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A miniaturized bismuth-based sensor to evaluate the marine organism *Styela plicata* bioremediation capacity toward heavy metal polluted seawater

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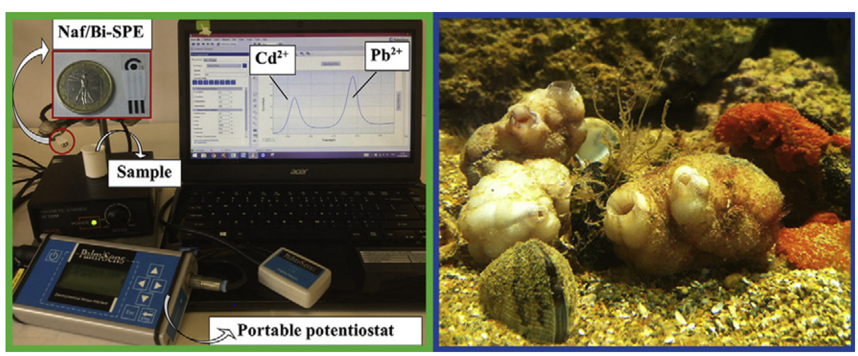
HIGHLIGHTS

- Disposable sensor for sustainable detection of Cd^{2+} and Pb^{2+} in natural samples
- Bioremediation experiment showed *S. plicata* bioconcentration capacity.
- *S. plicata* can be used for bioremediation of Cd^{2+} and Pb^{2+} polluted seawaters.

GRAPHICAL ABSTRACT



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ABSTRACT

Cadmium and lead are highly toxic heavy metals which cause a severe worldwide pollution. In addition to the toxic effect produced by the direct exposure, they can be bioconcentrated and accumulated in living organisms, including humans. Herein, a miniaturized and disposable electrochemical sensor was improved for the simultaneous detection of cadmium and lead ions to study the bioremediation of polluted seawater in presence of the filter-feeding marine organism *Styela plicata*. A screen-printed electrode modified *in situ* with a bismuth film was selected using the anodic stripping analysis as detection technique. This sensor was coupled with a portable potentiostat and the detection of cadmium and lead ions was carried out by Square Wave Anodic Stripping Voltammetry, allowing the simultaneous detection of both heavy metals at ppb level (LOD = 0.3 ppb for lead, 1.5 ppb for cadmium).

This analytical tool was then applied to assess the bioremediation capacity of *S. plicata* through a bioremediation experiment, in which the organism has been exposed to seawater artificially polluted with 1000 ppb of Cd^{2+} and Pb^{2+}. The matrix effect of both seawater and acid digested biological samples was evaluated. A bioconcentration phenomenon was observed for both heavy metals through the analysis of *S. plicata* tissues. In details, Pb^{2+} resulted to be about 2.5 times more bioconcentrated than Cd^{2+}, giving an effective bioremediation level in seawater of 13% and 40% for Cd^{2+} and Pb^{2+}, respectively. Thus, our results demonstrate the capability of *S. plicata* to

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bioremediate Cd^{2+} and Pb^{2+} polluted seawater as well as the suitability of the electrochemical sensor for contaminated marine environment monitoring and bioremediation evaluation.

1. Introduction

Heavy metals are among the most dangerous pollutants because of their high toxicity for living organisms and for their low biodegradability, due to their persistence and accumulation in the environment (Glorennec, 2006; Valls and Lorenzo, 2002). Among heavy metals, cadmium and lead are non-essential elements with well-known toxic effects on humans (Godt et al., 2006; Gracia and Snodgrass, 2007; Fewtrell et al., 2003; International Agency for Research on Cancer, 2006; Satarug et al., 2010). They are ubiquitous, according to their diffuse utilization in human activities, such as industrial processes, mining and metallurgic sectors, incinerators, batteries, pigments, antifouling and anticorrosive coatings (Glorennec, 2006; Kustin et al., 1983). Although some countries banned many products that have been important sources of these metals, they are still widely present in the world. Thus, cadmium and lead pollution is an important problem that needs to be faced by elaborating innovative strategies for the monitoring and the remediation of the contaminated environment. In this scenario, innovative approaches, which exploit different scientific disciplines, are designed to study and furnish sustainable solutions in bioremediation field.

Aquatic environments are worthy of note because they simultaneously represent accumulation reservoirs and secondary sources of pollutants, such as heavy metals. The Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC of the European Parliament and of the Council), which is the European regulation for assessment of the good environmental status of marine system (European Commission, 2008), promotes an ecosystem-based approach, considered as important requirement for sustainable management and environmental monitoring programs. Thus, some recent studies are based on a combination of chemical contaminant measurements and biological response (Hagger et al., 2008; Thain et al., 2008). There are many strategies to remediate contaminated waters, based on mechanical, chemical, and biological techniques. The biological approach involves the *bioremediator* organisms, which can naturally concentrate in their tissues some kinds of pollutants (process known as *bioconcentration* phenomenon) and remove them from the surrounding environment (Gifford et al., 2007). Furthermore, they may be able to give recognizable responses about the contamination level (*bioindicators* organisms) or even degrade the pollutants (Patron et al., 2013; Aresta et al., 2015). The use of bioremediators for the decontamination of the environment is known as *bioremediation*: it is an ecosystemic approach as it takes into consideration the role that each living and non-living component of an ecosystem plays in it, giving information on the bioavailability of pollutants, on their direct effects on organisms and on trophic relations.

In this work, the bioremediation capacity of *Styela plicata* (Tunicata, Ascidiacea) for lead and cadmium contaminated seawater was investigated. This organism belongs to the group of benthic filter-feeding organisms, which live anchored to the hard bottom and retain nutrients from waters to feed. *S. plicata* grows abundantly as a fouling organism on natural seabeds, as well as on submerged man-made structures and on artificial substrates for shellfish and fish culture. These suspension-feeding organisms are known to show a high bioconcentration capacity, due to their well developed gill structure, the presence of vanadocytes and the absence of kidneys, and to have a high filtering activity with respect to other ascidian organisms (Thiyagarajan and Qian, 2003). The possibility of exploiting the combination of their abundant growth in polluted coastal areas and their ability to remove contaminants from seawaters has generated considerable interest. *S. plicata* is characterized by good properties as bioremediator and has been the subject of many studies for the bioconcentration of cadmium, lead, arsenic, mercury and heavy metal compounds in natural environments (Aydın-Önen, 2016; Bellante et al., 2016; Choi et al., 2014; Philp et al., 2003; Radford et al., 2000;), as well as bacteria (Stabili et al., 2016) and for the biodegradability of some organic chemicals (Cestone et al., 2008). It has been reported that they can bioconcentrate persistent compounds present in the aquatic environment, resulting quite resistant to their toxic effects (Petersen, 2007; Sumerel and Finelle, 2014; Abdul et al., 2015). However, to our knowledge, its capacity to bioconcentrate Cd^{2+} and Pb^{2+} under a time-controlled exposure to seawater artificially contaminated with known quantities of both the heavy metals has never been evaluated.

Herein, we present for the first time a time-controlled bioremediation experiment, in which *S. plicata* specimens were exposed to known concentrations of Cd^{2+} and Pb^{2+}. In addition, the bioremediation study was carried out using both reference methods and an innovative and miniaturized electrochemical sensor. In details, a cost-effective, portable and easy-to-use device based on a bismuth modified screen-printed electrode associated with a portable potentiostat was used as an environmentally friendly analytical tool. This experimental setup was successfully employed by our research group for the detection of cadmium and lead in fresh water and for phytoremediation study using *Lemna minor* aquatic plant (Neagu et al., 2014). The use of bismuth permits the measurement of cadmium and lead without the removal of dissolved oxygen, necessary for the more traditional mercury-based electrodes, providing a wide negative potential window (until −1 V), suitable for the detection of these cations (Economou, 2005; Wang, 2005; Kokkinos and Economou, 2008; Svancara et al., 2010); moreover bismuth is considered much less toxic than mercury (Armenta et al., 2008; Economou, 2005; Wang, 2005; Arduini et al., 2010a,b). Such electrodes can be obtained either by *in situ* or *ex situ* deposition of Bi^{3+}, or even by using bismuth-based materials, and many kinds of substrates can be employed (Arduini et al., 2010a,b). In this work, screen-printed graphite electrodes modified with Bi^{3+} by *in situ* electrodeposition and Nafion polymeric films have been chosen since characterized by high sensitivity (Calvo Quintana et al., 2011). Nafion/bismuth-modified sensor performances (Naf/Bi-SPE) were firstly investigated and then used to measure Cd^{2+} and Pb^{2+} both in seawater and in biological tissues of *S. plicata*, after the exposure of the organisms to artificially contaminated seawater. To evaluate the responses obtained by the sensor, benchmark methods have been used for comparison (ICP-MS and ICP-OES). In this way, the bioremediation ability of *S. plicata* with respect to Cd^{2+} and Pb^{2+} has been estimated within a time-controlled bioremediation experiment.

2. Experimental section

2.1. Reagents and apparatus

Nitric acid (HNO_3 ≥ 69%), sodium hydroxide (NaOH ≥ 30%), sodium chloride (NaCl) and sodium acetate anhydrous (CH_3CO_2Na) were obtained from Fluka (Trace Select). Bismuth ($BiCl_3$ 1 mg/mL in 1% p/v HCl) and lead standard stock solutions ($Pb(NO_3)_2$ 1000 μg/mL in 1% p/v HNO_3) were acquired from Carlo Erba, whereas cadmium standard stock solution ($Cd(NO_3)_2$ 1007 μg/mL in 1.2% p/v HNO_3) was acquired from Sigma Aldrich. Commercial available Nafion® 117 (5% v/v in ethanol) was obtained from Aldrich. All the solutions were prepared using double-distilled water (by a Milli-Q system, Millipore). Teflon or polyethylene were chosen for containers (beakers, vessels, tubes, pipettes, round-bottomed flasks). All containers were soaked with HNO_3 3% v/v