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BIOLOGICAL ACTIVITY AND ELEMENTOLOGY OF BENTHIC ALGAE FROM KARACHI COAST

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Abstract

Forty species of seaweeds were collected from Karachi coast and several biological tests conducted on them inorder to investigate their antibacterial, antifungal, phytotoxic and insecticidal activities. Brown seaweeds showed greater antibacterial activity than the green and red ones. *Chaetomorpha antennina, Gracilaria foliifera, Jolyna laminarioides* exhibited greatest antifungal activity and *Codium shameelii* the poorest. The highest phytotoxic activity (100 %) was displayed by *Asparagopsis taxiformis* at 1000 µg/mL concentration, while *Osmundea pinnatifida* showed the low insecticidal activity as compared to the other investigated species. Certain elements *e.g.* Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Pb, Zn were determined quantitatively. Among them Ca, Cr and Pb were found to occur in largest amount in green seaweeds, Co, Cu, Fe and Zn in greatest quantity in brown seaweeds, while Cd, K, Mg and Na in highest proportion in the investigated red seaweeds, indicating that various phyla of algae behaved differently in their elemental composition.

Introduction

The extracts of a number of marine plants were found to display a variety of antimicrobial activities (Baslow, 1969; Naqvi *et al.*, 1980; Rao *et al.*, 1991, Alam *et al.*, 1994). The ethanol extracts of *Codium dwarkense* Børg. and *Bryopsis plumosa* J. Ag. exhibited antifungal activity (Aliya & Shameel, 1999). Seaweeds selectively absorb elements like Na, K, Ca, Mg, I and Br from marine water and accumulate them 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. Marine algae generally contain Na, K, Ca, Mg and Fe in large quantities up to 15-25 % of dry weight (Kaur, 1997). The inorganic content appears very high when compared with 5-6 % in hay or nearly 4 % in cereals.

The coastal areas of Karachi offer a variety of sandy beaches, rocky ledges, swampy wetlands and few islands. They are inhabited by a variety of marine benthic algae (Shameel & Tanaka, 1992). Although a lot of work has been done on their taxonomy (Anand, 1940, 1943; Nizamuddin, 1964; Afaq-Husain & Shameel, 1991; Shameel, 2000) distribution (Saifullah, 1973; Shameel *et al.*, 1996, 2002; Hameed *et al.*, 2001) and phycochemistry (Shameel, 1990; Usmanghani & Shameel, 1996), but little work has been done on the biological activity of these organisms (Usmani *et al.*, 1991; Siddiqui *et al.*, 1993) as well as their elemental constitution (Rizvi & Shameel, 2001). Therefore, this work was initiated to comprehend the bioactivity and elemental composition of Karachi seaweeds.

Materials and Methods

Collection of marine algae: The healthy and mature specimens of different species of marine benthic algae were collected (1kg each) from various coastal areas of Karachi, Pakistan during December 1997 and November 2002 (Table I). The sub–littoral algae were

picked up as drift material. The collected seaweeds were brought to the laboratory, where they were washed immediately with running water to remove epiphytes, epizoans and attached debris. Later on they were washed with distilled water and soaked in MeOH for biological testing.

Table 1. Investigated marine algae and the area of their collection.

No.	Marine algae	Code no. used	Area of collection	Date of collection
	Chlorophyta:			
1.	*Bryopsis pennata* Lamour.	BP-01	Buleji	07.02.1998
2.	*Caulerpa racemosa* (Forssk.) J. Ag.	CR-02	Buleji	07.02.1998
3.	*Caulerpa scalpelliformis* (R. Br.) C. Ag.	CS-03	Paradise Point	25.01.1998
4.	*Caulerpa taxifolia* (Vahl) C. Ag.	CT-04	Manora	31.01.1999
5.	*Chaetomorpha antennina* (Bory) Kütz.	CA-05	Buleji	12.08.2002
6.	*Codium iyengarii* Børg.	CI-06	Buleji	07.01.1998
7.	*Codium shameelii* Nizam.	CS-07	Paradise Point	25.01.1998
8.	*Enteromorpha intestinalis* (L.) Nees	EI-08	Sandspit	15.05.1998
9.	*Ulva fasciata* Delile	UF-09	Buleji	30.07.2000
10.	*Ulva lactuca* L.	UL-10	Paradise Point	25.01.1998
	Phaeophyta:			
11.	*Colpomenia sinuosa* (Roth) Derb. *et* Sol.	CS-11	Manora	27.12.1997
12.	*Cystoseira indica* (Thivy *et* Doshi) Mairh	CI-12	Buleji	02.03.1998
13.	*Dictyota dichotoma* (Huds.) Lamour.	DD-13	Buleji	27.12.1997
14.	*Dictyota hauckiana* Nizam.	DH-14	Manora	06.02.2001
15.	*Iyengaria stellata* (Børg.) Børg.	IS-15	Manora	27.12.1997
16.	*Jolyna laminarioides* Guimareãs	JL-16	Buleji	27.07.2002
17.	*Padina pavonica* (L.) Thivy *in* Taylor	PP-17	Paradise Point	25.01.1998
18.	*Padina tetrastromatica* Hauck	PT-18	Buleji	19.11.1998
19.	*Sargassum boveanum* J. Agardh	SB-19	Buleji	16.02.2000
20.	*Sargassum ilicifolium* (Turn.) C. Ag.	SI-20	Buleji	16.02.2000
21.	*Sargassum swartzii* (Turn.) C. Ag.	SS-21	Paradise Point	25.01.1998
22.	*Sargassum tenerrimum* J. Ag.	ST-22	Manora	27.12.1997
23.	*Sargassum vulgare* C. Ag.	SV-23	Buleji	02.03.1998
24.	*Stoechospermum marginatum* (C. Ag.) Kütz.	SM-24	Buleji	20.01.1997
25.	*Stypopodium shameelii* Nizam. *et* Aisha	SS-25	Manora	27.12.1997
	Rhodophyta:			
26.	*Asparagopsis taxiformis* (Delile)Trevisan	AT-26	Buleji	16.02.2002
27.	*Botryocladia leptopoda* (J. Ag.) Kylin	BL-27	Manora	19.11.1998
28.	*Champia compressa* Harv.	CC-28	Buleji	30.07.2000
29.	*Cystoclonium purpureum* (Huds.) Batt.	CP-29	Manora	27.07.2002
30.	*Dermonema abbottiae* Afaq., Nizam *et* Shameel	DA-30	Buleji	27.07.2002
31.	*Gelidium usmanghanii* Afaq. *et* Shameel	GU-31	Buleji	03.11.2002
32.	*Gracilaria corticata* (J. Ag.) J. Ag.	GC-32	Buleji	01.12.1998
33.	*Gracilaria foliifera* (Forssk.) Børg.	GF-33	Manora	27.07.2002
34.	*Hypnea musciformis* (Wulf.) Lamour.	HP-34	Buleji	01.12.1998
35.	*Hypnea valentiae* (Turn.) Mont.	HV-35	Paradise Point	27.02.1999
36.	*Melanothamnus afaqhusanii* Shameel	MA-36	Buleji	27.07.2002
37.	*Osmundea pinnatifida* (Huds.) Stack.	OP-37	Buleji	30.07.2000
38.	*Sarconema furcellatum* Zanard.	SF-38	Manora	25.01.1998
39.	*Scinaia saifullahii* Afaq. *et* Shameel	SS-39	Buleji	07.02.1998
40.	*Solieria robusta* (Grev.) Kylin	SR-40	Buleji	24.10.1999

***Antibacterial bioassay*:** It was performed against a variety of Gram positive and Gram negative bacteria (Table 2) using agar well diffusion technique. The samples (6 mg) of each crude methanol extract was used for antibacterial tests. All pathogenic microbes were clinical isolates and provided by the Department of Microbiology, University of Karachi, except *Staphylococcus aureus* and *Candida albicans* which were obtained from Liaquat National Hospital, Karachi. The pure bacterial cultures were inoculated in nutrient broth and incubated at 37 °C for 2-8 h till the turbidity developed. The turbidity of Nutrient Broth (NB, Oxoid Uni Path Ltd., England) as obtained in the test tubes was compared with McFarland turbidity standard. Test samples of 200 μg/100 mL concentration as well as dimethyl sulphoxide (DMSO) were added in their respective wells (Carran *et al.*, 1987; Atta-ur-Rahman *et al.*, 2001). The zones of inhibition were measured in mm and compared with reference antibacterial drug: Tetracycline (Sigma, USA).

***Antifungal bioassay*:** The fresh algal material (1 kg each) 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 35 °C in rotary evaporator. The crude gummy methanolic extract (24 mg) was dissolved in 1 mL sterile dimethyl sulphoxide (DMSO) serving as stock solution. Sabouraud dextrose agar (SDA Merck, Germany) 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 into screw-caped tubes and autoclaved at 121 °C for 15 min. Tubes were allowed to cool to 50 °C and non-solidified SDA medium was poisoned with 66.6 μL of stock solution giving final concentration of 400 μg/mL of SDA. Tubes were then allowed to solidify in slanting position at room temperature. Each tube was inoculated with 4 mm diameter piece of inoculum removed from a 7-day old culture of fungi, for non-mycelial growth an agar surface streak was employed. For various fungal organisms used see Table 2. The tubes were incubated at 27-29 °C for 7-10 days (Paxton, 1991; Atta-ur-Rahman *et al.*, 2001). Growth inhibition was calculated in percentage and compared with standard antibiotic drugs: Miconazole and Ketoconazole (Johnson & Johnson Pak, Ltd.) and sometimes Amphotericin-B (Oxiod, USA).

***Lemna bioassay*:** The plant *Lemna aequinoctialis* Welw., was used to study the phytotoxic activity of each MeOH extract. The stock solutions were prepared by dissolving 30 mg of the crude extract in 1.5 mL of the methanol. Nine flasks (three for each concentration) were inoculated with 1000 μL, 100 μL and 10 μL of the stock solution for 1000, 100, and 10 μg/mL. The solvent was evaporated overnight in sterilized condition. To each flask, 20 mL of E-medium at pH of 5.5-6.0 was added. Then 10 plants of *Lemna aequinoctialis* having a rosette of three fronds were added to each flask. Two other flasks were supplemented with solvent and reference plant growth inhibitor as well as promoter, they served as negative and positive controls, respectively. For positive control Paraquat (ICI Pak. Ltd.) was used. The flasks were plugged with cotton and placed in growth cabinet for 7 days. On the seventh day the number of fronds per flask were counted (Atta-ur-Rahman, 1991). Interpretation of the result was made by analyzing growth regulation in percentage which was calculated with reference to the negative control.

***Insecticidal assay*:** It was used to assess the insecticidal activity of each MeOH extract (200 mg) of the seaweeds. The insects were exposed to the test compound by direct contact toxicity method using filter paper impregnated with test sample (1571.33 μg/cm²). Afterwards 10 adult insects of different types and of same age were transferred to Petri dishes. A check batch of negative control was treated with solvent for determination of

solvent effect. Another batch supplemented with reference insecticides *i.e.* Mortein Coopex (Reckitt Benckiser Pak. Ltd.) was used. All of them were kept without food for 24 hours (Farhana, 2000). Mortality counts were carried out after 24 hours' exposure period.

Table 2. Test organisms used for bioassays.

No.	Test organisms	Abbr. used	Procurement
	***Bacteria*:**		
	Gram positive		
1.	*Bacillus subtilis* (Ehrenberg) Cohn	B.s	Department of
2.	*Corynebacterium diphtheriae* (Kruse) Lehmann *et* Neumann	C.d	Microbiology, Univ. of Karachi
3.	*Staphylococcus aureus* Rosenbach	S.a	Liaquat National
4.	*Streptococcus pyogenes* Rosenbach	S.p	Hospital
	Gram negative		
5.	*Pseudomonas aeruginosa* (Schroeter) Migula	P.a	Department
6.	*Proteus mirabilis* Hauser	P.m	of Microbiology,
7.	*Klebsiella pneumoniae* (Schroeter) Trevisan	K.p	University
8.	*Shigella dysenteriae* (Shig) Castellani *et* Chalmers	S.d	of Karachi
9.	*Salmonella typhi* (Schroeter) Warren *et* Scott	S.t	
	***Fungi*:**		
	Human pathogens		
10.	*Aspergillus flavus* Link	A.f	Clinical isolate
11.	*Aspergillus niger* van Tieghem	A.n	Clinical isolate
12.	*Candida albicans* (Robin) Berkhout	C.a	LN Hospital
13.	*Candida glabrata* Saito	C.g	Clinical isolate
14.	*Pseudallescheria boydii*	P.b	Department of
15.	*Trichophyton longifusus* Malmsten	T.l	Microbiology,
16.	*Trichophyton schoenleinii* Lebert	T.s	Univ. of Karachi
	Animal pathogens		
17.	*Microsporum canis* Bodin	M.c	Department of
18.	*Trichophyton mentagrophytes* Blanchard	T.m	Microbiology,
19.	*Trichophyton simii*	T.s	Karachi University
	Plant pathogens		
20.	*Fusarium moniliforme* Sheldon.	F.m	Department of
21.	*Fusarium solani* (Mart.) Sacc.	F.s	Botany, University
22.	*Mucor* sp. Mich. *ex* Fr.	M.s	of Karachi.
	***Common stored grain pests*:**		
23.	*Callosobruchus analis* Fabricius	C.a	MAHQ Biological
24.	*Rhyzopertha dominica* Fabricius	R.d	Research Center,
25.	*Sitophilus oryzae* (L.) Fabricius	S.o	University of
26.	*Tribolium castaneum* Herbst	T.c	Karachi
27.	*Trogoderma granarium* Everts	T.g	

Abbr. = abbreviation

Table 3. Antibacterial activity of crude methanol extract of different algae shown as zone of inhibition in mm.

Organisms	Gram positive				Gram negative				
	B.s	C.d	S.a	S.p	Pea	P.m	K.P	S.d	S.t
Cholorophyta:									
Codium iyengarii	x	x	x	x	x	x	x	x	x
Codium shameelii	x	13	12	13	12	x	x	x	x
Enteromorpha intestinalis	x	x	x	x	x	x	x	14	x
Ulva fasciata	x	x	x	x	x	x	x	x	x
Phaeophyta:									
Colpomenia sinuosa	17	14	x	17	13	x	14	x	13
Cystoseria indica	x	13	x	14	x	x	16	x	14
Dictyota hauckiana	x	11	x	11	x	x	x	x	x
Iyengaria stellata	14	15	x	14	12	x	14	x	15
Padina tetrastromatica	x	x	x	x	x	x	x	x	x
Sargassum ilicifolium	x	x	x	x	x	x	13	12	x
Stoechospermum marginatum	x	12	x	x	x	x	x	x	x
Rhodophyta:									
Botryocladia leptopoda	x	13	x	14	x	x	14	15	14
Champia compressa	x	x	x	x	x	14	x	15	x
Osmundea pinnatifida	x	x	x	x	x	x	x	13	x

B.s = *Bacillus subtilis*, C.d = *Corynebacterium diphtheriae*, K.p = *Klebsiella pneumoniae*, S.a = *Staphylococcus aureus*, S.d = *Shigella dysenteriae*, S.p = *Streptococcus pyogenes*, S.t = *Salmonella typhi*, P.a = *Pseudomonas aeruginosa*, P.m = *Proteus mirabilis*, x = no inhibition. (Conc. of sample 200 µg/100 µL DMSO; values refer inhibition caused by methanolic extract, standard drug: tetracycline; activity: 9-12 = non-significant, 12-15 = low, 15-18 = good, >18=significant).

Ashing and digestion of the seaweeds: The algal material was initially dried under shade at room temperature and later on in an oven at 60 – 80 °C for 1 h. It was then powdered through grinder, 1 g of the grinded sample was taken in a porcelain crucible and ashed at 500 °C in a muffle furnace to constant weight for 2 h. The ash was cooled at room temperature, moisted with 10 drops of distilled water and carefully dissolved in 3 mL HNO_3 (1 : 1). The acid solution of the sample was then heated gently on a hot plate at 100-120 °C till nearly dry. The crucible was returned to muffle furnace and ashed again for 1 h at 500 °C. It was then cooled and dissolved in 10 mL HCl (1:1), and the solution was filtered through Whatman filter paper No. 42 (Schleicher & Schuell, Germany) into a 100 mL volumetric flask (Jones, 1984). If the concentration is very high (*e.g.* Ca, K, Na, Mg) it is further diluted, mixed well and made ready for AAS reading.

Elemental assay: The flame Atomic Absorption Spectrometry (AAS, Model Perkin-Elmer 3100, USA), was used at Hamdard University, Karachi for the purpose of estimating Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Pb and Zn. Instructions for instrument setting, calibration and assay for specific elements were strictly followed as laid down in the operational manual.

Results and Discussion

The results of antibacterial tests indicated that ten algal extracts inhibited the growth of both Gram +ve and Gram –ve bacteria. *Enteromorpha intestinalis*, *Stoechospermum marginatum* and *Osmundea pinnatifida* prevented the growth of only one bacterium, while *Sargassum ilicifolium* and *Champia compressa* inhibited only two bacteria (Table 3). *Colpomenia sinuosa* and *Iyengaria stellata* showed positive activity against 6 bacteria

including 3 G +ve and 3 G -ve bacterial strains and appeared to be most active, while *Cystoseira indica* exhibited positive activity against 4 bacteria, indicating that brown seaweeds are more active than green and red ones. *Codium iyengarii*, *Ulva fasciata*, and *Padina tetrastromatica* showed no inhibition against all of the investigated 9 bacteria. Similar observations have been made on a variety of algae such as *Enteromorpha compressa*, *Padina gymnospora*, *Sargassum wightii* and *Gracillaria corticata* which were found active against Gram positive culture of *Bacillus* sp. (Rao *et al.*, 1991). *Gracilaria corticata* and *Padina gymnospora* showed antibacterial activity against *Bacillus megaterium* and *Staphylococcus aureus* Rosenbach (Ahmad & Parveen, 1993).

Thirty species of green, brown and red seaweeds were tested against 7 species of human pathogens and 3 species of animal as well as 3 species of plant pathogens for *in vitro* fungicidal bioassay (Table 4). Most of them were active and some were either inactive or showed a very poor activity against these pathogens. Only 10 species exhibited moderate activity (45-50 %). *Botryocladia leptopoda* exhibited a good activity while *Codium shameelii* showed poor activity. *Chaetomorpha antennina*, *Jolyna laminarioides* showed significant activities against *Trichophyton longifusus* and *Microsporum canis*. *Gracilaria foliifera* displayed a significant activity against *Trichophyton longifusus*, while in another observation it also showed significant antifungal activity against a variety of pathogens (Ali *et al.*, 2000). The crude methanolic extract of *Stoechospermum marginatum* was found to inhibit the growth of *Micrococcus pyrogenes* var. *aureus* (Anonymous, 2000). The ethanolic extracts derived from seven seaweed species when tested, showed no detectable antifungal activity against *Epidermophyton floccosum*), *Microsporum canis* and *Trichophyton rubrum* (Alam *et al.*, 1994). It appears that different seaweeds behave variably against a variety of fungal species.

The results of the *Lemna*-bioassay showed that methanolic extract of many seaweeds inhibited the growth of plant while that of the others helped to stimulate the growth of *Lemna aequinoctialis* (Table 5). A very interesting observation was made on the methanolic extract of *Enteromorpha intestinalis* which displayed 95 % inhibition of the fronds at middle concentration *i.e.* 100 µg/mL. *Iyengaria stellata* showed moderate activity (47.36 % growth inhibition) at lowest concentration *i.e.* 10 µg/mL. *Aspargopsis taxiformis* exhibited significant activity (100 % inhibition) at highest concentration *i.e.* 1000 µg/mL, moderate activity (66.66 % inhibition) at middle concentration *i.e.* 100 µg/mL, while poor activity (12.5 % inhibition) at lowest concentration *i.e.* 10 µg/mL. *Melanothamnus afaqhusanii* exhibited good activity (100 % inhibition) at highest concentration *i.e.* 1000 µg/mL, while negative activity helped to promote growth (-15.7 %) at lowest concentration *i.e.* 10 µg/mL. *Sargassum boeanum* displayed a poor activity, while in another observation the ethanolic extract of *S. tenerrimum* showed 100 % inhibition of the fronds at highest concentration *i.e.* 500 µg/mL (Ali *et al.*, 2000). It appears that different species of the same genus act as variably, probably they accumulate different natural products which may be responsible for phytotoxic activity.

For insecticidal bioassay 12 marine algae were chosen and their crude extracts tested against five different insects *vis a vis Callosobruchus analis*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum* and *Trogoderma granarium* (Table 6). *Osmundea pinnatifida* was active against most of the tested insects and appeared to be the most active seaweed. It was found to contain a variety of natural products (Ali *et al.*, 2000) which might be responsible for such activity. *Jolyna laminarioides* exhibited moderate activity against *Tribolium castaneum*. All green and brown seaweeds were active but most of the tested red seaweeds showed no activity against any of the above mentioned insects.

Table 4. Antifungal activity of crude MeOH extract of different marine algae shown as % inhibition.

	Human pathogens							Animal pathogens			Plant pathogens		
Name of objects	**A.f**	**A.n**	**C.a**	**C.g**	**P.b**	**T.l**	**T.s**	**M.c**	**T.m**	**T.s**	**F.m**	**F.s**	**M.s**
Chlorophyta:													
Caulerpa racemosa	-	-	7.9	x	63	-	40	33	30	55.5	-	50	-
Caulerpa taxifolia	-	-	x	x	56	-	50	13.3	30	54	-	55	-
Chaetomorpha antennina	x	-	x	x	x	90	-	90	-	-	-	x	-
Codium iyengarii	-	46	x	x	54	-	50	50	x	50	-	56.3	-
Codium shameelii	x	-	x	x	-	x	-	x	-	-	-	x	-
Enteromorpha intestinalis	x	-	x	x	-	65	-	85	-	-	-	x	-
Ulva fasciata	x	12.22	49.7	x	-	-	-	38.33	24.32	-	-	55.71	20
Ulva lactuca	x	50	x	x	40	-	26	33.2	x	x	-	4.2	-
Phaeophyta:													
Colpomenia sinuosa	x	x	x	x	-	x	-	00	-	-	-	x	-
Cystoseria indica	x	-	x	x	-	26	-	28	-	-	x	x	-
Dictyota hauckiana	x	x	x	x	x	x	x	x	x	x	50	x	-
Iyengaria stellata	x	-	x	x	-	43	-	60	-	-	x	x	-
Jolyna laminarioides	x	-	x	x	-	85	-	90	-	-	-	10	-
Padina tetrastromatica	x	-	x	x	60	x	40	16	11	50	x	51.6	-
Sargassum boveanum	-	x	3.43	40	-	-	-	61.66	36.48	-	-	x	8.00
Sargassum ilicifolium	-	3.33	52.68	x	x	-	-	5.00	63.51	x	-	44.28	4.00
Sargassum vulgare	-	56	x	x	50	-	5.7	50	x	45	-	4.2	-
Stoechospermum marginatum	-	-	x	x	30	-	60	50	46	64	-	58.3	-

Table 4 (Cont'd.)

Name of objects	Human pathogens							Animal pathogens			Plant pathogens		
	A.f	A.n	C.a	C.g	P.b	T.l	T.s	M.c	T.m	T.s	F.m	F.s	M.s
Rhodophyta:													
Botryocladia leptopoda	-	-	x	x	52	-	60	50	55	60	66.6	60	-
Champia compressa	-	6.66	1.94	46.6	-	-	-	43.33	39.18	-	-	60	2.66
Cystoclonium purpureum	x	-	55	x	-	15	-	10	-	-	-	x	-
Dermonema abbottiae	x	-	x	x	-	50	-	45	-	-	-	x	-
Gracilaria corticata	-	50	x	x	16	x	14.2	16	x	x	-	14.2	-
Gracilaria foliifera	70	-	x	x	-	80	-	65	-	-	-	x	-
Hypnea musciformis	-	-	37.16	x	36	x	30	43	58.91	46	-	70	6.66
Hypnea valentiae	-	-	x	x	50	-	40	50	55	40	x	58.3	x
Melanothamnus afaqhusanii	60	-	x	-	-	50	-	45	-	-	-	x	-
Osmundea pinnatifida	-	23.3	3.43	30	-	-	-	x	8.10	-	-	28.57	x
Sarconema furcellatum	-	-	x	x	56	x	64	50	57	56	x	60	x
Solieria robusta	-	-	x	x	27.2	x	10	16	x	28.8	-	40	x
Standard drugs:													
Miconazole	-	100	110.8	110.8	100	70	100	98.4	100	100	100	73.25	100
Ketoconazole	-	-	-	-	100	-	100	-	-	100	-	-	-
Amphotericin-B	20		-	-	-	-	-	-	-	-	-	-	-

A.f = *Aspergillus flavus*, A.s = *Aspergillus niger*, C.a = *Candida albicans*, C.g = *Candida glabrata*, F.m = *Fusarium moniliforme*, F.s = *Fusarium solani*, M.c = *Microsporum canis*, M.s = *Mucor* sp., P.b = *Pseudallescheria boydii*, T.l = *Trichophyton longifusus*, T.m = *Trichophyton mentagrophytes* T.s = *Trichophyton schoenleinii*, , T.s = *Trichophyton simii*, - =Not tested, x =No inhibition, concentration of sample 400 μg/mL of medium, values refer in % inhibition, incubation temp. 27-29 °C; incubation time: 7-10 days; activity: 0-40= non-significant, 40-60= moderate, 60-70 = good, >70 = significant.

Table 5. Phytotoxic activity of marine algae in % growth regulation.

	Conc. of sample µg/mL		
Name of Alga	**1000 µg/mL**	**100 µg/mL**	**10 µg/mL**
Cholorophyta:			
Caulerpa racemosa	50	41.66	12.5
Codium shameelii	22.23	-5.88	-13.33
Chaetomorpha antennina	47.3	10.5	26.3
Enteromorpha intestinalis	63.6	95.0	22.2
Ulva fasciata	78.9	31.5	5.2
Phaeophyta:			
Colpomenia sinuosa	29.41	7.14	31.57
Cystoseira indica	23.52	14.28	31.57
Dictyota hauckiana	16.6	29.4	6.6
Iyengaria stellata	23.52	-21.22	47.36
Jolyna laminarioides	68.4	52.6	36.8
Sargassum boveanum	9.9	8.8	-22
Rhodophyta:			
Asparagopsis taxiformis	100	66.66	12.5
Botryocladia leptopoda	29.41	-14.2	10.52
Champia compressa	63.63	41.66	-11.11
Cystoclonium purpureum	100	36.8	31.5
Dermonema abbottiae	100	31.5	5.2
Gracilaria corticata	42.1	36.8	31.5
Gracilaria foliifera	-15.7	10.5	-10.5
Hypnea musciformis	45.4	16.6	0.0
Melanothamnus afaqhusanii	100	21.0	-15.7
Osmundea pinnatifida	0.0	0.0	11.11

Standard drug: paraquat, growth period: 7 days.; + ve regulation = growth inhibition, - ve regulation = growth promotion.

Different species of seaweeds were analyzed for the composition of eleven elements (Table 7 A, B, C). Among them Ca, Fe, K, Mg and Na were found in large amounts (on the average 2411.38-76714.25 ppm), Co, Cu, Pb and Zn were present in small quantities (5.88-53.28 ppm), while Cd and Cr were detected in extremely small amounts (1.61 – 5.13 ppm). Iron was also found in large quantity, Cr and Zn in medium amount and Co in small proportion in several brown seaweeds of the Saronic Gulf, Greece (Kanias *et al.*, 1991). It was also detected in very small quantity in several other species of green, brown and red seaweeds (Hasni & Sarwar, 1985, Rizvi & Shameel, 2001). In general, Ca, Cr and Pb were found to occur in largest amount in green seaweeds, Co, Cu, Fe and Zn in greatest quantity in brown seaweeds, while Cd, K, Mg and Na in highest proportion in the investigated red seaweeds.

Table 6. Insecticidal test for marine algae in % mortality.

Name of Alga	Control	C.a	R.d	S.o	T.c	T.g
Chlorophyta:						
Enteromorpha intestinalis	100	x	x	x	20	x
Ulva fasciata	100	x	x	x	x	10
Phaeophyta:						
Jolyna laminarioides	100	x	x	x	40	x
Sargassum boveanum	100	20	x	x	x	20
Sargassum ilicifolium	100	x	x	x	x	10
Rhodophyta:						
Botryocladia leptopoda	100	x	x	x	x	x
Champia compressa	100	10	x	x	x	x
Cystoclonium purpureum	100	x	25	x	x	x
Dermonema abbottiae	100	x	x	x	x	x
Hypnea musciformis	100	x	x	x	x	x
Melanothamnus afaqhusanii	100	x	x	x	x	x
Osmundea pinnatifida	100	20	20	x	x	20

C.a = *Callosobruchus analis*, R.d = *Rhizopertha dominica*, S.o = *Sitophilus oryzae*, T.c = *Tribolium castaneum*, T.g = *Trogoderma granarium*, x = no activity, standard drug: pyrethroids conc. of Coopex = 235.71μg/cm^2.), incubation temperature: 37 °C, humidity: 50 %, sample solvent MeOH, sample concentration 1571.33 μg/cm^2: Pyrethriod & Permethrin (1:1).

Ulva lactuca from China and 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* sp. and *Grateloupia* sp. contained medium amount (Chapman & Chapman, 1980). The trace metal distribution in seaweeds of Indian coast has also been well documented. Metal concentration in the seaweeds were in the order Fe, Mn, Zn, Cu with the exception of a few seaweeds *e.g. Ulva reticulata, Sargassum wightii* and *Sarconema* sp. which concentrated more Zn than Mn (Zingde *et al.*,1976). Seaweeds from other tropical areas exhibit a similar trend. Ganesan *et al.* (1991) reported that the tropical seaweeds tend to accumulate more Fe than Mn, Zn and Cu.

The present study was designed to obtain preliminary research information on the biological activity as well as elemental composition of seaweeds from Karachi coast and research work is to be continued in order to investigate the distribution of various elements in different thallus parts of marine benthic algae for the sake of comparison.

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Table 7. Elemental composition in marine algae from Karachi coast.

Alga*	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Na	Pb	Zn
A:	**36669.75**	**1.38**	**4.39**	**7.60**	**11.89**	**2827.7**	**50457.87**	**10753.06**	**70200.87**	**17.60**	**35.26**
BP-01	80800	3.15	8.05	9.925	12.9	3795	10855	6660	28535	43.875	37.425
CR-02	70300	2.2	6.8	12.525	11.25	2542.5	19625	764.5	155950	23.675	21.725
CS- 03	80750	BDL	BDL	BDL	7.75	8427.5	3900	3725	1909	BDL	37
CT-04	14755	0.975	4.0	10.425	8.5	2840	15810	6870	110400	19.1	25.05
CI- 06	14730	1.925	9.55	2.825	5.65	862.5	231700	9605	169350	26.075	18.25
CS-07	18733	BDL	BDL	BDL	28	1076.6	70633	8100	52833	BDL	42
EI- 08	4745	0.5	3.675	23.325	14.0	2695	18590	13400	17470	19.6	81.55
UL-10	8545	2.3	3.05	1.85	7.125	382.5	32550	36900	25160	8.55	19.15
B:	**30857.12**	**1.18**	**7.03**	**1.91**	**13.87**	**3410.62**	**75704.78**	**10073.87**	**26266.16**	**10.40**	**96.09**
CS-11	43943.33	2.33	16.65	B.D.L	19.93	4064.33	125736.66	7156.66	29216.66	15.93	123.1
CI-12	19050	3.95	5.125	4.7	8.125	249	118125	9425	80562.5	8.1	33.625
DD-13	21080	BDL	13.4	BDL	29.6	1220	5400	7120	10800	4	213.6
IS-15	16810	1.05	14.5	BDL	15.3	767	269000	6060	56520	4.8	64.1
PV-17	24900	BDL	BDL	BDL	12.5	5000	47075	11425	1999	BDL	76
PT-18	46950	2.875	6.375	16.15	12.375	3105	26620	24500	20530	15.25	44.475
SS-21	87950	BDL	BDL	BDL	1.65	391.5	76820	7565	37740	40.7	16.85
ST-22	13605	0.2	9.9	BDL	12.2	3465	39350	10000	19500	6.5	118.2
SV-23	16055	1.2	7.0	0.18	8.6	1740	60666	14166	9902.2	6.8	274.8
SM-24	18835	0.7	4.4	BDL	10.5	1570	50750	6450	8902.5	9.6	41.3
SS-25	30250	0.7	BDL	BDL	21.8	15945	13210	6945	13255	2.75	50.95
C:	**2713.14**	**2.27**	**6.23**	**5.87**	**9.86**	**995.82**	**82442.85**	**13206.07**	**133675.71**	**12.29**	**28.50**
BL-27	9055	2.9	8.05	3.4	13.6	499.5	65925	27040	202375	7.2	26.375
GC-32	11725	1.875	5.55	7.1	8.375	1105	114750	4580	26290	13.32	35.3
HM-34	7977.5	1.975	5.1	2.325	6.675	230.5	62125	4930	129687.5	3.85	15.675
HV-35	10350	2.7	7.75	10.625	11.625	1825	112937.5	15260	154187.5	14.1	29.925
SF-38	7447.5	2.55	7.45	5.425	12.7	340.75	136375	19820	220187.5	16.05	20.775
SS-39	8350	1.15	2.85	4.55	4.2	1070	14925	12350	18690	13.45	47.875
SR-40	34087	2.8	6.9	7.7	11.9	1900	70062.5	8462.5	184312.5	18.1	23.6
Average amount	**26746.67**	**1.61**	**5.88**	**5.13**	**11.87**	**2411.38**	**69535.17**	**11344.34**	**76714.25**	**13.43**	**53.28**

ers under the column of ''Alga'' refer to the taxonomic names of algae given in Table I. BDL= below detection level.
A = Chlorophyta, B = Phaeophyta, C = Rhodophyta.

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