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Elemental Composition and Bioactivity of Seaweeds from Coastal Areas of Karachi, Pakistan

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ABSTRACT. Sixteen species of green, brown and red seaweeds from Pakistan were analyzed for their elemental composition by Atomic Absorption Spectrometry and several significant elements like Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Pb and Zn were detected quantitatively. Among these elements Ca, Fe, K, Mg, and Na, were found in large amount (13646-97006 ppm), Cr, Cu, Pb and Zn were present in small quantities (7.37-15.96 ppm) while Cd and Co, were detected in extremely small amounts (2.16-5.89 ppm). The average quantity of Na was found to be the highest among these algae (97006.543 ppm) followed by K (64119.75 ppm) and Ca (22735.47 ppm). The average amounts of Co (5.89 ppm) and Cr (7.372 ppm) were quite low, Cd was detected in the lowest quantity (0.5-3.95). Eight species of seaweeds were also tested for antifungal activity showing moderate activity against *Aspergillus niger*, *Microsporum canis*, *Pseudallescheria boydii*, *Trichophyton simii*, poor activity against Fusarium solani and *Macrophomina phaseolina. Sargassum vulgare* appeared to be most active while *Gracilaria corticata* was the least active, *Codium iyengarii, Ulva lactuca* occupied intermediate position. In general, human pathogens were most sensitive against the above four seaweeds and plant pathogens proved to be the least sensitive, while animal pathogens appeared to be as intermediate.

Introduction

Research and utilization of seaweeds have increased markedly day by day. The great significance of elements for human nutrition has been confirmed in the New Millennium by the acquisition of new knowledge. Seaweeds constitute a rich source of agar, algin, proteins, minerals and trace elements. Trace elements such as manganese, iron, copper and zinc are significant in pollutions as well as in nutrition. The trace elements are accumulated in the cell matrix and increased in percentage when extractable polysaccharides were removed. This shows that trace elements in residue are mostly in their organic forms, which are easily assimilated by animals (llyas, 1997). Marine algae have been found to be active against the fungi *Trichophyton mentagrophytes, T. rubrum*, *Candida albicans* and number of other fungal organism (Baslow, 1969). It is an established fact that the seaweeds are full of innumerable wealth of bioactive properties (Naqvi *et al*., 1980 and Rao *et al.*, 1991). Kanias *et al.* (1991) determined trace elements in the dry matter of marine brown algae of

Saronic Gulf. Some species of marine algae from the coast of Goa, India were analyzed for Co, Cu, Fe, Mn, Ni, Pb and Zn (Hoppe and Levring,1982). *Ulva lactuca* from China, South East Asia has been reported to be rich in Iron (Ahmad *et al*., 1989). The edible green alga *Codium intricatum* (Mosure-miru) was found to contain a considerable quantity of Iodine (0.13-0.16% of the dry wt) whilst red algae such as *Gelidium* & *Grateloupia* contained medium amount (Chapman and Champan, 1980).

Many types of seaweeds have been used as food since they are not poisonous and usually have soft tissues. When used as food, seaweeds have many indirect medicinal effects. Seaweeds selectively absorb from the seawater the elements like Na, K, Ca, Mg, Cl, I and Br, which are accumulated in their thalli. The accumulated elements vary from species to species. For example, large quantities of K and I are taken up by many brown seaweeds and Ca and Br by red algae. Seaweeds generally contain Na, K, Ca, Mg, and Fe in large quantities up to 15-25% of dry weight. The inorganic content appears very high when compared with 5-6% in hay or nearly 4% in cereals. Seaweeds are known as alkaline food since their inorganic components play a very important role in preventing blood acidosis (Kaur,1997).

Karachi, the capital of Sind province and the biggest city of Pakistan has a coastline of about 100 km at the northern boundary of the Arabian Sea. It includes beaches and numerous islands. The coastal waters around Manora, Sandspit, Hawkesbay, Buleji, Paradise Point, Pacha, Nathiagali and Cape Monze inhabit a variety of marine benthic algae (Shameel and Tanaka, 1992). Although a lot of work has been done on their taxonomy, distribution, morpho-ecological studies, and phycochemistry (Anand, 1940 & 1943 and Usmanghani & Shameel,1996), but not much data is available in literature related to their elemental composition and bioactivity. Therefore, this work was undertaken in this connection for the first time from the coastal areas of Karachi, Pakistan.

Materials and Methods

Collection of Marine Algae

The healthy and mature specimens of different species of marine algae were collected from sand-bottom bays, large and shallow sand bottom flats, small and large pools with rocky and sandy bottom at the leges on various coastal areas of Karachi, Pakistan, *e.g.* Buleji, Manora and Paradise Point during September 1998 to December 1999.The sublittoral algae were picked up as drift material. The collected seaweeds were brought to the laboratory, where they were washed immediately with distilled water to remove epiphytes and attached debris.

Ashing and Digestion of the Seaweeds

The algal material was dried under shade and 1 g of it was oven dried, ground seaweeds were weighed into a porcelain crucible and ignited in a muffle furnace at 500ºC for 2 hs (Scheme 1). The ash was cooled at a room temperature, wet with 10 drops of distilled water and dissolved in HNO_3 (1: 1). The sample in acid solution was then heated gently on a hot plate at 100-120ºC till nearly dry. The crucible was returned to muffle furnace and ashed again for 1 hr at 500ºC. It was then cooled and dissolved in 10 ml HCl (1:1), and the solution was filtered through an acid washed filter paper into a 100 ml volumetric flask. The solution was now diluted to volume with distilled water, mixed well and made ready for AAS reading (Jones, 1984).

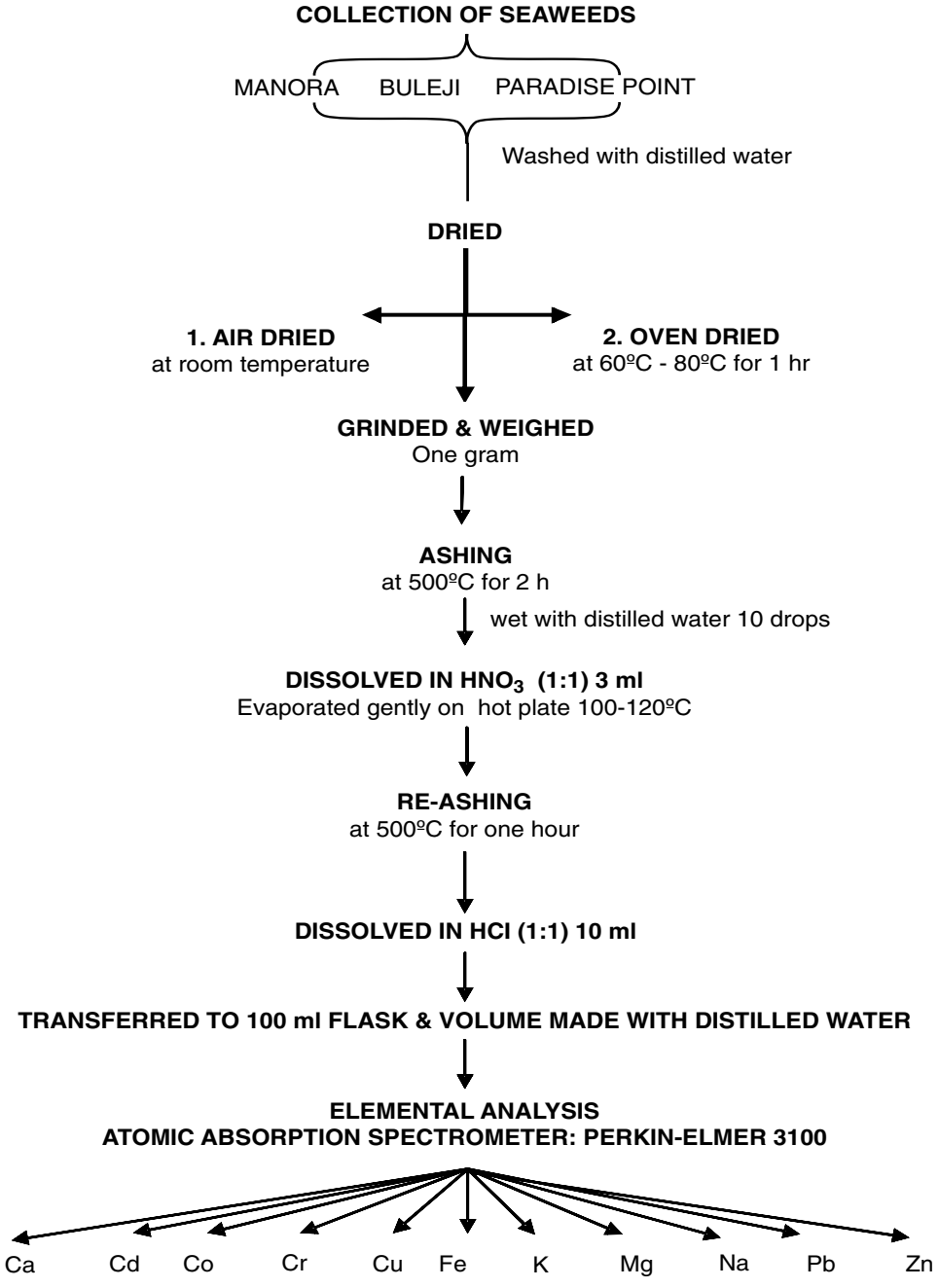
Elemental Assay

A flame atomic absorption spectrometer (AAS, Model Perkin-Elmer 3100, USA), was used for the purpose of estimating Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Pb and Zn. The various instrument parameters have been presented in Table 1. Instructions for instrument setting; calibration and assay for specific element as laid down in the operational manual were strictly followed.

Anti Fungal Bioassay

The fresh algal material (100 g) was soaked in MeOH for seven days at room temperature. The MeOH extract was filtered through Whatman filter paper and concentrated under reduced pressure at 35ºC in rotary evaporator. The crude gummy methanolic extract was partitioned between water and petroleum ether. The extract was used for antifungal activity.

The crude extract (24 mg) was dissolved in 1 ml sterile dimethyl sulphoxide (DMSO) serving as



Scheme 1. Scheme for the elemental composition of seaweeds of the Karachi coast.

TABLE 1. Instrument parameters.

Elements		Wave length (nm)	Slit (nm)	Sensitivity (mg/l)
Calcium	Ca	422.7	0.7	0.092
Cadmium	Cd	228.8	0.7	0.016
Cobalt	Co	240.7	0.2	0.078
Chromium	Cr	357.9	0.7	0.041
Copper	Cu	324.8	0.7	0.077
Iron	Fe	248.3	0.2	0.039
Potassium	K	766.5	0.7	0.043
Magnesium	Mg	285.2	0.7	0.008
Sodium	Na	589.0	0.2	0.012
Lead	Pb	283.0	0.7	0.079
Zinc	Zn	213.0	0.7	0.018

Recommended flame: Air - acetylene

stock solution. Sabouraud dextrose agar (SDA) was prepared by mixing 32.5 g sabouraud, 4% glucose agar and 4 g of agar-agar in 500 ml distilled water, then steamed to dissolve and dispense 4 ml amount in to screw caps tubes and autoclaved at 121ºC for 15 min. Tubes were allowed to cool to 50ºC and non-solidified. The SDA medium is poisoned with 66.6 µl of stock solution giving final concentration of 400 µg/ml of SDA. Tubes were then allowed to solidify in slanted position at room temperature. Each tube was inoculated with 4 mm diameter piece of inoculums removed from a 7-day-old culture of fungi, for non-mycelial growth on agar surface streak was employed. The tubes were incubated at 28-30ºC for 7-10 days. Humidity (40% to 50%) is controlled by placing an open pan of water in the incubator. Growth was measured in mm, inhibition was calculated in % and compared with standard antibiotics (Paxton, 1991 and Atta-ur- Rahman et al., 2001).

Results and Discussion

Sixteen species of marine benthic algae belonging to the Chlorophyta, Phaeophyta and Rhodophyta have been analysed for the composition of eleven elements (Table 2). Among these elements Ca, Fe, K, Mg and Na were found in large amounts (on the average13646- 97006 ppm), Cr, Cu, Pb and Zn were present in small quantities (on the average 7.37-15.96 ppm) while Cd and Co were detected in extremely small amounts (on the average 2.16-5.89 ppm). Iron was also found in large quantity, Cr and Zn in medium quantity and Co in small amount in several brown seaweed of Saronic gulf Greece (Kanias *et al*., 1991). Research study has shown

TABLE 2. Elemental composition of marine algae from the coastal areas of Karachi in ppm.

Marine algae	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Na	Pb	Zn
Chlorophyta	**32312.5**	**1.84**	**5.92**	**8.65**	**9.9**	**2186.25**	**54855**	**12366.58**	**67977.5**	**23.47**	**33.93**
Bryopsis pennata Lamour.	80800	3.15	8.05	9.925	12.9	3795	10855	6660	28535	43.875	37.425
Caulerpa racemosa (Forssk.) J. Ag.	70300	2.2	6.8	12.525	11.25	2542.5	19625	764.5	155950	23.675	21.725
Caulerpa taxifolia (Vahl) C. Ag.	14755	0.975	4.0	10.425	8.5	2840	15810	6870	110400	19.1	25.05
Codium iyengarii BØrg.	14730	1.925	9.55	2.825	5.65	862.5	231700	9605	9605	26.075	18.25
Enteromorpha intestinalis (L.) Nees	4745	0.5	0.5	23.325	14.0	2695	18590	13400	13400	19.6	81.55
Ulva lactuca L.	8545	2.3	2.3	1.85	7.125	382.5	32550	36900	36900	8.55	19.15
Phaeophyta	**27351.6**	**2.67**	**6.16**	**7.01**	**9.7**	**673.5**	**68470.33**	**16030.33**	**36998.23**	**10.05**	**117.63**
Cystoseira indica (Thivy et Doshi) Mairh	19050	3.95	5.125	4.7	8.125	249	118125	9425	80562.5	8.1	33.625
Padina tetrastromatica Hauck	46950	2.875	6.375	16.15	12.375	3105	26620	24500	20530	15.25	44.475
Sargassum vulgare C. Age.	16055	1.2	7	0.18	8.6	1740	60666	14166	9902.2	6.8	274.8
Rhodophyta	**12548.2**	**2.22**	**5.81**	**5.51**	**9.14**	**70196.4**	**47923.57**	**13721.78**	**119177.78**	**12.06**	**29.47**
Agardhiella robusta (Grev.) GØrg.	10570	1.5	2.575	2.025	3.275	355	29025	8190	24805	11.675	42.075
Botryocladia leptopoda (J.Ag.) Kylin	9055	2.9	8.05	3.4	13.6	499.5	65925	2740	202375	7.2	26.375
Hypnea musciformis (Wulf) Lamour.	7977.5	1.975	5.1	2.325	6.675	230.5	62125	4930	129687.5	3.85	15.675
Hypnea valentiae (Turn.) Mont.	10350	2.7	7.75	10.625	11.625	1825	112937.5	15260	154187.5	14.1	29.925
Sarconema furcellatum Zanard.	7447.5	2.55	7.45	5.425	12.7	340.75	136375	19820	220187.5	16.05	20.775
Scinaia saifulahii Afaq. *et* Shameel	8350	1.15	2.85	4.55	4.2	1070	14925	12350	18690	13.45	47.875
Solieria robusta (Grev.) Kylin	34087.5	2.8	6.9	7.7	11.9	1900	70062.5	8462.5	184312.5	18.1	23.6
Average amount	22735.47	2.165	5.893	7.372	9.531	1527.015	64119.75	13646.44	97006.543	15.965	47.646

that seaweeds containing Ca & Zn display a good intestinal uptake, but the Fe present in seaweeds seems to be taken up at lower rate. In human beings seaweeds intake is thought to restore fitness (Bougle *et al*., 1996). The average quantity of Na was found to be highest among these algae (97006.543 ppm) followed by K (64119.75 ppm) and Ca (22735.47 ppm). The average amounts of Co (5.89 ppm) and Cr (7.372 ppm) were quite low. The Cd was detected in the lowest quantity (0.5-3.95 ppm). Sodium was found to be in highest amount in the Chlorophyta (67977.5 ppm) and Rhodophyta (119177.78 ppm) while K was detected in highest quantity in the Phaeophyta (68470.33 ppm) on the average. However Cd was present in smallest amount in Chlorophyta (1.84 ppm), Phaeophyta (2.67ppm) as well as Rhodophyta (2.22 ppm). It was also detected in very small quantity in several species of green, brown red seaweeds (Hasni and Sarwar, 1985).

Eight species of seaweeds such as *Codium iyengarii, Ulva lactuca, Dictoyta ciliata, Iyengaria stellata, Sargassum vulgare, Botryocladia leptopoda, Gracilaria corticata, Hypnea musciformis* were tested against 6 species of human pathogens 3 of animal pathogens and 4 species of plant pathogens for *in vitro* fungicidal bioassay. Most of them were either inactive or showed a very poor activity against these pathogens. Only four species (Table 3) exhibited moderate activity (45-50%), among them were included two representatives of green, one of brown and one from red seaweeds, while working on the anti-fungal activity of *Codium intertextum* Febles *et al.* (1995) emphasized the considerable action of the methanolic extract against *Saccharomyces cerevisiae*, strong against *Candida albicans* and moderate action against *Candida tropicalis* and *Candida guilliermondii*.

Sargassum vulgare appeared to be most active while *Gracilaria corticata* was the least active species. The extract of *Srgassum tenerrimum* was not found to have any chemotherapeutic activity (Naqvi *et al*., 1980).The species of this genus have been reported to possess antibiotic substances, Sarginin A and Sarginin B (Conover and Sieburth, 1964).

The two green seaweeds occupied intermediate position (Table 3). In general human pathogens were most sensitive against all the four seaweeds and plant pathogens proved to be least sensitive, while animal pathogens appeared to be intermediate. Recently, the MeOH extract of red alga *Sarconema furcellatum*, appeared to be most active against *Trichophyton schoenleinii, Pseudallescheria boydii, Aspergillus niger Microspermum canis, Trichophyton mentagrophyles, T. simii* and *Fusarium solani* (Rizvi *et al*., 2000).

Conclusion

This type of research work, which is still in continuation, will help us in understanding the Elementology of Seaweeds. We have the admirable treasure house of seaweed resources hidden under our Coastal and high tide regions, the drifts of what we receive of our sandy beaches, on our coast and on the natural rocky ledges we have in our beloved country. The need of the time is adaptation of the exact method of exploration of our seaweed wealth, the use of which can eliminate Iodine deficiency in our areas of higher altitude, it can help

TABLE 3. Fungicidal bioassay of certain seaweeds from Karachi coast showing inhibition in mm.

Name of fungi	Chlorophyta		Phaeophyta	Rhodophyta
	Codium iyengarii	*Ulva lactuca*	*Sargassum vulgare*	*Gracilaria corticata*
Human pathogens:				
Aspergillus niger	46	50	56	50
Pseudallescheria boydii	20	40	50	16
Animal pathogens:				
Microsporum canis	50	33	50	16
Trichophyton simii	50	00	45	0
Plant pathogens:				
Fusarium solani	03	04	4	14
Macrophomina phaseolina	25	11	5	0

our nutritional industry and it can help us in the contest of becoming developed from the developing stage.

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التركيب العنصري والنشاط الحيوي للطحالب في المناطق الساحلية لكراتشي - باكستان

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المستخلص. قيست تراكيز عناصر كلاً من الكالسيوم ، والكادميوم ، والكوبلت ، والكروم ، والنحاس ، والحديد ، والبوتاسيوم ، والمغنسيوم ، والصوديوم ، والرصاص ، والزنك ، بجهاز امتصاص الطيف الذري في ستة عشر نوعًا من الطحالب الخضراء والبنية . وقد وجد أن كلاً من الكالسيوم ، والحديد ، والبوتاسيوم ، والمغنيسيوم ، والصوديوم بتراكيز عالية تتراوح ما بين (١٣٦٤٦-٩٧٠٠٦ جزء من المليون) ، في حين يوجد الكروم ، والنحاس ، والرصاص ، والزنك بتراكيز قليلة تتراوح ما بين (٧,٣٧-١٥,٩٦ جزء من المليون) . بينما وجد الكادميوم ، والكوبلت بتراكيز قليلة جدًا تتراوح ما بين (٢,١٦-٥,٨٩ جزء من المليون) . وقد كان متوسط تركيز عنصر الصوديوم هو الأعلى في تلك الطحالب (٩٧٠٠٦,٥٤٣ جزء من المليون) يليه عنصر البوتاسيوم (٦٤١١٩,٧٥ جزء من المليون) والكالسيوم (٢٢٧٣٥,٤٧ جزء من المليون) . أما متوسطا تراكيز الكوبلت (٥,٨٩ جزء من المليون) والكروم (٧,٣٧٢ جزء من المليون) فكانا منخفضين ، في حين أن تركيز عنصر الكادميوم هو الأقل (٠,٥-٣,٩٥ جزء من المليون) .

فحصت ثمانية أنواع من الطحلب ضد نشاط الفطر وأظهرت نشاط متوسط ضد *Aspergillus niger, Microsporum canis, Pseudallescheria boydii, Trichophyton simii* ، ونشاط منخفض ضد *Fusarium solani, Macrophomina phaseolina* وكان *Sarassam vulgare* هو الأكثر نشاطًا ، بينما *Gracilaria Corticata* هو الأقل نشاطًا ، في حين احتلت *Codium iyengarii, Ulva lactuca* موقعًا متوسطًا . وبشكل عام فقد كانت الكائنات الممرضة للإنسان هي الأكثر حساسية لأنواع الطحالب الأربعة السالفة الذكر ، بينما الكائنات الممرضة للنبات هي الأقل حساسية في حين أن الكائنات الممرضة للحيوان متوسطة .