ACTA BIOLÓGICA COLOMBIANA
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Facultad de Ciencias
Departamento de Biología
Sede Bogotá

UNIVERSIDAD NACIONAL DE COLOMBIA

ARTÍCULO DE INVESTIGACIÓN / RESEARCH ARTICLE

Metal concentrations in *Isognomon alatus* by stages and climatic seasons in San Andrés Island, Colombian Caribbean

Concentraciones de metales en *Isognomon alatus* por estadios y épocas climáticas en San Andrés Isla, Caribe colombiano

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Received: 12st July 2021. **Revised**: 24th April 2022. **Accepted**: 09th December 2022
Editor asociado: Omar Ávila

Citation/ citar este artículo como: Romero-Murillo, P.; Campos Campos, N.H, and Orrego, R. (2023). Metal concentrations in *Isognomon alatus* by stages and climatic seasons in San Andrés Island, Colombian Caribbean. *Acta Biol Colomb*, *28*(3), 415-427. https://doi.org/10.15446/abc.v28n3.97227

RESUMEN

Este estudio evalúa el potencial de la ostra plana *Isognomon alatus* como especie biomonitora de contaminación por metales, a partir de los patrones de bioacumulación en adultos y juveniles presentes en la Reserva de la Biosfera Seaflower de la Isla de San Andrés (Colombia) en tres épocas climáticas. El análisis por Espectrometría de Absorción Atómica de especímenes de *I. alatus* y muestras de agua recolectadas en SAI (2009 y 2010) permitió observar concentraciones más altas (Max: 2,235 µg/g peso seco) de Zn en seston, en comparación con Cd (Max: 0.078 µg/g peso seco) y Cu (Max: 0,069 µg/g peso seco). Estas concentraciones variaron estacionalmente en relación con la intensidad de las lluvias en la zona. Por medio de un ANOVA a dos vías ($p<0.05$), se determinaron diferencias estadísticamente significativas entre sitios y épocas de muestreo para las concentraciones de metal (Cd, Cu, Zn) en organismos y para el Factor de Bioconcentración (FBC), que corresponde a la relación entre la concentración de metal en ostras y seston. Con respecto al efecto de edad/tamaño, la concentración de Cd obtenida y evaluada por rangos en juveniles es significativamente menor a la observada en adultos durante la época seca, similar a lo registrado en otras investigaciones en ostras. Una comparación de resultados obtenidos con los antecedentes disponibles para *I. alatus* indicó que SAI ha sido impactada por contaminación con Zn. Los resultados confirman biodisponibilidad de Cd previamente reportado; y la bioacumulación de metales, especialmente Cu y Zn, de acuerdo con las estimaciones de FBC.

Palabras clave: Contaminación Marina, Invertebrados, Bivalvo, Material particulado, Manglares.

ABSTRACT

This study evaluates the potential of the flat oyster *Isognomon alatus* as a biomonitor species for metal contamination, based on bioaccumulation patterns in adults and juveniles present in the Seaflower Biosphere Reserve on San Andrés Island (Colombia) in three seasons climatic. The analysis by Atomic Absorption Spectrometry of *I. alatus* specimens and water samples collected in SAI (2009 and 2010) allowed us to observe higher concentrations (Max: 2,235 µg/g dry weight) of Zn in seston, compared to Cd (Max: 0.078 µg/g dry weight) and Cu (Max: 0.069 µg/g dry weight). These concentrations varied seasonally in relation to the intensity of rainfall in the area. By means of a two-way ANOVA ($p<0.05$), statistically significant differences were determined between sampling sites and seasons for metal concentrations (Cd, Cu, Zn) in organisms and for the Bioconcentration Factor (BCF), which corresponds to the relationship between the metal concentration in oysters and seston. Regarding the effect of age/size, the Cd concentration obtained and evaluated by ranges in juveniles is significantly lower than that observed in adults during the dry season, like what was recorded in other oyster studies. A comparison of the results obtained with the available data for *I. alatus* indicated that SAI had been impacted by Zn contamination. The results confirm the bioavailability of Cd previously reported; and the bioaccumulation of metals, especially Cu and Zn, according to FBC estimates.

Keywords: Marine Pollution, Invertebrates, Bivalve, Particulate Material, Mangroves.

INTRODUCTION

Anthropogenic activities as wastewater treatment, agriculture, boat transport, etc., generate various types of waste in coastal ecosystems, which in turn, modify the presence and bioavailability of metals (Beraldi et al., 2019). Several factors as urban development with poor wastewater management, presence of ports and industries without adequate waste management processes, etc., are at the Colombian coasts and affect the contributions in the metal concentrations for environmental matrices, including water, sediments and organisms (Moncaleano-Niño et al., 2022).

Water matrix analysis, used as a reference of quality on coast, is constantly changing due to physical-chemical processes of runoff and seawater mixtures, tidal action, winds, and changes in redox conditions, particularly in the coastal marine environment (Beraldi et al., 2019; Villagran et al., 2021). Therefore, the bivalves filter-feeder organisms are widely used in short and long-term monitoring programs, as biomonitors of metal bioavailability (Lu et al., 2019). They have constant contact with water and suspended material (Lebordais et al., 2021). They are highly tolerant to salinity/temperature changes and contamination and have the ability to record such environmental variations in their tissues (Zhou et al., 2008). Furthermore, in these organisms the bioaccumulation processes may be associated to individual's age, size, sex, or reproductive cycle (Belcheva et al., 2006; Sokolowski et al., 2004; Spann et al., 2011), as it can provide an integrated measurement of the presence of metals and their effects over time and space as a relative measure of the bioavailable fraction, as well as their potential for bioaccumulation (Weng and Wang, 2014).

The results of research in metal accumulation in oyster soft tissue showed differences in metal concentration among small and large sizes, and these are related to age. In addition, these studies indicated metal concentration has temporal variability with differences among seasons evaluated, higher metal concentration in dry season relationship with metal availability (Otchere, 2022; Shakouri and Gheytasi, 2018) (Table 1).

In particular, the oyster *Isognomon alatus* (Isognomonidae), a bivalve associated with red mangrove roots (*Rhizophora mangle*) at the Colombia Caribbean including insular areas such as San Andres Island (SAI), is abundant especially in areas of mangrove exposed to water streams (Suarez-Ulloa et al., 2019). The bivalve *I. alatus* has a sessile lifestyle considered an appropriate characteristic for studies of metal bioaccumulation (Yap et al., 2011) in comparison to other currently less frequent species in the region such as *Crassostrea rhizophorae*, *Donax denticulatus* or *I. bicolor* (Campos, 1988).

SAI, Colombian insular Caribbean, has a solid waste treatment and sewage wastewater systems with inadequate sanitation. Leachate from pig farms, remnants from an old power plant, harbor activity, and tourism among others, which go directly and continuously into mangrove ecosystems (Romero-Murillo and Polanía, 2008). The "Red de Vigilancia de la Calidad Ambiental Marina en Colombia-Surveillance Network for the Conservation and Protection of Marine and Coastal Waters of Colombia" (REDCAM) performs environmental monitoring on metals in SAI, for example, during 2000 has registered 1.7 and 1.8 µg/L of cadmium (Cd) in surface water (Vivas-Aguas and Marín, 2004). However at present, water metal concentrations (Cd, Cu, Zn) are below of reference values used for REDCAM (Buchman, 2008, CONAMA, 2005; EPA, 2022; INVEMAR, 2019).

This study focused on evaluating the levels of essential copper (Cu) and zinc (Zn) and non-essential (Cd) metal concentrations in adults and juveniles stages of *I. alatus*, under the dry, transition, and rainy climatic seasons at mangrove ecosystems of SAI, Colombia during 2009 to 2010, in order to understand the bioaccumulation of metals in a bivalve as a biomonitor of the degree of metal pollution in the coastal area in the Colombian insular Caribbean.

MATERIALS AND METHODS

SAMPLING SITES

San Andres Island has been part of the San Andres, Providencia, and Santa Catalina archipelago, Seaflower Biosphere Reserve, as declared by the Man and Biosphere Program of UNESCO (Howard, 2006). It is an insular area of 25 km^2 with more than 70,000 inhabitants, whose main activity is based on tourism. It is located northwest of the Colombian Caribbean coast (12°28'N, 81°40'W; Fig. 1).

San Andres Island is characterized by being subjected to the action of the northeast and east-northeast trade winds, which in a cyclical periods every year determine two periods of precipitation or rain (R), during April to June and September to November, with a precipitation ranging 100- 285 mm, interspersed between a dry period (D) during December to March, with precipitation register less than 13 mm and a transition period (T) between July and August with daily precipitation ranged 0-100 mm (Bernal et al., 2006; IDEAM, 2011; Ruíz-Ochoa and Bernal-Franco, 2009). In this study the sampling was conducted during August 2009 representing transition season, November 2009 representing rain season and February 2010 representing dry season.

Table 1. Metal concentration (µg/g dw) in the soft tissue of juveniles and adults of bivalves.

Site	Specie	Stage/ Size (mm)	Cd	Cu	Zn	References
Ghana*	*Crassotrea tulipa*	Small (36) (ds)	0.99 (0.8 – 1.20)	70 (45 -98)	360 (280 - 740)	Otchere, 2022
		Large (55)	0.88 (0.4 – 1.80)	21.5 (6.6 - 90)	1310 (260 - 3030)	
		Small (36) (ws) §	0.27 (0.04 – 0.30)	27.1 (9.0 - 66)	2230 (483 - 3000)	
		Large (55)	0.14 (0.04 – 0.20)	34 (12.3 - 79)	510 (390 - 3540)	
Iran	*Sacostrea cuccullata*	Class I (<30)	2.340 ± 0.455	6.227 ± 1.946	8.976 ± 2.851	Shakouri and Gheytasi, 2018
		Class II (30-50)	2.220 ± 0.455	6.442 ± 2.090	10.335 ± 0.294	
Corsica (Mediterranean)	*Mytilus galloprovincialis*	Class I (43-54)	0.390 ± 0.200	5.55 ± 1.40	79.7 ± 37.8	Richir and Gobert, 2014
		Class III (65-74)	0.357 ± 0.089	4.98 ± 1.55	75.7 ± 43.8	
San Andres Island (Colombia)	*Isognomon alatus*	Juvenil (16)	1.158 ± 0.601	3,988 ± 2.332	2166.050 ± 1318	This study
		Adult (33)	0.833 ± 0.416	4.249 ± 2.695	4312.645 ± 2406.373	

* In Ghana registered small and large individuals were during dry season and wet season indicating maximum and minimal valor of metal concentration. § The rainy period is referred to as the wet season. ds: dry Season, ws: et season

Sampling sites were defined in two of the island's major bays: Hooker and Honda (Fig. 1), which have the largest extensions of red mangrove (*R. mangle*). Sampling sites were defined as E1: inside of Hooker Bay with a high population density), E2: mouth of Hooker bay, in front of the harbor, and E3: inside Honda Bay. During sampling in the rainy and dry season, physicochemical variables such as temperature and salinity were recorded using portable manual equipment (WTW LF330 / SET. ±0.2 °C). These variables showed that the temperature was higher in E2 and E3 during the rainy season in contrast to that observed for E1. Furthermore, E1 showed an increment in salinity during dry season (Table S1).

SESTON AND OYSTER SAMPLES

A total of 27 water samples (three per season, 2 L each) were collected by hand from prop roots of mangrove during each climatic season. Two of the three water samples obtained per station (4 L) were combined for the analysis of metal associated with seston. Samples were homogenized and filtered with a vacuum pump through glass fiber filters (Whatman, GF/C, 47 mm diameter), pre-cleaned with ultrapure 0.7 % HNO_3, and stored in plastic containers (Beltrame et al., 2009). Subsequently, filters were dried at 60 °C in order to reach a constant weight. The third sample (2 L) was used for organic matter (OM) and inorganic matter (IM) determination, filters were weighted and incinerated in a muffle furnace at 500 °C for 12 h, and IM content was calculated by weight difference from initial dry value (total seston) and ash (OM). The same incineration procedure was performed for filters without particles used as procedural blanks.

A total of 374 individuals of *I. alatus* were also collected manually, according to availability by sites sampled, 101 juveniles and 45 adults in E1, 105 juveniles and 50 adults in E2 and 73 adults in E3. 69 individuals in August of 2009 during the transition season, 54 individuals in November of 2009 in the rainy season and 55 individuals in February of 2010 during the dry season. These were stored in plastic bags and cooled at - 4 °C for transportation (Darrigran et al., 2007). The juvenile stage was defined according to Nawawi (1993), as individual immature <20 mm with thin and whitish gonads under macroscopic analysis, covering only a portion of the flesh, mantle relatively thick, while the adult stage was characterized as individual mature with >30 mm. Adult and juvenile stages were separated according to size (juveniles 16.0 ± 0.2 mm length; adults 34.3 ± 0.63 mm length). In each site and climatic season, adults and juveniles were manually extracted and an absence of juvenile individuals in E3 was observed in all three climatic periods.

All individuals were washed with MilliQ water to remove sediment and particles that could interfere with metal analysis. Groups of organisms of 3-8 adult individuals and 15-20 juveniles per group were randomly separated and the entire soft tissue was extracted from the bivalves. Each group was a pool of individuals, which was weighed and stored in plastic vials. This pool was dried in an oven at 60 °C until constant weight and macerated for metal analysis (considering 0.5 g soft tissue as a minimum dry weight). Pools were obtained by triplicate for each station and climatic season. Valve width was measured for each individual with a 0.05 mm precision, from the inner margin of the soft tissue as well as total weight using an analytical balance (Sartorius BP221S 0.1 mg).

METAL QUANTIFICATION

The particles organic and inorganic known as seston are retained on the membranes, in order to determine

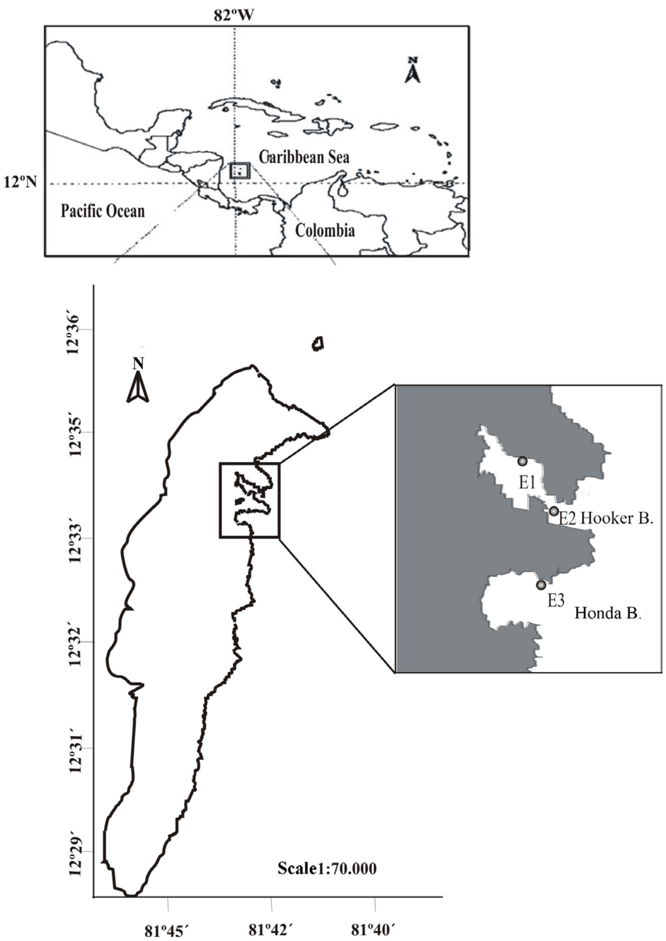

Figure 1. Sampling sites in San Andres Island, Colombia. Based in Romero-Murillo and Polanía (2008).

metals a soft extraction was performed with 25 mL of HCl 0.1N for 24 h under stirring. To determine metals in the soft tissue of oysters, an extraction was performed with 0.5 g of dry macerated mixture in a teflon recipient with a 2:1 mixture of concentrated HNO_3: HCl of analytical grade for 24 h at room temperature. Subsequently, the mixtures were heated at 80 °C for 12 h with adding 5 mL of the acid mixture and filtered to remove impurities. The final extract was diluted to 10 ml with HCl 0.1 N (Garay-Tinoco et al., 2003).

A metal content analysis was performed using the methodologies proposed by Garay-Tinoco et al. (2003) and using Atomic Absorption Spectrometry Shimadzu AA-6300 at Instituto de Investigaciones Marinas y Costeras, INVEMAR (Santa Marta, Colombia). Content analyses were subjected to quality control consisting in a reference material sample TORT-1 (lobster hepatopancreas) from the National Research Council of Canada (NRCC), to determine the detection limit and recovery percentage. The analytical values obtained were within the expected range and certificate (Table S2). The final concentration was expressed in μg/g dry weight (dw).

BIOCONCENTRATION FACTOR ANALYSIS

Using the metal concentrations measured in organisms and seston, the bioconcentration factor (BCF) defined as the ratio of the metal concentration of the bivalve tissues compared to the metal concentration associated to seston, was calculated using the following formula BCF = concentration of the metal in the organism/concentration of metal in the seston (Jonathan et al., 2017). This considers the seston as the main source of metals intake for the organism (Wang, 2013).

STATISTICAL ANALYSIS

Statistical analyses were performed using Statistica 7.1 (StatSoft, 2005). Rank transformation was applied to meet assumptions of normality (Shapiro test p <0.05) as well as homogeneity of variances (Cochran test p <0.05). For metal concentration in soft tissue and bioconcentration values (three replicates per station and climate season), a two-way ANOVA was used to determine statistically significant differences among the concentrations of Cd, Cu and Zn in the soft tissue of *I. alatus* in the study sites: E1, E2 and E3 and in the different climatic seasons: transition, rainy and dry. A factorial ANOVA was also performed to compare the concentrations of Cd, Cu, and Zn in soft tissue and BCF with respect to age (adults and juveniles), sites (E1 and E2) and climatic seasons (rainy and dry) considering transformed data as mentioned above. Subsequently, statistically significant differences were verified using the Post hoc Newman-Keuls test (p <0.05). Factorial ANOVA results were presented using a Sigma-restricted parameterization as a coding strategy for age and by least square means bi-dimensional graphs.

RESULTS

METAL CONCENTRATIONS IN SESTON

Concentrations of Cd, Cu, and Zn were determined in seston. A high concentration of Zn (0.522- 2.235 μg/g dw) compared to Cd (<LOD - 0.0780 μg/g dw) and Cu (<LOD - 0.0690 μg/g dw) was observed during the three climatic seasons and sampling sites (Fig. 2). Increases in the three metals from transition to rainy season and a decrease during the dry season, except for E1, were observed. Contrastingly, the OM/IM pattern showed an increase of OM during the rainy season, which remained until the dry season, and an IM with few variations (Fig. 2). Station E1 showed the greatest differences among the sampling sites.

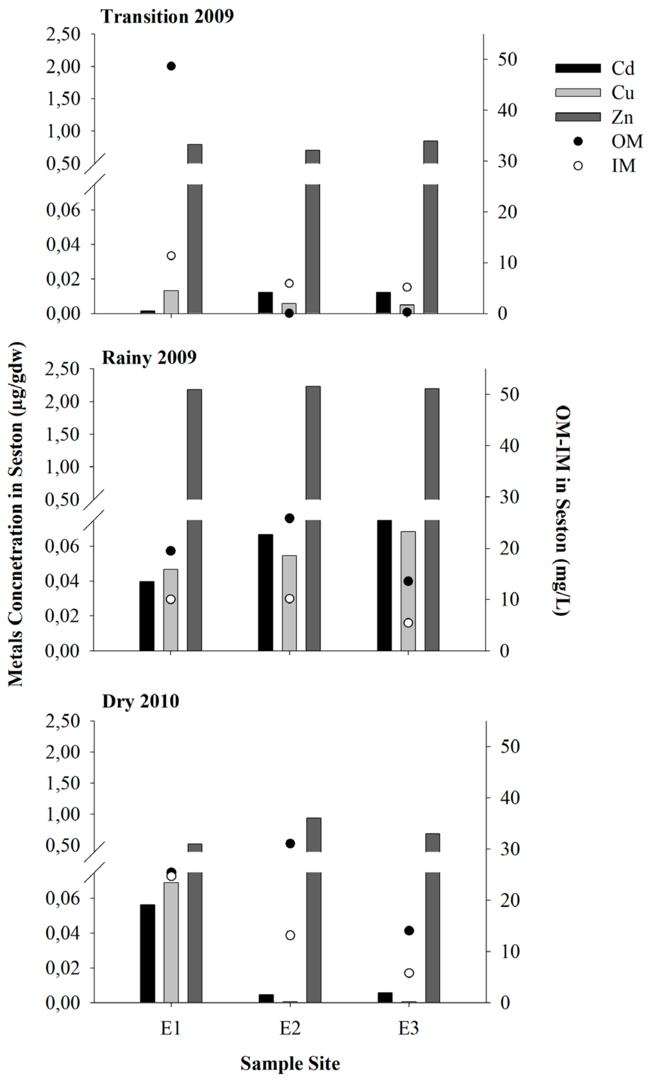

Figure 2. Total metals concentration in seston (bars: Cd, Cu y Zn) (µg/g dw), organic (OM) and inorganic matter (IM) content (mg/L) (points) by sampling season and station. August 2009 representing Transition season, November 2009 representing Rain season and February 2010 representing Dry season.

METAL CONCENTRATIONS IN SOFT TISSUE IN CLIMATIC SEASON

Average metal bioaccumulation for adults was 1.20±0.79 µg/g dw for Cd, 3.07±2.31 µg/g dw for Cu, and 3340.84±1743.90 µg/g dw for Zn. Figure 3a shows statistically significant differences for sites and seasons, in metal concentrations (Cd, Cu, Zn) in the oyster tissue analyzed with the two-way ANOVA ($p < 0.05$), and figure 3b respective BCF. For Cd concentration, the significant differences presented were given by the high concentrations in the transition season and the low concentrations in the dry season in E1. For Cu concentration, the significant differences were given by the high concentrations in the dry season in all the localities. And for Zn, significant differences were evidenced between the transition and dry season in E2, and the rainy and dry season in E3.

In contrast, average BCF was 266.5±549.5 for Cd, 2389.4±4612.9 for Cu, and 3193.1±2468.9 for Zn. Cadmium BCF showed significant differences among sites and seasons. The highest Cd BCF was found in E1 during the transition season followed by dry season in E2 and E3. Copper BCF was the highest of the three metals which together with Zn BCF showed significant differences mainly due to high concentrations in the dry season (Fig. 3b).

METAL CONCENTRATIONS RELATIONSHIP TO AGE/ SIZE

The Table 1 shows the average metal concentrations in soft tissue for juveniles and adults. The factorial ANOVA was used to identify the influence of the age on metal accumulation and BCF in E1 and E2, sampling sites where enough adults and juveniles were collected during rainy and dry seasons (Fig. 4, Table S3). This ANOVA test showed statistically significant differences since the Cd concentration rank in juveniles from E2 was significantly different to the one found for adults during the dry season. The Cd BCF rank showed significant differences for E2 juveniles during the dry season. As for the Cu concentration rank, the BCF did not show significant differences by age. For the Zn concentration rank, the metal concentration was significantly different for juveniles and adults during the rainy season.

DISCUSSION

The records of precipitation coincide with the increase in suspended solid materials during the rainy season, measured in the seston samples (OM-IM), especially in the amount of OM in E2 and E3 sampling sites which maintained their value until the dry season. The pattern of OM-IM in E1 showed a decrease during the transition to the rainy season and a subsequent increase to the dry season, mainly due to runoff and sediment removal. In SAI, a high surface runoff has been continuously reported, mainly during the rainy season (Vargas-Cuervo, 2004), causing *R. mangle* to flood constantly (Urrego et al., 2009).

Furthermore, these results have also shown a seasonal variability of metal concentration in the seston because of increased and decreased rainfall, with a Zn>Cd>Cu pattern in open sampling sites (E2 and E3) and Zn>Cu>Cd in a closed site (E1). A decrease in the metal concentration was identified, by two orders of magnitude from rainy to dry season mainly in E2 and E3, which was inverse to E1. The differences in the metal concentrations observed between open and closed sites might be related to the release of metal ions from seston caused by changes in salinity and/ or the effect of rainfall. It has been also demonstrated that an increase in salinity reduces the availability of metals due

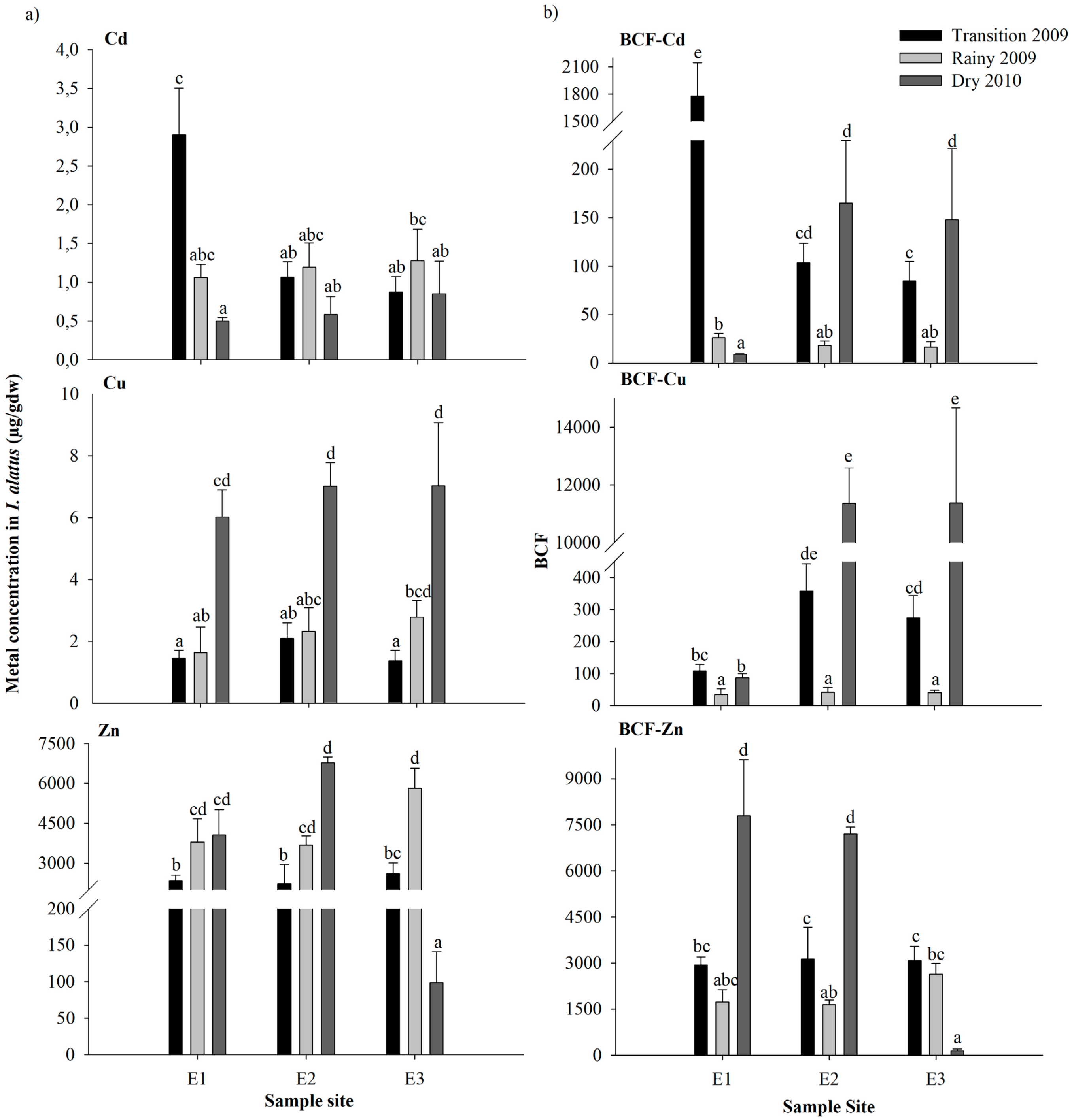

Figure 3. a) Metals average concentration in *I. alatus* µg/g dw ± SD by season and sampling site in adults. b) Bioconcentration Factor (BCF) average ± SD for *I. alatus* of San Andres Island by season. August 2009 representing Transition season, November 2009 representing Rain season and February 2010 representing Dry season. Statistically significant differences among the groups (a-d) by the two way ANOVA among seasons and sites of data with rank transformation in all cases with $p < 0.05$, confirmed by post-hoc Newman-Keuls test ($p < 0.05$).

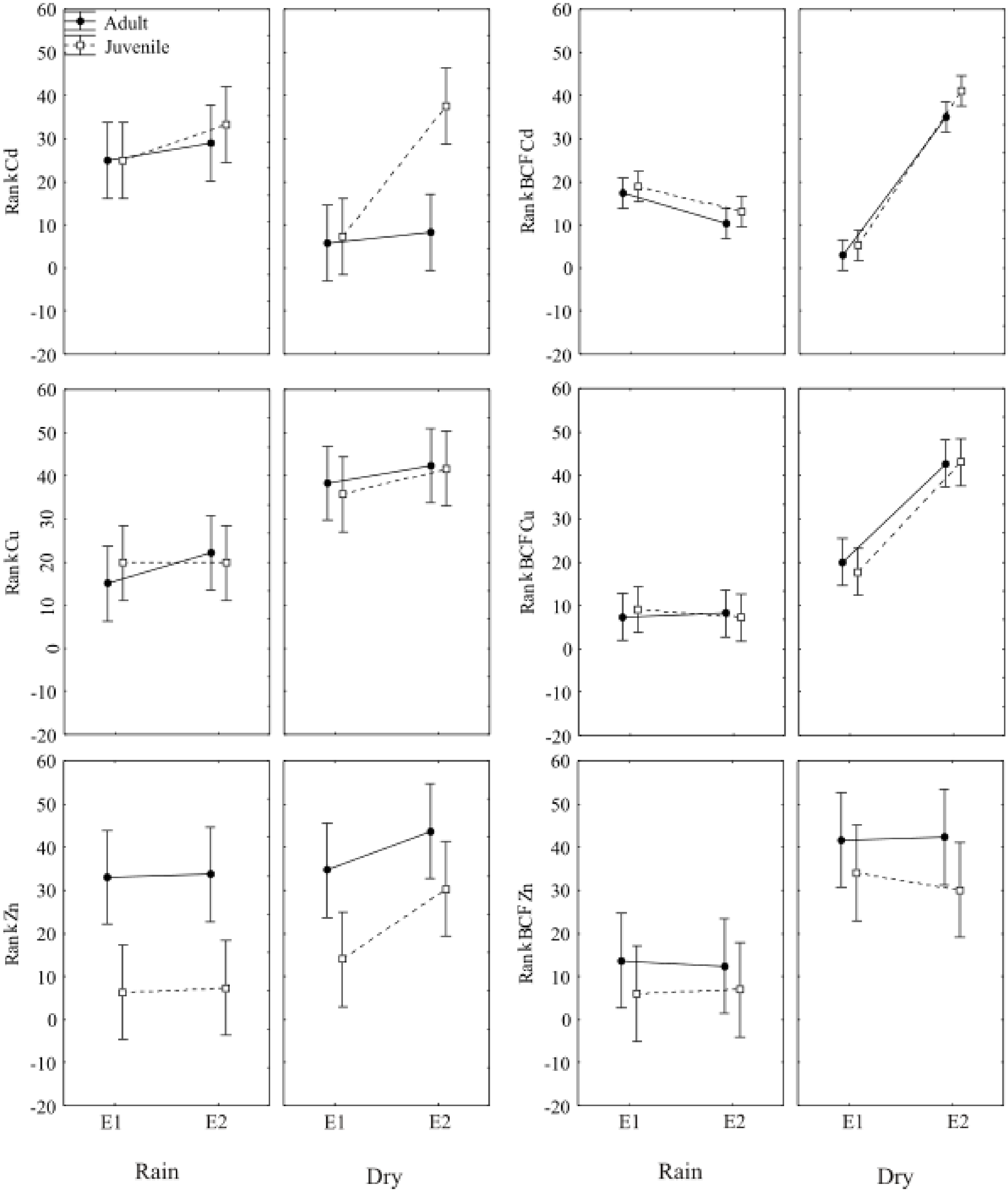

Figure 4. Least square means bi-dimensional graph of covariates (Cd, Cu, Zn, BCF range and their means. Vertical bars represent ±SE and denote 0.95 confidence intervals. Sigma-restricted parametrization as coding strategy with categorical predictor (adult: ●; juvenile: ●), comparing the concentration of Cd, Cu and Zn in soft tissue and BCF with respect to age (adults and juveniles), sites (E1 and E2) and climatic seasons (rainy and dry), considering transformed data.

to the formation of highly stable metal-chloro complexes which precipitate (Yao et al. 2016). According to Barletta et al. (2019) and Yao et al. (2016), these physical and chemical parameters may induce changes in the metals' environmental behavior added to the contribution of rainfall and hydrodynamic events (currents, winds) at local level, releasing metal ions from seston.

However, in contrast to the wide salinity range found by Barletta et al. (2019), in present study the salinity fluctuations showed a narrow range, indicating that salinity is not the main cause of the differences observed between open and closed sites for the metal concentrations. According to Yao et al. (2016) other factors such as sediment-particle transport and the input of contaminated particulate material would contribute to metal concentration. Therefore, a prolonged effect of runoff and sediment removal has been identified, which is related to the increment of Cd and Cu concentrations in seston during the dry season in E1.

For the dry season, the sampling site E1 was strongly influenced by the action of winds, which determined the resuspension of organic and inorganic material and the increase of metal presence in the seston. Research on suspended sediment has found that sediment with low oxygen concentrations and substantial amounts of iron, carbon, and organic sulfides, can bind to metals such as Cd in the form of CdS, which is more bioavailable to the organisms (Schmitz et al., 2015). However, few records exist for Hooker Bay's sediments and those that do exist connect these sediments to deposit activities of sewage water, runoff, and waste from the former power plant (Romero-Murillo and Polanía, 2008), which could most likely include iron or sulfides associated with Cd ions.

Regarding the metal concentration in seston, the amount of OM, the bioaccumulation of metal in the oysters and subsequent BCF, may indicate the importance of OM in the absorption of metals in these bivalves. According to Wu et al., (2012), metal accumulation and bioconcentration are reflected after a period of exposure to the seston, mainly of organic origin. This indicates that the absorption of metals like Cd is not immediate due to several factors such as the bond with the substrate, the digestive processes, the solubility of the substrate in the gastrointestinal system, and the retention time in the gut. In this study, this increase in the accumulation of metals during the dry season occurred due to a late effect of rainfall increasing the organic matter. This confirms that the particulate pathway plays an important role in the accumulation of metals and further absorption of particulate organic material, through gills specially with food intake, as determined by Hédouin et al. (2010). In addition, it shows differences in results among monitoring water vs organisms for effect determination on organisms.

On the other hand, the metal concentrations in oyster´s tissue showed a Zn>Cu>Cd pattern. The minimum and

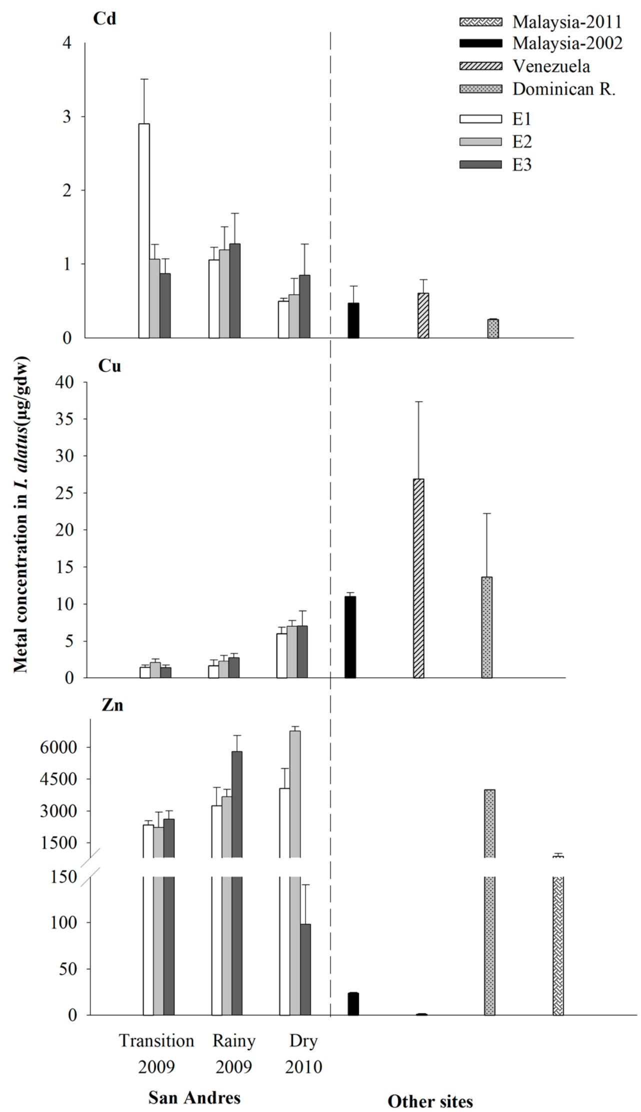

Figure 5. Comparative metal concentration (Cd, Cu y Zn) (µg/g dw) in *I. alatus* (average ± SD) between this study and Malaysia (Yap et al., 2011; Saed et al., 2002), Venezuela (Jaffé et al., 1998) and Dominican Republic (Sbriz et al., 1998). August 2009 representing Transition season, November 2009 representing Rain season and February 2010 representing Dry season.

maximum levels of Cd (0.0006-2.91 µg/g dw) and Cu (0.015-7.02 µg/g dw) found in the oysters were similar to ranges previously reported for *I. alatus* in other sites of Colombia: Cd (0.86-2.33 µg/g dw) and Cu (0.42-1.34 µg/g dw) during rainy seasons throughout the 80s (Campos, 1988).

This study compared with research in *Crassostrea tulipa*, oyster of coastline in Ghana is comparable by changes between seasons, especially in Zn. In both Ghana and SAI (Colombia), Zn concentration increased in rainy (wet season). This is most likely related with oyster affinity with Zn as showed by Liu et al. (2013) (Table 1).

A comparison of the obtained results in this study with those previously reported for *I. alatus* in countries such as Malaysia in impacted area in Rio Sepang Kecil and Besar (Yap et al., 2011; Saed et al., 2002), and areas impacted by metals in Venezuela (Jaffé et al., 1998) and the Dominican Republic (Sbriz et al., 1998) (Fig. 5), indicated that SAI have been highly impacted by metal concentrations, in particular by Zn.

The accumulation of essential metal Zn in *I. alatus* tissue is determined mainly by its role in physiological processes. A higher level of Zn is found in oysters compared to other bivalves, due to Zn being a vital part in the functioning of the metalloenzymes. (Liu et al., 2013; Pan and Wang, 2009). Beyond its function, it is likely that the efficiency of assimilation (absorption minus disposal) (Wang and Fisher, 1999) and the metal capture mechanisms in bivalves (metallothionein-like proteins, metal-rich granules, metal in cell debris, etc.) contribute to an increased accumulation of Zn in the tissues of *I. alatus* (Shi and Wang, 2004; Wang, 2011). This is also why a higher level of Zn is found in the gills as it has permanent contact with seston through it´s filter function.

Therefore, in SAI the poor waste management of human activities, such as urban sprawl (with the presence of pig farms), and others such as port, commercial, and tourist activities, continuously increase the sewer discharge resulting in leachate being dumped directly into the sea (Romero-Murillo and Polanía, 2008; INVEMAR, 2019). According to Barletta et al. (2019), the wastewater and the residues of antifouling paint from boats (which contains Cu and Zn), could increase metal concentrations in organisms.

Alternatively, results from the present study also confirm the presence of Cd previously reported by REDCAM (Vivas-Aguas and Marín, 2004) in seawater analysis and further demonstrates its bioavailability.

At the same time, BCF results showed that metals ranged Cu >Zn> Cd with an inverse relationship between metal concentrations in seston and organisms, as has been indicated by Jonathan et al. (2017). According to Kwok et al. (2014), BCF higher than 100 percent indicates bioaccumulation of the pollutant, as recorded in this study. This means that there is a high accumulation of the three metals with the highest BCF for Cu and Zn, then this BCF is associated with the bioavailability of these metals especially of Zn. Besides, according to Jonathan et al. (2017), high BCF values are also attributed to an efficient uptake of free metal ions through contact with an aqueous environment, in the case *I. alatus* in the gills tissue mainly.

According to Paul-Pont et al. (2010), organisms living in contaminated environments may modify their physiology and biochemistry, developing better detoxification mechanisms by quickly transferring metal from the gills to the visceral mass, thus increasing their level of tolerance. Present study suggests that *I. alatus* is an organism that would be able to live under variable environmental conditions in polluted environments with high suspended solids due to its accumulation capacity, especially of metals. Therefore, this species can be considered as a good biomonitor for spatio-temporal monitoring of the presence of metals (Cd, Cu and Zn), as well as an indicator of metal bioavailability.

INVEMAR with its Network of Vigilance for the Protection and Conservation of Sea and Coastal Water Quality Monitoring Program (REDCAM) has been constantly monitoring metals such as Cd, Pb, Cr, and more recently, Zn and Cu on Colombian coasts since 2001 without considered season (Bayona-Arenas and Garcés-Ordóñez, 2018). The monitoring results for SAI showed a range of between <0.42 and 5 μg/L for Cd, between <0.9 and 19.6 μg/L for Cu, and between 6.9 and 44.6 μg/L for Zn in water (Bayona-Arenas and Garcés-Ordóñez, 2018; Vivas-Aguas et al., 2011, 2014). Though these records do not show the presence of these metals in organisms, results from this study showed bioavailability of Cd, Cu, and Zn in *I. alatus*, mainly of the latter with high concentrations (3096.6±1945.22 μg/g dw), especially for E2 in dry season and E3 in rainy season.

Finally, the evaluation of differential effects caused by exposure to polluting agents during the different stages of ontogenetic development is scarcely discussed and rarely reported. In this study to fill this gap, a comparison was conducted between adults and juveniles. The results established differences observed between both stages in the sampling sites for Cd and Zn concentrations (Fig. 4). The juveniles showed higher Cd bioaccumulation than adults in E2 in the dry season, while adults showed higher Zn bioaccumulation than juveniles during the rainy season among E1 and E2, suggesting that juveniles have high sensitivity to Cd, a metal with high affinity for thiol groups found in molecules such as proteins (Martinez-Finley et al., 2012). This pattern agrees with observed by Otchere (2022) and Shakouri and Gheytasi (2018) in oysters (*Crassostrea tulipa*, *Anadara senilis Saccostrea cucullata*) in contrast to registered by Richir and Gobert, (2014) in mussel *M. galloprovincialis* without differences significally among metal (Table 1).

According to Duquesne et al. (2004) and Spann et al. (2011), it is likely that growing organisms require higher energy supplies under stress and employ additional energy sources such as free amino acids (with thiol groups) due to its low reserve of glycogen. Thus, small individuals lose more glycogen and experience a redistribution of energy reserves under increased stress by Cd this metal.

CONCLUSIONS

This study confirms the potential use of oyster *I. alatus* as a biomonitor species of metal levels in coastal marine environments in areas of the Colombian Caribbean, due to its capacity to bioaccumulation and bioconcentration, besides allowing the recording of spatio-temporal differences. The observed accumulation of Cd, Cu, and Zn in *I. alatus* was affected by climatic seasons, the age of the individuals and the sampling sites. Rain delayed the accumulation, mainly on the outside of Hooker Bay and Honda Bay, due to the drag of particulate material from the inside of Hooker Bay, which proved the intake of high Zn concentrations at this time and its impact on organisms. Finally, higher Cd bioaccumulation in juveniles compared to adults were observed in the sampling sites, indicating continuous Cd bioavailability.

Based on the analysis of these results and as a first approximation to the situation in San Andres, we recommend the development of a continuous biomonitoring program using oysters *I. alatus*, in order to evidence spatio-temporal variation over longer periods of time. Future research should also incorporate the evaluation of metals in all the environmental matrices (sediment, water, and other groups of organisms) and other elements of concern (As, Hg, Pb) as product of industry activity (including port activity) as well as the measurement of specific exposure biomarkers such as metallothioneins to determine the effects of metals in *I. alatus* in this environment.

AUTHOR'S PARTICIPATION

The authors contributed respectively in: Field work, P. Romero-Murillo, N.H. Campos Campos, statistical analysis; R. Orrego and P. Romero-Murillo; all authors writing—original draft preparation; All authors have read and agreed the published version of the manuscript.

ACKNOWLEDGEMENTS

The authors thank to staff in San Andres and Santa Marta of Universidad Nacional de Colombia, Caribbean Campus for the collaboration in all process and The Instituto de Estudios en Ciencias del Mar (CECIMAR) for the support in database and laboratories. The Instituto de Investigaciones Marinas y Costeras (INVEMAR), Programa de Calidad Ambiental Marina (CAM) for the support in database and laboratories.

FINANCIAL SUPPORT

This research is a part of the project "Determinación de la capacidad de acumulación de metales pesados (Cd, Cu, Zn) en *Isognomon alatus* de la isla de San Andres" led by Néstor Hernando Campos. This research was funded by Universidad Nacional de Colombia (UNAL), Caribbean campus, project Code 10491 awarded to Néstor Hernando Campos. The results presented here are part of the MS Thesis of Patricia Romero Murillo at Facultad de Ciencias, UNAL.

ETHICAL STATEMENTS

In this study, for the care and use of animals the specimens were collected according to protocol registered in Darrigran et al., 2007, which indicated to use refrigeration and isolation from sunlight for relaxation and subsequently to processing the sample.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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