

Assessment of metal pollution index, target hazard quotient and human health risk in some marine organisms collected from the extreme west coast of Algeria

by

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Abstract

Trace metals are among the most hazardous pollutants in marine environments due to their bioaccumulative properties and their high toxicity. The present study aims to assess the level of metallic contamination in Ghazaouet Bay (northwest Algeria) by analysing 10 trace metals (Mo, Zn, Cu, Mn, V, Ni, Co, Cr, Cd and Pb) in 2 seaweeds (*Enteromorpha linza* and *Corallina officinalis*) and in a marine gastropod (*Patella ferruginea*) collected respectively from three polluted sites (A, B and C). The metal pollution index (MPI) revealed significant contamination, particularly for V, Cr, Mo, Cd and Pb in algae and for Cd and Pb in limpets. The highest MPI values for *E. linza*, *C. officinalis* and *P. ferruginea* were recorded at station C, while station A appeared the least polluted site. Health risks associated with the consumption of these organisms were assessed using the target hazard quotient (THQ) and the hazard index (HI). Although all THQ and HI values remained <1, indicating a low overall risk, the relatively high HI for *C. officinalis* (0.695) and *E. linza* (0.416) raises moderate concerns, especially due to Pb and Cd levels exceeding Centre d'Étude et de Valorisation des Algues (CEVA) standards. These results highlight the need for continual monitoring of metallic contamination of these marine food resources.

Key words: marine organisms, trace metals, metal pollution index (MPI), target hazard quotient (THQ), hazard index (HI), extreme west coast of Algeria

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1. Introduction

Nowadays, the rapid progress of human activities has led to an increasing use of metals, some of which are considered serious pollutants in marine ecosystems as they are responsible for the contamination of sediments and water and pose significant threats to human life as well as to various marine organisms due to their accumulation and their transfer through food chains (Daby, 2006; El-Said, 2013; El-Said and Draz, 2010; Milošković et al. 2013; Ray & Vashishth, 2024). In aquatic environments, these persistent and poorly biodegradable chemical elements often tend to concentrate in sediments, which in turn become major sources of metals for the water column and biota (Díaz-de Alba et al., 2011; Huang et al., 2023; Tang et al., 2010). Furthermore, although some of these metal elements at low concentrations are essential for living organisms, others are toxic and can have harmful effects on both biodiversity and human health. In living organisms, the accumulation and toxicity of trace metal elements (TMEs) generally depend on several factors, including species-specific features, metal properties and environmental factors (Moiseenko & Gashkina, 2020). Metal contamination in marine environments is often assessed by analysing abiotic compartments, such as water and/or sediments, providing insight into pollution levels (Belhadj et al., 2017; Keria et al., 2024; Topcuoğlu et al., 2003). However, many researchers prefer to supplement or even replace this approach by analysing biological components, which can offer a better understanding of metal accumulation and its effects on marine life (Abdullah et al., 2007; Singh et al., 2016). To this end, bioindicator species used in such assessments must meet certain criteria, including a sedentary lifestyle to represent the region under study, abundance in the study area, ease of sampling and the ability to accumulate metals in measurable quantities (Conti & Cecchetti, 2003; El-Mahrouk et al., 2023; Zaidi et al., 2022; Zhou et al., 2008).

In this context, the present study aims to assess the levels of metallic contamination in the extreme west coast of Algeria (Ghazaouet) through the analysis of three marine organisms: two macroalgae, *Enteromorpha linza* (also known as *Ulva linza*) and *Corallina officinalis*, and a marine gastropod, *Patella ferruginea*. Marine algae are known for their high capacity to accumulate metals without suffering severe toxic effects (Bryan, 1971; Nowicka, 2022; Salt et al., 1995; Seregin & Kozhevnikova, 2023). Several studies have highlighted the usefulness of the green alga *E. linza* (Astorga-España et al., 2008; Malea & Haritonidis, 2000; Mohamed & Khaled, 2005; Siddique et al., 2022; Tabudravu et al., 2002; Topcuoğlu et al., 2003; Villares et al., 2001) and the

red alga *C. officinalis* (AbouGabal et al., 2023; Bouthir et al., 2006; Kaparapu et al., 2015; Kut et al., 2000; Strezov & Nonova, 2009; Topcuoğlu et al., 2010) for monitoring metal in marine ecosystems. Additionally, *P. ferruginea*, an endangered Mediterranean species, is widely recognised and has of great interest as a metal bioindicator due to its sedentary nature and ecological role. These species are often considered as excellent biosentinels by networks monitoring metal pollution in the Mediterranean (Benguedda & Dali youcef, 2012; Bergasa et al., 2007; Connan & Tack, 2010; Conti & Finoia, 2010; Duysak & Azdural, 2017; Hamed & Emara, 2006; Kelepertzis, 2013; Maatallah et al., 2014; Nakhlé et al., 2006; Storelli & Marcotrigiano, 2005; Tlig-Zouari et al., 2010; Türk Çulha et al., 2022).

The present study examines the concentration of 10 trace metals (Mo, Zn, Cu, Mn, V, Ni, Co, Cr, Cd and Pb) in marine organisms collected from three sites along the extreme western coast of Algeria. Statistical analyses and comparisons with regional values were performed to identify the most contaminated stations. The degree of metal contamination was evaluated using the metal pollution index (MPI), a widely applied indicator for assessing metal pollution in marine organisms. Additionally, the target hazard quotient (THQ) and the hazard index (HI) established by the EPA's IRIS database were applied to estimate the potential human health risks associated with the consumption of these contaminated organisms (Ben ameur et al., 2025; Rashid et al., 2024).

2. Materials and methods

2.1. Study area

Ghazaouet is situated in the north-west of Algeria, approximately 10 km from the Morocco border (Fig. 1). This region is often considered a pollution hotspot (M.A.T.E., 2006, 2007), as it is subject to multiple sources of pollution, including solid and liquid inputs transported to the marine environment by the region's wadis (Wadi Ghazaoua, Wadi Abdallah, etc.), which are laden with domestic and industrial effluents, and uncontrolled landfill sites. The bay is also affected by solid, liquid and gaseous discharges from the Alzinc plant, which has been in operation since 1974 and is dedicated to the production of zinc, other metals and sulphuric acid. The plant's immediate proximity makes Ghazaouet and its marine environment highly vulnerable to metal contamination (Belhadj et al., 2017). Additionally, pollution in the marine environment of Ghazaouet is aggravated by discharges from various activities within its mixed-use harbour,

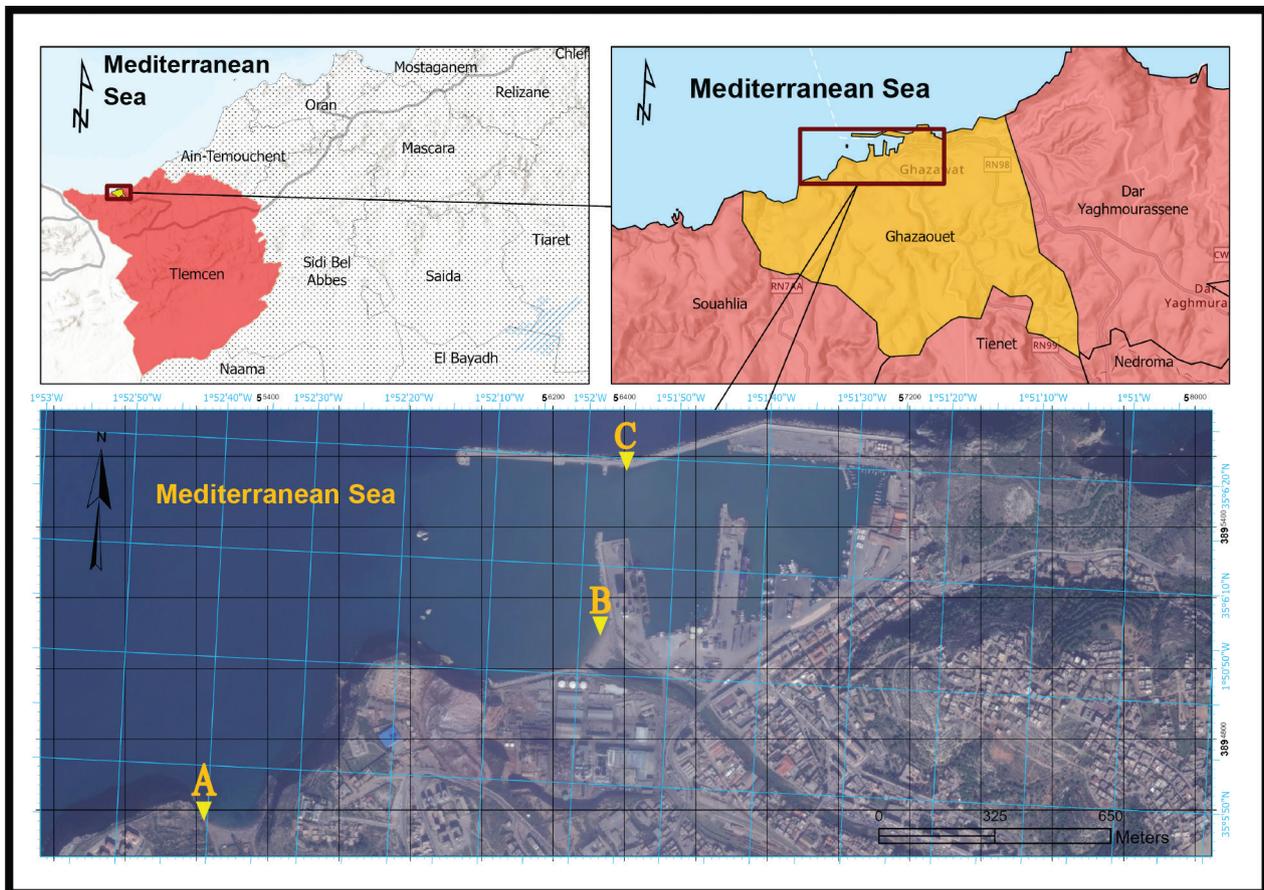


Figure 1

Study area and location of sampling points.

Symbols:

△ A: Sampling point

Grid:

- In blue: GWS84 geographic coordinates, format: DD°MM'.

- In black: coordinates in UTM31 North map projection

A: 35° 05' 44.73" N
1° 52' 40.02" W
B: 35° 06' 04.63" N
1° 51' 56.87" W
C: 35° 06' 19.12" N
1° 51' 55.16" W

including hydrocarbon pollution from transport and fishing boats and the discharge of wastewater from the town of Ghazaouet into this semi-enclosed marine structure (M.A.T.E., 2006, 2007).

2.2. Sampling

Sampling of marine organisms (seaweeds and gastropods) was conducted at three selected stations in the marine environment of Ghazaouet, near the main domestic and industrial discharge points (Fig. 1). Each month, from July 2010 to June 2011, samples were collected at the following sites:

- Station (A): site located west of Ghazaouet on the beach of Oued Abdallah (35°05' 44.73" N and 1°52' 40.02" W).
- Station (B): site located to the east of the first station, on the beach of the Oued Ghazouana about 1 km away (35°06' 04.63" N and 1°51' 56.87" W).
- Station (C): site located inside the port of Ghazaouet, 500 m east of the town (35°06' 19.12" N and 1°51' 55.16" W).

Seaweed thalli were hand-picked at all three sampling stations. For limpets, approximately 15 individuals of *P. ferruginea* were collected at stations



A and C, where the species had been found. The individuals (2.5 cm long on average) were carefully detached from rocks using a stainless steel knife. All samples were placed in plastic bags and transported in a cooler. In the laboratory, the thalli and limpets were rinsed with distilled water to remove salts and suspended particles. The gastropods' soft tissue was extracted. Both the algal thalli and the soft tissue of *P. ferruginea* were subsequently dried, crushed, labelled and stored until mineralisation.

2.3. Mineralisation and metal assay

All marine organisms' samples underwent mineralisation to determine the total concentrations of metallic elements.

The digestion procedure was adapted from standard microwave-assisted acid digestion protocols (Amiard, 2011; Benguedda et al., 2011; U.S. Environmental Protection Agency (EPA), 2007). Approximately 200 mg of dried algae and 150 mg of dried limpet (mole part) were transferred to Teflon tubes and digested with 6 mL of Suprapur HNO₃, added in two stages of 3 mL each. The tubes were then heated in a microwave digestion system (Anton Paar Multiwave 3000) at 80°C for 1 hr.

After evaporation and the appearance of black residues at the bottom of the tubes, the samples were removed, cooled and diluted to a final volume of 20 mL with ultrapure water. The resulting solutions were stored in polyethylene bottles.

The concentrations of metals were determined using inductively coupled plasma mass spectrometry (ICP-MS) with an Agilent Technologies 7700x instrument. The reliability of the analytical procedures

was verified using certified reference materials, which were also mineralised and analysed with all the samples. For algae, lichen reference material (IAEA-336) marketed by the International Energy Agency (IAEA) was used. For limpets, the measurements were validated using lobster pancreas reference material (TORT-2), provided by the National Research Council of Canada (NRC). The obtained results showed good agreement with the certified values (Table 1).

2.4. Assessment of trace metals

2.4.1. MPI

The MPI, as defined by Usero et al. (1997), was used to compare the total metal concentrations in the three marine organisms and to obtain an overview of the level of contamination. MPI was calculated using the following equation:

$$MPI = (M_1 \times M_2 \times \dots \times M_n)^{1/n} \quad (1)$$

where M_1 and M_2 are the concentrations of the first and the second metals, respectively, etc., while M_n is the concentration of the n metal and n is the number of metals (Usero et al., 2005). An MPI value <1 indicates a non-polluted ecosystem, whereas an MPI value >1 indicates metal contamination (Stanišić et al., 2021).

2.4.2. THQ and HI

The potential health risk, related to metal exposure dose, was assessed by using the THQ and the HI from the US EPA's IRIS database (Ali et al., 2021; Rahhou

Table 1

Measured and certified values of the concentrations of trace metals in the two reference materials (mg · kg⁻¹ d.w.)

Elements	Algae (IAEA-336)			Patella (TORT-2)		
	Measured (mg · kg ⁻¹)	Certified (mg · kg ⁻¹)	Recovery (%)	Measured (mg · kg ⁻¹)	Certified (mg · kg ⁻¹)	Recovery (%)
Mo	0.15 ± 0.09	NV	-	0.90 ± 0.07	0.95 ± 0.10	94.42
Zn	41.00 ± 3.69	30.40	134.86	210.53 ± 26.61	180.00 ± 6.00	116.96
Cu	3.48 ± 0.18	3.60	96.75	104.11 ± 3.24	106.00 ± 10.00	98.22
Mn	63.58 ± 1.97	63.00	101	14.51 ± 0.37	13.60 ± 1.20	106.69
V	1.08 ± 0.07	1.47	73.33	1.84 ± 0.05	1.64 ± 0.19	112.32
Ni	0.82 ± 0.09	NV	-	2.65 ± 0.17	2.50 ± 0.19	105.88
Co	0.25 ± 0.01	0.29	85.17	0.52 ± 0.01	0.51 ± 0.09	101.76
Cr	0.82 ± 0.02	1.06	77.08	0.83 ± 0.05	0.77 ± 0.15	107.66
Cd	0.11 ± 0.04	0.12	91.45	26.31 ± 0.12	26.70 ± 0.60	98.53
Pb	4.73 ± 0.45	4.90	96.61	0.76 ± 0.20	0.35 ± 0.13	216.57

d.w., dry weight; NV, no value.

et al., 2023; US EPA, 2013). The HI was calculated by comparing the average metal concentrations in several marine organisms' samples from this study to global standards established by the US EPA. The HI represents the sum of THQs for all analysed metals, and it was calculated using the following equations (Ali et al., 2021; Rahhou et al., 2023; US EPA, 2013).

$$\text{Exposure Dose (EDi)} = (C_i \times D_i \times \text{Ed}) / (\text{Bw} \times \text{At}) \quad (2)$$

$$\text{Target Hazard Quotient (THQ)} = \frac{\text{Exposure Dose}}{\text{Dose/RfD}} \quad (3)$$

$$\text{Hazard Index (HI)} = \sum \text{THQ} \quad (4)$$

where EDi represents the exposure dose ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) of the metal 'i', C_i is the average concentration of metal 'i' in marine organism samples ($\text{mg} \cdot \text{kg}^{-1}$) and D_i is the daily intake of marine organisms ($5.2 \text{ g} \cdot \text{capita}^{-1} \cdot \text{day}^{-1}$ for seaweeds); for molluscs in Algeria, the daily intake is estimated at $2.7 \times 10^{-6} \text{ kg} \cdot \text{capita}^{-1} \cdot \text{day}^{-1}$ (Bouiba et al., 2024; FAO, 2016), Ed represents the average exposure duration (e.g., 70 years), Bw is the average body weight (e.g., 70 kg), At means the average lifetime (e.g., 70 years) and RfD means the recommended reference dose ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$) (US EPA, 2013, 2016).

In case HI is <1 , no apparent risk to human health is detected. However, if HI is >1 , in this case, a moderate to high risk exists, suggesting potential adverse effects on human health (Ali et al., 2021; Rahhou et al., 2023; US EPA, 2013).

2.5. Statistical analysis

The statistical analysis of data was conducted using SPSS version 20 (IBM Corp., Armonk, NY, USA). The Kruskal–Wallis test, a non-parametric method, was applied to detect significant statistical differences in TME in the two seaweeds and the marine gastropod among the three sampling stations (A, B and C). Additionally, the correlation analysis of trace metal concentrations at the level of each matrix and between the three matrices

(seaweeds and the marine gastropod) was performed using Spearman's correlation coefficient (ρ) and partial correlation, respectively. Statistical significance was considered at $p < 0.01$ and $p < 0.05$.

3. Results and discussion

3.1. Metal concentrations in seaweeds

The concentrations of metals found in the two seaweeds, *E. linza* and *C. officinalis*, at the various study stations are presented in Tables 2 and 3. All the metals analysed were detected in the two algae in the present study.

For the green alga *E. linza* (Table 2), except for molybdenum, all the minimum mean concentrations of several metals were recorded at station A. The maximum mean concentrations of Mo, Zn, Cu, V, Ni and Cd were recorded at station C, while the maximum mean concentrations of Cr, Mn, Co and Pb were recorded at station B. The decreasing order of metal accumulation for this green algae for the three stations (A, B and C) in the present study is as follows: Zn > Mn > Pb > V > Cu > Cr > Ni > Co > Cd > Mo; Zn > Pb > Mn > Cu > V > Cr > Cd > Ni > Co > Mo; Zn > Pb > Mn > Cu > V > Cr > Cd > Ni > Co > Mo.

The Kruskal–Wallis test, used to detect differences in metal concentrations between the three study stations (A, B and C), revealed that Cu, Zn, Mo, Cd and Pb showed very highly significant spatial differences ($p < 0.001$), while Mn showed significant spatial variation ($p < 0.05$). For the remaining metals, no significant spatial difference was recorded, suggesting similar levels across the stations (Table 2).

Average concentrations of manganese, cobalt and nickel were 44.78 , 1.12 and $4 \text{ mg} \cdot \text{kg}^{-1}$ at station A; 105.38 , 2.22 and $5.95 \text{ mg} \cdot \text{kg}^{-1}$ at station B and 84.41 , 1.72 and $6.34 \text{ mg} \cdot \text{kg}^{-1}$ at station C, respectively. Average concentrations of copper were 7.34 , 57.41 and $61.11 \text{ mg} \cdot \text{kg}^{-1}$, respectively; average concentrations

Table 2

Metal concentrations in green algae (*E. linza*) samples ($\text{mg} \cdot \text{kg}^{-1}$ d.w.) and the results of the Kruskal–Wallis test

Stations	Values	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	MPI
A	Mean \pm SD	0.26 \pm 0.06	122.61 \pm 88.79	7.34 \pm 2.42	44.78 \pm 19.56	9.06 \pm 3.83	4.00 \pm 1.60	1.12 \pm 0.61	6.36 \pm 2.54	0.77 \pm 0.66	10.24 \pm 11.40	5.41
	Min–Max	0.19–0.43	51.56–304.22	4.27–11.91	19.97–95.59	4.43–16.14	1.95–7.79	0.46–2.83	3.16–12.15	0.20–2.34	2.58–41.92	
B	Mean \pm SD	0.24 \pm 0.08	766.37 \pm 390.89	57.41 \pm 11.11	105.38 \pm 61.41	14.12 \pm 9.21	5.95 \pm 5.64	2.22 \pm 1.69	13.74 \pm 13.44	6.58 \pm 8.91	130.00 \pm 41.50	17.33
	Min–Max	0.16–0.43	392.07–1782.11	34.70–70.54	41.99–253.84	6.38–38.40	1.88–20.97	0.78–6.64	5.22–50.71	1.99–31.28	71.55–205.14	
C	Mean \pm SD	0.48 \pm 0.29	2158.46 \pm 1904.34	61.11 \pm 4.95	84.41 \pm 50.04	15.70 \pm 11.37	6.34 \pm 4.95	1.72 \pm 1.22	11.20 \pm 8.84	7.41 \pm 6.38	124.33 \pm 105.70	19.84
	Min–Max	0.28–1.27	263.92–6015.70	18.56–153.21	32.00–173.17	4.76–44.05	2.00–18.30	0.55–4.10	2.79–30.08	1.29–23.00	24.91–378.83	
p		<0.001	<0.001	<0.001	0.004	0.159	0.628	0.079	0.072	<0.001	<0.001	

d.w., dry weight; MPI, metal pollution index; SD, standard deviation.



Table 3

Comparison of the concentrations of trace metals in green algae (*E. linza*) with values taken from the Mediterranean literature ($\text{mg} \cdot \text{kg}^{-1}$ d.w.)

Sites	Metals										Reference
	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	
Ghazaouet Bay (Algeria)	0.24–0.48	122.61–2158.46	7.34–61.11	44.78–105.38	9.06–15.70	4.00–5.95	1.12–2.22	6.36–13.74	0.78–7.41	10.24–129.90	Present study ^a
Galicia (Spain)	-	19.30–187.00	7.36–1372.00	13.49–496.00	-	7.64–339.00	-	2.17–421.00	-	-	Salvado (1992)
El-Mex Alexandria Coast (Egypt)	-	29.72	14.62	36.75	-	9.96	-	-	0.73	14.26	Mohamed Khaled (2005) ^a
South Eastern coast (Egypt)	-	42.51	16.92	-	-	-	1.19	-	-	-	Abdallah and Abdallah (2008) ^b
Ghazaouet Bay (Algeria)	-	7.87–105.91	2.97–7.80	-	-	0.40–0.66	-	0.71–1.20	0.02–0.13	0.87–2.28	Belhadj (2008) ^a
Ghazaouet Bay (Algeria)	29.50–34.33	2.41–10.90	-	-	-	-	-	-	-	0.29–1.92	Benguedda et al. (2011) ^c
Urla (Turkey)	-	97.50	4.43	-	-	-	3.15	42.70	1.04	-	Akali and Kucuksezgin (2011) ^b
Foça (Turkey)	-	74.80	10.70	-	-	-	4.45	12.60	9.98	-	-
Abu-Qir Bay (Egypt)	-	55.90	11.50	69.52	-	12.17	-	-	1.77	28.71	El-Nemr et al. (2012) ^d
Marsa Matrouh coast (Egypt)	-	4.95–58.47	4.02–65.72	-	-	3.15–17.01	-	-	0.19–1.34	37.78–159.39	Khaled et al. (2014) ^e
Abu-Qir Bay (Egypt)	-	31.10	4.60	76.84	-	11.73	7.00	-	3.16	17.76	Shams El-Din et al. (2014) ^f
Akyaka (Turkey)	-	7.20–68.22	1.50–38.95	2.94–39.23	-	1.02–16.81	-	0.19–22.71	0.01–0.19	2.49–8.02	Yozukmaz et al. (2018) ^g
West coast (Algeria)	-	5.50	2.60	0.03	18.00	2.00	8.10	2.00	<0.30	<0.40	Oucif et al. (2020) ^h

^a*Enteromorpha linza*.

^b*Enteromorpha* sp.

^c*Enteromorpha compressa*.

^d*Enteromorpha intestinalis*.

d.w., dry weight.

of zinc were 122.61, 766.37 and 2158.46 $\text{mg} \cdot \text{kg}^{-1}$, respectively; and average concentrations of molybdenum were 0.26, 0.24 and 0.48 $\text{mg} \cdot \text{kg}^{-1}$, respectively, for stations A, B and C. These six elements (Mn, Co, Ni, Cu, Zn and Mo) are essential to plants at low concentrations, but they can produce toxic effects when metal uptake is too high (Benabdallah et al., 2017; Devez, 2004; Nunes et al., 2024; Türkmen et al., 2005). For the other metals, the average concentrations of vanadium were 9.06, 14.12 and 15.7 $\text{mg} \cdot \text{kg}^{-1}$, respectively; those of chromium were 6.36, 13.74 and 11.2 $\text{mg} \cdot \text{kg}^{-1}$, respectively; those of cadmium were 0.77, 6.58 and 7.41 $\text{mg} \cdot \text{kg}^{-1}$, respectively; while the average concentrations of lead were 10.24, 130 and 124.33 $\text{mg} \cdot \text{kg}^{-1}$, respectively, for stations A, B and C. These four elements (V, Cr, Cd and Pb) are not essential for algal development and are toxic metals, even in trace amounts (Benabdallah et al., 2017; Devez, 2004) (Table 2).

Table 3 presents the mean minimum and maximum concentrations of metallic elements obtained in *E. linza*, in comparison with previous results for *Enteromorpha* from the Mediterranean region (Abdallah & Abdallah, 2008; Akali & Kucuksezgin, 2011; El-Nemr et al., 2012; Khaled et al., 2014; Mohamed & Khaled, 2005; Oucif et al., 2020; Salvado, 1992; Shams El-Din et al., 2014; Yozukmaz et al., 2018). According to this comparison, the Cr, Mn, Ni and Cu contents recorded in *E. linza* in the present study remain lower than those reported by Salvado (1992), who obtained the highest concentrations of these elements in

Enteromorpha sp. from Galicia (Spain). In addition, the work of Benguedda et al. (2011), Akali and Kucuksezgin (2011) and Khaled et al. (2014) showed higher levels in their green algae for Mo, Cd and Pb, respectively. The study carried out on *E. compressa* from western Algeria also showed the highest Co and V values in this green alga (Oucif et al., 2020). However, the concentrations of zinc in the present study were highest compared to the literature (Table 3).

For red seaweed *C. officinalis* (Table 4), apart from molybdenum and vanadium, minimum mean concentrations of all elements were recorded at station A. The maximum average concentrations of Zn, Cu, Ni, Co, Cr, Cd and Pb were observed at station B, while the maximum average concentrations of V and Mn were observed at station C. The decreasing order of metal accumulation for this red seaweed at the three sampling points is as follows: Zn > Mn > Pb > V > Cu > Ni > Cr > Cd > Co > Mo for station A; Zn > Pb > Mn > Cu > Cd > V > Cr > Ni > Co > Mo for station B; Zn > Pb > Mn > V > Cu > Cd > Cr > Ni > Co > Mo for station C. The results of the Kruskal–Wallis test revealed a significant difference ($p < 0.05$) for Cr accumulation, highly significant differences ($p < 0.01$) for V, Cu and Mo, and very highly significant differences ($p < 0.001$) for Cd and Zn between sampling points A, B and C. However, no significant differences were found for Co and Ni accumulation between the different sampling points (Table 4). With regard to the elements essential for plant

development (Mn, Co, Ni, Cu, Zn and Mo), the average concentrations of manganese, cobalt and nickel were 54.6, 0.68 and 2.65 mg · kg⁻¹ at station A; 112.77, 1.42 and 3.64 mg · kg⁻¹ at station B and 138.75, 1.11 and 3.19 mg · kg⁻¹ at station C, respectively. Average concentrations of copper were 10.8, 36.06 and 28.95 mg · kg⁻¹; average concentrations of zinc were 293.18, 1566.14 and 1331.52 mg · kg⁻¹ and average concentrations of molybdenum were 0.162, 0.11 and 0.29 mg · kg⁻¹ for stations A, B and C, respectively. For the metals considered non-essential (V, Cr, Cd and Pb), the average concentrations were 13.95, 2.52, 1.8 and 29.03 mg · kg⁻¹ at station A; 11.95, 7.36, 12.22 and 306.98 mg · kg⁻¹ at station B and 32.2, 5.54, 7.33 and 218.34 mg · kg⁻¹ at station C, respectively (Table 4).

Table 5 shows the average minimum and maximum levels of the metal elements monitored (Mo, Zn, Cu,

Mn, V, Ni, Co, Cr, Cd and Pb) in the red alga *C. officinalis*, compared with the metal levels reported in studies on coralline algae from the Mediterranean (Allam et al., 2016; Belhadj, 2008; Benabdallah et al., 2017; Benguedda et al., 2011; El-Nemr et al., 2012; Oucif et al., 2020; Salem et al., 2019; Topcuoğlu et al., 2010). According to Table 5, the results of the present study are higher compared to the previous work carried out in the Mediterranean, at least for Cu, Zn, Mn, V, Cd and Pb. Additionally, the mean Cr levels in the present study are higher than those reported in the literature, with the exception of those reported by Topcuoğlu et al. (2010) in *Corallina mediterranea* from Yumurtalik (Turkey). Cobalt is less concentrated in the corallines from this study compared to those from Abu Qir, Egypt (Salem et al., 2019) and from Yumurtalik (Turkey) (Topcuoğlu et al., 2010). Nickel levels in the present study remain lower than those

Table 4

Metal concentrations in red algae (*C. officinalis*) samples (mg · kg⁻¹ d.w.) and the results of the Kruskal–Wallis test

Stations	Values	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	MPI
A	Mean ± SD	0.16 ± 0.14	293.18 ± 92.61	10.80 ± 18.90	54.60 ± 26.03	13.95 ± 3.50	2.65 ± 1.54	0.68 ± 0.23	2.52 ± 2.02	1.80 ± 0.56	29.03 ± 10.18	6.26
	Min–Max	0.07–0.58	175.88–512.01	2.44–64.12	23.20–95.79	8.57–19.40	1.46–6.77	0.32–1.13	0.63–7.82	0.96–2.43	16.02–49.79	
B	Mean ± SD	0.11 ± 0.11	1566.14 ± 208.09	36.06 ± 11.15	112.77 ± 60.41	11.95 ± 9.06	3.64 ± 4.01	1.42 ± 1.14	7.36 ± 10.69	12.22 ± 0.93	306.98 ± 116.60	16.13
	Min–Max	0.00–0.29	1264.37–1797.86	15.85–47.23	55.13–198.48	7.06–30.36	1.42–11.76	0.71–3.68	2.29–29.16	11.36–14.06	173.00–476.41	
C	Mean ± SD	0.29 ± 0.15	1331.52 ± 656.66	28.95 ± 16.03	138.75 ± 94.11	32.20 ± 19.64	3.19 ± 1.75	1.11 ± 0.61	5.54 ± 3.90	7.33 ± 1.95	218.34 ± 89.55	16.58
	Min–Max	0.09–0.65	612.76–3146.27	7.53–69.29	15.64–361.88	10.86–75.10	0.44–5.67	0.63–2.03	0.73–13.39	4.29–11.35	106.87–432.00	
p		0.008	<0.001	0.002	0.021	0.002	0.752	0.070	0.047	<0.001	<0.001	

d.w., dry weight; MPI, metal pollution index.

Table 5

Comparison of the concentrations of trace metals in red algae (*C. officinalis*) with values taken from the Mediterranean literature (mg · kg⁻¹ d.w.)

Sites	Metals										Reference
	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	
Ghazaouet Bay (Algeria)	0.12–0.16	293.18–1566.14	10.8–36.06	54.60–138.75	11.95–32.2	2.65–3.64	0.69–1.42	2.52–7.36	1.80–12.22	29.03–306.90	Present study ^a
Ghazaouet Bay (Algeria)	-	12.67–211.44	2.60–15.22	-	-	0.69–0.75	-	1.07–1.89	0.18–0.54	1.78–6.72	Belhadj (2008) ^a
Fethiye Coast (Turkey)	-	31.50	4.25	43.40	-	2.50	<0.05	3.34	<0.02	<0.10	Topcuoğlu et al. (2010) ^b
Alanya (Turkey)	-	41.92	2.76	47.93	-	1.31	<0.05	3.39	<0.02	<0.10	Topcuoğlu et al. (2010) ^b
Yumurtalik (Turkey)	-	248.97	54.51	39.33	-	157.39	12.96	94.25	0.95	4.98	Topcuoğlu et al. (2010) ^b
Ghazaouet Bay (Algeria)	-	14.97–42.76	1.34–10.92	-	-	-	-	-	0.64–2.38	21.78–89.89	Benguedda et al. (2011) ^b
Abu Qir (Egypt)	-	40.16	9.89	46.33	-	32.76	-	-	10.34	41.85	El-Nemr et al. (2012) ^c
Honaine Coast (Algeria)	-	4.16–5.31	0.35–0.45	-	-	1.86–2.44	-	-	0.35–0.45	3.29–3.96	Allam et al. (2016) ^b
Rechgoun Beach (Algeria)	-	0.00–37.50	0.00–0.96	7.89–71.80	2.25–13.14	0.00–1.04	0.00–0.59	0.33–3.01	0.00–0.12	0.00	Benabdallah et al. (2017) ^b
Sidi Boucif Beach (Algeria)	-	11.65–47.04	0.00–2.62	19.18–115.81	2.30–16.05	0.00–1.45	0.02–0.91	1.17–4.51	0.00–0.11	0.00–2.55	Benabdallah et al. (2017) ^b
Abu Qir (Egypt)	-	37.11	0.62	44.08	-	29.89	14.99	1.55	1.00	42.95	Salem et al. (2019) ^b
West coast (Algeria)	-	21.60	<0.30	12.50	2.10	1.20	0.30	1.40	<0.30	1.20	Oucif et al. (2020) ^b

^a*Corallina officinalis*.

^b*Corallina elongata*.

^c*Corallina mediterranea*.

^d*Corallina* sp.

d.w., dry weight.



reported in other studies, notably those in Yumurtalik (Turkey) (Topcuoğlu et al., 2010) (Table 5).

These results reveal the high bioavailability of these metals in marine ecosystems of Ghazaouet (sediment and water) and the capacity of these two seaweeds to accumulate these metals, whether essential or not (Alahverdi & Savabieasfahani, 2012; El-Mahrouk et al., 2023; Salt et al., 1995). Metallic contamination in these two seaweeds (green and red) is significant at least for V, Cr, Mo, Cd and Pb, as these elements are not biologically essential for plants (Devez, 2004). This contamination is particularly marked in algae from station B, followed by algae from station C. The study of Denton et al. (2006), Güven et al. (2007) and El-Moselhy et al. (2016) has already shown different accumulation trends in algae, but with specific priority given to essential elements such as Fe, Mn, Zn and Cu. However, the high levels of certain metals (Zn, Mn and Pb) in our algae reflect not only the anthropogenic abundance of these metals in the sampling environment (Mohamed & Khaled, 2005; Shriadah & Emara, 1991) but also the affinity of algae for certain metals, as has already been observed in certain macroalgae from temperate regions, which accumulate Fe, Zn, Mn and Pb abundantly (Rajendran et al., 1993; Storelli et al., 2001). More specifically, the genus *Ulva* (*U. lactuca*) is known for its particular affinity for Fe, Mn, Cu, Zn, Pb and Cd (Ghoneim et al., 2014; Ho, 1990). Taking the case of Pb in the present study (a non-essential metal), which is always among the top three elements accumulated in both algae, along with Zn and Mn, it is well known that this metal can be taken up by some marine algae in very high concentrations from marine waters and sediments (Fritioff, 2005; Lafabrie et al., 2007). This variability in accumulation may be linked to several factors, such as the availability of the element in each station, the specificity of the biochemical composition, the metabolic activities of each alga and the affinity of the

algae itself for each element (Haritonidis & Malea, 1995; Stengel et al., 2004; Tabudravu et al., 2002). However, in the literature, fluctuations in element concentrations in algae are often also associated with seasonal variations in the growth rate (Carlson & Erlandsson, 1991; Ghosn et al., 2020; Tomlinson et al., 1980). Kaimoussi et al. (2005) added that these growth processes alone are insufficient to fully explain the differences in variability between elements, since Haritonidis and Malea (1995) have already shown that in addition to growth dynamics and the age of the algal tissue, it is also necessary to take into account the physicochemical factors of the environment (temperature, pH, salinity, light, etc.) that affect the bioavailability of elements for algae and their variations in the environment. In fact, we have often observed similarities between changes in the concentration of a metal in marine algae and changes in sediments (Belhadj et al., 2017; El-Said, 2013). This leads us to assume that these variations are also indicative of temporal changes in metal concentrations in seawater, according to Pohl et al. (1993), in addition to the variations already observed in sediments. This further confirms the capacity of algae to absorb heavy metals from water and sediments (Davis et al., 2003). Several studies (Akcali & Kucuksezgin, 2011; Haritonidis & Malea, 1995, 1999; Phillips, 1994; Żbikowski et al., 2006) have concluded that it is not easy to determine the main factors influencing seasonal variations in metal accumulation in macrophytes, as these changes can result from interactions between several factors. Despite this, because of their short lifespan and rapid response to environmental changes, these algae remain good indicators of pollution (Domingues and Galvao, 2007).

3.2. Metal concentrations in marine gastropods

Table 6 presents the metal concentrations obtained in limpets from stations A and C. All the metals analysed in

Table 6

Metal concentrations in gastropod (*P. ferruginea*) samples (mg · kg⁻¹ d.w.) and the results of the Kruskal–Wallis test

Stations	Values	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	MPI
A	Mean ± SD	0.72 ± 0.18	248.9 ± 100.66	21.83 ± 28.67	22.88 ± 17.09	6.93 ± 4.48	8.76 ± 5.65	1.73 ± 0.73	4.47 ± 6.22	8.94 ± 2.29	28.83 ± 13.81	10.08
	Min–Max	0.50–1.04	122.08–467.76	8.67–104.62	8.31–64.36	2.65–15.88	3.33–20.93	0.47–2.9	0.85–23.56	6.27–15.03	12.31–58.73	
B	Mean ± SD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Min–Max	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
C	Mean ± SD	0.66 ± 0.12	959.48 ± 363.77	89.71 ± 28.29	20.34 ± 8.59	7.28 ± 5.673	5.48 ± 0.94	0.97 ± 0.36	1.39 ± 0.68	10.02 ± 3.51	113.09 ± 47.36	12.16
	Min–Max	0.46–0.84	229.64–1637.72	19.07–132.95	13.59–44.28	2.69–22.73	4.22–6.94	0.72–2	0.77–2.86	6.2–18.32	19.63–18.32	
<i>p</i>		0.301	<0.001	<0.001	0.651	0.868	0.60	0.004	0.102	0.383	<0.001	

d.w., dry weight; MPI, metal pollution index; NA, not analysed. Standard deviation (SD).

the present study were found in the soft tissues of limpets collected in the marine environment of Ghazaouet.

Minimum mean concentrations of V, Cu, Zn, Cd and Pb were recorded in limpets from station A, while those of Cr, Mn, Co, Ni and Mo were observed in limpets from station C. The maximum average concentrations of the latter metals were recorded in limpets from station A, while those of V, Cu, Zn, Cd and Pb were recorded in limpets from station C. The decreasing metal gradient in limpets from each study station is as follows: Zn > Pb > Mn > Cu > Cd > Ni > V > Cr > Co > Mo; Zn > Pb > Cu > Mn > Cd > V > Ni > Cr > Co > Mo for points A and C, respectively. The results of the Kruskal–Wallis test showed no significant difference ($p > 0.05$) in the accumulation of V, Cr, Mn, Ni, Mo and Cd in relation to the study stations. On the contrary, the difference in accumulation was statistically significant ($p < 0.05$) for Co and very highly significant ($p < 0.001$) for Cu, Zn and Pb accumulation in relation to the different sampling points (Table 6).

Regarding the elements essential or indispensable for animal life (Zn, Mn, Cu, Ni, Co and Mo), the mean concentrations of Mn, Co and Ni were 22.88, 1.73 and 8.76 mg · kg⁻¹ at station A and 20.34, 0.97 and 5.48 mg · kg⁻¹ at station C, respectively. The average concentrations of Cu were 21.83 and 89.71 mg · kg⁻¹, those of Zn were 248.9 and 959.48 mg · kg⁻¹ and those of Mo were 0.72 and 0.66 mg · kg⁻¹ at stations A and C, respectively. The mean concentrations of non-essential metals (V, Cr, Cd and Pb) were 6.93, 4.47, 8.94 and 28.83 mg · kg⁻¹ at A and 7.28, 1.39, 10.02 and 113.09 mg · kg⁻¹ at C, respectively (Table 6). The metal levels recorded in our limpets are too high to be due solely to natural concentrations. Moreover, they indicate the same anthropogenic multi-contamination as that observed in sediments (Belhadj et al., 2017) or in the seaweeds of this study (Tables 2 and 4). This tends to prove that these concentrations have, in fact, been brought about by the presence of these contaminants in the surrounding abiotic (water and sediment) and biotic (algae) environment and not by biological requirements for these metals (Bordin et al., 1992; El-Nemr & El-Said, 2017), at least for Cd and Pb, as these elements are not biologically essential for these molluscs. This leads us to say that, in addition to the influence of the abiotic environment (water and sediments), our limpets could be influenced by the consumption of local algae (Boucetta et al., 2019; Mbandzi et al., 2021; Shiber, 1980). In fact, Yüzereroğlu et al. (2010) have already demonstrated that the accumulation of TME in *Patella caerulea* is affected by heavy metals present in macroalgae sharing the same environment. According to El-Naggar et al. (2016), aquatic invertebrates absorb

Zn directly through their gills and mucous membranes. Davies & Cliffe (2000) and Nakhlé (2003) added that certain elements (Pb, Zn and especially Cd) adsorb onto the mucus deposited by limpets as they move. This mucus is thus found on rocky substrates as well as on bacterial and algal films, with high levels of metals, even in uncontaminated areas. The limpet's back-and-forth movement along its track forces it to graze on micro-algae already impregnated by this mucus. The content of metals therefore increases in the limpet's tissues, as well as in the new mucus secreted during its next passage. We are thus witnessing an increase in the concentration of metals, even in areas with little or no contamination. Nevertheless, it should be borne in mind that the actual mechanism of metal uptake in limpets is rather complicated and that fluctuations in metal concentrations result from a combination of factors that are directly correlated to physiological changes linked to the reproductive cycle (weight, sexual cycle, food abundance, temperature, etc.), but also to other independent factors, such as the bioavailability of metals and variations in environmental factors (Bergasa et al., 2007; Mbandzi et al. 2021; Nakhlé, 2003; Storelli & Marcotrigiano, 2005). Some studies, such as those by Davies et al. (2005), Hamed & Emara (2006) and Al-Homaidan et al. (2011), have highlighted the same observations, explaining that there is a global tendency for some metals, such as Zn, to be preferentially incorporated into limpet tissues. Furthermore, Ghosn et al. (2020) reported that the high levels of Zn and Cu in molluscs are often attributed to their essentiality. Pb is always in second place (stations A and C) in our limpets, although it is not biologically useful for living organisms. Mn is found in third place (station A) and fourth place (station C) in our limpets, while Cd is always in fifth place despite being one of the few elements with no known function in animals (Dabrin, 2009). Chiffolleau et al. (2001) showed that, as a general rule, the more concentrated an element is in the environment, the more concentrated it is in filter-feeders, although there are exceptions, such as copper, for which mussels have a regulatory system. In terms of the decreasing concentration gradient, Ni and V occupy positions 6 and 7; while Cr, Co and Mo are in the last positions, the same positions for Co and Mo have been observed in algae of this study and in sediments (Belhadj et al., 2017). It should be remembered that these elements are all useful in low concentrations for animals (Casas, 2005; Dabrin, 2009; Devez, 2004).

Furthermore, with regard to the limpet, these average metal levels obtained, when compared with the other samples, show that this species can have a very high bioaccumulation capacity, even exceeding that of sediments (Belhadj et al., 2017) (case of Cd



for station A) or that of the two algae (Cu and Mo for stations A and C; Co and Ni for station A and Cd for station C). In general, the results obtained highlighted the ability of the marine gastropod, *P. ferruginea*, to accumulate a wide range of metals in the study area. To this effect, our results are consistent with those reported by Türk Çulha et al. (2022), who demonstrated that *P. caerulea* is a sensitive bioindicator species, particularly for the bioaccumulation of nickel in marine environments. However, the absence of this species from station B is probably linked to the pronounced effect of metal pollution due to the immediate proximity of this station to the metal production plant (Alzinc Complex).

A comparison with other studies carried out on limpets from Mediterranean localities (Abdel-Halim et al., 2019; Belhadj, 2008; Belkhouja et al., 2010; Benguedda et al., 2011; Bordbar et al., 2015; Connan & Tack, 2010; Conti & Cecchetti, 2003; Cubadda et al., 2001; Kelepertzis, 2013; Türkmen et al., 2005; Yüzereroğlu et al., 2010) showed (Table 7) that the average concentrations of Cu and Zn recorded in limpets in the present study are higher than those in the works mentioned. For chromium and cobalt, the mean concentrations in limpets in the present study are lower than those found in *P. caerulea* in Iskenderun Bay (Turkey) (Türkmen et al., 2005). Similarly, for Mn, Ni, Cd and Pb, the average levels in the present study are lower than those found in *P. caerulea* from El-Mex Bay (Egypt) (Abdel-Halim et al., 2019) (Table 7).

3.3. Assessment of metal accumulation using quality indices

3.3.1. MPI

MPI is employed to assess and compare the quality of the sites studied in relation to the total metal content. It has been previously applied to assess metal contamination in different marine organisms and to compare its degree between different species (Abdel-Salam & Hamdi, 2014; El-Moselhy et al., 2016; El-Sikaily, 2008; Hamed & Emara, 2006; Ibrahim & El-Regal, 2014). The results obtained from MPI for all the samples are shown in Figure 2. All the MPI values recorded are >1 for all the sites studied in this research. The MPI values >1 indicate that the marine environment of Ghazaouet is, in fact, polluted. Clearly, metallic pollution of the sediments (Belhadj et al., 2017) has had an impact on marine algae as well as marine gastropods. Additionally, stations B and C always show values close to and higher than those of station A, whatever the sample considered, which shows once again that these stations are more contaminated than station A, which is the least contaminated by the metals analysed during this study.

The lowest MPI values for *E. linza*, *C. officinalis* and *P. ferruginea* were obtained at station A, with values of 5.41, 6.28 and 10.08, respectively. The highest MPI values for *E. linza*, *C. officinalis* and *P. ferruginea* were recorded at station C, with values of 19.84, 16.58 and

Table 7

Comparison of the concentrations of trace metals in gastropod (*P. ferruginea*) with values taken from the Mediterranean literature (mg · kg⁻¹ d.w.)

Sites	Metals										Reference
	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	
Ghazaouet Bay (Algeria)	0.66–0.72	248.90–959.48	21.83–89.71	20.34–22.88	6.93–7.28	5.49–8.77	0.97–1.73	1.39–4.47	8.94–10.02	28.83–113.09	Present study ^a
Sicilian coast (Italy)	-	2.20–19.10	0.47–3.75	-	-	-	-	0.10–1.01	1.70–11.80	0.06–2.18	Cubadda et al. (2001) ^f
Tyrrhenian coast (Italy)	-	87.40–117.10	10.20–19.20	-	-	-	-	0.72–0.96	2.89–4.06	0.51–1.50	Conti and Cecchetti (2003) ^e
Iskenderun Bay (Turkey)	-	23.10–46.60	1.58–4.02	1.39–4.31	-	3.60–12.20	2.31–5.15	4.77–8.33	2.39–4.97	4.28–14.50	Türkmen et al. (2005) ^f
Ghazaouet Bay (Algeria)	-	1.69	0.03	-	-	0.00	-	0.45	0.02	0.61	Belhadj (2008) ^a
North Cotentin (France)	-	46.50–66.80	4.30–4.90	4.70–20.20	-	1.70–2.60	<1.00	2.30–3.90	3.90–7.30	0.90–1.30	Connan and Tack (2010) ^b
North coast (Tunisia)	-	-	5.59–9.29	5.40–10.76	-	3.00–4.14	-	-	0.43–1.50	2.73–3.61	Belkhouja et al. (2010) ^f
Yumurtalik (Turkey)	-	3.70–9.84	1.09–2.12	-	-	0.39–1.05	0.05–0.14	-	0.24–0.44	0.05–0.31	Yüzereroğlu et al. (2010) ^f
Ghazaouet Bay (Algeria)	-	2.86–48.86	0.066–5.79	-	-	-	-	-	-	4.28–7.76	Benguedda et al. (2011) ^a
Stratoni and Artemida (North East Greece)	-	48.00–196.00	6–28	10.00–36.00	-	9.00	-	-	-	8.00–96.00	Kelepertzis (2013) ^b
Larymna Bay (Greece)	-	38.10–126.00	7.2–95.8	1.90–33.60	-	-	-	-	-	-	Bordbar et al. (2015) ^b
El-Mex Bay (Egypt)	-	71.33–82.83	20.15–59.47	72.00–98.77	-	16.70–27.16	-	-	18.99–24.57	139.81–168.42	Abdel-Halim et al. (2019) ^f

^a*Patella ferruginea*.

^b*Patella* sp.

^c*Patella caerulea*.

d.w., dry weight.

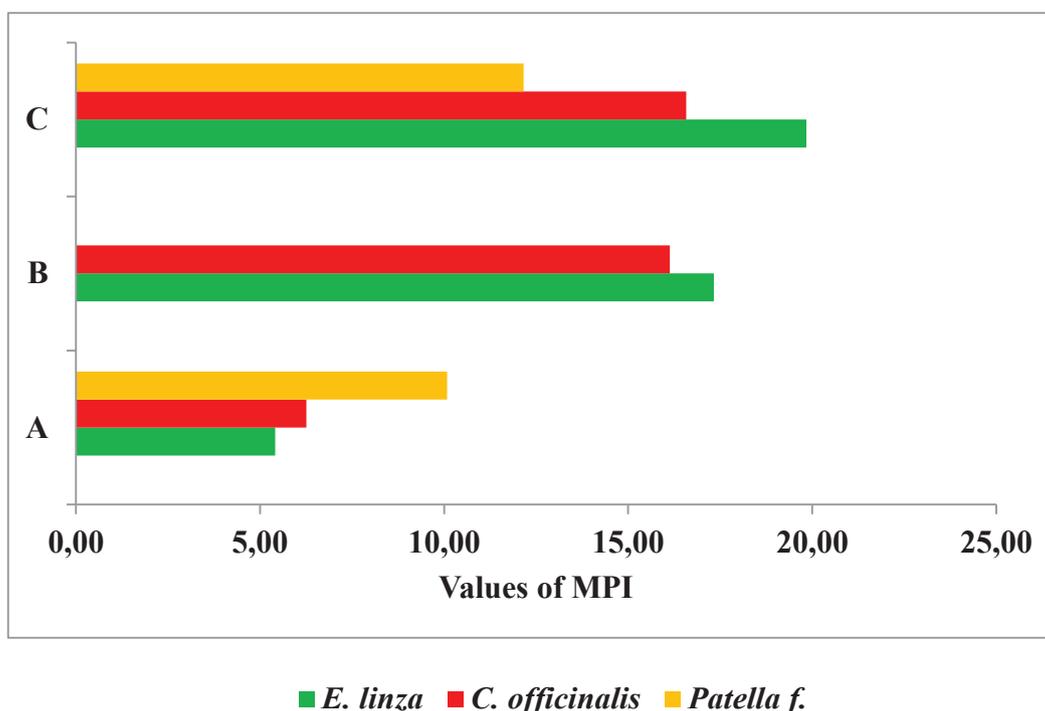


Figure 2

MPI for all the samples from the three stations of this study. MPI, metal pollution index.

12.16, respectively. The MPI value in *P. ferruginea* was higher than in the two algae at station A (Figure 2). The comparison of MPI for the two algal species, particularly at stations B and C, showed that MPI for *Enteromorpha* is slightly higher than that of *Corallina*. El-Nemr et al. (2012) carried out an analogous comparison between the MPI of *C. mediterranea* and the MPI of *E. intestinalis*. They showed that this difference is attributed to the fact that these two algae belong to the red and green, respectively, and that this difference is due to the calcareous structure of corallines compared to the filamentous structure of *Enteromorpha*. They conclude that MPI analysis revealed that the metal content depends on algal species. Based on the MPIs for the living marine organisms, we can conclude that station A was the least polluted location. Furthermore, Mokhtar et al. (2009) have already shown that MPI can provide a good representative image of the state of the environment as well as anthropogenic impacts on aquatic environments.

3.3.2. Health risk assessment

The THQ assesses the potential non-carcinogenic risks to human health from individual metal consumption, while the HI represents the combined

risk of all metals present in an organism. If THQ or HI is <1 , adverse effects on human health are unlikely. However, if the THQ or HI is ≥ 1 , there is a potential risk to human health. The THQ was determined for each metal using the estimated daily intake of *E. linza* and *C. officinalis* as seaweed sources and *P. ferruginea* as a mollusc (considered as a dietary item) with the Recommended Reference Dose (RfD) for the 10 metals as a reference value (Table 8).

For seaweeds, a daily intake of 5.2 g dry weight (d.w.) was used (Rahhou et al., 2023), while for *P. ferruginea*, the estimated daily intake was $0.0274 \text{ g} \cdot \text{capita}^{-1} \cdot \text{day}^{-1}$ (Bouiba et al., 2024; FAO, 2016). Table 8 shows that the THQ values for all metals in *E. Linza* and *C. officinalis* were <1.0 , indicating that the predicted exposure doses were below the RfD established by the US EPA. Similarly, *P. ferruginea* showed THQ values close to zero for all metals, suggesting negligible risk. However, when the HI was calculated using the mean metal concentrations, both *E. linza* (HI = 0.416) and *C. officinalis* (HI = 0.695) had values <1.0 , indicating a low risk. By contrast, *P. ferruginea* had an extremely low HI value (0.003), confirming an insignificant risk. Among the studied elements, lead (Pb) contributed the most to the HI values, particularly in *C. officinalis* (HI = 0.515), which may suggest a potential concern related to this metal. Moreover, the HI values of



Table 8

The reference dose for metals in foods (RfD) and mean values of the THQ and HI for the investigated elements

RfD (mg · kg ⁻¹ · day ⁻¹)											
Elements	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	
	0.005	0.3	0.04	0.14	0.009	0.02	0.02	0.003	0.001	0.004	
THQ and HI in studied marine organisms											
THQ	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb	HI = ΣTHQ
<i>E. linza</i>	0.001	0.038	0.012	0.006	0.016	0.003	0.001	0.039	0.055	0.246	0.416
<i>C. officinalis</i>	0.000	0.040	0.007	0.008	0.024	0.002	0.001	0.019	0.079	0.515	0.695
<i>P. ferruginea</i>	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.001	0.003

HI, hazard index; THQ, target hazard quotient.

examined marine species decreased in the following order: *C. officinalis* > *E. linza* > *P. ferruginea* (Table 8).

For seaweeds, Pb and Cd showed the highest THQ values. It was 0.246 and 0.055, respectively, for Pb and Cd for *E. linza*. THQ was again 0.515 and 0.079, respectively, for Pb and Cd for *C. officinalis*, making these elements the primary contributors to potential health risks for these marine organisms. The THQ for Zn also had an important contribution with 0.038 and 0.040 for green algae (*Enteromorpha*) and red algae (*Corallina*), respectively, compared to other metals, which have relatively low THQ values (<0.05), indicating minimal health risks individually. However, the Pb and Cd levels (mg · kg⁻¹ · d.w.⁻¹) in the two seaweeds studied (Tables 2 and 3) greatly exceeded the maximum contaminant levels for these two elements, respectively, limited by CEVA (2019) to 0.5 mg · kg⁻¹ and 5 mg · kg⁻¹. The marine environment of Ghazaouet is the 'natural' outlet for most of the direct and indirect discharges from the various potential sources of contamination in the region. The Alzinc plant, located in the municipality, is a major producer of industrial waste. Its residues are stored at the top of the cliff overlooking the sea and the plant, within the complex. Zinc, cadmium and lead are among the major components of industrial waste in terms of concentration and effects (Belhadj et al., 2017; MATE, 2007).

For mollusc *P. ferruginea*, the THQ values are extremely low (≈0), indicating a negligible risk from consuming this marine gastropod.

3.4. Correlations between metals in each sample

The results for Spearman's correlation coefficient (ρ) are shown in Table 9. The Spearman correlation coefficients (ρ) between the 10 metals in each separate matrix (two seaweeds and the marine gastropod) reveal a strong correlation for some metals, whereas others show an inverse correlation.

In *Enteromorpha*, numerous significant positive correlations ($p < 0.01$ and $p < 0.05$) were observed between trace metals (Table 9). These correlations, particularly involving V, Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb, suggest that these elements may share common anthropogenic or natural sources, or that their accumulation in algae is influenced by synergistic interactions (Haritonidis & Malea, 1995; Yozukmaz et al., 2018).

In *Corallina*, compared to green algae, metal correlations were generally weaker (Table 9). Nevertheless, several significant positive correlations ($p < 0.01$ and $p < 0.05$) were detected, particularly between Cr, Mn, Co, Ni, Cu, Zn, Cd and Pb. These relationships may reflect factors, such as competition between metals for binding sites and their relative bioavailability in red algae (El-Adl & Bream, 2015).

In *Patella*, metal correlations were more variable than in algae (Table 10). Significant positive correlations ($p < 0.01$ and $p < 0.05$) were observed between several elements, particularly involving V, Cr, Co, Ni, Cu, Zn, Pb and Cd, whereas some negative correlations were also recorded. These results highlight the complex relationships among trace metals, which can influence the ability of limpets to bioaccumulate them in their tissues (El-Adl & Bream, 2015; Idrissi Azzouzi et al., 2024).

3.5. Partial correlations between different samples

The results of the partial correlation analysis ($p < 0.05$) of the metals studied between seaweeds (*E. linza* and *C. officinalis*) and the marine gastropod (*P. ferruginea*) are presented in Table 10.

Statistically, very strong positive correlations are recorded for almost all metals, as shown in Table 10, between the green alga *E. linza* and the marine gastropod *P. ferruginea*. This can be explained

Table 9

Spearman correlations between the concentrations of trace metals in all the samples of the present study

	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb
<i>E. linza</i>										
Mo	1.000	0.523**	0.392*	0.247	0.407*	0.399*	0.215	0.341*	0.505**	0.283
Zn	0.523**	1.000	0.876**	0.733**	0.638**	0.545**	0.619**	0.676**	0.910**	0.855**
Cu	0.392*	0.876**	1.000	0.781**	0.583**	0.456**	0.639**	0.658**	0.923**	0.948**
Mn	0.247	0.733**	0.781**	1.000	0.860**	0.815**	0.943**	0.930**	0.778**	0.877**
V	0.407*	0.638**	0.583**	0.860**	1.000	0.912**	0.898**	0.936**	0.651**	0.678**
Ni	0.399*	0.545**	0.456**	0.815**	0.912**	1.000	0.896**	0.895**	0.504**	0.540**
Co	0.215	0.619**	0.639**	0.943**	0.898**	0.896**	1.000	0.962**	0.659**	0.738**
Cr	0.341*	0.676**	0.658**	0.930**	0.936**	0.895**	0.962**	1.000	0.732**	0.737**
Cd	0.505**	0.910**	0.923**	0.778**	0.651**	0.504**	0.659**	0.732**	1.000	0.884**
Pb	0.283	0.855**	0.948**	0.877**	0.678**	0.540**	0.738**	0.737**	0.884**	1.000
<i>C. officinalis</i>										
Mo	1.000	0.356	0.462*	0.495**	0.654**	0.740**	0.388*	0.706**	0.217	0.153
Zn	0.356	1.000	0.792**	0.549**	0.190	0.230	0.557**	0.604**	0.878**	0.838**
Cu	0.462*	0.792**	1.000	0.470*	0.126	0.466*	0.616**	0.733**	0.700**	0.657**
Mn	0.495**	0.549**	0.470*	1.000	0.370*	0.454*	0.777**	0.496**	0.544**	0.531**
V	0.654**	0.190	0.126	0.370*	1.000	0.368*	0.386*	0.527**	0.023	0.128
Ni	0.740**	0.230	0.466*	0.454*	0.368*	1.000	0.532**	0.711**	0.142	0.025
Co	0.388*	0.557**	0.616**	0.777**	0.386*	0.532**	1.000	0.740**	0.568**	0.494**
Cr	0.706**	0.604**	0.733**	0.496**	0.527**	0.711**	0.740**	1.000	0.543**	0.448*
Cd	0.217	0.878**	0.700**	0.544**	0.023	0.142	0.568**	0.543**	1.000	0.861**
Pb	0.153	0.838**	0.657**	0.531**	0.128	0.025	0.494**	0.448*	0.861**	1.000
<i>P. ferruginea</i>										
Mo	1.000	-0.148	-0.165	0.120	0.282	0.263	0.377	0.487*	-0.069	-0.151
Zn	-0.148	1.000	0.793**	0.218	0.031	-0.191	-0.437*	-0.376	0.114	0.878**
Cu	-0.165	0.793**	1.000	0.144	0.025	-0.051	-0.463*	-0.541**	0.038	0.684**
Mn	0.120	0.218	0.144	1.000	0.671**	0.283	0.323	0.222	0.420*	0.117
V	0.282	0.031	0.025	0.671**	1.000	0.358	0.463*	0.493*	0.474*	0.058
Ni	0.263	-0.191	-0.051	0.283	0.358	1.000	0.743**	0.488*	0.268	-0.461*
Co	0.377	-0.437*	-0.463*	0.323	0.463*	0.743**	1.000	0.720**	0.281	-0.590**
Cr	0.487*	-0.376	-0.541**	0.222	0.493*	0.488*	0.720**	1.000	0.233	-0.397
Cd	-0.069	0.114	0.038	0.420*	0.474*	0.268	0.281	0.233	1.000	0.017
Pb	-0.151	0.878**	0.684**	0.117	0.058	-0.461*	-0.590**	-0.397	0.017	1.000

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

by a similar metal bioaccumulation for these two organisms, or by the fact that *Patella* accumulates these metals in part by consuming *Enteromorpha*.

Compared with the correlations between *Enteromorpha* and *Patella*, the correlations between *Corallina* and *Patella*, as shown in Table 10, are generally weaker. However, certain metals, such

as Cr, Co, Ni and Zn, showed high correlations, as does Zn-Pb, which could suggest that these metals are accumulated by *Patella* through *Corallina*. Additionally, certain metals (V, Cr, Mn, Co and Ni) recorded strong correlations in both pairs, indicating a possible common source of these metals in the environment.



Table 10

Partial correlation coefficient ($p < 0.05$) measuring the relationship between trace metal concentrations in different samples of this study

	Mo	Zn	Cu	Mn	V	Ni	Co	Cr	Cd	Pb
<i>E. linza – P. ferruginea</i>										
Mo	1000	0.645	0.199	0.402	0.568	0.582	0.450	0.546	0.426	0.317
Zn	0.645	1000	0.679	0.535	0.701	0.461	0.450	0.508	0.592	0.747
Cu	0.199	0.679	1000	0.427	0.466	0.131	0.187	0.237	0.451	0.838
Mn	0.402	0.535	0.427	1000	0.823	0.756	0.899	0.876	0.428	0.681
V	0.568	0.701	0.466	0.823	1000	0.774	0.818	0.827	0.534	0.670
Ni	0.582	0.461	0.131	0.756	0.774	1000	0.837	0.852	0.336	0.315
Co	0.450	0.450	0.187	0.899	0.818	0.837	1000	0.904	0.413	0.454
Cr	0.546	0.508	0.237	0.876	0.827	0.852	0.904	1000	0.403	0.483
Cd	0.426	0.592	0.451	0.428	0.534	0.336	0.413	0.403	1000	0.552
Pb	0.317	0.747	0.838	0.681	0.670	0.315	0.454	0.483	0.552	1000
<i>C. officinalis – P. ferruginea</i>										
Mo	1000	0.088	-0.023	0.415	0.517	0.462	0.421	0.513	0.021	-0.040
Zn	0.088	1000	0.664	0.370	0.324	0.011	0.214	0.289	0.647	0.865
Cu	-0.023	0.664	1000	0.098	0.134	-0.130	-0.162	0.002	0.213	0.520
Mn	0.415	0.370	0.098	1000	0.369	0.349	0.475	0.370	0.324	0.282
V	0.517	0.324	0.134	0.369	1000	0.269	0.410	0.387	0.131	0.132
Ni	0.462	0.011	-0.130	0.349	0.269	1000	0.686	0.730	0.135	-0.051
Co	0.421	0.214	-0.162	0.475	0.410	0.686	1000	0.788	0.398	0.142
Cr	0.513	0.289	0.002	0.370	0.387	0.730	0.788	1000	0.274	0.185
Cd	0.021	0.647	0.213	0.324	0.131	0.135	0.398	0.274	1000	0.732
Pb	-0.040	0.865	0.520	0.282	0.132	-0.051	0.142	0.185	0.732	1000

*Correlation is significant at the 0.05 level.

4. Conclusions

The marine environment at Ghazaouet continues to be the site of a number of metal contaminations due to its role as a receptacle for the majority of anthropogenic discharges from the region. The results of the present study show that the levels of several metals analysed are a cause for concern, with abnormally high concentrations and significant variations. These metal contaminants are found in all the samples of living organisms, such as seaweeds and marine gastropod, with particularly marked concentrations in stations B and C compared with station A. The presence of trace metals in the two matrices from this marine environment sometimes exceeds the levels observed in other regions of the Mediterranean.

Concerning seaweeds, *Enteromorpha* from station B accumulated the highest average levels of Cr, Mn, Co

and Pb during this study. Those from station C had the highest average levels of V, Ni, Cu, Zn, Mo and Cd. As for *Corallina*, those at station B had the highest average levels of Cr, Co, Ni, Cu, Zn, Cd and Pb, while the highest average concentrations of V, Mn and Mo were found at station C. These results are sufficient to demonstrate metallic contamination in these two seaweeds, particularly for V, Cr, Mo, Cd and Pb, as these metallic elements are non-essential and have no biological role in plants.

For limpets, it is clear that those from station A are the most concentrated in Cr, Mn, Co, Ni and Mo, while the other elements are more abundant in limpets from station C. The metal concentrations found in the limpets in the present study are too high to be solely of natural origin, particularly for Cd and Pb, as these elements are not essential for these organisms. In addition, they reflect the same multi-contamination observed in algae. Concerning the MPI values calculated for the three marine organisms, the highest

values for *E. linza*, *C. officinalis* and *P. ferruginea* were recorded at station C. The MPI value in *P. ferruginea* was higher than in the two algae at station A. Based on the MPI results for marine organisms, station A proved to be the least polluted location compared with stations B and C.

Concerning the risk to human health following consumption of these marine organisms, the calculation of THQs and HIs showed that Pb and Cd are the metals that contribute most to the total risk. However, even if the HIs found are <1, indicating the absence of an immediate risk, the high levels of these two metals in our seaweed studied highlight a significant concern if consumption is frequent and high. *P. ferruginea* poses no significant health risk, making it a safer choice compared to the seaweeds of this study.

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