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Accumulation of trace metals in the digestive gland and muscle of the common octopus (*Octopus vulgaris*) from the northwestern Atlantic coast of Morocco

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ABSTRACT

The present study aimed to assess trace metal (TM) accumulation in the digestive gland and muscle of *Octopus vulgaris*, collected along the northwestern Atlantic coast of Morocco. Essential TMs (Cu and Zn) were the most abundant in tissues, with the following sequence: Cu > Zn > Cd > Cr in the digestive gland and Zn > Cu > Cr > Cd in muscle. In addition, TM concentrations were consistently and significantly higher in the digestive gland. For non-essential TMs, Cd was significantly more elevated in digestive glands than in muscle, with concentrations up to 702 times higher, and exhibited greater interindividual variability. Also, significant relationships were found in the digestive gland between Cd and Cu levels and the size of samples. In contrast, sex and maturity stage had no effect on TM content or distribution pattern among tissues. Metal ratios (Cd/Zn and Cd/Cu) in the digestive gland clearly correlated with biometric traits and TM levels, indicating a complex interactive behavior between these pollutants during the bioaccumulation process. These findings enhance our understanding of metal partitioning in *O. vulgaris* and highlight their potential as bioindicators.

1. Introduction

The intensification of industrialization and urbanization over the past few decades has resulted in the discharge of huge quantities of trace metals (TMs) into marine environments often without appropriate treatment. These TMs enter marine environments through direct input, atmospheric deposition, and soil erosion via runoff (Veena et al., 1997; Kojadinovic et al., 2007; Wang et al., 2013; Radhalakshmi et al., 2014; Qi et al., 2023). They accumulate in marine organisms such as fish, crustaceans, and bivalves (Schüürmann and Markert, 1998; Feng et al., 2020; Guendouzi et al., 2020) and tend to biomagnify through the food web (Sun et al., 2020; Aarif et al., 2023; Huang et al., 2024; Jishnu et al., 2024; Aarif et al., 2025). Elevated concentrations of TMs in water, sediments, and marine biota are highly hazardous, pose significant ecological risks and can adversely affect marine life (De Boeck et al., 2010; Storelli et al., 2011). TM loads in marine ecosystems have consequently received much attention, and their bioaccumulation levels in edible products such as fish and mollusks have raised serious concerns regarding food safety and human health. Monitoring these xenobiotics has become a top priority to assess the sanitary state of the marine environment and to provide effective tools for implementing suitable policies and conservation strategies to prevent potential irreversible damage.

TMs can be categorized into essential TMs (copper, zinc, iron, chromium, calcium, potassium, manganese, magnesium, sodium, phosphorus, fluoride, iodine, selenium, cobalt), which play important roles in biological functions of living organisms and are required in small levels (Hossen et al., 2015), and non-essential TMs (mercury, cadmium, lead, aluminum, nickel), which have uncertain biological functions and are potentially toxic even at low concentrations (Kamaruzzaman et al., 2011). Among metals of greatest concern in marine ecosystem are cadmium (Cd), chrome (Cr), copper (Cu), and zinc (Zn). Cu is a respiratory pigment in hemocyanin that makes up 98 % of blood proteins (Villanueva and Bustamante, 2006). The essential characteristics and

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functions of each element in cephalopods are not well understood (Villanueva and Bustamante, 2006). Cu accumulation has been shown to cause histopathological alterations in intestinal tissue, leading to malabsorption, which hinders the process of food intake and conversion (Yang et al., 2022; Zheng et al., 2023). At concentrations above safe limits, metals impact the functions of aquatic organisms by interfering with enzymes and cellular components (Copat et al., 2013). Studies have demonstrated that TMs can negatively impact the respiratory function (Chai et al., 1993), immune function (Anderson et al., 1989; Rougier et al., 1996), physiological and biochemical functions, and embryonic development of aquatic organisms (Zheng and Pu, 1997; Jia and Chen, 1998).

In Morocco, several species have been used for biomonitoring metal contamination in the marine environment, following the criteria outlined by Rainbow and Phillips (1993) and Zhou et al. (2008). Bivalve mollusks like mussels are commonly used to assess the spatiotemporal trends in metal pollution (Kouali et al., 2020, 2022a). However, relying on a single taxon may be problematic, as metal bioaccumulation varies by species due to ecological (e.g., diet, feeding behavior, seasonality) (Metian et al., 2013; Renieri et al., 2014) and physiological factors (e.g., metal uptake, detoxification) (Storelli et al., 2005; Penicaud et al., 2017). As a result, species from the same area can show different contamination levels, possibly skewing assessments. Using diverse bioindicator models can provide a more accurate picture of marine metal pollution (Rainbow, 1995).

The common octopus (*Octopus vulgaris*) is a benthic and cosmopolitan species that lives in shallow coastal waters and continental shelf areas of the tropical, subtropical, and temperate waters of the Atlantic, Indian, Pacific Oceans, and Mediterranean Sea. *O. vulgaris* typically inhabits rocky, sandy, and muddy seabeds, ranging from the intertidal zone to the edge of the continental shelf, a depth of 200 m (Mangold, 1983; Belcari et al., 2002). Its diet consists of crustaceans, teleost fish, mollusks, and polychaetes (Smith, 2003). This species is semelparous, characterized by post-spawning mortality (Faraj and Bez, 2007). *O. vulgaris* is most abundant in the Atlantic waters off Morocco, particularly along the Safi coastline. This abundance is related to the upwelling system (Balguerías et al., 2002). Due to their predator-prey role in a marine benthic ecosystem, sedentary lifestyle, short life span, capacity to adapt to changing environmental demands (Mangold, 1983), *O. vulgaris* possesses potential as a bioindicator of marine environment quality (Boyle and Knobloch, 1982). Despite their vast expanse and diversity of biotopes and pollution sources in Moroccan coastal waters, as well as their growing importance in the national fisheries economy, the use of *O. vulgaris* in the biomonitoring of metal pollution remains limited in time and space (Karim et al., 2016). These reasons underscore the need for systematic monitoring to assess metal bioaccumulation and the ecological health of Moroccan coastal ecosystems.

The digestive gland of a cephalopod accounts for 6–10 % of its total body weight. Besides its major physiological function in the digestive process (synthesis of digestive enzymes, digestive absorption, storage of essential nutrients, and excretion of part of the digestive residues) (Boucaud-Camou et al., 1976), this organ is also recognized for its role as a bio-concentrator of metals entering the body (Penicaud et al., 2017; Rodrigo and Costa, 2017). The digestive gland accumulates high levels of essential and non-essential elements and is involved in various detoxification mechanisms (Miramand and Guary, 1981; Bryan, 1984). The detoxification strategy developed by *O. vulgaris* in response to oxidative stress caused by pollution pressure in coastal areas includes the production of metallothionein (MT) proteins (Raimundo et al., 2010; Semedo et al., 2012; Sillero-Ríos et al., 2018) and the induction of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), GST (Glutathione S-transferases) activities (Semedo et al., 2012; Sillero-Ríos et al., 2018).

O. vulgaris is a cephalopod inhabiting the continental shelf along the northwestern coast of Morocco. The species lives in direct contact with the seabed, with adult mobility guided by the search for prey (Smith, 2003). Nevertheless, individuals seem to be clustered in well-defined areas, making the load of metal contaminants in their tissues as an effective indicator of the level of metal contamination occurring in their proximal environment (Miramand and Bentley, 1992; Bustamante et al., 2002a; Napoleão et al., 2005). The study area was selected based on the abundance of *O. vulgaris* and its proximity to pollution sources. In this context, *O. vulgaris* captured in the coastline around Safi coast (northwestern Morocco) was investigated in order to highlight its suitability as a bioindicator of TM bioavailability. The specific aims of the present work were: (i) to evaluate the potential of *O. vulgaris* species as a suitable bioindicator of metal contamination in the Moroccan coastal environment; (ii) to determine the pattern of distribution of Cd, Cr, Cu, and Zn levels among two key organs, the digestive gland and the muscle; (iii) to investigate the relationships between TM concentrations and individual size (length and weight), as well as variations in sex and maturity; (iv) to examine potential interactions between TMs as a function of biological parameters; and (v) to compare this model with others bioindicators.

2. Material and methods

2.1. Study area

The northwest Atlantic coast of Morocco, located between 32°5′9.35" N and 32°32′56.8" N, hosts a major agglomeration with large ports, a sanitation network, and significant industrial activity. Sewage wastewater is discharged into the proximate coastal seawater without any prior treatment, making this area a highly polluted hotspot (Chafik et al., 2001). A stretch of this coastline, extending from Cap Beddouza (32°32′56.8" N, 9°16′41.6" W) to Souiria Kdima (32°5′9.35" N, 9°34′18.16" W), and located 34 km north and 36 km south of Safi city, respectively, was investigated (Fig. 1). The study area is bordered by the Oued Tensift, a large river that flows into the Atlantic Ocean. The region has a semi-arid climate, with hot summers from May to November and humid, temperate winters from November to April. The region receives an average of 397 mm of annual rainfall, with a mean temperature of 18 °C (Benhamdoun et al., 2023). The area is close to several industrial discharge points, including phosphate plants, a thermal power plant, agro-food units, and tanneries. Notably, it serves as one of the world's major hubs for phosphoric acid and fertilizers production, with discharges primarily composed of mineral products (Dahbi et al., 2024; El-Azzouzi et al., 2024). The characterization of domestic and industrial effluents reveals metallic contamination in wastewater by Cd and Pb, in seawater by Cd, Pb and Cu, and in sediment by Cd and Cu, exceeding the allowed values. This contamination affects the quality of intertidal seawater and sediments along the Safi coast (Kouali et al., 2022b; Rafiq et al., 2022). Furthermore, Cheggour et al. (1999), relate the decrease in biological diversity and macrobenthos species densities in this area to the proximity of industrial effluents heavily laden with Cd and Cu.

2.2. Sampling design and sample processing

Sixty specimens of *O. vulgaris* were collected from March to July 2022 during the fishing season (Table 1). The species was caught at low tide by fishermen using traditional methods. The fishermen operated on foot or from small boats, employing clay pots, traps, and hand jigs. Notably, *O. vulgaris* inhabits rocks and coastal crevices, making it difficult to catch. Consequently, the collection period was extended from March to July based on the availability of the species at the sampling sites. The collected individuals were placed in a cold box at +4 °C and transported to the laboratory for further analysis. The samples were subsequently subjected to various biometric measurements, including total weight (TW) and dorsal mantle length (DML), measured with a VWR precision balance (0.01 g) and a 0.01 mm precision vernier caliper, respectively. The sex of the collected specimens was determined after evisceration, and the gonadal maturity stage for each individual was evaluated according to the scale of Sánchez and Obarti (1993), which

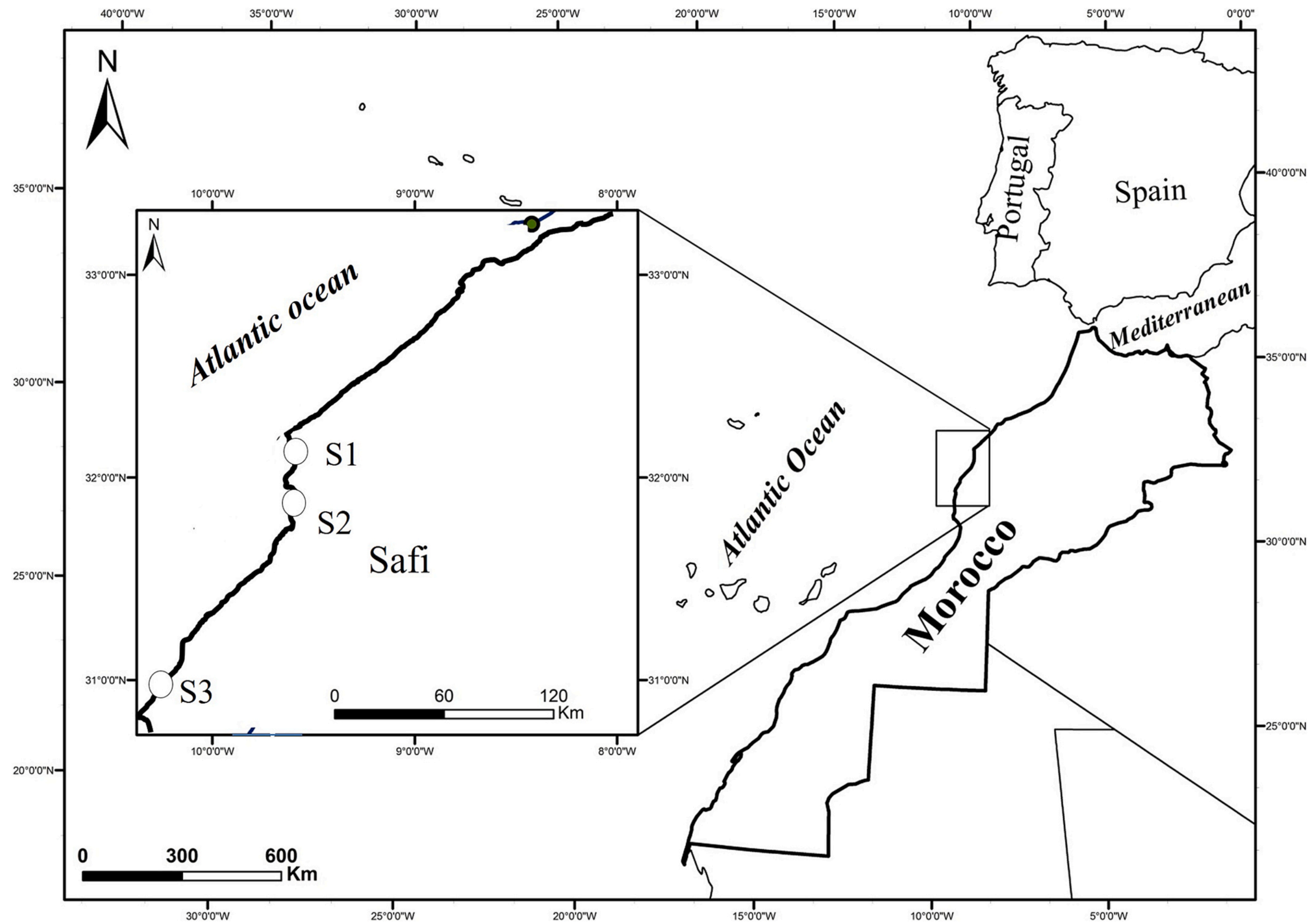

Fig. 1. Geographical position of the study area.

Table 1
Biometric characteristics of *O. vulgaris* samples (mean ± standard deviation; range).

	TW (g)	DML (cm)	Number
All individuals	593.01 ± 374.14 (130.76–1544.74)	11.34 ± 2.94 (6.00–17.77)	60
Females	612.73 ± 378.44 (153.20–1544.74)	11.41 ± 3.11 (6.50–17.77)	37
Males	561.26 ± 373.26 (130.76–1335.48)	11.21 ± 2.71 (6.00–16.00)	23
Immatures	549.23 ± 352.59 (130.76–1544.74)	10.94 ± 3.08 (6.00–17.77)	38
Matures	668.62 ± 405.91 (191.26–1461.32)	12.02 ± 2.60 (7.50–16.60)	22

includes four macroscopic maturity stages: immature, maturing, mature, and post-spawning. The muscle (the mantle-tentacle mixture) and digestive gland were removed separately and individually from each specimen sampled.

To individually determine the TM contents (Cd, Cr, Cu, and Zn) in the soft tissues of *O. vulgaris* individuals, the digestive gland and part of the muscle were dried at 135 °C for 24 h to obtain the dry weight (d.w). Afterward, the samples were ground for subsequent acid digestion (mineralization). 0.25 g of the digestive gland and 3 g of the muscle were placed in a digestion vessel and exposed to a mixture of 4 ml of nitric acid (HNO_3, SAJ first grade, 69 % purity, Sigma-Aldrich) and 6 ml of hydrochloric acid (HCl, ACS reagent, 37 % purity, Sigma-Aldrich). The mixtures were left to stand overnight at ambient temperature. The digestion vessels were then placed on a hotplate and digested for 2 h (95 °C for 75 min). After cooling, the acid solutions were filtered through 0.45 µm Whatman filters and adjusted by adding bi-distilled water to 10 ml. The Cd, Cr, Cu, and Zn contents were determined using an Atomic Absorption Spectrophotometer with flame (AI 1200, Aurora Instruments Limited, Canada). The absorption wavelengths used for Cd, Cr, Cu, and Zn were 228.8, 375.9, 324.7, and 213.9 nm, respectively. The limits of detection (LOD) and quantification (LOQ) ranged from 0.024 to 2.700 µg/g and 0.080 to 9.010 µg/g, respectively.

2.3. Quality control and assurance

The TM analysis was conducted in triplicates to assess analytical accuracy. The obtained data were averaged. SRM 2976 mussel tissue (Report, National Institute of Standard and Technology NIST, Canada) was used as a reference material to assess the accuracy of the analytical procedure. The results showed good agreement with the certified and measured TM contents, with recovery proportions ranging from 96 to 106 %. The precision and trueness ranged from 3 to 8 %, and from 2 to 5 %, respectively. The standard errors of the analytical methods used in the TM analyses were < 5 % (Dahbi et al., 2024; Benhamdoun et al., 2025). All chemicals and reagents were from Sigma-Aldrich (Switzerland), and glassware was thoroughly cleaned with deionized and acidified water.

2.4. Data processing and statistical analyses

In this study, the TM contents in *O. vulgaris* tissues were first tested for normality and homogeneity of variance at the $P > 0.05$ level using the Kolmogorov-Smirnov and Levene tests, respectively, prior to subsequent statistical analyses. Non-normal variable data were log-transformed.

In general, the means ± standard deviations of the TM contents in each tissue were calculated either for all the individuals studied ($n = 60$) or for the groups of *O. vulgaris* established based on defined sex and gonadal maturity stages. According to the normality of data, an independent-sample Student's *t*-test was performed to compare the TM contents between the digestive gland and muscle tissues, as well as between different sexes (females and males) and maturity stages (immature and mature). Moreover, the Pearson correlation test was performed to determine the relationships of the TM contents in the tissues with the biometric parameters (DML and TW), as well as between the TM contents in both tissues. Furthermore, to assess the relative importance of the digestive gland versus muscle in the metal bioaccumulation process and its dynamics, we analyzed the evolution of the TM ratio between digestive gland and muscle contents as a function of biological parameters, using Pearson's correlation test.

It is well established that Cd has the ability to replace Zn and Cu ions in metal-binding metalloproteins (MTs) due to the similarities of their ionic properties (e.g., ionic valence) (Viarengo, 1989; Cosson et al., 1991). As the rate of biosynthesis of these MTs may be limited in the cells of the digestive gland, on the one hand, and the number of metal-binding sites in these proteins is limited (Li et al., 2023), on the other, competitive interactions between different TMs are to be expected. Calculating TM ratios as a function of metal concentrations could therefore prove highly informative in assessing these interactions (Bustamante et al., 1998a; Raimundo et al., 2005, 2010). In addition, the relationships of the Cd/Zn and Cd/Cu ratios in the digestive gland with the biometric parameters of samples were further explored to highlight the potential interactions between metals throughout the life of *O. vulgaris*, when the bioaccumulation process occurs in this organ (Raimundo et al., 2005). All statistical analyses were performed using SPSS 20 and JASP software.

3. Results

3.1. Trace metal contents in tissues

The concentrations of Cd, Cr, Cu, and Zn in the digestive gland and muscle in the *O. vulgaris* are presented in Table 2. The essential metals Cu and Zn were the most abundant in the species tissues. The digestive gland contained higher levels of metals than the muscle, following the decreasing order: Cu (2066.96) > Zn (1591.35) > Cd (188.13) > Cr (59.93) (μg/g d.w). In contrast, the sequence of metal contents in the muscle differed considerably, presenting the decreasing order: Zn (79.55) > Cu (21.98) > Cr (13.62) > Cd (0.61) (μg/g d.w). TM concentrations showed high interindividual variability between the 60 individuals analyzed, even for the same organ and the same metal. This variability was even more marked for Cd, ranging from 29.40 to 411.60 μg/g d.w in the digestive gland and from 0.06 to 3.16 μg/g d.w in the muscle (Fig. 2). Furthermore, several significant correlations between metal contents within the same tissue were observed (Fig. 3). In the digestive gland, Cu showed a very strong correlation with Cd ($r = 0.72$, $P < 0.001$), whereas in muscle, this metal (Cu) was significantly correlated with Zn ($r = 0.42$, $P < 0.001$). It should be noted that Cr displayed slight negative correlations with Cd in the digestive gland ($r = -0.32$, $P < 0.05$) and Cu in muscle ($r = -0.26$, $P < 0.05$).

Table 2
Trace metal concentrations (μg/g dry weight) in the tissues of the soft body of *O. vulgaris* from the sampling locations (mean ± standard deviation; range).

Trace metals	Digestive gland	Muscle	Student *t*-test and *P*-value
Cd	188.13 ± 78.37 (29.40–411.60)	0.61 ± 0.65 (0.06–3.16)	−18.53 (**0.000**)
Cr	59.93 ± 32.47 (13.14–169.20)	13.62 ± 9.44 (1.00–36.73)	−10.60 (**0.000**)
Cu	2066.96 ± 1415.57 (111.90–6709.80)	21.98 ± 24.50 (6.22–156.50)	−11.18 (**0.000**)
Zn	1591.35 ± 518.64 (401.10–3329.00)	79.55 ± 13.37 (50.23–102.93)	−22.57 (**0.000**)

Bold values denoted very highly significant difference ($P < << 10^{-6}$) between groups according to the independent-samples Student's *t*-test.

Fig. 4 summarizes the potential relationships between metal bioaccumulation in tissues and biometric parameters in *O. vulgaris*. Among metals bioaccumulated in the digestive gland, only Cd and Cu were highly correlated with TW ($r = 0.46$ and $r = 0.40$, respectively, $P < 0.01$) and with DML of *O. vulgaris* ($r = 0.44$ and $r = 0.45$, respectively, $P < 0.001$). In contrast, no significant correlation was observed between muscle metal concentrations and biometric parameters.

3.2. Metal partitioning between digestive gland and muscle

Metal concentrations in the digestive gland were much higher than those recorded in muscle (Table 2) (Student's *t*-test, $P < 0.05$). The extent of inter-tissue variation in metal content varied greatly depending on the metal in question, with averages ranging from 9-fold and 21-fold for Cr and Zn, respectively, to 177-fold for Cu. However, the quantitative shift in favor of the digestive gland is even greater for Cd, reaching an average factor of 702 times. In addition, for each metal, the ratio of the digestive gland content to muscle content conceals major variations between individuals, ranging from 2 to 42 for Cr, 5 to 40 for Zn, 6 to 724 for Cu and 57 to 5461 for Cd. However, with the exception of Cr ($r = 0.69$, $P < 0.001$), none of the other metals under investigation showed significant correlation between the digestive gland and muscle concentrations (Table 3). Moreover, the ratio between the digestive gland concentrations and muscle concentrations for metals has not varied significantly with biometric parameters of *O. vulgaris*, except for Cu, which has clearly evolved with TW ($r = 0.42$, $P < 0.001$) and DML ($r = 0.40$, $P < 0.01$), and Cd demonstrated a weaker correlation with DML ($r = 0.36$, $P < 0.05$).

3.3. Variation of trace metal contents with sex and maturity stages

Table S1 shows the TM concentrations (μg/g d.w) in females and males, as well as in immature and mature groups, in the digestive gland and muscle of investigated *O. vulgaris*. Statistical tests revealed no significant differences in TM concentrations between sexes in both tissues (Student's *t*-test, $P > 0.05$). Therefore, there seems to be no variation in the contents of bioaccumulated metals in the digestive gland and muscle related to sex and maturity.

Further comparisons involving the ratio of digestive gland contents to muscle contents for each metal revealed no significant differences between females and males and between immature and mature groups (Student's *t*-test, $P > 0.05$) (Table S2). Therefore, the allocation process of metals between the digestive gland and muscle in *O. vulgaris* seems not to be influenced by reproductive factors such as sex and the level of gonadic maturation.

3.4. Relative abundance between Cd and Cu/Zn within the digestive gland

Most of the ratios between metals in the digestive gland and biometric parameters of *O. vulgaris* significantly correlated. Indeed, the ratio of Cd to Zn concentrations (Cd/Zn) showed strong positive correlations with TW and DML ($r = 0.34$, $P < 0.01$ and $r = 0.40$, $P < 0.01$, respectively). In contrast, the ratio of Cd to Cu concentrations (Cd/Cu) showed very strong negative correlations with TW and DML ($r = -0.37$, $P < 0.01$ and $r = -0.44$, $P < 0.001$, respectively). Furthermore, the similar trend was confirmed between the metal ratios and the metal concentrations found in the digestive gland. In fact, the ratio of Cd to Zn

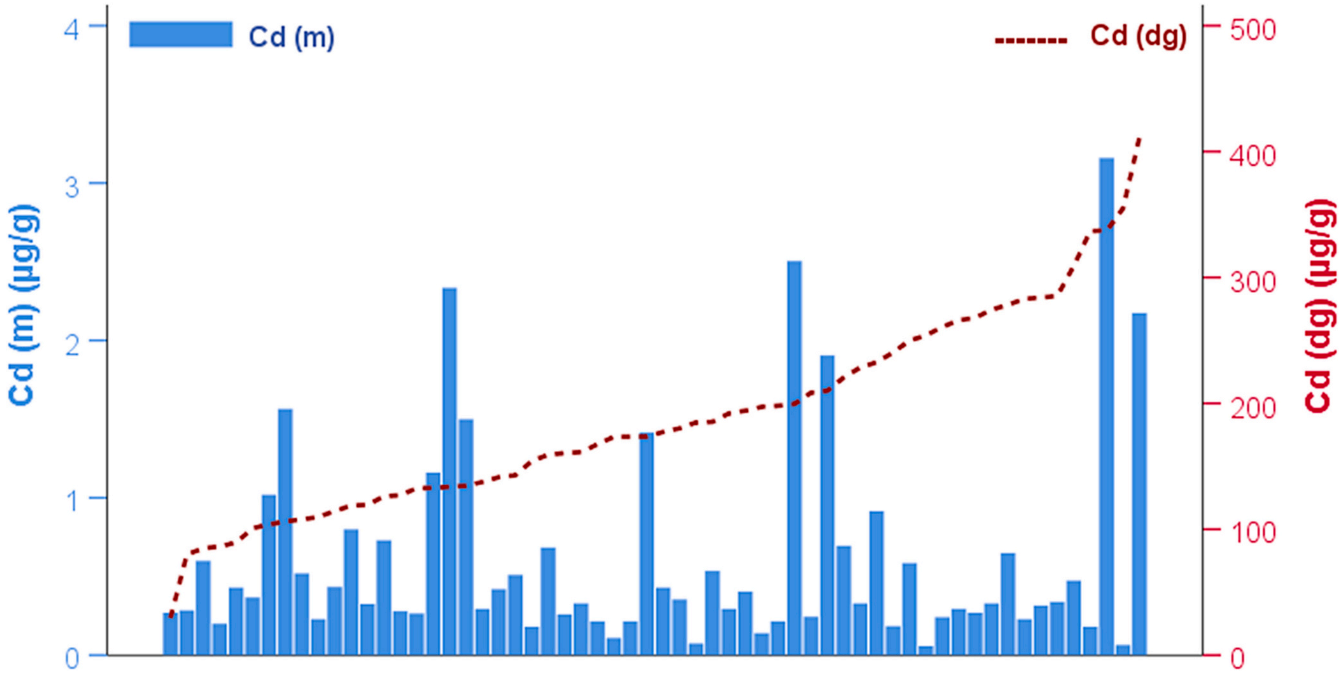

Fig. 2. Interindividual variability of Cd contents (µg/g dry weight) in the digestive gland (dg) and muscle (m) of *O. vulgaris*.

concentrations (Cd/Zn) was positively correlated with Cd contents in the digestive gland ($r = 0.75$, $P < 0.001$) (Fig. 5). However, the ratio between Cd and Cu concentrations (Cd/Cu) was negatively correlated with Cd concentrations in the digestive gland ($r = -0.29$, $P < 0.05$).

4. Discussion

4.1. *O. vulgaris* as a suitable bioindicator for metal pollution in Moroccan coastal areas

Several studies carried along the northwestern Atlantic coast of Morocco have focused on metal contamination in seawater (Rafiq et al., 2022), sediment (Kouali et al., 2022b; Minoubi et al., 2023), and the use of bioindicators, such as algae (Boundir et al., 2021), mussels (Kouali et al., 2020, 2022a), barnacles (Dahbi et al., 2024), and gastropods (Sif et al., 2024). The present study is the first report to validate *O. vulgaris* as a bioindicator of metal pollution in Moroccan Atlantic waters. It is an opportunistic and voracious predator which occupies higher trophic level compared to mussels, barnacles, and gastropods. This enables individuals to accumulate high concentrations of TMs (Olmedo et al., 2013; Penicaud et al., 2017; Huang et al., 2024). In addition, the short life span and territorial nature allow *O. vulgaris* to quickly reflect the quality of its habitat (Boyle and Knobloch, 1982). Indeed, the specimens of *O. vulgaris* samples randomly collected and individually analyzed were all heavily contaminated with TMs under investigation, reflecting, therefore, a metal contamination in the monitored coastal ecosystem. Moreover, the concentrations of TM in the tissues analyzed had a concentration pattern comparable to that observed in seawater (Cu 6.00 mg/l > Zn 1.00 mg/l > Cd 0.54 mg/l; Rafiq et al., 2022), and sediment (Zn 73.78 µg/g > Cr 54.52 µg/g > Cu 38.23 µg/g > Cd 3.37 µg/g; Kouali et al., 2022b; Zn 36.54 µg/g > Cr 11.34 µg/g > Cu 3.85 µg/g > Cd 0.39 µg/g; Minoubi et al., 2023) where *O. vulgaris* lives. This overall correspondence indicates that bioaccumulated concentrations in *O. vulgaris* accurately reflect ambient metal pollution in the seawater and sediment. Such a correlation between metal levels in the biotope and tissues of bioindicators is a common trend that has been well reported worldwide (Ben Salem and Ayadi, 2017; Feng et al., 2020; Marrugo-Negrete et al., 2021; Lozano-Bilbao et al., 2022; Duysak et al., 2023; Al Jufaili et al., 2024; González-Delgado et al., 2024), including NW coastal area in Morocco (Kouali et al., 2020, 2022b; Dahbi et al., 2024). In addition, given the high concentrations of TMs recorded in tissues, particularly in the digestive gland, *O. vulgaris* may tolerate and prosper in highly polluted environments (Ahmed et al., 2022). Taking into consideration these criteria and other prerequisites, *O. vulgaris* is definitely a suitable bioindicator of metal pollution in NW seawaters in Morocco, deserves to be more frequently used in monitoring studies. The sedentary lifestyle of *O. vulgaris* in the upper part of the continental shelf (Mangold, 1983), combined with its benthic behavior in direct contact with the substrate, make it possible to be permanently and directly exposed to local metal pollution during its short lifetime (Napoleão et al., 2005). TMs can be dispersed or accumulated in various compartments like the water column, sediment, and prey, which can serve as potential sources of contamination for *O. vulgaris* (Penicaud et al., 2017). Several studies have revealed that the food pathway is the main route for TMs bioaccumulation in *O. vulgaris* (Martin and Flegal, 1975; Bustamante et al., 1998a; Koyama et al., 2000; Bustamante et al., 2002a; Penicaud et al., 2017). TMs can also be transferred from the aquatic compartment through the skin and gills to *O. vulgaris*, which are considered additional uptake pathways (Bustamante et al., 2004; Penicaud et al., 2017).

4.2. Comparison of metal contents in *O. vulgaris* with recent Moroccan literature

O. vulgaris is a cosmopolitan species. Its suitability in biomonitoring metal pollution in coastal environments has been well reported around the world (e.g., in Egypt, Nessim and Riad, 2003; in Portugal, Raimundo et al., 2004; in Tunisia, Rjeibi et al., 2014; in Spain, Lozano-Bilbao et al., 2018 and Escánez et al., 2021; in Italy, Ariano et al., 2019). In Morocco, however, although this species is abundant and widely distributed in Atlantic and Mediterranean coastal areas (approximatively 3500 km), studies recording metal accumulation are still completely lacking. The unique study assessing metal concentrations in this cephalopod was conducted several years ago in the Mediterranean Sea in northern off Morocco (Karim et al., 2016). The geographical factors, environmental conditions (such as presence of upwelling zones), and industrial activities are key determinants the metal exposure and pollution in the

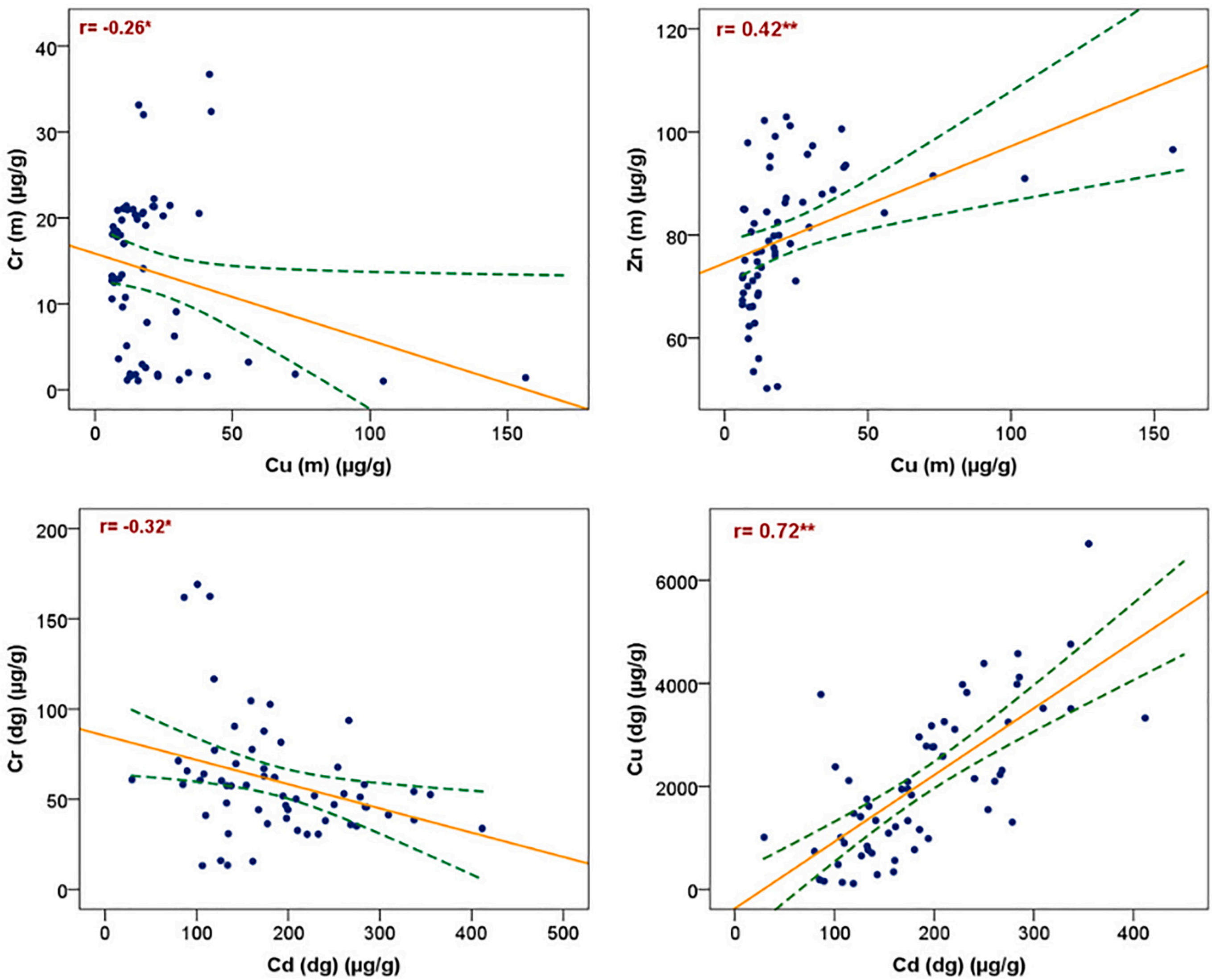

Fig. 3. Relationships between trace metal contents (µg/g dry weight) within the digestive gland (dg) and muscle (m) in *O. vulgaris* (Cd vs. Cr and Cd vs. Cu in the dg, and Cu vs. Cr and Cu vs. Zn in m).

marine ecosystem. These factors influence the bioavailability and uptake of TMs by marine biota (Sun et al., 2020; Aarif et al., 2023; Lozano-Bilbao et al., 2024a). In this context, the present study contributes to understanding the bioaccumulation of TMs in *O. vulgaris*. In Mediterranean Moroccan waters, higher concentrations of TMs in *O. vulgaris* were reported in the digestive gland for Cu (152.00–187.00 µg/g) and Cd (1.35–1.95 µg/g), and in the muscle for Zn (280.00–298.00 µg/g) (Table 4). Nevertheless, even if conducted on the same species, comparing these data with ours entails comparing two fundamentally distinct biotopes, the Atlantic and the Mediterranean environments, whose physical and chemical properties, as well as the human impact, differ significantly (Menemenlis et al., 2007; Lozano-Bilbao et al., 2024a). Indeed, for various anthropogenic reasons, the NW Atlantic coastal area in Morocco is more impacted by metals as it concentrates the major cities with important harbors and industrial facilities (Kouali et al., 2020; Minoubi et al., 2023; Dahbi et al., 2024). In addition, the physicochemical parameters differ markedly between the two biotopes, as Mediterranean waters have higher salinity, temperature, and density and lower nutrient with specific water circulation (Tanhua et al., 2013). Such differences might influence the bioavailability and uptake of TMs in biota. Variations in bioaccumulation between the Mediterranean and Atlantic populations of *O. vulgaris* may result from differences in trophic resources, food chain complexity, and physiological traits that affect metal regulation and homeostasis (Lacoue-Labarthe et al., 2016; Pouil et al., 2016). To shed light on some of these hypotheses, further investigations have to be conducted in both coastal marine sides in Morocco to gather deeper insights on ecological, biological and physiological aspects of each of the two *O. vulgaris* populations.

Along the Atlantic coastline of Morocco, the use of macroinvertebrates as bioindicators of metal pollution has been largely restricted to a well-defined taxa, with a notable absence of cephalopods. This study is, therefore, novel and provides a baseline for future related studies on cephalopod taxa in these areas. Nevertheless, a comparison can be drawn with recent reports on two sedentary biofilter species in these coastal areas, the blue mussel *M. galloprovincialis* and the goose barnacle *P. pollicipes* (Kouali et al., 2022a; Dahbi et al., 2024). For consistency, only TM concentrations measured in *O. vulgaris* muscle were considered. As expected, *O. vulgaris* exhibited a metal concentration profile markedly different from those of the two biofilters (Table 4), as this species is a carnivorous and opportunistic species (Smith, 2003). In fact, Cr and Zn were present in similar orders of magnitude in *O. vulgaris* (0.99–36.73 and 50.23–102.93 µg/g) and *M. galloprovincialis* (8.45–68.30 and 65.25–259.05 µg/g), respectively, while Cd occurred in much lower contents in *O. vulgaris* (0.06–3.16 µg/g) than in mussels and

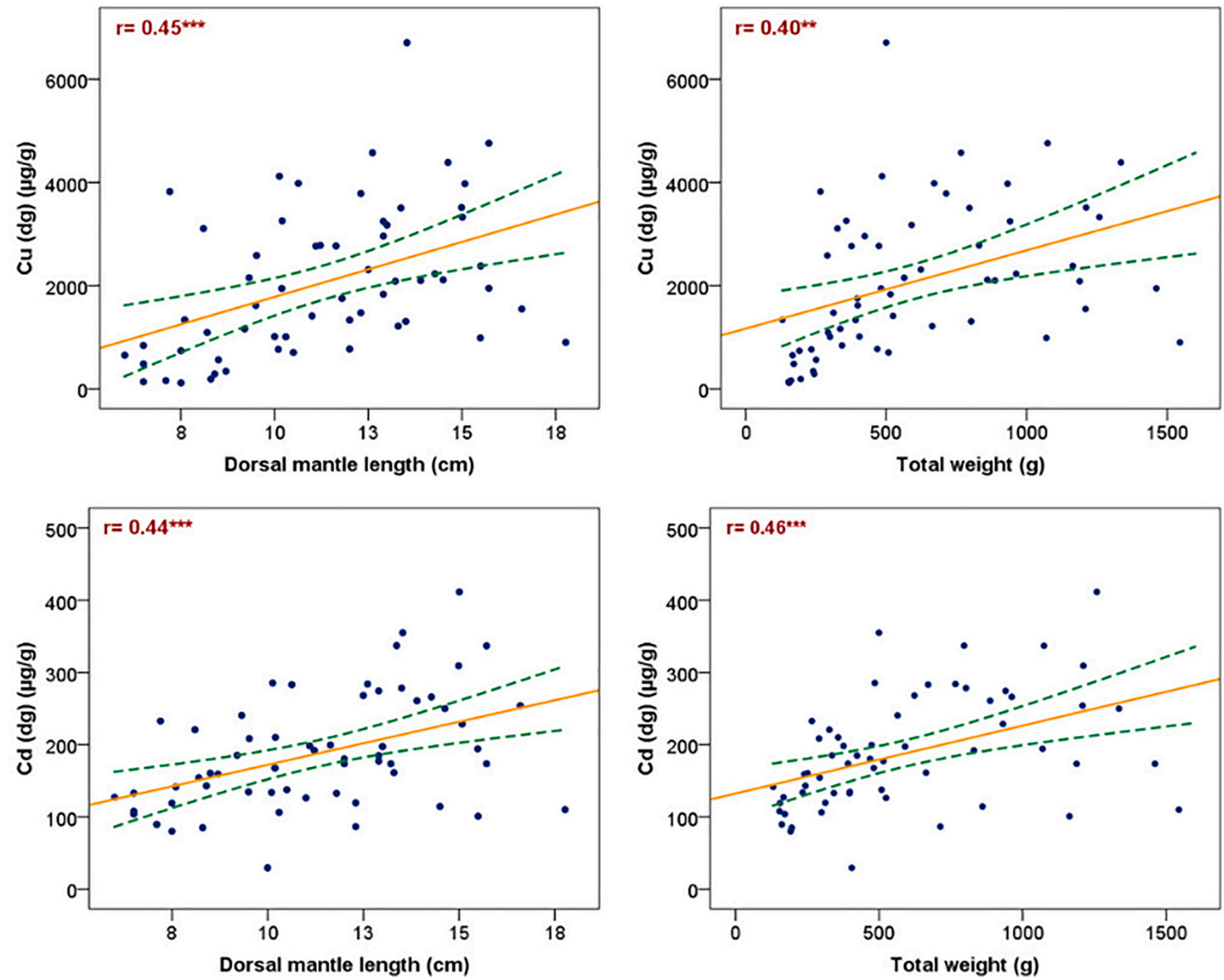

Fig. 4. Relationships between trace metal contents (µg/g dry weight) and biometric parameters (TW and DML) within the digestive gland (dg) in *O. vulgaris* (Cd vs. TW, Cd vs. DML, Cu vs. TW and Cu vs. DML).

Table 3
Pearson correlation between trace metal concentrations in the digestive gland and muscle tissues of *O. vulgaris*.

	Cd_{dg}	Cr_{dg}	Cu_{dg}	Zn_{dg}
Cd_m	0.131 (0.320)	**−0.295 (0.022)**	−0.019 (0.883)	−0.024 (0.857)
Cr_m	−0.241 (0.064)	**0.694 (0.000)**	−0.161 (0.220)	**0.464 (0.000)**
Cu_m	0.007 (0.954)	−0.149 (0.254)	−0.197 (0.130)	−0.208 (0.109)
Zn_m	−0.088 (0.501)	0.119 (0.362)	−0.084 (0.525)	−0.005 (0.967)

P-value between parenthesis. Significant correlation is in bold.

barnacles (2.30–41.40 and 7.65–39.10 µg/g, respectively). The opposite situation was observed for Cu, which was present in higher quantities in the *O. vulgaris* (6.22–156.50 µg/g) than in the two biofilters (2.90–47.80 and 5.55–9.80 µg/g, in mussels and barnacles, respectively) (Kouali et al., 2022a; Dahbi et al., 2024). The pattern of bioaccumulation of metals in the tissues of a given species, particularly muscles, is influenced not only on the extent of metal pollution in the ambient environment but also on the sensitivity of the species to each metal. This sensitivity may result from a combination of several ecological and physiological parameters such as feeding behavior, metal absorption, detoxification strategies, and depuration rates, which vary according to the taxon in question and its intrinsic capacities (Horvat et al., 2011; Sevillano-Morales et al., 2015; Dey et al., 2024). As suggested by several authors (e.g., Rainbow and Phillips, 1993), to obtain a comprehensive assessment of the extent of metal pollution in a given area, a wide range of suitable bioindicator models should be used simultaneously.

4.3. *Effects of biometric parameters, sexual pattern and interindividual variability in metal contents*

As with many bioindicators, metal pollution load in the surrounding environment significantly impacts the levels of accumulated metals in *O. vulgaris*. However, these levels may not solely reflect the extent of metal contamination of coastal waters, sediment and food items, as high interindividual variation in metal concentrations occurs between analyzed samples in both organs. Therefore, metal pollution of coastal waters does not seem to affect all individuals in the same way, even within the same species and habitat. Such variability was not linked to the growth of *O. vulgaris*, as no relationships were found between accumulated levels in tissues and body length or size. More subtle intrinsic parameters related to the biology and physiology of individuals may also interfere with the levels of accumulated metals in the tissues of *O. vulgaris*. The lack of growth-dependent metal bioaccumulation levels

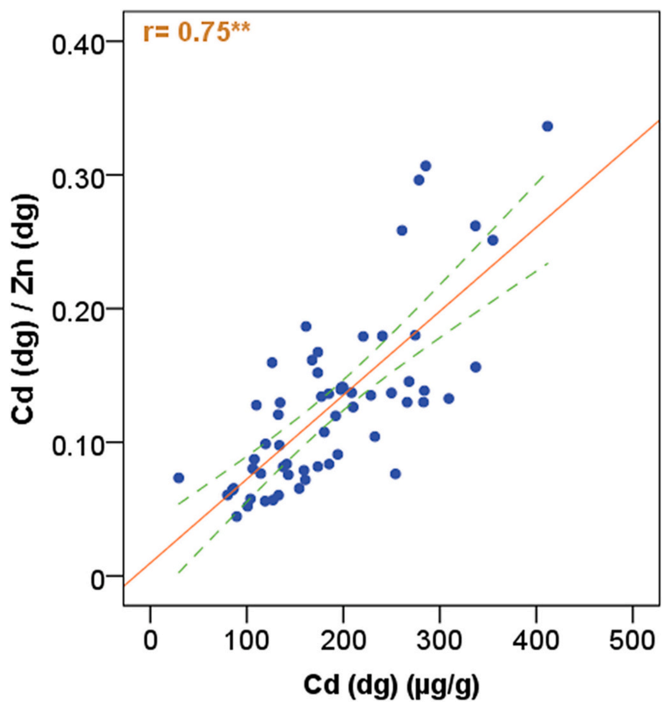

Fig. 5. Relationship between the Cd/Zn ratio and Cd contents (µg/g dry weight) in the digestive gland (dg) of *O. vulgaris*.

in *O. vulgaris* is consistent with most related results provided in the literature, which show no significant relationships between growth parameters and metal concentrations in cephalopods (Bustamante et al., 1998a; Seixas et al., 2005; Bustamante et al., 2008; Raimundo and Vale, 2008). However, the situation is more complex than it may appear, as other contradictory findings have also been reported. In the case of the muscle of *O. vulgaris*, the relationship between TM levels and body weight is rarely observed (Raimundo et al., 2005). Conversely, a positive correlation between Cd and Cu levels and body size and weight has been observed in the digestive gland. Additionally, mercury concentrations show a linear positive correlation with size in the muscles of *O. vulgaris* and squids (Monteiro et al., 1992; Rossi et al., 1993; Lischka et al., 2020). According to Nessim and Riad (2003), the effect of body weight is inversely proportional to the concentrations of Cr, Cu, and Cd in the muscle, meaning that as body weight increases, the concentrations of these TMs in muscle decrease.

As for biometric parameters, no relationships were revealed between metal concentrations in *O. vulgaris* and the reproductive status of individuals, including sex and sexual maturity. This result is also in consistent with previous reports on *O. vulgaris* species (Miramand and Bentley, 1992; Raimundo et al., 2005; Roldán-Wong et al., 2018). However, regarding growth parameters, previous results obtained on cephalopods, including *O. vulgaris*, have always been contradictory. According to several authors, the concentrations of TMs did not show any relation to sex and/or sexual maturity (Miramand and Bentley, 1992; Raimundo et al., 2004; Raimundo et al., 2005; Seixas et al., 2005; Lourenço et al., 2009; Rjeibi et al., 2014; Roldán-Wong et al., 2018). In contrast, an inverse pattern was obtained by Nessim and Riad (2003) for *O. vulgaris* caught along the Egyptian coasts, with immature individuals exhibiting higher TM concentrations than mature conspecifics. The authors argued these variations were due to a higher metabolic rate in immature specimens because of faster growth combined with weaker detoxification abilities. The bioaccumulation of metals may derive from a more complex combination that also involves intrinsic physiological properties, including the metabolic rates and detoxification capacities of each individual. All these factors may be more or less efficient according to Rossi et al. (1993) and Canli and Atli (2003), significantly modulating the extent of metal load in tissues, leading to significant variations between individuals. Under the same environmental conditions, certain individuals, regardless of their age, sex, or reproductive status, may be more prone to uptake and/or to depurate metals than conspecifics (Bustamante et al., 2002a; Sillero-Ríos et al., 2018). Therefore, even within the same size range and sexual status, *O. vulgaris* sampled in the same coastal area could exhibit different TM contents in their tissues. Conversely, similar metal contents could be observed in different size and sexual categories. In addition, the short life span of individuals of *O. vulgaris* experiencing similar environmental conditions could also be a determining factor in bioaccumulated metal levels and, therefore, explain such interindividual disparities. We hypothesize that the inter-individual variability in metal bioaccumulation is inversely proportional to the life span of individuals in the population. *O. vulgaris* is characterized by a high prey consumption rate and efficient assimilation of nutrients, increasing its consumption intensity as the temperature rises (Mangold, 1983). Therefore, as the digestive tract is the primary pathway through which metals to enter the body (Bustamante, 1998), the short life span of *O. vulgaris* may be a factor contributing to the heterogeneity in bioaccumulation among individuals. Indeed, during their brief lives, *O. vulgaris* may consume different types of prey, influencing the nature and quantity of metals absorbed. In contrast, for other species, differences between items consumed within populations may tend to level out as individuals live longer. This hypothesis is difficult to verify given the lack of reliable measurements and information on the life history of each sampled individual, such as the individual trophic history, including the types of prey ingested, the frequency of prey capture, and the specific habitats they frequented. To delve deeper into this phenomenon, future studies should aim to track individual of *O. vulgaris* over their life span, gathering detailed data on their feeding habits and environmental conditions. Understanding these factors will be crucial to elucidating the ultimate mechanisms underlying metal bioaccumulation and its ecological implications.

4.4. Metal partitioning among organs and potential interactions in digestive gland

Our results revealed major quantitative differences between the

Table 4
Concentrations of trace metals (µg/g dry weight) in various bioindicators of metallic pollution as reported in the Moroccan literature.

Bioindicators	Species (tissues)	Zone	Cd	Cr	Cu	Zn	References
Cephalopods	*Octopus vulgaris* (muscle)	Safi	0.06–3.16	0.99–36.73	6.22–156.50	50.23–102.93	Present study
		Nador	0.24–0.58	–	133.00–171.00	280.00–298.00	Karim et al. (2016)
	Octopus vulgaris (digestive gland)	Safi	29.40–411.60	13.14–169.20	111.90–6709.80	401.10–3329.00	Present study
		Nador	1.35–1.95	–	152.00–187.00	17.90–80.30	Karim et al. (2016)
Bivalves	*Mytilus galloprovincialis*	Safi	2.30–41.40	8.45–68.30	2.90–47.80	65.25–259.05	Kouali et al. (2022a)
		Agadir	1.05–4.20	–	16.36–44.93	158.32–308.20	El Mourabit et al. (2024)
Gasteropods	*Phorcus lineatus*	El Jadida	9.40–10.68	1.61–4.01	157.60–184.47	94.87–107.36	Sif et al. (2024)
Crustaceans	*Pollicipes pollicipes*	Safi	7.65–39.10	1.65–4.40	5.55–9.80	179.75–749.00	Dahbi et al. (2024)
Algae	*Ericaria selaginoides*	Safi	0.13–0.43	0.12–0.32	0.44–4.04	11.46–27.67	Boundir et al. (2021)

–: not measured.

digestive gland and the muscle. Although both organs are considered sites of bioaccumulation for metal pollutants, most metals were stored in the digestive gland (on average, 106 times more than in the muscle). Once these pollutants enter the general circulation, mainly via the digestive and respiratory tracts, they accumulate preferentially in the digestive gland, where they trigger the synthesis of specialized chelating proteins (Penicaud et al., 2017). The existence of such a bio-concentrator organ (the liver in fish and the digestive gland in most macroinvertebrates, such as mussels and octopuses) represents a universal adaptive pattern present in most animal taxa, enabling them to survive and adapt in highly polluted areas (Rodrigo and Costa, 2017). In some animal models, the metal content of the digestive gland (or liver) reflects a multi-year accumulation corresponding to the animal's life span (Marques et al., 2021; Bilgin et al., 2023; Sow et al., 2023; Lozano-Bilbao et al., 2024b). However, the digestive gland contents of *O. vulgaris* can only reflect a short-term history, as individuals in this species have a life span of little more than two years (Otero et al., 2007). The very short life span, combined with rapid growth and high metabolic activity, suggests that *O. vulgaris* may reflect recent environmental changes and exposure to pollutants more sensitively than longer-lived species.

In the present study, the pattern of metal accumulation differed markedly between the digestive gland (Cu > Zn > Cd > Cr) and the muscle (Zn > Cu > Cr > Cd). Quantitatively, the digestive gland concentrated Cd, Cr, Cu and Zn at levels 308, 4, 94 and 20 times higher than muscle, respectively. The high potential capacity of the digestive gland in accumulating metals is a common phenomenon, attributed to the selectively induced MTs in this organ (Viarengo, 1989; Viarengo and Nott, 1993; Ahmed et al., 2022). These proteins are known to have a high affinity for metals and therefore bind large quantities of these chemicals, thus enhancing sequestration and detoxification mechanisms (Visnjic-Jeftic et al., 2010). High concentrations of essential elements such as Cu, Cr, and Zn are likely due to the storage of these metabolizable elements in the digestive gland of cephalopods (Finger and Smith, 1987). In addition, the accumulation of potentially toxic TMs, such as Cd in this organ is probably related to the detoxification process already observed in other marine mollusks (Coombs and George, 1978).

Two types of proteins have been identified as potential binding sites for TMs in cephalopods: low molecular weight (LMW) and high molecular weight (HMW) proteins (Finger and Smith, 1987; Craig and Overnell, 2003). Moreover, the concentration of metals in the digestive gland is closely dependent on the qualitative and quantitative composition of the induced proteins (Rocca, 1969; Bustamante et al., 2002b; Raimundo et al., 2010). In their investigations conducted on the Portuguese coasts, Raimundo et al. (2010) attempted to correlate metal concentrations with induced protein fractions in the digestive gland of *O. vulgaris*. These authors aimed to better understand the binding mechanisms and highlight specific affinities between categories of proteins and metals. Although the results were not entirely conclusive, some deductions proved interesting and highlighted specific associations. Cd, Cu and Zn showed strong positive correlations with LMW protein categories, suggesting preferential associations between these metals and LMW proteins. This finding should lead to a strong correlation between levels of these metals in the digestive gland. Our results are partially consistent with Cd and Cu, as these elements appear to evolve in synergy in this organ, showing a strong positive correlation between their respective levels. However, Cd was not correlated with Zn, reflecting their independent evolution. Such a result suggests the involvement of other binding proteins in Zn sequestration and/or the implication of regulatory homeostasis mechanisms for this metal in the digestive gland. This could be explained by specific mechanisms through which metal-protein associations occur for each metal. Zn is known to bind strongly to LMW proteins in the digestive gland of *O. vulgaris* (Raimundo et al., 2010). However, high concentrations of this metal are also likely to bind other classes of proteins such as HMW present in the gland, a greater extent than Cd and Cu. Differences in chemical affinity with sequestering proteins could also explain our results concerning Cd and Zn content profiles in the gland. Indeed, the sequestration rate, which reflects one of the major aspects of the animal's sensitivity to a given metal, does not appear to be similar for Cd and Zn. This could lead to distinct variations in Cd and Zn levels in the digestive gland. Similar results were recently reported for *M. galloprovincialis* and *P. pollicipes* inhabiting the same area on the northwestern Atlantic coasts in Morocco (Kouali et al., 2022a; Dahbi et al., 2024). These authors reported differential patterns of metal accumulation in soft tissues. Barnacles appeared to be more sensitive to Zn and somewhat less so to Cd, whereas mussels accumulated Cr more effectively, while barnacles showed a lower degree of bioaccumulation for this metal. According to these authors, variations in sequestration rates between barnacles and mussels are likely associated with differences in biochemical and cellular detoxification processes, as well as species-specific differences in metal metabolism. Moreover, crustaceans have regulatory mechanisms that allow for greater tolerance to higher metal levels and toxicity, such as the induction of metal-binding proteins and the presence of cytosolic granules (Rainbow and White, 1989).

In addition to the preferential interactions between metals and protein categories induced in the digestive gland, metal substitution phenomena are also likely to occur within this organ, as metal binding is a highly dynamic process. Indeed, the similarity of the chemical behavior based on the electronic configuration between Cd on the one hand and the essential elements Zn and Cu on the other may favor competition between these three elements in protein binding (Viarengo, 1989; Cosson et al., 1991), therefore, rendering the presence of Cd in large quantities a serious danger for organisms (Miramand and Bentley, 1992; Carvalho et al., 2005). With this in mind, we adopted an antagonistic approach based on metal content, analyzing the possible substitution of the essential metals (Zn and Cu) by the non-essential metal (Cd) in the binding molecules produced in the digestive gland. Our results provided clear evidence in this regard. The evolution of ratios involving Cd to the essential metals Cu and Zn showed variable patterns of change as Cd concentration increased in the digestive gland. For the couple Cd and Zn, the Cd/Zn ratio clearly increased with the rising Cd concentrations rose in the digestive gland. A kind of interaction between these two metals in this organ may therefore be expected. On the other hand, the MTs synthesis and the subsequent metal binding to MTs can become limiting as exposure concentrations increase, leading to increased competition between metals for binding to proteins that are already present. Therefore, substitution of Zn by Cd ions may occur when Cd levels in the digestive gland exceed the molecular (transcription) and/or physiological (all the subsequent steps) cellular limits for triggering the production of metal-binding proteins (Castaldo et al., 2021). Another explanation could be suggested to explain such an increasing trend of the ratio Cd/Zn. As an essential and more easily metabolized metal, Zn excretion in situations of Cd overtoxicity can be enhanced by removing Zn already bound to MTs and its excretion throughout the ink sac (Bustamante et al., 1998b), thus, freeing up potential binding sites for Cd chelation. A reverse tendency seems to occur in the digestive gland for the couple Cd and Cu. Indeed, Cd/Cu ratio slightly decreased when Cd levels increased. At this stage of the study, we have no plausible explanation for the Cd—Cu pair. Furthermore, the evolution of Cd/Zn and Cd/Cu ratios in the digestive gland as a function of *O. vulgaris* size proved to be more informative in understanding the interactions between these metals relative to biometric parameters. The Cd/Zn ratio increased with growth parameters, suggesting that larger individuals are more likely to sequester Cd in the digestive gland, progressively replacing Zn ions at metal-binding sites. Interestingly, the opposite situation was once again observed for the couple Cd and Cu, since the Cd/Cu ratio decreased as DML and TW increased. Here again, we do not have sufficient arguments to explain such a trend. Nevertheless, the fact that Cu is critically required in hemocyanin synthesis for respiratory processes in mollusks (Van Holde and Miller, 1995) must not be unrelated to these trends.

5. Conclusion

This study gives an overview of the state of the environment along the northwest Atlantic coast of Morocco, and provides valuable insights into TM contamination levels in a major fisheries resource. As expected, the findings revealed relatively high concentrations of TMs (Cd, Cr, Cu, and Zn) in *O. vulgaris*, which suggests its potential as a reliable bioindicator species for monitoring metal pollution in Atlantic coastal ecosystems of NW Morocco. Notably, the digestive gland exhibited higher concentrations compared to muscle, highlighting its role in sequestrating metal contaminants. The sex, maturity stage and biometric parameters had no effect on TM accumulation in organs, with exception for Cd and Cu concentrations which are being increased in the digestive gland as bigger the individuals were. The results also provided several insights into the interactive behavior between particular TMs within the digestive gland, since the ratios involving certain pairs of TMs clearly evolved as a function of certain biometric parameters. That said, the scope of the study deserves to be extended and should encompass the seasonal pattern and include surrounding waters and sediments data, as well as other relevant tissues, such as gills and skin. This would enable a better understanding of *O. vulgaris* exposure pathways to TMs and their distribution pattern among organs. Also, future investigations focused on understanding the mechanisms underlying *O. vulgaris*' ability to accumulate and tolerate high levels of toxic metals, such as Cd, would provide a better understanding of the species' resilience to metal contamination and help to give a more accurate idea on the health risks associated with consumption of contaminated seafood. Beyond these immediate scientific contributions, this research is also crucial for promoting sustainable fishing and improving the monitoring of metal pollution in Moroccan Atlantic coastal areas.

CRediT authorship contribution statement

Mohamed Techetach: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation, Conceptualization. **Hafid Achtak:** Writing – review & editing, Resources, Methodology, Formal analysis. **Enrique Lozano-Bilbao:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Hassnae Kouali:** Visualization. **Fatima Rafiq:** Formal analysis. **Maha Kerkich:** Investigation. **Abdallah Dahbi:** Writing – review & editing, Visualization, Validation, Data curation, Conceptualization.

Declaration of competing interest

The authors 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.2025.118037.

Data availability

Data will be made available on request.

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