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Use of the sea hare (*Aplysia fasciata*) in marine pollution biomonitoring of harbors and bays

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ABSTRACT

Our study evaluated heavy metal concentrations in soft tissues of sea hare, *Aplysia fasciata*, from the Lower Laguna Madre, Texas. Heavy metals in tissues followed Se > As > Pb > Cd. Concentrations ranged As (BDL-28.08), Cd (BDL-5.50), Pb (BDL-12.85) and Se (4.25–93.43 ppm). Median As, Cd, Pb, and Se tissue levels exceeded exposure levels. Significant relationships occurred in metal-metal (As—Cd, As—Pb, Cd—Pb, Cd—Se, and Pb—Se), metal-tissue (significant Se uptake by inhalant and exhalant siphons and As in the hepatopancreas), and metal-metal within tissue (As—Pb in the hepatopancreas and Cd—Pb in the digestive cecum) analyses ($p < 0.05$). Bioaccumulation factors (BAF) suggested the inhalant siphon, hepatopancreas, and digestive cecum function as macroconcentrators of Cd, hepatopancreas and digestive cecum as macroconcentrators of Pb, and all tissues were deconcentrators for As and Se. As a bioaccumulator of heavy metals, *Aplysia* was evaluated as a bioindicator of marine pollution in harbors and bays.

1. Introduction

The evaluation of marine ecosystem health involves the use animal bioindicator species, whose spatial and temporal occurrence and metal accumulation in their tissues can identify pollutant contamination and the presence of environmental stressors (Boening, 1999; McCarthy and Shugart, 1990; Yusof et al., 2004). The most common targeted metals of marine pollution monitoring include, arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg) (Chiarelli and Roccheri, 2014). Other metals monitored in marine ecosystems include copper (Cu), selenium (Se) and tin (Sn) (Kennish, 1998). Marine invertebrates are particularly useful bioindicators of heavy metal pollution, and the ability to bioaccumulate metals can vary among taxonomic groups (Rainbow, 1995, 2002).

Whereas, molluscs need to bioaccumulate and maintain essential metals in their tissues (e.g., Cu and Zn), other non-essential metals (As, Cd, Pb, and Se) disrupt their metabolism and physiology (Jakimaska et al., 2011a, 2011b). Such heavy metals are the focus of marine pollution monitoring, and marine molluscs are reliable bioindicators of these metals (Gupta and Singh, 2011; Rittschof and McClellan-Green, 2005; Zhou et al., 2008). One potential mollusk includes the sea hare, *Aplysia sp*. (Gastropoda, Anaspidea), which has received intensive research on its neurobiology, toxicology, chemical defense, and bioactive molecules (Kamiya et al., 2006; Kicklighter et al., 2005; Nusnbaum and Derby, 2010; Rittschof and McClellan-Green, 2005; Takeda, 1992). The ability of the sea hare to be used in monitoring marine ecosystem health has been explored, and more recent studies involve investigations with *Aplysia maricultures* (Jarvis et al., 1995; Patel et al., 1973).

An early study by Phillips (1917) found *Aplysia* "liver" (i.e., hepatopancreas) to contain traces of Cu, Fe, Mn, and Zn. Additionally, *Aplysia* tissues (e.g., gill and hepatopancreas) are known to accumulate polychlorinated biphenyl (PCBs), Ag, and As in the laboratory (Bianchini et al., 2007; Jahan-Parwar et al., 1990; Shiomi et al., 1988). Similar to polychaetes, gastropods, and bivalves, the sea hare also is known to biomethylate arsenic as tetramethylarsonium ion (Me_4As^+) and differentially store metals among tissues (Bianchini et al., 2007; Fattorini and Regoli, 2004; Morita and Edmonds, 1992). For example, Co, Cu, Fe, Mn, Ni, Sr, and Zn differentially accumulate in tissues (e.g., reproductive, intestine, and hepatopancreas) of field collected *A. benedicti* (Patel et al., 1973). Most recently, Jarvis et al. (1995) reports metal (Cd, Cu, Ni, Pb, and Zn) accumulation effects on *A. californica* growth for laboratory-cultured individuals. When sea hares were fed different metal concentrations of green (*Ulva lactuca*) and red (*Agardhiella subulata*) seaweed exposed to metals, they found variation in

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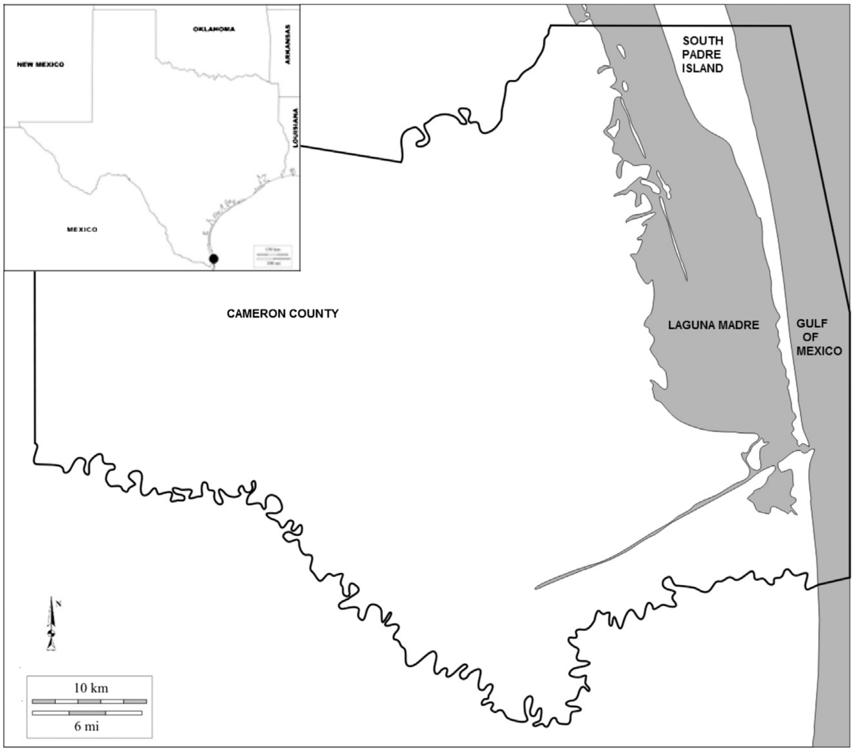

Fig. 1. Location of the Lower Laguna Madre and Gulf of Mexico, Texas.

Aplysia organ bioaccumulation.

The ability of *Aplysia* to bioaccumulate metals and pollutants in its tissues warrants its consideration as a potential marine bioindicator animal of bays, inlets, and harbor areas of the Laguna Madre of south Texas, USA. This hypersaline lagoon is identified as one of the region's most susceptible estuaries to marine pollution (Biggs et al., 1989). In a harbor polluted with As, Cd, Pb, and Se, and where sea hares regularly congregate, we investigated the bioaccumulation of these non-essential and borderline metals in *A. fasciata* tissues through its saltwater exposure pathways. Through our examination, we evaluated sea hare potential as a biomonitor of marine harbor ecosystem health.

2. Methods and materials

A. fasciata ($n = 5$) were collected in May 2012 from the entrance to Port Isabel Harbor, Cameron County, Texas, USA (26.063079, − 97.209248) (Fig. 1). This harbor exists in the Lower Laguna Madre (LLM), which "comprises the south Texas estuary bounded by the barrier island, South Padre Island, and extending from near Brownsville north to the Land Cut, and intersected by Mansfield Pass at Port Mansfield" (BBEST, 2012:3-1). The Laguna Madre is one of the few hypersaline estuaries in the world and has an average salinity of 35 to 45 ppt (Tunnell and Judd, 2002). The high salinity of the lagoon results from low freshwater inputs, high evaporation, shallow water, and its small connectivity with the Gulf of Mexico (BBEST, 2012; TDWR, 1983).

The harbor collection site was chosen based on its greater than eighty years of use supporting commercial fishing, petroleum industry, and recreational boating and tourism. Based on these activities and surrounding land use, the major and potential sources of metals in LLM waters includes agricultural use of As in herbicides and cotton desiccants, Cd from fertilizer, municipal wastewater, industrial releases, or landfills, Pb released from regional refineries and petroleum companies, and Se from industrial waste and sediment transfer (ATSDR, 2007a, 2007b, 2012; GESAMP, 1988; National Research Council, 1977). Additionally, our field observations found this harbor location to contain a large, regularly occurring gathering of sea hares in the LLM. This is similar to Audesirk (1979), who also reported changes in sea hare gatherings and seasonal abundance.

Physiochemical field measurements (temperature, pH, salinity) were taken at the collection site using a Hydrolab Quanta Multi-probe sonde (OTT Hydromet, Germany). Surface ocean water grab samples were also taken and placed in new, pre-cleaned, high-density polyethylene bottles and stored on ice during transport. At the lab, water samples were filtered at 0.45 μm and stored unacidified and frozen at − 20 °C until tested at room temperature (22.5 °C) (see Avenzino and Kennedy, 1993; Batley and Gardner, 1977; USEPA, 1983).

All collected *Aplysia* specimens were placed in separate polyethylene bags, transported on ice, and stored at − 20 °C until thawed and dissected. On dissection, sea hare bodies were defrosted to room temperature, and all dissection blades were rinsed with distilled water to avoid contamination. Soft tissues extracted included the inhalant (IS) and exhalant (ES) siphons, hepatopancreas (HP), and digestive cecum (DC). Tissue sample preparation included freeze-drying and pulverization. Approximately 0.2 g of dried tissue was digested on a hot block with Trace Metal grade 70% nitric acid (NHO_3) for 40 min at 95 °C. After digestion, samples were diluted with Millipore (Millipore Sigma Aldrich, Billerica, MA) purified water and stored in 50 mL polypropylene vials until analysis. At the least, three replicates per each of the four tissue types from each sea hare body were analyzed. Concentrations of As, Cd, Pb, and Se were determined using inductively couple plasma optical emission spectroscopy (ICP-OES) (PerkinElmer Optima 8300). Analyses were performed in triplicate using calibration curves maintained at a R^2 value of 0.99 or better. Limits of detection were As 0.01, Cd 0.0001, Pb 0.0015, and Se 0.0088 ppm, and values below detection limits were identified as BDL.

Statistical analysis was conducted using JMP 11.2 (SAS, Cary, NC). Summary statistics (mean, standard deviation, median, standard error) were calculated for each metal analyzed for a total of 80 measurements. Median dissolved metal values for water and tissue samples were compared to the saltwater chronic and acute criteria of the Texas Aquatic Life Surface Water Risk-Based Exposure Limits (SWRBEL) (TCEQ, 2014a, 2014b). Additionally, comparisons were made to the saltwater criterion continuous concentration (CCC) and criteria