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Anthropogenic Pressure Impact in the Annaba Gulf on the Heavy Metal Concentrations Variation Measured in Water and *Posidonia oceanica* (L.) (Delile 1813) Leaves

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ABSTRACT

Human activity increasingly impacts coastal marine ecosystems through urban discharges and the use of the ocean as a dumping ground, leading to elevated pollution levels. This research aimed to determine trace metal element (TME) concentration profiles (Cd, Cu, Pb, and Zn) in the water and leaves of the *Posidonia oceanica* seagrass bed in the Gulf of Annaba (Algeria). Water and plant samples were collected at four sampling sites selected for their hydrodynamics and proximity to effluents flowing into the Gulf. Flame atomic absorption spectrophotometry was used to identify TMEs. The results showed that Cd, Pb, Zn, and Cu are present in all seawater samples. The measured TME concentrations are listed in descending order where Pb is followed by Zn, Cu, and Cd. The order of enrichment in both leaf types (adult and intermediate) of *P. oceanica* is Pb > Zn > Cu > Cd. S2 exhibited the highest lead concentrations, while S4 had the lowest. The BCF calculation revealed that our plant concentrates Cd, Pb, Cu, and Zn in various leaves within the biotope. The Fisher test (one-factor ANOVA) results showed significant differences in the concentrations of measured TMEs in the three compartments (water, FI, and FA) for Cu, Cd, and Pb and significant differences for Zn. However, for the four measured TMEs, the two-factor ANOVA test revealed no significant differences between the station-compartment groups.

INTRODUCTION

Acting as receptacles for atmospheric and land pollutants, anthropogenic activities are at the root of serious pollution problems in various ecosystems, particularly aquatic ecosystems. In reality, contamination of an aquatic ecosystem is not limited to just one of its compartments (water, sediment, or flora and fauna), nor is it confined to the area close to the pollutant source; the contaminant can be found at various levels of the aquatic ecosystem due to exchanges between compartments **(Belabed *et al.*, 2008; Boutabia-Trea *et al.*, 2015; Kadri *et al.*, 2015)**.

Marine coastal systems are under pressure from a variety of anthropogenic activities, and they serve as a sink for potential pollutants such as trace metal elements (TMEs) **(Abdennour *et al.*, 2000; Islam & Tanaka, 2004; Belabed *et al.*, 2013; *Ouali et al.*, 2018)**.

TMEs are potential pollutants of the aquatic ecosystem due to their toxicity, persistence in habitats, difficulty in biodegrading, and above all, their tendency to concentrate in aquatic organisms **(Ikem & Egiebor, 2005; Otansev *et al.*, 2016; Pejman *et al.*, 2017)**. The *P. oceanica* is a Mediterranean-endemic marine phanerogam capable of colonizing large areas of seabed, where 350 animal and plant species coexist, according to **Campanella *et al.* (2001)** and **Conti *et al.* (2007)**. According to **Boudouresque *et al.* (2006)**, seagrass meadows are an engineered ecosystem that plays important ecological, geological, and economic roles; however, this ecosystem is sensitive to human disturbances, such as coastal development, pollution, high water turbidity, and trawling. Due to the role they play in the Mediterranean coastal ecosystem, seagrass beds have been designated as a priority habitat by the European Union directive on the conservation of natural habitats, as well as the wild fauna and flora **(H & SD, 1992; Moreno *et al.*, 2001; Gobert *et al.*, 2005)**. Given their significance, sea grass beds are an excellent tool for assessing the quality of the Mediterranean coastal waters and detecting disturbances within them **(Pergent-Martini *et al.*, 2005)**.

Tourist activity has an increasing impact on the waters of the Gulf of Annaba, in addition to the effects of population growth and anthropogenic impacts linked to agriculture, fishing, industry, and shipping **(Belabed *et al.*, 2013; Ouali *et al.*, 2018)**, as well as domestic discharges and their impact on the bacteriological quality of the waters and aquatic fauna **(Kadri *et al.*, 2015, 2017; Boufafa *et al.*, 2021)**. Despite this growing pressure, the problem of metallic contamination in *P. oceanica* has gained little attention in northeastern Algeria; the only work assessing metallic contamination in *P. oceanica* has been undertaken by **Boutabia *et al.* (2015, 2017)** in the Gulf of Annaba, and by **Zeghdoudi *et al.* (2019)** in the Gulf of Skikda. In this context, we measured the concentrations of the TMEs (Pb, Cd, Cu, Zn) in surface water, as well as in adult and intermediate leaves of *P. oceanica* collected from four different sites in the Gulf of Annaba. The analysis of metal accumulation profiles would provide information about the health of the seagrass and its environment. It would allow us to compare the metal contamination levels in the Gulf of Annaba to those reported in other Mediterranean locations.

MATERIALS AND METHODS

1. Study area

Annaba is located on the southern shore of the Algerian-Provence basin in northeastern Algeria. Its bay is bounded to the East by Cap Rosa (8°15'E and 36°58'N) and to the west by Cap de Garde (7°47'E and 36°58'N) (Fig. 1). Freshwater enters the Bay of Annaba through two wadis: Oued Mafrag to the East and Oued Seybouse to the southeast, with highly irregular flows depending on the season. These wadis also transport organic and mineral matter from various sources (agricultural, terrestrial, industrial, and domestic) **(Ounissi *et al.*, 2014)**. It receives direct discharges from various industries along the coast, most notably the plant protection products industry (Fertial) and urban wastewater, which is only treated briefly. Numerous other wastewater sources are scattered along the coast (Oued Boukhmira, Oued Bedjimâa, Rizi Amor, Lacaroube) **(Belabed *et al.*, 2017; Ouali *et al.*, 2018)**.

The Gulf of Annaba is subject to various types of pollution, whether biological, chemical or organic. Pollution in the coastal ecosystem comes from industrial, agricultural, and urban sources. The main polluting industries in the Annaba region, according to **Grimes (2010)** include the power plant, the Fertial fertilizer factory, the SIDER Arcelor Mittal complex, the food industry (ORELAIT), vegetable oil and soap production (ENCG), the brewery and lemonade factory (EMIB), the metallurgical unit (Ferrovial), and the cement factory (hydro-canal).

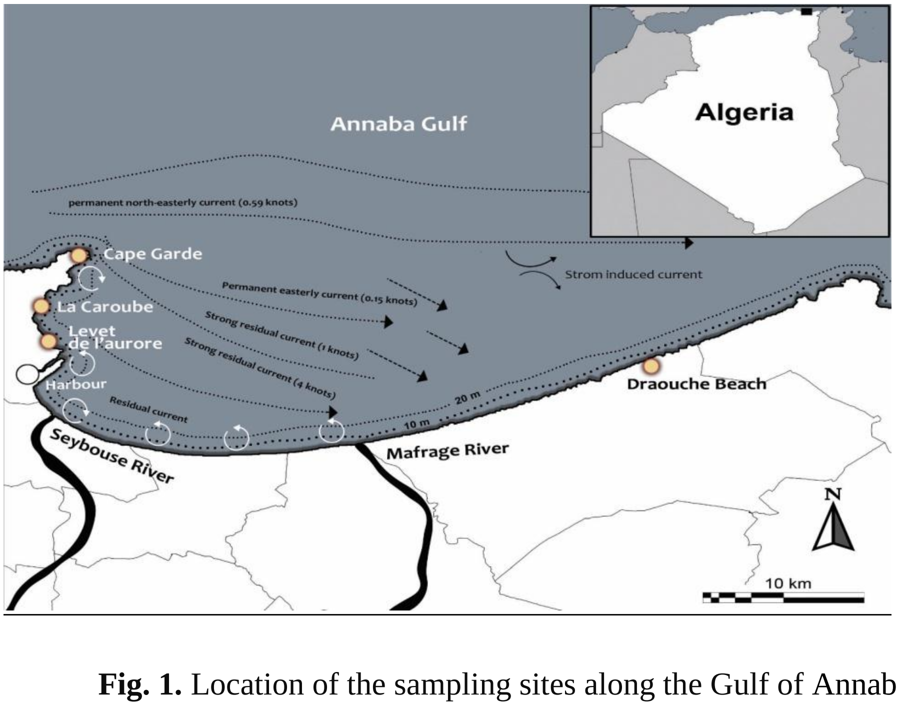


Fig. 1. Location of the sampling sites along the Gulf of Annaba

The Seybouse, Bedjima, Boukhemira, and Mafragh wadis, as well as numerous urban effluent discharges and the port, are the primary “point” sources that drain the vast majority of contaminants produced in their catchment areas into the Gulf of Annaba. Due to certain issues (access difficulties, lack of Posidonia at mouths), the sites were chosen based on their proximity to the various discharges and pollution sources (urban discharges, proximity to the port of Annaba) (Fig. 1). To cover a larger area of the Gulf of Annaba, four sampling stations were chosen. Table (1) summarizes the information on the location and characteristics of each zone.

Table 1. Sampling station characterization

Station	GPS coordinates	Depth	Nature of background	Other characteristics
S1 Draouche	36°52’ 15.03’’N 8°02’ 34.75’’ E	10 m	Meuble	Presence of a power station
S2 Lever de l’aurore	36°54’ 35.47’’N 7°46’ 21.48’’ E	12 to 15 m	Rocky	Urban waste + Proximity to the port; seaway
S3 Lacaroube	36°55’38.06’’N 7°45’ 41.07’’E	5 m	Meuble	Urban discharges; beaching (fishermen and yachtsmen)
S4 Cap de garde	36°58’01.1"N 7°47’17.2"E	5 to 8m	Rocky	Relatively less affected by human activity

2. Collection and preparation of samples

In addition to TMEs, complementary analyses were conducted to better understand the environment’s physicochemistry. In the case of water, we used a HORIDA multi-parameter (U-5000G) to measure the environmental parameters (dissolved oxygen, salinity, pH, and conductivity) monthly *in situ*. Surface water was sampled and frozen until analysis. To ensure optimal preservation, samples were packaged in 250mL glass vials, filled to the brim, and topped with 2mL 65% nitric acid. According to the method outlined by **Pergent (1987)**, we collected thirty orthotropic *P. oceanica* rhizomes, each bundle about a meter apart. The clusters were extracted by hand from the sediment, rinsed with seawater, and divided into three batches of ten. They were then placed in plastic bags and kept in a freezer at -20°C until processed, following the method of **Kantin and Pergent-Martini (2007)**.

2.1 Analytical procedure and analysis

Samples of *P. oceanica* were dissected to separate the leaves into juvenile (fJ), intermediate (fI) and adult (fA) leaves, according to the method of **Giraud (1979)**. Only the last two samples were cleaned of epiphytes and rinsed with distilled water. They were then dried to constant weight at 105°C, ground and mineralized by wet grinding. Nitric acid and hydrogen peroxide (5/2ml each of HNO_3 and H_2O_2) were added to the crushed material and then heated to 100°C until a clear solution was obtained. The solution was then filtered (using Wattman No. 41 paper filters), and the filtrate obtained was transferred to volumetric flasks and made up to 25ml with 2% $HNO_{3,}$ following the method described by **Boutabia-Trea (2017)**. The product thus obtained was stored in hermetically sealed polyethylene flasks until analysis.

2.2 Determination of metallic contaminants

The metal concentrations in all samples were determined using the flame metal absorption spectrophotometry (FMS) method, according to the desctiption of **Boutabia-Trea (2016)**. Furthermore, all elements are measured in micrograms per gram of dry weight (for leaves) and microliters per liter (for water).

2.3 Bioconcentration factor (BCF)

to We calculated the bioconcentration factor based on the TME concentration in seawater and tissue samples of *P. oceanica,* following the formula used by **Lewis *et al.* (2007)**, as follows:

BCF = Ca/Cb(1)

Where, Ca is the metal concentration in *P. oceanica* tissues (µg/ g dw), and Cb is the metal concentration in seawater (µg/ g dw).

3. Statistical analysis

The results are presented as mean ± standard deviation (m±s). The data were statistically analyzed using R statistical software version 3.6.3.

One-way and two-way ANOVA were used to compare several parameters (stations, compartments (water and Posidonia leaves), and metal concentration). Pearson correlation analysis with significance levels ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$) was used to assess the relationship between metal concentrations in *P.oceanica* and surface water. We described and condensed as much information as possible into a table of complex quantitative data using the principal component analysis (PCA). It also provided us with a graphical representation that is easier to interpret.

RESULTS

1. Environmental characterization

Calculating the mean value of each physicochemical parameter measured in surface waters at the four study sites (Table 2) revealed that pH is slightly higher than 8, and conductivity fluctuated between 44. 88± 13.16 (S3) and 49.99± 7.19ms/ cm (S4). Moreover, the salinity oscillated between 30.12± 0.04(S2) and 32.32± 0.22‰ (S1). Regarding the dissolved oxygen, its levels oscillated between 8± 0.5 (S2) and 9.22± 0.17mg/ l (S1).

Table 2. Spatial variation of physicochemical parameters in the four sampling stations of the Gulf of Annaba (Mean values ± SE)

Parameter	S1	S2	S3	S4
CE (ms/cm)	48.71 ± 8.7	48.15 ± 6.72	44.88 ± 6.16	49.99 ± 7.19
S (‰)	32.32 ± 4.96	30.12 ± 4.71	31.58 ± 6.37	31.55 ± 5.02
pH	8.44 ± 0.22	8.62 ± 0.04	8.62 ± 0.17	8.71 ± 0.03
O_2(mg/l)	9.22 ± 0.17	8 ± 0.51	8.27 ± 0.72	9.2 ± 0.79

2. TME concentrations in the Gulf waters

The TME concentrations vary from station to station in the Gulf of Annaba. The waters showed the following decreasing order of enrichment based on the average levels of TMEs measured: Pb is followed by Zn, Cu, and Cd. Furthermore, the enrichment of water in the four stations varieed by metal in the following decreasing order: S4> S3> S2> S1 for lead, S1> S4> S2> S3 for zinc, S3> S2> S1> S4 for copper, and S4> S2> S1> S3 for cadmium. Copper had a minimum level in S4, while lead had a minimum level in S1, in addition cadmium and zinc had a minimum level in S3 (Fig. 2).

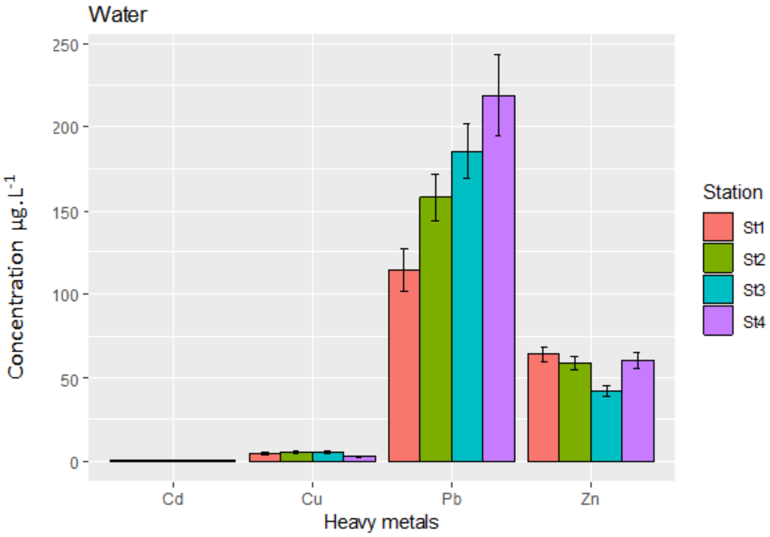


Fig. 2. MTE measured in the Gulf of Annaba waters

3. MTE concentrations in adult leaves of *P.oceanica*

The TME content in adult leaves was measured in the following order of decreasing enrichment: Pb> Zn> Cu> Cd. However, we noted that the enrichment of adult *P. oceanica* leaves varied from station to station, following the decreasing order listed below: for lead and zinc, it is S2> S3> S1> S4, for copper, it is S1> S3> S2> S4, and for cadmium, it is S4 > S3 > S1 > S2. Adult leaves in S2 appeared to accumulate more Pb and Zn, whereas those in S2 and S4 appeared to accumulate more Cu and Cd, respectively. The adult leaves in S3 appeared to have the same level of enrichment for all TMEs measured (Fig. 3).

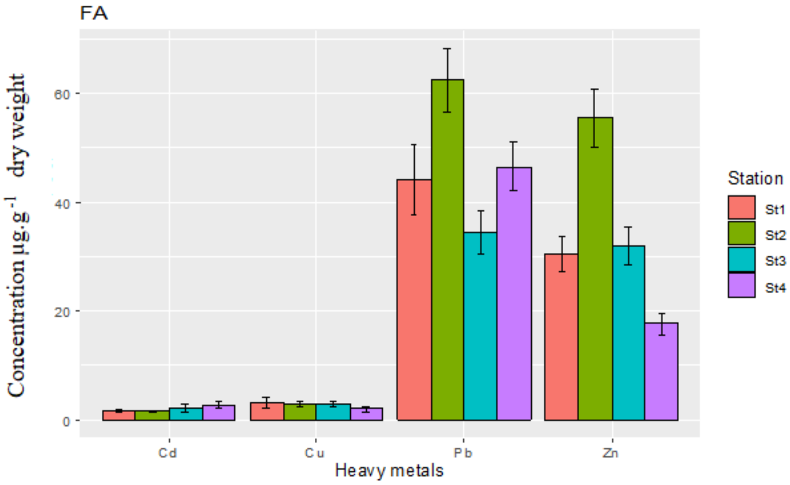


Fig. 3. MTE measured in adult leaves of *P. oceanica* from the Gulf of Annaba

4. MTE concentrations in intermediate leaves of *P. oceanica*

The average MTE content of *P. oceanica* followed the order of decreasing enrichment according to Pb> Zn> Cu> Cd. However, the enrichment of intermediate leaves by TMEs differed from station to station; it occured in the following decreasing order S2> S1> S3> S4 for lead, and S2> S3> S1> S4 for zinc. Moreover, copper enrichment followed the order S1> S2> S3> S4, while cadmium enrichment followed a different order S4> S3> S1> S2. These findings indicate that intermediate leaves in S2 accumulate the most lead and zinc, whereas intermediate leaves in S1 and S4 appeared to concentrate copper and cadmium (Fig. 4).

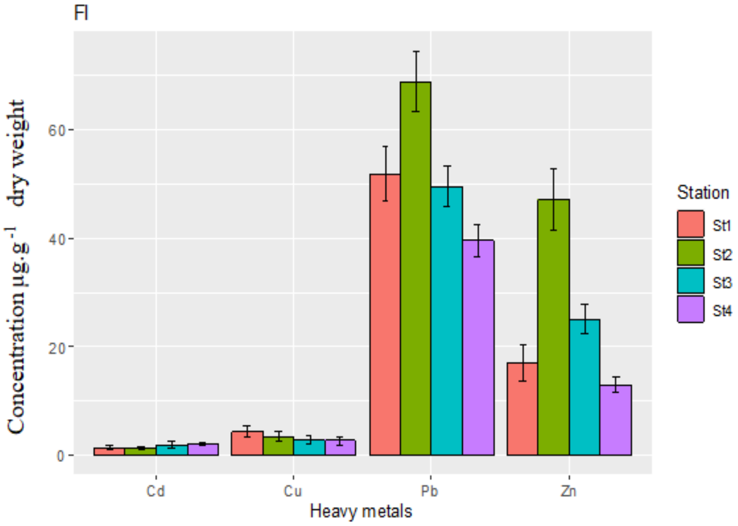


Fig. 4. MTE measured in intermediate leaves of *P. oceanica* from the Gulf of Annaba

5. BCF distribution in both tissue compartments

Zinc had slightly higher BCF values in adult leaves; the zinc transfer rate was significantly higher for both FI and FA in S2 and S3. Copper BCF values were low; only FI at S4 had a BCF value of around 1.3.

Whereas, lead had a maximum value of no more than 0.86, except for FIs in S2, which recorded a BCF value of around 1.22. Cadmium exhibited the highest BCF values (between 1.96 and 4.75) among the TMEs measured (Fig. 5); however, the values recorded in the FAs were higher than those noted in the FIs; additionally, in the two plant compartments, S1 and S3 recorded the highest Cd transfer rates.

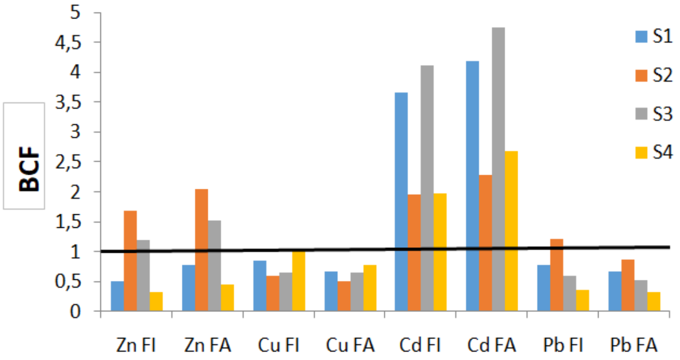
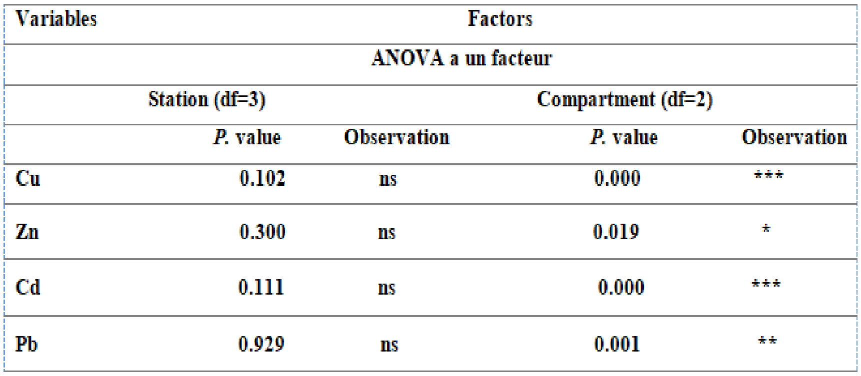


Fig. 5. BCF spatial distribution of TMEs measured in intermediate (FI) and adult (FA) leaves

The Fisher test (one-way ANOVA) results (Table 3) showed that the concentrations of TMEs measured in the three compartments (Water, IF, and AF) differed very significantly for Cu and Cd (*P*= 0.000), highly significantly for Pb (*P*= 0.001), and significantly for Zn (*P*= 0.019).

Table 3. One-way ANOVA results testing the effect of station and compartment interaction on variation in TME concentration in *P. oceanica.*



Variables	**Factors**			
	ANOVA a un facteur			
	Station (df=3)		**Compartment (df=2)**	
	***P.* value**	**Observation**	***P.* value**	**Observation**
Cu	**0.102**	**ns**	**0.000**	*****
Zn	**0.300**	**ns**	**0.019**	*
Cd	**0.111**	**ns**	**0.000**	*****
Pb	**0.929**	**ns**	**0.001**	****

NB: *(*P*≤ 0.05). ** (*P*≤ 0.01). ***(*P*≤ 0.001). ns (*P*> 0.05)

However, since these results do not indicate which group pairs differd, we used the Tukey test to determine the precise positions of these differences. The Tukey test revealed highly significant differences for Zn and Cu between adult leaves and water, as well as for Cd and Pb between plant tissues (adult and intermediate leaves) and water (Fig. 6).

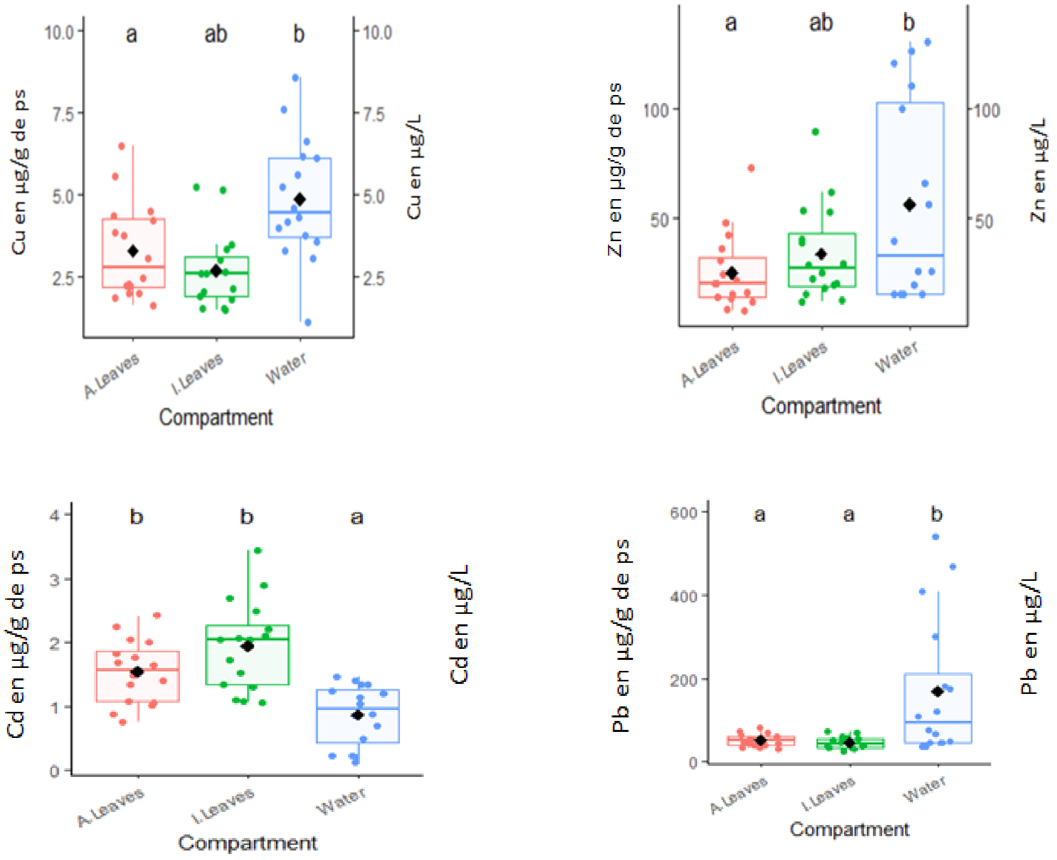
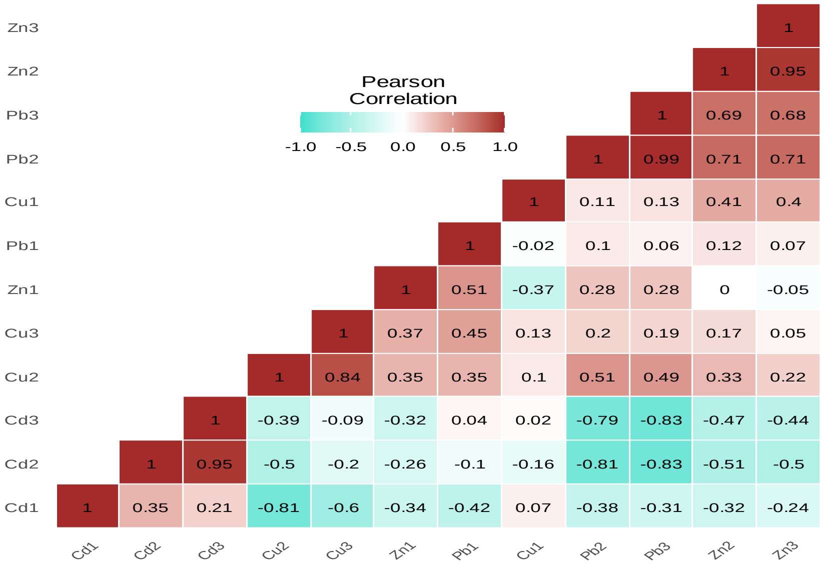


Fig. 6. Heavy metal accumulation in different compartments

At *P*< 0.05 (Tukey test), a, b, and ab indicate that the spatiotemporal variation issignificant. Whisker boxes labeled with the same letter do not differ significantly (*P* 0.05). The central box boundaries represent the interquartile range (IQR) with the first (lower bound) and third (upper bound) quartiles. Small circles represent the outliers. Pearson's parametric correlation coefficient calculation reveals positive correlations between Zn2 - Zn3 (r= 0.95), Zn3 - Pb2 (r= 0. 71), Zn3 - Pb3 (r= 0.68), Zn2 - Pb2 (r= 0.71), Zn2 - Pb3 (r= 0.69), Pb3 - Pb2 (r= 0.99), Pb2 - Cu2 (r= 0.51), Pb1 - Zn1 (r= 0.51), Cu3 - Cu2 (r= 0.84), and Cd3 - Cd2 (r= 0.95). This test also indicates the existence of negative correlations between Zn3 - Cd2 (r=-0.5), Pb3 - Cd2 (r= -0. 81), Pb3 - Cd3 (r= -0.83), Pb2 - Cd2 (r= -0.81), Pb2 - Cd3 (r= -0.79), Cu2 - Cd1 (r= -0.81), Cu2 - Cd2 (r= -0.5), and Cu3-Cd1 (r= -0.6) (Fig. 7).



	Cd1	Cd2	Cd3	Cu2	Cu3	Zn1	Pb1	Cu1	Pb2	Pb3	Zn2	Zn3
Zn3												1
Zn2											1	0.95
Pb3										1	0.69	0.68
Pb2									1	0.99	0.71	0.71
Cu1								1	0.11	0.13	0.41	0.4
Pb1							1	-0.02	0.1	0.06	0.12	0.07
Zn1						1	0.51	-0.37	0.28	0.28	0	-0.05
Cu3					1	0.37	0.45	0.13	0.2	0.19	0.17	0.05
Cu2				1	0.84	0.35	0.35	0.1	0.51	0.49	0.33	0.22
Cd3			1	-0.39	-0.09	-0.32	0.04	0.02	-0.79	-0.83	-0.47	-0.44
Cd2		1	0.95	-0.5	-0.2	-0.26	-0.1	-0.16	-0.81	-0.83	-0.51	-0.5
Cd1	1	0.35	0.21	-0.81	-0.6	-0.34	-0.42	0.07	-0.38	-0.31	-0.32	-0.24

Fig. 7. Pearson correlation coefficient analysis for TME tree compartments with stations

6. Principal components analysis (PCA)

The multi-variate analysis aims to structure the presence of heavy metals (Cu, Zn, Cd, Pb) in the four stations' three compartments (water, adult leaves, intermediate leaves). The application of PCA indicates that 83.30% of the total variability (inertia) of our variable matrix is explained by the first two principal components (Figs. 8, 9).The first PCA axis explains 55.7% of total variability; it is positively correlated with Zn (r= +0.81, $\cos^2$= 0.64), Pb (r= +0.87, $\cos^2$= 0.75); in contrast, this axis is negatively correlated with Cd (r= -0.81, cos2= 0.67). The second axis of the PCA explains 27.6% of the total variation; it is characterized by a strong negative correlation with Cu (T) (r= -0.86, $\cos^2$= 0.73). The projection of the concentrations measured in the four stations onto the two PCA axes reveals a strong presence of Cu in S1, S2, and S3, and of Cd in S3 and S4, along with a strong presence of Zn and Pb in S1 and S2 (Fig. 8).

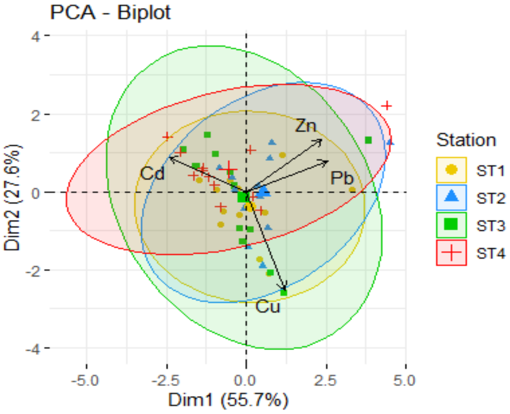


Fig. 8: PCA of metals at *P.oceanica* stations studied

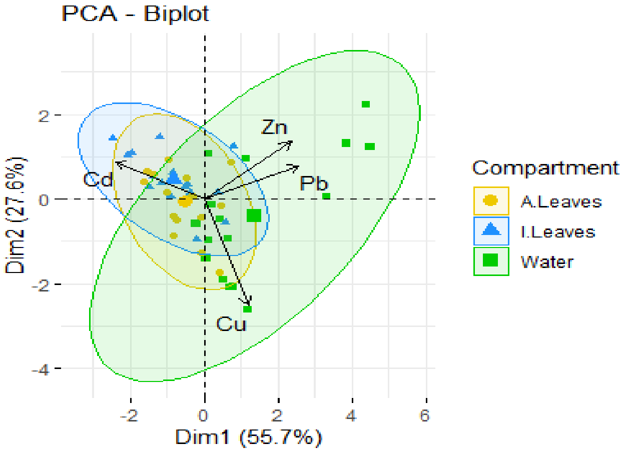


Fig. 9. PCA of metals in the various *P. oceanica* compartments studied

Projecting the MTE concentrations found in the 3 compartments onto the 2 axes of the PCA, we could see that Zn and Pb were strongly present in water, and Cu in both water and adult leaves; Cd, on the other hand, was strongly present in adult and intermediate leaves (Fig. 9).

DISCUSSION

The Gulf of Annaba is heavily contaminated with a variety of substances transported by air and land linked to human activity; these xenobiotics, contained in urban, industrial, and agricultural discharges, most often end up in this coastal ecosystem, heavily contaminating it **(Ounissi *et al.* 2021)**. The accumulation of contaminants in the aquatic ecosystem by living organisms reflects the quality of its habitats and pollution levels **(Belabed *et al.*, 2008; Kadri *et al.*, 2017; Trea-Boutabia *et al.*, 2017; Ouali *et al.*, 2018; Boufafa *et al.*, 2021)**. The average content of the four measured TMEs reveals that the enrichment of the Gulf of Annaba waters occurs in the following decreasing order: Pb> Zn> Cu> Cd. The high presence of cadmium and lead is recorded in S4 (1.03± 0.3 and 219± 220.88µg/ l, respectively) and that of copper and zinc is registered in S3 (5.86± 2.62µg/ l) and S1 (64± 53.76µg/ l), respectively.

Lead has a highly significant positive correlation with zinc (r= 0.71, *P*≤ 0.01) and a significant negative correlation with cadmium (r= -0.52, *P*≤ 0.05) among the TMEs measured in the gulf waters. **Stanisic *et al.* (2021)** in their study reported a positive correlation between zinc and lead (r= 0.86) in Montenegrin coastal waters. The sime authors also found a correlation between water and lead content with that of *P. oceanica* (r= 0.78), as reported in studies investigating the use of *P. oceanica* as a bioindicator species **(Rybak *et al.*, 2012; Bonanno *et al.*, 2017)**. It is generally accepted that water contamination depends on environmental conditions and metal availability.

Contamination is widely accepted to be influenced by environmental factors and metal availability. **Richir *et al.* (2013)** in their study reported that, during experimental (in situ) exposure of *Posidonia* to 15 trace elements, seawater enrichment by TMEs decreases differently depending on whether the level of contamination is moderate or high, contrary to our findings. The authors also found that zinc comes first, followed by lead, copper, and cadmium in moderately contaminated environments. In the case of acute contamination, however, copper comes first, followed by lead and cadmium. **Stanisic *et al.* (2021)** reported the following order of decreasing enrichment in Montenegrin coastal waters: Cu> Pb> Zn> Cd. Similarly, these authors noted a highly significant positive correlation between zinc and lead in this study.

The average TME content measured in *P. oceanica* tissues follows the same decreasing order of enrichment found in water (Pb> Zn> Cu> Cd) for both AF and IF. In the Gulf of Skikda (Algeria), a locality close to the study area, **Zeghdoudi *et al.* (2019)** recorded the same order of decay of these TMEs in *P. oceanica* leaves. Compared to the four MTEs measured in this study, zinc and copper tend to come before lead and cadmium in *P. oceanica* inhabiting the Montenegrin coastal ecosystem **(Stanisic *et al.*, 2021)**. The Ionian Sea **(Bonanno *et al.* 2017; Bonanno and Borg, 2018)**, the Tyrrhenian Sea **(Bonanno & Racuia, 2018)**, and the Aegean Sea **(Malea *et al.* 2019)** all have the same decreasing order of studied metals. **Joksimovi *et al.* (2011)** and **Stankovi *et al.* (2014)** reported an order of heavy metal decay in this marine plant on the Montenegrin coast that differs from the previous by the presence of lead, copper, and finally, cadmium after zinc. The decreasing order of heavy metals in the northwestern Mediterranean area **(Tovar-Sanchez *et al.*, 2010; Luy *et al.*, 2012; Di Leo *et al.*, 2013)** still includes zinc and cadmium in first and last places, respectively; however, copper comes after zinc, thus preceding lead.

However, the content of each assayed metal varies from station to station; in the AFs, the highest zinc and lead contents are found in S2, while the highest copper and cadmium contents are found in S1 and S4, respectively. Maximum levels are found in S1 for copper, S4 for cadmium, and S2 for zinc and lead in the IFs. The minimum levels of the TMEs measured evolve similarly in the two tissue compartments; for cadmium, they are found in S2 and S4 for the other three TMEs. **Malea *et al.* (2019)** in their study proposed that differences in TME accumulation between compartments, as described in various studies, could be due to differences in TME bioavailability in sediments and the water column. Averaging the TMEs measured in the 2 tissue compartments, it can be noted that FAs accumulate more zinc (33.92± 15.80µg.g^{-1}dw) and cadmium (1.94± 0.48µg.g^{-1}dw) than FIs (25.51± 15.22 and 1.55± 12.21µg.g^{-1}dw for zinc and cadmium, respectively). On the other hand, FIs accumulate more copper (3.27µg.g^{-1}dw) and lead (52.49± 12.21µg.g^{-1}dw) than FAs (2.62µg.g^{-1}dw for copper and 46.51±11.7µg.g^{-1}dw for lead).

Sanz Lazaro *et al.* (2012) discovered very high cadmium, fairly high zinc, and low lead concentrations in the leaves. The low concentration of Cu in adult leaves can be explained by its use in metabolic activity during the growth period of intermediate and juvenile leaves; copper is used where it is needed, according to **Romero (2007)**. The TME uptake and translocation in seagrass vary depending on the TMEs and plant tissue. The chemical properties of each TME influence this specificity. Cadmium recorded BCF≥ 1 values in all stations and in both compartments; values fluctuated between 1.96 and 4.75, but maximum values were recorded in S1 and S3 (3.66, 4.12 for FI, and 4.17, 4.75 for FA). Zinc showed BCF≥ 1 values in S2 and S3 for both FI (1.69, 1.19) and FA (2.04, 1.52); lead and copper showed the lowest BCF values; BCF≥ 1 values were noted in FI of S4 (1.02) for copper and in FI of S2 (1.22) for lead.

Several authors have reported differences in the accumulation of specific TMEs in different *P. oceanica* compartments. According to **Lewis and Devereux (2009)**, Pb accumulates preferentially in the aerial parts of the plant compared to the roots. According to **Richir *et al.* (2013)**, the absorption kinetics of *P. oceanica* leaves vary depending on the elements and the age of the leaves. Young leaves form new tissues and incorporate MET more quickly than older, senescent leaves due to their growth phase. Lead has a positive correlation with zinc and copper in the two tissue compartments (FA and FI) but a negative correlation with zinc content in all three compartments (seawater, FA, and FI). Cadmium, unlike lead, has a negative correlation with lead in all three compartments, as well as with zinc and copper in FA and FI. The content of each element recorded in FA correlates positively with FI. The projection of the TME concentrations measured in the three compartments onto the two axes of the PCA reveals the presence of Zn and Pb in water and Cu in both water and adult leaves; Cd, on the other hand, is found in adult and intermediate leaves.

This high level of lead contamination in the waters of the Gulf of Annaba is linked in part to the heavy road and sea traffic that has impacted the area for decades. **Ancora *et al.* (2004)** in their study found a decrease in lead contamination levels in the Gulf of Naples between 1989 and 1999, following a ban on its use as an anti-knock additive in gasoline (lead alkyls) and as a primary component of water pipes. Furthermore, **Tranchina *et al.* (2005)** in their study discovered that Pb concentrations measured in *P. oceanica* scales were statistically correlated with Pb emissions into the air, reflecting the level of Pb pollution in the coastal marine environment. These TMEs could also come from port activities, which have been linked to water contamination by lead, cadmium, and mercury; additionally, the presence of the fishing port represents a potential source of pollution, since cleaning and maintaining water from boats (trawlers, sardine boats) is discharged directly into the Gulf waters. Using agricultural fertilizers and pesticides contributes significantly to increased copper, zinc, and cadmium concentrations in water. Due to their untreated discharge into water, these TMEs are thought to have an industrial origin (steel complex, fertilizer manufacturing complex). Except for lead, the TME contamination levels in *P. oceanica* leaves reported in this study are among the lowest in the Mediterranean (Table 4).

Pb levels in the Gulf of Annaba are among the highest in the Mediterranean, rivaling those reported in Greece's contaminated Gulf of Antikyra **(Malea *et al.*, 1994)**. Cadmium levels are slightly higher than those reported in the Gulf of Skikda (Algeria) and Tunisia **(Zakhama-Sraib *et al.*, 2016; Zeghdoudi *et al.*, 2019)**. Moreover, they are close to those reported in the Tyrrhenian Sea **(Warnau *et al.*, 1995)**; and they are in the lower half of the Mediterranean scale range (Table 4). Copper levels in the Gulf of Annaba are comparable to those found in the Ionian Sea by **Di Leo *et al.* (2013)** and in the same location by **Boutabia-Trea *et al.* (2017)**; copper levels in the Gulf of Annaba are the lowest in the Mediterranean. Zinc levels in *P. oceanica* leave in the Gulf are among the lowest, ranking third only to northern Corsica **(Gosselin *et al.* 2006)** and the Gulf of Skikda **(Zeghdoudi *et al.* 2019)**. According to **Luy *et al.* (2012)** and **Sanz-Lázaro *et al.* (2012)**, the spatial variation in metal concentrations recorded along Mediterranean coasts is caused by either specific anthropogenic pressures or natural heterogeneity of environmental facies.

Table 4. TME concentrations (µg.g^{-1} dry weight) in the leaves of *P. oceanica* from the Mediterranean Sea

Location	Cd	Cu	Pb	Zn	Reference
Antikyra Gulf (Greece)	**2.7 - 44**	2.8 – 148	**10.5-123**	27.1-97.7	**Malea *et al.* (1994)**
France	0.30-1.50	**6.00 - 45.0**	**1.60-55.0**	15.0-147	**Roméo *et al.* (1995)**
Thyrrheniansea (France-Italy)	2.10-2.40	10.02-16.25	5.96-8.35	**144-179**	**Warnau *et al.* (1995)**
Favignana Island (Italy)	0.63-6.12	5.00-23.9	0.23-12.7	79.0-171	**Campanella *et al.* (2001)**
Sicily (Italy)	1.90-4.00	2.50-13.0	7.40-14.0	**170-310**	**Tranchina *et al.* (2004, 2005)**
Northcorsica (France)	0.40-1.20	9.00-16.0	6.00-25.0	14.0-31.0	**Gosselin *et al.* (2006)**
Ustica Island (Italy)	**3.60-7.50**	**19.8-53.2**	1.10-5.0	**142-260**	**Conti *et al.* (2007)**
Italy, France, Spain	*1.4-4.39*	6.93-15.1	0.6-3.83		**Lopez y Royo *et al.* (2009)**
Linosa Island (Italy)	0.95-5.49	3.12-17.7	0.59-13.8	16.2-156	**Conti *et al.* (2010)**

French coast	2.40	12.8	2.14	98.0	**Luy *et al.* (2012)**
IonianSea (Italy)	0.09-0.57	0.97-6.95	0.23-1.71	15.0-193	**Di Leo *et al.* (2013)**
Sicily (Italy)	0.53-2.02	8.24-18.7	1.69-5.21	55.7-165	**Bonanno *et al.* (2017)**
Tunisia	0.63-1.31	5.48-14.6	0.22-4.08	43.9-116	**Zakhama-Sraib *et al.* (2016)**
Gulf of Annaba (Algeria)		0.76-6.54		10.67-87.53	**Trea-Boutabia *et al.* (2017)**
Skikda gulf (Algeria	0.53-2.33	6.07-52.44	7.73-71.23	8.52-40.17	**Zeghdoudi *et al.* (2019)**
Gulf of Annaba (Algeria)	1.19-2.58	1.92-4.30	**34.35-68.93**	12.93-55.58	Present study

Values in bold type correspond to stations considered as impacted by authors.

CONCLUSION

The biomonitoring study in the Gulf of Annaba using adult leaves of *P. oceanic* to assess the level of metal contamination yielded significant results. Zinc, copper, lead, and cadmium were measured in three different compartments: seawater, adult leaves (FA), and intermediate leaves (FI) of *Posidonia*. S4 has the highest concentrations of lead and cadmium in the water compartment, while S1 has the highest zinc concentration. Meanwhile, copper is more concentrated in S3. The results for *Posidonia* leaves reveal that adult leaves have higher lead and zinc concentrations in all four stations in a decreasing order from S2 to S4 for lead and S1 to S3 for zinc.

S1 displys the highest copper concentrations, followed by S3 and S2, and finally, S4. The cadmium concentrations in mature leaves are higher than in water, with S4 being the highest and S2 being the lowest. Intermediate leaves, which have a weaker defense system than adult leaves, absorbed more lead and zinc in S2, while copper is more concentrated in S1 and cadmium in S4. Cadmium contamination was found in adult and intermediate seagrass leaves in all stations using the BCF (Bioconcentration factor) calculation, with higher concentrations in S3 and S1. The highest zinc concentrations were found in S2 and S3, with a trace of lead in intermediate leaves in S2.

Finally, *Posidonia* meadows, particularly *P. oceanica* leaves, have proven useful bioindicators for assessing chemical contamination in the Gulf of Annaba. Due to their ecological importance, year-round presence and widespread distribution, they are ideal candidates for establishing networks to monitor metal pollution in the Mediterranean.

These findings highlight the critical importance of continuing to study and protect these marine ecosystems, which are critical for preserving biodiversity and the health of our environment.

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