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Sediment–biota coupling of macro- and trace elements with limited morphological integration in Ruditapes decussatus

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Abstract

Understanding whether morphological traits reliably reflect contamination gradients remains a central challenge in coastal biomonitoring. We investigated sediment–biota coupling of macroelements (Ca, Mg) and trace metals (Al, Fe, Pb, Zn, Hg) in the grooved carpet shell *Ruditapes decussatus* across five Mediterranean coastal sites characterized by contrasting but overall low contamination levels. We tested the hypothesis that, under sub-threshold trace metal exposure, phenotypic variability would be more strongly associated with geochemical gradients than with contamination intensity. Element concentrations in sediments differed significantly among sites and were reflected in shell and soft tissue composition, confirming tight benthic–biotic coupling. However, despite spatial variability in trace metals, no significant relationships emerged between metal concentrations and morphological traits. In contrast, macroelement patterns were significantly associated with morphological variation, suggesting that geochemical background conditions and environmental factors override trace metal exposure in shaping phenotypic variability under low-contamination regimes. These findings highlight the limited sensitivity of morphological traits as early-warning indicators of trace metal contamination and emphasize the need to disentangle natural geochemical variability from pollution signals in biomonitoring programs.

Introduction

Coastal marine environments play a key role in providing ecological goods and services, including biogeochemical cycling, carbon sequestration and the maintenance of biodiversity (Littles et al., 2018; Pérez-Ruzafa et al., 2019). However, these systems are exposed to multiple anthropogenic stressors (Halpern et al., 2007), such as pollution by hydrocarbons, industrial organic pollutants, domestic and industrial affluents. Chemical contaminants originating from these sources can impair important ecosystem functions (Johnston et al., 2015); heavy metals, in particular, may be toxic even at low concentrations and can accumulate along marine food chains (Penicaud et al., 2017; Chovanec et al., 2003; Rainbow 2017), ultimately impacting ecosystems' integrity, quality, and diversity (Fleeger et al., 2003; Shahidul Islam and Tanaka 2004; Häder et al., 2020).

The Mediterranean Sea is a biodiversity hotspot hosting 7–8% of all marine species globally known (Cuttelod et al., 2009); nonetheless, the basin is currently one of the most polluted European marine areas (EEA 2018). Pollution is mainly caused by anthropogenic inputs, reaching the sea through rivers, air, drainage of coastal areas, or by direct discharge, which primarily impacts coastal environments, including lagoons and other transitional systems (Newton et al., 2014; Richir and Gobert 2016). Among Mediterranean countries, Tunisia contributes remarkable amounts of chemical pollutants to marine environments, especially along coastal areas where urban effluents and industrial wastes, produced by chemical and mining activities, are discharged after incomplete treatment (Zaghden et al., 2016 and literature cited). In particular, the Gulf of Gabès is increasingly suffering the consequences of trace metals contamination resulting from industrial discharges (Annabi et al., 2018a; Annabi et al., 2018b; Naifar et al., 2018).

Biological monitoring of contaminants and other xenobiotics is to date acknowledged as an effective approach compared to chemical analysis of seawater or sediments. Indeed, pollutants may accumulate in biological tissues, reaching concentrations higher than those in abiotic matrices, in which the concentrations may be below instrumental detection limits, thereby limiting their determination by significant methodological and analytical drawbacks (Tzafriri-Milo et al., 2019).

Several invertebrate taxa are commonly used to assess the quality of coastal marine ecosystems; however, among these, bivalves have long been acknowledged as particularly effective biomonitors, due to their eco-biological characteristics (Boening 1999; Zuykov et al., 2013; Richir and Gobert 2014). Bivalves are considered good bioindicator species, because of their high distribution in the marine environment, accessibility for sampling (Rainbow and Phillips, 1993; Zhou et al., 2008) and economical value. Moreover, due to their sessile habits, accumulating pollutants, they reflect local pollution.

We focused on the grooved carpet shell *Ruditapes decussatus*, a widely distributed Mediterranean bivalve of ecological and economic relevance(Hmida et al., 2019) that has been frequently proposed as a biomonitor species in coastal environmentsBilgin et al., 2017; Uluturhan et al., 2019).. Building upon previous contamination assessments in the region, we investigated whether spatial variability in sediment geochemistry is consistently reflected in elemental accumulation patterns and phenotypic traits of this species.

Specifically, we quantified the concentrations of two macroelements (Ca and Mg) and five trace metals (Cu, Fe, Hg, Pb, and Zn) in sediments, shells, and soft tissues collected from five coastal sites characterized by contrasting environmental conditions and generally low to moderate contamination levels. We examined sediment–biota coupling to evaluate the extent to which elemental variability in sediments is transferred to biological matrices, and whether such variability translates into measurable morphological differentiation.

Given that macroelements may respond to natural environmental gradients and potentially modulate trace metal incorporation,(Liu and Wang, 2015). We further explored the relationships between macro- and trace-element concentrations. Hypothesized that, under relatively low contamination regimes, morphological variability would be more strongly associated with macroelement-driven geochemical gradients than with trace metal concentrations.

To test this hypothesis, we assessed key morphological traits related to shell growth and siphonal development, evaluating whether spatial differences in elemental composition correspond to phenotypic variation across populations.

Materials and Methods

Study area and sample collection

Sediment samples and *Ruditapes decussatus* specimens were collected in February 2017 from the Bizerte Lagoon (Fig. 1A), a transitional system located in the northeast of Tunisia (37°8'- 37°14'N 9°48'-9°56'E). The basin has a diameter of 11–13 km, a surface area of 150 km^2, and an average depth of 7 m with maximum depths (12 m) in the central area (Ouakad, 2007). It is connected to the Mediterranean Sea in its north-eastern sector through the Bizerte Canal, which is 7 km long and 12 m deep (Soussi 1981). The lagoon lays in a geological depression where several perennial and ephemeral water courses converge, discharging industrial, agricultural, and urban wastewaters (Garali et al., 2009). Indeed, the basin is surrounded by a number of densely populated urban areas (i.e., Bizerte, Zarzouna, Menzel Jemil and Menzel Abderrahmen on the North side and Menzel Bourguiba and Tinja on the West and South West side) and by cement, petrochemical, textile, and metallurgic industrial activities. The most important industrial facilities include: the "Ciments de Bizerte" factory, created in 1952; the "El Fouledh" metallurgical factory in Menzel Bourguiba, established in 1965 and the "Tunis Acier" factory (Bizerte), created in 1991 (ANPE 1990). In addition, an important diversified agricultural activity is developing in the plains surrounding the lagoon, reaching the surface of 12,700 hectares (VV.AA. 2003). Nonetheless, the exploitation of halieutic resources in the lagoon supports an important economic sector, ranking second in terms of export value of Tunisian agricultural and food products. Specifically, increasingly efforts are currently made for the development of shellfish farming, and mainly for clams' production (Abidli et al., 2019).

Six sediment samples were randomly collected in the northern sector of the lagoon close to the town of Menzel Jemil (37°13'11"N, 9°55'23"E) at a depth of approx. 50 cm using a hand-held cylindrical Perspex sampler (i.d. = 8 cm). Surface sediments (0–5 cm) were removed and transported to the laboratory in a refrigerated container, where they were air-dried at room temperature for one week, mixed, crushed with a clean pestle and mortar, and sieved on 2-mm mesh screen nested inside a 63-µm sieve. The finest fraction (< 63µm) was collected and used for elemental analyses (Salomons and Förstner, 2012). In addition to sediments, a batch of clams (55 individuals) were obtained from local fishermen. Samples were immediately placed in polyethylene bags and cold-stored, in the dark, during transportation to the laboratory. Once in laboratory, all specimens were carefully cleaned with distilled water, to remove epibionts and inorganic particles before chemical analyses. Between February and March 2017 sediment samples and *R. decussatus* individuals were collected from four locations along the coastal area of the Gulf of Gabès (Fig. 1A). Due to its very favourable geomorphological and climatic conditions, the Gulf of Gabès is one of the most productive areas of the Mediterranean. Indeed, this area contributes over 42% of the fish production in Tunisia (Hattab et al., 2013; Halouani et al., 2016). The benthic flora is mainly represented by the marine phanerogams *Posidonia oceanica* and *Cymodocea nodosa*. These seagrass beds provide shelter, food, and nesting sites for a number of fish and invertebrate species. Seagrass also contributes to the oxygenation of seawater, increasing the stability of loose seabed and shores, and enriching surficial sediments with organic matter (Ben Brahim et al., 2010; El Zrelli et al., 2018). However, the area subjected to several anthropogenic pressures due to the discharge of partially treated wastewater of urban, industrial, and mining origin in the areas of Sfax, Gabès, and Skhira. The pollution of the gulf has determined substantial modifications in both biotic and abiotic characteristics

of coastal waters, leading to the alteration of the biodiversity of coastal benthic assemblages (Rabaoui et al., 2014; El Kateb et al., 2018; Annabi et al., 2018a; Béjaoui et al., 2019).

Specifically, sample collection was performed at four sites characterized by different sources of contamination:

- - Gargour: (33°31'N, 10°42'E) an intertidal zone on the north coast of the Gulf of Gabès, located 15 km south of the city of Sfax and 10 km south of SIAPE I (Industrial Company of Phosphoric Acid and Fertilizers). Discharging effluents containing high concentrations of metals, deriving from industrial activities, affect this area (Ghribi et al., 2019). The site is enriched in nutrients, salts and especially in phosphate (derived from the phosphogypsum discharges) leading to phytoplankton blooms (Salem et al., 2015);
- - Hchichina: (34°25'0"N, 10°10'60"E) a coastal city in the Governorate of Sfax located 66 km from the Gabès region and close to the mining area of Skhira;
- - Zarrat: (33°40'0"N, 10°21'0"E): a coastal city in south-eastern Tunisia located about 30 km south of the urban and industrial agglomeration of Gabès;
- - Sidi Salem (33°53'28" N, 10°49'35"E): a city located on the Djerba Island (indicated as "Djerba" in the following analyses), and characterized by significant urban pollution caused by mass tourism.

Sediment samples and *R. decussatus* individuals were processed in the laboratory adopting a procedure identical to that described for the Bizerte Lagoon location.

Morphological analyses

For each bivalve, the total wet weight and the shell wet weight (after dissection and removal of soft tissues by a ceramic scalpel) were measured to the nearest 0.01 g using a digital balance. In addition, six linear measures were determined to the nearest 0.01 mm using a digital calliper: total length (TL), shell width (SW), shell thickness (ST), distance between anterior and posterior adductor muscle scar (DA), length of exhalant siphon (LES), and length of inhalant siphon (LIS) (Fig. 1B).

Elemental analysis in sediments and bivalves

Six and ten clam specimens per location were randomly selected for the determination of element concentrations in shells and soft tissues, respectively. Sediment and clam samples were oven-dried for 48 h at 100°C and 100 mg of each sample were mineralized in a Hot Blok for 1 h 15 min at 96°C in 1.5 ml of nitric acid and 4.5 ml of hydrochloric acid and adjusted to 10 ml with deionized water. Clams' soft tissues were subsequently dried to a constant weight at 55°C for 48h, mineralized with 3 mL of nitric acid (1 M), and then stored for 48h (Annabi et al., 2013). The product was adjusted to 30 mL with deionized water and analysed for the macroelements, Ca and Mg, and for the trace elements, Al, Cu, Fe, Hg, Pb, and Zn, in triplicate (variability < 10%), using Inductively Coupled Plasma Optical-Emission

Spectroscopy (Jobin Yvon Horiba JY2000 Ultrace ICP-OES). Elemental concentrations were given as µg g^{-1} of dry weight.

Data analysis

Values in the text are expressed as mean ± 1 SE. Prior to analyses, data were Log_{10}-transformed and checked for normality and homoscedasticity (Shapiro-Wilks and Levene's tests, respectively). Morphological data met the assumptions; accordingly, univariate parametric statistical procedures (i.e., ANOVA and Pearson product-moment correlation) were used for among-location comparisons and for assessing the strength of co-variation among parameters (α = 0.05). Conversely, elemental concentrations were both non-normal and heteroscedastic, and thus non-parametric univariate procedures (i.e., Mann-Whitney U test and Spearman rank correlation) were generally adopted.

For both sediments and biological samples, Euclidean distance similarity matrices were constructed with Z-scaled macroelement and trace metal concentration data. Exploratory 3d non-metric multi-dimensional scaling (nMDS) was subsequently performed, while a type III permutational multivariate analysis of variance (PERMANOVA; Anderson, 2005), based on 9,999 unrestricted permutations of raw data, was used to test the null hypothesis of no difference in elemental concentrations among the five locations. nMDS and PERMANOVA were also applied to assess differences in *R. decussatus* morphological features across the five locations. Principal Component Analysis was used to analyse covariation among morphological variables and to reduce their number. To test the congruence among the multivariate structure of morphological data and elemental concentrations in sediments, bivalve shells and soft tissues, centroids were calculated for all datasets. The strength concordance among datasets was assessed by estimating the Spearman coefficient Rho via Mantel tests with 9.999 permutations.

All statistical procedures were implemented using the R package (R Development Core Team, 2020). Specifically, nMDS, PERMANOVA, and congruence analyses were performed using the *metaMDS*, *adonis*, and *bvStep* functions of the *vegan* package, respectively (Oksanen et al.,, 2019).

Results

Sediments

Sediments samples of the five locations showed significant differences in macroelements contents (PERMANOVA, Pseudo-$F_{4,10}$ = 4.25, *P*(MC) = 0.01). Further bivariate comparisons showed that Hchichina differed significantly from Bizerte, Djerba, and Gargour, the latter in turn being different from Zarrat (Tab. S1 in online information). Differences were mostly due to variations in Mg, that showed in Hchichina and Zarrat concentrations respectively three times and twice higher than in the remaining locations, while Ca showed negligible among-site variations (Fig. 2).

Trace metals concentrations also varied significantly among locations (PERMANOVA, Pseudo-$F_{4,10}$ = 16.11, *P*(MC) = 0.001; see also Fig. 3A), with significant bivariate differences among all locations with the exception of Zarrat vs. Bizerte, Gargour, and Djerba (Table S1). Hg concentrations were always below the instrumental detection limits, while Pb showed negligible inter-site variations. In contrast, maximum and minimum concentrations of Al, Fe, and Zn were determined at Hchichina and Djerba respectively, while at the remaining locations the three elements showed comparable concentrations or characterized by relatively minor differences (Fig. 3B). Ca was negatively correlated with Zn (Spearman r = -0.9, $P < 0.05$); conversely, Al, Fe, and Zn showed comparable but non-correlated variation patterns (Fig. 4B; max Spearman $r = 0.7$, Fe vs. Zn).

Shells and soft tissues of R. decussatus

Macroelement contents in shells varied significantly among locations (PERMANOVA, Pseudo-$F_{4,10}$ = 8.64, *P*(MC) = 0.001). As observed for sediments, Ca showed negligible differences among locations (Fig. 2). While considering Mg significant differences were highlighted between Zarrat and all the remaining sites, and between Bizerte and Djerba (Table S1). In fact, Mg showed a pattern of inter-location variation, generally reflecting that observed for sediments, and varied significantly in particular between Zarrat, characterized by the lowest concentration of the element, and the other four sites (Fig. 2).

Hg and Pb concentrations in shells were always below instrumental detection limits. For the remaining trace metals, significant inter-location differences were observed (PERMANOVA, Pseudo-$F_{4,10}$ = 39.4, *P*(MC) = 0.001 see also Fig. 4A for the relative nMDS plot). Further bivariate comparisons showed that significant differences occurred among all sampling sites (Table S1). Similarly to what was observed for sediments, Al, Fe, and Zn showed significant maxima at Hchichina, followed by Bizerte, while at Gargour and Zarrat the three elements showed similar contents or with relatively minor differences. Notably, Al was not detected in the shells from Gargour (Fig. 4B). Negligible relationships were observed between Ca and trace element concentrations; however, Fe was significantly correlated with Mg and Zn. Moreover, Fe concentrations in shells were significantly correlated with Fe, Al, and Zn concentrations in sediments (min Spearman r = 0.9, $P < 0.05$). In general, macroelements in *R. decussatus* soft tissues varied significantly among locations (PERMANOVA Pseudo-$F_{4,45}$= 6.16, *P*(MC) = 0.001); Ca and Mg showed a similar pattern of variations across locations, with Gargour showing the highest concentrations, followed by Djerba, Bizerte, Hchichina and Zarrat (see also Table S1 for bivariate comparisons).

Regarding trace metals, mercury was undetectable in the soft tissues of all samples. The remaining metals varied significantly among sites (PERMANOVA, Pseudo-$F_{4,45}$= 5.76, *P*(MC) = 0.001; see also Fig. 4C-D for an nMDS plot and for metal-specific patterns), with significant bivariate differences among all the locations with the exception of Gargour vs. Hchichina and Bizerte (Table S1). Bivalves from Djerba showed Al contents one order of magnitude higher that in the other locations (Fig. 4D). In addition, maximum and minimum Fe and Zn contents were observed at Gargour and Zarrat, respectively, while the

remaining locations showed intermediate values; Pb was not detected in samples from the Bizerte Lagoon while negligible concentrations were observed in the rest of the locations (Fig. 4D).

R. decussatus morphology and relationships with elements content

In general, morphological anomalies were not observed in the samples collected (shells and siphons). The morphological traits of *R. decussatus* showed a remarkable inter-location heterogeneity, with individuals' total and shell wet weights varying by four times from minima observed in the Bizerte Lagoon and maxima at Djerba and Zarrat (Table 1). Shells' linear measurements were characterized by a similar, but less pronounced, variability (Table 1). In contrast, the length of the exhalant and inhalant siphons, showed a different pattern of variation, with the highest and lowest values determined in the Gargour and Djerba populations, respectively (Table 1).

Table 1 Morphological features (means ± SE in brackets, n = 55) of *Ruditapes decussatus* from the five locations included in the study. TW = total weight, SW shell weight (both expressed in g), SWi = shell width, TL = total length, ST = shell thickness, DAM = distance between the anterior and posterior adductor muscle scar, LIS = length of inhalant siphon, LES = length of exhalant siphon (all expressed in mm). The parameters used in further analyses are reported in bold, after testing for correlation among the eight morphological measures (Tab. S2); the results of a permutational ANOVA followed by bivariate between-location comparisons are also reported.

	Bizerte (1)	Gargour (2)	Hchichina (3)	Zarrat (4)	Djerba (5)	Pseudo-$F_{4,275}$
TW	2.71 (0.2)	1.4 (0.09)	17.22 (0.28)	26.61 (0.39)	10.86 (0.2)	126.9***; (1) < (3) < (2) = (4) < (5)
SW	9.07 (0.4)	4.62 (0.2)	24.45 (0.33)	35.39 (0.49)	16.49 (0.27)	
SWi	7.18 (0.45)	4.49 (0.3)	23.24 (0.48)	33.3 (0.74)	15.58 (0.4)	
TL	10.64 (0.73)	6.02 (0.41)	24.97 (0.66)	37.04 (0.82)	17.03 (0.43)	
ST	12.09 (0.34)	6.45 (0.17)	27.02 (0.23)	39.74 (0.37)	17.67 (0.2)	
DAM	2.71 (0.2)	1.4 (0.09)	17.22 (0.28)	26.61 (0.39)	10.86 (0.2)	
LIS	9.07 (0.4)	4.62 (0.2)	24.45 (0.33)	35.39 (0.49)	16.49 (0.27)	11.2***; (5) < (1) = (3) = (4) < (2)
LES	7.18 (0.45)	4.49 (0.3)	23.24 (0.48)	33.3 (0.74)	15.58 (0.4)	

A Principal Component Analysis indicated that only two factors explained 94.7% of the total variance in the dataset (Factor 1 = 73.2%; Factor 2 = 21.4%). While the lengths of the exhalant and inhalant siphons contributed significantly to the second factor (factor loadings = 0.97 and 0.98, respectively), all the remaining morphological variables contributed to the first factor (minimum factor loading = 0.93 for the

distance of the adductor muscle). A correlation analysis performed for each location generally confirmed the results of the PCA (Table S2); accordingly, subsequent analyses focused only on two parameters, i.e. the total individual wet weight and the length of the inhalant siphon. The high inter-location variability suggested by the NMDS plot (Fig. 5A) was confirmed by a PERMANOVA followed by bivariate contrasts (Pseudo-$F_{4,275}$ = 51.1, *P*(MC) = 0.001; Tab. S3 for bivariate contrasts; see also Table 1 for univariate comparisons).

Figure 5B) summarizes the results of the tests assessing the congruence between the multivariate structure of morphological data and those of macro- and trace element concentration data in sediments, bivalve shells and soft tissues, as summarized by their respective centroids. Inter-location variations in *R. decussatus* morphology were negatively related with those characterizing macroelements' concentrations in the bivalves' soft tissues, the latter in turn reflecting that observed in sediments (Fig. 5B). In addition, morphology was positively related with shell macroelements content, the latter showing a positive, yet statistically non-significant (Rho = 0.46, *P* = 0.12) relation with soft tissues. In general, *R. decussatus* morphology showed negligible relationships with trace metals, either in sediments or in bivalves' shell and soft tissues max Rho = -0.19, *P* = 0.56). However, trace metals in sediments were positively related to those in shells, but not to those in soft tissues (Fig. 5B).

Discussion

Overall, the present study highlights a clear spatial coupling between sediment elemental composition and bioaccumulation patterns in *Ruditapes decussatus*, while revealing a limited integration between trace metal variability and morphological traits. Elemental concentrations in sediments were consistently reflected in clam shells, confirming the strength of benthic–biotic interactions in these coastal systems. However, despite significant spatial differences in trace metal concentrations, no corresponding morphological alterations were detected (Table S3). This pattern suggests that, under the relatively low contamination levels observed across the study sites, trace metals may accumulate without inducing detectable phenotypic responses, whereas macroelements—particularly Mg—appear more closely associated with inter-population morphological variability (Table S4).

Macroelements and trace metals in sediments and in R. decussatus

Sediment analyses revealed significant spatial variability in macroelement composition, primarily driven by differences in magnesium content, whereas calcium showed comparatively lower differentiation among sites. The predominance of carbonate-rich sediments likely shapes the geochemical background of these coastal systems, influencing elemental distribution and availability. In biological matrices, macroelement concentrations were consistently higher than trace metals, as expected given their fundamental role in biomineralization and physiological processes. In particular, magnesium may contribute to shell structural variability, as Mg incorporation can affect crystal formation and shell

properties. These findings suggest that natural geochemical gradients, rather than contamination per se, may contribute to inter-population differences observed in shell traits.

In general what has been obtained by results of the present studies was common with previous studies. Indeed, Missaoui et al., (2016) revealed a high natural water content was observed in the Gulf of Gabes sediment with 17% of organic matter content. According to the standard (NF P94-048, 1996), Gabes sediment can be considered as moderately organic soil and the calcium carbonate content classified this sediment as slightly calcareous.

Trace metals in sediments

race metal concentrations exhibited clear spatial heterogeneity across sites,

with higher values generally observed in areas influenced by industrial activities. However, measured concentrations remained within the lower range of surface sediment concentrations reported for the Mediterranean region (1.7 to 6200 ppm for Zn; 3 to 3300 ppm for Pb; and 0.6 to 1890 ppm for Cu), (according to the MAP Technical Reports Series, 1996). Mercury concentrations in our samples are undetectable and therefore lower than those obtained in the northern Lake of Tunis which varied from 0.17 to 2.6 ppm (Anonyme 1996) and lower than the Dutch standards for surface sediment quality (15 ppm of total Hg).

Among all five sites analysed, the Gargour site, located 17 km south of Sfax, exhibited the highest concentrations of the analyzed metals (Al, Fe, Mg, Zn, and Pb). Previous studies have shown high levels of copper, zinc and cadmium at this site due to the high industrial activity in the area and the transport of effluents from streams (Hamza-Chaffai et al., 1999, 2003).Moreover, the carbonate phases, commonly present in sediments, have the capacity to retain heavy metals.Copper (Cu) and zinc (Zn) are often associated with these mineral phases. Therefore, variations in calcium (Ca) content may indirectly reflect differences in sediment geochemical properties influencing metal retention (Plassard et al., 2000; Yong et al., 1990).

In shells and soft tissues

Similarly, previous studies have shown that metals preferentially associate with the finest fraction of the sediment (Solodov et al., 1998; Coulibaly et al., 2008). The trapping of heavy metals in fine sediments could pose a more or less long-term ecotoxicological concern for aquatic life. According to François et al., (2002), there is a risk of remobilization and their absorption by living organisms when physico-chemical conditions allow it.

Macro- and micro-elements in clams

The shell of bivalves is a complex structure of proteins and calcium carbonate crystals made from chemical elements extracted from seawater. In our study, a relatively low concentration of iron, aluminum and zinc in the shells of *R. decussatus* was found, whereas for the macro elements, magnesium and calcium, the concentrations were higher. Relatively high concentrations are expected for

calcium since the shell structures of the bivalves are mainly composed of calcium carbonate (Der Sarkissian et al., 2017). Our results are consistent with other works carried out on *R. decussatus* in the Gulf of Gabès (Hamza Chaffai et al., 2003; Smaoui Dammak et al., 2003) and in northern Tunisia (Chouba et al., 2001). These studies show that metal content in this species is lower than those observed in another bivalve species (*Cerastoderma glaucum*). The work of Rabaoui et al., (2012) also revealed higher levels in different species of mollusks collected in the Gulf of Gabès. Indeed, for the gastropod *Gibulla ardens* the lowest concentrations of mercury and lead were 0.042 µg/g in the Elbibene Lagoon and 0.633 µg/g in the Chebba region, respectively, while the highest values were found in the region of Zarzis (0.181 µg/g) and in the island of Djerba (2.543 µg/g). For the *Pinna nobilis* samples, the average mercury concentrations were of the order of 0.027 µg/g (Elbibane Lagoon), reaching a maximum of 0.312 µg/g in Louata. Lead concentrations ranged from 0.691 ± 0.1435 µg/g in the Elbibane Lagoon to 1.682µg/g in the island of Djerba.

Trace metals were detected in both shells and soft tissues, with spatial variability consistent with sediment patterns. However, accumulated concentrations remained within relatively low ranges and did not correspond to detectable structural alterations. Metal bioaccumulation is known to depend on speciation, bioavailability, and organismal traits; thus, under mild exposure scenarios, accumulation may occur without exceeding physiological thresholds necessary to induce morphological disruption.

The higher concentrations observed at the Gargour site likely reflect local anthropogenic inputs, consistent with previous reports identifying the northern Gulf of Gabès as one of the most impacted areas due to industrial and urban discharges, including phosphogypsum deposits (Machreki-Ajmi and Hamza-Chaffai, 2006). Nevertheless, even at this site, the contamination gradient appears insufficient to trigger overt phenotypic effects in *R. decussatus*.

The ecological implications of metal exposure depend strongly on speciation, mobility, and bioavailability (Boust et al., 1999), as well as on organism-specific traits such as age, sex, developmental stage, and tissue distribution (Rand et al., 1995; Amiard-Triquet & Rainbow, 2009). Consequently, the absence of detectable morphological alterations in the present study does not necessarily imply the absence of ecological relevance, but rather indicates that contamination levels likely remained below thresholds capable of inducing structural impairment. In this context, although measured concentrations did not raise immediate concern, the persistence of anthropogenic discharges and the well-documented capacity for metal bioaccumulation in marine organisms support the need for continued environmental monitoring (Machreki-Ajmi and Hamza-Chaffai, 2006).

Relationship with morphology

The present study contributes to assessing the variability of selected morphological and physiological traits of the European clam *Ruditapes decussatus*, a species for which detailed morphometric investigations remain relatively limited (Gérard, 1978; Maître-Allain, 1983). The analysis of specimens collected from the five study sites revealed significant inter-population differences in several morphological parameters.

Although all analyzed individuals were sexually mature (maximum length > 25 mm), specimens from the Bizerte Lagoon were clearly distinct from those collected at the other locations, showing the lowest total and shell wet weights (Table 1). This lagoon ecosystem is subject to multiple anthropogenic pressures, including domestic and industrial discharges (ANPE, 1990), which may influence environmental conditions and resource availability. Due to limited lagoon–sea water exchange, released pollutants may accumulate in sediments and become integrated into the ecosystem (Essid, 2008). However, despite these environmental constraints, no clear morphological deformities were observed.

Although chemical characteristics of sediments were investigated as potential determinants of morphological differences, trace metal concentrations did not show detectable effects on shell structure or siphonal development. This absence of morphological anomalies is consistent with the relatively low contamination levels measured in both sediments and biological tissues. Previous studies have documented shell malformations and anatomical anomalies in bivalves exposed to higher levels of environmental stress or disease (Buschbaum and Saier 2001; Trigui El Menif et al., 2006; El Bour et al., 2002; Jaafar et al., 2004; Paillard et al., 2004; Trigui El Menif et al., 2005). In the present case, however, macroscopic examination of shells and siphons did not reveal epibionts, brown ring disease, or structural deformities.

Overall, the results suggest that the morphological variability observed among populations of *R. decussatus* is more plausibly associated with local environmental and trophic conditions than with trace metal contamination. Under the relatively low contamination gradient detected across the study sites, trace metals may accumulate in shells and tissues without inducing measurable phenotypic alterations. The absence of temporal replication limits our ability to account for seasonal variability, which is known to influence bivalve growth dynamics and metal bioaccumulation patterns. Future research integrating temporal replication would help refine the interpretation of sub-threshold contamination effects.

Conclusions

This study investigated the relationships between sediment chemistry, elemental accumulation, and morphology of the grooved carpet shell *Ruditapes decussatus* across contrasting coastal environments in Tunisia. Our results show that spatial variability in sediment composition is clearly reflected in the elemental profiles of clam shells and soft tissues, confirming a strong benthic–biotic coupling in these ecosystems.

Despite significant spatial differences in trace metal concentrations, overall contamination levels were low and did not induce detectable morphological alterations in *R. decussatus*. This finding suggests that, under current environmental conditions, trace metal exposure is insufficient to exert measurable effects on shell growth or siphonal development. In contrast, macroelements and particularly Mg displayed a significant congruence between sediments, clam tissues, and shell morphology, indicating that element availability linked to local geochemical and trophic conditions plays a more prominent role in shaping phenotypic variability.

The relation among macro- and micro elements in sediment and in clams' tissues was not significant, trace metals in sediments were positively related to those in shells, but not to those in soft tissues. While the study of the relation between the chemical concentrations in the different matrices (biotic and abiotic) and the morphological variations in clams showed that: In general, *R. decussatus* morphology showed negligible relationships with trace metals, either in sediments or in bivalves' shell and soft tissues, while a significant congruence between *R. decussatus* morphology and macroelements in bivalves' shells and soft tissues was observed. Considering trace metals, they have no effects, likely due to the fact that their concentrations in sediments and bivalves are relatively low and inadequate to induce significant variations in the morphology of the grooved carpet shell.

Overall, *R. decussatus* proves to be an effective biomonitor of spatial variation in sediment chemistry, particularly for macroelements and bioavailable trace metals recorded in shells. However, morphological traits alone appear to be poor indicators of trace metal contamination when pollution levels remain below ecotoxicological thresholds. Future studies should integrate seasonal sampling, higher contamination gradients, and complementary physiological or molecular biomarkers to better resolve the relative contributions of chemical stressors and environmental drivers to bivalve phenotypic plasticity.

Declarations

Ethical Responsibilities of Authors

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work.

This article does not contain any studies involving human participants or animals performed by any of the authors. Ethics approval and consent to participate: Not applicable.

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Author Contribution

AA conceived the presented study and carried out the biological analysis. WBA and AA contributed to sample collection and preparation for chemical analysis. GM conceived the statistical analysis and wrote the manuscript with support from AA. FF and MC: Supervision, Writing – review & editing. All authors discussed the results and contributed to the final manuscript.

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Data Availability

All data generated or analyzed during this study are included in this published article. The additional statistical analysis is available in the supplmenetary materials.

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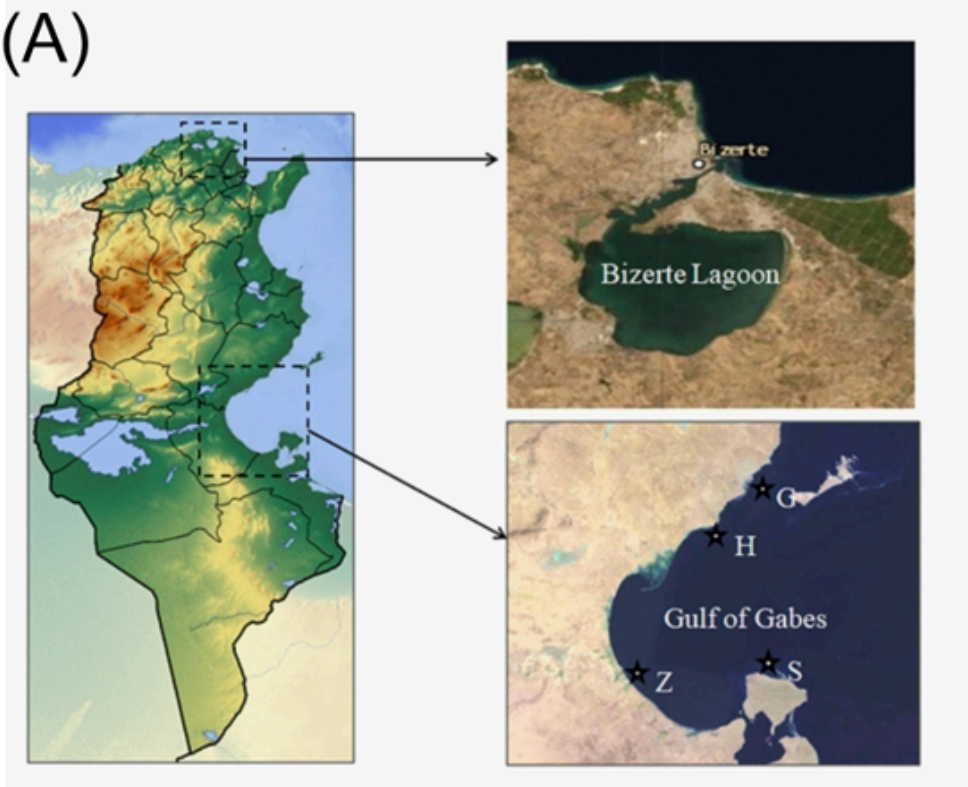
Figures

Fig. 1



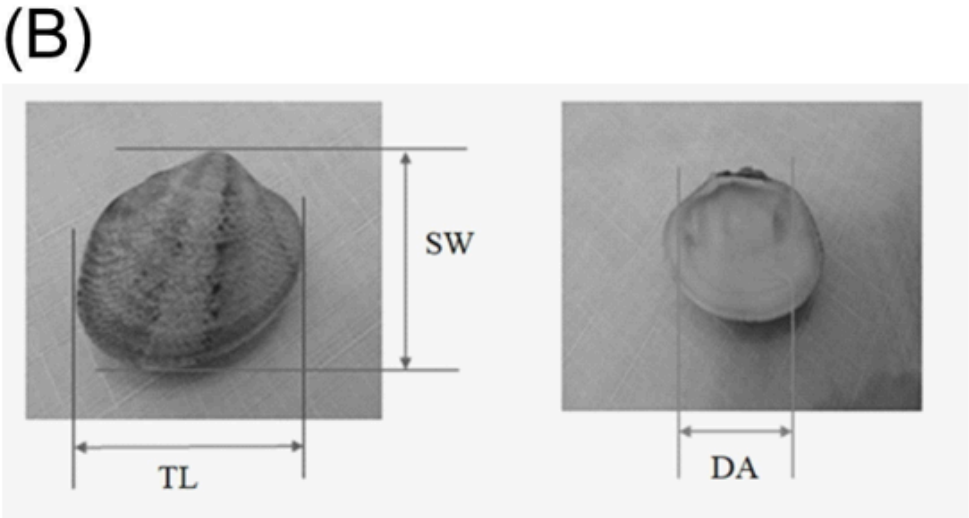


Figure 1

(A)Map of the studied locations: Bizerte Lagoon (Northern Tunisia) and the Gulf of Gabés (Southern Tunisia); for the latter, the insert shows the four coastal locations where sampling operations were performed. (B) *Ruditapes decussatus*: linear measurements included in the morphometric analysis. TL = Total Length; SW = Shell Width; DA = distance between adductor muscle (anterior and posterior).

Fig. 2

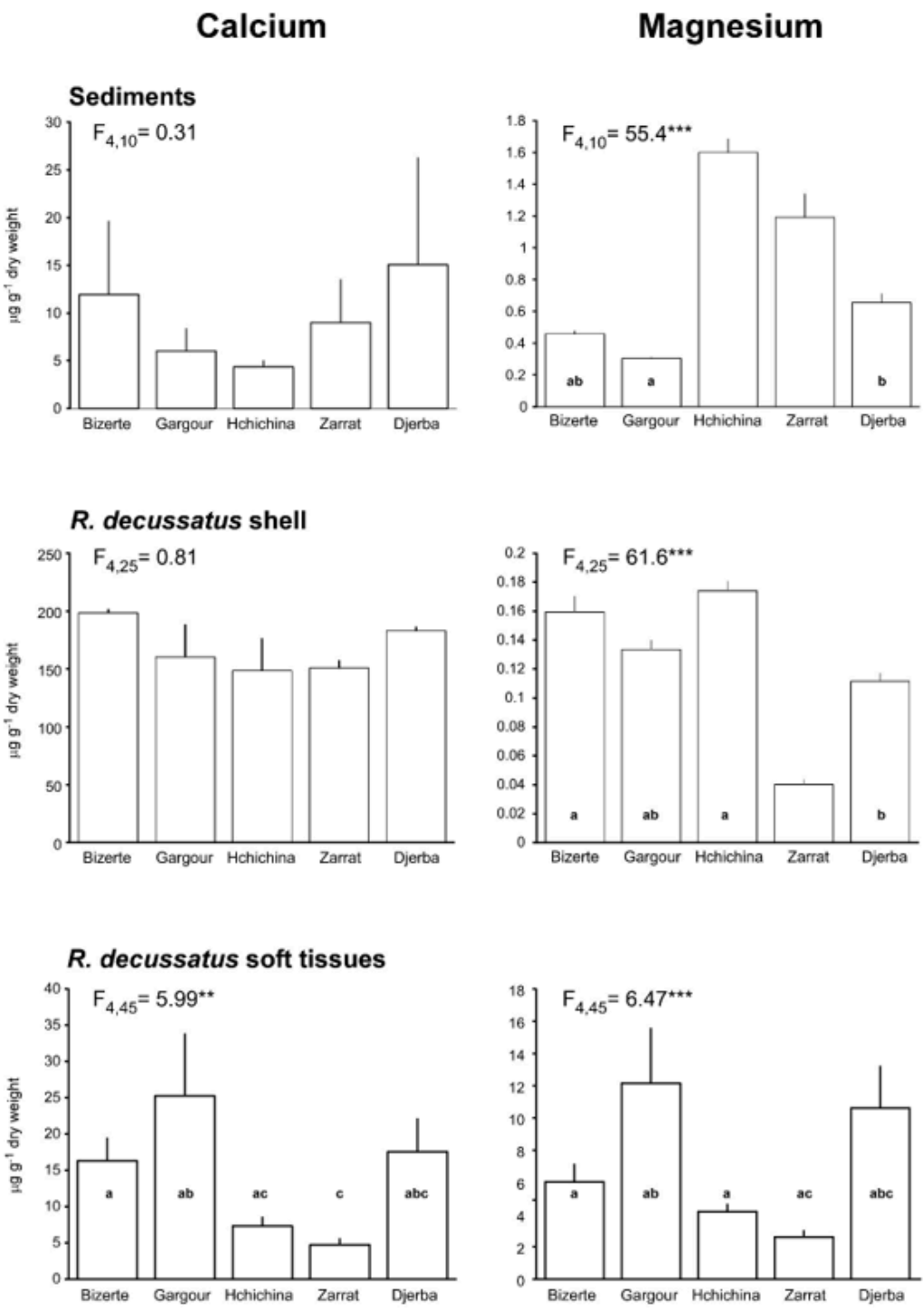


Figure 2

Calcium and Magnesium contents (expressed as mg g^{-1} dry weight) in sediments and in *Ruditapes decussatus* shells and soft tissues. Results of 1-way ANOVA (factor = location) are included; * = *P*< 0.05; ** = *P*< 0.01; *** = *P*< 0.001. Locations showing the same letters are not statistically different (Mann-Whitney U test, a = 0.05). Bars = 1SE.

Fig. 3

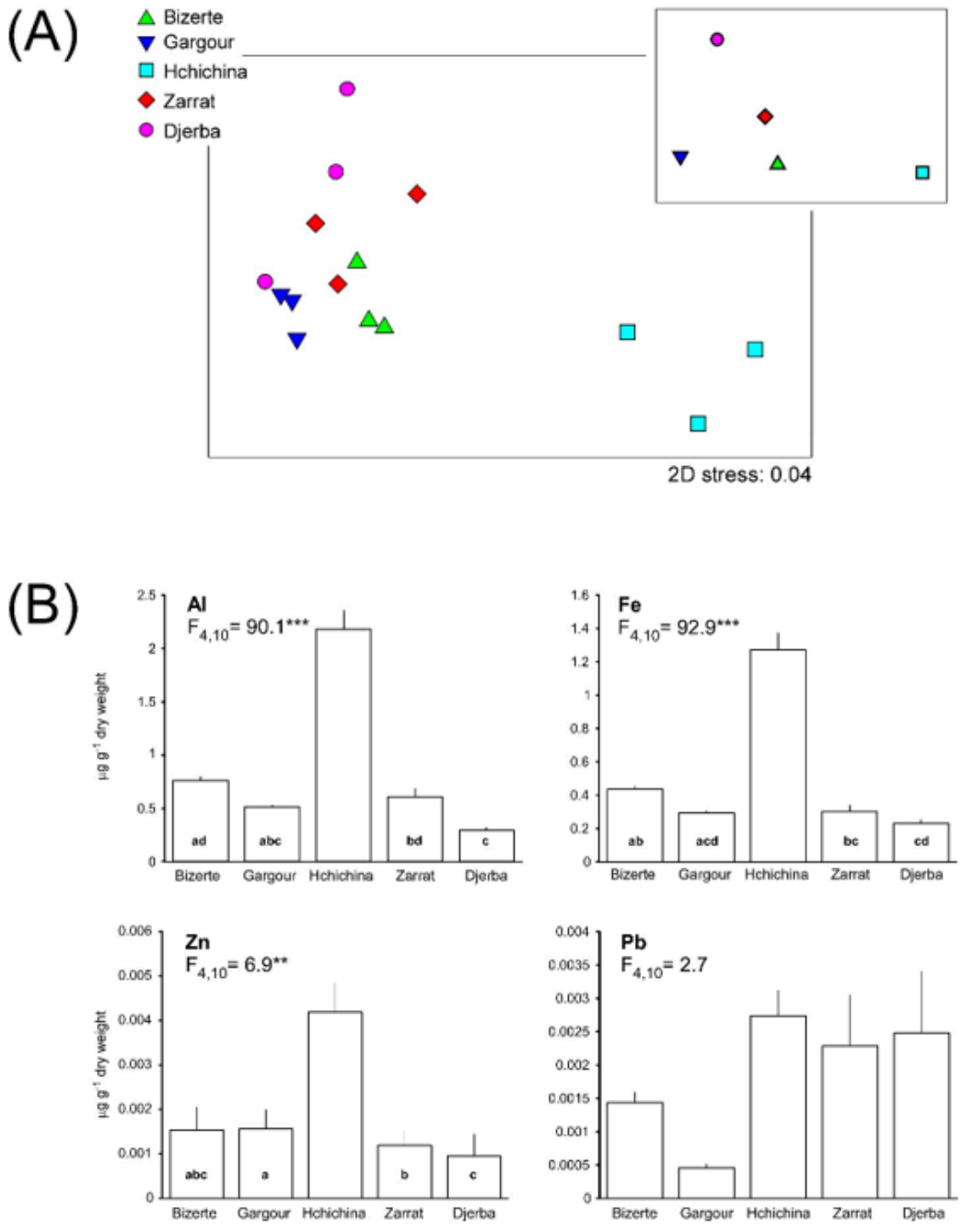


Figure 3

(A) nMDS plot of trace metal contents in sediments from the five sampling locations; in the insert, the nMDS plot of centroids is included. (B) Al, Fe, Pb, and Zn concentrations (expressed as mg g^{-1} dry weight) in sediments of the five locations. Results of 1-way ANOVA (factor = location) are included; * = $P< 0.05$; ** = $P< 0.01$; *** = $P< 0.001$. Locations showing the same letters are not statistically different (Mann-Whitney U test, a = 0.05). Bars = 1SE.

Fig. 4

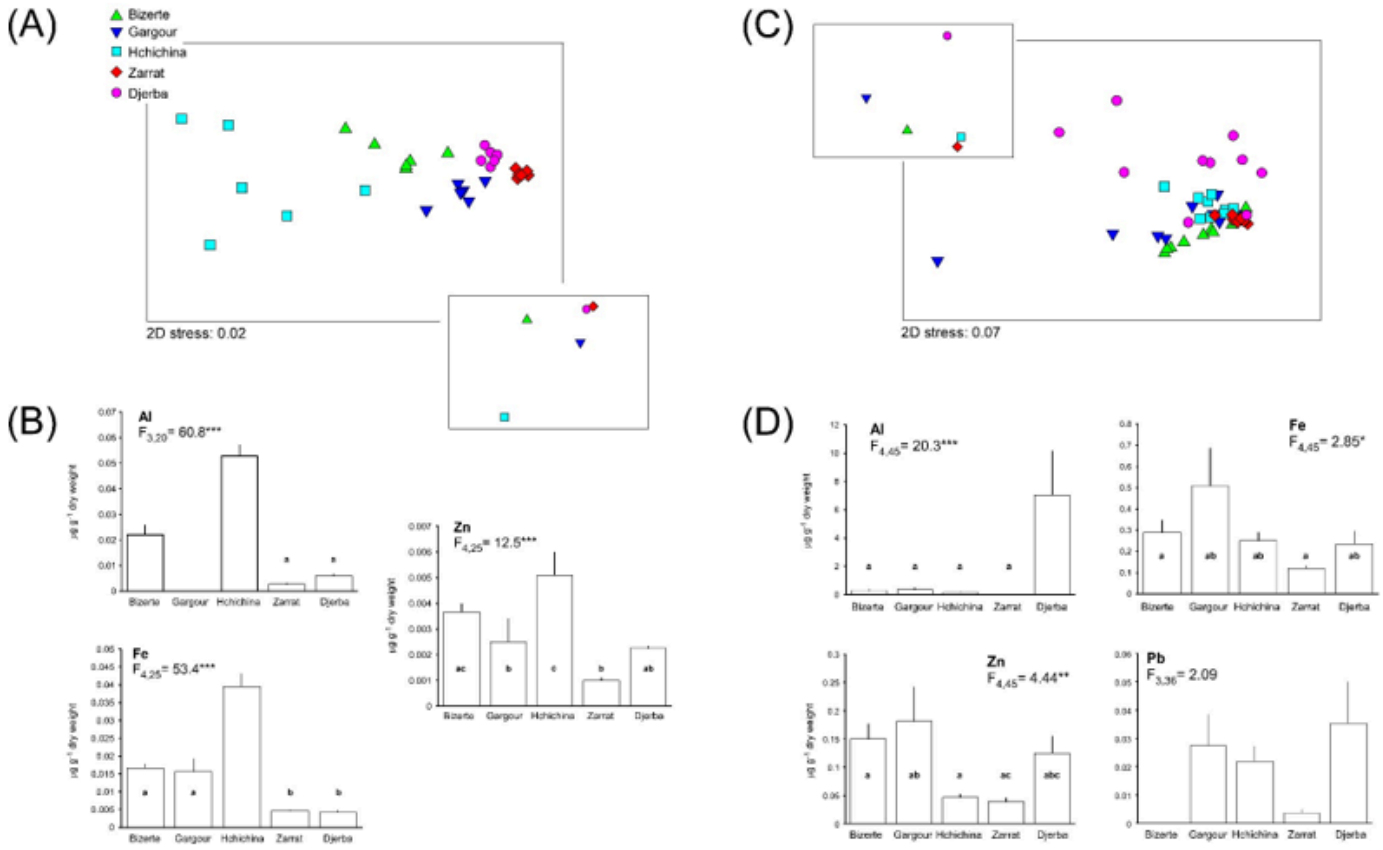


Figure 4

Top:nMDS plot of trace metal contents in shells (A) and soft tissues (C)of *Ruditapes decussatus* from the five sampling locations; in the inserts, the nMDS plot of centroids is included. Bottom: Al, Fe, Pb, and Zn concentrations (expressed as mg g^{-1} dry weight) in shells (B) and soft tissues (D) of *R. decussatus* from the five locations. Results of 1-way ANOVA (factor = location) are included; * = *P*< 0.05; ** = *P*< 0.01; *** = *P*< 0.001. Locations showing the same letters are not statistically different (Mann-Whitney U test, a = 0.05). Bars = 1SE.

Fig. 5 (A-B)

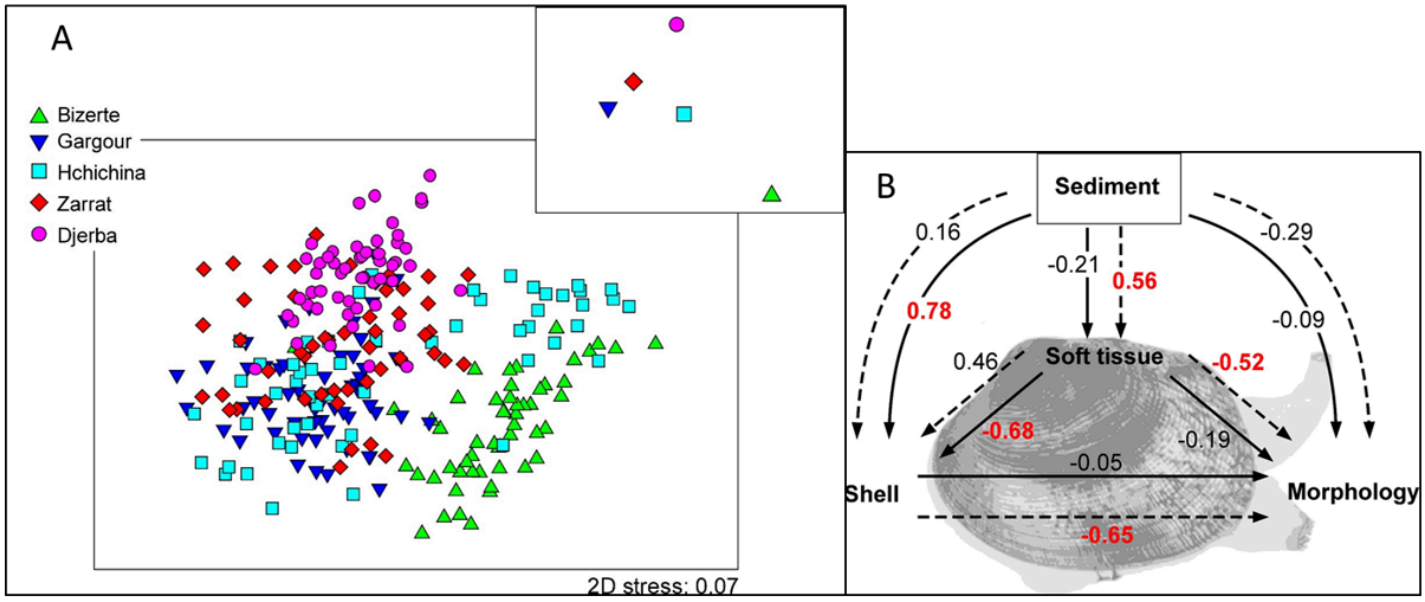


Figure 5

(A) nMDS plot of morphological parameters (total wet weight and length of the inhalant siphon) of *Ruditapes decussatus*from the five sampling locations; the inset shows the nMDS plot of centroids.

(B) Summary of the results of the congruence analysis among the multivariate structure of morphological data and macro- and trace element concentrations in sediments and in the shells and soft tissues of *Ruditapes decussatus*, as summarized by their respective centroids. Dashed arrows indicate macroelements, continuous arrows indicate trace metals. Values refer to Spearman's rho coefficients estimated via Mantel tests (9,999 permutations); significant values are reported in bold.

Supplementary Files

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- Supplementarymaterialsedit.docx