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HEAVY METALS IN SEDIMENTS OF TWO NEOTROPICAL ESTUARIES AND USE OF BRAIN ACETYLCHOLINESTERASE FROM THREE FISH SPECIES AS A BIOMARKER

Mikele Cândida Sousa de Sant'Anna[1,2]; Elida Virna Rodrigues Barbosa[2]; Maria Priscila Sá Matos Ribeiro[2]; Ranilson de Souza Bezerra[3]; Danilo Francisco Corrêa Lopes[2,4]*

Abstract

This study aimed to assess the levels of heavy metals (As^{3+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+}) in the sediments of two neotropical estuarine complexes (Santa Cruz Channel and Sirinhaém River) and to investigate the effect of these concentrations on the activity of Acetylcholinesterase (AChE) in the brains of *Centropomus undecimalis*, *Diapterus auratus* and *Diapterus rhombeus*, during the dry and rainy seasons. For each site and seasonal period, sediment samples were collected, and five individuals of each species were acquired from artisanal fishermen. Cu and Zn were determined by neutron activation, while Hg was analyzed by atomic absorption spectrometry with cold vapor generation; Cd and Pb were quantified by atomic absorption spectrometry with electrothermal atomization. Brain AChE activity levels were measured in the three species. The enzyme's activity was lowest in the dry season, coinciding with the highest concentrations of Hg, which exceeded the limits established by current legislation. These results indicate that the mercury in the sediments has been affecting the species' neurophysiology, constituting a relevant environmental impact with potential implications for human health due to bioaccumulation and consumption of these fish. The variation in AChE activity proved sensitive to seasonal Hg concentrations, highlighting its potential as a biomarker for environmental monitoring of this metal.

Keywords: Acetylcholinesterase. Biomarkers. Heavy metals. Environmental impact.

[1]Federal University of Maranhão (UFMA), Coordination of the Post-Graduate Programme in Aerospace Engineering (PPGAERO), Cidade Universitária Dom Delgado, Avenida dos Portugueses, 1966, Vila Bacanga, 65080-805 São Luís, Maranhão, Brasil

[2]Federal University of Maranhão (UFMA), Research Center for Bioeconomy, Environment, Innovation, Intelligence, Technology, Education and Health (BAITES), 65085-580 São Luís, Maranhão, Brasil

[3]Federal University of Pernambuco (UFPE), Center for Biological Sciences (CB), Department of Biochemistry and Biophysics (DBR), Avenida Reitor Joaquim Amazonas, Cidade Universitária, 50740-570 Recife, Pernambuco, Brasil

[4]Federal University of Maranhão (UFMA), Coordination of the Post-Graduate Programme in Transportation Engineering, Avenida dos Portugueses, 1966, Vila Bacanga, 65080-805 São Luís, Maranhão, Brasil

*Corresponding author: maria.priscila@academico.ufs.br

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1 Introduction

Coastal regions suffer from intense human occupation, causing strong pressure on marine ecosystems (DO CARMO; ABESSA; MACHADO NETO, 2011). However, the scenario was aggravated by the industrial revolution and anthropological activities, which boosted industrialization and urbanization, a strong influence on the contamination of the environment by heavy metals, with their mobilization rates in the environment increasingly accelerated since 1940 (ALI; KHAN; ILAHI, 2019). This pollutant load includes a variety of xenobiotic agents due to their chronic toxicity, non-biodegradability, and environmental bioaccumulation. Heavy metals (MPs) are incredibly harmful environmental pollutants affecting waters, soils, and marine life (SHARMA et al., 2022).

At high concentrations in the aquatic environment, these metals exhibit a strong accumulative potential in biota (PASQUAL et al., 2024). Among these organisms, fish stand out as the focus of several studies on metal contamination, as they absorb both essential and non-essential metals from water and food, retaining them in their muscle tissues (KHASHROUM, 2024).

Due to these factors, identifying and quantifying these metals is essential. In Brazil, CONAMA - National Environment Council Resolution No. 454/2012 regulates heavy metal levels in sediments, establishing guidelines. This resolution defines two classification levels: Level 1, below which adverse effects on biota are unlikely, and Level 2, above which such effects are probable. For arsenic (As), for example, the limits for freshwater sediments are 5.9 mg/kg (Level 1) and 17 mg/kg (Level 2). In contrast, the values for saline/brackish water sediments are 8.2 mg/kg and 70 mg/kg, respectively (YIN et al., 2024).

In the aquatic environment, sediments are the compartment considered most significant in the concentration of metals. Sediments play a fundamental role in bioavailability, as they can internally and/or superficially retain several chemical elements (MAO et al., 2024). The direct or indirect introduction of toxic substances, including heavy metals, can lead to increased concentration in the aquatic environment, enabling their assimilation by fish and living organisms present in the environment, including humans (SOLIMAN; YOUNIS; ELKADY, 2024).

Fish have been widely used as biomonitoring organisms of environmental quality, becoming a valuable tool in diagnosing and monitoring the environment and the health of organisms that inhabit polluted ecosystems (BANCEL et al., 2024).

Since fish can be collected directly from impacted areas (*in vivo*) or their tissues or biomolecules can be exposed to different concentrations of chemical compounds in the laboratory (*in vitro*), several studies have explored these approaches (LOPES et al., 2019; ALBUQUERQUE et al., 2021; DOS SANTOS et al., 2024).

Bioindicators and biomarkers are biological explanations for pollutants and have become a valuable and practical instrument in monitoring environmental quality, as they provide information not only about the intensity, tolerance limits, and adverse effects of pollutants on organisms, but also about how they occur, the transfer and accumulation of these substances throughout the food chain (ZHANG, 2021a). In this context, biomarkers are also efficient in monitoring at a biochemical level. They can identify the presence of contaminants in the environment, including heavy metals, even before these elements reach higher organizational levels (AHMAD; IMRAN; AHSAN, 2023). Another alternative is the use of enzymes present in discarded tissues of fish species that can be used as a specific biomarker for monitoring aquatic environments impacted by heavy metals (NUNES et al., 2020) since these pollutants bind to peripheral sites and trigger conformational changes or can interfere with the hydration state of the active center, altering the rate of hydrolysis of the substrate by acetylcholinesterase (AChE) Brain, which can cause severe neurological problems and even death.

AChE is most commonly found in nervous tissues and the membrane of erythrocytes (ASSIS et al., 2018). The main functions of AChE are the breakdown of the neurotransmitter acetylcholine in cholinergic synapses, preventing postsynaptic overestimation, and participation in the development of neuronal tissues (ASSIS et al., 2018). The activity of this enzyme is inhibited by pesticides, such as organophosphates and carbamates, as well as heavy metals (LOPES et al., 2019). In this way, AChE has been proposed as a biomarker for the presence of these compounds in several studies due to its sensitivity and specificity, mainly in aquatic organisms (LOPES et al., 2019). The inhibitory effects of these agents on enzymes and other associated functions of marine organisms may be essential to understanding the concentration and bioavailability of such agents in ecosystems (DE SOUZA et al., 2018). The inhibitory effects of these agents on enzymes and other associated functions of marine organisms may be essential to understanding the concentration and bioavailability of such agents in ecosystems (AMIN et al., 2023).

Nonetheless, to understand the potential risk of metals to fish fauna and their consumers in a region, it is necessary to determine the concentration of metals in fish and the environment in which they live.

The State of Pernambuco (Northeast region of Brazil) has 17 estuarine areas (MÉRIGOT et al., 2017; SILVA-JÚNIOR et al., 2016) with a high degree of degradation, especially those close to the metropolitan region of its state capital city (Recife), caused by domestic sewage and industrial waste.

The Santa Cruz Channel Estuarine Complex (CECSC) has historically suffered several anthropogenic impacts, such as the study involving a chlorine and caustic soda factory (which used mercury electrodes) released its industrial waste over 24 years (1963 to 1987) into the Botafogo River, one of the main rivers that make up this estuarine complex (LIMA et al., 2009; MEYER, 1996).

Furthermore, the lack of basic sanitation in urban areas located on the banks of the Santa Cruz Channel generates the daily release of all domestic effluents, most of which are untreated (SINGH,2024).

The Sirinhaém River Estuarine Complex (CERS) is an area impacted by agricultural pollution and the dumping of waste from sugar mills and alcohol distilleries in its basin (BRAGA, 1986). Furthermore, this complex significantly contributes to domestic and industrial waste and large shrimp farms (LIRA; FONSECA, 1980).

Heavy metals in sediments can disturb the ecological balance by affecting the growth and survival of aquatic organisms, reducing biodiversity. In addition, these alterations can occur in the biochemical and physiological changes resulting from the presence of heavy metals (KHUSHBU et al., 2022).

The species *Centropomus undecimalis* (Bloch 1792), *Diapterus auratus* Ranzani 1842, and *Diapterus rhombeu*s (Cuvier 1829) are widely distributed in the estuaries of Pernambuco, including the CECSC and CERS (MÉRIGOT et al., 2017; SILVA-JUNIOR et al., 2016). In these regions, they are an essential source of food and income and are commonly targeted by artisanal fishermen. The species *C. undecimalis* is classified as piscivorous (LIRA et al., 2017), while *D. auratus* and *D. rhombeus* are characterized as zoobentivorous and zooplanktivorous, respectively (TEMÓTEO, 2015). Due to the differences in their trophic levels, these species can present different degrees of contamination due to the bioaccumulation of pollutants along the food chain (NANINI-COSTA et al., 2016).

Considering the factors above, the species *C. undecimalis*, *D. auratu*s, and *D. rhombeus* were selected in this study to assess the levels of heavy metals (As^{3+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+} , and Zn^{2+}) in the sediments of the two main estuarine complexes in the region, as well as to investigate the influence of these metals on the activity of acetylcholinesterase (AChE) in the brains of these species.

2 Material and Methods

2.1. Study Area

The present study was conducted in 2012 in two seasonal periods, dry (October to March) and rainy (April to September), in two areas, one located on the North coast and the other on the South coast of the state of Pernambuco, Northeast region of Brazil. On the North coast, the study area was the estuarine complex of Santa Cruz Channel (CECSC), municipality of Itapissuma (Figure 1). This estuary receives water from an important water network composed of the Igarassu, Botafogo, Arataca, Carrapicho, and Catuama rivers (CPRH, 2003).

The community of Barra de Sirinhaém is located in the municipality of Sirinhaém and has a high density of mangroves. This area is located in the Sirinhaém River estuarine complex (CERS) on the South Coast of Pernambuco. The estuarine complex of the Sirinhaém River is supplied by several branches, such as the Arrumador, Trapiche, and Aquirá rivers, where several lagoons, numerous islands, and an extensive mangrove are found (LIRA et al., 2010). This area is of extreme biological importance and strongly influences the Guadalupe Environmental Protection Area (Figure 1).

2.2. Material Collection

2.2.1. Specimens

The specimens were acquired (already cooled in ice) from artisanal fishermen in the municipalities of Itapissuma and Sirinhaém soon after landing in their respective harbors (Figure 1). After identification, five individuals of each species (*C. undecimalis*, *D. auratus*, and *D. rhombeus*) were randomly separated and, still in the field, brain tissues were extracted. The tissues were stored in plastic pots, labeled, and in isothermal boxes containing dry ice. Then, they were taken to the laboratory and kept in the freezer (-20 °C) for further analysis.

2.2.2. Sediment collection

Surface sediments were collected in both estuaries during the two seasonal periods. The samples were collected in triplicate at points close to where the fishermen captured the selected species. The samples were identified and kept in plastic containers, stored in isothermal boxes containing ice, until they were kept in freezers (-20 °C) for analysis.

2.3. Materials (for AChE assay)

Acetylthiocholine iodide, bovine serum albumin (BSA), 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), Tris (hydroxymethyl) aminomethane, and dimethylsulfoxide were purchased from Sigma.

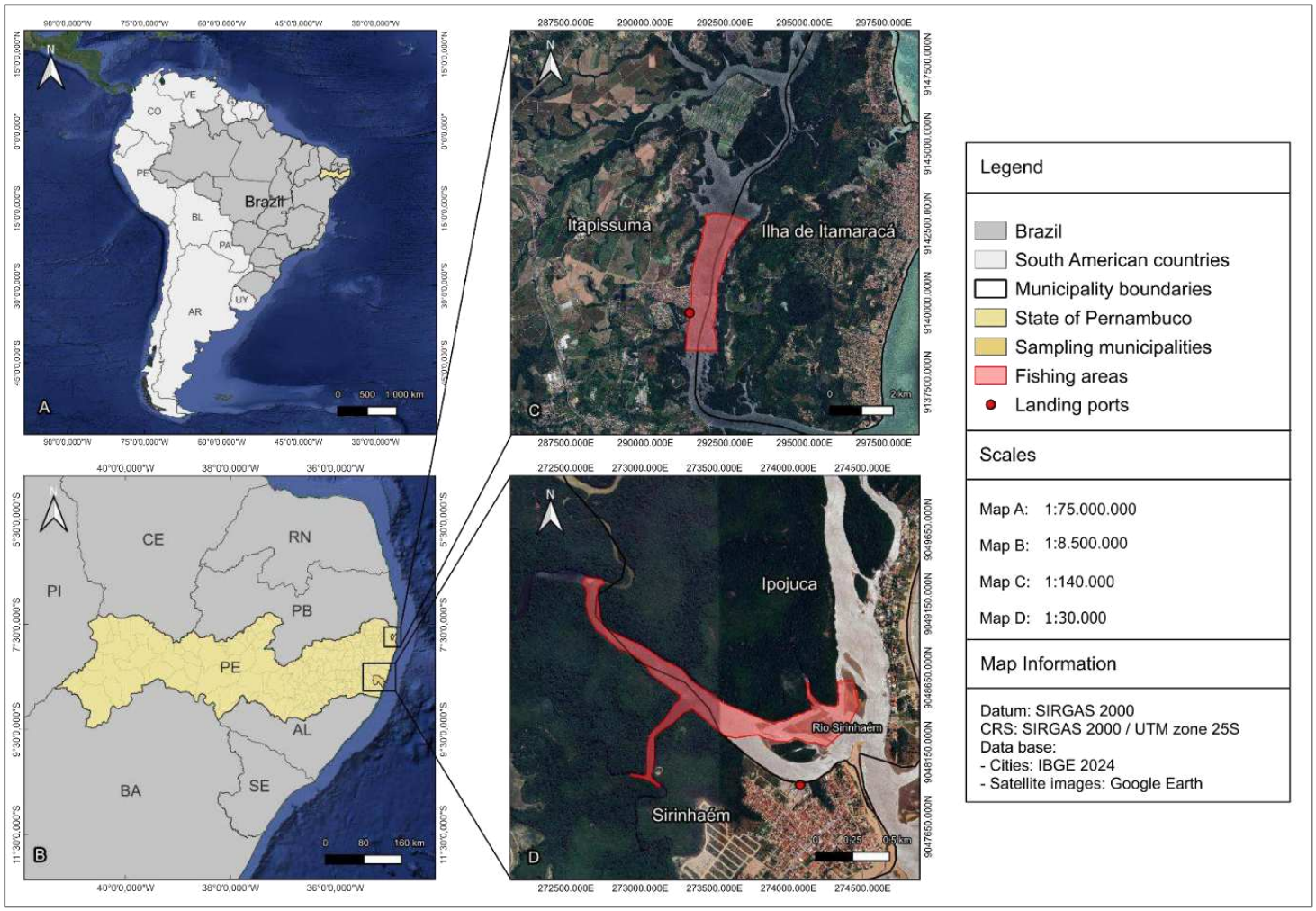

Figure 1. Location of fishing landing harbors where specimens were acquired. On the left, the Estuarine Complex of the Sirinhaém River (CERS) and on the right, the Estuarine Complex of the Santa Cruz Channel (CECSC).

Disodium hydrogen phosphate and HCl were obtained from Merck. Trisodium citrate was purchased from Vetec. Glycine was purchased from Amersham Biosciences.

The spectrophotometer used was the Bio-Rad Smartspec TM 3000.

2.4. AChE extraction

Brain tissues from specimens of each species were homogenized in 0.5 mol L^{-1} Tris-HCl buffer, pH 8.0, maintaining a ratio of 20 mg of tissue per ml of buffer, using a Potter-Elvehjem tissue disruptor (IKA RW-20 digital).

Homogenates were centrifuged for 10 min at 10,000 x g (4°C), and supernatants (crude extracts) were frozen at 20°C) (ASSIS et al., 2014).

2.4.1. Enzyme activity and protein determination

Two hundred µL of DTNB (0.25 mmol L^{-1}) dissolved in 0.5 mol L^{-1} Tris-HCl buffer, pH 7.4, was added to the crude extract (20 µL), and the reaction started after the addition of 62 mmol L^{-1} of acetylthiocholine iodide (ASSIS et al., 2010; ARAÚJO et al., 2016; ARAÚJO et al., 2018; DE SOUZA et al., 2018; DOS SANTOS et al., 2022). Activity was determined in quadruplicate by spectrophotometry (Bio-Rad xMark™) following the absorbance at 405 nm at times 0 and 180 s, where the reaction exhibited a first-order (linear) kinetics pattern. An activity unit (U) was defined as the enzyme capable of converting 1 µmol of substrate per minute per mL of solution. A blank test was similarly prepared. In this assay, the crude extract sample was replaced by 0.5 mol L^{-1} Tris-HCl buffer, pH 8.0. According to Sedmak and Grossberg (1977), protein content was determined using bovine serum albumin as a standard.

2.4.2. Analysis of heavy metals in sediments

The sediments were thawed, dried at 60 ºC in an oven, macerated, and sieved (60 mesh). Arsenic (As^{3+}), Copper (Cu^{2+}), and Zinc (Zn^{2+}) were determined using the neutron activation method. Mercury (Hg^{2+}) was determined by atomic absorption spectrometry with cold vapor generation. Cadmium (Cd^{2+}) and lead (Pb^{2+}) were determined by atomic absorption spectrometry with Electrothermal Atomization.

Neutron activation analysis is an analytical method that measures gamma radiation induced in a sample by neutron irradiation (BODE, 1996). It was carried out with the Regional Center for Nuclear Energy (CRCN-PE) and the Institute for Energy and Nuclear Research (IPEN/CNEN-SP).

Sediment samples (200 mg) were packed in cylindrical aluminum capsules, specifically for irradiation. The analytical quality control was carried out with reference materials certified by the International Atomic Energy Agency (IAEA), namely: Soil7, SL1 (lake sediment), and SDN1/2 (sediment).

The samples were sent for activation, where they were irradiated under a thermal neutron flux of 5×10^{12} $cm^{-2} s^{-1}$ in the IEA-R1 research nuclear reactor. After cooling for 72 hours, the samples were transported for the detection of radiation induced by high-resolution gamma spectrometry, using hyperpure germanium detectors, models GEM-10195 and GEM-45190P (ORTEC® - Oak Ridge, Tennessee, USA). The electronics associated with the detectors comprised the Bias Supply, model 659, amplifier 672, and data accumulator MCB-919, with an analog-digital converter connected to a microcomputer.

The MAESTRO II software (EG&G ORTEC®) performed spectral acquisition monitoring. The spectra were analyzed using the Quantu-MCA program (BACCHI; FERNANDES, 2003). The data obtained were electronically transported to the Quantu-INAA program (BACCHI; FERNANDES, 2003). The program allowed the determination of elemental concentrations using the K_0 method.

The Hg in the samples was determined by atomic absorption spectrometry with cold vapor generation CV AAS in the Perkin Elmer CV AAS FIMS Equipment, using stannous chloride as a reducing agent. About 350 mg of samples and standard reference materials (SRMs) were dissolved by adding concentrated nitric acid over 8 hours, followed by the addition of 30% hydrogen peroxide. The flasks were shaken and kept at rest for 15 hours. To finish the digestion, the closed flasks were placed in an aluminum block at 90°C for 3 hours.

Cadmium and lead were determined by Electrothermal Atomization Atomic Absorption Spectrometry (ETAAS), using a Perkin Elmer Analyst 800 acid digestion of the samples, which was performed according to the procedure described for Hg analysis.

After quantification, the concentrations of metals were compared with the Resolution no. 454/2012 of the Brazilian National Council for the Environment (CONAMA, 2012). This resolution is based on international data and establishes general guidelines and reference procedures for managing material dredged (sediment) in waters (fresh and salty/brackish) under national jurisdiction. This resolution contains two levels of classification of the material to be dredged, specifically in brackish/saline waters (estuarine and/or marine environments), namely: Level 1, which presents the threshold below which there is less probability of a given metal causing adverse effects to the biota and Level 2, which refers to the threshold above which there is a greater probability of adverse effects to the biota.

2.5. Data Analysis

The AChE activity, separately for each species, and the concentrations of metals, were tested through two factors Analysis of variance (two-way ANOVA) between areas (CECSC x CERS) and seasonal periods (Dry and Rainy), following the necessary premises (normality and homogeneity of variances) to the use of this statistical test. Differences were considered significant when $p < 0.05$.

3 Results

3.1 AChE activity

The AChE activity present in the brains of individuals of the species *C. unidecimalis* showed a significant difference ($p < 0.05$) between the seasons (dry and rainy). However, there was no significant difference ($p > 0.05$) between the areas (CERS and CECSC), and there was no significant interaction ($p > 0.05$) between the factors (area x seasons) (Table 1). In both areas, the lowest activities were observed in the dry period (CECSC: 18.3 ± 1.9 mU/mg; CERS: 17.4 ± 2.6 mU/mg). The lowest and highest AChE activities were observed in CERS in the dry and rainy seasons, respectively.

Significant differences ($p < 0.05$; two-way ANOVA) between the mean values of AChE activity in individuals of the species *D. auratus* were observed both between seasonal periods and between areas (Table 2), observing a significant interaction ($p < 0.05$; two-way ANOVA) between the factors (Table 3). In both periods, individuals captured at CERS showed higher AChE activities than individuals captured at CECSC.

Table 1. Two-way ANOVA analysis of AChE activity present in the brain of the species *Centropomus undecimalis* about the seasonal period (dry x rainy), collection area (Estuarine Complex of Santa Cruz Channel - CECSC x Estuarine Complex of the Sirinhaém River - CERS), and interaction between these factors. D.F. - degrees of freedom; F - table value; P - probability; * - significant difference ($p < 0.05$); ns - not significant ($p > 0.05$).

	D.F.	Sum of Squares	Medium Square	F	P-value
Area	1	510.83	510.83	0.49	p > 0.05 ns
Season	1	130222.52	130222.52	126.53	p < 0.05 *
Area: Season	1	402.99	402.99	0.39	p > 0.05 ns
Residues	16	16466.58	1029.16		

Table 2. Two-way ANOVA analysis of AChE activity present in the brain of the species *Diapterus auratus* about the seasonal period (dry x rainy), collection area (Estuarine Complex of Santa Cruz Channel - CECSC x Estuarine Complex of the Sirinhaém River - CERS), and interaction between these factors. D.F. - degrees of freedom; F - table value; P - probability; * - significant difference ($p < 0.05$); ns - not significant ($p > 0.05$).

	D.F.	Sum of Squares	Medium Square	F	P-value
Area	1	8238.92	823.92	175.38	p > 0.05*
Season	1	70988.23	70988.23	1511.15	p < 0.05*
Area: Season	1	2210.04	2210.04	47.04	p > 0.05*
Residues	11	516.73	46.976		

Table 3. Two-way ANOVA analysis of AChE activity present in the brain of the species *Diapterus rhombeus* about the seasonal period (dry x rainy), collection area (Estuarine Complex of Santa Cruz Channel - CECSC x Estuarine Complex of the Sirinhaém River - CERS), and interaction between these factors. D.F. - degrees of freedom; F - table value; P - probability; * - significant difference ($p < 0.05$); ns - not significant ($p > 0.05$).

	D.F.	Sum of Squares	Medium Square	F	P-value
Area	1	0.05	0.05	0.85	p > 0.05 ns
Season	1	14.67	14.67	242.65	p < 0.05 *
Area: Season	1	0.05	0.05	0.84	p > 0.05 ns
Residues	10	0.60	0.06		

However, the two areas showed low mean activity values in the dry season (CECSC: 8.3 ± 0.3 mU/mg; CERS: 19.1 ± 2.4 mU/mg) when compared to the rainy season (CECSC: 124 ± 5.6 mU/mg; CERS: 183.7 ± 9 mU/mg).

The AChE activities for the species *D. rhombeus* showed a significant difference ($p < 0.05$) between the seasons (dry and rainy) (Table 4). However, there was no significant difference ($p > 0.05$) between the areas (CERS and CECSC), and there was no significant interaction ($p > 0.05$) between the factors (Table 4). The two areas showed low mean activity values in the dry season (CECSC: 13.8 ± 1.8 mU/mg; CERS: 25.5 ± 7.2 mU/mg) when compared to the rainy season (CECSC: 120.2 ± 16.9 mU/mg; CERS: 161.5 ± 0.9 mU/mg). The results were represented graphically (Figure 2).

Table 4. Mean levels of metals in sediment samples in two seasonal periods (dry and rainy), in the Estuarine Complex of the Sirinhaém River (CERS) and the Estuarine Complex of the Santa Cruz Channel (CECSC), coastal region of the state of Pernambuco, Northeast Brazil. Data expressed as average ± standard deviation.

	CERS		CECSC		Res. 454/2012 - CONAMA	
Metal	**Dry**	**Rainy**	**Dry**	**Rainy**	**Level 1****	**Level 2*****
Arsenic (‖: †)	10.353 ± 0.35	9.601 ± 0.930	17.850 ± 2.75	6.731 ± 1.37	19 [4]	70 [2]
Cadmium (ns)	0.108 ± 0.033	0.044 ± 0.012	0.014 ± 0.008	0.046 ± 0.014	1.2 [2]	7.2 [4]
Copper (ns)	2.262 ± 0.665	2.017 ± 0.335	2.372 ± 0.101	2.619 ± 1.241	34 [2]	270 [2]
Mercury (‖)	1.620 ± 0.257	0.301 ± 0.034	1.556 ± 0.068	0.307 ± 0.060	0.3 [4]	1.0 [5]
Lead (ns)	5.620 ± 0.292	3.831 ± 0.731	4.227 ± 0.354	4.502 ± 0.8955	46.7 [2]	218 [2]
Zinc (‡)	86.266 ± 8.960	74.233 ± 1.355	65.100 ± 3.333	69.200 ± 11.800	150 [2]	410 [2]

(‖): Significant difference ($p < 0.05$) between seasons; (‡): Significant difference ($p < 0.05$) between areas; (†): Significant interaction ($p < 0.05$) between factors (area and season); (ns): non-significant difference ($p > 0.05$); All concentrations are expressed in mg Kg^{-1}. **Level 1: threshold below which there is a lower probability of adverse effects on the biota; ***Level 2: threshold above which there is a greater probability of adverse effects on the biota.

3.2. Quantification of heavy metals in sediment

The average concentrations of Hg^{2+} present in the sediments did not show significant differences ($p > 0.05$; two-way ANOVA) between the areas, and the factors considered in the model did not show significant interaction ($p > 0.05$; two-way ANOVA).

However, there was a significant difference ($p < 0.05$; two-way ANOVA) between the dry and rainy seasons. Using the Reference Levels proposed by Resolution n. 454/2012 - CONAMA, the Hg^{2+} concentrations reported for the dry period (CERS: 1.620 ± 0.257 mg Kg^{-1}; CECSC: 1.556 ± 0.068 mg Kg^{-1}) were higher than Level 2 in both areas, and, for the rainy season, the concentrations were similar to Level 1 (CERS: 0.301 ± 0.034 mg Kg^{-1}; CECSC: 0.307 ± 60.060 mg Kg^{-1}).

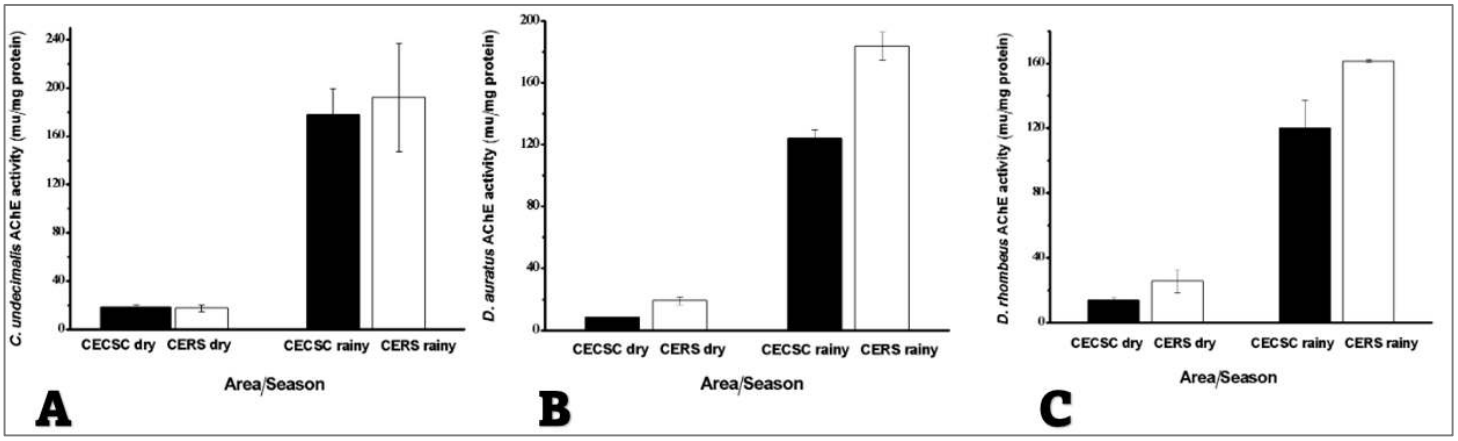

Figure 2. Acetylcholinesterase activity present in the brain of the species *C. undecimalis* (A), *D. auratus* (B) and *D. rhombeus* (C) captured in two seasonal periods (dry and rainy), in the Estuarine Complex of the Sirinhaém River (CERS) and in the Estuarine Complex of the Santa Cruz Channel (CECSC), coastal region of the state of Pernambuco, Northeast Brazil.

Arsenic concentrations showed significant differences ($p < 0.05$; two-way ANOVA) between the seasons, and the two factors (area and period) showed considerable interaction ($p < 0.05$; two-way ANOVA). However, no significant difference ($p < 0.05$; two-way ANOVA) was observed between the areas (Table 4). Similar to Hg^{2+}, both regions found the highest arsenic concentrations in the dry season. However, they were lower than Level 1 (Resolution n. 454/2012 – CONAMA) (Table 4). Zinc did not present significantly different concentrations ($p > 0.05$; two-way ANOVA) between areas and seasons. Nevertheless, the factors showed significant interaction ($p < 0.05$; two-way ANOVA) (Table 4). Cadmium, Copper, and Lead concentrations also showed no significant differences between areas and seasons, as well as no significant interactions ($p > 0.05$; two-way ANOVA) between the factors (Table 4). The concentrations of these four metals were lower than Level 1 (Table 4).

4 Discussion

The results corroborate research conducted by Moura and De Lacerda (2022), which suggests that regional and global environmental changes modulate the impact of anthropogenic sources on Hg bioavailability and emerge from an interaction between biological, ecological, and hydrological determinants.

Ultimately, the intensification of aridity linked to global climate change, as highlighted in northeastern Brazil and comparable semi-arid coastal regions around the world, in conjunction with the increased exploitation of water resources, intensifies the bioavailability of Hg and increases the environmental risks and exposure levels encountered by local biota, as well as affecting the traditional practices of human communities dependent on the estuary's biological resources.

In the estuaries of the Santa Cruz Channel (CECSC) and the Sirinhaém River (CERS), sediment samples from both seasons (dry and rainy) contain concentrations of As^{3+}, Cd^{2+}, Cu^{2+}, Hg^{2+}, Pb^{2+} , and Zn^{2+} below Level 1, which, according to CONAMA (2012), means reduced probability of the relevant metals affecting the health of the biota. The article presented similar contributions to Level 1 in the rainy season in both estuarine complexities. However, in the dry period, concentrations were higher than Level 2, indicating that in both estuarine complexes, there is a high probability of Hg interfering with the health characteristics of the organisms that inhabit these estuaries (CONAMA, 2012).

Variations in metal concentrations between seasonal periods can probably be associated with the intensity of the polluting load released by factories and residences located along these estuaries, including the Santa Cruz Channel, or also by the variability of geochemical vectors (e.g., suspended solids) and the dilution of river sediments by marine sediments that occur in the rainy season (PRABAKARAN, 2020).

High concentrations of Hg are associated with lower solubility of the metal in water during dry periods, according to the theory proposed by Lacerda et al., (2013), who state that when evaluating the dynamics of Hg in terms of concentration and flow, in the dry and rainy seasons, during a period of five years (2005 to 2009) in the estuarine complex of the Jaguaribe river, state of Ceará, northeast region from Brazil, observed that there is an increase in the residence time of water masses in the dry period (average value of 3.1 days) due to the reduction in river supply, thus causing a greater retention of suspended material, including Hg that adheres to this suspended material within the estuary. This theory may explain why Hg showed higher concentrations during the dry period in both estuarine complexes evaluated in the present study.

According to Marins et al. (2004), the chlorine and caustic soda industries, along with other diffuse sources such as illegal dumps and domestic/urban effluents, are the human activities that most contribute to the increase in Hg concentrations in Brazilian estuaries. Therefore, the high Hg concentrations in the CECSC are justified because the complex receives a strong pollution load from various human sources along its tributaries. Meyer (1996) states that the increase in Hg levels at CECSC is associated with effluents from the chlorine and caustic soda industry, installed in 1963 and deactivated in 1987, which released approximately 35 tons of Hg into the Botafogo River (one of the main rivers that make up the CECSC) during its period of operation.

High concentrations of Hg may also be associated with the large aquaculture farms that are installed around the two estuarine complexes (CECSC and CERS), corroborating the theory proposed by Chou (2002) and Berntssen et al. (2004), who stated that Hg is present in aquaculture effluents due to its presence as a natural element in feed (fishmeal) and as impurities contained in fertilizers and other chemical products used in the activity. With the growth of these enterprises (mainly intensive farming of marine shrimp that settles close to estuaries), Hg has been increasing its concentrations in estuary sediments over the years (MOURA; DE LACERDA, 2022).

The high concentrations of Hg and the low AChE activities in the three species evaluated show that the two estuarine complexes have been under intense anthropogenic impact over the years. Hg^{+} is a highly toxic contaminant and is the only metal capable of undergoing biomagnification in practically the entire food chain, as its concentration increases as the trophic level of the species increases (WU et al., 2024). The biomagnification of this metal is a worrying factor, as the negative effects caused to ecosystems can last decades (as is the case with CECSC - a chlorine and caustic soda company) or even centuries (ZHANG et al., 2021b).

Furthermore, Hg^{+} can be remobilized from deeper sediments (CROWTHER et al., 2021) and reach the water column, associated with organic matter, being reincorporated by biota (MILLARD et al., 2023). Metals in various orders of magnitude concentrations found in the water column are highly absorbed by biota, remaining for a long period due to the slow metabolization process. The bioaccumulation of Hg causes serious problems for fish fauna and humans (RAY; VASHISHTH, 2024). *In vitro* tests prove that, even at low concentrations, Hg has an AChE inhibitory potential present in fish that inhabit freshwater and marine/estuarine environments (HENRIQUES et al., 2023), as was the case of the three species evaluated in this study.

The high concentrations observed in the two estuarine complexes under study suggest that Hg has been directly interfering with the health of individuals belonging to the species evaluated, since all three showed a significant reduction in AChE activity in the dry period, when there was a higher concentration of Hg in both estuarine complexes. The FAO (2007) states that the first signs and symptoms of AChE inhibition are noticed with inhibitions ≥ 50%, and death, with inhibitions greater than 90%. Heavy metals can be present in samples from different sources (water, sediments, and domestic/urban and industrial effluents). They can cause false positives or negatives in analyses of pollutants such as pesticides (organophosphates and carbamates) and other anticholinesterase agents. However, the study of AChE inhibition caused by heavy metals associated with different methods, such as quantifying these elements in sediments and water, makes this enzyme an essential tool for monitoring the quality of aquatic environments.

The results of the present work indicate that Hg detected in sediments, in concentrations above the limits established by national and international legislation, has been altering the neurological physiology of the species under study, representing an environmental impact. It can, through the food chain, interfere with human health since this metal is bioaccumulated, and people commonly consume fish as a source of protein. However, new research related to the identification and spatial-temporal distribution of pesticides – mainly organophosphates and carbamates (LOPES et al., 2019; WANG et al., 2022), as well as the development of research focused on the effect of microplastics associated with Hg, both with high inhibitory power on AChE (already observed in the work carried out by (BARBOZA; VIEIRA; GUILHERMINO, 2018) for the species *Dicentrarchus labrax* (Linnaeus, 1758)) must be carried out.

5 Conclusions

Finally, it was concluded from the analysis that, during the intense rainy season, Hg concentrations remained consistently below the established regulatory limits in both estuarine complexes studied, thus suggesting a temporary pause in potential contamination. However, during the subsequent dry period, it was observed that Hg levels exceeded the Level 2 limit, as defined by CONAMA Resolution No. 454/2012, indicating a worrying high probability of negatively affecting the health characteristics of the diverse organisms that inhabit these intricate ecosystems. This concern is further intensified by the fact that Hg is classified as one of the top ten chemicals posing significant risks to public health, as described by the World Health Organization (WHO), in the document Ten chemicals of public health concern, thus highlighting the urgency of addressing this problem.

The elevated presence of Hg in estuarine environments during the dry season may be intrinsically related to various anthropogenic activities taking place in the region, including, but not limited to, industrial discharges and the widespread use of agricultural pesticides.

This widespread contamination poses a direct and immediate risk to local biodiversity, particularly affecting commercially significant species such as *Centropomus undecimalis*, *Diapterus auratus* and *Diapterus rhombeus*, all of which are prone to bioaccumulating this toxic metal and, as a consequence, pose a threat to the integrity of the food chain and, ultimately, to human health through their consumption, causing neurological and cognitive alterations.

For the evaluated estuarine environments, the competent authorities must intensify monitoring of industrial effluents (including those from agro-industries), domestic wastewater, and aquaculture discharges. In addition, the identification and elimination of illegal pollution sources are necessary.

These actions are critical to mitigate contamination by Hg and other pollutants, thereby reducing their impacts on aquatic biota and human health.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

All authors declare that they contributed significantly to the development of this manuscript as follows: Author Mikele Cândida Sousa de Sant'Anna was responsible for the conceptualization, methodology, formal analysis, and original draft preparation; Author Elida Virna Rodrigues Barbosa contributed through data curation, validation, investigation, and critical review of the manuscript; Author Maria Priscila Sá Matos Ribeiro participated in the methodology, visualization, project administration, and review and editing; Author Ranilson de Souza Bezerra carried out the software development, statistical analysis, verification, and additional technical contributions; and Author Danilo Francisco Corrêa Lopes was responsible for supervision, resource acquisition, final review, and approval of the submitted version. All authors state that they have read and approved the final version of the manuscript and assume full responsibility for its content.

DECLARATION OF INTEREST

The authors declare that they have no financial interests or personal relationships that could have influenced the work reported in this manuscript.

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