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Heavy Metals in the False Mussel, *Mytilopsis domingensis*, from Two Tropical Estuarine Lagoons

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The San Juan Bay Estuary System (SJBES) includes the Bahía de San Juan, and Lagunas (lagoons) Condado, San José, Torrecilla, and Piñones. SJBES, located on the northern coast of Puerto Rico, was declared an Estuary of National Importance that merits protection and environmental quality improvement by the United States Environmental Protection Agency (USEPA) in 1992 (USEPA, 2000) (Figure 1). Laguna San José (LSJ), located at 18º 25'48" North latitude and 66º 01'42" West longitude, is the largest body of water of the SJBES comprising a surface area of 550 ha (CEER, 1988). LSJ has two major inputs of freshwater creeks (Quebradas San Antón and Juan Méndez) and one major tide-dependent water exchange channel known as Canal Suárez which receives storm water run-off from a nearby airport (Figure 2). Another tide-dependent flow channel, Caño Martín Peña, is located west of LSJ (Figure 2). However, water circulation is restricted through Caño Martín Peña channel due to large quantities of natural and domestic debris that limits its size and depth (Webb and Gómez-Gómez, 1998). LSJ faces serious environmental pollution, as shown by the fish kill episodes due to frequent anoxic conditions, poor water circulation, and by the presence of industrial and domestic inorganic and organic toxic chemical wastes (CEER, 1988; Webb and Gómez-Gómez, 1998). Metals are among the contaminants detected in sediments of LSJ with concentrations of lead and mercury as high as 548 μg/g and 4.9 μg/g (dw), respectively (Acevedo et al., 2000). In an effort to ameliorate the impact of pollutants, Commonwealth and Federal agencies are planning to dredge certain areas of LSJ to improve water circulation (Webb and Gómez-Gómez, 1998). However, dredging activities could alter the speciation of elements, thus making them more water soluble and bioavailable, posing a potential health risk to aquatic biota and humans (Bryan and Langston, 1992; Wong and Yang, 1997).

The use of organisms to monitor bioavailability of metals in aquatic environments has been applied worldwide (Rainbow, 1995). Bivalves, such as mussels from the Mytilidae family, have been utilized as indicators of heavy metal pollution. These aquatic animals are sedentary filter feeders that readily accumulate heavy metals, providing information on the extent of contamination in aquatic environments (Goldberg, 1975; Szefer and Szefer, 1985; Micallef and Tyler, 1989; Rainbow, 1995; Andersen et al., 1996; Park and Presley, 1998; Ruangwises and Ruangwises, 1998). The false mussel *Mytilopsis domingensis,* which belongs to the family Dreissenidae (Morris, 1973), is widely distributed through LSJ and Laguna Piñones (LP) which is a less polluted site located within the largest remaining mangrove forest of Puerto Rico (Webb and Gómez-Gómez, 1998). Although this species does not belong to the mussel family, it is characterized as a small bivalve that resembles the mussel, but with a shelflike platform that serves for

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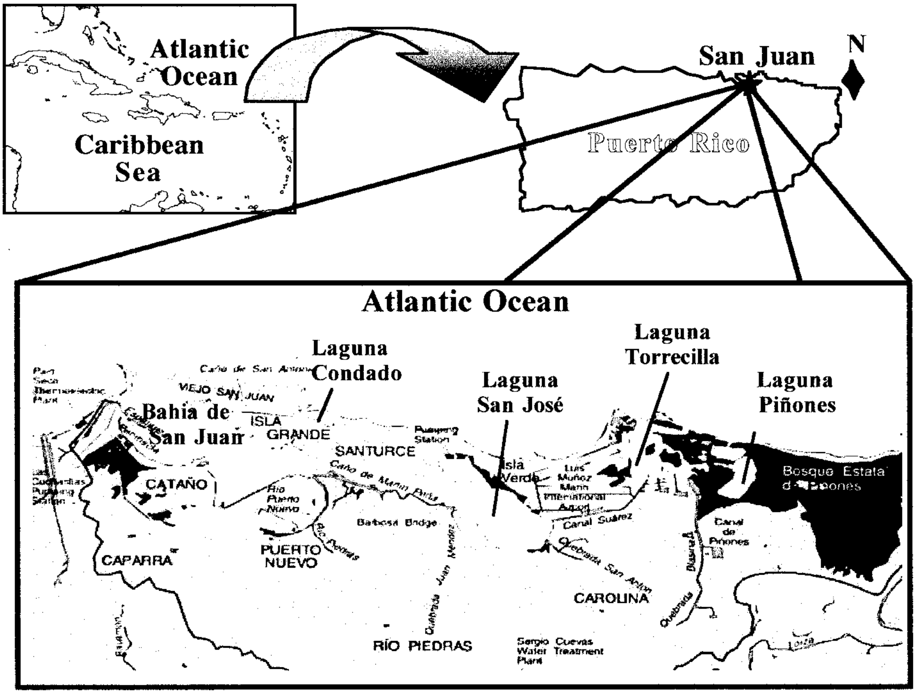

Figure 1. The San Juan Bay Estuary System which includes sampling locations of Laguna San José and Laguna Piñones, where the false mussel (*Mytilopsis domingensis*) was collected in Puerto Rico (modified from Webb and Gómez-Gómez, 1998).

attachment of cluster formations on hard surfaces (Morris, 1973). The potential use of *Mytilopsis domingensis* as a bioindicator of heavy metal pollution in LSJ has not been previously studied. Although this species is not consumed by humans due to its small size (<16 mm long), *Mytilopsis domingensis* serves as a food source for other aquatic organisms from this system which are consumed by man, such as the fish *Diapterus plumierri* and the blue crab *Callinectes spp*. The objective of this study was to compare concentrations of metals in the false mussel, *Mytilopsis domingensis,* from a polluted aquatic system, LSJ, to a relatively non polluted lagoon, LP.

MATERIALS AND METHODS

The false mussel, *Mytilopsis domingensis,* was collected from seven stations in LSJ (identified as M1 through M7) and four locations in LP (Figures 1 and 2). The LP was considered a reference site. False mussels attached to submerged roots of the red mangrove, *Rhizophora mangle*, or to concrete pilings from a bridge that crossed the LSJ were collected using sterile plastic gloves. Organisms were placed inside disposable plastic bags and placed on ice in a cooler. Samples were transported to the laboratory, where they were thoroughly rinsed with distilled deionized water (ddw) and stored at –20 ^{0}C, until further analysis. Because mussels are relatively small in size (11-16 mm length; 0.05 to 0.10 g wet weight), 100-200 individuals of similar size were pooled per location in LSJ. False mussels

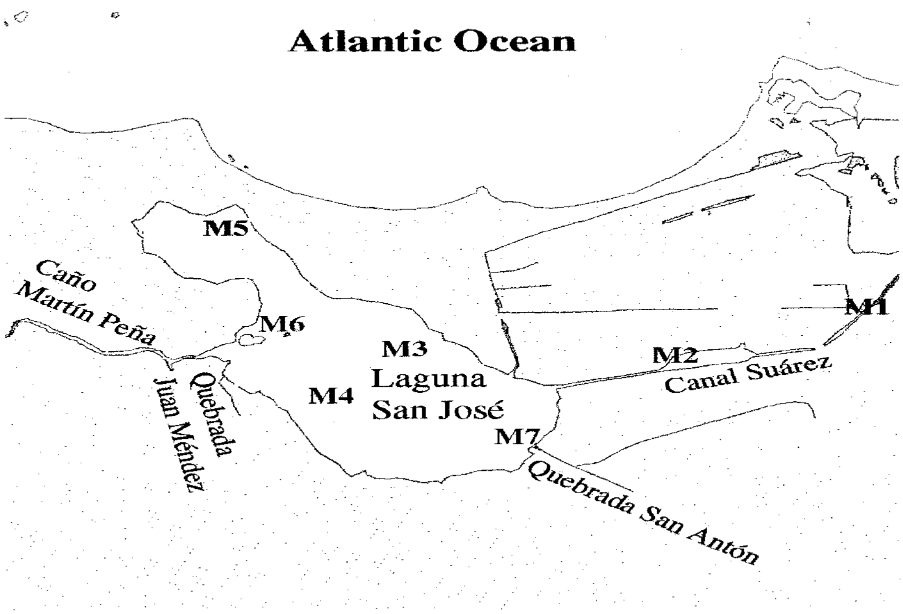

Figure 2. Sampling stations (M1 through M7) in Laguna San José (LSJ) where false mussels (*Mytilopsis domingensis*) were collected.

collected from four locations in LP were pooled into one large composite. Soft tissues were removed using Teflon-coated spatulas and homogenized in a parafilm covered beaker. Metal extraction of arsenic (As), cadmium (Cd), copper (Cu), lead (Pb), selenium (Se) and zinc (Zn) was conducted in triplicate with 2 mL of ddw and 5 mL HNO_3 (CEM, 1994) which was added to 3.0–3.5 g wet weight (equivalent to 0.30-0.35 g dry weight). The acidified sample was digested in a Model 1000 CEM microwave oven for 32 min and re-digested with 2 mL of 30% H_2O_2 for an additional 10 min (CEM, 1994). After digestion, samples were filtered through a Whatman 41 paper-filter, diluted to 50 mL with ddw and transferred to 60-mL polypropylene bottles. The Hg digestion was performed in a water bath at 95 ^{0}C following the EPA method 7471A (USEPA, 1996).

Metals of As, Cd, Pb, and Se were analyzed using a Perkin Elmer Atomic Absorption Spectrophotometer Model 1100B with a HGA-700 graphite furnace using USEPA methods 7060A, 7131A, 7421 and 7740 (USEPA, 1996). Cu and Zn were analyzed with the direct aspiration flame mode, (EPA Methods 7210 and 7950, respectively), while Hg was analyzed by cold vapor method using a sodium borohydride generation system (Perkin Elmer, 1987). Quality control was achieved by (i) checking sample containers and reagents for background contamination, (ii) using standard reference lobster hepatopancreas tissues of TORT- 2, (National Research Council, Ontario, Canada), ULTRA-check Blind QC Standard (Fisher Scientific, Pittsburgh, PA), and spiked samples to determine percent recoveries and validate the analytical procedure.

Data were tested by simple linear regression analysis between metals, and Tukey's

Studentized range test (p=0.05) for comparison among means using the statistical program StatView (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

A total of 168 metal analyses were performed on soft tissue of the false mussel, ***Mytilopsis domingensis***, from seven different LSJ composite samples and one composite sample from LP. The percent recovery for metals ranged from 90%-109% using lobster hepatopancreas-Tort 2 as a reference material. Spatial differences were observed in metal concentrations among the eight different stations (Figure 3), with the exception of Hg which was not detected in any of the tissue examined.

The highest concentration of As from LSJ (3.63 μg/g), was found in false mussels collected at the mouth of the Quebrada San Antón (station M7), while the lowest level (1.78 μg/g) was obtained in false mussels from northwest LSJ (station M5). Both stations demonstrated significant differences with As values in false mussels of LP (2.89μg/g) (Figure 3). Levels of As in false mussels from LP as well as in other false mussels in LSJ may be present in the organic form. Most of As in seafood (e.g. shellfish) is in the less toxic organic form, accounting for more than about 90% (USFDA, 1993). The other 10% of As is found in the toxic inorganic form of arsenate and arsenite (USFDA, 1993).

Concentrations of Cd were significantly higher in LSJ (except for station M5) than in organisms obtained from LP (Figure 3). Concentrations of Cd in LSJ varied from 0.11μg/g (station M5) to 0.24μg/g (station M3), while the average Cd concentration for LP was 0.10μg/g. Lower levels of Cd in false mussels from LP could be partly due to less contamination by the metal as reflected in Cd levels in analyzed bottom sediments. Webb and Gómez-Gómez (1998) did not detect Cd in LP sediments, while significant amounts (1-3 μg/g) of this element were found in sediments of LSJ (Webb and Gómez-Gómez, 1998; Acevedo et al., 2000). In addition, LP has higher average salinity values (38,606 μS/cm) than LSJ (2770μS/cm) as measured in the first 2m (Webb and Gómez-Gómez, 1998). As salinity increases, less uptake of Cd by aquatic organisms has been observed (Ray, 1984; Broman et al., 1991).

Pb is considered an ubiquitous toxic metal due to its many anthropogenic uses. Concentrations of Pb in false mussels from LSJ ranged from 0.52 μg/g (station M3) to 1.75 μg/g (station M6). Station M6, with the highest Pb concentrations, is located near the entrance of the Caño Martín Peña channel (Figures 1 and 2), a Pb-contaminated sediment site (Webb and Gómez-Gómez, 1998). Organisms collected at five LSJ stations showed significantly higher Pb concentrations when compared to those from the reference site of LP (0.43μg/g). Organisms from station M3, a bridge with concrete pilings, and station M5 did not exhibit differences in Pb concentrations from those obtained in organisms from LP.

Levels of Cu were statistically higher in false mussels from LP (11.7μg/g) than most stations in LSJ. Only stations M1 and M6 showed similar Cu levels to LP (Figure 3). Concentrations of Cu varied from 13.9 μg/g in station M1 (located in Canal Suárez) to 6.9 μg/g in station M5. Values of Zn in false mussels from stations M1, M4, and M6 from LSJ were significantly higher than in false mussels from LP (58.5 μg/g). The highest Zn concentration (86.3 μg/g) was obtained from organisms living on the concrete pilings of the bridge (station M4). However, false mussels sampled from station M5 exhibited the lowest Zn concentration (40μg/g)

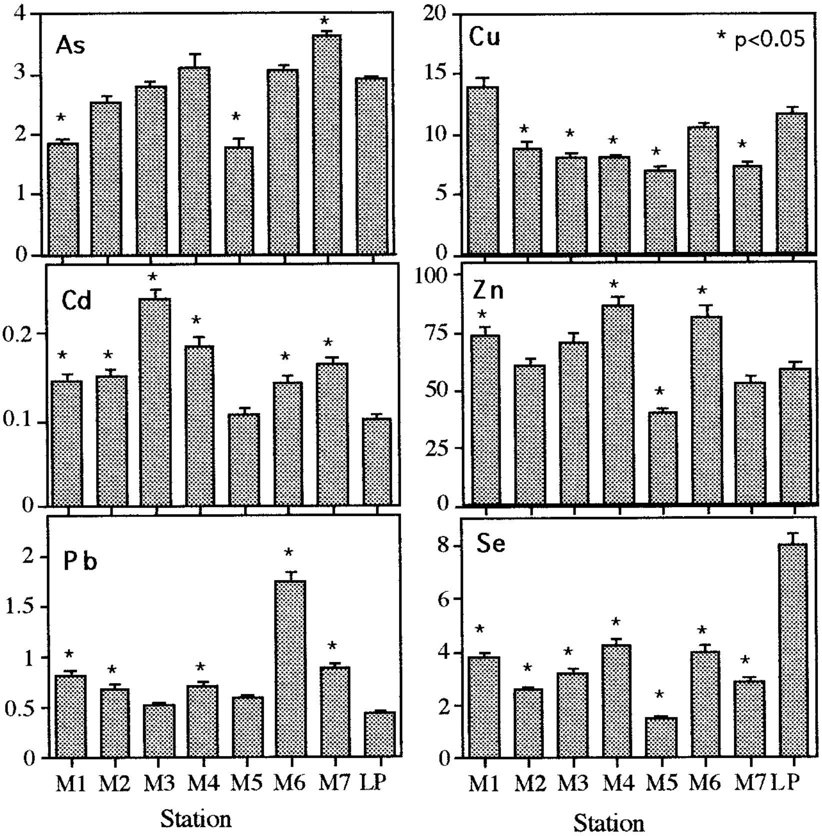

Figure 3. Average metal concentrations (μg/g dw) in the false mussel (*Mytilopsis domingensis)* collected from LSJ (stations M1through M7) and LP (reference site). Average values represent triplicate (n=3) ± SE. The symbol * represents significant differences in metal concentrations in false mussels between LSJ stations and LP.

throughout the LSJ system, a value significantly lower than those obtained from the LP (Figure 3).

False mussels obtained at the reference site had an average Se concentration of 8.0 μg/g which was significantly higher than any of the concentrations found in organisms from LSJ (Figure 3). The metal Se is known for reducing the effects of toxic metals, probably by forming insoluble complexes of metallic selenide (Goyer, 1996). Micallef and Tyler (1989) found a positive correlation between Se-Cd, Se-Hg, and Zn-Cd in soft tissues of *Mytilus edulis*. We did not find significant correlation between Se and Cd. Instead, Cd and Pb were significantly correlated ($p<0.05$) with Zn, using a simple linear regression, although the relationship showed low correlation coefficients (Table 1). The positive relationship between Cd, Pb and Zn could be due to similar, (i) natural and/or industrial sources, (ii) distribution in the dissolved or particle-bound phase, and (iii) uptake mechanisms by organisms (Preston at al., 1972; Phillips, 1976; Broman et al., 1991). Whether

Table 1 . Correlation of Zn with Cd, Pb, and Se in soft tissues of false mussels.

	linear regression equations	r^2	p-value
Zn-Cd	y = 0.061 + 0.001 x	0.246	0.0137
Zn-Pb	y = 0.041 + 0.012 x	0.198	0.0295
Zn-Se	y = -0.555 + 0.056 x	0.896	<0.001

the higher level of Se in the false mussels collected in the reference site had an antagonistic effect on the concentrations of the other toxic metals such as Cd and Pb is not known. A very significant correlation existed between Se and Zn when the data points from LP were not included, achieving a coefficient of correlation (r) of 0.947, unlike Micallef and Tyler (1989) that did not observe a correlation between these two elements.

In general, metal levels found in *Mytilopsis domingensis*, specially Cu and Zn, were similar to those found in other mussels species such as *Perna viridis*, *Mytilus edulis*, *Modiolus demissus*, *Tagelus dombeii*, *Semelle solida* and *Perumytilus purpuratus* (Szefer and Szefer, 1985; Micallef and Tyler, 1989; Andersen et al., 1996; De Gregori et al., 1994; De Gregori et al., 1996; Park and Presley, 1997; Ruangwises and Ruangwises, 1998). However, comparisons of metal concentrations in false mussels with other studies involving bivalves is limited by the fact that prior studies had been conducted with different species of bivalves that do not include *Mytilopsis domingensis.* Metal accumulation is known to be specie-dependent (Szefer and Szefer, 1985; Rainbow, 1995). Although spatial differences in metal concentrations were observed in *Mytilopsis domingensis,* levels tended to be significantly higher in the more contaminated LSJ when compared to the reference site of LP, particularly for Cd and Pb (Figure 3). Interestingly, false mussels from station M5 in LSJ frequently showed lower levels of metals when compared to other stations within LSJ, despite being collected in the vicinity of one of the most metal-contaminated sediment sites of LSJ. For instance, higher sediment concentrations were found near station M5 for Cu (211µg/g), Hg (4.9µg/g), Pb (548µg/g), and Zn (1530µg/g) with some metal exceeding sediment guideline concentrations (Long et al., 1995; Acevedo et al., 2000). Station M5 happened to be situated in a site with the highest sediment organic content of LSJ with 110 g/kg as C (Webb and Gómez-Gómez, 1998), probably rendering these metals from becoming bioavailable.

In conclusion, the false mussel, *Mytilopsis domingensis,* showed the potential to bioaccumulate heavy metals, thus becoming a useful biomonitor. The pattern of average metal concentrations in false mussels was Zn > Cu > Se > As >Pb > Cd. Further studies are necessary to understand how the life history (eg., sexual cycle, life span), metal kinetics, and variations in environmental physico-chemical characteristics may affect metal accumulation in this species, as it has been documented for other biomonitors of heavy metals (Andersen et al., 1996; Rainbow, 1995).

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REFERENCES

Acevedo D, Rodríguez-Sierra CJ, Reyes DR, Jiménez B (2000) Heavy metals in sediments and water from San Jose and Joyuda lagoons in Puerto Rico. In: Centeno JA, Collery P, Vernet G, Finkelman RB, Gibb H, Etienne JC (eds) Proceedings of the Sixth International Symposium on Metal Ions in Biology and Medicine. John Libbey Eurotext, Paris, p 169.

Andersen V, Maage A, Johannessen PJ (1996) Heavy metals in blue mussels (*Mytilus edulis*) in the Bergen Harbor area, Western Norway. Bull Environ Contam Toxicol 57: 589-596.

Broman D, Lindqvist L, Lundbergh I (1991) Cadmium and zinc in *Mytilus edulis* L. from the Bothnian Sea and the Northern Baltic proper. Environ Pollut 74: 227-244.

Bryan GW, Langston WJ (1992) Bioavailability, accumulation and effects of heavy metals in sediments with special reference to United Kingdom estuaries: A review. Environ Pollut 76: 89-131.

CEER (1988) Assessment and mitigation of ecological impacts from improvements to highway PR-26: San Jose Lagoon, Puerto Rico. Final Report to Puerto Rico Highway Authority. Center for Energy and Environmental Research, University of Puerto Rico, US Department of Energy, 123 pp.

CEM Corporation (1994) Operational manual for the microwave digestion applications, MSP 1000. Mathews, NC.

De Gregori I, Pinochet H, Delgado D, Gras N, Muñoz L (1994) Heavy metals in bivalve mussels and their habitats from different sites along the Chilean coast. Bull Environ Contam Toxicol 52: 261-268.

De Gregori I, Pinochet H, Gras N, Muñoz L (1996) Variability of cadmium, copper and zinc levels in molluscs and associated sediments from Chile. Environ Pollut 92: 359-368.

Goldberg ED (1975) The mussel watch- A first step in global marine monitoring. Mar Pollut Bull 6: 111.

Goyer RA (1996) Toxicology of metals. In: Klaassen CD (ed) Casarrett and Doull's Toxicology: The Basic Science of Poisons. McGraw-Hill, New York, p 691.

Long E, MacDonald D, Smith S, Calder F (1995) Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ Manage 19: 81-97.

Micallef S, Tyler PA (1989) Levels and interactions of selenium with group IIB metals in mussels from Swansea Bay, South Wales, U.K. Bull Environ Contam Toxicol 42: 344-351.

Morris PA (1973) A field guide to shells of the Atlantic and Gulf coasts and the West Indies. In: Clench WJ (ed) A Field Guide to Shells. Houghton Mifflin Company, Boston, 330 pp.

Park J, Presley BJ (1997) Trace metal contamination of sediments and organisms from the Swan Lake area of Galveston Bay. Environ Pollut 98: 209-221.

Perkin Elmer Corporation (1987) Analytical methods for atomic absorption spectrometry using the mercury/hydride system. Norwalk, CT.

Phillips DJH (1976) The common mussel *Mytilus edulis* as an indicator of pollution by zinc, cadmium, lead, and copper. II. Relationship of metals in the mussel to those discharged by industry. Mar Biol 38: 71-80.

Preston A, Jefferies DF, Dutton JWR, Harvey BR, Steele AK (1972) British Isles coastal waters: the concentration of selected heavy metals in sea water, suspended matter and biological indicators: a pilot survey. Environ Pollut 3: 69-82.

Rainbow PS (1995) Biomonitoring of heavy metal availability in the marine environment. Mar Pollut Bull 31: 183-192.
Ray S (1984) Bioaccumulation of cadmium in marine organisms. Experientia 40:14-23.
Ruangwises N, Ruangwises S (1998) Heavy metals in green mussels *(Perna viridis)* from the Gulf of Thailand. J Food Protect 61: 94-97.
Szefer P, Szefer K (1985) Occurrence of ten metals in *Mytilus edulis* L. and *Cardium glaucum* L. from the Gdansk bay. Mar Pollut Bull 16:446-450.
USEPA (2000) National estuary program. US Environmental Protection Agency - Office of Water. www.epa.gov/OWOW/estuaries/sjb.htm#summary.
USEPA (1996) SW-846 Test methods for evaluating solid waste, physical/chemical methods. US Environmental Protection Agency-Office of Solid Waste and Emergency Response. Washington, DC.
USFDA (1993) Guidance document for arsenic in shellfish. US Food and Drug Administration-Center for Food Safety and Applied Nutrition, 44 pp.
Webb R, Gómez-Gómez F (1998) Synoptic survey of water quality and bottom sediments, San Juan Bay Estuary System, Puerto Rico, December 1994-July 1995. U.S. Geological Survey Report 97-4144, San Juan, PR., 69 pp.
Wong JW, Yang CL (1997) The effect of pH and redox potential on the release of nutrients and heavy metals from a contaminated marine sediment. Toxicol Environ Chem 62: 1-10.