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Determination of Heavy Metals in *Sargassum angustifolium* Marine Alga of South West Coasts of Iran for Using in Animal Nutrition

S. A.T. Sadeghi*[1], A.A. Kamali[2], A.M. Kabirifard[1]
1- Scientific Members of Research Center of Agriculture and Natural Resource of Bushehr Province, Iran
2- Research Center of Agriculture and Natural Resource of Bushehr Province, Iran
*Email author:Talebsadegh55@yahoo.com

ABSTRACT
This study was conducted to determine the level of heavy metals such as Pb, Cd, Cr, Ni, Co, As and Hg in *Sargassum angustifolium* alga collected from the South West coasts of Iran. First three areas were selected for sampling. Then, at the time of growth peak of *Sargassum angustifolium* alga, the under study areas were referred and three alga samples were collected from each area at distances of 100 meters. The samples were washed with sea water and dried using the sun and incubator device. They were then ground and sent to the laboratory to be measured by atomic adsorption spectrometry of heavy metals. The results showed that the highest concentration of heavy metals in *Sargassum angustifolium* alga of south west coasts of Iran is as follow: Ni> Pb> Co> Cr> Cd> As> Hg. The level of these heavy metals is not in the range of livestock poisoning. Also, the *Sargassum angustifolium* alga can be utilized as a biosorbtion.
Key words: Heavy metals, *Sargassum angustifolium* alga, South West coasts of Iran, Iran.

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INTRODUCTION

The south west of Iran (Bushehr) belongs to arid regions and poor pastures exist there. On the other hand, many animals graze in these regions which provide their required nutrients from these pastures which are not enough. Persian Gulf is placed in south of Iran and contains large sources of nutrients such as marine algae which can be used for animal nutrition and even human [12]. The noticeable point which should be considered in use of alga as a nutrient is that the algae are one the most important biosorption of heavy metals which may poison animals and human. The algae catch heavy metals from the surrounding environment and accumulate them in their cell wall which their concentration may be 20000-40000 times higher than the surrounding water [10]. Therefore, the algae are very good biosorption [16]. Heavy metals are one of the major pollutants of seas and rivers environment which can ultimately poison animals and humans and cause various diseases [19], [6]. *Sargassum angustifolium* alga is one of the dominant algae of south west coasts of Iran which is noteworthy for ranchers to be utilized as animal nutrition because of their high growth speed, large size and their ease to collect. Investigation of heavy metals concentration in *Sargassum angustifolium* alga of the Strait of Hormuz of Iran showed that the highest concentrations were for Pb (13.8±3.8), Ni (21.5±6.9), Cu (6.4±1.6) and Cd (4.7±1.4) ppb [4]. Studying of the concentration of heavy metals in marine algae of Kuwait coasts demonstrates that the level of these metals has considerably increased during the years 1982 to 1992 due to the Persian Gulf war (for example, the amount of Ni has increased from 91 to 185.5 ppb [2]. In investigation of heavy metals level in different kinds of Sargassum alga in Ramswaram coasts (In the South of India), it was found that the concentration of these metals are significantly different among various kinds of algae and also in different parts of one alga. The heavy metals concentration was the highest in leaves, stems, receptacle and airbladder, respectively. Also, the *Sargassum weghtii* alga can be used effectively as an biosorbent in contaminated regions [7]. In the investigation of heavy metals concentration of Mannar Gulf algae of India, it was found that the highest levels of Cd (0.082±0.004), Cr (4.16±0.28), and Ni (3.66±0.05) ppm exist in Sargassum weghtii alga(Kartikai et al, 2009). In many populations, various species of algae including Sargassum alga are utilized as animal nutrition. Using of 25% of *Sargassum angustifolium* alga in the goat nutrition increased its daily gain of 140 g/day [3], and improved the feed conversion ratio[15]. Some researchers have introduced 4% of alga in broiler diets as the best treatment for weight gain in chicken [5]. Persian Gulf is one of the most contaminated seas in the world due to the largest oil spill in 1991 as a consequence of Persian Gulf War, existence of oil equipment in seas, ships haunting, arrival of municipal and industrial swages and also taking too long for water replacement which takes 3 to 5 years [1], [20]. On the other hand, because of the existence of arid regions in the coasts of this sea and the need to new nutrient sources including *Sargassum angustifolium* alga for animal nutrition, the investigation of heavy metals in this alga is the aim of this study.

MATERIALS AND METHODS

In the first step, three regions were selected in the coasts of Bushehr (in south west of Iran) for sampling of Sargassum alga. These areas includes the coasts of air bases, nuclear power plants and Rostami Port. At the time of the growth peak of *Sargassum angustifolium* alga, these regions were referred and three samples of this alga were collected from each region in distances of 1000 meters. These samples were washed by using sea water (according to the point that the aim is to use them for animal nutrition and the rancher don't have the time and money to wash the alga with fresh water) and then dried using the sun. They were then transferred to incubator device and were completely dried at temperature of 65 ºC for 24 hours and after that they were ground. The ground samples of every region were completely mixed with each other and the three samples were sent to the laboratory. The Pb, Cd, Cr, Ni, Co, Hg and As heavy metals were measured through atomic adsorption spectrometry by using "Younglin AAS 8020, made in Korea, 2010" device equipped with furnace and flame. The obtained data were analyzed using SAS software in a completely randomized design (CRD).

RESULTS

The level of heavy metals in *Sargassum angustifolium* alga in the sampling areas (south west coasts of Iran) are given in table 1. According to this table, the highest concentrations of heavy metals in *Sargassum angustifolium* alga of south west coasts of Iran are in the order of Ni> Pb> Co> Cr> Cd> As> Hg. The Ni concentration is much higher in comparison with the other elements in this alga species. Considering the sampling regions, the highest concentrations of Pb and Cd has been observed in Rostami port which shows a significant difference with the two other regions ($p<0.01$). The amounts of Cr in every three regions have significant differences with each other. The maximum amount of Cr was observed in the nuclear power plant region and the minimum amount of it was found in the air base region. The air base region showed the lowest level of Ni which has a significant difference with both nuclear power plant and Rostami port regions ($p<0.01$). The amounts of Ni in Rostami port and nuclear power plant regions were almost similar. The amount of Co in the air base region was much higher than the other two sites so that its level was 6.5 times higher than the nuclear power plant region and 11.5 times higher than Rostami port region. Although Rostami port region showed the maximum amount of Hg but there was no significant difference for this element in the studied regions. The concentrations of As in *Sargassum angustifolium* alga in every three studied regions showed significant differences ($p<0.01$) so that the concentration of this element in the air base site was 2.3 times higher than its concentration in the nuclear power plant and 5 times higher than its concentration in Rostami port site.

DISCUSSION

Because of the existence of alginate in the cell wall, the algae have the ability to adsorb heavy metals. Furthermore, the environmental status of an area, temperature, pH, the level of salinity, the waves of the sea, sun light, the algae grow step and its age, season changes and laboratory sample analysis method, all affect the level of heavy metals concentration [7], [14].

Except the amount of Hg which did not show any significant difference in the sampling areas, other heavy metals showed significant differences in the studied regions ($p<0.01$). Considering the sampling areas, no clear trend was observed about the concentration changes of all elements in *Sargassum angustifolium* alga, but in the case of individual elements, some areas showed more concentration. For example, the highest level of Pb was found in Rostami port region which was twice the other areas or the concentration of As in the air base region was twice than the nuclear power plant region and 5 times higher than Rostami port region (Table 2).

The oil specks were observed only in Rostami port region and this area was the nearest area to the wharf among the other sampling areas. The maximum amount of the urban sewages arrival was in the air base region and its minimum amount was found in Rostami port region and hence, the concentrations of As and Co in this region was several times higher than the other regions. At the time of the collection of alga

samples, the activity of the nuclear power plant was not start but the high concentration of some heavy metals in this area is due to the arrival of urban sewages into this area and the adjacency of Bushehr solid waste landfill area to this region. In general, the heavy metal concentration in *Sargassum angustifolium* alga in south west coasts of Iran (This study) is much more than the contamination of this alga in the Kuwait coasts [2] and Strait of Hormoz, Iran [4]. One of its major causes may be the existence of oil regions in the south west coasts of Iran such as Khark oil terminal and urban sewages arrival which increase the contamination level. In the south west coasts of Iran, the highest concentration belongs to Ni while in Kuwait coasts and Strait of Hormoz-Iran the maximum concentration of heavy metals belongs to Pb. Also, the concentrations of Cr and Pb in Sargassum weghtii alga in Mannar Gulf are much more than the concentrations of these elements in this study and the concentrations of Ni and Cd are much less than the concentrations of these elements in this study(Kartikai et al, 2009).
As repects the purpose of this study is to use *Sargassum angustifolium* alga in animal nutrition, therefore the investigation of the tolerable level of livestock to heavy metals is essential. The normal concentration of Pb in the nutrition of cow and sheep is 1-6 ppm and its toxicity level in the nutrition occurs in concentrations higher than 2000 ppm [21]. Therefore, the Pb level in *Sargassum angustifolium* alga of south west coasts of Iran is not in the range of toxicity and even in the normal range of Pb in the nutrition of cow and sheep.
The normal level of Cd in the nutrition of cow and sheep is 0.1-0.2 mg/Kg of dry matter and its toxicity occurs at concentrations above 50 mg/Kg of dry matter of the nutrition. The minimum tolerable concentration of Cd in the cow nutrition is determined to be 0.5 mg/kg. If the ruminant diets contains more than 30 mg/kg Cd, then anorexia, growth reduction and reduced milk production occur and causes abortion [18]. The maximum amount of Cd in *Sargassum angustifolium* alga in Rostami port region was observed to be 0.59 ppm which is better not to use in diet of cow and sheep due to the points mentioned above. There is a very different range in the required and toxicity level of Ni in ruminants. For these animals, the range is different depends on the rumen microflora and the solubility of Ni compounds. For example, for nickel carbonate which have low solubility, its concentration up to 250 mg/kg of dry matter is tolerable for ruminants [21]. In general, Mc Dowel (1985) has reported the Ni requirement of the ruminants to be 0.03-0.3 mg/kg, their strength level to be 50 mg/kg and the amount of this element in grasses forage to be 0.5-3.5 mg/kg. As respects the maximum Ni concentration in *Sargassum angustifolium* alga is 29 ppm and the resistance level of ruminants is 50 ppm, therefore, the amount of this metal in this alga is not in the range of poisoning of ruminants. Co is one the important elements in synthesis of B_{12} vitamin and its deficiency in ruminants causes various diseases. For cows, Co poisoning occurs when the daily dose reaches 1 mg /Kg of body weight. Sheep is more resistant than cow and stands up to 3.5 mg/Kg of daily dose per kilogram of live weight [10]. The normal amount of Co in the forage for sheep and cow health is 0.07-0.08 mg/Kg of dry matter [13]. The sheep need to Co is 0.1-0.2 ppm [17] and the maximum amount of Co was in *Sargassum angustifolium* alga collected in the air base region which its value was 3.15 ppm. Considering the above points, the digestibility of alga and the amount of alga in the animal diet which is a maximum of 25%, its consumption in the animal nutrition would not be problematic.
A dose of Mercury dichloride of 8 g was toxic to cows. The calves fed with milk containing 10 mg Hg of methylmercury per kilogram of milk, perished in the ages of 36 to 81 days old while the calves which received 2 to 4 mg Hg in the form of methylmercury per kilograms of milk as clinical remained normal [18]. The maximum tolerable concentration of Hg proposed in the sheep diet, in the form of organic or inorganic, is 2 mg per kilogram [17] Due to the low concentration of Hg in *Sargassum angustifolium* alga in the south west coasts of Iran, using of this algae in the animal nutrition is not problematic.
The maximum tolerable As concentrations in the cows diet are 50 and 100 mg/kg for its organic and inorganic forms, respectively [18]. Due to the low concentration of As in *Sargassum angustifolium* alga especially in Rostami port region, using of this alga in the animal nutrition is not problematic.
The important point is that according to the researches done, *Sargassum angustifolium* alga can be used in the animal nutrition up to a maximum value of 25% [15], and by considering the digestibility of 65% of the alga dry matter [9] and the level of heavy metals adsorption in animal body, it does not seem that using of *Sargassum angustifolium* alga collected in the south west coasts of Iran makes problem in longtime.

CONCLUSION

According to the high concentration of some heavy metals such as Ni, Pb and Cd in *Sargassum angustifolium* alga, this alga can be used as a biosorbtion. Furthermore, the air base region has the lowest concentration for many of the studied heavy metals. Since the concentrations of these metals in *Sargassum angustifolium* alga are not in the range of the livestock poisoning, it is proposed to use the alga collected from this region for the animal nutrition, although this alga species in the south west coasts of Iran can be used certainly in the animal nutrition.

Table 1- Mean(± SE) and range of heavy metals level in *Sargassum angistifolium* algae in south west coasts of Iran

	Pb	Cd	Cr	Ni	Co	Hg	As
Air Bas area							
Mean	1.3212[a]±0.3097	0.1014[a]±0.0292	0.3127[a]±0.0737	24.23[a]±0.445	3.1582[a]±0.1857	0.1243±0.0053	0.3818[a]±0.0439
Min	0.9594	0.0453	0.2298	21.49	2.8911	0.1155	0.3130
Max	1.9376	0.1435	0.4597	29.108	3.5152	0.1339	0.4634
Nuclear Power Plant area							
Mean	1.5956[a]± 0.0944	0.1102[a]±0.051	1.0575[b]±0.27	29[b]±4.83	0.4877[b]±0.225	0.1454±0.016	0.172[b]±0.012
Min	1.4970	0.0550	0.5639	20.148	0.2435	0.1259	0.1262
Max	1.7842	0.2117	1.4929	36.764	0.9368	0.1768	0.1953
Rostami Port							
Mean	2.2869[b]±0.3082	0.5959[b]±0.0350	0.7526[c]±0.1232	28.29[b]±1.58	0.2762[b]±0.1015	0.149±0.009	0.0755[c]±0.0182
Min	1.6945	0.5455	0.5353	25.404	0.1036	0.1326	0.0480
Max	2.7309	0.6633	0.9618	30.872	0.4552	0.1636	0.1098
Total mean	1.7345±0.1931	0.2692±0.0840	0.7076±0.1395	27.17±1.7887	1.3074±0.4722	0.1395±0.0067	0.2097±0.0474

Significant level= 5%

Table 2- Rank of sampling area on base content of heavy metals in *Sargassum angustifolium*

	Rank of area		
Element	1	2	3
Pb	Rostami Port	Nuclear Power Plant area	Air Bas area
Cd	Rostami Port	Nuclear Power Plant area	Air Bas area
Cr	Nuclear Power Plant area	Rostami Port	Air Bas area
Ni	Nuclear Power Plant area	Rostami Port	Air Bas area
Co	Air Bas area	Nuclear Power Plant area	Rostami Port
Hg	Rostami port	Nuclear Power Plant area	Air Bas area
As	Air Bas area	Nuclear Power Plant area	Rostami Port

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