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Effect of Wastewater on Heavy Metal Accumulation in *Cystoseria sp.* (Brown algae) and *Enteromorpha. sp.* (Green algae) in Derna Coast, Libya

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Abstract: In this work was studied effect of wastewater pollution on heavy metal accumulation in *Cystoseria sp.*, (brown seaweed) and *Enteromorpha sp.* (Green algae), in an effort to gain some insight into the level of metal contamination which might exist in the coastal marine environment along the Derna coast. Assessed by measuring the concentration of heavy metals as Pb, Zn, Mn and Cd, in the algae tissue and seawater. The results indicate concentrations of metals were invariably slightly higher in *Enteromorpha sp*., than in *Cystoseria sp.,* at all sampling stations. The metals concentrations recorded for the different tissues and sites of the present study confirm the higher concentrations usually observed in summer. The average MPI was highest (0.86) for the both species inhabiting S6 station and least (0.29) at S1 station. In general, the all of heavy metals show no detrimental effects on the domestic aquatic environment of Derna coast. However, must be monitored continuously to ensure that they stay at harmless levels.

Keywords: pollution, heavy metal, bio-indicators, wastewater, macroalgae.

INTRODUCTION

The wastewater discharge consists primarily of untreated human wastes and domestic wastes. Heavy metals are common types of toxic substance present in sewage sludge that affect aquatic life. The outfall has been in operation for over many years and discharges into the Derna coast area. Wastewater is any water that has been adversely affected in quality by anthropogenic influence. Wastewater can originate from a combination of domestic, industrial, commercial or agricultural activities, surface runoff or storm water, and from sewer inflow or infiltration(Beijer & Jornelo, 1979). The rationale for using organisms to assess levels of contamination in the marine environment, and the criteria by which these organisms are selected as suitable biomonitors are well documented (Al-Homaidan et al., 2011; Alkhalifa et al., 2012; Dokulil, 2003; Khaled et al., 2014; Kureishy et al., 1995; Li & Huang, 2012). The value of algae as bio-indicators has already been recognized in the mid of 19th century (Wu et al., 2014). Seagrass or macroalgae can be used as bio-monitors to give information on concentrations of heavy metal or changes in metal availabilities in the surrounding environment, besides their abundance in various environmental systems(Campanella et al., 2001; Capiomont et al., 2000). In general, algae are widely distributed in the aquatic environment and are sedentary, easy to collect, identify, and the bioaccumulation of trace metals occur in high degrees; satisfying all the fundamental requirements for bioindicators (Campanella et al., 2001). Brown algae species can accumulate high concentrations of metals in contaminated ecosystems, and as a result, they are chosen as heavy metal bio-monitors in coastal areas(Amado-Filho et al.,

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1997; Andrade et al., 2010; Astorga-España et al., 2008; Karez et al., 1994; Villares et al., 2002). Green algae are considered an indicator of contamination (Brown et al., 1999). These algae were chosen to conduct the investigation as bio-indicators for heavy metals pollution of Derna city coastal area at the east of Libya. Due to their they were almost available in all sites during field study. Due to their short life cycle, algae respond quickly to environmental changes and are thus a valuable indicator of water pollution(Domingues & Galvão, 2007). Many of the chemical tested are basic parts of components of domestic and sewage sludge. The nature of any potentially toxic substances depends mainly on the types of wastes entering the sewage system. Heavy metals are common types of toxic substance present in sewage sludge that affect aquatic life.Libya is located in lies the Mediterranean Sea, it is ranked 124th out of 142 countries on an Environmental Sustainability Index, which places the country well down the list signifying a country with serious environmental degradation (WEF, 2002). This region is very important, due to it having distinct environmental characteristics associated. Also it has an environment similar to other regions in Southern Europe such as Italy, the Greek islands and Turkey(Azzawam, 1984). The study area is located along the Derna coast, in Jabal Akhdar region the north east part of Libya at latitude 32° North and 22° East. This area which are exposed to different degrees of pollution. Marine pollution in Derna coastal, due to the enormous increment of sewage discharged into the aquatic environment, has drawn the attention in the last decades, see figure 2. Little quantitative data are available on the concentration of metals in seaweeds general pollution of this area. The main aim of this work was to investigate the effect of wastewater on heavy metal accumulation in *Cystoseria sp.,* (Brown algae) and *Enteromorpha sp.,* (Green algae), which might exist in the coastal marine environment along the Derna beaches.

MATERIALS AND METHODS

Study Area : The Derna city is a part of the Jabal Akhdar, were is situated in North east of Libya. Climatically, the study area of the Derna coast is influenced by the Mediterranean Sea to the north. The rainfall is erratic in quantity, frequency and distribution. It attracts considerably more reliable rainfall than other coastal regions of Libya between autumn to early spring, with the mean annual rainfall ranges between 450 and 650 mm, 24-30 % falling in January. The temperature is 8-13°C in winter and 22-27°C in summer Winds are northern in winter but southern and eastern southern in other seasons. Six stations were chosen including the Derna Harbor (S1), Shahat company (S2), Post station (S3), Republic station (S4), Algarod station (S5) and Desalination station (S6), see figure 1.

Fig. (1).Locations of the six study sites, which are located along the Derna coast. The inset map shows the location of the study area in Libya

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Fig. (2). Marine pollution in Derna coastal, due to the sewage discharged into the aquatic environment

Measurements: The concentrations of metals were measured in macroalgae species in triplicates in two seasons at six coastal stations along the Derna city coast. Seawater samples were also collected to detect their metal contents in order to gain more information on the environmental conditions of the area and possible bioaccumulation patterns, were cleaned with distilled water and all algal samples were air dried at 90°C (Al-Homaidan et al., 2011), were collected during two seasons in spring and summer, 2016. The two collected algae were identified as *Cystoseria sp.,* (Brown algae) and *Enteromorpha sp.,* (Green algae) and then kept to analyze for heavy metals (manganese (Mn), zinc (Zn), cadmium (Cd), and lead (Pb). The concentrations of manganese, zinc, cadmium and lead were determined in the collected algal samples according to previously reported methods (Al-Homaidan et al., 2011). 500 mg (dry weight) of each algal dried sample were placed in acid washed digestion tubes. 25 ml of concentrated nitric acid was added to each tube and the contents were evaporated to about dryness. After cooling, 20 ml of double deionized water was added to each tube and the contents were filtered through 0.45 μm millipore filters. The solutions were then transferred to 25 ml acid washed volumetric flasks and the volumes were completed to 25 ml with double distilled deionized water. All samples were analyzed in triplicates and the concentrations were expressed in μg (metal) per gram dry weight (alga) and the mean values were recorded (Al-Homaidan et al., 2011). All analysis was made in three replicates. Statistical analysis was based on SPSS (Version 11.0) program (Al-Homaidan et al., 2011).

RESULT AND DISCUSSION

Heavy Metals in Algae: The present data revealed of *Cystoseria sp.*, recorded a relatively high (Pb) concentration in summer at the S6 station 1.50±0.3 (μg/g), while recorded their lowest values in both summer and spring seasons at the S3, S5 stations with 0.78±0.02 and 0.40±0.08 (μg/g) respectively, table 1. The *Cystoseria sp.,* recorded a rela-

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tively high (Mn) concentration in summer and spring at the S1 station with 1.74±0.1 and 1.16±0.02 (µg/g) dry weight), while recorded their lowest values in summer and spring at the S2 station 0.72±0.02 and 0.36±0.02 (µg/g) respectively. The *Cystoseria sp.,* collected from the S6 station in both summer and spring seasons recorded the highest concentration of (Zn) level 1.15±0.06 and 0.81±0.01 (µg/g), respectively. Whereas the lowest levels were found at S3 station 0.69±0.03 (µg/g) in summer, followed by at S1 station 0.27±0.007 (µg/g) in spring, see figures 3 and 4. This results are in good agreement with those found by (Strezov & Nonova, 2003) who reported concentrations of (Pb) level between in two *Cystoseira sp*., in most stations from the Bulgarian Black Sea coast. On the other hand, the *Cystoseira sp.,* in the present study, recorded lower values of (Pb) compared than that reported by many authors as; (Al-Masri et al., 2003; Schintu et al., 2010) for algae collected from the south-western Sardinia, Italy; (Khaled et al., 2014) for *Cystoseira sp.,* in Marsa-Matrouh beaches, Egyptian in Mediterranean Sea; (Strezov & Nonova, 2007). Average while, the present data revealed a lower values of (Mn) than that recorded by (Strezov & Nonova, 2007). To compare the present data with those previously studied by many authors revealed that *Cystoseira sp.,* recorded lower values for (Zn) compared than that reported by many authors as; (Akcali & Kucuksezgin, 2011; Al-Masri et al., 2003; Culha et al., 2013; Khaled et al., 2014; Schintu et al., 2010; Strezov & Nonova, 2007)in Kastamonu station in Black Sea. In the present study recorded lower values of (Cd) compared than that reported by many authors as; (Akcali & Kucuksezgin, 2011; Al-Masri et al., 2003; Khaled et al., 2014; Schintu et al., 2010; Strezov & Nonova, 2007). On the other hand, the value of average concentration for (Pb) was in this study, higher to those found by (Culha et al., 2013) for *Cystoseira sp.,* in marine algae samples of all sampling stations in Black Sea, Marmara Sea and Mediterranean Sea. Also by (Sawidis et al., 2001) for *Cystoseira sp.,* in the Aegean Sea, Greece. As well, the value of average concentration for (Zn) was in this study, higher than to those found by (Culha et al., 2013) for *Cystoseira sp.,* in marine algae sample of samsun station in Black Sea. While, the value of average concentration for (Cd) was in this study, comparable to those found by (Culha et al., 2013) for *Cystoseira sp.,* in marine algae samples of all sampling stations in Black Sea, Marmara Sea and Mediterranean Sea. The present data revealed of *Enteromorpha sp.,* recorded a relatively high Pb concentration in summer and spring seasons at the S6 station 1.92±0.3 and 1.63±0.03 (µg/g) respectively.

Table (1). Average concentrations (µg g^{-1} dry weight) with standard error of means in *Cystoseira sp.,* collecting during spring and summer seasons 2016, along stations of Derna coast.

Stations	Metals								Ave.
	Pb		Mn		Zn		Cd		
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	
S1	0.61±0.01	1.20±0.03	1.16±0.02	1.74±0.1	0.27±0.007	0.75±0.008	0.007±0.01	0.007±0.01	0.72
S2	0.41±0.02	0.85±0.03	0.36±0.02	0.72±0.02	0.41±0.02	0.71±0.04	ND	ND	0.58
S3	0.60±0.05	0.78±0.02	0.58±0.03	0.93±0.02	0.59±0.06	0.69±0.03	0.001±0.002	0.001±0.001	0.52
S4	0.69±0.1	0.96±0.03	0.59±0.06	0.81±0.03	0.77±0.01	0.93±0.03	ND	ND	0.79
S5	0.40±0.08	0.84±0.03	0.57±0.03	0.89±0.03	0..68±0.03	0.86±0.01	ND	ND	0.71
S6	1.13±0.03	1.50±0.3	0.95±0.09	1.12±0.09	0.81±0.01	1.15±0.06	ND	ND	1.11
Ave.	0.831		0.868		0.718		0.004		

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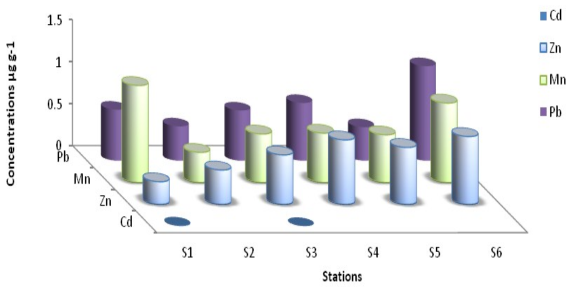

Fig (3) . Average concentrations (µg g^{-1} dry weight) in *Cystoseira sp.,* collecting during spring season 2016, along stations of Derna coast.

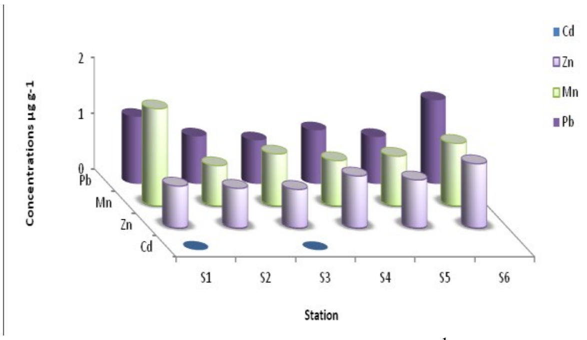

Fig (4) . Average concentrations (µg g^{-1} dry weight) in *Cystoseira sp.,* collecting during summer season 2016, along stations of Derna coast.

while recorded their lowest values in summer and spring at the S4 0.96±0.03 and 0.69±0.1 (µg/g) respectively, table 2. While, *Enteromorpha sp.,* recorded a relatively high Mn concentration in summer and spring at the S4 station 1.91±0.03 and 1.85±0.06 (µg/g), while recorded their lowest values in summer and spring at the S3 station 1.12±0.02 and 0.88±0.03 (µg/g) respectively. In *Enteromorpha sp.,* we also have data for Zn, which was at a maximum at S6 station, 1.82±0.06 µg/g in summer. Minimum levels of Zn in this alga 0.05±0.01 (µg/g) in spring, were found at S4 station, see figures 5 and 6.Comparison of the present data with those previously studied by many authors revealed that the concentrations of Zn, Pb and Cd for *Enteromorpha sp.,* lower than that recorded by (Schintu et al., 2010) along the south-western Sardinia, Italy; (Khaled et al., 2014) in the Marsa-Matrouh beaches in Mediterranean Sea; (Strezov & Nonova, 2007) for *Enteromorpha sp*. The present data revealed a lower values of Mn and Zn for the *E. compressa* than that recorded by (Villares et al., 2001), also for Zn with those previously studied by many authors as(Culha et al., 2013; Say et al., 1990). While, the value of average concentration for (Pb) was in this study, higher to those found by (Culha et al., 2013) in Black Sea, Marmara, Sea and Mediterranean Sea; (Sawidis et al., 2001) in the Aegean Sea, Greece. The value of average concentration for (Zn) was in this study, higher than to those found by (Culha et al., 2013) for *Enteromorpha sp*., in marine algae in Ordu station in Black Sea. As well, the value of average concentration for (Cd) was in this study, comparable to those found by (Culha et al., 2013) for *Enteromorpha sp*. in marine algae samples of all sampling stations in Black Sea, Marmara Sea and Mediterranean Sea. Many authors reported that the expected levels of Zn in *Enteromorpha sp*., are in the range 10-50 µg g^{-1} and 95-130 µg g^{-1}, for uncontaminated and contaminated sites respectively (Phillips, 1990; Stenner & Nickless, 1975).

Table (2). Average concentrations (µg g^{-1} dry weight) with standard error of means in *Enteromorpha sp.,* collecting during spring and summer seasons 2016, along stations of Derna coast.

Stations	Metals								Ave.
	Pb		Mn		Zn		Cd		
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	
S1	0.86±0.01	1.20±0.03	1.22±0.02	1.72±0.1	1.74±0.007	1.25±0.008	0.001±0.01	0.003±0.01	1.00
S2	0.95±0.02	1.26±0.03	1.21±0.02	1.35±0.02	0.20±0.02	0.39±0.04	0.06±0.01	0.04± 0.00	0.68
S3	1.08±0.05	1.55±0.02	0.88±0.03	1.12±0.02	0.60±0.06	0.88±0.03	0.05±0.002	0.006±0.001	0.77
S4	0.69±0.1	0.96±0.03	1.85±0.06	1.91±0.03	0.05±0.01	0.19±0.03	0.002±0.0	0.006±0.001	0.71
S5	1.13±0.08	1.55±0.03	0.92±0.03	1.60±0.03	1.07±0.03	1.14±0.01	0.004±0.0	0.007±0.02	0.93
S6	1.63±0.03	1.92±0.3	1.24±0.09	1.56±0.09	1.53±0.01	1.82±0.06	0.007±0.0	0.007±0.0	1.21
Ave.	1.23		1.38		0.91		0.02		

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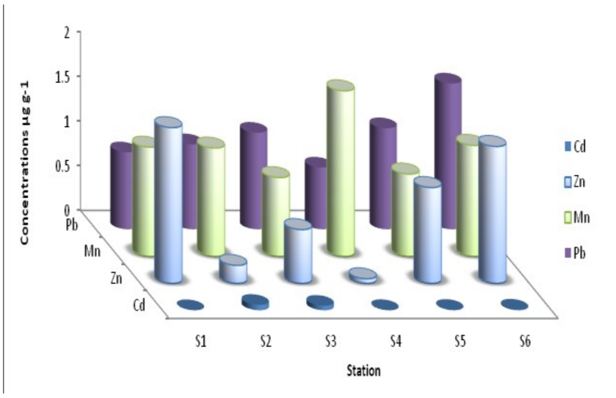

Fig (5) . Average concentrations (µg g^{-1} dry weight) in *Enteromorpha sp.,* collecting during spring season 2016, along stations of Derna coast.

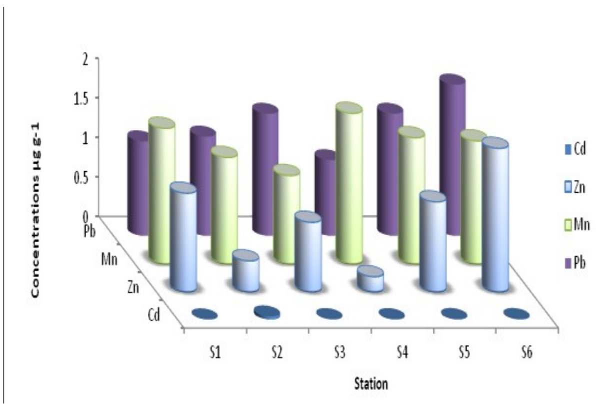

Fig (6) . Average concentrations in *Enteromorpha sp.,* collecting during summer season 2016, along stations of Derna coast.

Heavy Metals in Water: Determination of heavy metal concentrations in marine algae samples is usually preferred in the seawater and sediment samples. Heavy metal concentrations in seawater are very low and show wide fluctuation. At the same time, heavy metal levels in the sediment samples can be changed by organic matter content, grain size composition, pH and oxidation-reduction potential, etc. (Farías et al., 2002; Förstner, 1985). In our study, low metal contamination was found in S1 for Pb, Mn, Cd and Zn in seawater compared with contamination metals accumulated in the tissues of algae. The concentration of the metals in the six sampling sites followed the order of Mn>Zn>Pb>Cd. The average of heavy metals concentrations in sea water were lower at site S2 and relatively higher at site S1 (Table 3, Figures 7 and 8).

Mn was the most abundant metal, whereas Cd was the least abundant. The other metals exhibited intermediate concentrations and variability among the six sites. Largely, metals contents in seawater, from S1 obtained in this work are intermediple, site S1 in our study were the most contaminated for Pb in summer and spring seasons. This site is seem heavily affected by human activities and was the closest to the wharf. Pb concentrations from seawater in this study ranged between 0.12 and 0.54 (µg l^{-1}), were this result agree with (Chakraborty et al., 2014) for water sea along the coast of the Gulf of Kutch in India. While site S4 were the most contaminated for Mn in both summer and spring seasons, between 0.24 and 0.52 (µg l^{-1}). As well, the maximum values of Cd metal were also determined at site S5 in both summer and spring seasons between 0.003-0.05 (µg l^{-1}). These were much similar results obtained by (El-Adl et al., 2017), along Al-Hanyaa coastline, Libya, as well as the standard limits of Environmental Protection (EPA, 2014).

In addition, results in this study show that the site S6 were the most contaminated for Zn in summer and spring seasons. Despite of the discharge of wastes in sea water, our results were much lower than those reported from along Al-Hanyaa Coastline, Libya 5.4–7.4 (µg l^{-1}) (El-Adl et al., 2017).

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Table (3). Average heavy metals concentrations (μg l^{-1}) with standard error of means in the selected marine water collecting during spring and summer seasons 2016, along stations of Derna coast.

Stations	Metals								Ave.
	Pb		Mn		Zn		Cd		
	Spring	Summer	Spring	Summer	Spring	Summer	Spring	Summer	
S1	0.47±0.01	0.54±0.05	0.37±0.03	0.42±0.08	0.17±0.01	0.21±0.008	ND	0.002±0.0	0.31
S2	0.15±0.08	0.20±0.05	0.24±0.03	0.31±0.01	0.11±0.2	0.17±0.05	ND	0.001±0.0	0.20
S3	0.16±0.08	0.21±0.05	0.36±0.01	0.41±0.08	0.28±0.2	0.35±0.05	ND	0.002±0.0	0.22
S4	0.12±0.01	0.20±0.06	0.41±0.01	0.52±0.01	0.29±0.05	0.31±0.01	ND	0.004± 20.0	0.23
S5	0.37±0.01	0.41±0.01	0.36±0.02	0.45±0.08	0.28±0.01	0.36±0.01	ND	0.005± 20.0	0.29
S6	0.12±0.01	0.21±0.08	0.28±0.01	0.31±0.08	0.41±0.01	0.52±0.01	0.002±0.0	0.004±0.01	0.23
Ave.	0.23	0.30	0.34	0.40	0.26	0.32	0.00	0.00	
	0.26		0.37		0.29		0.00		

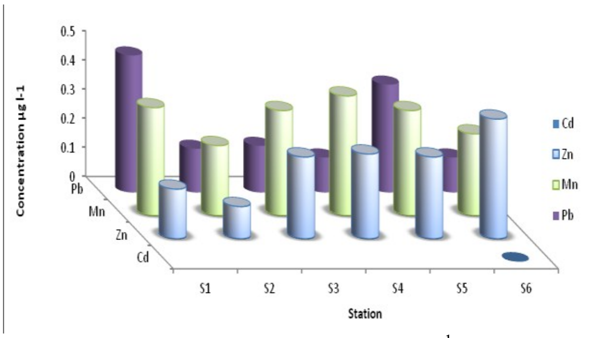

Fig (7) . Average concentrations (μg l^{-1}) in water collecting during spring season 2016, along stations of Derna coast.

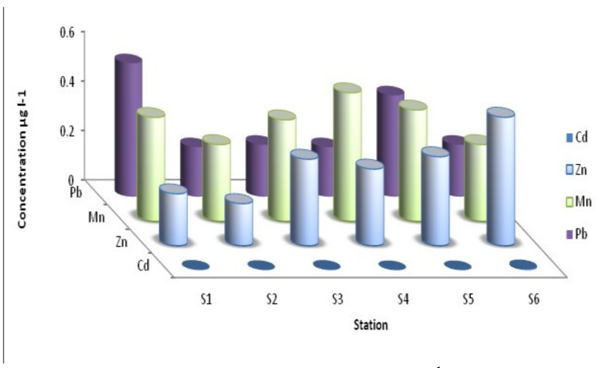

Fig (8) . Average concentrations (μg l^{-1}) in water collecting during summer season 2016, along stations of Derna coast.

Metal Pollution Index (MPI): The average MPI was highest for the algae species inhabiting S3 and S6 stations, intermediate for the algae species of S4 and S5 stations, and least for the algae species of S1 and S2 stations (Table 4 and Fig. 9 and 10). Metal pollution index (MPI) can be used to compare the average heavy metal content of different algal species within the same site or among different sites (El-Adl et al., 2017).The ability to accumulate heavy metals was highest in *Chaetomorpha sp.,* which was substantially higher than *Enteromorpha sp.,* at most stations. This points to a marked genotypic variability in heavy metal accumulation and agrees with the findings of (Khan et al., 2015) who reported that some macroalgae can concentrate heavy metals in their tissues to several times higher than those in the ambient water.

Table (4). Metal Pollution Index (MPI) of algae species along six stations of Derna coast

Algae	S1	S2	S3	S4	S5	S6	Average
Enteromorpha sp.	0.32	0.37	0.37	0.16	0.31	0.41	0.32
Chaetomorpha sp.	0.32	0.36	1.09	1.21	1.06	1.16	0.87
Average	0.32	0.37	0.73	0.69	0.69	0.79	

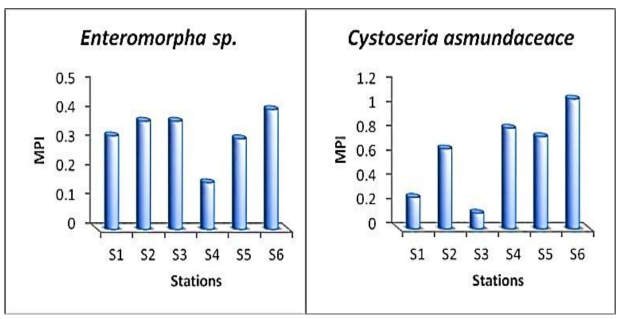

Fig (9) . Metal pollution index (MPI) of *Enteromorpha sp.,* and *Chaetomorpha sp.*, algae along the six stations of Derna coast.

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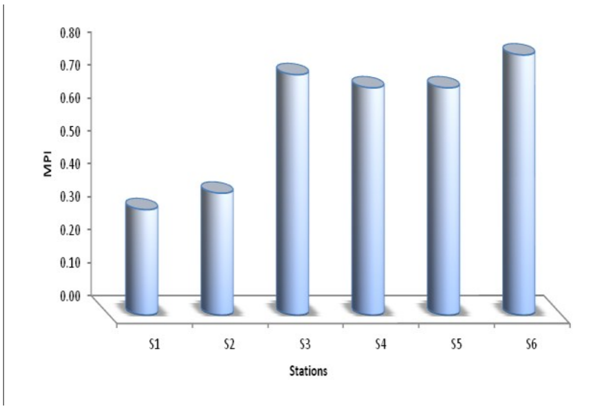

Fig (10). Metal pollution index (MPI) of algae along the six stations of Derna coast.

Heavy Metals in Two Seasons: The metals concentrations recorded for the different tissues and sites of the present study confirm the occurrence of significant seasonal variability, with maximum concentrations usually observed in summer. Such seasonal variability in trace metal concentrations have been reported by other authors for Pb and Cu in Posidonia oceanica (Malea et al., 1994), for Cd, Cu, Pb and Zn in Posidonia australis (Ward, 1987), and for Cd, Cu, Pb and Zn in Zostera marina (Lyngby et al., 1982). The latter authors found that maximum concentrations of heavy metals were recorded when the growth has ceased, and decline of these metals occurred at the beginning of the growth season. In this study, the concentrations of zinc, lead and manganese in algae varied seasonally, the concentrations collected in summer show exhibiting significantly higher metals levels than those of individuals collected during the spring. Generally, as previously reported by many authors as(Akcali & Kucuksezgin, 2011; Brown et al., 1999); there may be a number of reasons for the seasonal differences found, including: environmental factors, such as variations in metal concentrations in solution, interactions between metals and other elements, salinity, pH etc., metabolic factors, such as dilution of metal contents due to growth; or they may be due to interactions between both kinds of factors, different genetic capacities for metals concentration.

CONCLUSION

The obtained heavy metal contents indicated that different species demonstrated various degrees of metal accumulation. High levels of Mn were detected in the both species of algae. Drainage of waste into the coast of city are probably the main cause of this problem. The information compiled from the above recommendations can then direct strategies for initial environmental surveys to investigate contamination of heavy metals in Derna coast.

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تأثير المياه العادمة على تراكم المعادن الثقيلة في الطحالب البنية (*Cystoseria sp*) و الطحالب الخضراء (*Enteromorpha sp.*) في ساحل درنة، ليبيا

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المستخلص : في هذا العمل تم دراسة تأثير تلوث المياه العادمة على تراكم المعادن الثقيلة في *Cystoseria sp.,* (الطحالب البنية) و *Enteromorpha sp.,*. (الطحالب الخضراء)، في محاولة للحصول على بعض التبصر لمستوى التلوث المعدني الذي قد يكون موجودا في البيئة البحرية الساحلية على طول ساحل درنة. تم التقييم من خلال قياس تركيز المعادن الثقيلة كالرصاص، الزنك، المنغنيز و الكادميوم، في أنسجة الطحالب ومياه البحر. تشير النتائج إلى أن تركيزات المعادن كانت أعلى قليلا في *Enteromorpha sp.,* مما كانت عليه في *Cystoseria sp.,* في جميع محطات أخذ العينات. وتركيزات المعادن المسجلة للأنسجة والمواقع المختلفة لهذه الدراسة تؤكد ان التركيزات أعلى عادة في الصيف. وكان متوسط مؤشر التلوث أعلى (0.86) لكل من النوعين في المحطة S6 وأقل (0.29) في المحطة S1. بشكل عام، لا تظهر جميع المعادن الثقيلة أي آثار ضارة على البيئة المائية المحلية لساحل درنة. ومع ذلك، يجب رصدها بشكل مستمر لضمان بقاءها في مستويات غير ضارة.

الكلمات المفتاحية : التلوث، المعادن الثقيلة، المؤشرات الحيوية، مياه الصرف الصحي، الطحالب الكبيرة.

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