding condition D, the reduction in LPO levels and the increase on GPx activity may indicate that *U. lactuca* has not undergone oxidative stress from the SIL still present in solution. To effectively eliminate ROS, oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) by the enzyme GPx is needed. Hence, according to the results obtained for conditions of exposure to Hg or Hg + SIL (B and C), the content in GSH and GSSG did not indicate significant differences compared to condition A (control), corroborated with LPO absence, showing that the macroalgae was able to eliminate the excess of ROS. Nevertheless, there was an increase in GSSG levels, which may be due to the increase in ROS in the presence of SIL but proved insufficient to produce cellular damage or increase antioxidant activity of *U. lactuca*. In condition C, the absence of significant differences in the content of GSH and GSSG corroborates the absence of oxidative stress in this condition. Therefore, oxidative stress appears to be due to the presence of Hg and not SIL or SIL-remediated water. Sharaf et al. (2019) observed that *Chromera velia*,

exposed to Hg increased the production of ROS which triggered an antioxidant response, namely the activity of the enzymes SOD, CAT and ascorbate peroxidase (APX). Likewise, the activity of the antioxidant and photosynthetic systems of *U. pertusa* was not inhibited by multiple-contamination of PTEs in seawater, suggesting that adaptation is a biological mechanism for *Ulva* sp. (Ge et al., 2017). Therefore, antioxidant responses at the particular subcellular sites where oxidative stress is triggered could contribute to the overall tolerance of algae during conditions of contaminant stress (Pinto et al., 2003; Torres et al., 2008).

If, photoinhibition occurred, the simultaneous presence of O_2 and an excess of excitation energy would produce ROS through the transferred energy from chlorophyll triplet states to O_2 , resulting in photo-oxidative damage and ultimately leading to photoinhibition of photosystem II (PSII) and photosystem I (PSI) as well as of other several cellular functions (Zhang et al., 2020). Such cascade of events would lead to alteration in Chl *a*, Chl *b* and Chl *a*/Chl *b* values as well as in antioxidant enzymes' activity and even visible signs of stress on the macroalgae tissue as decolouration. Thus, it could be assumed that the treatment with SIL and its presence in aqueous environment does not pose toxicity levels capable of inhibiting the metabolism or causing cellular damage to *U. lactuca*.

5. Conclusion

In this study, the method proposed for the decontamination of Hg from saline waters, based on silica-supported ionic liquids (SIL), in particular [Si][C3C1im][SCN] proved to be very promising, with removals up to 98 % in 6 h, with just 250 mg/L SIL.

The ecotoxicological evaluation of the proposed methodology using the macroalgae *U. lactuca* showed that organism growth rates and photosynthetic rates were not affected by exposure to SIL, compared to the control condition. Even the SIL-remediated water (with or without the presence of SIL) did not hinder the natural development of *U. lactuca*. The biomarkers analysis did not show significant alterations in the biochemical performance of *U. lactuca* with observed minor effects of oxidative stress being associated to the presence of Hg. The presence of SIL had no toxicity levels that could inhibit metabolism or cause cellular damage in *U. lactuca*.

Overall, the results support the use of [Si][C3C1im][SCN] as an alternative methodology to remove the top-priority contaminant Hg from water, which is a global concern. However, for the actual implementation of the technology, it is important to conduct toxicological assays with other model species to attest its safety, since macroalgae, despite being widely used for this purpose, are known to be resilient organisms. Furthermore, it will be important to validate the sustainability of the technology on a larger scale, in environmental and economic terms. (Table 3)

Funding

This work received financial support from the European Union (FEDER funds through the Operational Competitiveness Program (COMPETE2020) and PT national funds (FCT/MCTES, Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) through the projects REQUIMTE (UIDB/50006/2020 and UIDP/50006/2020), CESAM (LA/P/0094/2020) and CICECO-Aveiro Institute of Materials (UIDB/50011/2020, UIDP/50011/2020 & LA/P/0006/2020).

CRediT authorship contribution statement

Thainara Viana: Formal analysis, Writing – original draft, Writing – review & editing. **Raquel Almeida:** Methodology, Investigation, Formal analysis, Writing – original draft. **Paula Figueira:** Methodology, Investigation, Formal analysis, Writing – original draft. **Luciana Rocha:**

Methodology, Investigation, Formal analysis. **Márcia C. Neves:** Methodology, Investigation, Formal analysis. **Rosa Freitas:** Methodology, Formal analysis, Validation, Supervision. **Mara Freire:** Methodology, Validation, Resources, Supervision. **Bruno Henriques:** Methodology, Validation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Funding acquisition, Project administration. **Eduarda Pereira:** Methodology, Validation, Resources, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Bruno Henriques and Márcia C. Neves thanks FCT (Fundação para a Ciência e Tecnologia) for funding through the Scientific Employment (Ref. CEECIND/03511/2018 and CEECIND/00383/2017 respectively), while Thainara Viana and Paula Figueira acknowledge FCT/MCTES (Fundação para a Ciência e Tecnologia and Ministério da Ciência, Tecnologia e Ensino Superior) and ESF (European Social Fund) through CENTRO 2020 (Programa Operacional Região Centro) for their PhD grant ref. 2022.13015.BD and SFRH/BD/139077/2018, respectively.

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