

Biomonitoring of Heavy Metals in Some Brown Algal Species in the Red Sea Area (Saudi Arabia and Egypt)

الرقابة الحيوية على العناصر الثقيلة بواسطة بعض أنواع الطحالب البحرية البنية في منطقة البحر الأحمر (المملكة العربية السعودية ومصر)

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Abstract: The concentration of Iron (Fe), Zinc (Zn), Cobalt (Co), Lead (Pb), Cadmium (Cd) and Copper (Cu) were determined in specimens of four marine brown algae, *Turbinaria decurrens*, *Sargassum dentifolium*, *Sargassum latifolium* and *Padina pavonia*. The four algal species were collected from two sites: The Red Sea coasts at Hurghada in Egypt, an area slightly influenced by anthropogenic activities, and Gizan in the Kingdom of Saudi Arabia, an area which requires intensive study, especially in ecological terms. In order to gain a complete picture of the quality of the aquatic environment in both studied sites, heavy metals concentrations were determined in the selected algal species. The picture of bio-available metal loads in the different sites of the selected areas provided by the four algal species was rather univocal. An overall trend of increased all-determined metal concentrations in Gizan was clear. Lead (Pb) was considered to be the influenced pollutant in Gizan, where its concentration in (T). *decurrens*, (S). *dentifolium*, (S). *latifolium* and (P). *pavonia* was (54.5), (45.17), (55.28) and (56.8) fold than that in the same species in Hurghada. On the other hand, the metal concentrations recorded at Hurghada, a site expected to be uncontaminated, generally fall in the lowest values available in the literature and may be considered as a useful reference for intraspecific comparison within the Red Sea area.

Keywords: Brown algae, Biomonitoring, Heavy metals, Red sea area, Gizan, Hurghada.

المستخلص: تم تعيين تركيز العناصر الثقيلة مثل الحديد (Fe) - الزنك (Zn) - الكوبلت (Co) - الرصاص (Pb) - الكاديوم (Cd) و النحاس (Cu) في أربعة عينات طحالب بحرية بنية هي، تريناريا ديكورانس - سارجاسم دنتيفوليوم - سارجاسم لاتيفوليوم و بادينا بافونيا. كذلك تم حصاد الطحالب البحرية من منطقتين على البحر الأحمر، هما مدينة الغردقة بجمهورية مصر العربية، وهي منطقة قليلة التعرض لمصادر التلوث و التدخلات البشرية. و منطقة جازان بالمملكة العربية السعودية والتي لا توجد بحوث سابقة للتعرف على طبيعة البيئة البحرية فيها. تم تعيين تركيز العناصر الثقيلة المذكورة أعلاه في العينات الأربعة من الطحالب في كلتا المنطقتين، و كذلك عدد التسكر المسئول عن ادمصاص العناصر الثقيلة بها بهدف التوصل إلى معرفة مدى تلوث البيئة البحرية بالعناصر أعلاه. أوضحت الدراسة أن هناك زيادة كبيرة في تركيز كل العناصر الثقيلة في طحالب جازان، مقارنة بطحالب الغردقة. أظهرت الدراسة أيضاً أن تركيز عنصر الرصاص (Pb) وهو العنصر الأكثر خطورة، في الطحالب تريناريا ديكورانس - سارجاسم دنتيفوليوم و سارجاسم لاتيفوليوم و بادينا بافونيا بمنطقة جازان يكافئ (54.5) - (45.17) - (55.28) و (56.8) ضعف تركيزه في نفس الطحالب بمنطقة الغردقة. كما أوضحت النتائج أن تركيز العناصر الثقيلة في منطقة الغردقة كانت في نطاق التراكيز الدنيا المذكورة في بحوث سابقة، و بذلك يمكن التأكيد على أن البيئة البحرية بمنطقة الغردقة تعتبر مقياساً يمكن الرجوع إليه للمقارنة بينها وبين مواقع أخرى في منطقة البحر الأحمر لتحديد درجة تلوثها.

كلمات مدخلية: الطحالب البنية - مراقبة حيوية - العناصر الثقيلة - منطقة البحر الأحمر - جيزان - الغردقة - مصر - السعودية.

Introduction

Heavy metals pollution is an environmental problem of worldwide concern. The heavy metals lead (Pb), copper (Cu), cadmium (Cd), (Zn) zinc and cobalt (Co) are among the most common pollutants found in industrial effluents. Even at low concentrations, these metals could be toxic to organisms, including humans. For instance, lead is extremely toxic and can damage the nervous system, kidneys, and reproductive system, particularly in children. Lead has been found in at least (1026 of 1467) national priorities list sites identified by the USA Environmental Protection Agency (EPA). Chronic exposure to elevated levels of cadmium is known to

cause renal dysfunction, bone degeneration, liver damage, and blood damage. The USA Department of Health and Human Services has reported that there is sufficient evidence in humans for the carcinogenicity of cadmium and cadmium compounds. Although copper and zinc are essential trace elements, high levels can have harmful health effects. Copper is also toxic to a variety of aquatic organisms, even at very low concentration. Cobalt is also known to be toxic where large amounts of cobalt cause lung cancer and skin allergy, and also has a harmful effect on the ears. The (EPA) requires lead, copper, cadmium, and zinc in drinking water not to exceed (0.015), (1.3), (0.005) and (5 mg/L), respectively (Sheng, *et.al.* 2004).

Heavy metals in the aquatic environment can remain in solution or in suspension and precipitate on the bottom or be taken up by organisms. The analysis of metal concentrations in biota samples at the same locations can indicate the transfer of metals through food chains (Topcuoglu, *et al.* 2002).

The determination of heavy metal levels in marine organisms is usually preferred, rather than the measuring of the metal concentration in sea water and sediment samples. Metal concentrations in sea water are very low and show wide fluctuation. Heavy metals concentrations in sediment samples can be changed by organic matter content, grain size, composition, pH and oxidation-reduction potential, and so on. At the same time, the metal levels in sediments are relatively invariant with time (Topcuoglu *et al.* 2003). Also the analysis of environmental matrices such as water or sediments provides a picture of the total contaminant load rather than of that fraction of direct eco-toxicological relevance. Thus the use of biomonitors eliminates the need for complex studies on the chemical speciation and (hence presumptive bioavailability) of aquatic contaminants. As even closely related species may exhibit different accumulation strategies for trace elements, there is a need to identify widespread biomonitors to allow intraspecific comparison of accumulated metal concentrations over a large geographical area (Campanella *et al.* 2001).

Ideal biomonitors should be sedentary, easy to identify, abundant, long lived, available for sampling throughout the year, large enough to provide sufficient tissue for analysis, resistant to handling stress caused by laboratory studies of metal kinetics, and tolerant of exposure to environmental variations in physico-chemical parameters such as salinity (Rainbow 1995).

Macro algae species are usually used to indicate heavy metal levels in both estuarine and coastal waters through the world. In benthic food webs, macro algae are key links and they act as time-integrators of pollutants. Their sedentary nature is another reason why algae species are well fitted as monitor organisms in heavy metal monitoring (Topcuoglu, *et al.* 2003). Among the three main fleshy algae groups, the brown species were one of the most abundant along the Red Sea coasts in both Gizan and Hurghada.

The alginate, the predominant chelating agent, from both *Sargassum dentifolium* and *Sargassum latifolium* in the Egyptian Red Sea area was studied by (Larsen *et al.* 2003). The study showed that alginate in both species contains more guluronic

acid (G) than mannuronic acid (M). Therefore, these two species are considered to be ideal biomonitors for heavy metals where they have a high affinity towards any traces of the divalent heavy metals in the aquatic environment, and can be used as a means of comparison between different sites in the Red Sea in which they grow.

The algal species selected in this study are *Turbinaria decurrense*, *Sargassum dentifolium*, *Sargassum latifolium*, and *Padina pavonia*, as common species throughout the year. Also, the aforementioned *Sargassum* species has a high affinity towards heavy metals, so it could provide a useful picture concerning the environment in both test sites in the Red Sea area.

Materials and Methods

(I) Samples of algae

Samples of algae collected from Egypt were harvested at Hurghada (Al Ahiaa Station), while algal samples collected from Kingdom of Saudi Arabia (KSA) were harvested at Gizan (Haras El Hedood Station). All algal species were collected in Spring. The four algae are hand-pickable in the sub-tidal zone at a depth ranging from (1m to 2 m). The samples were washed in seawater at the sampling station. Then the samples were washed with tap water to remove sand and any other epiphyta. Lastly, samples were rinsed in distilled water. They were sun-dried, homogenized and kept away from metallic materials and dust to avoid contamination.

(II) Alkaline extraction of alginate

Alginate was extracted according to the method described by (Abdel Zaher 1994). (5 gm) of dried algae was suspended in (100 ml) of (0.2 N H₂SO₄) at (30°C), with shaking followed by filtering. The residue was suspended in (200) ml distilled water and (200) ml (6%) sodium carbonate at (50°C) with stirring for (2) hours. It was then left over night with continuous stirring at room temperature. In excess of sodium carbonate, the alginic acid was converted to a sodium alginate and was solubilized. The resulting sodium alginate solution was separated from the solid phase by filtration. This step is followed by the precipitation of the alginic acid by the addition of dilute hydrochloric acid (5%) and the conversion of sodium salt to the insoluble acid (pH 1.0). The alginic acid precipitate was pelleted by centrifugation and washed with a (95%) aqueous ethanol solution, then dried and weighed.

(III) Extraction of sulphated polysaccharide from the algal samples:

(5 ml) of (HCl) were added to (5 gm) of the dried algal sample. Next, the pH was adjusted to be (1 to 3). The mixture was left for (3 hrs) at 80°C, and then filtered. The filtrate was neutralized by sodium carbonate before being dialyzed against tap water for (48 hrs). The polysaccharide was precipitated by adding (4) volumes of the solution ethanol. The precipitate was dried and weighed (Abdel Zaher 1994).

Heavy metal analysis was carried out according to the method described by (Cottenie *et al.* 1982). (1 gm) of the algal samples was transferred into a (100 - ml) round bottom glass flask. (10 ml) of a mixture of perchloric acid, nitric acid and hydrochloric acid were added. Heating the mixture at (70-80°C), using the method of (Topcuoglu *et al.* 2003), showed better digestion of the algal sample. After complete digestion, the sample was filtered, and metal concentrations were determined by an atomic absorption spectrophotometer (Varian, Model Spectra AA. 220). The values are expressed as means in the analysis of three subsamples for each sample. The procedural blank samples were used inside each batch. Results are given in (g g⁻¹) of dry weight.

Results

(1) As shown in (Table 1), alginic acid content in *Turbinaria decurrense* at Hurghada was higher than in the same species at Gizan. Conversely in the other three species, there was a higher concentration of alginic acid content in both *Sargassum* species and a marked elevation in the concentration of alginic acid in *Padina pavonia* at Gizan rather than that at Hurghada.

(2) Sulphated polysaccharides represented the

Table (1): The Concentration of Alginic Acid (gm % of the dry weight) in the tested brown algae.

Algal species	Collection site in the Red Sea Area	
	Gizan (KSA)	Hurghada (Egypt)
<i>Turbinaria decurrense</i>	43.25	45.50
<i>Sargassum latifolium</i>	40.14	39.80
<i>Sargassum dentifolium</i>	38.55	36.00
<i>Padina pavonia</i>	36.80	32.69

second most abundant acidic polysaccharide containing sulphonic acid function groups. The sulphated polysaccharides content in the algal species are shown in (Table 2). The results showed that the sulphated polysaccharide was higher in algal species *Turbinaria decurrense*, *Sargassum dentifolium*, *Sargassum latifolium* at Hurghada than that at Gizan, but in *Padina pavonia* it seems to be nearly equal in both Gizan and Hurghada.

(3) The concentrations of heavy metals in algae

Table (2): The Concentration of sulphated polysaccharides (gm % of the dry weight) in the tested brown algae.

Algal species	Collection site in the Red Sea Area	
	Gizan (KSA)	Hurghada (Egypt)
<i>Turbinaria decurrense</i>	11.35	12.8
<i>Sargassum latifolium</i>	9.63	11.90
<i>Sargassum dentifolium</i>	8.95	11.38
<i>Padina pavonia</i>	9.2	9.98

collected from both sites, Gizan and Hurghada, are shown in (Table 3). The comparison showed a variation of heavy metals concentration in the two sites. An overall trend of increased metal concentrations at Gizan was clear. (Pb) is considered to be the most pollutant and its concentration in *Turbinaria decurrense*, *Sargassum dentifolium*, *Sargassum latifolium* and *Padina pavonia* was (54.5), (45.17), (55.28) and (56.8) fold than that in the same species at Hurghada. Depending on the same arrangement of the algal species, (Fe) concentration represents (52.3), (17.34), (10.35) and (10.15) fold than that in the same algal species at Hurghada. (Co) was also present in high concentrations: its concentration at Gizan was (36.6), (31.5), (41.05) and (63.5) fold than that in the same species at Hurghada. (Zn) and (Cu) showed a slight elevation at Gizan where (Zn) concentration was (6.77), (5.56), (3.06) and (3.99) fold than that in the same species at Hurghada. (Cu) concentration showed the lowest values, it was (3.21), (7.48), (1.88) and (3.44) fold than that in algal species at Hurghada.

Table (3): Heavy metals concentrations (g g⁻¹ dry weight) in the algal species collected from two different sites, Gizan and Hurghada, in the Red Sea area.

Metals	Turbinaria decurrens			Sargassum dentifolium			Sargassum latifolium		Padina pavonia		
	Gizan	Hurghada	Published results(*)	Gizan	Hurghada	Published results(*)	Gizan	Hurghada	Gizan	Hurghada	Published results(#)
	4530.0	86.6		1778.5	102.56		1190.3	115.0	1005.3	98.97	
Fe	65.37	9.65	65	49.43	8.89	85	46.52	15.18	49.29	12.35	Nd
Zn	38.43	1.05	6.66	28.39	0.90	7.25	36.54	0.89	35.57	0.56	53.00
Co	168.57	3.09	Nd	129.64	2.87	Nd	161.43	2.92	152.86	2.69	Nd
Pb	0.964	1.15	Nd	0.214	0.72	Nd	0.643	0.93	2.464	0.97	6.36
Cd	12.96	4.03	Nd	9.36	1.25	Nd	8.75	4.65	9.57	2.78	1.00
Cu			2.5			0.84					11

* Nd = not determined

* Published results (#): Heavy metal concentration in *Padina pavonia* at the clean station in the Mediterranean. Ref.: (Campanella et al., 2001)

* Published results (*): Heavy metal concentration in the algae at Hurghada in the period, 1980-1981. Ref.: (Hamdy AA, 1982).

Discussion

The mechanism of metals biosorption is described in the review by (Davis et al. 2003a). The carboxylic groups are generally the most abundant acidic functional group in brown algae. The adsorption capacity of the algae is directly related to the presence of these sites in the alginate polymer and also on the structure of the alginate itself. Regions with alginate rich in guluronic acid residues (G-blocks), which display a high selectivity for divalent cations, provide a multi-dentate environment for complexation, whereas in regions rich in mannuronic acid (M-blocks), complexation would be predominantly mono-dentate and therefore weaker, so the higher the (G) ratio the higher the divalent metal ion chelation. The second most abundant acidic functional group in brown algae is the sulfonic acid of fucoidan, which play a secondary role in binding with heavy metals at low pH.

In this study, choosing the same four algal species to make comparison between the two studied sites means that they have the same chemical composition of the alginate, where the chemical composition depends on the systematic position (Topcuoglu et al. 2003), but where the concentration may differ from one environment to another due to the change in the salinity, temperature, (pH), light and oxygen. Therefore, it was necessary to determine the content of alginic acid and sulphated polysaccharides in all algal

species in both studied sites. Alginic acid content, as shown in (Table 1), was slightly different between the same species at Hurghada and Gizan. Comparing this result with the level of the heavy metals in the same species in the two studied sites, as shown in (Table 3), we found that at Hurghada, although *Sargassum* species had high affinity towards the heavy metals, it showed a very low concentration of all of the determined heavy metals. This result reflects the low level of pollution in Hurghada. This result was confirmed by the other two algal species, *Turbinaria decurrens* and *Padina pavonia* where they showed a low level of all of the measured heavy metals. Although the concentration of (Fe), (Zn) and (Cu) in *Turbinaria decurrens* and *Sargassum dentifolium* at Hurghada measured in this study is higher than that detected before by (Hamdy 1982), as shown in (Table 3), the level (in the period 1982-2004) is still in the normal range cited in the literature. When comparing between the level of (Zn), (Pb) and (Cu) in *Padina pavonia* at Hurghada and in the same species at the clean station in the Mediterranean Sea, as reported by (Campanella et al. 2001), we noticed that the level of the three metals at the reference site in the Mediterranean is (4.29), (2.36) and (3.95) fold than that in the same species at Hurghada. This result showed that the marine environment at the Hurghada site showed a lower pollution level.

Concerning the level of the heavy metals in the algae at Gizan, compared with the same species at Hurghada, as shown in (Table 3), there is a high concentration of all the determined heavy metals at Gizan to differing degrees. (Pb) is the most polluting heavy metal where its concentration in *Padina pavonia* at Gizan is (56.8) fold than that in the same species at Hurghada. Since the same species in the two studied sites has a high difference in concentration of (Pb), this means that the

difference is related to neither the content of the chelating agent, alginic acid (because it is nearly equal), nor to the composition of the chelating agent (because it is similar in the same species). So the difference in concentration of heavy metal is only related to the elevation of this metal in the aquatic environment at Gizan.

Concerning the difference in the concentration of individual metals between the four algal species collected at Gazan, this observation could be explained on the basis of the different affinity of each algal species towards a specific metal. (Pb) concentration was in the following order:

Turbinaria decurrens > *Sargassum latifolium* > *Padina pavonia* > *Sargassum dentifolium*.

This result showed that *Sargassum dentifolium* has lower affinity towards (Pb). (Fe) concentration was higher in the order

Turbinaria decurrens > *Sargassum dentifolium* > *Sargassum latifolium* > *Padina pavonia*.

At the same time (Zn) and (Cu) showed a different pattern where their concentrations were in the order

Turbinaria decurrens > *Sargassum dentifolium* = *Padina pavonia* > *Sargassum latifolium*.

This result showed a different pattern where both *Sargassum dentifolium* and *Padina pavonia* have the same affinity towards these two metals and *Sargassum Latifolium* has the lowest affinity towards them. These results could be explained on the basis of the ion (Fe) exchange mechanism by which the heavy metals present in the aquatic environment replace the light metals

(Na⁺, K⁺, Ca²⁺ and Mg²⁺)

binding with alginate in the brown algae. Since the alginate composition differs from one species to another, the affinity of different brown algae towards a specific metal is expected to be also different even if they grow in the same aquatic environment (Davis *et al.* 2003a).

Another important observation in this study is that *Turbinaria decurrens* at Gizan has a higher affinity towards all types of determined heavy metals. This observation could be explained on the basis of the chemical composition of alginate. Since no data are available about the chemical composition of alginate from *Turbinaria decurrens*, the best biomonitor in this study, more studies in this field are needed.

Conclusion

The brown algae, *Turbinaria decurrens*, *Sargassum dentifolium*, *Sargassum latifolium* and *Padina pavonia* have considerable potential as

cosmopolitan biomonitors of trace metals in the Red Sea area. These algae are available in every season all over the Red Sea coastal area and are easy to sample and identify. The four selected algal species were collected from two stations, Gizan in Kingdom of Saudi Arabia and Hurghada in Egypt. An overall trend of increased metal concentration, especially (Pb), was observed at Gizan station. Conversely, the metal concentrations recorded at the Hurghada reference station fall in the range of the lowest values available in the literature, thus confirming the hypothesis of an experimental area virtually uninfluenced by anthropogenic activities, and which may be considered as a useful reference point for intraspecific comparison within the Red Sea area. *Turbinaria decurrens* at Gizan showed a high affinity towards all types of the tested heavy metals. Algae are considered to be a source of food and may be a source for heavy metals for many marine animals, including fish, feeding on it. Therefore, the investigation of trace metal concentrations in the tissue of different animals may provide useful information on the transfer of potentially toxic elements from biotic compartments (water and sediments) to higher consumers, including man. However, further studies are needed in this area.

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