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Experimental Mercury Bioaccumulation Trends in Sea Anemone *Actinia equina* Exposed to Chlor-Alkali Industry Effluent Contaminated Water

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Abstract—Trends of experimental mercury bioaccumulation by the sea anemone *Actinia equina* were investigated. Sea anemones specimens were collected in two sampling stations on the Iberian coast presenting different mercury profiles. Sampling stations had been chosen by its geographic differences and also by its level of exposition (displayed beach/sheltered and diversity types of pollutants). The experimental procedure take special attention with regards to the mechanisms of processes driving measured sediment-organisms fluxes. Local Hg concentration on sediments (fine, <63 μm, and coarse, >63 <1000 μm), were also analyzed for laboratorial and field comparisons. In the experimental assay aquariums with the collected sea anemones were exposed to a mercury contaminated water 0.016 $\mu g \cdot L^{-1}$ sampled in a chlor-alkali industry effluent, in order to discuss trends in Hg contamination. The results showed that even within similar specimens a considerable variability on patterns of Hg accumulation is observed. The experiments showed the potential of sea anemone *Actinia equina* as Hg contamination and bioaccumulation indicator and the importance of the exposure period required for the equilibration in the case of high mercury contamination exposition in comparison with non contaminated areas.

Keywords: mercury, *Actinia equina*, bioaccumulation, chlor-alkali

INTRODUCTION

Mercury (Hg) is a priority pollutant due to its persistence, bioaccumulation and toxicity in the marine environment (Tam and Wong, 2000; Ikem *et al.*, 2003). The major factor that determines the concentration of mercury in the biota is the methylmercury concentration in water. Methylmercury is a common seafood contaminant that illustrates the complex interactions between marine pollution, accumulation in food chains, and human health (Mason *et al.*, 2006). That concentration is controlled by the relative efficiency of methylation/demethylation. Demethylation is both photochemical and biological. Methylmercury is better

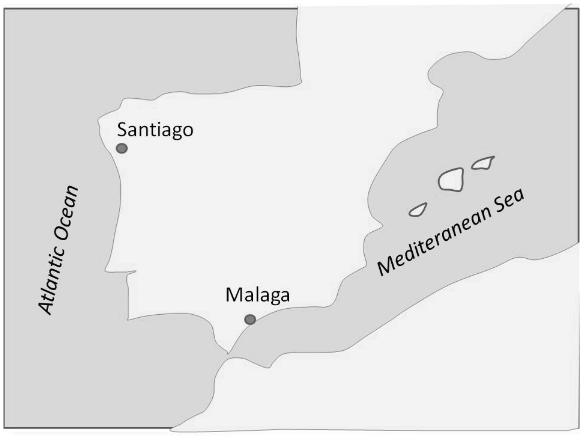

Fig. 1. Sampling sites in the Iberian coast during September 2008 and March 2009.

retained than the inorganic form by organisms (Ebinghaus *et al*., 1999). For that reason methylmercury is bioconcentrated instead of inorganic mercury. Anoxic waters/sediments are important sources of methylmercury, likely the result of the methylating activity of sulfate-reducing bacteria. In surface water methylcercury may come from anoxic layers (Davis *et al*., 2003). Data regarding mechanism of mercury transference and transport between sediment and biota, in unstable conditions, are very limited (European Commission, 2001). In mercury-contaminated sites, the general improvement of water quality could potentially increase the bioavailability of mercury, while sulphide-rich marsh sediments may reduce. Ecotoxicological risk posed by contaminated sediments will depend on metal availability as well as of the ability of living organisms to assimilate metals (Amiard *et al*., 2006). Anemones inhabit virtually all marine environments and are abundant and ecologically diverse and in different contaminated regions. Cnidarians, like other soft-bodied marine invertebrates so far studied, are able to utilize organic substances dissolved in the sea for their metabolism (Howard and Brown, 1984; Brown, 1987; Scott, 1990; Harland *et al*., 1990; Harland and Nganro, 1990; Guzman and Jimenez, 1992; Mitchelmore *et al*., 2003). *Actinia equina* was chosen because it might possibly be effective bioaccumulation indicator here since are very common and live in an environment which is continually exposed to garbage dumping, untreated sewage inflow, land and river runoff, and atmospheric fallout from heavy traffic and various small-scale industries scattered in coastal areas. Several studies have showed that this species possess morphological and physiological adaptations to environmental forcing factors, which include eventual pollutants (Denton and Burdon-Jones, 1986; Hanna and Muir, 1990). These species present a wide geographic distribution and plays important ecological key role in the marine environment since the North Sea (Scandinavia: Sweden, Norway and Denmark) passing for the English

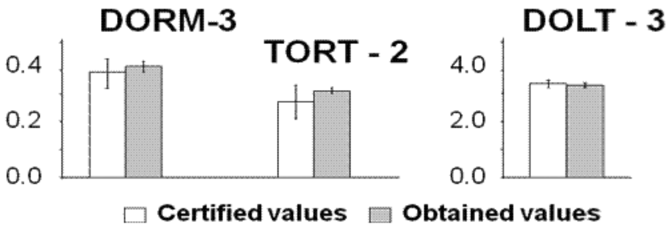

Fig. 2. Certified values (CRMs), obtained values and respective standard deviations.

Channel (Great-Britain), until the Atlantic Northwest of the Iberian Peninsula, with a strong representation in the Portuguese coast, and also in the Mediterranean sea and Morocco (Solé-Cava and Thorpe, 1992). The experimental design tries to understand the potential of sea anemone *Actinia equina* as Hg contamination and bioaccumulation indicator and the importance of the exposure period required for the equilibration in the case of high mercury contamination exposition in comparison with non contaminated areas. The present experiments address the question of the exposure period required for the equilibration in the case of high mercury contamination exposition. Laboratorial experiments were conducted to compare mercury bioaccumulation trends in tentacle and peduncle of the sea anemone *A. equina* collected in several areas of the Iberian coast and exposed to high contaminated water. The specific objectives are: (i) total mercury bioaccumulation in different parts of anemone body (tentacle and peduncle) after and before exposition to contaminated water; (ii) biota powerful of anemones bioaccumulation vs. environment mercury accumulation comparison (Water Hg concentration); (iii) period required for the equilibration.

MATERIALS AND METHODS

Biological material

Actinia equina specimens were collected in two sampling stations in the Iberian Coast, Santander and Malaga (Fig. 1). Sampling stations had been chosen by its geographic differences and also by its level of exposition (displayed beach/ sheltered and diversity types of pollutants). Mean size and weight of the collected specimens were, respectively, 8.21 g and 2.37 cm.

Sediments

Surface sediments were collected in all the sampling stations. Sediments were dried during three days in oven at 40°C. After dry, sediment were homogenizer and sieved for coarse (1000–63 μm) and fine (<63 μm) fractions.

Mercury analysis and quantification

Hg tissue concentrations ($\mu g \cdot g^{-1}$, dry weight) were determined with a

Table 1. Physicochemical parameters of the water in the two sampling places on the Iberian coast.

	SANTIAGO	MÁLAGA
T (°C)	13.7	16.3
pH	8.4	7.82
Cond. (μg/s)	33.4	54.9
D.O. (mg/l)	9	8.8

Mercury Analyser (Leco AMA 254), by Atomic Absorption Spectrometry (AAS) after sample thermal decomposition. This methodology quantifies mercury directly from the combusted sample, requiring small amounts of sample. The entire analytical procedure was validated by analysing three standard biological reference materials (DORM-3, DOLT-3 and TORT-2) at the beginning and end of each set, thereby ensuring that the instrument remained calibrated throughout the study (Fig. 2).

Acclimation and experimental conditions

All field collected sea anemones were maintained at the laboratory in a 22°C temperature, pH 7.5 ± 0.5 and conductivity, 750 ± 50 μS/cm, in a period of 12h/12h light/dark. Organisms were fed twice a day at 2% of its body weight with commercial fish food (TetraMin). Specimens for studies were acclimated for 1 month in 20 L glass aquarium containing 18 L of aerated (DO = 5.04 mg/L), dechlorinated water (pH = 7.5–7.7, $NaHCO_3$ = 192 mg/L, $CaSO_4$ = 120 mg/L, $MgSO_4$ = 120 mg/L, and KCl = 8 mg/L) (17). The stocking rate for the *in vivo* study were 4 anemones/aquarium (each aquarium had 40 cm length, 35 cm width and 20 cm depth). Two aquaria were used per treatment. Anemones were not fed for 24 h prior to the experiments and no food will be provided during the test period. Six anemones per condition were removed every 7-days interval, killed and dissected on ice. In the experimental assay aquariums with the collected sea anemones were exposed to a mercury contaminated water 0.016 $\mu g \cdot L^{-1}$ sampled in a chlor-alkali industry effluent.

RESULTS AND DISCUSSION

Data regarding mercury trapping particulate matter and associated contaminants including particulate mercury are very important. In mercury-contaminated sites, the general improvement of water quality could potentially increase the bioavailability of mercury due to cinnabar or coating contaminated particles. These particles may resuspend lately from bed sediments providing contaminated particles back to the water column. Mercury bioaccumulation process in *Actinia equina* suggests a relation with the local Hg contamination profile, due to its suspensivore behaviour, showing to be sensible to sediment particles (resuspended particles).

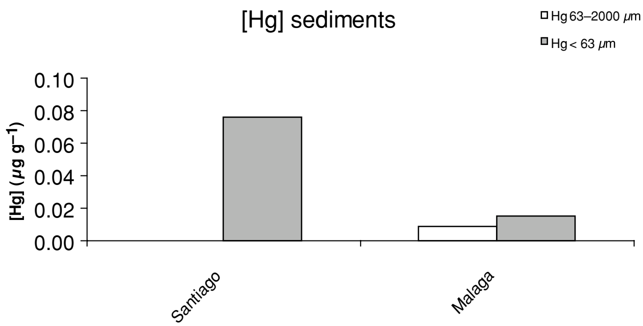

Fig. 3. Mercury concentration (μg·g^{-1}) in fine and coarse sediments on the two sampling stations along the Iberian coast.

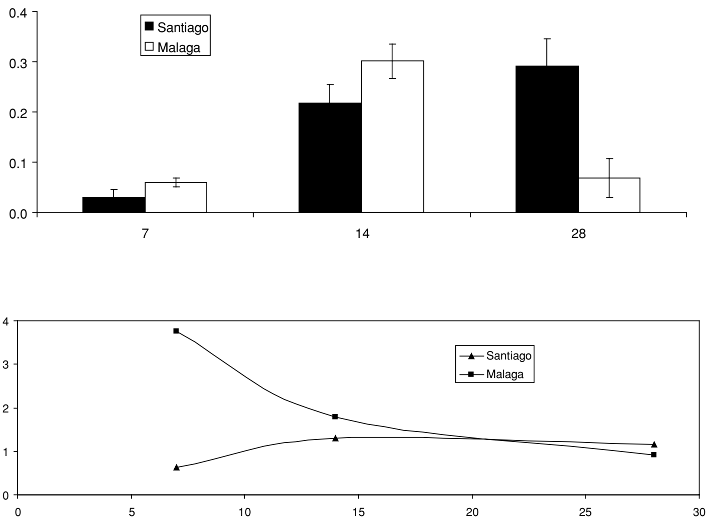

Fig. 4. Comparison of trends and values of mercury concentrations (μg·g^{-1}) accumulated by *Actinia equina* specimens from the two sampling sites along the Iberian Coast, during the experimental period (28 days) (error bars represent standard error).

The results of the physicochemical parameters of the water from the two sampling sites on the Iberian coast showed that Santiago present lower water temperature and conductivity and higher pH and dissolved oxygen than Malaga (Table 1). The total mercury concentration present at the surface (5 cm deep) of the fine coarse sediments (<63 μm) from the sampling stations varied between 0.149 and 0.8 μg·g^{-1}. The highest value was obtained in samples from Malaga. The Total mercury concentration present in coarse sediments varied between

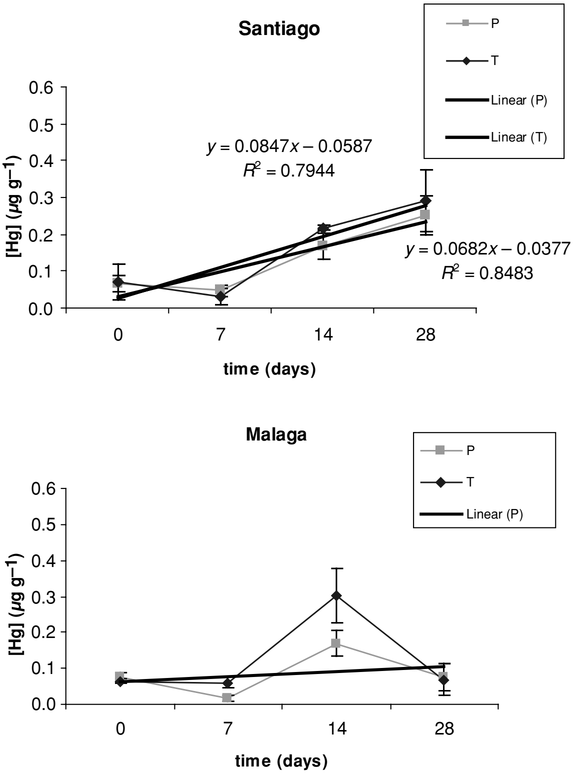

Fig. 5. Mercury concentrations ($\mu g \cdot g^{-1}$) accumulated by *Actinia equina* specimens from the two sampling sites along the Iberian Coast, during the experimental period (28 days) (error bars represent standard error).

0.0003 and 0.012 $\mu g \cdot g^{-1}$. The lowest value is observed in Santiago de Compostela (Fig. 3). The higher relation observed with the fine sediments (<63 μm) confirm the previous statements and suggest that species morphology and habitat location may be important in the mercury bioaccumulation process and that like other suspension-feeding invertebrates (bryozoans, sponges, brachiopods and ascidians) accumulate contaminants from water in dissolved and particulate forms (Lobban and Harrison, 1997; Hurd, 2000).

The observed important differences observed on physicochemical parameters and Hg sediment concentrations between the two sampling stations suggests that these variables may be important to the *Actinia equina* bioaccumulation trends and to the different temporal patterns of behaviour. The relation between anemones bioaccumulation and sediment fractions mercury contents, showed a consistent higher pattern of association with the thin sediments (<63 μm), both in tentacle and peduncle (respectively $R^2 = 0.4315$ and 0.533) (Fig. 2). Mercury concentration in certified (CRMs) and obtained values) were not significantly different (Fig. 2).

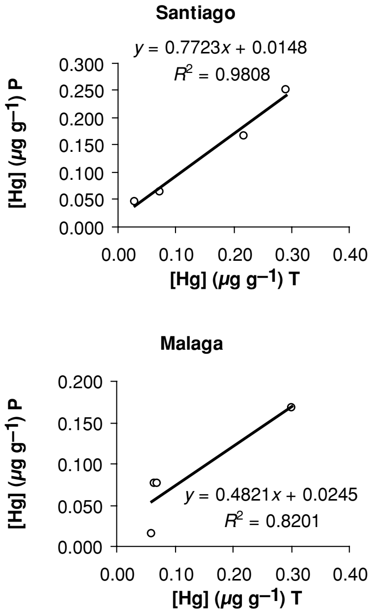

Fig. 6. Correlation between Hg concentration in tentacles ($\mu g{\cdot}g^{-1}$) and peduncles of *Actinia equina* from the two sampling sites of the Iberian Coast, Santiago and Malaga.

Experimental mercury bioaccumulation values

When compare the trends of the tentacle/peduncle Hg bioaccumulations, in the 7th day of experiments low mercury bioaccumulation values were observed in specimens from Santiago (0.6 $\mu g{\cdot}g^{-1}$) and very high in specimens from Malaga (3.8 $\mu g{\cdot}g^{-1}$) (Fig. 4). In the 14th day of experiments bioaccumulation values were almost similar between the specimens from the two sampling sites, but the specimens from Malaga presented higher values (1.8 $\mu g{\cdot}g^{-1}$) (Fig. 4). In the 28th day of experiments bioaccumulation values of the specimens from Santiago continue to increase and in the specimens from Malaga a substantial decrease was observed (Fig. 4). In mercury-contaminated sites, the general improvement of water quality could potentially increase the bioavailability of mercury, while sulphide-rich marsh sediments may reduce its bioavailability (Turner and Southworth, 1999). Tentacles and peduncles Hg bioaccumulations trends during the period of experiment were significant in tentacles ($r^2 = 0.848$) and peduncles ($r^2 = 0.791$) for the specimens from Santiago, and for tentacles ($r^2 = 0.97$) of specimens from Malaga (Fig. 5).

When compare the relation between tentacle and peduncle Hg bioaccumulations trends, it was observed that, in the 28th day of experiments, in the Santiago specimens, the Hg bioaccumulation ratio almost double and in the Malaga specimens Hg bioaccumulation ratio were almost half, when compared to the starting values (Fig. 4). This relation presented a significant correlations to the specimens from Santiago (r^2 = 0.98) and Malaga (r^2 = 0.82) (Fig. 6). The tentacles and peduncles can be appears to act as a reservoir for accumulation of Hg to protect the gastro vascular cavity. Mercury uptake occurs mainly via ingestion of contaminated food and mercury accumulation and depuration processes, can occur during sea anemone life cycle depending on the diet, water conditions and individual susceptibility for environmental contaminants. The tentacles may have the ability to accumulate large quantities of Hg from the environment, and also plays an important role in the storage, redistribution and detoxification, so, it is the target part of body for Hg accumulation in sea anemones from heavily contaminated environment (Harland *et al.*, 1990; Lobban and Harrison, 1997; Hurd, 2000). At present, the majority of the chlor-alkali industry has been converted to membrane technology banning the use of mercury in the process (OSPAR Commission, 2005). When the industrial plants are converted or shut down, mercury still has the potential to be released into the global environment. Although mercury and other contaminants in coastal and marine aquatic systems are of widespread interest and concern, data regarding mercury flux estimation and Hg transport mechanism in unstable conditions are very limited.

The experimental procedure showed the importance of the sediment-organisms fluxes to the Hg bioaccumulation trends. The results showed that even within similar specimens a considerable variability on patterns of Hg accumulation is observed. The experiments also showed the potential of sea anemone *Actinia equina* as Hg contamination and bioaccumulation indicator and the importance of the exposure period required for the equilibration in the case of high mercury contamination exposition in comparison with non contaminated areas.

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