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Levels of Heavy Metals (Zn, Pb, Cd and Cu) In *Ulva* sp. and *Enteromorpha* sp. macrophytic Green Algae Along Benghazi Coast Line

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***Abstract*—** Pollution with heavy metals is one of the most severe marine environmental problems in the world. In this study concentrations of heavy metals (Zn, Pb, Cd and Cu) tested in tow marine green algae Ulva sp. and *Enteromorpha* sp. Collected from four Sites, Elsaberry, Juliana, Eshbellia and Benghazi port (Elmina) along of Benghazi city Coast. The coast extends to about 18 km at the north east of Libya. The algae samples were collected during the winter and summer seasons 2011 and summer 2012. The results obtained shows that the concentration were found to range from 18.66 mg/kg (Pb), 1.93 - 5.5 mg/kg (Cd) and 2.36 - 6.13 mg/kg (Cu). There was a significant difference in heavy metals concentrations found between the sites and also with seasons. The *Enteromorpha* sp. showed ability to accumulate heavy metals more than Ulva sp. this could be attributed to the large surface area of the *Enteromorpha* sp. Generally, the summer concentrations of metals were found to be higher than in winter concentrations and this may be due to high temperature levels that affect the uptake of metals and to the availability and presence of nutrient mainly Nitrogen and phosphorous due to the increase in sewage discharge into the sea during summer time.

Keywords— Heavy metals, Accumulation, Green algae, Pollution monitoring.

I. INTRODUCTION

Algae is an outotrophic protists that can be both unicellular or multicellular organisms. These organisms are found in sub groups chromosomal (dinoflagellates, diatoms, golden algae and brown algae) and Archaeplastida (red algae and green algae) [1]. They are important ecologically and environmentally because they are responsible for the production of approximately 70% of the oxygen and organic matters in aquatic environments [2], [3]. Algae are widely found in the environment and adopted to fresh and marine water [4] and have an outstanding ability to absorb and accumulate heavy metals from the surrounding environment [5].

Algae and *Posidonia oceanica* has been considered among the different species selected as an important tool for monitoring in (IMAP) implementation for the Mediterranean countries [6]. It can be easily sampled and monitored and is widely distributed along the Libyan coastal water. These weeds Sargassum *hemiphyllum*, *Petalinia fascia* and *Colpomenia sinusa* have characterized by their number of binding sites. Due to their high number of binding sites *Sargassum* and *Petalinia* which fond to be promising for bio sorption application. Many investigations have been carried out for bio sorption of heavy metals by the other important divisions of algae Green algae (Chlorophyta) and Red algae (Rhodophyta). *Ulva reticulate* was the focus of the study of [7]. The influence of several factors such as pH, initial metal concentration and contact time were analyzed. Green algae *Cladophora fascicularis* was determined by [8] to be an effective and economical bio sorbent material for the removal of heavy metal ions.

The United Nation Environment Programme (UNEP) has recommended the use of algae to monitor contaminates especially heavy metals that can be measured in algae following the UNEP biota protocols for contaminant determinations. The term heavy metals have been used widely without having prises definition.

Heavy metal present in the marine environment from different sources mainly from industrial sources, such as metal plating facilities, mining operations, tanneries and sewage discharge. Heavy metals are not biodegradable and tend to accumulate in living organisms. The tendency of metals to accumulated in the organisms as well as their bio magnification problem has been the focus to many researchers specially the issue of its affect through the food chain. The bio magnification phenomenon has been clearly illustrated in Mercury Hg^{+} and DDT. The metal pollution problems, needs an, effective monitoring program. Algae and *Posidonia oceanica* has been considered among the different species suggested by [6] as an important tool for monitoring in IMAP implementation for the Mediterranean countries, since it can be easily sampled and monitored, these species of the algae, is widely distributed along the Libyan coast and could be collected in a yearly basis and the contaminants measured following the biota protocols for contaminant

determinations. This study was aimed to find out the level of heavy metals (Zn^{+2}, Pb^{+2}, Cu^{+2} and Cd^{+2}) in green algae *Ulva* and *Enteromorpha* present within four coastal area of Benghazi city.

II. METHODOLOGY

Samples collection

Samples were collected randomly from four different areas namley AL-sabri area (A), Juliana area (B), Eshbilla area (C)and Alminah area (D) from the east coast of Benghazi city (Figure 1).

Figure 1: Map illustrate the study areas (A.B.C and D)

Preparation of the Samples

The Samples were collected (48 sample from each species 24 for winter season and 24 for summer season) and put in a clean plastic bags. The bags were closed labeled with all the information needed and placed in the field box with ice and taken to the laboratory. The samples were then washed and cleaned, sand and any foreign materials were removed. The samples were then dried on a Algal Herbarium paper 41.25 cm x 28.75 cm at room temperature which was around 25 C^{o}. It took about one week for the summer samples and between 2-3 weeks for the winter samples to dry, after that the samples were grounded using a grounder. The powder was then kept in plastic test tube with cup until digestion.

Samples Digestion

Samples were digested using concentrated nitric acid HNO_3 according to [9,10] for the analysis of heavy metals. All the steps regarding the quality control and quality assurance has been considered and implemented. Digestion has been done in an acid cleaned digestion test tube. Half gram (0.5 g) of dry grounded algae were placed in the digestion tube and then 5ml of the concentrated nitric acid HNO_3 was added. The test tubes were then put on a Shaker for 24 hours. The samples were filtered using Wattman paper no 42. The samples were made up to volume in 100 ml volumetric flask and the samples become ready for measurements.

Preparation of Standards and Determination of the Metals

Standard solutions have been prepared for all the metals studied (Cd, Pb, Cr, Zn and Cu). The calibration curve was established for each element using the prepared standard. Glass tubes were washed well 20 % nitric acid and with distilled water. The determination of the metals studied were done according to [10]. The Atomic Absorption Spectrophotometer (AAS), Analytic Jeha Nov AA300) at the laboratories of Ras Lanuf petro chemicals Company.

Statistical analysis

The data was subjected to statistical analysis using computer program of (SPSS) verion 11.0 including the analysis of variance (one-way ANOVA) for testing of the differences between sites.

III. RESULTS AND DISCUSSION

In order to facilitate the illustration of the results and its interpretation and discussion, the data obtained has been dealt with for each site separately, as follows: from table (1)for site(A) Alsabry that the concentration of Zn in *Ulva sp*. were found to range from 5.45-18.03 mg/kg the lowest level reported for the winter season and the highest value was for the summer season , while the level for Pb found to be ranging from 2.61 mg/kg in winter season to 16.0 mg/kg for summer season. The concentration of Cd showed a very low concentration infact below detection limits, while for the winter time the concentration was 5.56 mg/kg, while the level for Cu were also below detection level to 3.80 mg/kg. The concentration of metals in *Enteromorpha sp*. showed almost similar pattern as for the *Ulva sp.* were the highest concentration of Zn 24.60 mg/kg observed in the summer season and lowest 4.27 mg/kg reported for the winter season, while the concentration for Pb found to be range from 4.94 mg/kg in winter season to 11.33 mg/kg for summer season. The values obtained for Cd were below detection limits in summer season to 4.20 mg/kg for winter season, while the levels obtained for Cu were below detection limits in summer season and 6.13 mg/kg for the winter time.

Table (1) Concentrations of Heavy metals (mg/kg) in *Ulva* sp. and *Enteromorpha* sp. for the two seasons for Alsabry Area.

Metals	Seasons	*Ulva sp.*	*Enteromorpha sp.*
Zn	Winter	5.45	4.27
	Summer	18.03	24.60
Pb	Winter	2.61	4.94
	Summer	16.00	11.33
Cd	Winter	5.56	4.20
	Summer	*BDL	*BDL
Cu	Winter	3.80	6.13
	Summer	*BDL	*BDL

*BDL = Below Detection Limits

Table (2) shows the levels of metals at site (B) Juliana area. The results for the Ulva sp. Indicate that the highest concentration of Zn was observed in summer season 21.33 mg/kg. The lowest concentration 4.63 mg/kg was reported for the winter season, while in the case of Pb the levels ranges from 6.65 mg/kg for the winter season to 13.83 mg/kg for summer time. In the case of the Cd the the levels observed were from undetected in summer to 1.93 mg/kg

in winter, while for Cu it was almost the same case as for Cd were the levels observed were from undetected in summer to 3.86 mg/kg in winter. The concentration of Zn *Enteromorpha* sp. were found to range from 2.0 -20.66 mg/kg in which lowed reported for the winter time and the highest reported for the summer season, while the values for Pb were found to range from 2.0 mg/kg in winter season and 18.66 mg/kg for the summer season. The concentration of Cd and Cu were undetected for the summer season and very low concentration in winter season 2.16 and 2.73 respectively.

Table (2) Concentrations of Heavy metals (mg/kg) in Ulva sp. and Enteromorpha sp. for the two seasons for Juliana Area.

Metals	Seasons	*Ulva sp.*	*Enteromorpha sp.*
Zn	Winter	4.63	2.0
	Summer	21.33	20.66
Pb	Winter	6.65	2.00
	Summer	13.83	18.66
Cd	Winter	1.93	2.16
	Summer	*BDL	*BDL
Cu	Winter	3.86	2.73
	Summer	*BDL	*BDL

*BDL = Below Detection Limits

From Table (3) for site (C) Eshbellia Area. The levels of Zn in the Ulva sp. were ranging from 7.76-21.33 mg/kg the lowest reported for the winter season, while the highest level recorded for the summer samples, while the levels for Pb found to be ranging from 4.33 mg/kg in winter season to 15.33 mg/kg for the summer season. The concentration for Cd showed a very low concentration in fact below detection limits for the summer season and 2.0 mg/kg for the winter time. The concentration for Cu was 4.33 mg/kg for the winter samples and below the detection level in summer samples.

Table (3) Concentrations of Heavy metals (mg/kg) in Ulva sp. and *Enteromorpha* sp. For the two seasons for Eshbellia Area

Metals	Seasons	*Ulva sp.*	*Enteromorpha sp.*
Zn	Winter	7.76	4.80
	Summer	21.33	23.16
Pb	Winter	4.33	1.86
	Summer	15.33	13.66
Cd	Winter	2.00	5.30
	Summer	*BDL	*BDL
Cu	Winter	4.33	3.66
	Summer	*BDL	*BDL

*BDL = Below Detection Limits

The concentration of metals in the Enteromorpha sp. showed almost similar pattern as for the Ulva sp. were the highest concentration 23.16 mg/kg for Zn was observed in the summer season and lowest 4.80 mg/kg reported for the winter season, while the concentration for Pb it was found to range from 1.86 mg/kg in winter season and 13.66 mg.kg for the summer season. The values obtained for Cd were below detection limits in summer season to 5.30 mg/kg for winter season, while the levels obtained for Cu were also below detection limits in summer season and 3.66 mg/kg for the winter samples.

Table (4) illustrate the levels of the metals in Ulva sp at site (D). The highest concentration 27.16 mg/kg of Zn was observed in the summer samples and the lowest concentration 7.03 mg/kg for the winter season, while in the case of Pb the lowest level 1.76 mg/kg was observed for winter season and the highest 12.00 mg/kg was reported for summer season. In the case of Cd, the levels observed were from undetected in summer to 2.80 mg/kg in winter, while for Cu it was the levels observed were from undetected in summer to 2.63 mg/kg in winter.

For *Enteromorpha sp.* The highest concentration 20.66 mg/kg for Zn was observed in the summer 20.66 mg/kg and the lowest concentration 2.96 mg/kg for winter time, while the values for Pd were found to range 1.73 -14.00 mg/kg the high value reported for the summer samples and the lower value reported in the winter time. The observed levels for Cd ranges from undetected in summer to 5.05 mg/kg in winter, while for the levels for Cu were from undetected in summer to 4.91 mg/kg in winter.

Table (4) Concentrations of Heavy metals (mg/kg) in Ulva sp. and Enteromorpha sp. For the two seasons for Benghazi port (Elmina Area).

Metals	Seasons	*Ulva sp.*	*Enteromorpha sp.*
Zn	Winter	7.03	2.96
	Summer	27.16	20.66
Pb	Winter	1.76	1.73
	Summer	12.00	14.00
Cd	Winter	2.80	5.05
	Summer	*BDL	*BDL
Cu	Winter	2.63	4.91
	Summer	*BDL	*BDL

*BDL = Below Detection Limits

All metals studied were tend to high in the summer season regardless of the locations with the exception of the Cd an Cu in which their levels were undetectable during the summer time. The high levels obtained could be attributed to the increase of sewage input into the marine environment in in the summer season as this source could be most important source of heavy metals in the marine environment. The low levels for the winter season could be due to the high dilution and mixing during the winter time. The levels obtained were comparable. The high levels found also reflect on the non availability of the metals and the strong biosorption of the algae. The highest value reported in the port area site (D) were possibly due to the Marietime activity around this area, however the values were relatively lower than those reported by [11], for the coastal area of Saudi Arabia. The levels obtained were found to be comparable with those reported by [12], in Alexandria where *Enteromoroha sp.* showed great ability to accumulate metals in which the concentration of

Zn reported to range 4.77-30.94 mg/kg, while in another study [13] for Tunisian coastal area were the concentration of Zn for the winter was very low compared the results obtained in this study. The finding of [14] study in Italy has reported levels ranged 1.3-31.3 mg/kg and the study by [15] in Libya coast were similar to the levels finding of the current study.

The difference is also mainly due to the differences in physio - chemical treatments such as (pH) and mineral contaminants between sites and seasons. The Largest concentration of Lead and thus is more polluted than other areas. Significant differences also appeared between site (B)and site (A) the reason for this difference is that pollution sources of the area entering these different regions. In addition to the sabri region site (A) is an open, relatively renewable beach, while the area of Juliana site (B) is a closed beach similar to the Gulf.

The findings are relatively consistent with those of [12] in the coastal region of Saudi Arabia, while the load concentration ranged 13.90-30.50 mg/km. Also agreed with [16] in India, the Lead concentration ranged 5.68-33.53 mg/kg , as well as almost identical study [15] in Libya, Lead Values were high ranging between 28.52-64.26 mg/kg. in winter this is due to monsoons, torrential rains and wave movement the cause pollutants to be emitted and are responsible for the increasing when compared to other seasons as well [17]. In Egypt in ULva sp. high lead values were up to 22.30 mg/kg [18] in Turkey the values of lead are up to 34.7 mg/kg in *ULva* sp. As for [19] in Pakistan in *ULva* sp. had very few lead values 0.34 mg/kg, Also the study of [20] in India during the summer season in green algae had very few Lead values 0.37 mg/kg. When comparing the general average lead element of *ULva* sp. In the summer 14.31 mg/km. while *Enteromorpha* sp. 14.41 mg/kg, as for in winter season *ULva* sp. 3.81 mg/kg while *Enteromorpha* sp. 2.63 mg/kg.we note that the absorption of lead element in the summer by algae is greater than the winter season, Also we note that *Enteromorpha* sp. Has the ability to absorb minerals more than *ULva* sp. due to the Large surface area of the algae .while *Enteromorpha* sp. 14.41 mg/km, as for in winter season uLva sp. 3.81 mg/kg while *Enteromorpha* sp. 2.63 mg/km. we note that the absorption of lead element in the summer by algae is greater than the winter season, Also we note that *Enteromorpha* sp. has the ability to absorb minerals more than *ULva* sp. due to the large surface area of the algae.

Cadmium(Cd) the concentration of cadmium in green algae during the winter ranged between 2.08-5.56 mg/km. The heavy rainy season leads to the presence of pollutants from various sides such as sanitation, road washing and industrial and agricultural wastes that may be responsible for the increased concentration of minerals in the period of monsoons. in addition, the increased level of organic compounds, nutrients and decreasing salinity during monsoon period improved the composition of the mineral compound and its sedimentation in sediments by reducing the possibility of mobility [21].

AL-Sabri area, site (A) carries the greatest value for cadmium concentration and the results of statistical analysis. Table (7) showed the existence of significant differences sources of pollution entering the marine environment in those areas and because of the monsoons and the abundance of rain that led to different sources of pollution, these results are consistent with a study [16]. In India which ranged from cadmium concentration 4.31-21.02 mg/kg, the results also agree with [15]. On the Libyan west coast, the concentration of cadmium in green algae ranged 0.14-3.18 mg/kg. as for the study Kamala *et al.,* (2009) In India the concentration of cadmium was high up to 64.21 mg/kg. In general, the element cadmium showed negative relationship between it and the rest of the element, perhaps this is attributed to the monsoons and the abundance of rain that Led to difference of their source. The general average cadmium element for *ULva sp.* In winter 3.07 mg/kg, while *Enteromorpha sp.* 4.17 mg/kg. we note the ability of the *Enteromorpha sp*. To be greater than *ULva sp.* in absorbing minerals.

Copper(Cu) the concentration of copper in green algae during the winter ranged between 2.73-6.13 mg/kg. The highest concentration was seen in al Elsberry area (A) 6.13 mg/kg among the results of the statistical analysis table (7) there were no significant differences between the sites. This study is consistent with the results Wael *et al.,* (2016) in Egypt concentration of copper 6.4 mg/kg in green algae *ULva sp.* , it differs from this study as it showed that a high copper concentration of up 40mg/kg in green algae *Enteromorpha sp.* When comparing the general mean concentration of copper in *ULva sp.* for winter season 3.07 mg/kg. while for *Enteromorpha sp.* 4.17 mg/kg. This reflect on the ability of *Enteromorpha sp*. to uptake Cu more probably due to its larger surface area.

Table (5) and Table (6) shows that the levels obtained in the current study were compared with others reported around the world. From the comparison it can be seen that the heavy metal concentrations were higher and lower than others reported levels as been inducated in table (5).

From the results of the statistical analysis for the ANOVA test, the data for the summer results indicates that there were no significant differences between sites for in Ulva sp. while for the winter results there were a significant difference between sites (p= 0.049). Likewise for the *Enteromorpha* sp. there were no significant differences found in the concentration of Zn between sites for both seasons. With regard to the concentration of Pb in Ulva sp. no significant differences between the sites and seasons were found while for summer results in the *Enteromorpha* sp. There were significant differences between sites (p=0.043). The concentration of Cd in Ulva sp. in winter showed significant differences between sites (p=0.047). As well as for *Enteromorpha* sp. there were significant differences between sites (p=0.043). The copper

concentration showed no significant differences between sites in both seasons.

Table (5) Comparison of Heavy Metals Concentrations (mg/kg) of Ulva sp. in the present Study and other studies.

Metals					
Reference	Location	Zn	Pb	Cd	Cu
Fekry *et al.,*(2019)	Egypt	13.5 – 22.3	7.18 – 22.3	0.53 – 0.61	5.33 – 8.91
Quratulan *et al.*, (2017)	Pakistan	20.3 – 85.7	0.34 – 0.68	0.23 – 0.54	2.2 – 4.7
Wael *et al.,*(2016)	Egypt	-	6.8 – 8.3	6.0 – 8.1	6.4 – 8.4
Samir and Yasser (2013)	Egypt	12.63	4.96	0.31	11.71
ALP *et al.,* (2012)	Turky	3.3 – 5.4	0.70 – 1.11	0.02 – 0.05	0.22 – 2.23
Sudallah *et al.,* (2012)	India	0.06 – 0.48	0.04 – 0.42	0.02 – 0.024	0.01 – 1.03
Abdallah(2010)	Egypt	10.10	4.5 – 6.6	0.18 – 1.6	2.0 – 8.6
Fauzi et Al(2001)	Libya	8.5 – 26.6	1.10 – 21.09	0.14 – 3.18	0.44 – 5.4
Present Study	Libya	20.2 – 27.16	11.5 – 16.00	2.08 – 5.56	2.73 – 6.13

Table (6) Comparison of Heavy metals Concentrations (mg/kg) of Enteromorpha sp. in the present Study and other studies

Metals					
Reference	Location	Zn	Pb	Cd	Cu
Aykut *et al.,*(2018)	KadinGreec	31.95	4.91	0.10	15.13
Prosenjit *et al.,* (2015)	India	6.30	1.00	-	55.8
Azza *et al.,* (2012)	Egypt	1.01 – 55.9	0.76 – 28.71	0.16 – 1.77	1.06 – 11.50
Abdallah, (2007)	Egypt	4.77 – 30.94	5.68 – 33.53	-	53.47
Villare *et al.,*(2002)	Spain	29.76	-	-	53.47
Present Study	Libya	2.1 – 24.60	1.73 – 14.00	2.16 – 5.30	2.73 – 6.13

IV. CONCLUSION

In conclusion, the four selected areas for the study (Elsberry, Julianaa, Eshbellia and Benghazi port Elmina) showed high level of contamination with marked seasonal changes in the concentrations of the metals examined (Zn, Pd, Cd and Cu) in both algae species *ULva sp.* and *Enteromorpha sp.* The ability of the both species to accumulate this metals suggest its use as bio indicators for the monitoring of metals pollution along the Libyan coast.

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