



Interspecific and Size-related Differences in Cadmium Accumulation in Gill Tissues of Three Marine Fish Species from the Adriatic Sea (Albania)

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ABSTRACT

Background: Fish gills, due to their direct contact with water, represent a primary site of metal uptake and are sensitive indicators of environmental metal exposure. This study aimed to quantify cadmium (Cd) concentrations in the gill tissues of three ecologically and commercially important marine fish species (*Solea solea*, *Dicentrarchus labrax* and *Mugil cephalus*) collected from the Adriatic Sea near the Ishëm River estuary (Albania) and to evaluate interspecific and size-related differences in cadmium accumulation.

Methods: Fish samples were collected between February and April 2023 and classified into juvenile (<150 g) and adult (>150 g) size classes. Cadmium concentrations in gill tissues, seawater and marine sediments were determined by graphite furnace atomic absorption spectrometry (GFAAS). Statistical analyses were performed using heteroskedasticity-robust linear models (HC3), robust Wald tests, Welch's tests and Games-Howell post hoc comparisons.

Result: Cadmium concentrations in seawater were below the limit of quantification (<0.07 µg/L), whereas marine sediments contained measurable Cd concentrations (50±5.4 µg/kg dry weight). Significant interspecific differences in gill Cd concentrations were observed, with *Solea solea* exhibiting the highest concentrations, *Mugil cephalus* intermediate concentrations and *Dicentrarchus labrax* the lowest overall concentrations. Although the overall effect of fish size was not significant, size-related differences varied among species, resulting in a significant species × size interaction. These findings demonstrate that fish gills are sensitive biomarkers of cadmium exposure and provide baseline data for future environmental monitoring of the Adriatic coastal ecosystem influenced by riverine pollution.

Key words: Adriatic sea, Bioaccumulation, Cadmium, Environmental contamination, Gill tissue, Marine fish.

INTRODUCTION

Cadmium (Cd) is a non-essential heavy metal of major environmental concern due to its persistence and toxicity (Caress and Steinemann, 2003; Järup and Åkesson, 2009). Unlike essential trace elements, cadmium has no physiological role and can exert adverse biological effects even at very low concentrations by inducing oxidative stress, disrupting cellular metabolism and interfering with enzymatic activity (Bhardwaj *et al.*, 2022; Liu *et al.*, 2009). Cadmium enters aquatic systems through both natural processes and human activities. Natural inputs include rock weathering and volcanic activity, whereas anthropogenic sources include mining operations, industrial effluents, phosphate fertilizers, urban wastewater discharges and atmospheric deposition (Alloway, 2013; Birch, 2018; Nriagu and Pacyna, 1988; Tchounwou *et al.*, 2012). In coastal ecosystems, river estuaries play a critical role in transporting cadmium from terrestrial catchments to marine environments, where the metal can partition between the dissolved phase and bottom sediments (Förstner and Wittmann, 1981). Sediments often act as long-term sinks for cadmium, but resuspension processes can reintroduce bioavailable fractions into the water column (Chapman and Wang, 2001; Förstner and Salomons, 1980). Fish are widely used as bioindicators because they accumulate metals in specific tissues (Ahmed *et al.*, 2016; Authman, 2015; Burger, 2006). Among fish

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organs, gills represent a key interface between the organism and the aquatic environment. They play essential roles in respiration, osmoregulation and ion regulation and are continuously exposed to the surrounding water, making them a primary route for the uptake of dissolved metals (Evans *et al.*, 2005; Hollis *et al.*, 1999). Cadmium uptake across fish gills occurs mainly via calcium transport pathways because of the chemical similarity between Cd²⁺ and Ca²⁺ ions (Niyogi and Wood, 2004; Roesijadi and Unger, 1993).

Once absorbed, cadmium can induce structural and functional damage to gill epithelia, impair gas exchange and disrupt ionic balance, ultimately affecting fish health and survival (Lakshmi *et al.*, 2024; Thophon *et al.*, 2003). As a result, gill tissue is considered a sensitive biological matrix for assessing metal exposure (Souid *et al.*, 2013). Cadmium accumulation in fish is influenced by a range of biological and ecological factors, including species, body size, age, trophic level, feeding strategy and habitat preference (Dural *et al.*, 2006; Squadrone *et al.*, 2013). Benthic and demersal species generally exhibit higher cadmium concentrations due to their close association with contaminated sediments, whereas pelagic or detritivorous species tend to reflect exposure primarily to dissolved metal fractions (Luoma and Rainbow, 2005; Marzali *et al.*, 2021). The Adriatic Sea, particularly along the Albanian coastline, is vulnerable to heavy metal contamination as a result of riverine inputs and insufficient wastewater treatment infrastructure (Pan and Wang, 2012). The Ishëm River is considered one of the most polluted rivers in Albania and represents an important source of metal discharge into the adjacent marine environment (Keci, 2021). Despite its ecological significance, data on cadmium accumulation in marine fish from this region remain limited (Kljaković-Gašpić *et al.*, 2002). Therefore, the objectives of the present study were to (i) quantify cadmium concentrations in the gill tissues of three marine fish species (*Solea solea*, *Dicentrarchus labrax* and *Mugil cephalus*) collected near the Ishëm River estuary and (ii) assess interspecific and size-related differences in cadmium accumulation in relation to cadmium levels measured in seawater and marine sediments.

MATERIALS AND METHODS

Study area and sample collection

The study was conducted in the coastal zone near Shetaj village, Durrës, Albania, in proximity to the estuary of the

Ishëm River (Fig 1). The sampling area is located at approximately 41°36'36.8"N and 19°29'48.8"E. Seawater, sediment and fish samples were collected from the same coastal area near Kepi i Rodonit, close to the Ishëm River estuary. Fish were obtained from the local fishing zone within an offshore area of approximately 3 km, whereas seawater and sediment were collected at the marine station shown in Fig 1. The Ishëm River drains a catchment area of approximately 673 km², flowing through urban, agricultural and industrial zones before discharging into the Adriatic Sea. Due to limited wastewater treatment and industrial regulation, the river is considered an important source of heavy metal contamination to the adjacent marine environment (Keci, 2021).

Sample collection

Fish samples were collected between February 2023 and April 2023 from local fishermen operating within approximately 3 km of the central sampling location shown in Fig 1. The specimens were purchased directly from local fishermen upon landing. Sampling depended on fishing activity and target species availability throughout the study period. The study focused on three marine fish species with distinct ecological characteristics: *Solea solea*, a benthic feeder, *Dicentrarchus labrax*, a predatory demersal species and *Mugil cephalus*, a detritivorous species. Fish were categorized into two size classes, juvenile individuals under 150 g and adult individuals over 150 g. The 150 g threshold was selected to create two clearly distinguishable size groups and to ensure adequate representation of both smaller and larger individuals within each species. For each species and size class, six composite samples were prepared, each consisting of seven individuals, resulting in 42 fish per species and size class and 252 fish in total. Composite sampling was used to obtain analytically sufficient tissue mass and to reduce the influence of individual biological variability on estimates of mean Cd

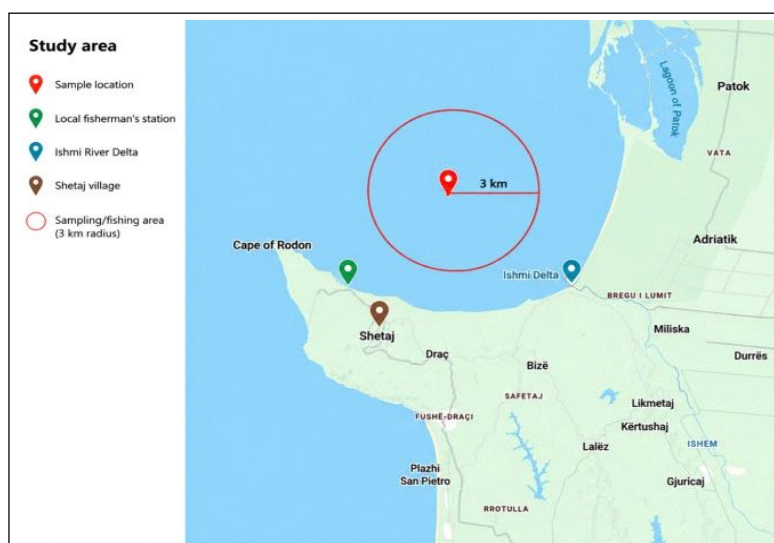


Fig 1: Study area and sampling locations for fish, seawater and marine sediment.

concentration (Luoma and Rainbow, 2005; Marzali *et al.*, 2021). Gill tissues were excised using stainless steel instruments, rinsed with deionized water to remove external debris and stored at 4°C until analysis. Seawater and marine sediment samples were collected from the marine station shown in Fig 1 using pre-cleaned polyethylene containers to assess environmental cadmium levels.

Sample preparation and cadmium analysis

Fish gill samples were prepared and analyzed at the Department of Pharmacology, Toxicology and Environmental Protection, University of Life Sciences in Lublin, Poland, using the following analytical procedure. Approximately 3 g of fresh gill tissue (wet weight) were accurately weighed and placed into ceramic crucibles. Samples were pre-dried on a hot plate at 100°C to remove residual moisture and subsequently incinerated in a muffle furnace at 435°C for 8 h. After cooling, 5 mL of 30 wt. % hydrogen peroxide (H₂O₂) was added to each crucible to facilitate further oxidation of residual organic material. The samples were heated on a hot plate at 75°C and then returned to the muffle furnace at 435°C until complete mineralization was achieved. The resulting inorganic ash was dissolved in 10 mL of 65 wt.% nitric acid (HNO₃) and quantitatively transferred to a 50 mL volumetric flask through a 0.45 µm membrane filter. Each flask was filled to the mark with deionized water and thoroughly mixed.

Seawater samples were filtered directly through 0.45 µm membrane filters immediately after sampling to remove suspended particulate matter and they were acidified by adding 65 wt. % HNO₃ (the final concentration of nitric acid in the samples was 0.1 mol L⁻¹). The prepared samples were analyzed without further digestion, allowing determination of dissolved cadmium concentration.

Marine sediment samples were air-dried at room temperature until constant weight was achieved, homogenized and cleared of coarse debris. A subsample of 1 g (dry weight) was heated with 32 mL of the aqua regia (8 mL of 65 wt. % HNO₃ and 24 mL of 35 wt. % HCl) at 100°C for 2 hours. The extract was transferred through a 0.45 µm membrane filter to the volumetric flask and filled up to the final volume (50 mL) with the deionized water.

Cadmium concentrations in gill tissue digests, seawater and sediment extracts were determined using graphite furnace atomic absorption spectrometry (GFAAS, SpectrAA 220Z, Varian, Australia) equipped with an electrothermal atomizer and a Zeeman background correction system. Measurements were performed at a wavelength of 228.8 nm using a cadmium hollow cathode lamp (Varian, Australia) operated at 4 mA. A volume of 20 µL of sample solution and 5 µL of a palladium-magnesium (Pd/Mg) chemical modifier (0.3 g Pd and 0.9 g Mg per litre) were injected into the graphite furnace. The temperature programme included a pyrolysis step at 600°C followed by atomization at 1600°C. The spectral slit width was set to 0.5 nm. Calibration was performed using a series of aqueous Cd standard solutions. Each

analytical result was calculated as the mean of three instrumental replicates. The accuracy and precision of the analytical procedure were verified using the certified reference material DORM-3 (NRC Canada). The following analytical parameters were obtained: recovery, 90%; repeatability, 5.3%; linearity range of the calibration curve, 0.1-1.0 µg L⁻¹; limit of detection, seawater 0.02 µg L⁻¹, sea sediment 0.02 µg L⁻¹, fish gills 0.03 µg L⁻¹ and limit of quantification, seawater 0.07 µg L⁻¹, sea sediment 0.08 µg L⁻¹, fish gills 0.10 µg L⁻¹.

Statistical analysis

Statistical analyses were performed using Jamovi software version 2.7.26. Cadmium concentrations are presented as mean±standard deviation (SD) for each composite sample. Preliminary assumption checks included the Shapiro-wilk test for normality of residuals, Levene's test for homogeneity of variances and graphical inspection using Q-Q plots and boxplots. Because Levene's test indicated heterogeneity of variances among groups, inference was based on an ordinary least squares linear model with species, size and their interaction, using heteroskedasticity-consistent HC3 standard errors. The significance of model terms was evaluated with robust Wald tests. To aid interpretation of the interaction, size classes were compared within each species using Welch's t-tests and species were compared within each size class using Welch's one-way ANOVA followed, where appropriate, by Games-Howell post hoc comparisons. Statistical significance was set at p<0.05. The composite sample (n=6 per species-size group) was considered the experimental unit for all statistical analyses. Instrumental triplicate measurements were used exclusively for analytical quality control and were not treated as independent observations.

RESULTS AND DISCUSSION

Cadmium (Cd) concentrations in the gill tissues of the studied fish species are presented in Table 1 as mean values of composite samples. Values are presented as mean±SD of instrumental triplicate measurements for each composite sample. Statistical analyses were performed using the composite samples as independent observations. Clear differences were observed among species and, in some cases, between size classes. *Solea solea* showed the highest Cd concentrations overall, whereas *Dicentrarchus labrax* had the lowest values in the large-size class. *Mugil cephalus* showed intermediate concentrations.

Preliminary assumption checks indicated approximate normality of residuals according to the Shapiro-wilk test (Table 4; p= 0.240), whereas Levene's test indicated significant heterogeneity of variances among groups (Table 3; p<0.001). Therefore, inferential analysis was based on a heteroskedasticity-robust linear model with HC3 standard errors. Robust Wald tests showed a significant effect of species on Cd concentrations and a significant species × size interaction, whereas the overall main effect of size

was not significant (Table 2). To interpret the interaction, size classes were compared within each species using Welch's t-tests. No significant size-related difference was detected for *Solea solea* ($p = 0.629$), whereas Cd concentrations were lower in small than large *Mugil cephalus* composites ($p = 0.045$) and higher in small than large *Dicentrarchus labrax* composites ($p = 0.008$).

Among large fish, species differed significantly according to Welch's ANOVA ($F_{2,7.06} = 27.23$, $p = 0.00048$). Games-Howell post hoc comparisons (Table 5) showed that *Solea solea* had significantly higher Cd concentrations than *Mugil cephalus* ($p = 0.0176$) and *Dicentrarchus labrax* ($p = 0.0030$), whereas *Mugil cephalus* also showed higher concentrations than *Dicentrarchus labrax* ($p = 0.0116$).

The Q-Q plot (Fig 2) indicated approximate normality of residuals. The interaction between species and size is shown in Fig 3. The lines are not fully parallel, which is consistent with the significant species $\times$ size interaction detected in the robust model. This indicates that the direction and magnitude of size-related differences were not the same across the three species.

The distribution of Cd concentrations across species and size groups is illustrated in Fig 4. The boxplot highlights marked differences among species in the large-size class and considerable variability in small *Solea solea* composites, which contributes to the lack of a clear overall size effect.

Environmental measurements are summarized in Table 6. Cadmium in seawater was below the limit of quantification ($<0.07 \mu\text{g/L}$), while marine sediments contained measurable levels of Cd ($50 \pm 5.4 \mu\text{g/kg}$ dry weight). These findings confirm the presence of cadmium in the study area, primarily in the sediment fraction, but the limited number of environmental samples does not allow firm conclusions regarding exposure pathways.

The present data show that Cd concentrations in fish gill composites differed among species, but size-related patterns were not uniform. After accounting for heterogeneity of variances by using a heteroskedasticity-robust model, the species effect remained significant and the species $\times$ size interaction also reached significance, whereas the overall main effect of size was not significant. Accordingly, the dataset does not support a blanket statement that body

size is irrelevant. Rather, it suggests that any size-related effect is species-dependent under the sampled conditions. Similar species-associated differences in metal accumulation have been reported previously in marine fish (Luoma and Rainbow, 2005). Among the investigated species, *Solea solea* showed the highest Cd concentrations overall. This pattern is consistent with, but does not by itself prove, an influence of benthic habitat use and closer contact with sediment-associated contaminants (Birch, 2018; Förstner and Salomons, 1980). The elevated Cd concentrations observed in *Solea solea* may be related to its benthic lifestyle and closer association with bottom sediments, which frequently act as long-term reservoirs of trace metals. Benthic fish are generally more exposed to sediment-associated contaminants through direct contact with the substrate and through feeding activities occurring at the sediment-water interface. Although the present study was not designed to determine exposure pathways directly, the detection of measurable Cd concentrations in marine sediments supports the possibility that sediment-associated exposure contributed to the observed accumulation patterns (Birch, 2018; Chapman and Wang, 2001; Förstner and Salomons, 1980; Lee *et al.*, 2024).

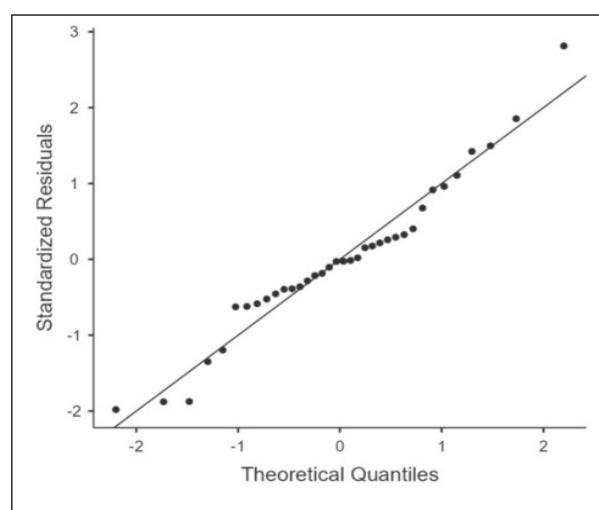


Fig 2: Q-Q plot of model residuals for assessment of normality.

Table 1: Concentration of Cd in gill tissues ($\mu\text{g/kg}$ wet weight).

Sample	Cd content in gills $\mu\text{g/kg}$ wet weight					
	<i>Solea solea</i>	<i>Solea solea</i>	<i>Mugil cephalus</i>	<i>Mugil cephalus</i>	<i>Dicentrarchus labrax</i>	<i>Dicentrarchus labrax</i>
	Large group	Small group	Large group	Small group	Large group	Small group
1	34.99 $\pm$ 0.94	14.70 $\pm$ 0.22	17.32 $\pm$ 0.35	17.14 $\pm$ 0.15	2.72 $\pm$ 0.01	18.15 $\pm$ 0.33
2	28.42 $\pm$ 1.56	14.66 $\pm$ 0.60	10.49 $\pm$ 0.59	6.08 $\pm$ 0.79	5.94 $\pm$ 0.08	8.91 $\pm$ 0.67
3	63.56 $\pm$ 2.54	13.48 $\pm$ 0.49	13.18 $\pm$ 0.47	4.11 $\pm$ 0.03	3.63 $\pm$ 0.17	26.73 $\pm$ 0.32
4	52.75 $\pm$ 1.69	68.71 $\pm$ 2.54	21.01 $\pm$ 1.24	12.32 $\pm$ 0.01	5.69 $\pm$ 0.20	11.10 $\pm$ 0.17
5	46.84 $\pm$ 1.26	53.54 $\pm$ 0.91	13.48 $\pm$ 0.01	6.88 $\pm$ 0.08	3.76 $\pm$ 0.05	19.40 $\pm$ 0.08
6	26.66 $\pm$ 0.43	52.69 $\pm$ 1.90	30.41 $\pm$ 1.64	9.58 $\pm$ 0.54	1.79 $\pm$ 0.03	9.62 $\pm$ 0.10

Values are presented as mean $\pm$ SD of instrumental triplicate measurements performed for each composite sample.

Mugil cephalus exhibited intermediate Cd concentrations and relatively low variability, particularly in the small-size class, whereas *Dicentrarchus labrax* showed low concentrations in the large-size class but higher values in the small-size class. They may reflect differences in feeding ecology and habitat use, although the underlying mechanisms remain uncertain. Because the data are based on composite samples rather than individual fish, the results should be interpreted as group-level differences in mean Cd burden rather than as evidence of individual biological responses.

Environmental measurements provide context but should not be overinterpreted. Cadmium was below the limit of quantification in seawater and measurable in marine sediment, which is compatible with sediment acting as a local reservoir of Cd (Birch, 2018; Förstner and Wittmann, 1981). However, the limited number of environmental samples precludes a robust assessment of transport, resuspension, or uptake pathways. Therefore, the present results support the use of gill tissue as a practical indicator of recent Cd exposure, but they do not establish the dominant source or mechanism of accumulation in this coastal system.

Several limitations should be acknowledged. First, the study relied on six composite samples per species and size class, which improves analytical representativeness but reduces information on individual variability. Second, the environmental component was limited and does not permit detailed source apportionment. Third, fish were collected over an extended period, so seasonal or temporal variability cannot be excluded. Future studies should include

more extensive environmental sampling and individual-level fish measurements to clarify cadmium dynamics more precisely in this coastal ecosystem. In addition, information regarding fish age, sex and reproductive status was not available and therefore the potential influence of these biological factors on Cd accumulation could not be evaluated.

Table 2: Heteroskedasticity-robust linear model results for cadmium (Cd) concentrations in fish gill tissues.

Robust wald tests (HC3)				
Term	F	df1	df2	p
Species	10.17	2	30	0.00042
Size	0.032	1	30	0.859
Species × Size	8.15	2	30	0.00149
Residual df			30	

Table 3: Levene's test for homogeneity of variances of cadmium (Cd) concentrations among species and size groups.

Homogeneity of variances test (Levene's)			
F	df1	df2	p
20.1	5	30	<.001

Table 4: Shapiro-wilk test for normality of model residuals.

Normality test (Shapiro-wilk)	
Statistic	p
0.962	.240

Table 5: Games-howell post hoc pairwise comparisons of species within the large-size class.

Games-howell post hoc comparisons, large-size class					
Species comparison	Mean difference	SE	df	t	p
<i>Solea solea</i> - <i>Mugil cephalus</i>	24.56	6.67	7.86	3.68	0.0176
<i>Solea solea</i> - <i>Dicentrarchus labrax</i>	38.28	6.02	5.12	6.36	0.0030
<i>Mugil cephalus</i> - <i>Dicentrarchus labrax</i>	13.73	3.03	5.50	4.52	0.0116

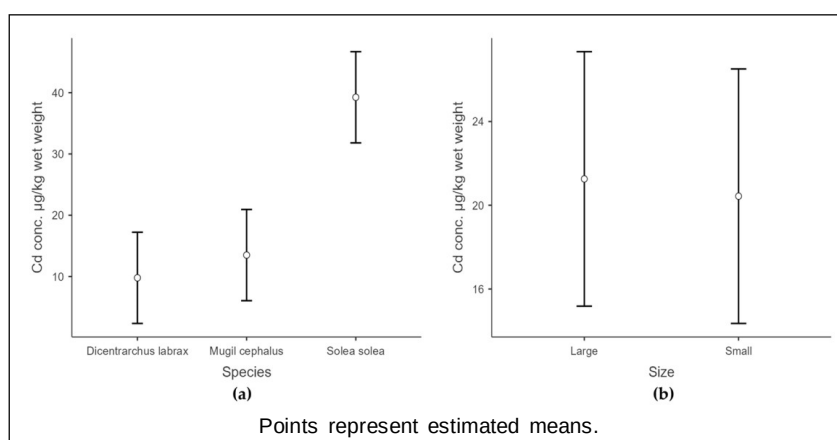


Fig 3: Estimated marginal means of cadmium (Cd) concentrations in fish gill tissues by (a) species and (b) size class based on the heteroskedasticity-robust linear model.

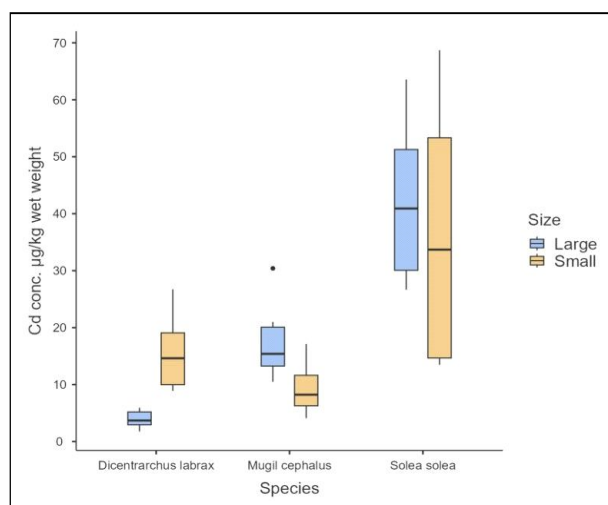


Fig 4: Distribution of Cd concentrations across fish species and size classes. Boxes represent interquartile range, whiskers represent variability and dots represent individual samples.

Table 6: Cd concentration in seawater and marine sediment.

Sample	Cd concentration
Seawater	<LOQ (<0.07 µg/L)
Marine sediment	50±5.4 µg/kg dry weight

CONCLUSION

This study showed species-related differences in cadmium concentrations in the gill tissues of three marine fish species collected near the Ishëm River estuary. After accounting for heterogeneity of variances, the species effect remained significant, whereas size did not show a consistent overall effect and size-related patterns varied among species. The data support species-related differences in mean gill Cd burden but do not establish the underlying mechanism. Cadmium was detectable in marine sediment but remained below the limit of quantification in seawater; however, the limited number of environmental samples precludes firm conclusions about exposure pathways. Overall, the study provides site-specific baseline data that may support future environmental monitoring in coastal areas influenced by riverine inputs.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and integrity of the data presented in this study.

Ethics statement

Not applicable. Fish specimens were obtained from commercial catches and no live animal experiments were conducted specifically for this study.

Conflict of interest

The authors declare that they have no conflicts of interest regarding the publication of this manuscript. The study was conducted independently and no funding agency or commercial organization influenced the study design, data collection, analysis, interpretation of the results, or preparation of the manuscript.

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