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# Trace metal Biomonitoring of algae (*Ulva lactuca*), and Mollusks (*Patella caerulea* ; *Stramonita haemastoma* ; *Phorcus turbinatus*) along the Eastarn-Algerian coast.

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## Abstract

A total of six trace metal elements (Cd, Cr, Cu, Pb, Zn and Ni) were analyzed in the green algae *Ulva lactuca* (Linnaeus, 1753) and in the tissues of three gastropods: *Stramonita hemastomas* (Linnaeus, 1766), *Phorcus turbinatus* (Born 1778) and the limpet *Patella caerulea* (Linnaeus, 1758)sampled from four stations located along the coastal areas of eastern Algeria (North-Eastern Mediterranean Sea). Data were processed through multivariate approaches such as Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) on PCA factors. The results obtained showed a very het- erogeneous distribution of pollutants. Highly significant correlations were determined for the different species studied, depending on heavy metals, stations and seasons. In green algae, *U. lactuca* has been associated with higher levels of Ni, Cr and Zn in the sampling station 'St.T', while an accumulation of Pb and Cu was detected in St.2. Cr was significantly higher in St.3. In winter and spring, mol- lusks had the highest contamination levels. Monodonta and Patella in the stations St.1, St.2 and St.3 showed higher contents in Ni, Zn and Cu, respectively. In addition, Thais and Patella in stations St.T and St.3had a higher contamination with Pb, Crand Cd, respectively. Statistical comparison was performed using the non-parametric Kruskall Wallis and Mann-Whitney tests. This work confirms that *U. lactuca*, *S. haemastoma*, *P. turbinatus* and *P. caerulea* are good biomonitoring tools of trace metal contamination in Mediterranean marine areas

***Keywords****: Algae, Mollusks, Biomonitoring, PCA and LDA, Eastern Coast of Algeria.*

## 1. Introduction

Much attention has been paid to the use of marine organisms as bio-indicators for trace metal pollution in sea waters. Among these, macroalgae and mollusks are the most prominent, as they takeup and accumulate dissolved and particulate metals from seawater [1- 2]. Metal accumulation in marine seaweeds operates via passive and active mechanisms. These processes also involve ion exchange mechanisms by interacting with surface polysaccharides and metal sequestration within vacuoles rich in polar organic compounds [3-4]. Macroalgae can accumulate trace metals thou- sands of times higher than the lower trophic base levels of aquatic food chaine [5] . Algae bind only soluble metal ion, whose concentrations de- pend on the nature of suspended particulate matter, as both organic and inorganic complexes can be present [6-4] . Sea lettuce, *U. lactuca*, is a species of green marine algae, widely used in biomonitoring studies in the Mediterranean to evaluate its usefulness as a bioindicator of trace metals [7-13] . Recently, researchers have also identified other bioindicators of trace metal pollution in Mediterranean areas, such as the gastropod mollusks *Stramonita heamstoma*, *Phorcus turbinatus* (Born 1778) and *Patella caerulea* (Linnaeus 1758). These species are herbivorous and constitute the second link in the trophic chain. These gastropods often ingest epiphytic organ- isms, which live on aquatic algae and capture metals mainly from the plant matrix [14-17] . *Phorcus turbinatus*, commonly labelled as the turbinate monodont, is a marine gastropod mollusk from the family of Trochidae, the top snails. This species lives on the rocky sea eds of the intertidal belt, tideland, as deep as 5–10 m. It is very tolerant to salinity and high temperatures and can survive out of the water for several hours. *Patella caerulea* is a species of limpet form the family Patellidae. It is known by the common names Mediterranean limpet and rayed Mediterranean limpet. It is native to the Mediterranean Sea lives on rocky substrata of tidelands and can also survive for long time outside the water.It is only found in the Mediterranean, where it is a popular food [14-16] . *Stramonita hemastoma* (Thais) has been the subject of several studies as a bioindicator of pollution by tributylt in (phenomenon of imposex) ([18-20]. In Algeria, commonly known as Bakouma, this purple muricidae was used as a bio-indicator of metal pollution with an eco- toxicological profile [16]. The main objective of this study was to ob tain more information on the possible use of the species selected during this work as bio-indicators of metallic pollution all along the East-Algerian coasts. The second objective was to classify all the sampling stations according to the metal bioaccumulation degree in the species studied during the sampling period.

## 1. Materials and methods

***Sampling and Sample Pre-treatment***

Heavy metal contamination levels of Cd, Cr, Cu, Zn, Pb and Ni in seaweed *U. lactuca*, limpet (*Patella caerulea*), whelk (*Stramonita haemastoma*), and snail (*Phorcus turbinatus*). Samples collected from the eastern coasts of Algeria (**Fig** ure 1). To compare the spatio-seasonal variations, four different localities were chosen for the present study( Jan 11- feb19). Sampling station one (St.1) is El Djerda island, with a fishing harbor where anthropogenic wastes are released by local fishermen. Station two (St.2) is located at about 70 Km upstream from the Gulf of Skikda, where chemical wastes are released by a petroleum treatment plant. Station three (St.3) is located at about 200 km away from the Gulf of Annaba, also including anthropogenic wastes. The fourth is St. T, the control station located at about 80 km from the Chétaibi Bay. Algae pretreatment was performed by rinsing with filtered seawater and freeze storage in polyethylene bags at -20°C. Subsequent algae analysis only concerned mature thalli. Specimens were then pooled, divided into amounts of 0.7 g (dry weight) and

mineralized in a microwave oven (MDS 2000; CEM, Italy) with ultrapure$HNO_3$ and $H_2O_2$ (6 + 2 mL; Merck, Germany) [21]. Twenty adult Monodonta, Patella and purple individuals sized 30 to 40 mm we recollected manually in the intertidal-supralittoral zone (30dept). Purification was done by immersion in filtered seawater (of the corresponding station) for 24 h [22- 23] . After that, the soft parts were extracted from the shell using a hammer and a spatula in plastic, in order to avoid metal contamination, and then rinsed with deionized MilliQ water. Then samples were finally kept in polyethylene bags and deep-frozen. Mollusks were digested in the microwave oven and analyzed individually [21]. All microwave mineralization followed a similar procedure as for algae. Separate dry-weight (dw) determinations were performed on different biota by oven-drying at 105°C to a constant weight (20 replicates for each species). All chemicals used for treatments were ultra pure grade. Water for solution preparation and sample cleaning was obtained from a Millipore Milli-Q system. Glassware cleaning before use was performed by soaking in 10% $HNO_3$ for 24 h and rinsing with Milli-Q water.

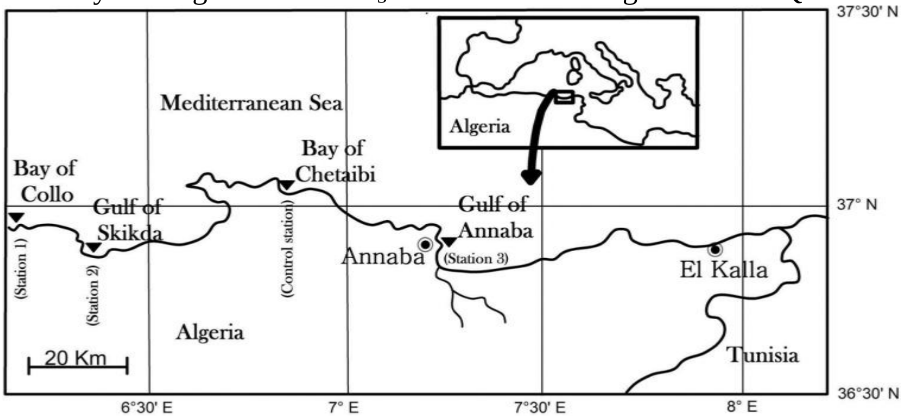


**Fig** .1. Map of the Four selected sampling sites.

***Trace metal***

Trace metal concentrations in the samples were evaluated using an appropriate method to each element. A Perkin Elmer Analyst 300 flame (FAAS) atomic absorption spectrometer served to measure Cu and Zn and a Graphite furnace atomic absorption spectroscopy (GFAAS)800 HGA with auto sampler for Cd, Cr and Pb. The matrix modifiers for the determination of Cd and Pb were phosphate mono ammonium $NH_4H_2PO4$ (10%) and the phosphate PO4 (0.2 mg), respec- tively. For Cr measurement, we used magnesium nitrates Mg $(NO_3)_2$.

**Table** 1: Limits of detection (LoD)[a] and precision (CV)[b] for analyses performed by the two techniques used.

| Metal | Algae | | Mollusks | |
|---|---|---|---|---|
| | LoD (pg $g^{-1}$ d.w.) | CV | LoD (pg $g^{-1}$ d.w.) | CV |
| Cd | 0,04 | 2,7 | 0,05 | 4,3 |
| Cr | 0,71 | 3,9 | 0,94 | 3,6 |
| Cu | 0,006[c] | 4,5 | 0,01[c] | 4,5 |
| Pb | 0,07 | 2,0 | 0,09 | 1,4 |
| Zn | 0.13[c] | 2,5 | 0.09[c] | 2,5 |

*d.w: dry weight; [a] Calculated on the basis of 10 determinations of blanks as three limits the standard deviation of the blank ; [b] Percentage referred 10/ 10 de1ennina1ions prefund on the same sample*

The whole analytical procedure was checked for accuracy by certified reference materials: CRM 279 (sea lettuce) and ERM CE 278 (mussel tis- sue). The analytical performances of the techniques used in terms of limits of detection (LoDs), precision, and accuracy are reported in **Table** 1 and **Table** 2 .

**Table** 2 : Analysis of certified reference materials (CRMs; mean ± SD)[a]

| Me1al | CRM 279 (sea lettuce) (µg $g^{-1}$ d.w.) | | ERM-CE 278 (mussel tissue) (µg g-1 d.w.) | |
|---|---|---|---|---|
| | Certified | Found | Certified | Found |
| Cd | 0.274 ± 0.022 | 0.272 ± 0.031 | 0.3 ± 0.007 | 0.34 ± 0.011 |
| Cr | (10.7)[b] | 10.75 ± 0.64 | 0.7 ± 0.06 | o.79 ± 0.03 |
| Cu | 13.14 ± 0.37 | 12.54 ± 0.59 | 9.4 ± 0.13 | 9.40 ± 0.21 |
| Pb | 13.48 ± 0.36 | 13.41 ± 0.22 | 2.0 ± 0.04 | 2.10 ± 0.05 |
| Zn | 51.3 ± 1.2 | 53.6 ± 1.5 | 83.1 ± 1.7 | 82.1 ± 1.6 |

*d.w: dry weight; [a] : Number of replicates ;[b]: Not certified values.*

***Statistical analysis***

Data were standardized and analyzed through several techniques such as principal component analysis (PCA) and linear discriminant analysis (LDA) on PCA factors [21-17] . PCA was applied for the mean of the metal concentrations in the algae (*Ulva lactuca*) (first data set) and the Monodonta Patella mollusks and the whelks (second data set), in order to verify possible bioaccumulation patterns and to detect possible contamination levels among sites in the area of study. LDA was applied to discriminate sites and organisms according to their metal content. For a grouping variable with more than two classes, we represented the LDA results showing (a) The structure matrix: correlations between the discriminate variables and the canonical discriminant functions plotted on the correlation circle; and (b) Hierarchical classification (CAH) was carried out to find groups or classes of homogeneous varieties [23- 22]. Data analysis was performed using the XL STAT 2019 package and program Facto Mine R.2.4.1. Extra.

## 2. Results

The metal concentrations (mean SD) in the biota are reported in **figures** 2, 6, 7 and 8.

***Ulva lactuca :***

The lowest concentrations recorded for Cd, Cu, Cr, Pb and Ni were detected in seaweeds harvested at St.T with the exception of a high rate of Zn during the spring (28.91± 2.4 $\mu$g.g-1) (**Fig** . 2). However, the highest Zn concentrations were ob- served in the St.3 and St.2with a maximum value in autumn and in summer (26.20±0.27 $\mu$g.g $^{-1}$, 25.53 ± 0.49 $\mu$g.g $^{-1}$) respectively. Two peaks of Cr characterized St.3 in autumn (23.13 ± 0.02 $\mu$g.g $^{-1}$) and summer (19.65± 0.04 $\mu$g.g $^{-1}$), followed by the St.1 in summer (17.99± 0.3 $\mu$g.g $^{-1}$) (**Fig** . 2). Data for *Ulva lactuca* of the four coastal sites (**Fig** . 2) were submitted to PCA and LDA analyses. Results are reported in **Fig** ure 3 (a, b, and wherein the first two components of the PCA (dim1 * dim2) revealed account for 78.1% of the total variance

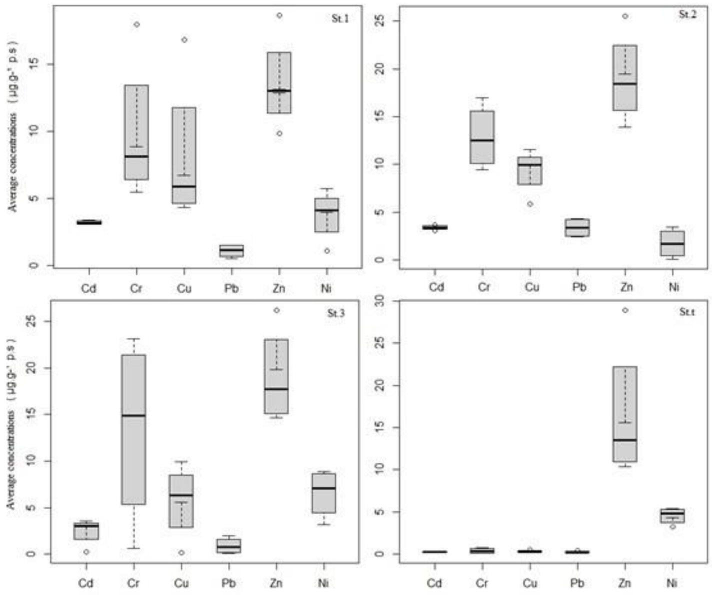

**Fig**.2. Boxplot showing the Spatio-temporal variations of metal contents (Cd, Cr, Cu, Pb, Zn and Ni) in *U. lactuca* in the four stations (St.1: El Djerda beach, St.2: Military beach, St.3: Rizi Amor beach and St.t: Oued El Ganem beach (Jan11-Feb19).

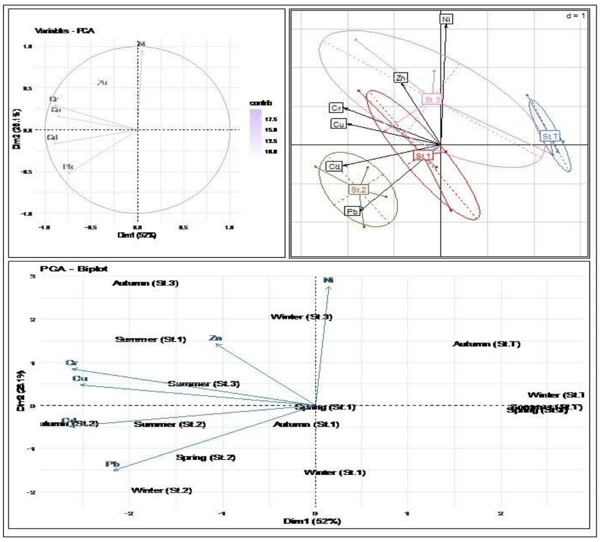

**Fig**.3. LDA of PCA results of sampling station factors (*U. lactuca* data). Left (a): correlations between independent variables and discriminant functions (correlation circle). Right (b): projection of canonical scores with ellipses and gravity center of classes. Down (c): The analysis explained 78% of the total variance (52% and 26.1% for the first and second factors, respectively).

Hierarchical class analysis (CAH) revealed three groups of macroalgae **Fig** 4. The first was loaded with Ni and Zn, the second with Pb, and the third with Cd, Cr, and Pb. The analysis clearly showed that Chetabi Bay (Wadi El Ganem Beach: St.T) was not considerably different from the other stations, contrary to what we expected.

The green alga species variations according to stations and seasons were compared through cluster analysis **Fig** 4. Results allowed us to identify four different trends: (i) Ni was higher in the St.T site (winter and summer) than in St.3 (spring) and St.T (autumn and spring). (ii) This additional study also showed significant differences between Pb and Cu accumulation for St.2, (iii) bioaccumulation being higher in winter and summer

respectively.(iv) In winter St.1 had the highest levels of Cr. In St.3, the Zn and Cr were significantly higher in autumn and summer.

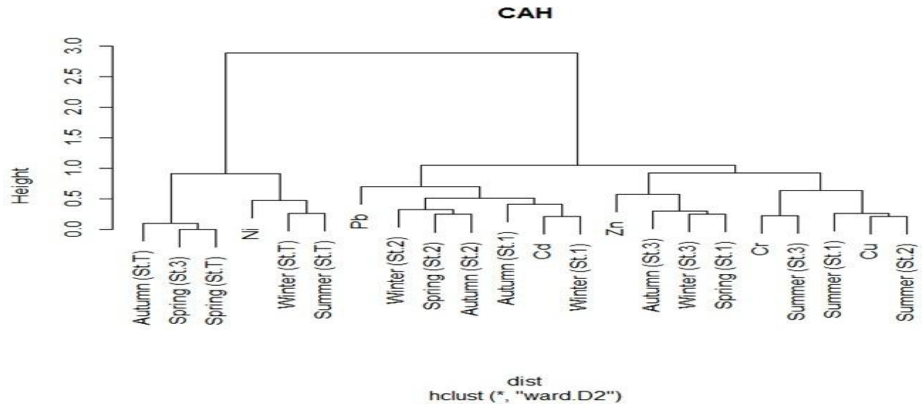


**Fig . 4.** Classification of sample sites and Macroalgae data (cluster analysis) associate with the heavy metals and sample seasons data.

***Patella, Stramonita and Phorcus***

***Patella***

Chromium recorded the lowest metal concentrations in St.3 in autumn (0.01 ±0.4 $\mu$g.g-1), and St.T in winter (0.05 ± 1.01 $\mu g.g^{-1}$). The highest concentrations of Pb were observed in limpets found in stations St.1 and St.2 (28.49 ± 0.26 $\mu g.g^{-1}$ in autumn; 0.54 $\mu g.g^{-1}$ in spring, respectively ), while Zn values were the most important in limpets from St.3 in winter (44.995 ±1.34 $\mu$g.g- 1), in St.2 in spring (37.77 ± 0.58 $\mu$g.g-1) and in St.1 in autumn (36.86 ± 0.24 $\mu$g.g-1) **Fig** 5.

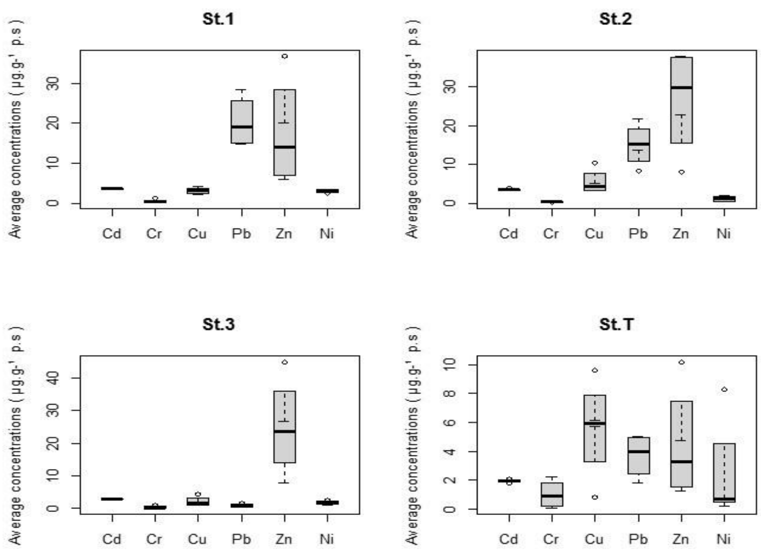


**Fig** .5. Boxplot showing the Spatio-temporal variations of the metallic contents (Cd, Cr, Cu, Pb, Zn and Ni) in *P. caeruela* in the four stations (St.1: El Djerda beach, St. 2: Military beach, St.3: Rizi Amor beach and St.t: Oued El Ganem beach. (Jan11-Feb19).

***Thais (Stramonita haemastoma)***

A summer peak of Cu was registered in whelks from St.3 (87.2 ± 1.01 $\mu g.g^{-1}$) and from St.1 (16.25 ±0.35 $\mu g.g^{-1}$). In autumn, we collected copper-loaded purple from St.2 (15.96 ± 0.97 $\mu g.g^{-1}$). As shown in

Fig 6, values of Zn increased in autumn and winter in thais at St.3 (53.543±1.02 µg.g$^{-1}$, 50.563±0.9 µg.g$^{-1}$), St.1 (51.974± 0.56 µg g$^{-1}$; 40.317 ± 0.57 µg.g$^{-1}$) and St.2 (39.694±0.66 µg.g $^{-1}$, 34.085± 0.57 µg.g $^{-1}$), respectively. Despite the heterogeneous metal distribution results, Zn and Cu were very widely bioaccumulated in Thais.

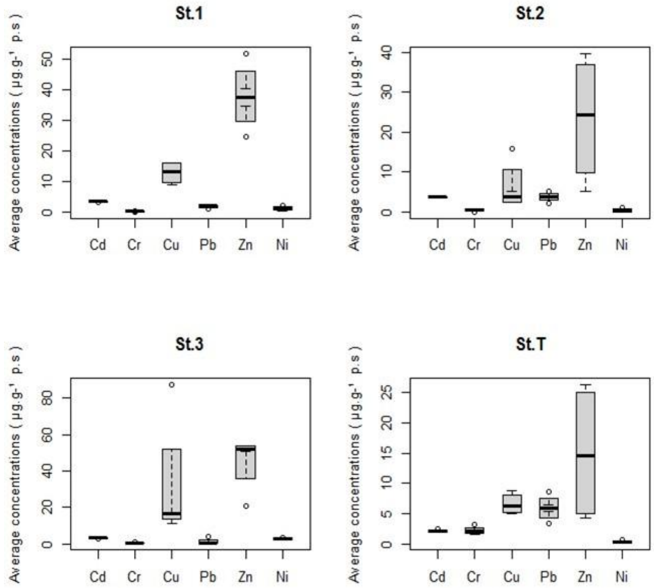


**Fig** .6. Boxplot showing the Spatio-temporal variations of the metallic contents (Cd, Cr, Cu, Pb, Zn and Ni) in *S. stramonita* in the four stations (St.1: El Djerda beach, St. 2: Military beach, St.3: Rizi Amor beach and St.t: Oued El Ganem beach. (Jan11-Feb19).

***Phorcus turbinatus***

A heterogeneous spatiotemporal variation of metal concentration found in *P. turbinatus* through Boxplots (Box and whiskers plots) is displayed in **Fig** 7 . The lowest concentrations recorded for Cd, Cu, Zn, Cr, Pb and Ni were at St.T and St.2 (The highest concentrations for these elements were observed at St). Data for mollusks for the coastal sites were submitted to PCA and LDA.

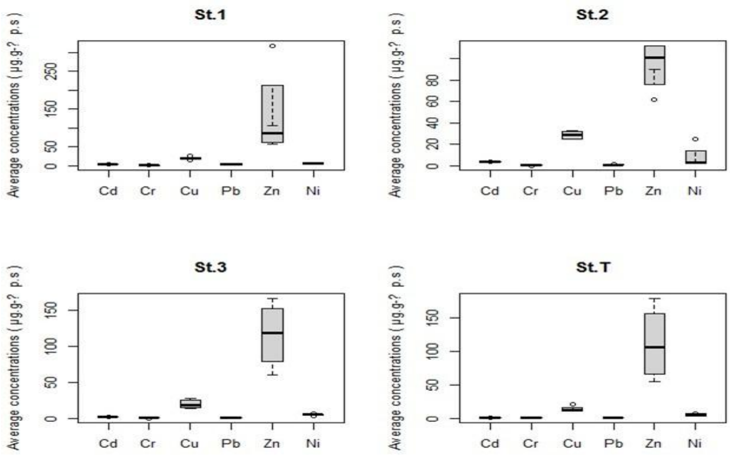


**Fig** .7. Boxplot showing the Spatio-temporal variations of the metal contents (Cd, Cr, Cu, Pb,Zn and Ni) in *P. turbinatus* in the four stations (St.1: El Djerda beach,St. 2: Military beach, St.3: Rizi Amor beach and St.t: Oued El Ganem beach (Jan11-Feb19).

Results are reported in **Figs**. 8 and 9. The factor loadings are reported in **Fig** 8; the first two PCA components explain 72.09% of the total variance. This study shows that (i) : Cr, Ni and Pb are positively correlated, (ii) :Zn is positively correlated with Ni, and (iii) :Cu, Cr and Cd are not correlated. PCA data for heavy's metals and species are reported in **Fig** 8.

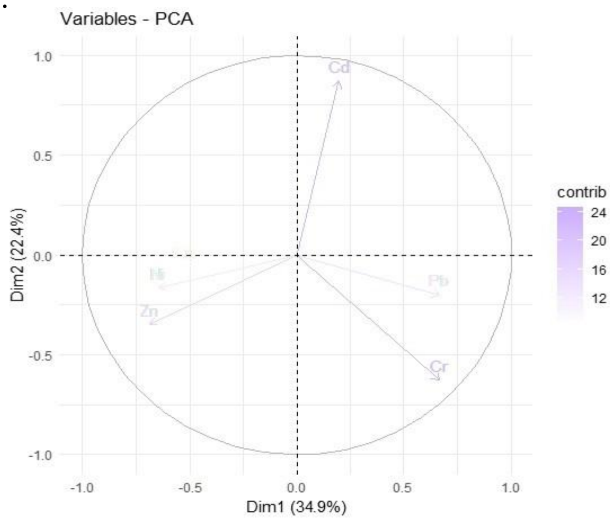


**Fig** . 8. Canonical graph of factor loadings of PCA (correlation circle) for Molluscs data.

The classification of the sampling stations according to metal concentrations in mollusks shows a visible spatio-temporal distribution pattern: (i) : Cd define the spatial distribution of St.1 and St.2, (ii) : high levels of Cu, Ni and Zn, at St.3 and (iii) : Cr and Pb characterize the St.T.

Analogously, by comparing with PCA applied to species and seasons **Fig** 9, we see that (i) Patella and *Thais haemastoma* species have high concentrations of Cd, Pb and Cr and (ii) Phorcus species have high levels of Zn, Ni and Cu. All the species studied revealed that major bioaccumulation occurs in autumn . From this study, we can draw additional conclusions: (i) St.1 and St.2 (Djerda peninsula and Skikda Gulf) are strongly associated with Cd concentrations in limpet and Thais; (ii) St.3 (the Gulf of Annaba) is characterized by concentrations of Cu, Ni and Zn in Phorcus; and (iii) St.T (Chétaibi Bay) is influenced by Cr concentrations in the limpet and Thais.

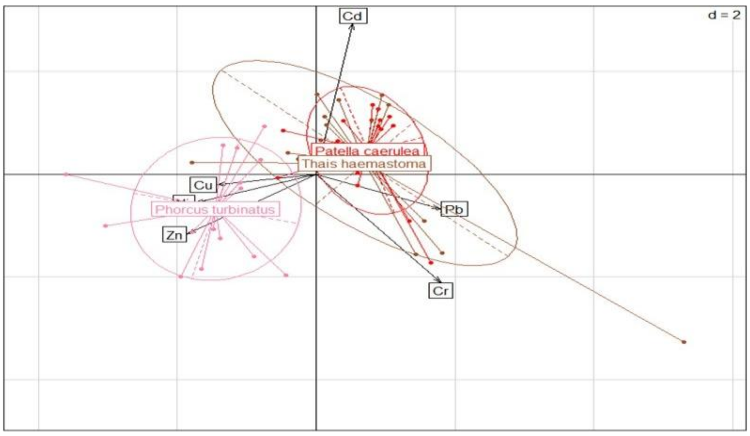


**Fig** . 9. Plot of factor scores on the factorial plane. Projection of the rows with ellipses and the gravity center of Molusck's data are reported from sample sites.

## 3. Discussion

As a first step in the analysis of heavy metals in algae (**Fig.1**) (*Ulva lactuca*), the accumulation sequence was St.1 : Zn >Cr> Cu >Cd> Ni >Pb ; St.2 : Zn> Cr> Cu >Cd> Pb> Ni ; S.T3: Zn> Cr> Cu> Ni >Cd> Pb and St.T : Zn> Ni >Cd ≥ Cr ≥ Cu ≥ Pb . Those orders are in agreement with findings in *U. lactuca* from Coasts of Algeria [24-25] . In Chlorophyta from coastal waters of Yemen, the Gulf of Aden [26], also, those sequences are slightly different from those in previous studies from Mediterranean sea, with Cd and Cr being inverted [27-29]. This is consistent with recent findings suggesting that the accumulation of several trace elements in seaweeds is closely related to thallus morphology and growth strategy. Filamentous and sheet-like macroalgae, exhibiting a high surface area for element absorption, have been reported to accumulate higher contents of several elements than coarsely branched and thick-leathery macroalgae [30]. A reverse pat- tern could be attributed to the fact that those elements Cd and Cr accumulation might not be closely related to thallus morphology and growth strategy, but to species phylogeny [30].The values of Kruskal-Wallis and the analysis of variance in two-ways (stations, season) reveals a very highly significant ($p< 0.001$) station effect, and very highly significant station-season interaction ($p<0.001$). This relative absorption of metals in *U. lactuca* was lower than that found in the same species from Hong Kong Island [31] and in *U. fenestrata* from Peter the Great Bay, Japan [32] . There are several attributing factors for such differences in metal accumulation by macro algae, including life span, morphology, contact surface area, growth rate and specific affinity for selective metals by particular species [33] . These positive correlations may be explained by a common origin for the elements or by synergic interaction among them [31-35]. The negative correlations between pairs of metals in macro algae may be due to different origins, to environmental behavior, or to competition for uptake sites [35] . During this study, mean levels of Cr and Zn in *U. lactuca* collected at St.T and St.2 were respectively 0.4 ± 0.77 and 19.08± 0.64 $\mu$g.g-1 dry weight (**Table** 4). Our results are similar to those found in Cystoseira of Linosa Island, Sicily [4] and they are higher than the rate found in *U. rigida* of the lagoon of Venice, Italy [28] and at Cystoseira of the island of Ustica, Italy [36] . High concentrations of Zn in *U. lactuca* were observed in autumn (St.T: 28.91± 2.4, St.3: 26.20 ± 0.27, and St.2: 25.54 ± 0.49 $\mu g.g^{-1}$ in dry weight). A similar pattern was observed in Ulva in coastal areas of the eastern Aegean Sea, but harvested in spring and summer [25] and in *U. Fenestrata* from Great Bay, Sea of Japan [32]. In addition, our results are much higher than those previously found in *U. lactuca* from Algerian coasts [37-24].

Overall, the data in the literature indicate that heavy metal accumulation in *U. lactuca* follows the order Zn> Cu>Cd [13, 37-38]. In addition, most *U. lactuca* had higher Zn concentrations than those found in Phaeophyta and Rhodophyta [33,39-40] .The high mean levels of Cd were found in *U. lactuca* from ST.2 (3.58 ± 0.20 $\mu g.g^{-1}$ dry weight). Similar data have been found in Chlorophyta from the Gulf of Aden, Yemen [26]. in the other hand, our results were much higher than those obtained in *U. lactuca* from Egypt [41- 43] , from the Atlantic coast of south-west Spain and Portugal [44] and from Kavala harbor (Greece) [34] . In addition, our results were higher in *U. Rigida* from Great Bay, Japan [32] and from the Gulf of Thessaloniki, Greece [30]. The average Cr concentrations were observed in autumn at St.3 (13.38 0.32 $\mu g.g^{-1}$ dry weight). Similar findings were reported in Ulva from Greece by [30] , from the Venice lagoon [28] and in *E. compressa* in the Gulf of Aden, Yemen [26] . The maximum Cu levels in Ulva at St.2 (9.32 ± 0.67 $\mu g.g^{-1}$ d.w) closely resemble the values observed for the same species from the Gulf of Suez, Red Sea [33-43] , and in *U. fenestrata* from Great Bay, Japan. Moreover, our results are similar to those found in *U.rigida* from the Venice Lagoon [28] they are superior to the values found in Ulva from the Algerian coast, the Honaïne beach [24] and Rabta Bay, Jijel [37] , Tyrrhenian coast [36] and Turkey coast [45] . However, our levels were lower than those in Ulva harvested from

Venice lagoon [28] and those from the Thermaikos Gulf [34]. Indeed, during this study, maximum Cu and Cd levels in *U. lactuca* were detected in the Gulf of Skikda. This is probably due to the mediocre environmental condition of these beaches seen their proximity to a source of diffuse pollution continuum of petrochemical origin. The maximum accumulation of Ni was observed in *U. lactuca* from St.3 (5.76 ± 0.20 $\mu g.g^{-1}$ dry weight). Our investigations are similar to those reported in the same species by [46] from Qatar, by [28] from the Venice Lagoon and by [34] Hari- tonidis and Malea, 1995) from the Gulf of Thermaikos. In addition, the Ni values in this study were higher than those found in previous studies of algae from the coast of Turkey ([47] and from the Gulf of Kutch, India [48] . In addition, our rates are lower than the results found in most algae of different biotopes from the Aegean Sea [49]. The average maximum value of lead in *U. lactuca* was 3.35 0.30 $\mu g.g^{-1}$ dry weight in St.2. The rates in this work are lower compared to those reported by [28] from the Venice Lagoon, by [34] and from the Thermaikos Gulf, by [33] from Egypt, by [50] along the Kuwaiti coast, by [51] from Alexandria and by [31] from Hong Kong. Results of the present investigation are in accor- dance with those reported by [32] in *Ulva fenestrata* from Peter the Great Bay, Sea of Japan and by [26] in *E. compressa* from the Gulf of Aden, Yemen. In this study, maxima of Ni, Pb, Cu and Cr were observed during winter and summer. However, the minima were found in spring. The observed seasonal differences may be due to several factors, including environmental factors (variations in metal concentrations in solution, inter- actions between metals and other elements, salinity, pH, etc.), metabolic factors (such as the dilution of metal content due to growth, or they may be due to interactions between the two types of factors mentioned above [52] . [34] Attributed the seasonal pattern of concentrations of several metals in *Ulva lactuca* to growth mechanisms. In macroalgae, bioaccumulation decreases during periods of growth and increases during the winter period [52] .The results obtained in winter differ from other seasons for all metals except Cr and Zn in Ulva (Tukey HSD post hoc test). This finding is in agreement with those obtained in *U. lactuca* in the coastal areas of the eastern Aegean [25] and in *U. rigida* on the delta coast, Evros [53]. With regard to Zn in *U. lactuca*, the gradual decrease in tissue contents during late summer to mid-autumn could be explained by a potential in- crease in growth rate induced by a decline in water temperature (from 27 to 18°C). The same result was found by [30] in *U.rigida*. Optimal growth of Green algae ulvacae in Mediterranean coastal areas has been observed at water temperatures between 12 and 23◦C, while temperatures higher than 24◦C are responsible for a halt in growth during summer [52]. In autumn, despite favorable temperatures, growth of this opportunistic ephemeral seaweed might have been moderated due to low nutrient availability [54] . Most element contents in this seaweed displayed no distinct seasonal trend from summer to early spring. This could be at least partly explained by a comparatively low variation in growth rate during this particular period of the year. [55] Suggested that seasonal variation in fluvial and terrestrial inputs may have induced marked changes in element load in the seawater/sediment which potentially influenced the seasonality of seaweed element contents. Tissue element concentrations generally showed a clear seasonal pattern, mainly characterized by a decrease during spring and/or summer with in- creasing water temperature and solar irradiance, which suggests that tissue element seasonality is markedly associated with the seasonal growth pattern of the macroalgae. Higher growth rates during spring and/ or summer induced from higher temperature and light conditions may have diluted the accumulated elements and, thus, reduced their concentrations. Therefore, in accordance with previous findings [52- 54] , the present data suggest that the relation- ships between trace element concentrations in sea- weed tissues are a function of environmental variables affecting seaweed growth, and this interferes with the use of macroalgae as biomonitors of trace element contamination.

***Spatio-temporal variation***

In this study, maxima of Ni, Pb, Cu and Cr were observed during winter and sum- mer.However,the minima were found in spring. The observed seasonal differences may be due to several factors, including environmental factors (variations in metal concentrations in solution, interactions between metals and other elements, salinity, pH, etc.), metabolic factors (such as the dilution of metal content due to growth, or they may be due to interactions between the two types of factors mentioned above) [52] .([34] , attributed the seasonal pattern of concentrations of several metals in *Ulva lactuca* to growth mechanisms. In macroalgae, bioaccumulation decreases duringperiods of growth and increases during the winter period [52] . The results obtained in winter differ from other seasons for all metals except Cr and Zn in Ulva (Tukey HSD post hoc test). This finding is in agreement with those obtained in *U. lactuca* in the coastal areas of the and in *U. rigida* on the delta coast, [53]egard to Zn in *U. lactuca,* the gradual decrease in tissue contents during late summer to mid-autumn could be explained by a potential increase in growth rate induced by a de- cline in water temperature (from 27 to 18°C). The same result was found by [30] in *U.rigida*. Optimal growth of Green algae ulvacae in Mediterranean coastal areas has been observed at water temperatures between 12 and 23°C, while temperatures higher than 24°C are responsible for a halt in growth during summer [52] . In autumn, despite favorable temperatures, growth of this opportunistic ephemeral seaweed might have been moderated due to low nutrient availability [54]. Most element contents in this seaweed displayed no distinct seasonal trend from summer to early spring. This could be at least partly explained by a comparatively low variation in growth rate during this particular period of the year. [55] suggested that seasonal variation in fluvial and terrestrial inputs may have induced marked changes in element load in the seawater/sediment which potentially influenced the seasonality of seaweed element contents.Tissue element concentrations generally showed a clear seasonal pattern, mainly characterized by a decrease during spring and/or summer with in- creasing water temperature and solar irradiance, which suggests that tissue element seasonality is markedly associated with the seasonal growth pattern of the macroalgae.Higher growth rates during spring and/ or summer induced from higher temperature and light conditions may have diluted the accumulated elements and, thus, reduced their concentrations. Therefore, in accordance with previous findings [52-54] , the present data suggest that the relationships between trace element concentrations in seaweed tissues are a function of environmental variables affecting seaweed growth, and this interferes with the use of macroalgae as biomonitors of trace element contamination.

***Pattern of metal occurrences in mollusks***

In the present study, metal bioaccumulation in *S. haemastoma* was as follows: St.1: Zn> Cu> Cd> Pb> Ni> Cr; St.2: Zn> Cu> Pb> Cd> Ni> Cr; St .3: Zn> Cu> Ni Pb Cd> Cr; St.T: Zn> Cu Pb> Cr> Cd> Ni). This distribution is in agreement with that found in *T. clavegera* from the East Coast-Malaysia [56] . Also, the essential metals such as Zn, and Cu showed the highest accumulation rates over non-essential metals Cd, Cr, Ni and Pb. This is expected as in soft tissue, these metals have been acknowledged as biologically essential for metabolism, [16]; [56] play- ing a vital role as co-factors in enzymatic processes [16-57] and a useful part of the respiration al protein haemocyanin [16-58]. Moreover, these metals cannot be instantly excreted or detoxified as they are required to play roles in metabolism. Non-essential metals are among less accumulated metals as they are not needed in metabolism and would have no prerequisite minimum concentration. However, they are taken up by all organisms in proportion to the degree of environmental contamination levels. The existence of Cu suggests the presence of haemocyanin, an oxygen-binding protein, as a major respiratory pigment. Confirmation has been found that *S.*

*haemastoma* and *T. clavigera* have haemocyanin [16- 59]. High Cu concentration supports the hypothesis of the presence of such respiratory pigments. It has been discovered that Cu and Zn are selectively localized in the tissue of *S. haemastoma*, the same results were found in *T. clavegera* from the East Coast of Malaysian peninsula [56] . The highest concentration of Zn was reached in hepatopancreas and gills while the highest Cu concentration was in the liver and hemocyanin in blood [16- 60]. Blakmore [59] , suggesting that *T. clavigera*, when exposed to low dissolved oxygen concentrations, may yield more hemocyanin to increase respiratory efficiency and consequently lead to increase in body concentration of Cu. Some authors supported the hypothesis linking genital malformations to the presence of organotin compounds and heavy metals in the aquatic environment [61]. Such selectivity could ex- plain the significance of Zn and Cu concentrations within soft tissues of *S. heamastoma* although specific tissues were analyzed. Bouzahouane [16] showed that the decrease in GSH levels from *S. heamastoma* clearly indicates the stress of GSH dependent detoxification pathways aimed at reducing peroxides to non-toxic and water-soluble primary alcohols. GSH is key in metal scavenging in the organism due to the high affinity of metals to its (eSH) group [62] .Also, CAT activity in *S. haemastoma* showed relatively the same profile as GST, with a significant increase in spring. The CAT activity induction can be attributed to higher levels of exogenous hydrogen peroxide, which is the major cellular precursor of the toxic hydroxyl radical (OH) [16] . According to Bouzahouane [16], the presence of large amounts of pollutants can overwhelm the antioxidant system, which caused lipid peroxidation of cell membranes by a high MDA level in *S. haemastom*a at a site exposed to industrial pollution in the Gulf of Annaba. The increase in MDA concentrations, a potency marker of the lipid peroxidation, can be explained by heavy metal contamination, which causes the overproduction of free radicals in the cell. For *P. caerulea*, metal concentrations increased in the following order: St.1 : Zn >Pb >Cu >Cd> Ni> Cr ; St.2: Zn> Pb >Cu> Ni> Cd> Cr ; St.3 Zn> Cu >Ni> Cd> Cr Pb and St.T: Zn >Cu> Ni >Pb> Cr Cd. This bioaccumulation pattern agrees with those of previous studies in the Gulf of Gaeta and Ustica Island, Italy [21] and disagrees slightly with those for Favignana Island [27]. Metal rates in mollusks studied through the scientific literature in the Mediterranean. Such a comparison should also provide more useful information on the degree of contamination of the St.T marine ecosystem (reference station). Generally, the Pb, Zn and Cu concentrations in limpets from Chétaibi Bay were not comparable to those reported by other researchers in clean coastal zones; this is due in particular to the amplitude of these metals (Pb, Zn and Cu) considered to be significantly higher compared to published reference levels for unpolluted Mediterranean coasts [4]; [10-13] . The presence of Pb, Zn and Cu concentrations in samples of limpet from St.1,St.2 and St.3 should be attributed to geological and mineralogical conditions of the marine environment, and they can be directly related to the contamination of the marine environment due to anthropogenic and industrial activities through metal contamination of sediment. This indicates that a significant part of these metals is readily available for limpets from Greece [63]. The mechanisms of absorption and assimilation of metals by limpets depend on much of the dis- solved form of metals and the metal bioavailability in green algae feeding limpets. This is in harmony with the work of [64] with *Patella piperata* in Canary Islands, Spain. In this work, metal concentrations in *P. turbinatus* showed high levels of Zn and Cu. Zinc is essential for normal growth and metabolism of animals exposed to strong accumulation in *P.turbinatus* (dry weight) in comparison with the other four metals. The high concentration of Zn is associated with the activity of enzymes that play a role in the enzymatic process in aquatic invertebrates.[65- 66]. Copper is an essential trace metal for the metabolism of animals, but at high levels, it is extremely toxic to aquatic life. [24- 35] reported that aquatic invertebrates usually regulate their body

concentrations of essential elements Zn, Cu at almost constant non-toxic levels. However, they consider that the non-essential elements Cd, Cr, Pb and Ni are always toxic irrespective of their accumulation level.

Chromium and Nickel are considered priority pollutants, due to their toxicities to aquatic organisms [2]. Cadmium is extremely dangerous. It acts as a poison, and may affect the blood pres- sure, the kidneys and destroy the red blood cells [67]. Lead is a neurotoxin that causes behavioral deficits in aquatic organisms and because of the growth rate, declines survival and metabolism. There is often little Pb accumulation in marine and freshwater species [68] . High concentrations may occur in aquatic organisms close to anthropogenic sources. Moreover, in the same genus, in the bay of Bakar (ex-Yugoslavia) [69] have found high levels of Cd (0.20 $ug.g^{-1}$ww) and Pb (0.61 $ug.g^{-1}$ww) in the soft body of *M. articulata*. While in the bays of Larymna, Vavrouna and Larouma (Greece), [70] indicated higher levels in *M. articulata*, *M . mutabilis* and *M. turbinata*. In addition, with the exception of Zn contents, Cd, Cr, Cu and Pb detected by [70] in *M. turbinata* and *M. mutabilis* in Sicily, were in perfect agreement with our results. Similarly, concordance was found for metal concentrations encountered in *M. labio* of Cape Aguilar (Hong Kong) [59]. Our results are also consistent with the work of [71] on *O.turbinatus* in the Bay of Iskenderun (Turkey) for Cr, Cu and Zn. Moreover, in the islands of Linosa and Ustica (Italy), rates of Cr, Cu, Cd, Pb and Zn reported in *M. turbinate*, are slightly lower than our results [36]; [4, 29] . Nickel is among the trace metals often measured in soft body of *M. articulata* and *M. mutabilis*. These levels are generally highly variable among trochus [70]. However, in the Bay of Iskendrum (Turkey), this metalloid was present in *M. turbinata* [71] with higher rates compared to those reported in our study. This element is affected by several factors such as housing, living conditions and the pretreatment and storage of samples after collection [7] .

## *Conclusion*

Statistical results, in particular those of the ACP and LDA, showed that the sequential pat- tern of metallic bioaccumulation (Cr, Cd, Cu, Zn, Pb and Ni) in marine aquatic species (algae and mollusk) all along the sampling stations during an annual cycle is well unambiguously illustrated. Some metal bioaccumulation patterns have been described here, but no one sampling site was more contaminated than the others. In this paper, most of the sequential distributions of heavy metals studied have been described. The latter are significantly correlated with species (algae and mollusks) more than with sampling stations.In green algae, *U. lactuca* was associated with higher levels of Ni in the reference station 'St.T', the maximum of Pb and Cu were observed in the Gulf of Skikda (St.2), and in Collo Bay (St.1). The Cr dominated the green lettuce of the Gulf of Annaba (St.3).For mollusks, Monodonta and Patella revealed that the Skikda Gulf, Collo Bay, and Annaba Gulf stations (St.2, St.1 and St.3,respectively)had higher metal contamination (Ni , Zn and Cu) in winter and spring. Thais and Patella indicated that Chetaibi Bay (St.T) and Annaba Gulf (St.3) had higher metal contamination (Pb, Cr and Cd, respectively) in winter and spring. The simultaneous use of several bioindicators in scientific biomonitoring studies is based on the possibility of obtaining more information on the different bioaccumulation patterns possible in the selected ecosystem. For *U. lactuca*, *P. caerulea*, *S. heamastoma* and *P. turbinatus*, this study confirms their consid- erable potential as cosmopolitan biomonitors of trace metals in the Mediterranean marine ecosys- tem. *U. lactuca* is at the base of the food web and is probably one of the major sources of trace metal contamination for many aquatic rocky inverte- brate grazers.

The selected species possess the necessary qual- ities to be good bioindcators: *U. lactuca* proved to be the most powerful Zn accumulator; *P. caerulea* was the most robust accumulator of cadmium; *heamstoma* and *P.*

*turbinatus* confirmed their high capacity to accumulate Cu. Moreover, it is worth noting that *P. caerulea* and *S. heamsatoma* are relevant to human food safety because it is commonly consumed seafood in many Mediterranean countries.

## References

[1] I. Y. Ahn, K. W. Kim, and H. J. Choi, *Marine pollution bulletin*, vol. 44, no. 5, pp. 424–431, 2002.

[2] P. S. Rainbow, *Environmental pol- lution*, vol. 120, no. 3, pp. 497–507, 2002.

[3] R. L. Veroy, N. Monta, M. L. B. de Guzman, E. C. Laserna, and G. J. Cajipe, *Botanica Marina*, vol. 23, no. 1, pp. 59– 62, 1980.

[4] M. E. Conti, M. Iacobucci, and G. Cecchetti, *International Journal of Environment and Pollution*, vol. 29, no. 1/2/3, p. 308, 2007. [Online]. Available: 10.1504/IJEP.2007.012808

[5] L. C. Rai, J. P. Gaur, and H. D. Kumar, *Biological Reviews*, vol. 56, no. 2, pp. 99–151, 1981.

*T.* C. Pergent-Martini, V. Leoni, V. Pasqualini, G. D. Ardizzone, E. Balestri, R. Bedini, A. Belluscio, Belsher, J. Borg, and C. F. Boudouresque, *Ecological Indicators*, vol. 5, pp. 213– 230, 2005.

[7] W. Boulajfene, E. Strogyloudi, V.-A. Catsiki, A. E. Mlayah, and S. Tlig-Zouari, *Marine pollution bulletin*, vol. 120, no. 1-2, pp. 274–285, 2017.

[8] V. A. Catsiki and P. Panayotidis, *Chemo- sphere*, vol. 26, no. 5, pp. 963–978, 1993.

[9] M. Warnau, G. Ledent, A. Temara, J.-M. Bouqueg- neau, M. Jangoux, and P. Dubois, *Science of the total environment*, vol. 171, no. 1-3, pp. 95–99, 1995.

[10] M. A. Schlacher-Hoenlinger and T. A. Schlacher, *Marine Bi- ology*, vol. 131, no. 3, pp. 401–410, 1998.

[11] Z. Kljaković-Gašpić, B. Antolić, T. Zvonarić, and A. Barić, *Fresenius Environmental Bulletin*, vol. 13, no. 11b, pp. 1210–1215, 2004.

[12] B. Bocca, M. E. Conti, A. Pino, D. Mattei, G. Forte, and A. Alimonti, *International Journal of Environmen- tal and Analytical Chemistry*, vol. 87, no. 15, pp. 1111–1123, 2007.

[13] A. Kaimoussi, A. Mouzdahir, and A. Saih, *Comptes rendus biologies*, vol. 327, no. 4, pp. 361– 369, 2004.

[14] S. Boucetta, H. Beldi, and B. Draredja, "Seasonal Variation of Heavy Metals in Phorcus (Osilinus) turbinatus (Gastropod, Trochidae) in the Eastern Al- gerian Coast," p. 17, 2016.

[15] "Effects of metal pollution on the activities of Acetylcholinesterase and glutathione-S-transferase in Phorcus (Osilinus) turbinatus (Gastropoda, Trochi- dae) of the coast East-Algerian," 2016.

[16] H. Bouzahouane, C. Barour, N. Sleimi, and K. Ouali, *Chemosphere*, vol. 207, p. 725e741, 2018.

[17] Q. Zhou, J. Zhang, J. Fu, J. Shi, and G. Jiang, *Analytica chimica acta*, vol. 606, no. 2, pp. 135–150, January 2008. [Online]. Available: 10.1016/j.aca.2007.11.018

[18] S. Chiavarini, P. Massanisso, P. Nicolai, C. Nobili, and R. Morabito, *Chemosphere*, vol. 50, no. 3, pp. 311–319, 2003.

[19] I. Lemghich and M. H. Benajiba, *Ecological Indica- tors*, vol. 7, no. 2, pp. 209–214, 2007.

[20] H. E. Mortaji, N. Elkhiati, A. Benhra, B. E. Haimeur, M. Bouhallaoui, S. Benbrahim, M. Kabine, and M. Ramdani, *Bulletin de l'Institut Scientifique, Rabat, section Sciences de la Vie*, vol. 33, pp. 13–18, 2011.

[21] M. E. Conti, B. Bocca, M. Iacobucci, M. G. Finoia, M. Mecozzi, A. Pino, and A. Alimonti, *Archives of Environmental Contamination and Toxicology*, vol. 58, no. 1, pp. 79–95, January 2010. [Online]. Available: 10.1007/s00244-009-9331-x

[22] W. Long and Q. Wang, *Procedia Com- puter Science*, vol. 18, pp. 1757–1763, 2013. [Online] Available:

10.1016/j.procs.2013.05.344
[23] H. Akoglu, *Turkish Journal of Emergency Medicine*, vol. 18, no. 3, pp. 91–93, September 2018.
[24] H. Allam, A. Aouar, W. Benguedda, and R. Bettioui, *Open Journal of Ecology*, vol. 06, no. 04, pp.159–166, 2016. [Online]. Available: 10.4236/oje.2016.64016
[25] I. Akcali and F. Kucuksezgin, *Marine Pollution Bulletin*, vol. 62, no. 3, pp. 637–645, March 2011.
[26] N. A. Al-Shwafi and A. I. Rushdi, *Environmental Geology*, vol. 55, no. 3, pp. 653–660, August 2008. [Online]. Available: 10.1007/s00254-007-1015-0
[27] L. Campanella, M. E. Conti, F. Cubadda, and C. Su- capane, *Environmental Pollution*, vol. 111, no. 1, pp. 117–126, 2001.
[28] M. Caliceti, E. Argese, A. Sfriso, and B. Pavoni, *Chemosphere*, vol. 47, no. 4, pp. 443–454, April 2002. [Online]. Available: 10.1016/ S0045-6535(01)00292-2
[29] M. E. Conti and G. Cecchetti, *Environmental research*, vol. 93, no. 1, pp. 99–112, 2003.
[30] P. Malea, S. Haritonidis, and T. Kevrekidis, *Hydrobiologia*, vol. 310, no. 1, pp. 19–31, August 1995. [Online]. Available: 10.1007/BF00008180
[31] Y. B. Ho, *BULLETIN OF MARINE SCIENCE*, vol. 47, p. 7, 1990.
[32] S. I. Kozhenkova, E. N. Chernova, and V. M. Shulkin, *Russian journal of marine biology*, vol. 32, no. 5, pp. 289–296, 2006.
[33] S. Chakraborty, T. Bhattacharya, G. Singh, and J. P. Maity, *Ecotoxicology and Environmental Safety*, vol. 100, pp. 61–68, February 2014. [Online]. Available: 10.1016/j.ecoenv.2013.12.003
[34] S. Haritonidis and P. Malea, *Environmental Pollution*, vol. 89, no. 3, pp. 319–327, 1995.
[35] S. Kamala-Kannan, B. P. D. Batvari, K. J. Lee, N. Kannan, R. Krishnamoorthy, K. Shanthi, and M. Jayaprakash, *Chemo- sphere*, vol. 71, no. 7, pp. 1233–1240, 2008.
[36] M. E. Conti and M. G. Finoia, *Journal of Hazardous Materials*, vol. 181, no. 1-3, pp. 388–392, September 2010. [Online]. Available: 10.1016/j.jhazmat.2010.05.022
[37] E. Laib and E. Leghouchi, *Environmental monitoring and assess- ment*, vol. 184, no. 3, pp. 1711–1718, 2012.
[38] M. A. Abdallah and A. M. Abdallah, *Environmental Monitoring and Assessment*, vol. 146, no. 1-3, pp. 139–145, 2008.
[39] D. Kut, S. Topcuoğlu, N. Esen, R. Küçükcezzar, and K. C. Güven, *Water, Air, and Soil Pollution*, vol. 118, no. 1-2, pp. 27–33, 2000.
[40] L. A. Mohamed and A. Khaled, *Chemistry and Ecology*, vol. 21, no. 3, pp. 181–189, 2005.
[41] A. E. Nemr, A. Khaled, and A. E. Sikaily, *Blue Biotechnology Journal*, vol. 1, no. 2, p. 273, 2012.
[42] K. M. El-Moselhy and M. N. Gabal, *Journal of Marine Systems*, vol. 46, no. 1-4, pp. 39–46, 2004.
[43] R. D. Stenner and G. Nickless, *Marine Pollution Bulletin*, vol. 6, no. 6, pp. 89–92, 1975.
[44] D. Kut, S. Topcuog, N. Esen, R. Küçükcezzar, K. C. Güven *et al.*, “Trace Metals in Marine Algae and Sed- iment Samples from the Bosphorus,” p. 7, 2010.
[45] T. W. Kureishy, *Marine pollution bulletin*, vol. 22, no. 8, pp. 414–416, 1991.
[46] S. Topcuoğlu, Ç Kırbaşoğlu, and N. Güngör, *Environment International*, vol. 27, no. 7, pp. 521–526, February 2002. [Online]. Available: 10.1016/S0160-4120(01) 00099-X
[47] J. N. Kumar, C. Oommen, and R. N. Kumar, *American-Eurasian J. Agric. & Environ. Sci*, vol. 6, no. 3, pp. 317–323, 2009.

[48] T. Sawidis, M. T. Brown, G. Zachariadis, and I. Sratis, *Environment International*, vol. 27, no. 1, pp. 43–47, 2001.

[49] H. A. Naser, *Marine pollution bulletin*, vol. 72, no. 1, pp. 6–13, 2013.

[50] M. El-Sayed and M. Dorgham, *Journal of King Abdulaziz University-Marine Sciences*, vol. 5, no. 1, pp. 13–24, 1994. [Online]. Available: 10.4197/ mar.5-1.2

[51] D. J. Phillips, *Biomonitoring of coastal waters and estuar- ies*, pp. 85–103, 1994.

[52] T. Boubonari, P. Malea, and T. Kevrekidis, *Botanica Marina*, vol. 51, no. 6, pp. 472–484, 2008.

[53] P. Malea and T. Kevrekidis, *Science of The Total Environment*, vol. 494-495, pp. 144–157, October 2014. [Online]. Available: 10.1016/j.scitotenv.2014. 06.134

[54] P. Malea, S. Haritonidis, and T. Kevrekidis, *Hydrobiologia*, vol. 310, no. 1, pp. 19–31, August 1995. [Online]. Available: 10.1007/BF00008180

[55] P. Timur, *Sains Malaysiana*, vol. 43, no. 4, pp. 529– 534, 2014.

[56] M. R. Rubio, P. Tineo, J. Diaz, and A. Torreblanca, *Marine Environmental Research*, vol. 35, no. 1-2, pp. 47–51, January 1993. [Online]. Available: 10.1016/0141-1136(93)90012-O

[57] D. L. Reish and P. S. Oshida, *Manual of Methods in Aquatic Environment Research*. Food & Agriculture Org., 1987.

[58] G. Blackmore, *Estuar- ine, Coastal and Shelf Science*, vol. 51, no. 2, pp. 127–139, 2000.

[59] H. A. Baumann, L. Morrison, and D. B. Sten- gel, *Ecotoxicology and Environmen- tal Safety*, vol. 72, no. 4, pp. 1063–1075, May 2009. [Online]. Available: 10.1016/j.ecoenv.2008.10.010

[60] T. E. Ayari, A. Mleiki, and N. T. E. Menif, *Journal of Aquaculture Research & Development*, vol. 08, no. 06, 2017. [Online]. Available: 10.4172/2155-9546.1000493

[61] M. A. Fernandez, A. M. Limaverde, I. B. de Castro, A. C. M. Almeida, and A. de Luca Rebello Wagener, *Cadernos de Saúde Pública*, vol. 18, no. 2, pp. 463–476, April 2002. [Online].Available: 10.1590/S0102-311X2002000200011

[62] N. Stamatis, A. S. Chinko, AchilleasChristoforidis, E. Nikolaos *et al.*, *environmental Monitoring and Assess- ment*, pp. 33–49, 2001.

[63] O. Bergasa, R. Ramírez, C. Collado, J. J. Hernández- Brito, M. D. Gelado-Caballero, M. Rodríguez-Somozas, and R. J. Haroun, *Environmental Monitoring and Assessment*, vol. 127, no. 1-3, pp. 127–133, April 2007. [Online].Available: 10.1007/s10661-006-9266-x

[64] J. C. Amiard, C. Amiard-Triquet, B. Berthet, *Marine Biology*, vol. 90, no. 3, pp. 425–431, 1986.

[65] H. Çoğun, T. A. Yüzereroğlu, F. Kargin, and Ö. Fi- rat, *Bulletin of Environmental Contamination and Toxicology*, vol. 75, no. 4, pp. 707–715, 2005.

[66] A. Matlack, *Introduction to green chemistry*. CRC Press, 2010.

[67] S. Mitra, S. Lukianov, W. G. Ruiz, C. C. Cosentino, S. Sanker, L. M. Traub, N. A. Hukriede, and G. Apo- daca, *PloS one*, vol. 7, no. 7, p. e41816, 2012.

[68] B. Ozretić, M. Krajnović-Ozretić, J. Santin, B. Medjugorac, and M. Kraš, *Marine pollution bulletin*, vol. 21, no. 12, pp. 595–598, 1990.

[69] A. Nicolaidou and J. A. Nott, *Marine Pollution Bul- letin*, vol. 36, no. 5, pp. 360–365, 1998.
O. Duysak and B. Ersoy, *Pakistan Journal of Zoology*, vol. 46, no. 5, 2014