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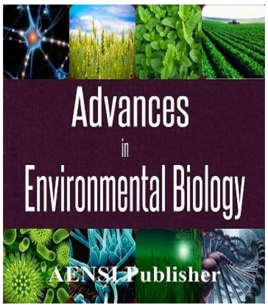

Evaluation of the Metallic Contamination Level in the Gulf of Annaba ((North-eastern Algeria) Using a *Posidonia oceanica* (L) Delile.

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ABSTRACT

The aim of this study is to evaluate the contamination level in waters of the Gulf of Annaba by the measurement of some elements metallic traces (MTE) (copper, zinc, nickel and chromium) in the marine phanerogam *Posidonia oceanica* in winter and summer periods. The collect of this plant was made in two stations: The first is the urban waste receptacle (Lacaroube) and the other is located quite far from the city center in a place called Ain-Achir. Heavy metals are assayed, using atomic absorption spectrophotometer, from various sections of the plant (root, rhizome, mature leaves). The assay results show that the levels of copper, zinc, nickel and chrome are different from one station to another, from one season to another and from one section of the plant into another. The contents of the MTE are, generally, higher in Lacaroube due to its proximity to urban areas and its hydrodynamics which is very different from the one of Ain-Achir. These differences are explained by the environmental conditions under which lies the *P. seagrass*.

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INTRODUCTION

The deterioration of aquatic environment and coastal waters is always attributed to domestic wastewater, industrial effluents and agricultural runoff [1]. Heavy metals such as zinc, copper and chromium or their derivatives are widely used in the industries of manufacturing of various alloys, chemical and metallurgical industries [2, 3].

Several metal ions are essential as elements metallic trace (MTE), but at high concentrations, they become toxic to aquatic fauna and flora [4]. The presence of "heavy metals" in living beings affects many metabolisms or development processes [5] mainly due to their toxicity, non-biodegradability, bioaccumulation and persistence in nature [6].

The use of living organisms to evaluate the environment's quality is not a new concept [7]; the use of marine plants by the scientific community is related to their sensitivity to the slightest changes in their environment and their quick reaction and for being the first link in the food chain [8, 9].

The *Posidonia oceanica* (L.) Delile meadow is a powerful integrator of the overall quality of marine waters [10, 11, 12, 13] since it exists in the bathymetric [0 - 40m] infralittoral range exposed directly to pollution [14]. *P. oceanica* was used in biomonitoring studies in the Mediterranean in order to assess its effectiveness as a trace metals contamination indicator [15, 16].

The Gulf of Annaba is subject to many sources of pollution such as wastewater of aglomerations within its watershed, port activities (market and fisheries) and industrial activity [17]. This study aims to assess, for the first time, the metal contamination of the Gulf of Annaba by the zinc, copper, chromium and nickel using the bioindicator species: marine phanerogam *P. oceanica*; and to know metal accumulation patterns and evaluate the relevance of the seagrass *P. oceanica* as potential bio-indicator of trace metals.

MATERIALS AND METHODS

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2.1. Study sites and methodology:

The Gulf of Annaba is located in the eastern coast of Algeria; It is limited by Cape Rosa from the east (8 ° 15 'E 36 ° 58'N) and by the Cap de Garde from the west (57 ° 16'E and 36 ° 58'N) (Fig. 1). The distance between the two capes is about 21.5 miles (40 km) and the maximum water depth is 65m. The continental shelf is narrow and sharply rugged northern the Cap de Garde (4.5 miles), then it widens into the Gulf until 14,05 miles (26,021 km) to shrink slightly to the east in the vicinity of Cape Rosa [18]. The sites receive domestic releases from important conurbations and untreated sewage contributing contamination by heavy metals.

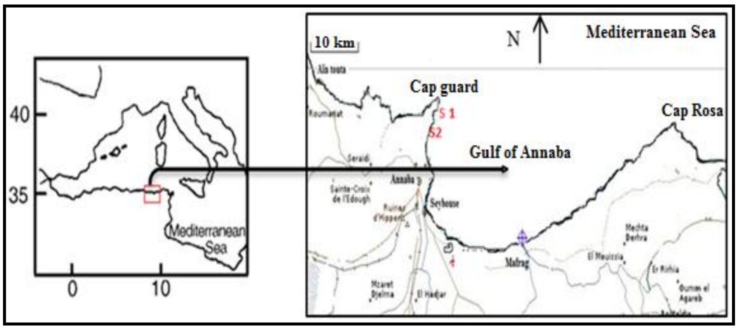

Fig. 1: Location of the Gulf of Annaba and sampling stations (S1: Ain-Ashir; S2: Lacaroube).

2.2. Biological model:

The marine plant *Posidonia oceanica* was randomly collected from individual diving by hand and at a rate of 30 complete Shoots per station (2 stations located in the western part of the Gulf) and per season (winter and summer) at a depth ranging between 5 and 15m depending on the station. Shoots collected were divided into three repetitions (N= 10).

2.3. Samples preparation:

P. oceanica samples were dissected in the laboratory to separate their roots (ra), rhizomes (rh) and leaves; these last have been separated using the Giraud method [19] in juvenile leaves (Jl), intermediate (Il) and mature ones (Ml). Only mature leaves have been cleaned of their epiphytes by a plastic ruler and rinsed with distilled water.

The samples were then dried at 105 ° C until a constant weight, then mashed and wet-mineralized. This consists in adding the nitric acid and hydrogen peroxide (HNO_3 and H_2O_2 in an amount of 5/2 ml) to the mash, and heating them at 100 ° C until a clear solution is obtained. The latter is then filtered through a filter paper (WhatmanN°41) and the filtrate obtained was transferred to the volumetric flasks and made up to 25 ml with 2% HNO_3. The resulting product was stored in polyethylene bottles tightly sealed until analysis.

2.4. Determination of metal contaminants:

The concentrations of Metallic Trace Elements (Zn, Cu, Ni and Cr) contained in the samples were determined using an atomic absorption spectrophotometer "AAS" with air acetylene flame (Shimadzu model AA- 6200). The metal content of the samples represent the average values obtained from three replicas and are expressed in micrograms per gram dry weight.

2.5. Statistical Analysis:

The results obtained by various tests were analyzed statistically using descriptive metric methods by giving the mean and standard deviation. They have been subjected to a comparison of means ("t" test) and variances at the level of significance $\alpha = 0.05$ via XLStat 2009 software.

Results:

3.1. Assessment of metal contaminants of the P. oceanica in Ain-Achir station (S1):

In roots, the assayed MTE concentrations collected during the two seasons are in descending order: Ni> Zn> Cr> Cu. MTE concentrations observed in winter are almost double of those observed in summer (Fig. 2). However, the comparison of average rates of Cr indicates highly significant differences ($t_{obs} = 6.65$; $p = 0.003$) (Fig. 2).

At rhizomes, the order of metal concentrations is not only different from that observed in the roots, but also differs from one season to the next. MTE concentrations order in winter is: Ni> Cr> Zn> Cu and Zn, while it becomes> Ni> Cr> Cu in summer (Fig. 2). MTE contents found in the rhizomes show a decrease in summer by 30 to 50% depending on the metal. The comparison of average amounts of Cr and Ni shows the existence of highly significant differences (Cr: $t_{obs} = 4.98$; $p = 0.008$; Ni: $t_{obs} = 7.74$; $p = 0.001$) (Fig. 2).

MTE concentrations found in mature leaves are higher than those in the roots and rhizomes. The MTE contents show descending order Ni> Zn> Cr> Cu in winter, and Ni > Cr> Zn> Cu in summer. The zinc and nickel witness a decrease of 50% and more in the summer. Regarding the chromium and copper, they showed very low decrease (Fig. 2). The comparison of means indicates the effect of the seasons on the MTE concentrations in this section (Fig. 2).

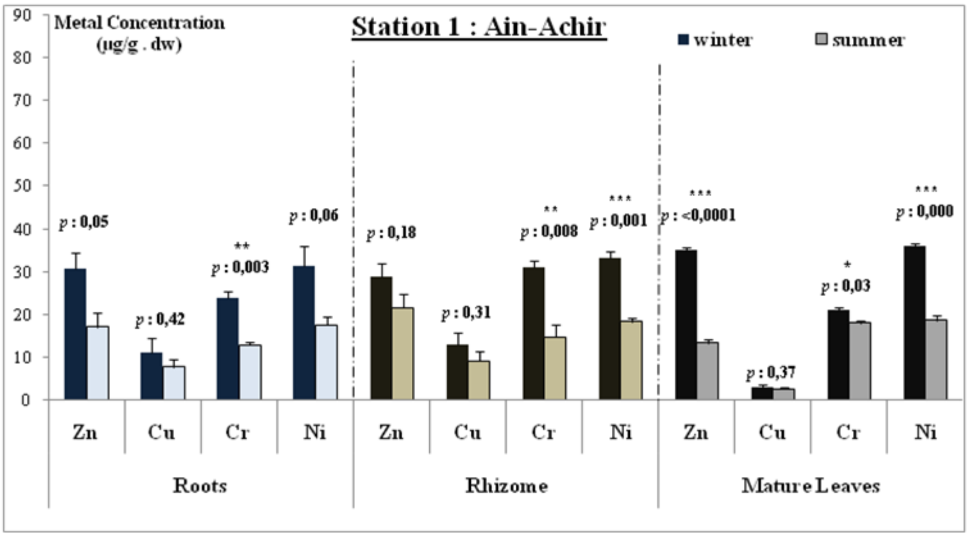

Fig. 2: Levels of MTE found in different studied compartments of *P. oceanica* in the station of Ain Ashir (S1)

(*: Significant differences exist between the means; **: highly significant differences exist between the means; ***: Very highly significant differences exist between the means)

3.2. Assessment of metal contaminants of P. oceanica in Lacaroube station (S2):

In Lacaroube station (S2), MTE concentrations witnessed in the roots show a variable decrease from one season to another. The orders Zn> Ni> Cr> Cu and Cr> Zn> Ni> Cu are noted in winter and summer respectively (Fig. 3). The chromium and copper found in the roots show a slight increase in summer, and only the zinc (Zn) and nickel (Ni) show high winter levels. Comparison of means of these two contaminants shows the existence of significant differences (Fig. 3).

In rhizomes, MTE contents show the same decreasing order in winter and summer: Zn> Ni> Cr> Cu; this distribution is similar to that found in the roots during the winter. It is also noted that during the summer, zinc contents fall by more than 60% and those of chromium and copper by more than 30% (Fig. 3). Means comparison shows highly significant differences in the four assayed contaminants (Fig. 3).

Regarding mature leaves, concentration order of the assayed MTE differs according on the season (Ni> Zn> Cr> Cu in winter and Zn > Ni> Cr> Cu in summer). However, it is noticed that nickel and copper show high levels in winter, unlike chromium and zinc where their contents are higher in the summer. Means comparison shows the existence of very highly significant differences of these two contaminants (Zn: t_{obs} = 20.56; p <0.0001; Ni: t_{obs} = 13.27; p <0.000) (Fig. 3).

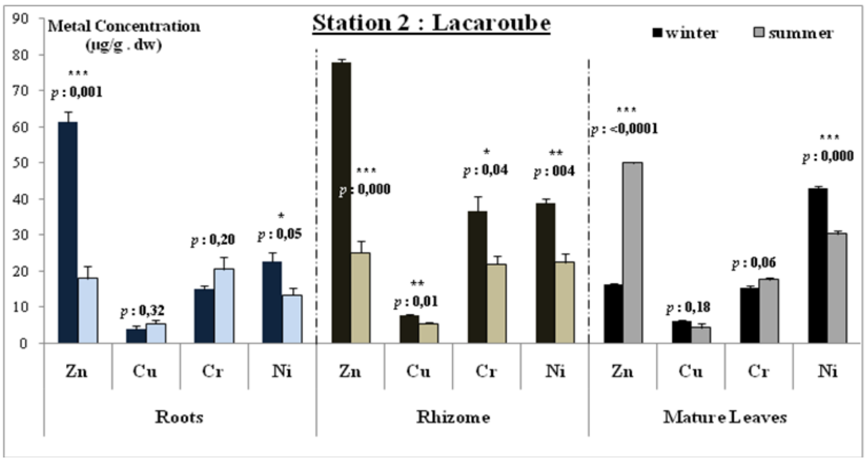

Fig. 3: Levels of MTE found in different studied compartments of *P. oceanica* in Lacaroube station (S2)

(*: Significant differences exist between the means; **: Highly significant differences exist between the means; ***: Very highly significant differences exist between the means)

The levels of zinc and copper contained in the roots and rhizomes vary depending on the season and the studied station; comparison of variances indicated significant differences (Tab. 1). As for chromium and nickel, statistical analysis (AV1) shows that the two studied effects (season and station) are not significant (Tab. 1). The test AV2 indicates a synergistic effect between the two factors (season and station) on the roots and leaves (Tab.1).

In leaves, the AV1 test shows only a season effect on the rate of chromium. However, the interaction of the two studied factors (season and station) influences significantly most of the measured metal contaminants (Tab. 1). It can be deduced that the station effect influences significantly the rate of Zn and Cu in roots and rhizomes (see Tab.1), the season effect influences significantly the rates of Zn in the roots and rhizomes and the rate of Cr in mature leaves (see Tab.1) and the interaction "season and station" influences significantly the rate of Zn, Cr and Ni (see Tab.1).

Table 1: Comparison of the station effect, the season effect and their interaction on the levels of MTE assayed in different sections of *P. oceanica* by the application of AV1 and AV2

	AV1				AV2	
	Station Effect		Seasonal effect		Interaction Stations & Season	
	F	*p*	F	*p*	F	*p*
Roots						
Zn	6,86	0,05* (S)	10,86	0,02* (S)	9	0,03* (S)
Cu	8,21	0,04* (S)	4,41	0,13 (NS)	5	0,18 (NS)
Cr	1,7	0,57 (NS)	1,19	0,86 (NS)	8,2	0,04* (S)
Ni	2,29	0,38 (NS)	3,62	0,18 (NS)	8,2	0,04* (S)
Rhizome						
Zn	20,52	0,005** (HS)	23,77	0,003** (HS)	6,6	0,09 (NS)
Cu	9,49	0,03* (S)	1,77	0,54 (NS)	6,6	0,09 (NS)
Cr	1,01	0,99 (NS)	1,02	0,99 (NS)	8,2	0,04* (S)
Ni	1,28	0,79 (NS)	1,11	0,91 (NS)	9	0,03* (S)
Mature Leaves						
Zn	2,35	0,37 (NS)	3,79	0,17 (NS)	9	0,03* (S)
Cu	3,96	0,16 (NS)	1,56	0,64 (NS)	7,4	0,06 (NS)
Cr	1,67	0,59 (NS)	10,33	0,02* (S)	8,2	0,04* (S)
Ni	1,83	0,52 (NS)	2,72	0,30 (NS)	9	0,03* (S)

[NS: $p: > 0.05$; * significant* : $p: \leq 0.05$; ** highly significant: $p: \leq 0.001$; *** very highly significant: $p: < 0.000$]

Discussion:

Various aquatic habitats of the Gulf of Annaba are exposed to high contamination by xenobiotics due to the air and wastewater rich of domestic, industrial and agricultural waste [20]. Consequently, contamination of the aquatic flora is inevitable and bioaccumulation potential of these micro-organisms reflects the water quality and more and more increasing pollution levels.

Spatio-temporal variation:

The results of this study show that the spatial and temporal variations are related to the degree of pollution in the Gulf of Annaba. The two stations of this Gulf have different concentrations compared to each assayed metallic element. The classification of MTE concentrations assayed in different sections of *P. oceanica* is as follows: Zn> Ni> Cr> Cu; this sequence of elements' classification corresponds partially to that found in the work of Campanella *et al*., [21] and Conti *et al*., [16] except for the location of Cu and Cr which are reversed.

This latter is present in both of the Gulf stations with a higher concentration than that at the French coasts (from 0.16 to 0.98 µg/g dry wt), but the concentrations of Zn and Ni are above the minimum values recorded in the same area [22]. The maximum value of Zn recorded in the Gulf of Annaba is of the order 77.88 µg/g dry wt; this concentration is lower than that found in *P. oceanica* collected in Marseille (107 µg/g dry wt) by Warnau *et al*., [23].

Ni with maximum content of about 43.05 µg/g dry wt; has a higher value compared to that found in Livormo, Italy (28.9µg / g dry wt) polluted by effluents of a chemical plant [24]. Cu is used in the manufacture of alloys, anti-fouling paints (as copolymer), enamel (stainless), fungicides, fertilizers etc... the maximum level determined in this study is of the order 13.23 µg/g dry wtin the station S1 (Ain-Achir).

This content is significantly lower than that reported in Sicily (31.9µg /g dry wt) by Conti *et al*., [25]. However, it is worth remembering that in the north west of the Mediterranean (area considered uncontaminated) background noise of copper equals the value of 15,3µg /g dry wt [21]. The levels of copper found in the two sampled stations are within the range of concentrations reported at the French coast (7.5 to 22.9 µg/g dry wt by Luy *et al*, [26]; which would suggest that the two stations can be considered of having a relatively low level of copper contamination.

The concentrations of measured metallic elements were higher in the S2 station, because it is close to urban centers. Regarding the station S1, an increase in the concentration of Zn is noticed in the summer.

The concentration of four assayed metal elements is high in winter compared to summer. Our results are supported by those of Schlacher-Hoenlinger and Schlacher [27] and Abdenour *et al*., [28] who cited that metallic pollution of water and sediments in the Gulf of Annaba is relatively higher during the winter compared to other seasons.

These authors explained that by the fact that at this time of year, storms act as a major restructuring force on seagrass by changing significantly the manner of granulometric distribution of sediments; this increases the rate of resuspension of sediments that can lead to higher rates of remobilization and consequently, a significant bioavailability of metals in sediments and pore water. We also note that the seasonal effect influences significantly the rate of Zn in the roots and rhizomes and on the rate of Cr in mature leaves (see Tab.1).

Variation between Sections of the plant:

Assayed MTE are present at very different levels of concentrations between compartments of *P. oceanica*. In this preliminary study, we note that the concentration of Zn is higher in the leaves than in the roots and rhizomes of *Posidonia* harvested in the station of Ain-Achir; this difference is shown in the work of Warnau *et al*., [23]. Variance analysis shows that the station effect influences significantly on the rate of Zn and Cu in the roots and rhizomes (see Tab.1).

Meanwhile, Copper is more present in the seagrass rhizomes of the two stations as reported by Campanella *et al*., [21]. The variation of this element follows a descending order: Rh> Ra>Fa; This result is the opposite of that found in a Warnau *et al*., [23] who noted that copper is present in the leaves more than in other parts of the plant. However, Cu and Zn are essential for the growth and metabolic processes in plants, and their accumulation is affected by metabolic regulation processes [29].

The results of our statistical analysis show the existence of a highly significant variation of Cu and Cr in the different compartments of *Posidonia*. These results are supported by those of Conti *et al*., [16] who reported that the Cu and Cr are negatively correlated, and that the concentration of Zn is independent from the concentrations of Cu and Cr. According to Lafabrie *et al*., [30], Ni is an element that accumulates preferentially in leaf blades of *P. oceanica* compared to the rest of the plant's sections. Regarding The Ni content identified in this study, a higher accumulation of this element is noticed in the mature leaves of both stations and during the two seasons.

Conclusion:

The distribution of metals in *P. oceanica* is very important for coastal waters monitoring since the species is located at the base of the food chain in the Mediterranean, and is probably the main source of metals for many animals that graze its leaves. It is clear, according to this study, that the assayed metallic trace elements in seagrass inhabiting the two sites show different behaviors illustrated by the existence of a highly significant difference in the content of each element from one section to another; from a station to another and from one season to another.

These differences are explained by the environmental conditions under which lies the *Posidonia seagrass*. Anthropization of the S2 station explains the high concentrations of MTE assayed at this location. While the winter contents are linked to weather conditions that cause disturbances of the plant's environment.

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