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Contaminant metal concentrations in holothurians: Seasonal, sex and tissue variations

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ABSTRACT

The main goal of this study was to investigate seasonal contaminant changes in three sea cucumber species caught in the North Atlantic. The risk associated with the consumption of these target species, taking into account the consumers age group, was also evaluated. For this purpose, the concentration of toxic metals cadmium (Cd), lead (Pb) and mercury (Hg) was analyzed during spring, summer, autumn and winter in two different tissues of female and male holothurians. Average concentration of the Cd, Pb and Hg were different among the studied species, and significant differences were observed considering sex, tissue and seasonality factors. The average values (dry weight) of the Cd and Hg of *Holothuria arguinensis* (Cd: 0.10 mg kg^{-1}; Hg: 0.10 mg kg^{-1}), *Holothuria forskali* (Cd: 0.10 mg kg^{-1}; Hg: 0.07 mg kg^{-1}) and *Holothuria mammata* (Cd: 0.19 mg kg^{-1}; Hg: 0.17 mg kg^{-1}) were lower than those regulated by the European Union. On the other hand, the average Pb values (dry weight) were high in *Holothuria arguinensis* (Pb: 2.97 mg kg-1) and *Holothuria mammata* (Pb: 1.77 mg kg-1). The risk associated with the consumption rate (CR_{lim}) of these species is low for Cd and Hg and high for Pb for adults and children, based on the maximum concentrations rate (CR_{lim}) of each metal. Results such as presented in this work are scarce or non-existent, potentially contributing to a database that allows for the future development of new regulations that limit the maximum concentrations of metals in the consumption of echinoderms.

1. Introduction

Toxic metal pollution has spread widely throughout the world mainly due to industrial human activity, where the amount and diversity of contaminants has increased exponentially, posing serious risks to human health (Brodziak-Dopierała and Fischer, 2023; Mititelu et al., 2025). One of the main pathways of human contamination by toxic metals is through food, making food safety issues a growing concern in recent decades (Scutaras and Trinca, 2023). Metals such as Cd, Pb and Hg are classified as non-essential metals because their metabolic or biological functions are not known (Munir et al., 2022; Kim et al., 2023). These metals are deleterious in several respects (Gall et al., 2015; Lamas et al., 2023) and therefore are listed among the top 20 hazardous substances by the US Environmental Protection Agency and the Agency for Toxic Substances and Disease Registry (ATSDR) (Xiong et al., 2017; Rehman et al., 2018; Rai et al., 2019).

Cadmium has been found in seafood, with the highest concentrations in crustaceans and molluscs (UK, 2006; Bonsignorea et al., 2018; Gu et al., 2021). Depending on the dose and duration of exposure, Cd can damage various organs such as the lungs, liver, kidneys and bones. However, the primary target organ of Cd toxicity is the kidney, being the main cause of kidney diseases in many regions of the world. In addition, it acts as an endocrine disruptor triggering changes in the immune system and being carcinogenic (Jarup and Akesson, 2009; Prozialeck and Edwards, 2012; Mahajan et al., 2022).

Lead is a toxic metal for both aquatic animals and humans, and its level tends to be higher in large predatory fish, crustaceans and molluscs (Garofalo et al., 2025). It is known that even small amounts of ingested Pb are harmful to organisms (Wani et al., 2015; Collin et al., 2022). Prolonged exposure can cause various pathological changes, including

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[1] Dr. Mercedes Gónzalez-Wangüemert passed away on 10th July 2025, before the publication of this manuscript. This work is dedicated to her memory.

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kidney, nervous system, liver, haematological, genetic, cytological and reproductive damage (Ashry et al., 2010; Flora et al., 2012; Álvarez-Lloret et al., 2021).

Mercury is among the most harmful contaminants representing a risk for the marine environment and to the people who use this resource (EFSA, 2012; Sheehan et al., 2014). Mercury concentration (under its most toxic form, methylmercury,) biomagnifies along the food chain, presenting the highest concentration in big marine predators (Fioravanti et al., 2025). As Hg is not an essential metal, it enters into organisms by molecular mimicry, promoting oxidative stress (Martinez-Finley et al., 2012; Kong et al., 2012). It can cause a wide spectrum of illnesses from infertility to low cognition in children (Hong et al., 2012; Charkiewicz et al., 2025).

The accumulation of metals in marine animals can be influenced by several factors, such as the animal's weight, size, and age, as well as its physical condition and environmental conditions (Aydın et al., 2017). Examples of these factors include the concentration and type of metal present in the sediment, time of exposure to the contaminated environment, the physicochemical characteristics of the environment, the metabolic rate of the tissues and/or the sex of the animals, and their feeding habits (Cutajar et al., 2024; Warnau et al., 2006).

Sediments are the main source of metals for benthic animals, as they feed by ingesting large quantities of substrate and these are a reservoir of metals in the marine environment (Zhang et al., 2015). When marine organisms are exposed to these metals, the bioaccumulation in their tissues can be variable, mainly depending on the environmental conditions in which they are thriving and trophic level (Rejomon et al., 2010; Jakimska et al., 2011; Worakhunpiset, 2018). This bioaccumulation is also linked to several endogenous factors, namely the physiological status, fat content, length, weight, and age (Charbonneau et al., 2022; Domingo, 2024). In addition, they represent a risk to human health due to their bioaccumulation and potential toxicity. These three metals can also cause disturbances in the marine environment, reducing the diversity and abundance of species (Hosono et al., 2010, 2011). Thus, the pollution of coastal marine environments raises the need to consider issues of food safety and environmental management.

The concentration of non-essential metals can be determined by several methodologies, including biota analysis; benthic animals are good biological indicators, as they maintain a dependent life cycle direct contact with the sediment (Ozkan et al., 2016; Bhuyan et al., 2024).

The European Union (EU) has established maximum levels for certain contaminants in foods such as seafood, where Cd, Pb and Hg are included (EU, 2023). However, EU does not limit the maximum amount of these metals for echinoderms, having a gap between legislation and food safety. On the other hand, sea cucumber and sea urchins are considered potential metal accumulators due to their dietary habit of ingesting large amounts of marine sediment (Wang et al., 2016; Boluda-Botella et al., 2024), being commonly used in toxicity bioassays (mainly sea urchins) due to their wide distribution, suitability for testing and sensitivity to environmental stressors (Morroni et al., 2018; Rakaj et al., 2021; Tamburini et al., 2022).

Sea cucumbers (Echinodermata: Holothuroidea) are benthic invertebrates characterized by limited mobility and that live in contact or buried in the sediment (Marrugo-Negrete et al., 2020). They have a wide global distribution, being present in almost all seas and oceans and in several ecological niches. More than 100 species have commercial value and have been consumed as a gastronomic delicacy for hundreds of years in Asia (Bruckner et al., 2003; Bruckner, 2005; Toral-Granda et al., 2008; Purcell et al., 2012, 2013; Purcell et al., 2023). The species *Holothuria arguinensis*, *Holothuria forskali* and *Holothuria mammata* feed on non-selective deposits and are commonly found on the shallow bottom of the Atlantic Ocean and Mediterranean Sea (*H. forskali* and *H. mammata*) and mainly Atlantic Ocean and colonizing Mediterranean Sea (*H. arguinensis*), presenting high abundance and density on the seafloor from these regions (González-Wangüemert et al., 2018a).

Most sea cucumbers are not traditionally consumed as food in Europe, except the species *Parastichopus regalis*, which is caught and consumed in NE Spain (Cataluña, Baleares islands and Castellón) having its internal muscle bands (espardeneyes) a high economical value (130–200 euros/kg) (Ramón et al., 2010, 2022; Maggi and González-Wangüemert, 2015). In recent decades, there has been a significant increase in the number of sea cucumber catches along the Mediterranean and Northeast Atlantic coasts for exporting to Asian countries, mainly China (González-Wangüemert et al., 2014, 2015). To date, there are few studies on the elemental profile, including non-essential metals in sea cucumbers off the coast of the Northeast Atlantic (Roggatz et al., 2016, 2017; González-Wangüemert et al., 2018b). Despite the small number of studies, there is evidence for the presence of the Cd, Pb and Hg of these species (Rehan et al., 2023). To the best of our knowledge, no information has been published about the metals composition of *H. arguinensis*, *H. forskali* and *H. mammata* of southwestern Portuguese Atlantic coast associating it with other factors such as tissue, sex and, season.

Therefore, the present study aims to determine the concentration of non-essential metals, Cd, Pb and Hg, in the spring, summer, autumn and winter in the tissues and male and female of *H. arguinensis*, *H. forskali* and *H. mammata* captured in the North Atlantic (Portugal). Also evaluating the risk associated with the consumption of these target species with regard to the consumers age group. Therefore, this study provides valuable information on the potential human health risk of sea cucumber consumption in relation to Cd, Pb, and Hg, contributing to the development of a more global assessment of the health risk of sea cucumber consumption in Europe.

2. Material and methods

2.1. Raw material and sample preparation

Sea cucumbers used in this study were caught on the coast of Sesimbra (Portugal) at 12 sampling sites according to similar distance for each point (Fig. 1) by SCUBA diving over, depth between 0 and 20 m, along one year (four seasons). No sampling was carried out in the Total Protected Marine Area (Parque Marinho Professor Luiz Saldanha), next to the sampling area. At each sampling site, all three species were sampled according to each species' substrate of occurrence (rock or sand), *H.arguinensis* and *H. mammata* were collected majority from sand, and *H. forskali* were collected from the rocky substrate only, as this species occurs predominantly in this substrate (Rehan et al., 2023; Silva et al., 2021). The species identification followed the approach described by Carletti et al. (2022). A total of 370 individuals were collected *H. arguinensis* ($n = 123$), *H. forskali* ($n = 124$), and *H. mammata* ($n = 123$). From individuals collected, it was choice to use for metal analysis 288 individuals (Table 1), these animals were chosen with sexual differentiation (gonads content) and similar weight and size.

The animals in each four seasons (spring, summer, autumn and winter) were classified by species. Total length (cm) and total wet weight (fresh – life weight and gutted; g) were recorded. The animals were transported alive to the laboratory where were slaughtered by immersion on ice: water slurry. After slaughtering, fresh sea cucumbers were gutted and separated in tissues: body wall, muscle band and gonads. The tissues, body wall and muscle band were cleaned and grinded. Then, were immediately freeze-dried and preserved at −80 °C until analysis. The gonads were immediately fixed in a buffered 4% formalin solution for 48 h to perform the visual and histological sex classification, followed the approach by Venâncio et al. (2022). After that, the material was separated into two subsamples: the first subsample was divided into season, species, tissues and sex; and the second subsample was a pool of these tissues for identification as *H. arguinensis*, *H. forskali* and *H. mammata*. The first subsample was used to measure the concentrations of Cd, Pb and Hg among seasons, tissues and sex. The second subsample was used to quantify the total metal content, the relationships between the metals with the weight and length, and to calculate

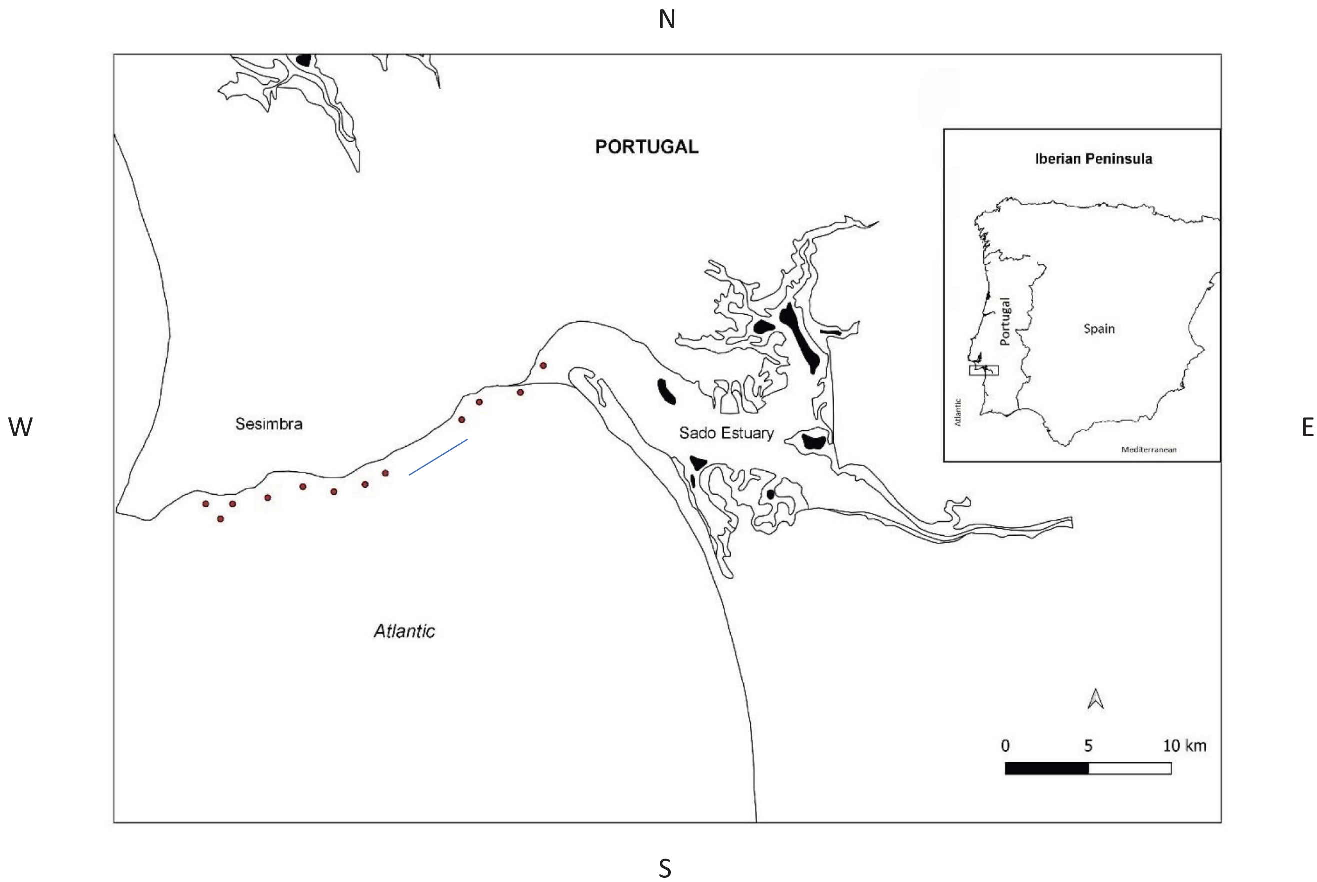

Fig. 1. Map showing dots as sampling areas at the Northeast Atlantic Coast (Sesimbra) in Portugal.
Marine Natural Reserve (Parque Marinho Professor Luiz Saldanha).
• Sites samples.

Table 1
Numbers of the individuals per species and seasons.

Species	Spring May	Summer July	Autum October	Winter January	*Total*
H. arguinensis	28	27	27	27	**109**
H. forskali	18	18	18	18	**72**
H. mammata	26	27	27	27	**107**
Total	**72**	**72**	**72**	**72**	**288**

the maximum permitted daily consumption rate (CR_{lim}).

Countries in Mediterranean (Turkey, Spain and Greece) consume body wall and muscle band from sea cucumbers (Cakli et al., 2004; Ramón et al., 2010; Antoniadou and Vafidis, 2011). In addition, these tissues are the most commonly consumed parts of sea cucumber species in worldwide, harbouring most of the total load of metals according to several studies of metal absorption biokinetics (Soegianto and Irawan, 2009; Mohammadizadeh, 2015; Tunca et al., 2016). Therefore, these were the parts used in this study.

2.2. Sample analysis

Mercury was determined by atomic absorption spectrometry according to the United States Environmental Protection Agency test method 7473 (U.S. EPA, 1998), using a mercury analyser (LECO, AMA 254, St. Joseph, MI, USA). The procedure was based on the decomposition about of 10.0 mg of fresh weight sample by combustion, preconcentration of Hg by amalgamation with gold and Hg detection at 254 nm.

Determination of Cd and Pb were performed according to EN 14084: 2003 (CEN, 2003). Samples (about 1.0 g of fresh weight) were digested in triplicate in a microwave oven (CEM, Mars 5, USA) using a mixture 4:1 of nitric acid (suprapur 65%, Merck) and hydrogen peroxide (30%, Merck). The content of Cd and Pb were then analyzed by atomic absorption in a graphite furnace spectrometer (Agilent, Spectra AA 240Z, USA) at 228.8 and 283.3 nm, respectively.

Laboratory material used in these methodologies was decontaminated with 200 g L^{-1} of HNO_3 for 24 h and rinsed with ultrapure water (18.2Mμ cm). The quantification of the three metals was performed using an external calibration method, with at least five different concentrations, using standard commercial solutions (1 g L^{-1}) for Hg, Cd and Pb (Merck, Darmstadt, Germany). To check calibration curves, two control standards (near the quantification limit and in the middle of the calibration curve) were analyzed in all work sessions. Methodology blanks were made using the same analytical procedure and reagents. The detection/quantification limits, calculated from the residual standard deviation of the response and the slope of the calibration curve for each element, were 0.004/0.011 (Hg), 0.002/0.006 (Cd) and 0.02/0.06 (Pb) mg kg^{-1} of dry weight, respectively. All analytical data are reported in mg kg^{-1} based on dry weight. To assess the accuracy of the methods, the certified reference material DORM-4 (fish protein from the National Research Council of Canada) was tested under the same conditions as the samples. The results obtained in this study are in good agreement with the reference values (Table 2).

Sea cucumbers are normally consumed dried (Purcell, 2014; Purcell et al., 2016), that's why conversion to dry weigh was *posteriori* calculate for Cd, Hg and Pb based on the moisture of *H. arguinensis* (87%), *H. forskali* (90%) (Sales et al., 2021) and *H. mammata* (73.6%)

Table 2
Methodology accuracy for Cd, Hg and Pb with certified reference material (DORM4 – National Research Council, Canada; mg kg^{-1} dry weight; $n \geq 4$).

Elements (mg kg^{-1})	Technique	Reference material	Certified	Obtained	Recovery (%)
Cadmiun	GFAAS	DORM-4	0.299 ± 0.018	0.296 ± 0.010	98
Lead	GFAAS		0.404 ± 0.062	0.394 ± 0.026	99
Mercury	AAS		0.412 ± 0.036	0.416 ± 0.017	101

Values are presented as average ± standard deviation.
DORM-4 - fish protein (National Research Council of Canada, Canada),
AAS (atomic-absorption spectroscopy), GFAAS (atomic absorption in a graphite furnace spectrometer).

(González-Wangüemert et al., 2018a). These studies were carried out with the same species and same region of the present work.

For the comparison of the results found in this present work was carried out in dry weight basis and all the values from the literature cited in this work, have been transformed, when necessary, from wet weight to dry weight by using a ratio of 10:1, according to Bordbar et al. (2011).

2.3. Maximum permitted rate of daily consumptions

Consumption of marine foods is significant in the diet of most European countries, mainly Portugal, Spain and France which are the main consumers (EEA Report, 2016). Several approaches were used to determine the risk of consumption of different species, classes and length/ weight of marine food. Currently, the World Health Organization (WHO) (WHO, 2016), the European Food Safety Authority (EFSA) (EFSA, 2015) and the United States Food and Drug Administration (FDA) (Evans, 2002) have developed a regulation to define the Maximum Quantity Limited (MQL) of contamination allowed in food. In the EU, the maximum permitted levels of Hg, Cd and Pb are not specified for echinoderms, however, the indexes indicated for the edible body wall of crustaceans, are 0.5 (mg kg^{-1}) of total Hg, 0.5 (mg kg^{-1}) of Cd and 0.5 (mg kg^{-1}) of Pb (EU, 2023) and were used for comparison. Crustaceans are the group with similar edible part of the sea cucumbers (muscle and body wall) if compared among seafood. Also, the MQL values to European Regulalation are lower than others groups. This approach aims to prioritize ensuring food safety for consumers by opting for the most restrictive scenario. The European Union legislation defines the MQL in wet weight, although environmental monitoring data is frequently reported on a dry weight basis to ensure the water content, which can change after collecting the animals, does not affect data interpretation (Rehan et al., 2023).

To calculate maximum allowable consumption rate of sea cucumber per day (CR_{lim}) that does not endanger the health of consumers, in terms of metal concentration, the U.S. EPA equation (U.S. EPA, 2000), was used according to Marrugo-Negrete et al. (2020). The formula used was as follows: $CR_{lim} = (RfD \times BW) / C_m$, where RfD (mg kg^{-1}) is the daily oral exposure of the human population likely to have no appreciable lifetime risk of non-cancerous harmful effects, BW (kg) is the consumer's body weight and C_m (mg kg^{-1}) is the average metal concentration in sea cucumbers. CR_{lim} values for *H. arguinensis*, *H. forskali* and *H. mammata* were expressed in grams per day (g day^{-1}). There is no oral RfD value for Pb, therefore a reference value for Pb (BMDL - Table 7) was obtained from the European Food Safety Authority (EFSA, 2010).

For these calculations, daily adequate intakes (AI), daily dietary reference intake (DRI) and daily tolerable upper intake levels (UL), set by the US Department of Agriculture (USDA) for adult females and males aged between 19 and 50 years (80 kg) and children (15 kg), were used (U.S. EPA, 2000, 2014, 2015). The contaminants Cd, Hg and Pb were compared with the maximum allowed level (ML) set by the European Commission as mentioned above.

2.4. Statistical analysis

All data were expressed as mean ± standard deviation. Analyses were performed in triplicate, and each measurement was taken in two replicates.

The described values (concentration of Cd, Hg, and Pb) were previously assessed for normality using the Shapiro-Wilk test (Shapiro and Wilk, 1965), and homogeneity of variance (homoscedasticity) using Levene's test. For non-parametric data, the Kruskal-Wallis test (Kruskal and Wallis, 1952) was used, and for parametric data, ANOVA *two-way* and GLM. Kruskal-Wallis was used just as *post hoc* parametric data.

For all statistical tests, the significance level (α) was 0.05 (95%). Spearman's correlation coefficient was calculated to determine the correlation between trace metals (concentration) and weight and length.

All data analyses were performed using STATISTICA software (Statsoft, Inc., Tulsa, OK, USA).

3. Results & discussion

3.1. Biometric data and their correlation with Cd, Hg, and Pb

The biometric data of the three sea cucumber species studied are presented in Table 3. Length (*L*) and weight (live - *LW* and gutted - *GW*) showed significant differences among the three species analyzed (Kruskal-Wallis test, $p < 0.05$), the highest values were observed for *H. arguinensis* (*L* 23.5 cm; *LW* 487.8 g; *GW* 212.0 g) and the lowest for *H. forskali* (*L*18.8 cm; *LW* 136.5 g; *GW* 76.3 g). *H. mammata* showed intermediate values (*L* 23.0 cm; *LW* 341.7 g; *GW* 144.4 g). Similar values have been previously reported by studies conducted with the same species in Mediterranean regions (González-Wangüemert et al., 2015, 2018b; Olaya-Restrepo et al., 2017) and are generally equivalent to those of other commercial species of the genus Holothuria from Indo-Pacific (Al-Rashdi et al., 2007a, 2007b; Glockner-Fagetti et al., 2016).

It is recognized that the physiological processes influencing the absorption, distribution, and elimination of trace metals are influenced by the length and weight of the animal. This observation is particularly valid for the absorption of Cd, Pb, and Hg, as the levels of these metals generally increase in heavier/older aquatic animal species, in contrast to the low concentration found in smaller or younger aquatic animals (Nathaniel, 2002; Raulinaitis et al., 2012). However, there are studies showing a higher concentration of trace metals in small animals, such as a study by Al-Najjar et al. (2018) that analyzed the concentration of seven metals in two sea cucumber species of different sizes. These authors found significantly higher values in small animals, namely Cd in *Actinophyga bannwarth* (small <25 cm 1.19 ± 0.26 μg/g; large >30 cm 0.93 ± 0.21 μg/g) and *Holothuria impatiens* (< 25 cm 0.86 ± 0.21 μg/g; large >30 cm 0.86 ± 0.21 μg/g) and Pb in *Actinophyga bannwarth* (small <25 cm 15.28 ± 1.81 μg/g; large >30 cm 14.69 ± 2.11 μg/g) and *Holothuria impatiens* (< 25 cm 17.51 ± 2.36 μg/g; large >30 cm 11.2 ± 1.33 μg/g). This fact may be associated with the environment where the animals were collected; that is, although there is a tendency for the concentration of certain metals to increase in older animals, if younger animals are in environments with higher levels of sedimentary contamination, the accumulation will be greater in them than in the former. In this study, only *H. mammata* showed a positive correlation between Pb concentration and animal weight ($p = 0.02$), *H. arguinensis* and *H. mammata* showed a positive correlation between weight and animal size, however, this correlation was not significant (*H. arguineneis*: $p = 0.06$; *H. mammata*: $p = 0.09$) (Table 4).

Table 3
Biometric data of *H. arguinensis*, *H. forskali* and *H. mammata*.

	H. arguinensis				*H. forskali*				*H. mammata*			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Life weight (g)												
Mean ± SD	479.6 ± 117.8^{bc}	456.3 ± 182.0^{c}	523.4 ± 129.0^{a}	492.0 ± 181.0^{ab}	152.9 ± 29.3^{a}	149.7 ± 43.1^{a}	111.3 ± 41.0^{c}	131.9 ± 27.9^{ac}	395.0 ± 93.5^{a}	341.4 ± 124.5^{b}	310.4 ± 90.5^{c}	320.1 ± 78.2^{bc}
Median	458.4	448.8	420.4	489.2	155.4	151.7	100.9	136.8	412.5	312.5	310.2	300.1
Range	184.0–911.9	144.8–913.2	123.9–1051.8	186.3–1007.7	87.2–216.9	74.5–260.4	73.5–292.1	85.9–195.7	193.6–558.9	169.8–719.7	126.9–540.0	151.31–508.51
Gutted weight (g)												
Mean ± SD	214.3 ± 75.5^{a}	218.2 ± 107.9^{a}	202.9 ± 93.5^{b}	212.7 ± 76.3^{a}	80.7 ± 12.9^{a}	84.4 ± 19.9^{a}	64.3 ± 13.1^{b}	75.8 ± 12.1^{a}	159.6 ± 32.4^{a}	138.5 ± 45.2^{b}	139.7 ± 30.8^{b}	139.8 ± 34.4^{b}
Median	199.3	199.4	180.5	215.7	82.57	88.7	62.1	77.5	162.6	125.4	145.9	136.4
Range	94.1–347.8	62.3–431.4	53.87–465.0	84.18–412.39	61.5–100.1	46.3–116.7	45.9–97.9	47.6–99.0	100.6–254.6	74.5–278.4	54.23–203.2	69.9–221.8
Length (cm)												
Mean ± SD	25.8 ± 4.0^{a}	21.7 ± 4.3^{a}	23.2 ± 5.2^{a}	23.2 ± 5.7^{a}	17.3 ± 2.6^{a}	15.5 ± 2.6^{a}	16.5 ± 2.1^{a}	17.9 ± 3.1^{a}	23.7 ± 3.4^{a}	22.1 ± 5.2^{a}	22.1 ± 3.7^{a}	24.0 ± 4.3^{a}
Median	26.2	21.8	23.8	22.3	17.3	15.6	15.8	17.6	23.9	21.2	22.6	24.5
Range	17.5–32.7	11.8–24.2	14.4–36.0	15.0–27.4	13.8–16.6	10.9–21.0	14.1–20.2	15.0–23.5	18.8–30.2	18.5–31.0	16.1–27.8	15.1–31.6

Mean ± standard deviation. Different letters in the same line for each species, denote significant differences ($p < 0.05$).

Table 4
Sperman-rho coefficients for relationships among trace metals, life weight and length of *H. arguinensis*, *H. forskali* and *H. mammata*.

	Weight	Length
H. arguinensis		
Length	0.471	–
Cd	0.350	0.226
Pb	0.274	−0.109
Hg	−0.253	0.147
H. forskali		
Length	0.094	–
Cd	−0.284	0.271
Pb	−0.029	0.224
Hg	−0.041	0.259
H. mammata		
Length	0.359	–
Cd	−0.174	−0.110
Pb	0.550*	−0.088
Hg	−0.062	−0.197

Positive correlation; -, negative correlation.
* $\rho < 0.05$.

3.2. Foraging habits and metal concentration of the sea cucumbers

The results obtained for Cd, Hg, and Pb levels are presented in Table 5. In general, the results show that the concentration of metals among the three species has significant differences (Cd: $p = 0.002$; Pb: $p = 0.004$; Hg: $p = 0.001$), with *H. arguinensis* being the species with the highest value, followed by *H. mammata*. *H. forskali* was the species with the lowest metal levels.

Since the three species of sea cucumbers are detritivores (Roberts et al., 2000) and were collected at the same sampling sites, since also varying sizes and weights, the differences in metal levels among theses species may be related, for example, to the characteristic feeding behavior of each one (Aydın, 2008; Aydın and Erkan, 2015; González-Delgado et al., 2024).

It is known the *H. arguinensis* does not exhibit foraging behavior at a specific time of day, and may search for food throughout the 24 h of the day (González-Wangüemert et al., 2015; Siegenthaler et al., 2015). *H. arguinensis* also frequently changes habitat and depth, moving in search of new locations and sediments.

On the other hand, although *H. mammata* shows a similar preference to *H. arguinensis* regarding habitat (sandy and rocky bottoms with seagrass), this species exhibits shelter behavior during the day and feeds mainly at night, generally showing fidelity to sediment zones near its shelter (crevice or hole in the rock where it remains during the day) (González-Wangüemert et al., 2015). Therefore, these two types of behavior can influence the results, since, if there is a higher concentration of metals in the sediment/feeding area of *H. arguinensis*, this species may present higher metal concentrations than *H. mammata*, which has a wider foraging area.

H. forskali, in turn, is associated with shallow zones and rocky environments (Hayward and Ryland, 2017; Félix et al., 2024). Studies in the feeding ecology of *H. forskali*, primarily focusing on intestinal content, show that this species is capable of actively selecting sediments rich in organic matter and fine particles suspended in the water column, not

Table 5
Total average (mg/ kg^{-1}) of trace metals in *H. arguinensis*, *H. forskali* and *H. mammata* (dw).

	H. arguinensis	*H. forskali*	*H. mammata*
Cd	0.10 ± 0.07^{b}	0.10 ± 0.04^{b}	0.19 ± 0.06^{a}
Pb	2.97 ± 2.90^{a}	0.19 ± 0.01^{c}	1.77 ± 1.30^{b}
Hg	0.10 ± 0.06^{b}	0.07 ± 0.04^{b}	0.17 ± 0.13^{a}

feeding exclusively on sediment (Mezali and Soualili, 2013; Belbachir et al., 2014; Belbachir and Mezali, 2018). This may explain why this species has the lowest metal content compared to the other two species, since sediment from deeper regions is the largest reservoir of metals. Another factor that may be associated with *H. forskali* is the lower contribution of algae to the organic matter of the ingested fine sediment, given that macroalgae are known to bioaccumulate higher levels of metals than the surrounding sediment (Bonanno and Orlando-Bonaca, 2018).

Therefore, these three distinct types of feeding behavior in the environment of the studied species can influence the results of contaminant levels in the tissues, with *H. arguinensis* expected to be the species most likely to encounter contaminated environments due to its larger foraging area compared to *H. mammata*, which moves less to feed. *H. forskali*, on the other hand, is the species with the most selective behavior and feeding habits.

Similar results were found in a study conducted by Rehan et al. (2023) with holothurians collected in three diferente sites in Sesimbra in Portugal, where *H. arguinensis* (1.7–2.3 mg kg^{-1}) and *H. mammata* (2.5–3.7 kg^{-1}) showed higher concentrations of metals (Pb) than *H. forskali* (1.3–3.9 kg^{-1}). However, *H. mammata* had higher Pb levels than *H. arguinensis*. This difference may be due to the time of year the animals were collected, since these results coincide with those of the present study for the summer - *H. arguinensis* (1.39 ± 0.76); *H. mammata* (2.55 ± 1.70) (Table 6).

Among the metals evaluated, Pb showed the highest concentration in the three species analyzed, followed by Cd and Hg (Table 5). Skordas et al. (2024) reported similar Pb and Cd concentrations, for *Holothuria poli* and *H. tubulosa* from Greece.

The region where the animals were collected comprises the coastal zone between the mouth of the Sado Estuary and the Sesimbra city. Possible sources of Cd, Pb, and Hg contamination in this area are the discharge of chemical contaminants in and around the Sado Estuary, such as the Port of Setúbal, copper mines in the Sado basin, and intensive agriculture, mainly rice cultivation and fish farming (Freitas et al., 2007). Despite this intense activity around the Sado Estuary, a study by Caeiro et al. (2005) have measured sediment contamination along the gradient at 153 sampling points and exhibit that only 3% were highly contaminated for Pb (50.1–69 mg kg^{-1}), Cd (6.1–8 mg kg^{-1}), and Hg (0.61–0.7 mg kg^{-1}). The study recently conducted by Almeida et al. (2023) shows the same proportion for Cd, Hg, and Pb in the water of the Sado Estuary, beging the highest average concentrations found for Pb (0.3705 μg/L), followed by Cd (0.242 μg/L), and lastly Hg (0.01 μg/L).

Therefore, the higher Pb values and lower Hg values found in both the sediment and the water of the estuary may be the best interpretation, reflecting the same proportion of these elements in the organisms analyzed. However, mainly due to cleaner technologies and improvements in industrial wastewater treatment, it is expected that the average concentrations of Cd, Pb, and Hg in the Sado Estuary and coastal area will decrease and/or stabilize at low concentrations (Moreira et al., 2009). In the last four decades, Pb has decreased in concentration by 83% and Cd by 11% (Almeida et al., 2023).

3.3. Metal concentration vs season

The second most significant factor in the concentration of metals in sedimentary animals appears to be seasonal variation (Warnau et al., 2006), preceded by variation in body compartments (tissues), which will discussed later, followed by geographic, bathymetric, and foraging behavioural factors already analyzed previously.

In this sense, seasonal variations are related to variations in the concentration of metals in the tissues of marine animals due to several factors. These factors may include increased flow during rainy seasons and the recirculation of marine sediments, which serve as natural repositories of mineral elements, or the flow of these elements to other

Table 6
Cd, Hg and Pb (mg/ kg^{-1}, dry weight) in sea cucumber caught from Sesimbra Portugal for one year. Mean ± SD (standard deviation). Different letters in the same line for each species, indicate significant differences ($p <$ 0.05).

Trace elements	*H. arguinensis*				*H. forskali*				*H. mammata*			
	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter
Mercury												
Mean ± SD	0.13 ± 0.07^{a}	0.08 ± 0.05^{a}	0.10 ± 0.06^{a}	0.09 ± 0.05^{a}	0.13 ± 0.08^{a}	0.05 ± 0.02^{b}	0.05 ± 0.03^{b}	0.05 ± 0.02^{b}	0.17 ± 0.10^{a}	0.15 ± 0.11^{a}	0.19 ± 0.13^{a}	0.18 ± 0.13^{a}
Median	0.13	0.08	0.07	0.08	0.14	0.05	0.05	0.05	0.12	0.10	0.18	0.15
Range	$<$ LoQ-0.29	$<$ LoQ – 0.12	$<$ LoQ – 0.20	$<$ LoQ – 0.15	$<$ LoQ – 0.23	$<$ LoQ – 0.07	$<$ LoQ – 0.10	0.05–0.07	$<$ LoQ – 0.34	$<$ LoQ – 0.38	$<$ LoQ – 0.36	$<$ LoQ – 0.47
Cadmium												
Mean ± SD	0.07 ± 0.04^{b}	0.13 ± 0.08^{a}	0.13 ± 0.09^{a}	0.05 ± 0.03^{b}	0.07 ± 0.02^{c}	0.09 ± 0.01^{bc}	0.10 ± 0.03^{b}	0.13 ± 0.08^{a}	0.22 ± 0.07^{a}	0.21 ± 0.08^{ab}	0.17 ± 0.07^{b}	0.17 ± 0.04^{b}
Median	0.07	0.13	0.11	0.03	0.08	0.09	0.09	0.12	0.22	0.21	0.16	0.16
Range	$<$ LoQ – 0.14	$<$ LoQ – 0.21	$<$ LoQ – 0.27	$<$ LoQ – 0.10	$<$ LoQ – 0.08	0.07–0.11	0.07–0.14	$<$ LoQ – 0.31	0.10–0.4	0.13–0.30	0.06–0.25	0.13–0.24
Lead												
Mean ± SD	5.43 ± 4.42^{a}	1.39 ± 0.76^{c}	1.69 ± 0.62^{c}	3.36 ± 2.48^{b}	0.15 ± 0.03^{a}	0.15 ± 0.03^{a}	0.29 ± 0.05^{b}	0.15 ± 0.08^{a}	1.20 ± 0.99^{c}	2.55 ± 1.70^{a}	1.43 ± 0.81^{c}	1.90 ± 1.32^{b}
Median	4.29	1.20	1.56	2.48	0.15	0.15	0.31	0.15	0.74	2.67	1.22	1.46
Range	1.44–12.01	$<$ LoQ – 2.70	0.97–2.51	1.27–7.51	$<$ LoQ – 0.18	$<$ LoQ – 0.18	$<$ LoQ – 0.33	$<$ LoQ – 0.24	$<$ LoQ – 3.36	0.75–5.55	0.61–2.46	0.78–4.01

areas due to increased water flow from the continent, as normally occurs in coastal areas (Smedley and Kinniburgh, 2002).

In this study, in general, *H. arguinensis* and *H. forskali* showed the same pattern throughout the year, with the lowest concentrations observed in summer, followed by autumn, winter, and spring. The pattern presented by *H. mammata* showed lower metal levels in spring, followed by autumn, winter, and summer (Table 6). Similar results were found for work carried out by Karadurmus et al. (2025) where this order was present for Cd and Hg in *Parastichopus regalis* from Turkey.

The concentration of Pb showed intra-annual variation with significant differences between seasons for *H. arguinensis* ($p = 0.01$) and *H. mammata* ($p = 0.02$), but not for *H. forskali* ($p = 0.9$) (Table 6).

Regarding Cd concentration, significant intra-annual variation was observed among the species *H. arguinensis* ($p = 0.00$) and *H. mammata* ($p = 0.01$) and *H. forskali* ($p = 0.04$). Total Hg was the most homogeneous metal throughout the year, generally showing no variations, *H. arguinensis* ($p = 0.19$) and *H. mammata* ($p = 0.86$) and *H. forskali* ($p = 0.08$) among seasons (Table 6).

For the period-analyzed (2018/2019), precipitation appears to have influenced the metal content in the animals, since periods of higher precipitation were followed by periods of greater accumulation of these metals in the animals (Fig. 2). The spring (March, April, and May), summer (June, July, and August), and autumn (September, October, and November) of 2018 were characterized as "*normal*" in terms of precipitation in Portugal. However, the winter (December 2018, January, and February) of 2019 was classified as extremely dry with below-normal precipitation, highlighting these months with very significant negative anomalies (IPMA, 2019, 2020). Under average annual rainfall conditions for winter, we would have an increase in metal concentrations in the spring. However, further multi-year monitoring studies are recommended to clarify the influence of metal levels in animals, water, and sediments over long periods.

To the best of our knowledge, there are no studies about the variation of metals in sea cucumbers and seasons at the area analyzed. However, a study carried out by Rehan et al. (2023) (July to August, summer 2018) with *H. arguinensis*, *H. forkali* and *H. mammata* at sampling points adjacent to the same region, showed similar concentrations of Cd and Pb for *H. arguinensis* and *H. mammata*, although slightly higher for *H. forskali*.

3.4. Metal concentration vs sex and tissue of sea cucumber

Sex of the species showed influence the affinity of metals in animal tissues. Although it is known that females frequently present higher levels of contamination than males, no clear pattern of variation in the concentration of the three metals were found in this work (Fig. 3). These results are in agreement with studies of other marine species, that report significant differences between males and females, thought the reasons for these differences are not fully understood (Bat et al., 2012; Lischka et al., 2020). Other factors than a hots pot effect were likely responsible for the observed sex differences in metals concentrations. It is suggested that the variation between males and females can be better explained to the tissue, where the analyzed metals show an affinity for tissues rich in mucopolysaccharides (Warnau et al., 2006). Although the captured animals were within a standardization of size and weight, the difference between individuals cannot be ruled out regarding of the mucopolysaccharides metabolic rate (reproductive, growth, disease, *etc.*) that directly influences the concentration rate of metals in tissues cannot be ruled out. Further analyses may be carried out in other years for a better understanding of the observed differences.

In general, the results presented here showed a significant higher concentration of Cd in females than males; on the other hand, Pb and Hg were present in greater quantities in males than females (Fig. 3).

Significant differences were found in Cd and Pb for *H. arguinensis* and *H. mammata*, but not for *H. forskali*. No significant differences ($p = 6.08$) were found in Hg concentration between males and females of the three species. As the Pb values for *H. forskali* were low close to the limit of detection (LoQ), it was not possible to verify the aforementioned differences for this species (Fig. 3). However, no significant differences were observed for Hg and Pb between male and female *H. forskali*.

At the tissue level, a higher concentration of metals was observed in muscle bands than body wall (Fig. 3). High variability in metal levels in the muscle bands was also observed throughout the seasons (Fig. 4). This result may indicate that the muscle bands function as a temporary reservoir of metals, probably due to faster incorporation into cells resulting from higher cellular energy expenditure (Gashkina, 2024). The concentration in the body wall is lower and more stable; therefore, these levels may be more permanent and better describe the metal levels in sea cucumber species.

A study by Tunca et al. (2016) analyzed the body wall and viscera of three species of Mediterranean Sea cucumbers (*H. polii*, *H. tubulosa*, and *H. mammata*) and found that the highest concentrations are in the viscera. Other studies also report that the highest levels of metals are found in the viscera of sea cucumbers (Liu et al., 2016; Lin et al., 2018; Tamburini et al., 2022), with the intestine being the most relevant due to its role in solubilization and bioaccumulation (McAloon and Mason, 2003).

A possible explanation for the lower concentrations of metals and the

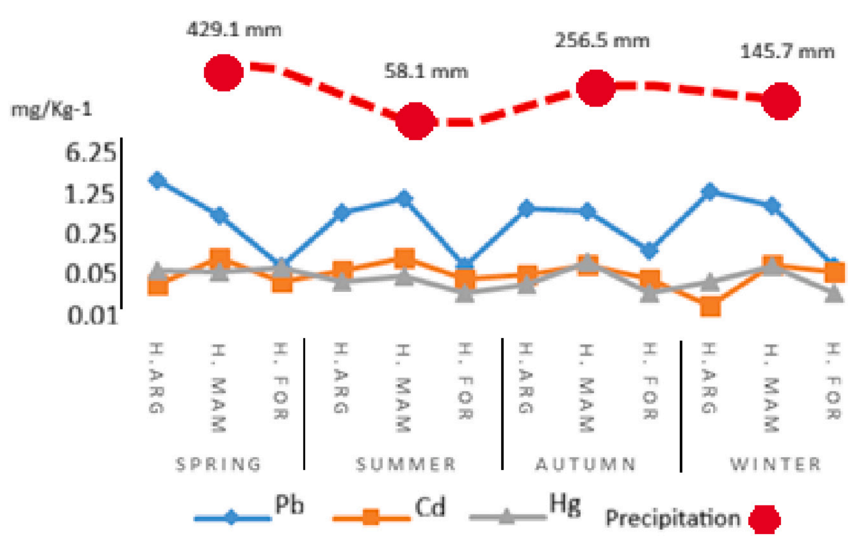

Fig. 2. Distribution of the species and metals (mg/ Kg^{-1}) along de year with precipitations average in 2018 (spring, summer and autumn) and 2019 (winter).

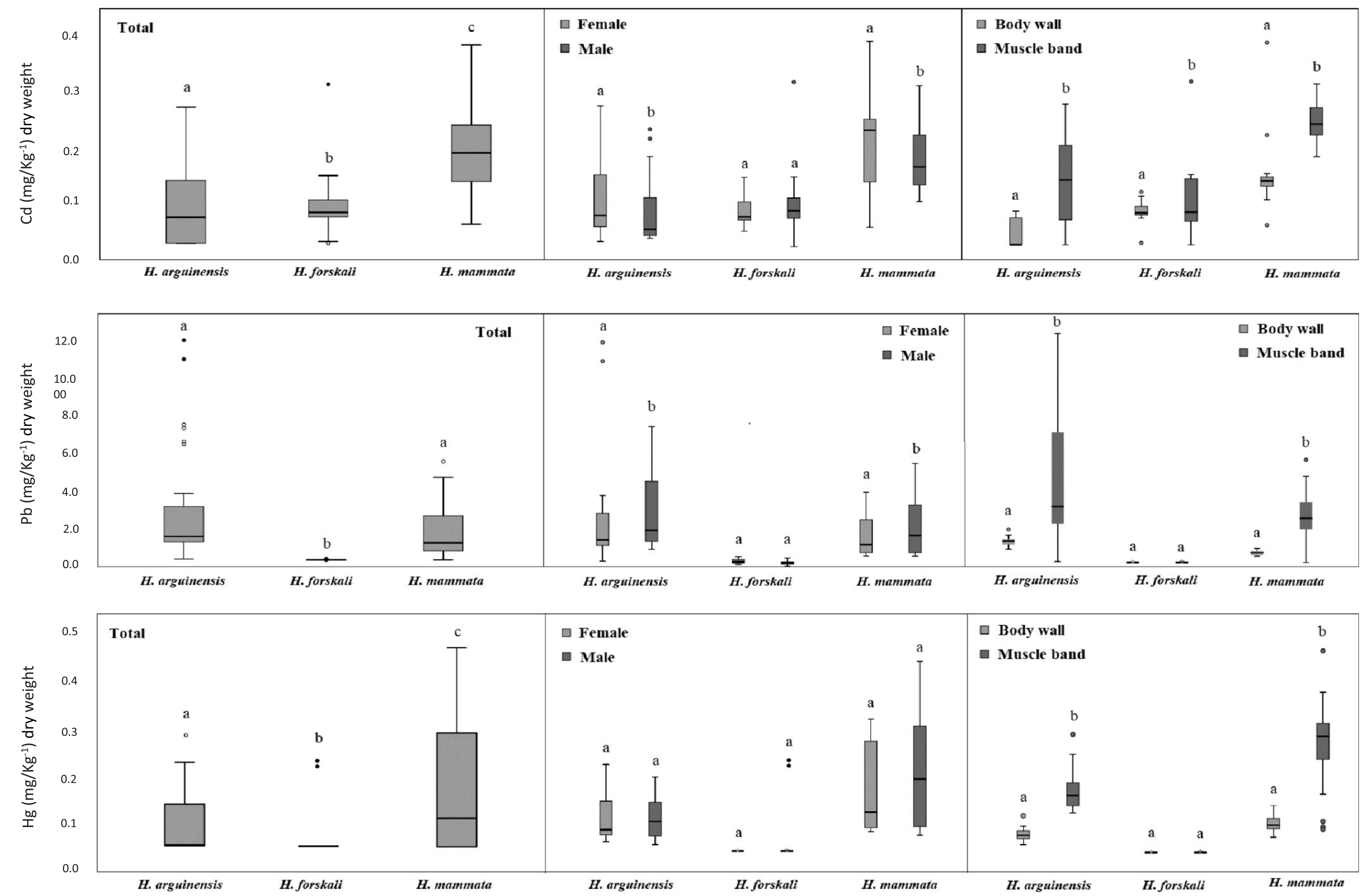

Fig. 3. Box plots of total Hg concentrations by *a)* species, *b)* sex for each species, *c)* body part, *d) H. arguinensis* for seasons and tissues, *e) H. forskali* for seasons and tissues, *f) H. mammata* for seasons and tissues. Each box plot presents the median, the 1st and 3rd quartiles, the maximum and the minimum. The circles correspond to the outliers and the black circles/colourfully are the extreme outliers. Total: same letters mean no significant differences between species ($p > 0.05$), different letters mean significant differences between species ($p < 0.05$); Sex (female and male): same letters between species means that there were no differences between sex ($p > 0.05$), different letters means that there significant differences between sex ($p < 0.05$); Tissue (body wall and muscle band): same letters between species means that there were no differences between tissues ($p > 0.05$), different letters mean that there significant differences between tissues ($p < 0.05$).

different profiles in the body walls may be the fact that muscle tissue contains a limited number of metal-binding proteins (Guner, 2007). Although the metal content was lower in the body wall of the sea cucumber, this tissue comprises 58 to 81% of the total amount of most metals, as it represents 85 to 90% of the entire body (Givianrad et al., 2014). These values may therefore be more representative than those found in the viscera (Liu et al., 2016).

3.5. Comparison with literature

Overall, the concentrations of Cd, Pb and Hg reported from several sea cucumbers species and regions worldwide are below the limits recommended by the European Union (Table 1 - Material Supplementary).

The oldest European study on the metal concentration in sea cucumbers was conducted by Augier et al. (1979), who verified the concentration of Hg in *H. forskali* from the Mediterranean Sea in the region of Marseille-France. It is interesting to note that only the result of one region was above the recommended limit (Calanques de Marseille - 2.90 mg kg^{-1}), the others showing levels below the recommended ones (Marseille Golf, Baie d Port-Cros, Port des Lecques, Port de la Ciotat, Cap d'Antibes - 0.31 mg kg^{-1}).

Most sea cucumbers species across different regions of the world exhibit low concentrations of contaminant metals. These findings are consistent with reported contaminant levels in other species of echinoderms, which also have lower metal contamination values than those observed in other marine animals (Shulkin et al., 2003; Mendil et al., 2010; Noël et al., 2011; Rocha et al., 2018; Branciari et al., 2020). A possible explanation for the results of the low metal content in sea cucumbers may be related to their low mobility, consequently, lower nutritional and O_2 requirements that are taken directly from the substrate or filtered from the water column.

Although sea cucumbers occur in areas with high metal concentrations, in the Atlantic coastal waters of Western Europe (Portugal), where *H. arguinensis*, *H. forskali* and *H. mammata* are found, the Hg index in commercially important fish species remais well below the limits recommended by the European Food Safety Authority and the World Health Organization for Food Safety (Costa et al., 2019).

3.6. Maximum allowable daily intake rate (CR_{lim})

Table 7 presents the maximum permitted daily intake rate (CR_{lim}) for *H. arguinensis*, *H. forskali*, and *H. mammata* (CR_{lim}). In general, the maximum permitted consumption rate of sea cucumbers from Sesimbra, in terms of Hg, ranged from 46.76 to 605.71 g/day, from 2.32 to 93.16 g/day for Pb, and from 78.95 to 300.00 g/day for Cd.

In general, consumption of these species caught in the Sado Estuary are a low risk for adults and children, since the quantity needed to reach the maximum limits is high for Cd and Hg. On the other hand are not recommended considering the high levels of Pb the exposure to *H. arguinensis* and *H. mammata*. Nevertheless, additional factors should be taken into account, including the bioacessibility, frequency of ingestion these metals, cooking procedures and, other indices for

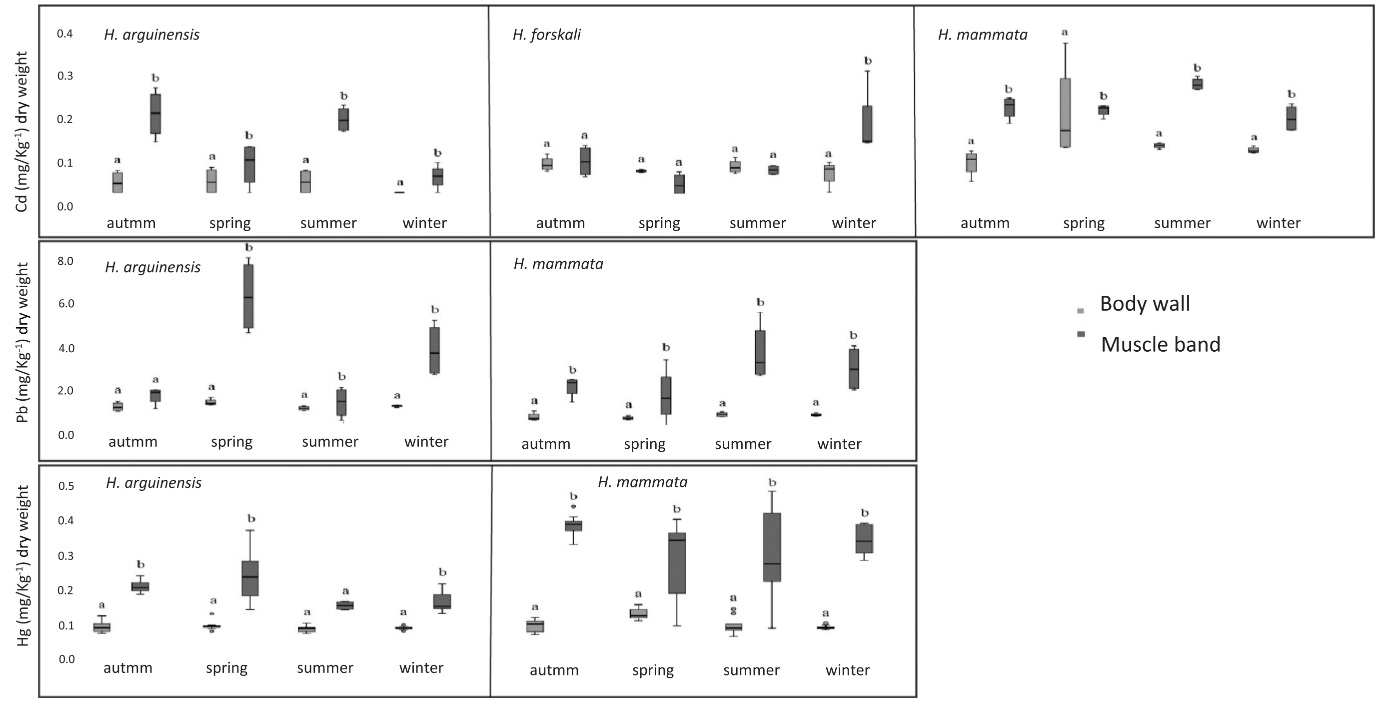

Fig. 4. Blox plots of cadmium, lead and mercury for the tissues (body wall and muscle) of sea cucumber *H. arguinensis*, *H. forskali* and *H. mammata* in season (autumn, spring, summer and winter). Each box plot presents the median, the 1st and 3rd quartiles, the maximum and the minimum. The circles correspond to the outliers. Same letters between element and species means that there were no differences between tissues ($p > 0.05$). Different letters means that there significant differences between tissue ($p < 0.05$).

Table 7
Maximum allowable daily consumption rate (CR_{lim}) of *H. arguinensis*, *H. forskali* and *H. mammata* by different age groups.

Metal	Concentration (mg/ Kg^{-1})	RfD[a] /BMDL[d,e,f] (mg/ Kg^{-1} BW/day)	CR_{Lim} (g/day)	
			Children[b]	Adults[c]
H. arguinensis				
Hg	0.10	5.7 × 10–4[a]	79.50	424.00
Pb	2.97	[d]6.3 × 10–4/[e]1.5 × 10–3/[f]5 × 10–4	2.32[f]	1.21[d]-5.96[e]
Cd	0.10	0.001	150.00	300.00
H. forskali				
Hg	0.07	5.7 × 10–4[a]	113,57	605.71
Pb	0.19	[d]6.3 × 10–4/[e]1.5 × 10–3/[f]5 × 10–4	36.32[f]	18.95[d] -93.16[e]
Cd	0.10	0.001	150.00	300.00
H. mammata				
Hg	0.17	5.7 × 10–4[a]	46.76	249.41
Pb	1.77	[d]6.3 × 10–4/[e]1.5 × 10–3/[f]5 × 10–4	3.90[f]	2.03[d] -10.00[e]
Cd	0.19	0.001	78.95	157.89

Values for Pb: [d] $BMDL_{10}$ for nephrotoxicity (adults), [e] $BMDL_{01}$ for cardiovascular effects (adults), and [f] $BMDL_{01}$ for developmental neurotoxicity (children) EFSA (2010).
[a] Values of oral reference dose (RfD) U.S. EPA (2015) and WHO (2018).
[b] BW = 15 kg (U.S. EPA, 2025).
[c] BW = 80 kg (U.S. EPA, 2025).

assessing risk to human health (Liu et al., 2018; Sales et al., 2023).

Similar values were found in other studies, for example, conducted by Marrugo-Negrete et al. (2020) which assessed the potential risk to human health of five metals in *Holothuria floridana* on the Colombian/ Caribbean coast. With the exception of Pb, Cd and Hg results were similar, where the recommended daily intake values for *H. arguinensis*, *H. forskali* and *H. mammata* for the group of children should be much lower than those found for *Holothuria floridana*.

As study carried out by Karadurmus et al. (2025) showed that the group most susceptible to health risk due to sea cucumber consumption, consists of individuals who consume it most frequently. In particular, those who consume sea cucumbers more than five times per week, especially in East Asia, present a higher risk of consumption associated with these metals. Although there is no increased risk an increase in daily consumption (depending on the biomass/weight of the animal) may lead to an increased risk of exposure to Cd and Hg.

4. Conclusions

The concentration of Cd, Pb, and Hg varied among the studied species. Significant differences were also observed between sex, tissue and season of sea cucumbers of these metals. In general, the risk of consuming these species is low for Cd and Hg, and the safe amount that can be consumed by children and adults, based on the maximum concentrations of each metal, is high. However, for Pb, the risk of consuming are high, making them unsuitable for children and adults. Further studies should be conducted in other regions to verify whether the patterns found here hold true. This study is important not only from a public health perspective but also for the identification and quantification of the main metals in sea cucumber species, as this data is scarce in Europe. The data presented here can serve as a basis for the future development of regulations to set that limit the maximum concentrations of metals in echinoderm consumption.

CRediT authorship contribution statement

Sabrina S. Sales: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mercedes González-Wangüemert:** Writing – review & editing, Conceptualization. **Narcisa M. Bandarra:** Writing – review & editing, Conceptualization. **André Breves:** Writing – review & editing, Conceptualization. **Helena M. Lourenço:** Validation, Supervision, Methodology.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Sabrina Sales reports was provided by Portuguese Institute for the Sea and Atmosphere. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2026.120017.

Data availability

Data will be made available on request.

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