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Marine macro-algae as a bio-indicator of heavy metal pollution in the marine environments, Persian Gulf

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The northern parts of the Persian Gulf are more affected by pollutants because of their low depth, limited rotation, salinity, and high temperature. The anthropogenic and non-anthropogenic contaminations caused by organic and inorganic pollutants in aquatic ecosystems will eventually lead to increase pollution in water, sediments, and aquatic organisms. It seems that, algae are the most suitable indicator for soluble heavy metals (HMs) in both active and passive states. Samplings were carried out on a monthly basis in four different stations in Bushehr Province, northwest of the Persian Gulf from March 2016 to March 2017. ICP-mass spectrometry was used to determine Pb, Cu, Cd, As, Cr, Zn, Al, Mn, Co, V, Ni, Mg, S, Fe, and Ca concentrations in some macroalgae. In this study *Padina gymnospora* (brown algae) and *Hypnea hamulosa* (red algae) had the highest absorption, while the *Cladophoropsis membranacea* (green algae) showed the least absorption in all the sampling areas.

[**Keywords:** Heavy metals, ICP-MS, Macro algae, Persian Gulf]

Introduction

The Persian Gulf is a shallow bay, with an average depth of 35-40 meters and a zone of almost 240 km. This area is connected through the Strait of Hormuz to international waters[1-2]. The time for changing the water in the gulf is between 3 and 5 years that shows that contaminants remain for a considerable time in the Persian Gulf[3]. The northern parts of Persian Gulf are more affected by pollutants because of their low depth, limited rotation, salinity, and high temperature[3]. Since there is an extensive exploitation of oil from huge reserves in the continental shelf of the Persian Gulf as well as the transportation of abundant oil and oil tankers, it has been estimated that the amount of pollution per square km of the sea is higher than those of the global average. About half of the crude oil and petroleum products to be exported by ships passing through the Gulf and pollution of oil in the Persian Gulf is estimated about 68 % of total petroleum pollution which is about 4 times the global contribution of pollution in the maritime transportation[4-6]. The anthropogenic and non-anthropogenic contaminations caused by organic and inorganic pollutants in aquatic ecosystems will eventually lead to increase pollution in water, sediments, and aquatic organisms[7-9]. Between the thousands of organic and inorganic substances entering aquatic ecosystems, heavy metals (HMs) mainly result in high toxicity[10-13]. Because of producing by a wide variety of resources, and also due to their sustainability and inseparability by biological processes as well as the ability to accumulate in many aquatic species. HMs have been attracted the ideas of many researchers[14]. Sediment is the ultimate place of heavy metal accumulations in aquatic environment. So, it is considered as a source of heavy metals in marine environments. Distribution and accumulation of HMs in sediments depends on many environmental factors such as acidity, partial size distribution, features of metal elements, metal concentration, and physical transfer[12]. It seems that, algae are the most suitable indicator for soluble HMs in both active and passive states, because the concentration of HMs in the body of plants can be several times higher than in the water around them[15]. Algae have the basic conditions as a biological indicator of HM pollutions, because they are sedentary, easily identified and collected and they are widely distributed. So, the accumulation of HMs in them is too high[16]. Since algae have a rapid growth rate and quick response to any changes in the environment, they can be used as an early warning of changes in the environmental conditions that may not be detectable by other methods, or difficult to use. Because algae exist at the bottom of food chain, they can be a good estimation of HMs in the environment without bioaccumulation affecting them. Algae are defined as an appropriate indicator of heavy metal pollutions[16]. Pollution of aquatic ecosystems to HMs can be confirmed through the investigation of water, sediment, algae and other aquatic organisms[17].

There are some reports on HMs pollution in Iran and some adjacent countries including Topcuoglu *et al*.[18]; Akcali and Kucuksezgin[19]; Areej *et al*.[20]; Amini *et al*.[21]; Chakraborty *et al*.[15]. Amini *et al*.[21] studied concentrations of metal in species of *Padina* and related sediment from Nayband Bay and Bostaneh Port, northern coastline of the Persian Gulf, Iran. They recorded the highest metal pollution index (MPI) value in *Padina borgessenii* (25.02) at Nayband Bay. They also found that the correlations was positive between metal contents in the sediment and nearly in all species, indicating that these algal could absorb metals from sediments and play role as bio-monitoring agents.

Akcali and Kucuksezgin[19] studied some heavy metals including Fe, Zn, Cu, Cr, Cd, Hg and Pb in macroalgae from eastern Aegean coastal waters and concluded that the *Cystoseira* sp. (brown algae), the *Ulva* sp. (green algae) and *Enteromorpha* sp. possess high potential as cosmopolitan bio-monitors for trace metals in the Aegean Sea. Areej *et al*.[20] studied macroalgae (brown algae) as bioindicators for HM pollution of Al-Jubail coastline of Saudi Arabia including *S. angustifolium, S. boveanum, S. latifolium,* and *Padina gymnospora.* Their results indicated the high pollution levels sampling sites with Zn and Cu that was generally due to uncontrolled removal of industrial waste into coastline. They concluded that the constancy of concentrations of Zn in all confirmed brown algae indicated the competence of the examined algae for bioaccumulation and bio-monitoring studies of Zn. The aim of present study was to investigate the effects of green, brown and red algae dwell in Persian Gulf on absorption rate of heavy metals.

Materials and methods

Study area

Bushehr Province encompasses 673.62 km from the north coast of the Persian Gulf. The coastline is determined on the basis of the latest progress of the water in the highest tide on land and the lowest water retardation affected by tides is determined based on the visual interpretation of satellite imagery. The Bushehr Province coordinate is 27 degrees and 14 minutes, to 30 degrees and 16 minutes north as well as 50 degrees and 6 minutes to 52 degrees and 58 minutes east. Sampling was carried out on the monthly basis from March 2016 through March 2017. The Emam Hassan portcity was chosen as the sampling area due to the existence of some oil refineries, ports and industrial factories. It was also true for Bushehr and Ameri ports as well as Nayband protected area which have docks and urban sewage discharges (Fig. 1).

Sampling and digest: Seawater, sediment and algae

Samples were obtained from the top of layer (5 cm depth) of sediments. 10 square meters of each area was sampled. The sediments were collected from the inter-tidal areas once low tides. The sediments were

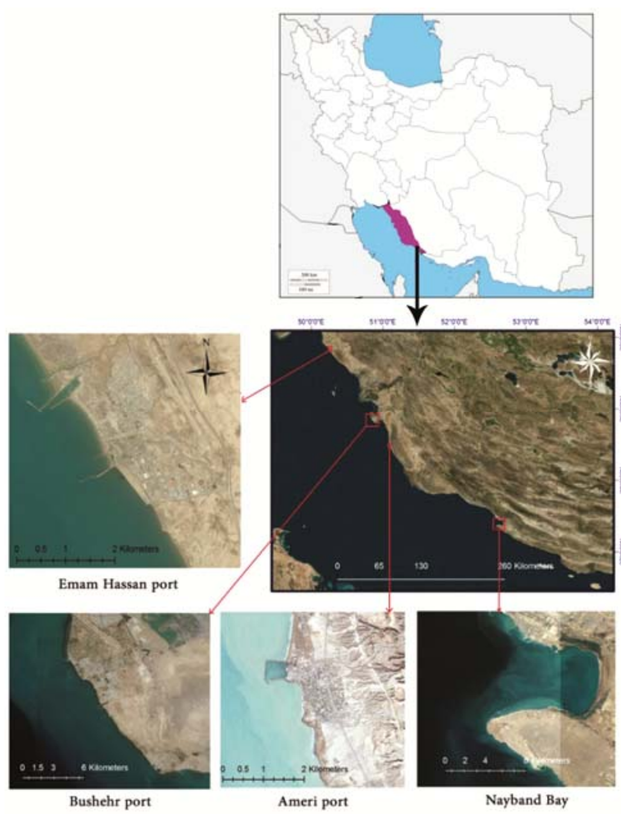

Fig. 1 — Sampling areas

placed in the polyethylene containers. Seawater samples were filtered and were collected in polyethylene bottles and stabilized by adding 65 % (v/v) nitric acid (HNO_3) before transfer to the laboratory. Algae samplings were carried out by hand in tidal areas at depths of 0.5 to 3 meters. From the all algae species collected from each station in the four areas, the similar species were selected and preserved (In general, three species were selected. 150 samples were collected from each of these three species). All of the samples (sediments, sea water and algae) transferred to the laboratory in the University of Persian Gulf by styrofoam cooler box at 4° C.

Sediment particle size is an important factor for assessing the extent of heavy metal pollutions. So, the samples were passed through the sieve with a 63 µm mesh size and then placed in oven at 105 °C for 16 hours until all moisture was wiped out. One gram of each sample was weighted and digested by 65 % nitric acid and then 60 % perchloric acid in a ratio of 1:4, firstly at 40° C for one hour, then at 140° C for 4 hours. All of the samples were washed out at the sampling site by sea water, then transferred to the laboratory in a zippered polyethylene bags. To extract HMs, 0.5 gm of algae was digested in 10 ml of 65 % nitric acid in a microwave oven, passed through the Whatman filter No. 40 and then diluted with distilled water to reach the exact volume. ICP-MS was used to determine the concentration of Pb, Cu, Cd, As, Cr, Zn, Al, Mn, Co, V, Ni, Mg, S, Fe, and Ca.

Statistical analysis

Minitab 16 software was used for data analysis. We used Kolmogorov-Smirnov (K-S) to determine the normality of the data and One-way ANOVA to study the significant difference between the variables, $p < 0.05$.

Results and Discussion

Identification of algae

The morphology and microscopic separation of algae was done and the common species were selected for all areas, including *Cladophoropsis membranacea* (green alga), *Hypnea hamulosa* (red alga) and *Padina gymnospora* (brown alga) (Fig. 2).

Concentration of heavy metals in seawater

The water pH values were determined to be 8.7 in the three areas, while 8.9 in Nayband Bay. The salinity was 38-40 ppt in all the areas. The HM concentrations in the seawater of all areas were recorded as Mg> S> Ca> Al> Fe> Zn> As> Mn> V> Cr> Ni> Cu> Cd> Co> Pb, respectively (Tables 1-2; Fig. 3). The highest concentration of Mg in water samples were measured between 1192 and 1497 mgl^{-1} ($p < 0.05$), with the maximum and minimum values in Bushehr and Emam Hassan ports, respectively. The maximum concentrations of S, Ca, Fe, As, Mn, V, Cr, Cu were recorded in Bushehr port, while maximum levels of Zn, Ni, Cd, Co, Pb were recorded in Emam Hassan port.

Concentration of heavy metals in sediments

The HM concentrations in sediments of all areas are presented in Tables 1-2 and Figure 4 with the order of Ca> Mg> Fe> Al> S> Mn> Ni> Cr> V> As> Zn> Pb> Cu> Co> Cd, respectively. The maximum

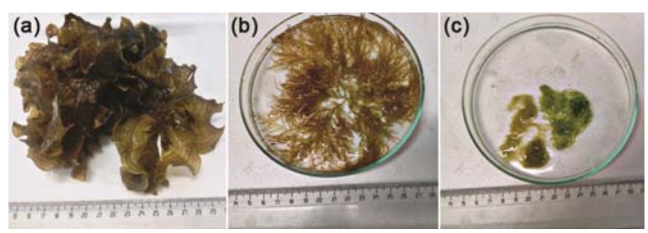

Fig. 2 — A: *Padina gymnospora*; B: *Hypnea hamulosa* and C: *Cladophoropsis membranacea*

Table 1 — Heavy metal (mean ± SD) in seawater (µg/g) and sediment (µg/g) (p ≤ 0.05).

Location	Pb	Cu	Cd	As	Cr	Zn	Co	V	Ni
Sea water									
Emam Hassan	0.0267±0.0267	0.00	0.220±0.220	21.44±5.23	1.600±0.987	31.2±24.0	0.133±0.133	1.96±1.26	1.34±1.34
Bushehr port	0.00	1.03±1.03	0.127±0.127	22.14±2.74	1.83±1.01	21.4±11.8	0.05±0.05	2.443±0.747	0.00
Ameri port	0.00	0.00	0.157±0.157	21.80±4.58	1.63±1.63	1.490±0.897	0.06±0.06	1.407±0.649	1.03±1.03
Nayband Bay	0.0133±0.0133	0.00	0.143±0.143	18.22±6.03	0.00	10.07±4.98	0.07±0.07	0.587±0.587	0.523±0.523
Sediment									
Emam Hassan	2362±666	4953±1188	167.5±65.5	3790±1183	14866±4136	5671±2862	2098±204	10895±1423	14622±3104
Bushehr port	2423±396	6694±1648	200.8±21.6	5248±170	15643±3456	7671±5068	2272±558	12082±3266	14293±2719
Ameri port	8692±4036	6254±832	99.2±37.1	12909±4886	17287±1329	12989±1865	2805±203	1276±131	17821±854
Nayband Bay	6513±2618	6239±2779	63.3±63.3	1548±351	11750±2889	3200±1617	1143±168	3491±1347	9052±1219

Table 2 — Heavy metal (mean ± SD) in seawater (mg/l) and sediment (µg/g).

Location	Al	Mn	Mg	S	Fe	Ca
Sea water						
Emam Hassan	0.443±0.328	0.003±0.003	1192±294	822±188	0.0367±0.0186	391.4±86.3
Bushehr port	0.557±0.322	0.01±0.01	1497±699	986±444	0.150±0.150	490±221
Ameri port	0.723±0.326	0.00	1366±508	940±349	0.02±0.02	434±152
Nayband Bay	0.783±0.130	0.00	1255±528	846±331	0.0367±0.0367	402±166
Sediment						
Emam Hassan	4088±382	152.5±11.4	8292±1321	1040±167	4897±800	177625±29192
Bushehr port	3612±1407	151.3±17.9	7525±2035	1268±187	5102±1375	205967±3336
Ameri port	4086±225	206.17±0.833	10871±465	1476.7±72.2	7824±954	199933±38167
Nayband Bay	1583±891	36.8±19.0	10132±1853	1999±116	1777±1006	225433±16444

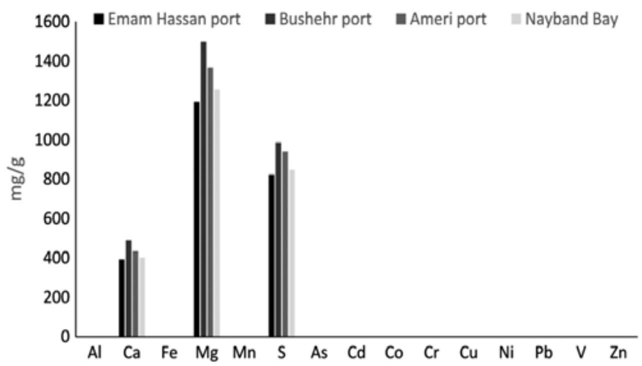

Fig. 3 — Heavy metal concentrations in seawater (mg/g), $p \leq 0.05$ by one-way ANOVA

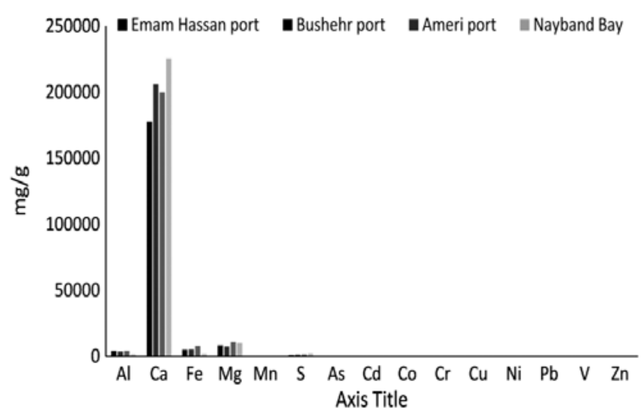

Fig. 4 — Heavy metals concentrations in sediments (mg/g), collected from different sites of Persian Gulf, Iran

level of Ca was determined in the sediments of Nayband Bay, while minimum was observed at Emam Hassan port. The maximum counteraction of Mg and Cu was detected in Bushehr port, while the maximum counteraction of Fe, Mn, Ni, Cr, V, As, Zn, Pb, and Co is reported from Ameri port. Furthermore, the maximum of alluminium counteraction was reported from Emam Hassan port.

Concentration of heavy metals in algae

The HMs concentration from selected algal species was determined and are presented in Tables 3-4 and Figure 5. The concentration of heavy metals in the examined species, *P. gymnospora* (Phaeophyta), *C. membranacea* (Chlorophyta) and *H. hamulosa* (Rhodophyta) are as follows: Al: 2246.35, 2093.04 and 2231.28 (mgg^{-1}); Ca: 75125, 66945 and 57564.9 (mgg^{-1}); Fe: 2255.26, 2185.35 and 2159.69 (mgg^{-1}); Mg: 10516.3, 5945.2 and 10820 (mgg^{-1}); Mn: 60.7, 48.8 and 40.10 (mgg^{-1}); S: 4363.4, 5535.4 and 15338.9 (mgg^{-1}); As: 6519.3, 2887.1 and 7179.1 (μgg^{-1}); Cd: 932.5, 220.2 and 773.7 (μgg^{-1}); Co: 2157.03, 1274.4 and 1554.05 (μgg^{-1}); Cr: 9728.4, 9149.05 and 10600.2 ($\mu g.g^{-1}$); Cu: 4869.6, 3990.6 and 5353.1 (μgg^{-1}); Ni: 10568.7, 8337.9 and 9488.6 (μgg^{-1}); Pb: 1247.1, 798.6 and 1186.2 (μgg^{-1}); V: 6329.7, 6703.2 and 8038.6 (μgg^{-1}); Zn: 9630.07, 9091.7 and 9898.7 (μgg^{-1}).

The results of this study undoubtedly show the high pollution ranks of Bushehr province coast that is generally due to unrestrained removal of industrial waste into coastline. Human activities, the entry of industrial and urban sewage and ports, caused a large amount of heavy metals reached the marine environments[22,23]. The amount of metals between sediments and seawater have a significant relationship with each other ($p < 0.05$), also the amount of metals in sediments and algae have a significant relationship with each other. However, the entry of heavy metals in different ways has created a lot of concerns in marine pollution; therefore, it seems very necessary to determine the biochemical index of pollution. Absorbing of inactive heavy metals for filtration requires cheap biomarkers which can accumulate heavy metals with low cost and high ability and remove from the environment[24]. In the past decades,

Table 3 — Heavy metals (mean ± SD, (µg/g)) in algae.

Place	Algae	Pb	Cu	Cd	As	Cr	Zn	Co	V	Ni
	P. gymnospora	1503±845	5577±1608	780.8±82.5	4179±1707	11210±3915	8421±4307	2678±1018	7683±2867	11969±4228
Emam Hassan	*C. membranacea*	239±239	3484±344	188±100	2224±2026	8994±1101	3932±3917	1265±410	5221±604	8382±1013
	H. hamulosa	863±380	6621±2641	1163±1040	10718±8218	16669±7416	7544±7531	1973±660	9703±4170	11974±3421
	P. gymnospora	981±223	6324±1067	753±260	6608±1020	13591±2632	12924±4345	2945±409	9358±1964	14043±2432
Bushehr port	*C. membranacea*	1389±728	6225±1348	223.5±67.9	3961±944	15417±2152	11371±5298	2259±342	10792±1782	14205±1987
	H. hamulosa	1106±334	5738±934	586±143	4827±499	11224±2172	13278±5257	1533±302	13086±7256	10829±2580
	P. gymnospora	1189±539	4755±1151	677±308	6606±1818	11253±2447	8675±4441	2118±501	7021±2014	13005±4216
Ameri port	*C. membranacea*	586±586	2933±460	98.8±33.8	1561±119	5988±3005	10000±1750	895±387	4218±2233	6843±3620
	H. hamulosa	801±181	4264±711	438±205	4145±1105	5769±1531	7261±7239	974±219	3731±199	5601±681
	P. gymnospora	1315	2822.5	1520	8685	2860	352.5	887.5	8500	3257.5
Nayband Bay	*C. membranacea*	981±528	3322±673	371±125	3803±277	6198±258	813±307	679.4±77.9	11064±5519	5523±586
	H. hamulosa	1975±994	4790±1978	909±555	9027±7037	8740±1398	1488±781	1737±1101	11511±7135	9550±2329

Table 4 — Heavy metals (mean ± SD, (mg/g)) in algae

Location	Algae	Al	Mn	Mg	S	Fe	Ca
	P. gymnospora	2496±527	70.7±15.6	8653±1612	4457±678	2566±405	70367±8507
Emam Hassan	*C. membranacea*	1857±546	65.8±40.5	5756±2994	4457±678	2125±953	90506±58994
	H. hamulosa	3261±574	54.75±8	15850±4950	11938±7130	3233±445	65750±30250
	P. gymnospora	3451±430	64.8±15.4	11749±1077	4784±768	3289±442	88325±11327
Bushehr port	*C. membranacea*	4171±985	82.7±21.2	9938±2493	5018±1607	4253±967	83994±25393
	H.hamulosa	2920±673	40.75±7.02	8984±1109	14653±1109	2588±480	28669±7440
	P. gymnospora	2686±666	79.6±19.2	14408±3503	5603±629	2846±607	83933±11810
Ameri port	*C. membranacea*	1531±541	33.4±14.1	2793±1208	4066±559	1540±600	19500±6200
	H. hamulosa	1256±326	26.0±14.0	7830±1220	30038±11102	1395±508	30638±12362
	P. gymnospora	352.5	27.75	7255	2610	320	57875
Nayband Bay	*C. membranacea*	813±307	13.69±3.51	5295±779	8479±3111	824±274	73781±23317
	H. hamulosa	1488±781	38.9±24.6	10617±4160	4728±2221	1424±692	105204±36864

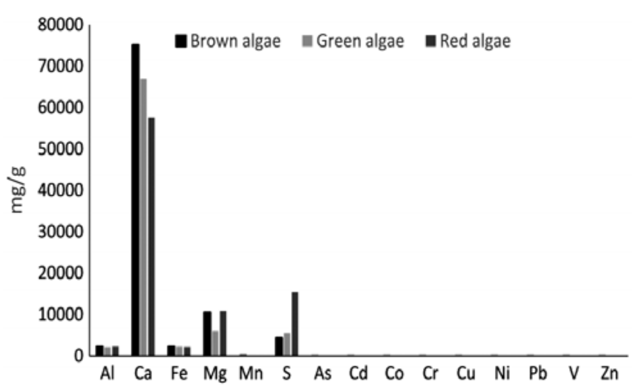

Fig. 5 — Heavy metal concentrations in algae (mg/g), collected from different sites of Persian Gulf, Iran

many cases were evaluated and they confirmed that algae are the most effective and talented case for purification of soluble heavy metals. The accumulation of HM in algae is very variable and different species of algae prefer specific elements. Different factors lead to changes in the accumulation of metals including longevity, morphology, level of contact, specific growth rate and the dependence of selected metals by different species.

The accumulation of heavy metals in algae

In the conducted research, with the aforementioned metals, brown and red algae had the highest absorption rate, while green algae showed the least absorption in all the sampling areas. Amini[21] stated that brown algae could be used as a bio- indicator in Persian Gulf, similar to results of the present study. As in our study, Areej[20] pointed out that the brown algae was a suitable bio-indicator in the south Persian Gulf, Some algal species and taxonomic groups show clear preferences for particular marine conditions, and can this act as potential bio-indicators.

Conclusion

All alga species were sampled from all over the 673.62 km from the north coast of the Persian Gulf. Similar species were selected in all regions. Including *Cladophoropsis membranacea* (green algae), *Hypnea hamulosa* (red algae) and *Padina gymnospora* (brown algae). The absorption rate of heavy metals was determined in all species. In the present study, we found that, the brown algae can be used to determine the amount of some metals (Mn, Fe, Al, Pb, Ni, Co, Cd) while red algae can be used to determine other kind of metals (S, Mg, Zn, Cu, Cr, As) and green algae showed the least absorption in all the sampling areas. These algae are plentifully present in the north of Persian Gulf and therefore can be used as bio-indicator for marine pollution studies. These data could also be useful for comparison with future information of marine pollution of the Persian Gulf.

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