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Original article

Are iron ore microparticles toxic for the European clam *Ruditapes decussatus*? Response elements from biomarker activities and *in silico* modeling

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ABSTRACT

Inevitably, high concentrations of iron, the most widely produced ore globally, can be found in aquatic environments. To assess the toxicity of iron on aquatic organisms, *Ruditapes decussatus* specimens were subjected to microparticles derived from two types of iron ore (hematite and magnetite) at four different concentrations (0.5, 1, 1.5, and 5 g/L). The findings revealed that both types of iron ore were absorbed by clams in a concentration-dependent manner. Biomarkers analysis demonstrated significant and organ-specific impacts on the health of the clams caused by these microparticles, which was further supported by computational analyses on bioavailability. Within seven days of exposure, changes were observed in the activities of several enzymes, including catalase, acetylcholinesterase, and glutathione S-transferases, as well as in the rate of lipid peroxidation in both the digestive gland and gills. This study provides an environmental perspective on the toxicological effects of iron ore microparticles.

1. Introduction

Global reports have extensively documented humans' uptake of heavy metals through food chains (Mulvad et al., 1996; Kumar and Achyuthan, 2007; Achary et al., 2017). These heavy metals, characterized by their persistent nature and inability to degrade, tend to accumulate in vital organs like the liver, stomach, and kidneys. Consequently, their entry into the body poses significant health risks (Duruibe et al., 2007). Each heavy metal exhibits specific toxicity: Cd primarily affects the kidneys and bones, Hg is particularly detrimental to the human nervous system, Pb has neurotoxic effects on children and pregnant women, and long-term exposure to As increases the risk of skin cancer, dermatomycosis, and hyperpigmentation (Khanam, 2014). The mining of metals contributes to environmental pollution, particularly the contamination of water caused by mining processes and waste discharge. Several studies, such as those conducted by Carlsson et al. (2002), Alvarez et al. (2008), and Mataís (2008), have highlighted the significance of mining waste deposits in close proximity to mining areas as a major and easily transportable source of contamination. The movement of these waste deposits depends on critical factors such as wind speed, direction, waste volume, waste type, sulfide concentration, porosity, degree of compaction, and more. The residues generated from metal mining have a substantial negative impact on water and sediment quality, as indicated by research conducted by Lottermoser (2010) and Younger et al. (2002). In addition to the physical disturbances caused by the high discharge and excessive deposition of mining waste on the seafloor, the presence of metals and metalloids can result in toxic effects, as evidenced by studies conducted by Ramirez-Llodra et al. (2015), Morello et al. (2016), and Jordi et al. (2021). Most studies on mining activities focus on sites affected by sulfide ore residues, as demonstrated by the works of Sternal et al. (2017) and Pedersen et al. (2018).

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The extraction of iron ore is crucial for the modern economy as it is an essential resource for many metal mines. Iron ore is extracted in various countries, including Australia, Brazil, Mauritania, and China (WA, 2019), is the latter being the world's leading producer. However, the iron mining industry is also characterized by significant waste production. In the Quadrilátero Ferrífero region of Brazil, approximately 40% of the extracted mass of iron ore is rejected for every metric ton extracted (Mendes et al., 2019). The National Mining Industries Company (SNIM), which operates iron ore mining, is mostly positioned in the wilayas of Tiris Zemour (Zouerate) and Dakhlet Nouadhibou in the southern part of the Cape Blanc in Mauritania. It has a mineral port where iron ore is loaded onto large ships for transportation to international markets. During this operation, significant amounts of mine dust invade the coast. The Cap Blanc site has the highest metal content, as it receives most of the discharge from the country's largest iron ore processing industry (SNIM). Several studies indicate that spatial variations of trace metal elements analyzed inside mussels' tissues (*Perna perna*) along the Mauritanian coasts show heterogeneous and relatively low trends in the concentration of different metals analyzed in this species (Bilal, 2017; IMROP, 2019; Sidoumou et al., 1999; Legraa, 2019).

Bivalves are commonly used as biological indicators in environmental programs and policies due to their bioaccumulation of various pollutants, particularly trace metals (Sellami et al., 2017; Oliveira et al., 2018). Ecotoxicological studies using molluscs often rely on monitoring biomarker responses (Volland et al., 2017). The biological material used in the current study was the benthic filter-feeding clam species *Ruditapes decussatus*, which is largely present in the Mediterranean Sea and Northeast Atlantic Ocean, and its usefulness in the quality assessment of water and sediments in coastal areas (Sobral and Widdows, 1997). In silico bioavailability and toxicokinetics, based on the physicochemical properties, were assessed to support the experimental findings.

2. Material and methods

2.1. Chemistry of iron ore

X-ray fluorescence analysis has been adopted by the SNIM company to determine the elements present (Iron, SiO_2, Al_2O_3, P, H_2O, and other elements that have not been disclosed) in the two types of iron ore, XFA (Hematite ore), and GMAB (Magnetite ore) (Table 1).

The exploitation of hematite (Fe_2O_3) and magnetite (Fe_3O_4) iron ore represents a significant source of heavy metal emissions such as Ni, Pb, Cu, Co, Cd, and Zn. These heavy metals, found in iron ore dust, are then deposited on surfaces near the mines at different distances depending on wind speed and particle size (Simpson and Spadaro, 2016; Embile et al., 2018; Mendes et al., 2019; WA, 2019). In particular, the high occurrence of iron is common in polluted sites (Berrow and Mitchell, 1991; Colbeck, 1995; Adamson, 2000; Manceau et al., 2002), and can cause significant interferences, such as:

- Fe/Cu: the iron line tends to absorb that of copper,
- Co/Fe: overestimation of the iron values when measuring those of cobalt and *vice versa* (Sammut, 2007).

In light of these interferences, it has been decided to perform heavy metal analysis on the fine fraction of the iron ore using Flame Atomic Absorption Spectroscopy (attacked by HCl + HF followed by HNO_3 + HCl and recovery by HCl, then analyzed by SAA-Flame) for Co, Cu, Ni, and Pb (Fig. 1).

Table 1
Composition of iron ore.

	Iron ore fine XFA (%)	Iron ore fine GMAB (%)
Iron	58	66
SiO_2	14	7
Al_2O_3	1	0.35
P	0.075	0.015
H_2O	1.5	1.5
Other elements	25.43	23.135

2.2. Sampling of animals and implementation of contamination experiments

European clams *R. decussatus* of approximately identical sizes (3.25–4.1 cm) were gathered next to Menzel Jemil City (GPS coordinates: 37°13′31.6″N 9°55′40.9″E) at Bizerte lagoon (Tunisia) because of its pristine aspect with no close iron discharges. In the field, bivalves were collected arbitrarily, nonetheless of sex and size, and placed in 2 L glass aquariums full of seawater collected previously at the collection biotope. The selected clams are transferred to coolers filled with seawater until arriving at the laboratory. Bivalves were first acclimatized for three days before exposure to nine different experimental conditions, with ten individuals per aquarium. The experimental conditions included a control group (20 clams not exposed to iron ores) and eight groups exposed to varying concentrations of hematite and magnetite ores.

The experimental conditions correspond to the following treatments for both hematite ore and magnetite ore:

- An untreated control (20 clams).
- A concentration C1 (10 clams exposed to 0.5 g/L of ore).
- A concentration of C2 (10 clams exposed to 1 g/L of ore).
- A concentration C3 (10 clams exposed to 1.5 g/L of ore).
- A concentration of C4 (10 clams exposed to 5 g/L of ore).

Clams exposed to iron ore were exposed for 7 days. The selected time intervals were determined to evaluate the impact of salinity over time. Following a 7-day exposure period, the water in each glass beaker was removed and replaced every 48 h with natural seawater containing equivalent salinity levels. This process aimed to refresh the water and maintain a stable salinity environment. During that period, the aquaria were full of natural seawater and is renewed every couple of days. A constant temperature was adopted during the experiment (18 ± 1 °C); the dissolved oxygen and the salinity were kept at 6.25 mg/L, and 32 PSU, respectively. No loss of life was noticed at the time of the experiment and bivalves were fed ordinarily.

2.3. Evaluation of biochemical markers' activities

Following the dissection of each group of five bivalves, their organs (gills and digestive glands) were carefully collected and placed in a stake box filled with ice to maintain their freshness. The total protein extraction process was then conducted using a buffer solution consisting of distilled water (500 mL), sucrose (500 mM), EDTA (1 mM), Tris/HCl (10 mM), and PMSF (1 mM), with a constant pH value of 7.4. The tissues were crushed with the assistance of an Ultra-Turrax homogenizer (IKA T18 Basic) in the prepared buffer, and subsequently, they were centrifuged for 30 min at 9000 rpm (4 °C) to separate the proteins, which were quantified in the resulting supernatant according to the method described by Bradford (1976).

Catalase is implicated in cell protection in opposition to Reactive Oxygen Species. Herein, the evaluation of catalase activities (CAT) was based on the method reported by Aebi (1974).

Glutathione *S*-transferase (GST) is an antioxidant biomarker that is a binding enzyme used in xenobiotics' detoxification. Its activity was evaluated using the protocol mentioned in Habig

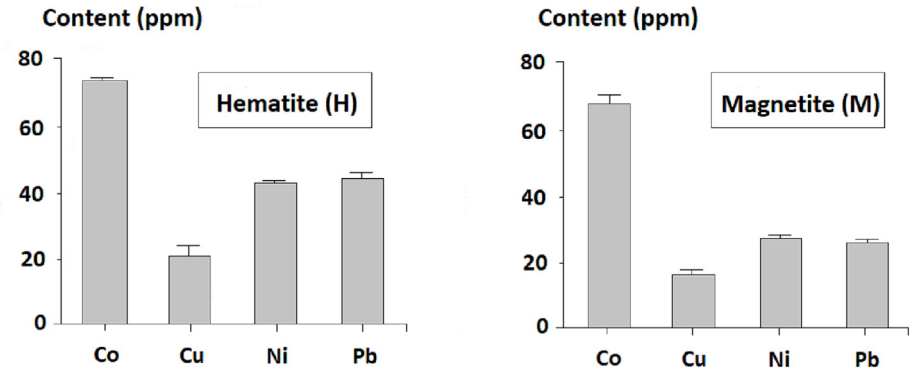

Fig. 1. Heavy metals' (Co, Cu, Ni, and Pb) contents measured in the two types of iron ore, namely Hematite ore and Magnetite ore.

et al. (1974). According to these authors, the substrates used are 1-chloro-2,4-dinitrobenzene and glutathione.

Malondialdehyde (MDA) rate is a lipid peroxidation marker of atherogenic lipoproteins. The measurement of MDA content was conducted as described by Haque et al. (2019).

Neurotoxicity was assessed by variations in acetylcholinesterase (AChE) activity, an enzymatic biomarker in charge of acetylcholine hydrolysis. AChE activity was evaluated by spectrophotometry after mixing 5,5′-dithiobis-2-nitrobenzoic acid and thiocholine to produce 5-thio-2-nitrobenzoic acid (Ellman et al. (1961).

2.4. Bioavailability and toxicokinetic properties

Bioavailability and toxicokinetic properties were investigated computationally, based on the physicochemical factors of hematite- and magnetite ore as reported earlier (Badraoui et al., 2023; Jedli et al., 2022; Zammel et al., 2022).

2.5. Data processing

The outcomes of the bioassay conducted were given as average ± SD. Significant differences between the controls and treatments were tested by one-way ANOVA. Whenever ANOVA outcomes are significant (p less than 0.05), multiple comparisons were made with the aid of Tukey's HSD test. All the data processing was accomplished using the program STATISTICA v.8.0.

3. Results

3.1. Chemistry of iron ore

The presence of high charges of iron, as shown in Table 1, was common in contaminated sites and can cause significant interference with heavy metals. Iron ores, such as hematite and magnetite, were analyzed using the SAA-Flame technique (Fig. 1). The Hematite (H) iron ore contains approximately 68.52 mg/kg of Co, 17.42 mg/kg of Cu, 28.28 mg/kg of Ni, and 26.75 mg/kg of Pb. On the other hand, the Magnetite (M) iron ore contains about 73.73 mg/kg of Co, 21.29 mg/kg of Cu, 43.51 mg/kg of Ni, and 44.59 mg/kg of Pb. The metal concentrations in Hematite-type iron ore microparticles follow thus the decreasing order of Co > Ni > Pb > Cu, with respective concentrations of 68.52 mg/kg, 28.28 mg/kg, 26.75 mg/kg, and 17.42 mg/kg. For Magnetite-type iron ore microparticles, the order is Co > Pb > Ni > Cu, with respective concentrations of 73.73 mg/kg, 44.59 mg/kg, 43.51 mg/kg, and 21.29 mg/kg.

3.2. Biomarker changes in R. decussatus

The activity variations of CAT, GST, and AChE, and levels of MDA, in both gills and digestive glands of *R. decussatus* contaminated by the two types of iron minerals (Hematite and Magnetite), are shown in Figs. 2, 3, 4, and 5, respectively.

3.2.1. Catalase (CAT) activity

Following exposure to two types of iron minerals (Hematite and Magnetite), the effect on CAT activity in clams is presented in Fig. 2. Compared to controls, CAT activity enhanced significantly in the gills and also the digestive glands of *R. decussatus* following the exposure to the two types of ore from concentration C3 (1.5 g/L). *R. decussatus* clams treated with 0.5 and 1 g/L (C1 and C2) revealed that microparticles of both types of iron ore had no significant impact on CAT activity in the two organs considered. However, the presence of in the experimental units 1.5 and 3 g/L (C3 and C4) was followed by a discernible augmentation in CAT activity in both organs for both types of iron ore microparticles.

The effect of magnetite microparticles at concentration C3, showing significant changes, was considerably superior in gills compared to digestive glands, while the effect of hematite microparticles at the same concentration C3 on both organs was similar.

3.2.2. Glutathione S-transferase (GST) activity

At the end of the bioassay, a significant increase was noticed in the GST activities (Fig. 3) in both organs considered for *R. decussatus* contaminated with hematite and magnetite iron ore microparticles compared to controls. For experimental units enriched with hematite microparticles, GST activity increased in comparison to controls in the gills and digestive glands of the European clams starting from C3 (1.5 g/L). For magnetite microparticles, a discernible increase in GST activity at gills was reported starting from C2 (1 g/L), while in the digestive glands, it was revealed starting from concentration C3 (1.5 g/L). The effect of both types of ore microparticles (hematite and magnetite) on GST activity was greater at gills compared to digestive glands.

3.2.3. Malondialdehyde (MDA) levels

For control specimens, the MDA levels showed no noticeable difference in the gills in comparison to the digestive glands (Fig. 4). These low MDA values in the controls confirm the stability of the experimental conditions during the experimental period. No significant difference was recorded in the clams contaminated with low doses (C1 and C2) compared to the controls. This result shows the absence of cellular damage in individuals contaminated with both types of microparticles of magnetite and hematite iron ore during the exposure period. Except for the gills of individuals

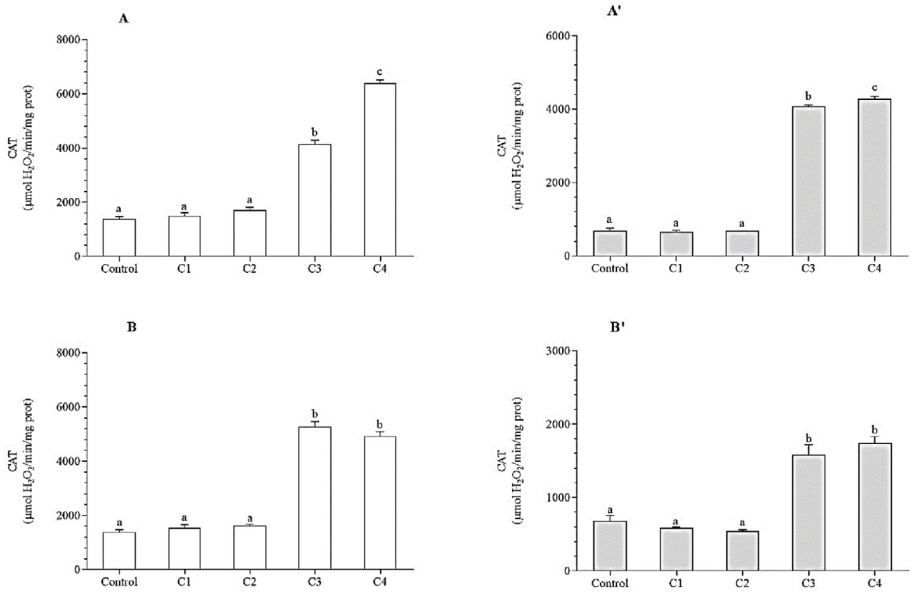

Fig. 2. Catalase (CAT) activities in gills (A and B) and digestive glands (A' and B') of *Ruditapes decussatus* contaminated with hematite and magnetite iron ore microparticles at four concentrations (C1-C4). Results are given as average values (n = 5) ± Standard Error (SE); Statistical significance is indicated by different letters (a, b, c).

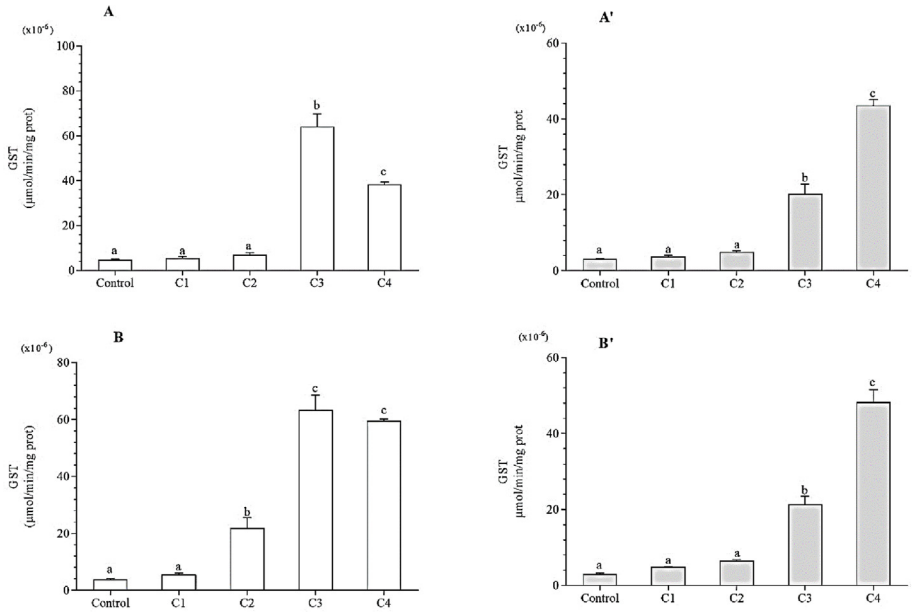

Fig. 3. Glutathione S-transferase (GST) activities in gills (A and B) and digestive glands (A' and B') of *Ruditapes decussatus* contaminated with hematite and magnetite iron ore microparticles at four concentrations (C1-C4). Results are given as average values (n = 5) ± Standard Error (SE); Statistical significance is indicated by different letters (a, b, c).

contaminated with magnetite-type iron ore where the MDA level began to increase from concentration C2 (1 g/L). Concentration C3 significantly modulated the MDA level at both gills and digestive glands after the two types of micro-particles of magnetite and hematite iron ore were introduced.

3.2.4. Acetylcholinesterase (AChE) activity

The exposure to hematite and magnetite microparticles caused a discernible inhibition in AChE activity (Fig. 5). The AChE activity measured after seven days from the start showed a significant inhibition for concentrations of iron ore microparticles equal to 0.5 g/L (C1), 1 g/L (C2), and 1.5 g/L (C3). Particularly, no significant modification was detected in comparison to the control under the treatment C4 (5 g/L) after a week of exposure.

3.3. In silico findings

The bioavailability and toxicokinetic features of iron ore are given in Table 2. The bioavailability polygons prove that hematite and magnetite ore possessed acceptable oral bioavailability and supported their potential toxicological outcomes. Fig. 6 exhibits that most of the physicochemical properties (flexibility, lipophilicity, etc.) allow iron ore to stand in the most suitable oral bioavailability, except unsaturation. While both pan assay interference structures and Brenk structural alert showed 0 alerts for hematite and magnetite ore, TPSA values were 43.37 and 36.92 respectively.

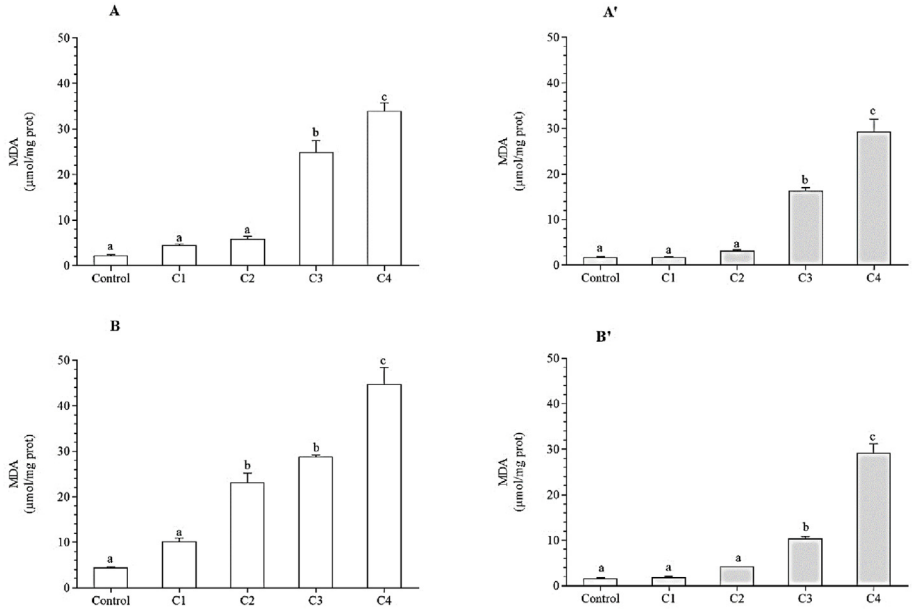

Fig. 4. Malondialdehyde (MDA) content in gills (A and B) and digestive glands (A' and B') of *Ruditapes decussatus* contaminated with hematite and magnetite iron ore microparticles at four concentrations (C1-C4). Results are given as average values (n = 5) ± Standard Error (SE); Statistical significance is indicated by different letters (a, b, c).

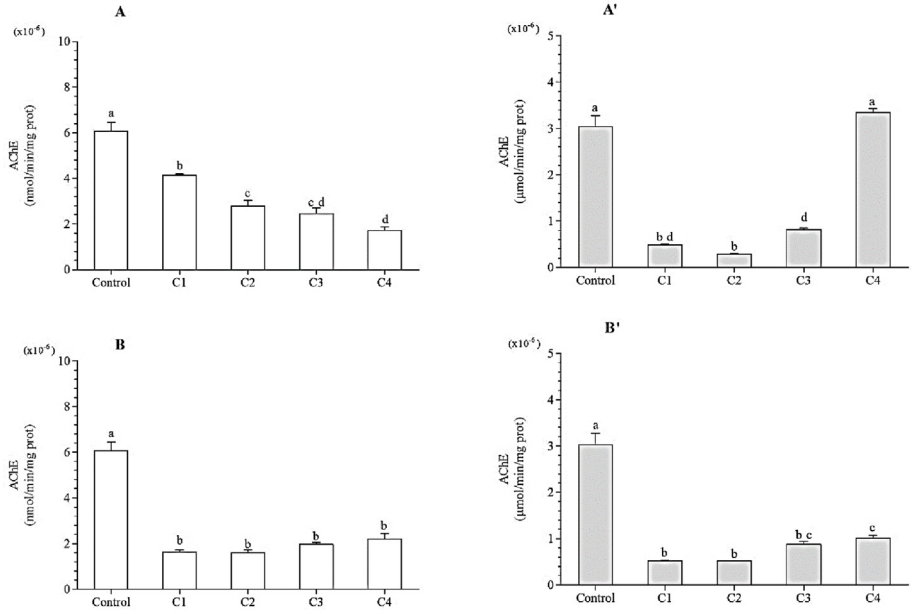

Fig. 5. Acetylcholinesterase (AChE) activities in gills (A and B) and digestive glands (A' and B') of *Ruditapes decussatus* contaminated with hematite and magnetite iron ore microparticles at four concentrations (C1-C4). Results are given as average values (n = 5) ± Standard Error (SE); Statistical significance is indicated by different letters (a, b, c).

Table 2
Bioavailability and toxicokinetic properties of hematite ore and magnetite ore.

Entry / Parameter	Hematite ore	Magnetite ore
Molar Refractivity	2.46	4.34
No. heavy atoms	5	7
No. rotatable bonds	2	0
TPSA (Å^2)	43.37	36.92
Pan Assay Interference Structures	0 alert	0 alert
Brenk Structural Alert	0 alert	0 alert

4. Discussion

The company SNIM exploits iron ore microparticles that have high metal contents for all measured metals. These results suggest that Magnetite-type iron ore microparticles contain higher metal concentrations than Hematite-type. Several research works confirmed already the detrimental impact of heavy metals on bivalves living in aquatic habitats. These filter-feeding organisms are known to absorb heavy metals from water, as evidenced by Chandurvelan et al. (2015) and Khazri et al. (2019). Once a high Reactive Oxygen Species (ROS) is observed, that is thought to be the cause of the harmful impact of heavy metals (Trevisan et al., 2014; Freitas et al., 2018). To mitigate the damage due to ROS,

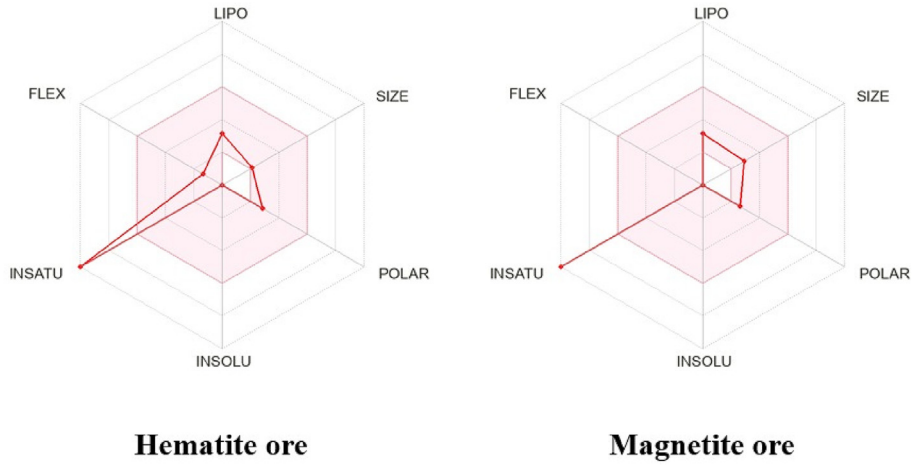

Fig. 6. Bioavailability hexagons of hematite ore and magnetite ore. LIPO: Lipophilicity, SIZE: Molecular size, POLAR: Polarity, INSOLU: Insolubility, INSATU: Insaturation, FLEX: Flexibility, The pink area represents the most suitable area for oral bioavailability. Note that regardless insaturation, both hematite ore and magnetite ore stand in the pink area, which is the most suitable for oral bioavailability.

organisms have developed defence tools such as antioxidant enzymes and non-enzymatic antioxidants. However, when ROS levels become too high, an oxidative stress status start, and biomolecule harm to proteins and lipids and have negative impacts on cells. According to the study of Shanker and Aschner (2003), the existence of metals in aquatic habitats may cause a significant modification of ROS and lead to oxidative stress. The current experiment aimed to examine the effects of exposure to hematite and magnetite iron ore microparticles on diverse biochemical markers linked to oxidative stress. For this purpose, the *R. decussatus* clams were considered as a biological model to evaluate the harmful impacts of iron ore microparticles on specific biomarkers. The detection of changes in markers of oxidative stress (enzymatic or not) within this bivalve indicates its exposure to this kind of pollutant, implying a potential hazard arising from its toxic properties. CAT, an enzyme, aids in the elimination of hydrogen peroxide (H_2O_2) that arises during oxidative stress in cells, according to multiple studies (Modesto and Martinez, 2010, *and references herein*). Its function is to accelerate the translation of H_2O_2 to H_2O and O_2 (Faria et al., 2010). Heavy metals can stimulate CAT and, thus, may be used as an oxidative stress biomarker (Ozkan et al., 2017). The results presented here indicate a discernible augmentation in the CAT activity in both organs tested (gills and digestive glands) of *R. decussatus* after the enrichment of its close environment with ore microparticles compared to controls (see Fig. 2). The noticeable susceptibility of mussels' gills, as opposed to their digestive gland, to stress caused by heavy metals, can be clarified by the fact that gills serve as the primary organs through which these invertebrates interact with water. They perform essential functions such as filtration, respiration, and ion exchange, and act as the primary interface for contaminants. The distinct reactions observed in this study among mussels exposed to varying concentrations provide a novel understanding regarding the species' environmental adaptability mechanisms, particularly concerning heavy metals, as well as the interplay between these changes and the levels of exposure. The outcomes of the bioassay conducted are in accordance with the conclusions of Valko et al. (2005) that the presence of metals can cause oxidative stress. Proportionality between the levels of copper present in the iron ore microparticles and catalase activity was also observed, suggesting that the augmentation in this activity followed exposure to copper. Copper has been previously identified as a toxic metal by Geracitano and Monserrat (2002) and Ferreira-Cravo et al. (2009). The discernible augmentation in CAT noticed at a C3 concentration resulted from the production of H_2O_2 as part of the antioxidant protection mechanisms that aim to defend cells against oxidative damage. Metal particles can cause oxidative stress by crossing the cell membrane barrier (Vale et al., 2016), leading to an inequality in pathways of oxidants and antioxidants (Falfushynska et al., 2016). The antioxidant phase II defence mechanism is characterized by the presence of GST enzymes, which are known for their substrates. According to the reports by Damiens et al. (2007) and Durou et al. (2007), GSTs are efficient to detoxify aquatic organisms with various xenobiotics (metals PAHs, PCBs, etc.). In *D. trunculus* living on the coast of Taghazout, a clear increase in GST activity was noticed, which is probably due to the presence of metals that stimulate the antioxidant protection processes (Radwan et al., 2010). This suggests that the detoxification pathway was induced by oxidative forces controlled by this enzyme, as reported by Elia et al. (2007). The presence of toxic metals and persistent organic xenobiotics can trigger the activation of GST, whether in the field- (Durou et al., 2007) or in laboratory conditions (Livingstone et al., 1990). Nevertheless, in the case of exposure to iron ore microparticles, the noticeable increase in GST activity compared to controls (Fig. 4) is considered a protective response against oxidative stress. According to Valavanidis et al. (2006), when there is too much ROS, it can overpower antioxidant systems and cause oxidative stress. This status can lead to an increase in lipid peroxidation at cell membranes, as noted in their study. The considerable elevation of MDA levels in the gills indicates that heavy metals induce cellular damage *via* a mechanism involving free radicals. The oxidative harm in the gills could additionally hinder the respiratory mechanisms by disrupting the normal functioning of these organs. Consequently, it implies that more energy is required to maintain homeostasis, particularly concerning osmotic balance, and there are alterations in the metabolic rate associated with the production of pro-oxidants. In relation to *R. decussatus* clams that were studied here within, it was found that exposure to both types of ore microparticles (hematite and magnetite) was followed by a discernible augmentation in lipid peroxidation at both organs considered after seven days (Fig. 5). The concentration of microparticles also correlated with an intensification in lipid peroxidation in these tissues. Couillard et al. (1995) found similar results when studying transplanted *P. grandis* in the field after exposure to heavy metals. Siwela et al. (2010) demonstrated that exposure to contaminated sediments and water with (Pb, Cu, Zn, Cd, Fe, and Ni) was followed by a significant augmentation in lipid peroxidation in the common pond snail *Lymnaea*

natalensis. Acetylcholinesterase plays a crucial role in nerve signal transmission in living organisms by breaking down acetylcholine in the synaptic gap and ending its excitatory effect on the postsynaptic membrane. This helps to ensure that nerve signals are transmitted normally. According to Soreq and Seidman (2001), AChE is an enzyme with a central character in the neurotransmission process. Inhibition of AChE, as stated by Kuhr and Dorough (1976), may result in a variety of behavioral changes, such as hyperactivity, loss of coordination, convulsions, and paralysis. The study assessed how the AChE activity in *Mytella charruana* was impacted by various ionic forms of heavy metals, including As^{3+}, Hg^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}. The results indicated that the inhibitory power of the metals on AChE in *M. charruana* followed a decreasing ranking: $Hg^{2+} > Pb^{2+} > Cd^{2+} > As^{3+} > Cu^{2+} > Zn^{2+}$, as evidenced by their respective IC_{50} values (Dos Santos et al., 2022). As shown in Fig. 3, exposure to hematite and magnetite microparticles caused a change in the AChE activity of the European clam. AChE activity measured a week from the start of the current experiment showed significant inhibition for concentrations less than 1.5 g/L (C1, C2, and C3). The reduction of AChE activity is triggered by the presence of heavy metals, which have been found to have an inhibitory effect on this enzyme in various species, including common carp*Cyprinus carpio* (Suresh et al., 1992) and the green crab *Carcinus maenas* (Elumalai et al., 2007), as consistent with the outcomes of the current bioassay. The negative impact on the health of clams, as reported by disruption in the assessed biomarkers, MDA levels, and decreased AChE activity might be the consequence of the bioavailability and toxicokinetic properties of iron ore. Such results might include interactions with the cytochrome P450 (Cyp) isoforms such as Cyp1A2, Cyp3A4, and CypC19. . . (Ishak et al., 2022, Badraoui et al., 2023). Interactions with Cyp are commonly assessed in toxicological approaches (Badraoui et al., 2023; Hedfi et al., 2022) as they are usually associated with mild to severe toxicological outcomes (Allouche et al., 2022; Hedfi et al., 2022).

5. Conclusions

This study provides a comprehensive summary of the initial findings regarding biomarkers observed in the gills and digestive glands of *R. decussatus* when the habitat was enriched with microparticles of iron ore, including hematite and magnetite. The results clearly demonstrate that exposure to these microparticles had detrimental effects on the health of the clams, as indicated by significant alterations in biomarker activities and the MDA rate. Specifically, concentrations C3 and C4 prominently stimulated GST, CAT, and MDA levels in both organs examined. However, a decrease in AChE activity was noticeable at the lowest tested concentration, C1, for both types of ore. The biomarker responses observed in clams exposed to both types of ore differed from those of the control group, and the impact of the ore was more pronounced on the gills compared to the digestive glands under both conditions.

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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