Changes in mercury content in oysters in relation to sediment and seston content in the Colombian Caribbean lagoons

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Abstract

Total mercury was evaluated in the mangrove oyster *Crassostrea rhizophorae*, in sediments and seston from the Ciénaga Grande de Santa Marta (CGSM) and Cispatá Bay (BhC) in two climatic seasons (rainy and dry). Composite samples of sediments, seston and oysters in juvenile and adult sizes were collected at six stations (three in each ecosystem) and Hg was quantified by atomic absorption spectrophotometry (EPA method 7473 PLTX-017). BhC had the highest Hg concentrations in sediment, seston and oysters compared to CGSM, with values close to the tolerable threshold for the ecosystem and associated biota (TEL) of 0.13 µg/g Hg and with a low risk of Hg contamination in the mangrove oyster. Although at CGSM Hg was below the TEL in sediment and was considered safe in the oyster, significant bioaccumulation was evident with the metal content in the seston, indicating a potential risk to the ecosystem and humans. The variables organic matter and temperature influenced metal availability in the sediment and seston, respectively; in contrast, they had no significant relationship in the oyster. In CGSM, higher Hg concentration was recorded in adult sizes, while in BhC the highest accumulation occurred in juveniles, especially during the dry season. These results emphasize the need for continuous monitoring of Hg contamination in both ecosystems. In addition, they highlight the importance of considering the size of oysters when assessing Hg contamination, as they may vary according to specific ecosystem and climatic conditions.

Subjects: Marine Biology, Aquatic and Marine Chemistry, Environmental Contamination and Remediation.

Comentado [chs1]: It would be very interesting to observe the molar ratio (Selenium:Mercury) in the oyster Crassostrea rhizophorae. Selenium has been documented to neutralize the toxic Hg effect if its Se/Hg molar ratio is > 1 (Burger and Gochfeld 2013). Selenium intervenes in the Hg demethylation process through the selenocysteine protein, transforming the metal to its inorganic and less toxic form. In this manner, Hg can be excreted more easily through pseudofeces and spawning (Medina-Morales et al. 2020; Vega-Sánchez et al. 2020).

Comentado [chs2]: It is recommended that after a period you do not start with an abbreviation.

Keywords: Mercury, *Crassostrea rhizophorae*, Bioconcentration factor, Pollution index, Sizes.

Introduction

Mercury (Hg) pollution is a global environmental problem due to its ability to bioaccumulate and biomagnify in food webs, with potentially devastating effects on ecosystems (Mountouris et al. 2002; Driscoll et al. 2013). Catastrophic events related to Hg pollution have been recorded throughout history, with the Minamata disaster in Japan being the most emblematic example. Mercury is extensively used in the gold amalgamation process, leading to its release into rivers, soils, and coastal ecosystems (Bolaños-Alvarez et al. 2024). Colombia ranks among the countries with the highest per capita Hg emissions globally, with annual discharges reaching up to 150 tons of Hg~~mercury~~ (Cordy et al. 2011). This widespread contamination has caused serious environmental and health problems, particularly in regions like Bolivar, Antioquia, Chocó, and the Bajo Cauca. In these areas, exposure to Hg~~mercury~~ has resulted in neurological damage and renal dysfunction in local communities that consume contaminated fish (Marrugo-Negrete et al. 2008; Alvarez et al. 2012). Additionally, contamination of major rivers such as the Atrato, Cauca and Magdalena has led to elevated Hg~~mercury~~ levels in sediments and fish, with adverse effects on biodiversity and fish populations due to reproductive and developmental toxicity (Güisa and Aristizábal 2013; Wesche 2021).

Comentado [chs3]: Update the value

In the Colombian Caribbean, the presence and impact of Hg on coastal ecosystems, particularly in bivalves, has only recently gained attention. Studies conducted near Cartagena Bay and Santa Marta, in areas such as Brujas Island, Barú Island, and Taganga, have documented seasonal variations in Hg concentrations in the mangrove oyster *Crassostrea rhizophorae*. In Cartagena Bay, higher Hg levels were observed during the rainy season compared to the dry season. Conversely, in Santa Marta, Hg concentrations were slightly lower in the rainy season compared to the dry season (Aguirre-Rubí et al. 2017). These fluctuations are likely influenced by environmental factors such as temperature, salinity, pH, dissolved oxygen, and sediment composition, which also affect the bioaccumulation process depending on the organism's size (Cogua et al. 2012; Valdelamar-Villegas and Olivero-Verbel 2018). Seasonal variations and environmental factors highlight the complexity of understanding Hg bioaccumulation in bivalves, particularly as these factors interact with the bivalve life cycle from juvenile to adult stages (Romero-Murillo et al. 2023).

Despite the ecological and socioeconomic importance of key areas like the Ciénaga Grande de Santa Marta (CGSM) and Cispatá Bay (BhC), there is a lack of data on Hg contamination in commercially important species such as *C. rhizophorae*. They are also impacted by various natural and anthropogenic pressures. In CGSM, untreated wastewater and pollutants from agricultural runoff degrade water quality and increase the risk of metal contamination (Alvarez et al. 2012). Additionally, illegal mining activities upstream of the Magdalena River introduce Hg into the aquatic system (Espinosa et al. 2011). In BhC, agricultural runoff, aquaculture, and urban development contribute to eutrophication, leading to the accumulation of contaminants such as Hg in sediments and biota (Marrugo-Negrete et al. 2020).

Mercury's impact on aquatic organisms varies based on species, the form of mercury, and local environmental conditions (Masson et al. 1995). Methylmercury disrupts the reproduction and development of aquatic species, causing detrimental effects on egg and larval formation, and leading to neurological impacts that affect behaviors such as feeding and predator avoidance (Richter et al. 2014). Bivalves, like oysters, are especially vulnerable to Hg accumulation due to their filter-feeding behavior, which can impact their growth, reproduction, and the quality of their edible tissues for human consumption (Gagnaire et al. 2004). These characteristics make bivalve's valuable indicator species for monitoring Hg contamination in marine ecosystems (Phillips 1977).

Based on the hypothesis that mercury bioaccumulation in bivalves is significantly influenced by environmental factors and organism size, leading to seasonal variations in Hg concentrations, this study aims to assess mercury bioaccumulation in *C. rhizophorae* in the coastal ecosystems of CGSM and BhC in the Colombian Caribbean. It will compare Hg concentrations across different climatic seasons and oyster sizes, evaluate the influence of key environmental variables on this process.

Materials and Methods

Study area

The Colombian Caribbean region is characterized by a bimodal climatic regime with a rainy and dry season influenced by the Intertropical Convergence Zone (ITCZ), generating periodic patterns (Restrepo and López 2008). Trade winds predominate from December to April (dry season), changing direction to the southeast between April and November (rainy season) (Vega-Sequeda et al. 2019).

The Ciénaga Grande of Santa Marta (CGSM) covers an area of 450 km² (González and Ricaurte-Villota 2023), and was declared a Ramsar Wetland and Biosphere Reserve (UNESCO 2001). Consiting of interconnected lagoons and a sandbar to the northeast separating it from the Caribbean Sea (Restrepo Martinez 2004) (Fig. 1A). The exchange of fresh and brackish water supports the development of *Rhizophora mangle* (red mangrove), providing substrate for the mangrove oyster (*C.~~rassostrea~~ rhizophorae*) (Rodríguez-Rodriguez et al. 2018). CGSM is a productive tropical ecosystem, yielding significant commercial catches of fish, crustaceans, and mollusks (Romero-Murillo et al. 2023).

Cispatá Bay (BhC), an estuarine system within the Sinú River delta, features fine to very fine sediments primarily influenced by the Sinú River (Ruíz-Ochoa et al. 2008). The 130 km² estuary is predominantly covered by mangroves (Castaño et al. 2010; Fig. 1A). Rainfall averages 66 mm in the dry season and 150 mm in the rainy season, with sediment discharge increasing from 3.1 kg/day to 11.5 kg/day during the rainy period (Rangel-Ch and Arellano 2010). BhC salinity fluctuates seasonally due to rainfall, droughts, and the mixing of fresh and brackish water (Cortés-Castillo and Rangel-Ch 2011).

Field phase

Oyster samples were collected from three stations in CGSM and BhC, selecting sites that represented gradients of water inflow from the sea and freshwater sources that might carry contaminants. A single sampling was conducted during each period: the rainy season (November 2021) and the dry season (March 2022). At each oyster collection site, in situ measurements of temperature, salinity, pH, and dissolved oxygen were taken at a depth of 0.5 m using WTW 3110 and YSI Pro1030 multiparameter probes.

277 individual oysters were collected from CGSM, and 237 from BhC under the collection permit for wild species specimens of biological diversity for non-commercial scientific research purposes, granted by the Autoridad Nacional de Licencias Ambientales (ANLA) through Resolution 1271 of October 23th 2014, modified by resolutions 1715 of December 30th 2015 and 00213 of January 28th 2021, to the University de Bogota´Jorge Tadeo Lozano (UTADEO). The samples were divided into six groups per station in each climatic season, with three groups consisting of juveniles (22.0–32.0 mm) and three groups of adults (35.0–56.5 mm) (Pacheco Urpí et al. 1983; Madrigal Castro et al. 1985). Juveniles were more abundant, allowing for a larger sample size to meet the dry biomass required for analysis. The result was a total of 71 composite samples across both ecosystems and climatic periods (Table 1).

At each station, specimens were primarily collected from the roots of mangrove trees. oysters were placed in plastic containers, were cleaned to remove any particles adhering to their shells (Fig. 1B), stored in pre-labeled, airtight polyethylene bag, and preserved in coolers with gel ice packs (~4 °C).

To determine mercury content in seston, and to serve as food for filter-feeding organisms like bivalves, three water samples were collected at each station in 2.8 L amber flasks and kept cold (~4°C). After homogenization, samples were filtered through two Whatman GF/C glass fiber filters (47 mm diameter) per sample using a manual vacuum pump. Filters were then stored in hermetically sealed, pre-labeled polyethylene bags, dried in an oven at 45°C for 24 hours, and weighed on an analytical balance (Cogua et al. 2012).

each station, three sediment samples were collected using a van Veen dredge. From each composite sample per station, 600 g of sediment was separated for mercury analysis, 75 g for organic matter determination, and 75 g for redox potential measurement. Samples were stored in airtight polyethylene bags using a silicone scoop to avoid contamination, ensuring no contact with the dredge edges. Samples were kept chilled (~4 °C) (Cogua et al. 2012).

Laboratory phase

To determine organic matter content, 5 g of dry sediment were placed in pre-weighed porcelain crucibles, subjected to calcination in a muffle furnace at 550 °C for 5 hours, and then left in a desiccator for 2 hours. Organic matter content was calculated based on the difference between the dry weight and the weight after calcination (Kenny and Sotheran 2013).

For redox potential quantification, sediment samples were dried at 40 °C for 24 hours. A portion of 25 g of sediment was then homogenized in 50 mL of deionized water using a VELP Scientifica magnetic stirrer for 30 min. Redox potential was measured with a YSI Pro1030 multiparameter probe equipped with an oxidation-reduction potential electrode, at a standard temperature of 25 °C (Aldridge and Ganf 2003).

For the chemical analyses, all materials were pre-treated by purging with 5% nitric acid (HNO_3) and deionized water for 24 hours to prevent contamination. Samples were handlered with gloves, glass, or plastic materials to avoid contamination. samples were then transferred to the Toxicology and Environmental Management laboratory at the University of Córdoba, preserved at ~4 °C, for mercury (Hg) quantification.

The anteroposterior length (APL) was measured on the inner side of the ventral valve using a Vernier caliper (precision of 0.05 mm). soft tissues were removed and weighed using an analytical balance (± 0.1 mg). For each sample, organisms of similar size were pooled, and soft tissues were placed in pre-weighed and labeled 30 mL glass vials. The vials were then lyophilized, and the final dry weight was recorded.

For mercury analysis, samples of seston, sediment, and oyster tissue underwent a pre-digestion process. Approximately 20-40 mg of dry material was subjected to calcination at 450 °C with a ramp of 50 °C over 8 hours. Following calcination, 1 mL of concentrated HNO_3 was added, and the sample heated on a hot plate to volatilize remaining residues. The sample was then subjected to microwave-assisted acid digestion at 180 °C for 20 minutes with 25 mL of deionized water to complete the digestion and prepare the sample for mercury quantification.

Comentado [chs4]: It is best to freeze-dry the samples, mainly the tissues of aquatic organisms, to perform mercury analyses. Remember that mercury is volatile, so when subjected to high temperatures it can be released, and this leads to errors in the technique.

Sediment and seston fractions smaller than 65 µm were digested with 5% nitric acid (HNO_3) for the subsequent determination of total Hg concentration using atomic absorption spectrometry

(EPA 2007). Hg analysis was conducted following EPA Method 7473 PLTX-017, which involves thermal decomposition, amalgamation, and atomic absorption spectrometry (Fernández-Martinez et al. 2015).

For analytical control, triplicate analyses of Hg solutions at different concentrations were performed. Calibration curves were established for three concentration ranges in all matrices: 0.005–0.02 µg Hg, 0.02–0.05 µg Hg, and 0.05–0.5 µg Hg. The coefficients of determination (R^2) were 0.9993, 0.9986, and 0.999 for sediment; 0.9973, 0.9962, and 0.9979 for seston; and 0.9996, 0.9996, and 0.9989 for oyster tissue. Error percentages were kept below 15% for all three matrices, and TORT-1 (lobster hepatopancreas) used as a reference material from the National Research Council of Canada (NRCC). Recovery percentages were 100 ± 1.4% for sediments (limit of detection, LOD = 0.00073 µg/g Hg), 100 ± 5.4% for seston (LOD = 0.000015 µg/g Hg), and 100 ± 1.4% for oysters (LOD = 0.00073 µg/g Hg) (Romero-Murillo et al. 2023).

Comentado [chs5]: They obtained very good values for the percentage of recovery using the TORT-1 (lobster hepatopancreas). However, it is convenient to use more specific reference materials, for example oyster or mussel tissue and another of sediments.

Data analysis

The bioconcentration factor (BCF) was calculated as the ratio of Hg concentration in oyster tissue to its presence in sediment (sd) and seston (st), expressed in parts per million (ppm, µg/g) in dry weight (d.w.). BCF was used to evaluate the efficiency of Hg accumulation in oyster soft tissue. Mountouris et al. (2002), BCF < 1 suggests no metal accumulation, BCF ≥ 1 and < 10 indicates accumulation and BCF ≥ 10 indicates hyperaccumulation of metal. The calculation was based on Mountouris et al. (2002) and Romero-Murillo et al. (2023).

$$BCF_{sd} = \frac{[Metal]_{organism}}{[Metal]_{sediment}}, BCF_{st} = \frac{[Metal]_{organism}}{[Metal]_{seston}}$$

Permutation analysis of variance (PERMANOVA) was applied to compare Hg concentration in oyster tissue and its BCF between the two ecosystems (k=2), the two climatic seasons (k=2), the six stations (k=6) and the two categorized size classes (k=2). 9 999 permutations were performed using Euclidean distance and type III sum of squares. *p*-values were computed using Monte Carlo (MC) permutation testing only when unique permutations were less than 100 (Anderson et al. 2008).

Relationships between mercury (Hg) concentrations in sediment and seston were examined using Pearson's and Spearman's correlation analyses based on data distribution. Normality tests (Shapiro-Wilk) were performed on each dataset prior to analysis (Zar 2010). Influences of environmental predictor variables on the oyster Hg concentration and Hg BCF in relation to seston were evaluated using a distance-based linear model (DistLM) with adjusted R^2 criterion and 9999 permutations (Anderson et al. 2008).

To evaluate mercury contamination in bivalves, the Nemerow integral contamination index -P_c- was used (Ding et al. 2022). Calculations of P_c are based on the average value of the individual pollution index (P_{avg}), the maximum value (P_{max}) and the minimum value (P_{min}). C_{avg} average concentration value, recorded in the data set evaluated, C_{max} and C_{min} are the maximum and minimum concentration values from the same data set, and S is the maximum concentration allowed in marine organisms (mollusks) with Hg (0.5 µg/g) (FAO/WHO 2010). The individual index values were calculated using the following formula:

$$P_{avg} = \frac{C_{avg}}{S}, \quad P_{max} = \frac{C_{max}}{S}, \quad P_{min} = \frac{C_{min}}{S} \qquad [1]$$

Once the historical values of P_{avg}, P_{max} and P_{min} were obtained for each country by year, the calculation of P_c was performed establishing (i) $P_c \leq 0.7$ considered no risk, (ii) $0.7 < P_c \leq 1$ low risk, (iii) $1 < P_c \leq 2$ medium risk, (iv) $2 < P_c \leq 3$ high risk and (v) $P_c > 3$ very high risk of contamination (Ding et al. 2022). ΣP_c is the sum of all P_c values divided by "n", the total number of years evaluated per country in its historical record, ensuring that the values of P_{max} and P_{min} do not overestimate or underestimate the calculation of the contamination index for each metal evaluated, respectively. It was calculated using the following equation:

$$\frac{\sum P_c}{n} = \sqrt{\frac{P_{avg}^2 + P_{max}^2 + P_{min}^2}{3}} \qquad [2]$$

A hierarchical clustering analysis was carried out using the squared Euclidean distance with Ward's linkage, minimizing variability and producing uniformly sized clusters (Tudor et al. 2002; Tudor and Williams 2004).

Human health risk assessment (Target Hazard Quotient)

Comentado [chs6]: In this manuscript they evaluated many very interesting variables, however, it is necessary to evaluate the Target Hazard Quotient to strengthen the work a lot. In the THQ formula, the concentration of the element (in this case mercury) is used converted into wet weight. They did not specify the percentage of moisture in the oyster; if they did not calculate this value, they can use 80%.

Results

Environmental conditions in both coastal lagoons

In CGSM, the temperature during the rainy season was 31.17 ± 0.48 °C (n=3), while in dry season it was 30.53 ± 0.84 °C (n=3). In BhC the temperature during the rainy season was 28.97 ± 0.43 °C (n=3), and in dry season it was 29.75 ± 0.03 °C (n=3) (Fig. 2A, Supplementary Table S1).

Comentado [chs7]: Do not specify the sample value (n=3) in each physicochemical parameter, that was explained in the methodology.

During the rainy season, CGSM had a higher pH value (8.77 ± 0.12, n=3) compared to BhC (7.84 ± 0.09, n=3), while during the dry season, were a decrease in pH in CGSM (8.44 ± 0.16, n=3) and an increase in BhC (8.19 ± 0.003, n=3). (Fig. 2A, Supplementary Table S1).

The average dissolved oxygen content was higher in CGSM during both climatic seasons, with values of 7.83 ± 0.66 mg/L (n=3) in rainy season and 7.39 ± 2.4 mg/L (n=3) during dry season. In BhC, the contents were 4.32 ± 0.5 mg/L (n=3) in rainy season and 5.52 ± 1.28 mg/L (n=3) along the dry season (Fig. 2A, Supplementary Table S1).

In CGSM, salinity values varied significantly between seasons, ranging from 2.47 ± 1.01 (n=3) during the rainy season to 18.53 ± 6.33 (n=3) in the dry season. In contrast, BhC showed less seasonal fluctuation, with average salinity levels from 24.9 ± 1.01 (n=3) to 30.83 ± 0.56 (n=3) across the two seasons (Fig. 2A, Supplementary Table S1).

Organic matter in CGSM was also seasonally variable, with contents during the rainy season at 11.67 ± 3.27% (n=3), approximately double the levels observed in the dry season (5.97 ± 2.4%, n=3). BhC, on the other hand, exhibited minor seasonal differences in organic matter, ranging from 5.6 ± 0.5% (n=3) to 6.06 ± 1.28% (n=3) (Fig. 2A, Supplementary Table S1).

In sediments the redox potential, both in CGSM and BhC, reducing conditions were recorded with a range of values from 28 to 77 mV between both sectors. Increases in redox potential were observed in BhC (52 ± 3, n=3 to 65 ± 4, n=3), and decreases in CGSM (50 ± 11, n=3 to 35 ± 4, n=3) from rainy season to dry season (Fig. 2A, Supplementary Table S1).

Concentration of Hg in sediments, seston and *C.~~rassostrea~~ rhizophorae*

Hg concentration in sediments and seston varied markedly between the two ecosystems. In BhC, in both sediments and seston, Hg concentrations are consistently higher than in CGSM in both climatic seasons (Fig. 2B, Supplementary Table S2).

In the rainy season, the highest concentration of Hg in sediments was found in CIS-1 (BhC) with 0.128 µg/g Hg dry weight (d.w.) which is double the highest content detected in CGSM (0.059 µg/g Hg $_{d.w.}$ in CGS-1). Hg content in sediments at BhC was slightly lower in the dry season but remained above 0.08 µg/g Hg $_{d.w.}$ indicating a possible constant source of Hg contamination. In CGSM, during the dry season, lower Hg was observed in CGS-1 and higher in CGS-2 (Fig. 2B, Supplementary Table 2).

The Hg available in the seston presented similar values in the stations of each ecosystem and in the two climatic seasons. However, as in sediments, a lower concentration was detected in the dry season. In BhC, the concentration went from 0.032 ± 0.005 µg/g Hg $_{d.w.}$ in the rainy season to 0.022 ± 0.001 µg/g Hg $_{d.w.}$ in the dry season. Lower concentrations were found in CGSM, with values ranging from 0.01 ± 0.001 µg/g Hg $_{d.w.}$ in the rainy season to 0.004 ± 0.001 µg/g Hg $_{d.w.}$ in the dry season (Fig. 2B, Supplementary Table S2).

In BhC the highest Hg content in seston was positively and significantly related to temperature (Pearson, r = 0.933, df = 11, *p*-value = 0.006) and in sediments Hg was significantly related to organic matter (Pearson, r = 0.845, df = 11, *p*-value = 0.039) (Fig. 2, Supplementary Table S3).

Hg concentrations in oyster tissue show distinct accumulation patterns in CGSM and BhC, varying as a function of climatic seasons. However, a pattern similar to that of sediment and seston was maintained, with a higher Hg content in oyster soft tissue in BhC (Fig. 2B). In the rainy season, Hg in the oyster was 0.083 ± 0.007 µg/g $_{d.w.}$ (n=17) in CGSM and of 0.135 ± 0.015 µg/g $_{d.w.}$ (n=18) in BhC showing significant differences between the two ecosystems (Permanova, *p*-value<0.01). In dry season, these differences were maintained, given the decrease in oyster Hg content in CGSM (0.066 ± 0.007 µg/g Hg $_{d.w.}$, n=18) and increased in BhC (0.154 ± 0.019 µg/g Hg $_{d.w.}$, n=18) (Table 1 and 2, Fig. 3A, Supplementary Table S4).

With respect to Hg BCF, both in sediments and seston, both ecosystems presented accumulation to hyperaccumulation of Hg in the oyster tissue, with the highest values in the dry season. In this same climatic season, at CGSM, the oyster presented an accumulation of Hg with the sediment (BCF≥1) and a hyperaccumulation of the metal with the seston (BCF≥10), as opposed to the accumulation condition in both matrices during the rainy season. In BhC, the oyster maintained the accumulation condition in both matrices (BCF≥1) as in rainy season (Table 1 and 2). These results notably emphasize the capacity of the oyster to accumulate Hg in its tissues, especially in CGSM through the seston in the dry season. Significant differences in BCF were determined between CGSM and BhC ecosystems, with higher concentrations in BhC (Permanova, *p*-value<0.05). However, no significant differences in concentrations were observed between climatic seasons, since the values measured at two of three stations in both CGSM (CGS-2 and CGS-3) and BhC (CIS-1 and CIS-3) were similar in both climatic seasons (Table 1 and 2, Fig. 3B).

In the two ecosystems evaluated, there were significant differences in the Hg contents between stations (Permanova, *p*-value<0.05; Table 2). In the rainy season at CGSM the differences occurred between stations CGS-1 and CGS-2 and at BhC between CIS-2 and CIS-3. In the dry season, significant differences were found between CIS-1 and CIS-2 in BhC, with the lowest concentrations in CIS-1 (Table 1, Fig. 2B, Supplementary Table S4 and S5).

There were significant differences in the length of juveniles and adults (Permanova, *p*-value<0.05) between stations (Table 2). In CGSM in the dry season, concentrations were higher in adult sizes at station CGS-3 (0.121 ± 0.016 µg/g Hg $_{d.w.}$) with respect to juveniles (0.039 ± 0.009 µg/g Hg $_{d.w.}$). In BhC, in both rainy and dry seasons, the highest concentration of juvenile lengths was at station CIS-2 (Table 1, Fig. 3A, Supplementary Table S4 and S5).

Importance of seston in the bioconcentration of Hg

High Hg bioconcentration factor values reflected a significant correlation with seston content (Pearson, r = 0.718, df = 11, p-value = 0.008), with conditions of accumulation (BCF≥1) in BhC where an average concentration between both climatic periods was observed in the seston of 0.027 ± 0.003 µg/g Hg $_{d.w.}$ (n=6) and in the oyster of 0.144 ± 0.012 µg/g Hg $_{d.w.}$ (n=36). While in CGSM hyperaccumulation conditions (BCF≥10) were reached with an average content in the seston of 0.007 ± 0.001 µg/g Hg $_{d.w.}$ (n=6) and in the oyster of 0.074 ± 0.005 µg/g Hg $_{d.w.}$ (n=35) (Table 1, Supplementary Table S4).

Differences in Hg bioconcentration between CGSM and BhC were determined (Permanova, p-value<0.05; Table 2). These differences were also observed as a function of climatic seasons, with an increase during the dry season in each CGSM season, and significant in the CIS-2 season in BhC compared to the rainy season. When the factors ecosystem and climatic season were combined, significant differences were still present, with higher values of Hg bioconcentration in CGSM in both climatic seasons compared to BhC (Table 1, Fig. 3B, Supplementary Table S5). These results indicate that the oyster in CGSM is accumulating higher concentrations of Hg in its tissues compared to the BhC oyster, although the accumulation is also considerably higher in the BhC oyster.

Significant differences between juvenile and adult sizes were determined with the Hg BCF, which was maintained when considering the climatic season (Table 2). In BhC, the highest values of Hg BCF were observed in juvenile sizes in both climatic seasons. In CGSM, the highest BCF occurred in adult sizes during the dry season, while they were similar in both sizes during the rainy season (Spearman, r = 0.25, p-value>0.05; Table 1, Fig. 3B, Supplementary Table S4).

Relationship between environmental variables and Hg bioconcentration in oysters

Between the Hg concentration in the mangrove oyster tissue and its BCF by oyster in relation to the metal content in the seston, it was not possible to find a positive or negative relationship with the environmental variables analyzed. The relationship between physicochemical variables with Hg concentration and BCF in the mangrove oyster were not significant (DistLM; p-value>0.05; Table 3). This suggests that environmental variables did not play a determining role in the differences in oyster Hg content and bioconcentration at CGSM and BhC (Fig. 3). Other factors, such as Hg content in the seston and local transport and sedimentation processes, may be playing a more influential role in Hg accumulation.

Hg contamination status of bivalves in a global context during the last decade

Considering Hg contamination levels in global monitoring studies over the past 12 years across different bivalve species, and using the adapted Nemerow comprehensive contamination index explained in the methodology, both ecosystems fall within Clade 1. CGSM shows no risk of Hg contamination for oyster consumption, similar to levels observed in Taganga. In contrast, BhC approaches a low risk of Hg contamination, comparable to levels found in Isla Cayo El Pigeon (Nicaragua); all these sites were monitored using the oyster *C.~~rassostrea~~ rhizophorae*. However, these values exceed those reported in regions such as China, Italy, and Montenegro, which presented the lowest risk of Hg contamination in bivalves like *Magallana gigas*, *Ruditapes philippinarum*, and. This underscores the importance of considering the potential risk of Hg contamination in the Colombian Caribbean, which has shown the highest risk of Hg contamination in bivalves globally over the past decade (Fig. 4, Table 4).

Discussion

The higher concentrations of Hg in sediments, seston, and oysters in BhC compared to CGSM (Fig. 2B, Supplementary Table S1) highlight significant concerns regarding environmental quality and ecosystem health. The elevated Hg levels in BhC are primarily linked to the Sinú River's input through the Sicará stream, which carries water and sediments contaminated by agricultural runoff (Campos et al. 2015). Among these, the application of fungicides containing phenylmercury (C_6H_5Hg) and the extensive spraying of rice fields with agrochemicals rich in mercury-based compounds (Marrugo-Negrete et al. 2020) are particularly significant sources. These practices contribute to the continual release of Hg into the estuarine system, exacerbating contamination levels over time. Additional sources of Hg in BhC include regional artisanal and small-scale gold mining operations, discharges of untreated municipal and industrial wastewater, the historical use of Hg-based paints as anti-corrosion agents on ships, and atmospheric deposition from regional emissions (Burgos et al. 2014, 2017).

In CGSM, the sources of Hg contamination are less well-defined. However, the entry of Hg into this system is associated with atmospheric deposition and anthropogenic activities, such as industrial discharges and gold mining in areas connected to the Magdalena River (Alonso et al. 2000; Mancera-Rodríguez and Álvarez-León 2006). Given CGSM's status as a Ramsar Wetland and Biosphere Reserve, identifying and mitigating potential contamination sources is crucial to preserving its ecological integrity and the socioeconomic benefits it provides to local communities.

While Hg levels in sediments in BhC and CGSM remain below the tolerable ecological threshold of 0.13 µg/g Hg $_{d.w.}$ (TEL) (Buchman et al. 2008), they are significantly lower than those observed in more heavily impacted regions, such as Cartagena Bay, Colombia (0.094–10.293 µg/g Hg $_{d.w.}$) (Alonso et al. 2000), and San Vicente Bay, Chile (0.37–0.95 µg/g Hg $_{d.w.}$) (Díaz et al. 2001). However, it is important to note that BhC exhibits a higher contamination risk compared to CGSM, as previous assessments have reported Hg concentrations exceeding the TEL threshold in sediments along the Sinú River and near the mouth of BhC by Feria et al.

(2010), Campos et al. (2015), and Marrugo-Negrete et al. (2020) reported Hg concentrations exceeding the TEL threshold in sediments along the Sinú riverbed and at the mouth of BhC. In contrast, in CGSM, Hg concentrations in sediments have remained below 0.11 µg/g Hg $_{d.w.}$, similar to the findings of this study (Fig. 2B, Supplementary Table S4).

The slight increase in Hg content in sediment and seston during the rainy season compared to the dry season (Fig. 2B), may be attributed to increased metal transport from terrestrial sources. Rainfall-induced sediment flushing and freshwater inflow during this period mobilize Hg from upstream sources into the estuarine systems, highlighting the critical role of hydrological dynamics in shaping contamination patterns (Baraj et al. 2003; da Silva Ferreira et al. 2013). This phenomenon is particularly pronounced in BhC, where contributions from the Sinú River amplify Hg loading (Marrugo-Negrete et al. 2020). Similarly, in CGSM, the Magdalena River serves as a major pathway for metal transport, emphasizing the interconnectedness of terrestrial and aquatic systems in driving contamination processes (Mancera-Rodríguez and Álvarez-León 2006; Table 3, Fig. 3A). These results highlight the importance of the sediment-seston interaction and local conditions in influencing Hg availability for oysters in the coastal areas of the Colombian Caribbean (Aguirre-Rubí et al. 2017).

Environmental factors, including temperature and organic matter content, appear to play a pivotal role in influencing Hg concentrations in sediments and seston. In BhC, significant correlations were observed between elevated Hg concentrations in seston and higher water temperatures during the rainy season (Fig. 2). This relationship aligns with findings that temperature accelerates chemical reaction rates, such as the methylation of Hg, thereby increasing its bioavailability (Richard et al. 2016). Additionally, higher Hg concentrations in sediments were associated with increased organic matter content during the rainy season, emphasizing the role of organic matter in retaining metals in sediments, particularly in fine sediments and sulfate-reducing environments (Cogua et al. 2012), as observed in both CGSM (Espinosa et al. 2021) and BhC (Fig. 2A).

Although no direct correlation was identified between pH and Hg concentrations in sediments and seston (Supplementary Table S3), slightly acidic to neutral pH conditions are known to enhance Hg precipitation in sediments (Para and Espinosa 2008). This mechanism likely contributes to the observed higher Hg concentrations in BhC sediments, which exhibited lower pH values compared to CGSM (Fig. 2). Furthermore, seasonal fluctuations in pH, particularly during the transition between rainy and dry seasons, may influence the solubility and availability of Hg, adding another layer of complexity to its distribution patterns.

Additionally, variations in sulfate to sulfide conversion processes may increase the flux of reactive phosphate and ammonium at the sediment-water interface (Uwah et al. 2013). This process favors the precipitation of Hg in sediments as insoluble hydroxides, oxides, carbonates, or phosphates (Volety et al. 2008; Azizi et al. 2018a). The interaction of these processes could account for the observed variations in Hg content in sediments, especially when considering seasonal pH fluctuations. This pattern is particularly evident in BhC due to the pH differences between the rainy and dry seasons (Fig. 2).

The role of salinity in modulating Hg methylation and sediment retention also warrants attention. Elevated salinity levels, such as those observed in BhC during the dry season and at CGS-3 in CGSM, can inhibit Hg^{2+} methylation through the production of hydrogen sulfide (H_2S). This compound forms mercury sulfide (HgS), a mineral that is poorly available for methylation processes, thereby limiting the bioavailability of Hg (Compeau and Bartha 1984). These findings highlight the intricate interplay of physicochemical factors in shaping Hg dynamics in estuarine ecosystems.

Regarding the mangrove oyster, the influence of environmental variables on Hg concentration and bioconcentration factor (BCF) was not significant (Table 1 and 2, Fig. 3). This aligns with findings from other studies where the effects of variables such as temperature, salinity, and pH on Hg uptake and accumulation in bivalves remain complex and not fully understood (Volety et al. 2008), unlike the clearer dynamics of Hg in sediments and seston, where processes such as accumulation, uptake, toxicity, and speciation are well-documented (Curtius et al. 2003; Suryanto Hertika 2021), the factors influencing Hg bioaccumulation in oysters are less understood and may be influenced by the organism's unique physiological and ecological traits.

Despite the lack of significant correlations, it is worth considering the potential role of high dissolved oxygen concentrations observed in CGSM. Elevated oxygen levels, coupled with variations in sediment chemical composition, can influence the metabolic activity of bivalves, potentially altering their ability to absorb and excrete Hg (Silva et al. 2003; Griscom and Fisher 2004). These findings suggest that while direct correlations may not be evident, indirect effects mediated through environmental and physiological interactions could still play a role in Hg bioaccumulation.

Mangrove oysters are known for their ability to filter large volumes of water during feeding (Restrepo and López 2008), capturing particulate matter from sediments (Coimbra 2003). Therefore, the Hg concentrations in oysters are closely related to the metal content in their environment (Fig. 2B). Hg bioconcentration was significantly associated with seston, which is expected given that oyster collection was primarily from mangrove roots submerged to depths greater than half a meter. As with seston, metal accumulation and hyperaccumulation were observed in relation to Hg concentrations in sediment (Table 1 and 2). These findings underscore the importance of measuring metal concentrations in sediments and seston to assess Hg availability and uptake by bivalves, providing a comprehensive understanding of the interaction between these organisms and their contaminated environments.

Interestingly, the bioconcentration patterns observed in BhC, where adult oysters showed lower Hg concentrations than juvenile oysters (Fig. 3B), align with previous studies. For example, Coimbra (2003) in Sepetiba Bay, Brazil, with *Mytela guyanensis* and Díaz et al. (2001) in San Vicente Bay, Chile, with *Tagelus dombeii*, reported inverse correlations between Hg content and species size. They suggested that metal assimilation rates decrease as the excretion rate increases in larger individuals, likely due to reduced metabolism and less water filtration with bivalve growth (Azizi et al. 2018b).

Several mechanisms regulate the accumulation of toxic metals such as Hg in bivalve tissues during their growth. One such mechanism is the formation of mineralized granules, which allows for Hg storage and potential detoxification (Cossa 1989). Bivalves also regulate Hg concentrations through excretion mechanisms via urine and feces, maintaining appropriate Hg levels (El-Moselhy and Yassien 2005). The development of new gill systems in bivalves plays a crucial role in filtering particles, including metals, from the aquatic environment (Kumar Gupta and Singh 2011). As bivalves grow, this gill development enhances their ability to capture and regulate Hg in their tissues.

Another important strategy for mitigating the accumulation of toxic metals in bivalves is the release of gametes during reproduction (Cossa 1989; Monsefrad et al. 2012). In both CGSM and BhC, the highest BCF values in oysters were observed during the dry season (Table 2, Fig. 3B). During gamete release, which typically occurs in the rainy season and includes several reproductive peaks in the Colombian Caribbean (López-Sánchez and Mancera-Pineda 2019), mineralized granules stored in lysosomes may be expelled along with the gametes (Costa et al. 2000). This exocytosis process releases lysosomal contents, including metals like Hg, into the aquatic environment (Cossa 1989). This possible release of Hg granules during gametogenesis may explain the lower BCF values observed in the rainy season.

Conversely, the highest BCF values were observed during the dry season, particularly in CGSM, where adult oysters exhibited greater Hg concentrations and BCF values than juveniles. These findings are consistent with previous research by Costa et al. (2000) and De Gregori et al. (1996), who documented similar trends in other estuarine ecosystems. The contrasting patterns between CGSM and BhC highlight the complexity of environmental and organismal factors that influence Hg bioaccumulation and bioconcentration dynamics.

Hg intake risk in *C. rhizophora*e at CGSM and BhC

Variability in contamination sources and Hg concentrations between CGSM and BhC stands out as a key factor influencing contamination risk. In particular, BhC shows values close to the permissible limit for Hg in bivalves for human consumption (0.5 µg/g Hg body weight)

(FAO/WHO 2010), raising concerns about the health of the ecosystem. This situation mirrors findings in Cayo el Pigeon Island, Nicaragua, and Santa Marta, Colombia, as reported by Aguirre-Rubí et al. (2017) (Fig. 4). However, both ecosystems remain far below the Hg levels reported for this species in coastal areas of the Dominican Republic, where Sbriz et al. (1998) recorded one of the highest Hg concentrations in the mangrove oyster (7.02 µg/g Hg $_{d.w.}$).

When comparing Hg concentrations in mangrove oysters with other bivalve species globally over the last decade, CGSM and BhC maintain low to no risk of Hg contamination. This contrasts with findings from coastal areas in China (Wang et al. 2018; Liu et al. 2019; Wang et al. 2021), Italy (Squadrone et al. 2016), and Montenegro (Perošević et al. 2018), which show lower Hg contamination risks. These comparisons serve as a reference point for assessing the potential risk of Hg contamination in CGSM and BhC (Fig. 4).

Nevertheless, the current state of Hg contamination in Colombia, particularly in Cartagena Bay, highlights the need for vigilance. Cartagena Bay has recorded some of the highest global Hg contamination risks for bivalves in the past decade (Aguirre-Rubi et al. 2017), attributed to historical discharges from industrial facilities like the Alcalis chlorine plant (Mancera-Rodríguez and Álvarez-León 2006; Bolaños-Alvarez et al. 2024). Similar trends have been observed in other regions, including San Vicente Bay, Chile (Díaz et al. 2001), and the Adriatic Sea, Croatia (Kljaković-Gašpić et al. 2010), underscoring the global relevance of monitoring Hg contamination in estuarine ecosystems.

The results of this study provide essential insights into Hg dynamics in CGSM and BhC. By identifying key drivers of contamination and assessing risk, these findings contribute to the development of targeted management strategies aimed at mitigating Hg pollution and safeguarding ecosystem health.

Conclusions

Cispatá Bay (BhC) had the highest concentrations of Hg in sediments, seston, and oysters compared to Ciénaga Grande de Santa Marta (CGSM) across both climatic seasons. However, the highest bioconcentration factor (BCF) values were observed in oysters from CGSM, especially with respect to seston, suggesting a higher potential for Hg accumulation in this ecosystem. Temperature in the water column and organic matter in sediments significantly influenced Hg concentrations in seston and sediments but showed no significant relationship with Hg bioconcentration in the oysters. Adult oysters accumulated more Hg in CGSM, whereas juvenile oysters accumulated more Hg in BhC, underscoring the importance of considering the bivalve size when assessing Hg contamination in different ecosystems. While the Hg levels in CGSM oysters remained below the permissible limit for human consumption (0.5 µg/g body weight), indicating no significant risk, while the oysters in BhC showed Hg concentrations more high, coming to consider a potential low risk of contamination. These findings highlight the need for continued monitoring of Hg levels in both ecosystems, with particular attention to oyster size and environmental conditions that may influence metal accumulation.

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