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Relationship Between Heavy Metal Concentrations in Certain Macroalge and Two Allied Mollusc Species Collected From Costal Waters of Tobruk City, Libya.

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Abstract

The relationship between heavy metals bioaccumulation ability of two algal species; *Polysiphonia opaca* (Rhodophyta) and *Ectocarpus siliculosus* (Phaeophyta), and two species of their allied gastropoda; *Monodonta turbinata* and *Patella caerulea* were studied. The samples were collected from two sites, clean (S1) and polluted (S2) during winter 2017from Tubrok coastline, Libya. Various degrees of Cu, Zn, Pb, Cd and Mn accumulation weredemonstrated depending on the investigated species. Epizoic *Poly.opaca* and *E. siliculosus* affected negatively the bioaccumulation ability of *P. caerulea* in both sites. The most tolerant *M. turbinata*, being have longevity than macroalgae, has better bioaccumulative properties towards most of the investigated metals. *Poly.opaca* was restricted to clean seawater, referring to its sensitivity to pollution, while *E. siliculosus* was restricted to polluted seawater, exhibiting its tolerance to pollution. The present results indicated that the presence or absence of macroalgal species was shown to be a good indicator for the quality of seawater. Based on the bioaccumulation factors (BAFs), the five heavy metals arranged in the descending order as Cd >Mn>Pb> Cu > Zn in clean site compared to Mn>Cd > Cu >Pb> Zn in polluted site. Whereas, the descending order of metal pollution index (MPI) was *M. turbinata* > healthy *P. caerulea*> infected *P. caerulea>poly. opaca* >*E. siliculosus*. Statistical analysis showed a significant correlation between species and the investigated Cu, Pb, Mn and Cd. *Poly. opaca*, *E. siliculosus*, cosmopolitan *M. turbinata* and *P. caerulea* are considered to be good environmentally friendly bioindicators for heavy metal pollution in the Mediterranean Sea.

Keywords: *Accumulation, Biomonitors, Heavy metals, Mollusca, Seaweeds.*

1. Introduction

Heavy metal toxicity to aquatic organisms and its ultimate effect on humans have gained serious attention from public and scientific community (Yayıntas et al., 2007). Essential heavy metals for regulation of metabolic activities may become toxic at higher concentrations (Canli and Atli, 2003). The elevated level of trace metals in different organs of microorganisms is used as an important tool or index of metal pollution in anaquatic ecosystem (Tarrio *et al*., 1991; Mendil *et al*., 2005). The last decades, water and sediment were used as indicators for pollution, but recently, organisms have been found to be a potential biotechnological alternative method than physicochemical methods (Zabochnicka-Swiatek and Krzywonos, 2014). The potentiality of organisms may be attributed to their ability to accumulate heavy metals thousands of times higher than the corresponding concentrations in seawater (Rainbow, 1995; Rai *et al*., 1981). The efficiency of metal bioaccumulation in algae depends on the bioaccumulation affinity of algae for a particular metal and the bioavailability and physical/chemical form for that metal, where they can concentrate and bind the free metal ions depending on their nature (Volterra and Conti, 2000; Zalewska and Saniewski, 2011). Mussels can accumulate and integrate concentrations of several metals in seawater for relatively long intervals. They also assimilate trace metals from their food and from the ingestion of inorganic particulate material (Struck *et al*., 1997). Mollusca, as filter feeders, obtain heavy metals from several sources such as food, water, inorganic particles (El-Sikaily et al., 2004).

Very little information is known about heavy metals content in aquatic biota in Libyan coastline. In order to fill this gap in knowledge, this investigation focused on analyzing of algal and their allied gastropods heavy metals Cu, Zn, Pb, Cd and Mn contents at Tobruk city, Libya. The study also aimed to determine the most appropriate metals accumulator to be potentially used as an indicator for pollution.

2. Materials and Methods

The study area (Tubrok City). is situated on the north–eastern coast of Libya at latitude (N - 32°‹06'‹59"N 32°‹04'‹01") and longitude (E24°‹00'‹30' - E23°‹ 53'‹36"). It is characterized by rocky shore, and suffered from sewage pollution in some sites. Two sites were chosen based on the status of water. The first site (S1) is clean **(used as a reference) seawater, the second site (S2) is** polluted seawater**,** and is far about 3Km easterly from the first site (Fig. 1).

Sampling

Water samples from reference and polluted sites were collected using sterile 1liter plastic container. Algal and gastropoda species with the same ages and size, where it is possible, were handpicked during low tide at winter 2017. They were rinsed thoroughly with seawater on-site, placed in plastic bags and transferred to the laboratory as soon as possible. Samples were initially washed under a jet of tap water and rinsed in distilled then de-ionized water to remove any mineral particles or organisms.

Identification of species

The investigated species were brought to the laboratory as soon as possible after collection. Macroalgal species were identified using different keys, such as the World Register of Marine Species (WoRMS Editorial Board, 2013; Abbott, 1999). Whereas, gastropod species were identified using the taxonomical keys cited in Borradaile *et al.* (1977) book.

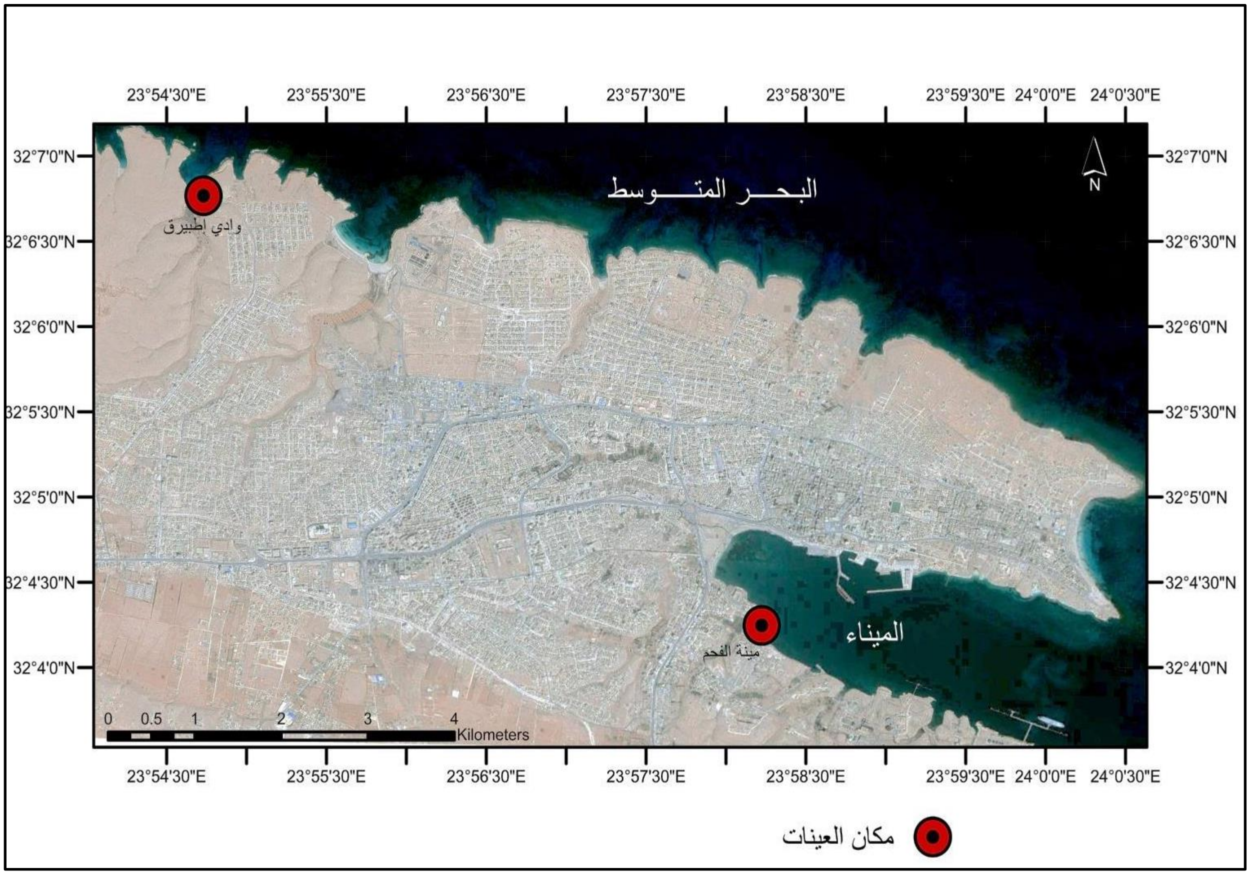

Figure.1 Map of ATobruk city, Libya located on the Mediterranean Sea.

Analytical methods:-

On sampling day, the studied organisms as well as seawater were immediately transported to the laboratory for analyses.

1-Sample Digestion

One gram of each sample was digested in 20 ml nitric acid and 3 ml perchloric acid in kjeldahlapparatus until white fumes appear and organism's tissues dissolved completely. The samples were allowed to cool and then after, 10 ml diluted HCl (1:1) were added and complete digestion process continued till the solutions turn clear. The digested samples made up to 50 ml by distilled H_2O, according to AOAC (1980).

2-Water analysis

Preparation of water samples

Five hundred ml of seawater were boiled and concentrated to 50 ml. The samples were allowed to cool and then after, 7.5 ml HCl (37%) were added and boiled for 10 minutes, after cooling, 7.5 ml nitric acid (69%) were added and boiled again till the solution volume becomes 25 ml, the solution volume made up to 50 ml by de-ionized H_2O. Sample concentration increases 10 times than the original concentration in seawater (Olowu *et al*., 2010).

Finally, the concentration of Zn, Cd, Pb, Cu and Mn in the water samples, macroalgal and mollusc tissues has been measured by graphite furnace atomic absorption spectrophotometry (A Analyst 800 Perkin Elmer).

Indices of pollution

1- Bioaccumulation factors (BAFs): BAFs of the studied samples were calculated based on the following formula (Barron, 1995).

BAFs=Metal concentration ($\mu gg-1$ fresh wt.) in the sample Metal concentration ($\mu g1-1$) in water

2- The metal pollution index (MPI):-MPI was used in order to compare the total content of heavy metals at locations studied (Usero *et al*., 1996)

$MPI=(Cf1xCf2x\ldots Cfnx)1/n$

Where, Cfn = concentration of metal in the sample (μgg-1 fresh wt).

n = number of metals

Statistical analysis

The data were analyzed using SPSS statistical program ver.22 **(2012)**. Duncan's test (ANOVA) for separating means and Pearson's Correlation were used to measure the strength of the interactions between heavy metals. P-value of less than0.05 indicates a significant variance. Also, Cluster analysis (Bray-Curtis similarity index) was employed.

3. Results and Discussion

Taxonomy of organisms

Macroalgae were represented by two species; Polysiphonia opaca belonging to Rhodophyta inhabiting clean site (S1) and Ectocarpus siliculosus belonging to Phaeophyta inhabiting polluted site (S2)(Table 1). Likewise, Mollusca were represented by two species of gastropoda; Monodonta turbinata and healthy and infected individuals of Patella caerulea in both sites. Rhodophyta and phaeophyta play vital ecological roles in marine communities (Wijesinghe and Jeon, 2011).

Table 1: Taxonomy of macroalgal and gastropoda species, Tobrukcoastline, Libya**.**

Taxonomy	**Macroalgae**		**Invertebrate**	
Division/ Phylum	Rhodophyta	Phaeophyta	Mollusca	Mollusca
Class	Florideophyceae	Phaeophyceae	Gostropoda	Gostropoda
Order	Ceramiales	Ectocarpales	Prosobranchiata (Streptoneura)	Vetigastropoda
Family	Rhodomelaceae	Ectocarpaceae	Patellidae	Trochidae
Genus	*Polysiphonia*	*Ectocarpus*	*Patella*	*Monodonta*
Species	*Opaca*	*siliculosus*	*caerulea*	*turbinata*
Locality	S1	S2	S1&S2	S1&S2

Pollution specificity of organisms

The flourished growth of *Poly. opaca* was found on *P. caerulea* as epizoic or on moist rock as epilithic in clean seawater only. It seemed to be specific to the clean seawater. On the other hand, *E. siliculosus* was found to be definitely limited to polluted water (S2). Seaweed cells have a large superficial area with sites that are able to provide fast and reversible bonding with cations (Amorim *et al*., 2003). Also, they have an ability to bind and accumulate micro-pollutants present in their surroundings which may be attributed to the unique structure of their cell walls, reflecting the impact of anthropogenic disturbance (Gopinath *et al*., 2011). All of these characters make algae good indicators for micro-pollution and the most reliable tools for monitoring biodiversity on the coast (Northon *et al*., 1996).

It is also interesting to note that *M. turbinata* and *P. caerulea* showed no preference for particular area; where they widely spread in clean site, moreover, they adapted to survive under various pollution levels in polluted site. This disagree with the findings of Traunspurger and Drews (1996) who reported changes in the diversity and community structure of gastropod through chronic and acute effects of sediment contamination by heavy metals. Generally, the pollution generated by heavy metals released from industrial and domestic sources causes serious changes in the aquatic ecosystem, resulting in a loss of biological diversity and the magnification and bioaccumulation of toxic agents in the food chain (He *et al.* 1998). Finally, the results indicated that, the macroalgal species clearly differ from clean to polluted seawater, so the presence or absence of macroalgal species would be a good indicator for the quality for seawater.

Metal concentrations in seawaters

Cu, Zn, Pb, Cd and Mn reached their maximum values in S2 (polluted site), with a remarkable elevation for Zn (9.3 µg l-1), this may be assigned to their concentration levels in nutrients and/or sewage discharges (Table 2).

Table 2: Heavy metal concentrations (µgl-1) in seawater at 2 studied sites at Tobruk city, Libya.

Sites	S1	S2	EPA (2017)
Cu	0.42	0.75	3.1
Zn	6.2	9.3	81
Pb	0.26	0.61	8.1
Cd	0.02	0.05	1.0
Mn	0.17	0.21	1.0

The results indicated that a similar pattern of metal concentrations in both sites was arranged in descending order as follow: Zn > Cu > Pb > Mn > Cd. Whatever, the metal concentration levels were lower than the maximum permissible values of EPA (2017). This may be interpreted by the contribution of Davies *et al.* (2006) who reported that, seawater metal concentrations are less than that accumulated in sediments, holding 99 % of total amount of metal present in the aquatic system, and Leal *et al.* (1997) who explained that the seawater metal concentrations affected by biological uptake, scavenging by particulate matter, release metals from bottom sediments, advection, mixing of water masses and the aeolian transportation of terrestrial materials.

Metal concentrations in organisms

Cu reached its maximum value (232.01 ± 10.52 µgg-1 fresh wt.) in *M. turbinata* at S2 and its minimum (14.60 ± 0.87 µgg-1fresh wt.) in *Poly. opaca* at S1Table (3).The concentration of Zn was found to fluctuatebetween92.59±8.33 µgg-1 fresh wt. for *M. Turbinata* at S1 and 8.32±0.09 µgg-1 fresh wt. for *E. siliculosus* at S2. According to Amin et al. (2009), who reported that Cu and Zn may be the most detrimental elements to gastropod populations in the mangrove area of Dumai coastal waters, the high concentrations of Cu and Zn in the investigated area may hurt the studied organisms.

Lead concentration level was found to oscillate between 106.68 ±7.39 µgg-1 fresh wt. for M. turbinata at S2 and 20.02 ± 4.69 for *E. siliculosus* in the same site. Coastal pollution and habits outdoor barbecue where forests and beaches of Libya, would lead to the emission of polluting elements such as Pb, this in partial agreement with Dadolahi-Sohrab *et al*. (2011) who stated that, high levels of Pb in marine organisms could be attributed to combustion of fossil fuels and oil pollution. Generally, brown algae accumulated heavy metal in considerable amounts due to their high content of binding polysaccharides and polyphenols (Hashim and Chu, 2004).

Concerning Cd bioaccumulation, healthy *P. caerulea* (S2) recorded the highest level of accumulation (28.23±4.10 µgg-1fresh wt.) whereas, *Poly. opaca* recorded the lowest concentration level (0.51± 0.20 µgg-1fresh wt.). According to Haug *et al*. (1974) the affinity of alginates in brown algae for divalent cations such as Pb2+, Cu2+, Cd2+, Zn2+ etc. increased with the guluronic acid content, but the affinity variation of divalent metals to the alginates depend on the different M/G ratios (β-D-mannuronic (M) and α-L-guluronic (G) acids).

Finally, Mn was found to be accumulated within the range 129.68± 10.50 μgg-1fresh wt for *M. turbinata* at S2 and 7.41±2.51 μgg-1fresh wt for *Poly. opaca* (S1). In general, heavy metals bioaccumulation pattern in tested organisms arranged in a descending order as follow: Cu>Zn>Pb>Mn> Cd in clean site, compared to Cu >Mn>Pb>Zn > Cd in polluted site. It is obvious that Cu recorded the highest rank whereas Cd recorded the lowest rank in both sites. The presence of Cu in high concentrations may cause great threat to all marine organisms, including fish crustacean, phyto- and zoo- plankton, macroalgae and filter feeders (Dadolahi-Sohrab *et al*., 2011). The obtained results exhibited that the infestation of *Patella caerulea* by *Poly. opaca* at S1 and *E. siliculosus* at S2 decreased its ability to accumulate metals, this may be attributed to negative effect of the parasites, *Poly. opaca* and *E. siliculosus*, on metal bioaccumulation and feeding activity of *P. caerulea* and / or the exhausting of some metals by the algal species from *Patella*. The potential indirect effects of algal parasites on a benthic community of *Patella* either in clean or polluted sites may be accounted for by the findings of Moore (2002) who reported that parasites affect the behavior of their hosts to become adaptive for the parasite.

Table 3: Metal concentrations ($\bar{X}$±SD μgg-1 fresh wt) in studied organisms inhabiting clean and polluted sites at Tubrok city, Libya. Data labeled with different letter are significantly different at $p \leq 0.05$.

Species	Cu	Zn	Pb	Cd	Mn
S1- Clean site 1-Poly. opaca	14.63± 0.87a	23.10±0.58b	25.74±5.71a	0.51±0.20a	7.41±2.51a
2- HealthyP.caerulea(S1)	97.82±9.41c	56.29± 11.54cde	59.17± 5.97c	15.91± 3.34b	54.55± 7.75d
3-Poly. infected Patella	55.75± 10.17b	51.33± 5.07cd	38.24± 7.25b	16.96± 4.08b	26.45± 3.44b
4-M. turbinata(S1)	98.92± 9.28c	92.59±8.33 f	63.34±9.94c	24.35±4.55c	37.61±4.98bc
S2- Polluted site 5- E. siliculosus	17.85± 2.14a	8.32±0.09 a	20.02±4.69a	0.77±0.21a	46.16±11.36cd
6- Healthy P. caerulea(S2)	97.85±15.67c	66.98±6.83e	66.10±6.64c	28.23±4.10c	48.94±8.86cd
7-E.infected Patella	65.52±8.25b	60.31±1.75de	46.10±6.64b	26.89±4.27c	28.94±4.18b
8-M. turbinata(S2)	232.01±10.52d	47.29±4.97c	106.68±7.39d	23.95±2.66c	129.68±10.50e

*The means in the same column followed with the same letter are statistically non-significant.

Similarly, G´erard and Poullain (2005) revealed that gastropod survival, growth and fecundity were predominantly affected by various abiotic and biotic stresses such as parasitism and natural or anthropogenic pollution. Moreover, Yüzereroğlu et al. (2010) demonstrated that the heavy metal accumulation in Patella caerulea is influenced by heavy metal in their allied macroalgae. These heavy metals have deactivated proteins, denature enzymes, and disturbed cell functions (Hall, 2002). They also elicit the production of free reactive oxygen species, which in turn impair the functions of proteins, lipids and DNA causing oxidative stress and ultimately cell death (Kannan and Jain, 2000).

The peculiar high levels of metals in M. turbinata were noticeable in both sites. This might be attributed to that gastropods have many resources to accumulate heavy metals beside their tolerance and longevity. This agrees with Struck et al. (1997) who reported that mussels can accumulate and integrate several metal concentrations for relatively long intervals from different sources of their food such as seawater, algal cells, and the ingestion of inorganic particulate material. In addition, Lobban and Harrison (1997) documented that the growth rates can affect accumulation patterns, where faster growing lower the concentration levels. The natural concentrations of metal ions are principally dependent on the ambient distribution, weathering and leaching of these elements from the soil in the catchment area (Tarvainen et al., 1997). The differences between the investigated species seemed to be significant in accumulating metals ($p < 0.01$). The fluctuation in Cu, Zn, Pb, Cd and Mn concentrations showed high significance between species inhabiting the two sites inspected, except Zn for Poly. opaca and Mn for E. siliculosus ($p> 0.01$) (Table 3).

Generally, the obtained results evidenced the abilities of the gastropods, M. turbinata and Patella caerulea to accumulate a wide range of heavy metals. On the other hand, the absence of Poly.opaca, which restricted to clean water, proved the contamination status of S2, emphasizing the pronounced effect of heavy metal pollution on algal diversity.

BAFs for different species at the clean site and the polluted site were calculated to compare their capability for metals accumulation regardless the differences between them Fig. (2).The BAF of Cd reached its maximum in M. turbinata (1217.61) and its minimum in Poly. opaca (25.64) in the clean site. Whereas, it reached its maximum in Relationship between Heavy Metal Concentrations in …

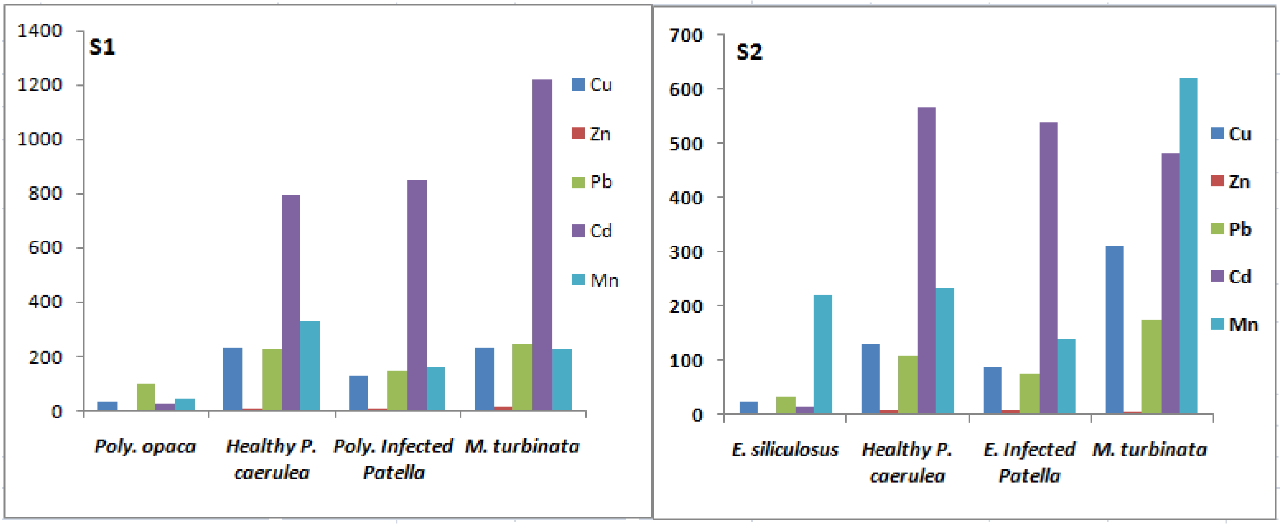

Figure.2 BAFs for the studied organisms at the clean (S1) and the polluted (S2) sites, Tobruk city, Libya.

healthy P. caerulea (564.5) and its minimum in E. siliculosus (15.42) in the polluted site. The highest and lowest Mn-BAFs were found in M. turbinata (617.53) and E. infected Patella (137.82) in the polluted site, respectively. While, in the clean site, the highest and lowest Mn-BAFs values were found in healthy P. caerulea (326.64) and Poly. opaca (44.36), respectively. The descending order of BAFs was Cd >Mn >Pb> Cu > Zn at S1 compared to Mn> Cd > Cu >Pb> Zn at S2. Cd and Mn displayed the highest BAFs in the clean and the polluted sites respectively. M. turbinata and P. caerulea, either healthy or infected, tended to accumulate more Cd and Mn at both sites.

In accordance with Mokhtar et al. (2009) heavy metal concentration in aquatic organisms increases several times over the environmental levels, demonstrating their potentiality as bioindicators. Moreover, BAFs increased with the size of the periwinkle (Davies et al., 2006) and could be affected by the passage of a contaminant through the trophic chain (Conti and Cecchetti, 2001).
M. turbinata got the highest metal pollution index (MPI)in both sites Fig. (3).It followed by healthy *P. caerulea*, infected *P. caerulea*, *E. siliculosus* and *poly. opaca* (Fig.3). Organisms surviving in S2 have a greater capacity for metals bioaccumulation. This may be attributed to that the longevity of *M. turbinata* and *P. caerulea* enables them to accumulate metals than ephemeral and short- lived algae. In spite of that; macroalgae are able to accumulate trace metals, reaching thousands of times higher than the corresponding concentrations in sea water (Rai *et al*., 1981).

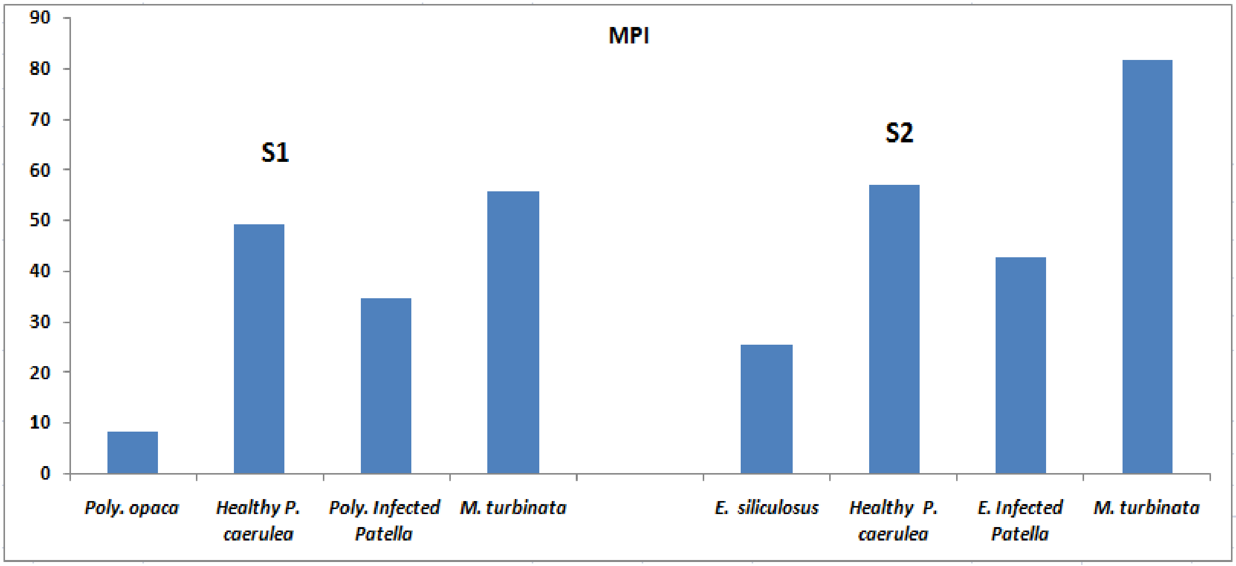

Figure.3 MPI values for the studied organisms in Tobruk coastline, Libya.

It is worthily mentioning that, if complexity with strong organic ligands takes place, the metals burden in aquatic organisms may not be proportional to their dissolved fraction in seawater, especially near industrial and sewage outfalls Luoma (1983). In this context, Mokhtar *et al*. (2009) explained that MPI provides a representative picture of the environmental state of any environmental impacts on the aquatic ecosystem.

ANOVA results verified that the variations in seawater metal levels are highly significant regardless the type of sites (F=25.86, df =4, P < 0.01, Table 4).The variance percentage for Zn (97.6 %) may be responsible for this increment in significance.

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Table 4: Two way ANOVA for seawater heavy metals at the studied sites, Tubrok city, Libya.

Source of Variati on	SS	Df	MS	F	P-value	F crit
Metals	88.9082	4	22.22705	25.8641	0.004054	6.388233
Sites	1.484561	1	1.484561	1.727482	0.259032	7.708647
Error	3.437514	4	0.859378			
Total	93.83027	9				

The two-way ANOVA analysis performed on the data (Table 5) indicates that the element ratios in species inhabiting different sites are statistically distinguishable from each other. Highly significant differences between species (F= 273.46, df=7, P < 0.01); between metals (F= 289.15, df=4, P < 0.01) as well as the interaction between species and metals (F= 2389.23, df = 28, P < 0.01) are recorded. The variance percentages of *M. turbinata* at S2 (65.12%), M. *turbinata* at S1 (10.53 %) and *P. caerulea* at S1, (8.24 %) were responsible for the peculiar elevation in this significance emphasizing their promising role in biomonitoring programs.

Table 5: Two way ANOVA for heavy metals detected in organisms inhabiting the studied sites, Al-Hanyaa city, Libya (n = 3).

. Source of Variation	SS	df	MS	F	P-value	F crit
Species	92103.55	7	13157.65	273.4643	4.16E-53	2.126324
Metals	55648.87	4	13912.22	289.147	1.04E-46	2.485885
Interaction	66898.36	28	2389.227	49.65691	1.11E-39	1.617112
Within	3849.175	80	48.11469			
Total	218500	119				

As shown in Table (6), species were significantly correlated with sites, Cu, Pb, Mn and Cd. Sites correlated with Mn. Copper correlated with Pb, Mn and Cd. Zn correlated with Pb and Cd. Finally, Pb was significantly correlated with Mn and Cd. These results shed some lights on the complicated relation between different elements control the abilities of different organisms to bioconcentrate contaminants in their tissues.

Table 6: Pearson's correlation analysis between species, sites, metals and their interactions.

	Species	Sites	Cu	Zn	Pb	Mn	Cd
Species	1						
Sites	.873**	1					
Cu	.594**	.283**	1				
Zn	.167	-.204	.392	1			
Pb	.561**	.249	.957**	.505*	1		
Mn	.633**	.461*	.891**	.056	.805**	1	
Cd	.582**	.257	.595**	.797**	.667**	.348	1

* Correlation is significant at 0.05 levels.

** Correlation is significant at 0.01 level.

Data in Table (7) showed that, a significant positive correlation (P <0.01) exists between Cu and Zn, Pb, Cd and Mn in the clean site and Pb and Mn in the polluted site. Zn positively correlated with Pb and Cd in the clean site and with Cd in the polluted site. The obtained results showed a significant positive correlation between Pb and Mn in both sites at p< 0.05.The competitions on binding sites and bioavailability may consider as important factors affecting the correlation between metals.

Table 7: Pearson's correlation analysis between species, sites, metals and their interactions.

	Clean site					**Polluted site**				
	Cu	Zn	Pb	Cd	Mn	Cu	Zn	Pb	Cd	Mn
Cu	1					1				
Zn	0.817*	1				0.381	1			
Pb	0.903**	0.843*	1			0.968**	0.504	1		
Cd	0.821*	0.892**	0.713	1		0.499	0.926**	0.634	1	
Mn	0.913**	0.608	0.782*	0.690	1	0.889**	-0.0171	0.809*	0.130	1

Cluster analysis was applied in order to determine the similarity between the species investigated, species classified into five groups depending on their similarity in metal accumulation efficiency (Fig. 4). Group A consists of the same species, healthy *P. caerulea* in S1 and S2 (with ≅ 95%), this may be attributed to their similarity in the efficiency of metal accumulation. Group A was similar with group C with more than 90%. Group A and C were similar with group B with ≅ 80%. Group A, B, and C were similar with E (*M. turbinata* atS2) with more than 60%. *Poly*. *opaca* and *E. siliculosus* have the similar pattern in metal accumulation. *Poly*. infected *Patella* and *E.* infected *Patella* seemed to be similar in their metals accumulation (with ≅ 60%). Likewise, healthy *P. caerulea* in S1 and S2 are similar in their efficiency for metal uptake. This may be attributed to *P. caerulea* stability and consistency for pollution and infection with any algal species. The cluster emphasized most of the previous results and facilitated data analysis and comparison between species.

4. Conclusion

The investigated species can be classified depending on their susceptibility to metal pollution as follow:

- *E. siliculosus* was limited to polluted seawater. It can be used as an indicator for water pollution.
- *Poly*. *opaca* was limited to clean seawater where it seems to be sensitive to pollution status.
- Gastropods have a higher longevity than macroalgae. So, they exhibit better bioaccumulative properties towards all metals except Mn accumulated by *E.* infected *Patella*. Generally, *M. turbinata* and *P. caerulea* were more tolerant and accumulator agents than the algal species *poly. opaca* and *E. siliculosus*.

- The descending order of BAFs was Cd >Mn>Pb> Cu > Zn in the clean site compared to Mn> Cd >Cu >Pb> Zn in the polluted site. Cd

and Mn displayed the highest BAFs in the clean and the polluted sites respectively.

- The descending order of MPI was *M. turbinata*> healthy *P. caerulea* have a greater capacity for metal bioaccumulation than infected *P. caerulea*>*poly. opaca*>*E. siliculosus* in both sites.
- Evaluation of heavy metal pollution using marine biomonitors and employing new techniques and accounts such as bioaccumulation factors (BAFs) and metal pollution index (MPI) has become more accurate, easy and clear.
- *Poly. opaca* and *E. siliculosus* are considered dependable tools for monitoring biodiversity on the coast referring to water quality. So, the presence or absence of macroalgal species would be a good indicator for the quality for seawater

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