Biological Sciences Section

Pak. j. sci. ind. res., vol. 37, no. 4, April 1994

VARIATION IN CHEMICAL CONSTITUENTS OF MANGROVE FOLIAGE *AVICENNIA MARINA* (FORSK.) VIERH. (AVICENNIACEAE)

Pirzada J. A. Siddiqui and Rashida Qasim*

Centre of Excellence in Marine Biology, University of Karachi, Karachi-75270, Pakistan

(Received January 26, 1991; reviseed December 23, 1993)

Seasonal variation in the organic and inorganic constituents of green, yellow and decomposing mangrove, *Avicennia marina,* leaves were studied. The leaf samples were collected from two different locations and analysed for total organic matter, water,protein, lipid, carbohydrate, nitrogen, organic carbon, and calorific content. Total inorganic content and the macro and micro-elements including sodium, potassium, calcium, magnesium, phosphorus, iron, manganese and copper were also determined. Results showed differences among leaf types and also between the sampling sites. The heavy metal pollution in the creek area was also reflected in the leaf samples from Bakran creek.

Key words: Mangrove, *Avicennia marina,* Chemical composition, Seasonal variation.

Introduction

Mangrove swamps occupy a considerable area of Pakistan coast. The estimated mangrove cover 0.26 million hectare on Pakistan coast [1], represents about 2.74% of the total mangrove covered area in the Indo-West Pacific region. A number of studies on chemical composition of fresh leaves [2-8], decomposing leaves [9-13] and on the seasonal variation in chemical constituents [14-15] have been reported from various parts of the world. From Pakistan studies pertaining to chemical constituents and other related aspects of mangrove foliage are scanty [16-18]. Seasonal variation in chemical composition of mangrove leaves is not yet reported from this area. Litter production in a mangrove stand [17] showed that enormous amount of litter enter into the coastal water, mineralize and recycle through the food web [18]. Analysis of biochemical constituents of mangrove litter will provide the data on the total energy released during the year and also help in understanding the mangrove ecosystem and physiological chemistry of the plant.

In the present study seasonal variation in organic and inorganic constituents of three types of leaves from two mangrove (*Avicennia marina*) swamps were measured. The effects of water and environmental conditions on the chemical constituents of leaves are also discussed.

Materials and Methods

Study area. Two sites were selected in the mangrove swamps near Karachi: (1) Karachi back waters near Baba and Bhitt Islands in the Manora channel (24°50' N and 60° 58') where Layari river, containing domestic and industrial wastes drains; and (2) Bakran Creek, a part of Gharo-Phitti Creek system located about 30 km southeast of the mouth of Manora channel (24°47'N and 55°20'E). This area recieves heavy metals and pollutants of agricultural origin. The condition of these sites have been discussed elsewhere [19,20].

Sample collection. Three physiological types of *Avicennia marina* leaves were randomly collected each month (July 1984 through June 1985) from the above sites. The leaf types included in this study were: (1) Actively photosynthesizing (green); (2) Senescent (easily detachable yellow leaves from the tree); and (3) Decomposing leaves (already fallen black leaves from the surface of the sediment). Leaves were first washed to remove mud and excessive salt or any other exotic material attached on them. Clean leaves were blotted dry, weighed and completely dried at 70°. Sub-samples were used to determine water content of leaves by taking the difference of wet and dry leaf weights. Dried leaves were ground for further biochemical estimations.

Chemical analysis. Analysis of organic content: Ash or total inorganic content was determined as weight remained after combusting organic matter at 550° for 8 hrs. Crude protein was evaluated by multiplying total ammonia nitrogen, measured by Microk Jeldhal method, by a conversion factor 6.25 [21]. Total lipid was extracted by soxhlation using chloroform-methanol mixture (2:1 v/v) and measured gravimetrically [22]. Carbohydrate including crude fiber was calculated by subtracting the sum of percent protein, lipid and ash content from 100. Organic carbon was determined as 'chromic acid oxidation value' according to the method of Holme and McIntyre [23]. Calorific content (energy) was measured from percent carbon according to the method given by Bhosle *et al.* [5]. Calorific content was also calculated using metabolite equivalent (5.56 for protein, 9.45 for lipid and 4.15 for carbohydrate).

Dried ground samples were wet ashed using nitric acid

* Department of Biochemistry, Karachi Univesity, Karachi, Pakistan.

and perchloric acid mixture. Sodium, potassium and calcium were analysed by flame photometery (Corning 400). Magnesium, manganese, iron, and copper were measured on atomic absorption flame emission spectrophotometer (Jarrel Ash AA - 782). Phosphorus was estimated according to Lowry *et al.* [24].

Results and Discussion

Seasonal variation in the chemical constituents of green, yellow, and black mangrove leaves was determined. Annual averages of organic content of all mangrove leaf types are given in Table 1, whereas seasonal variations are shown in Figs. 1-4. Annual average for water content was higher in green leaves compared to yellow leaves, and the highest water concentration was in black leaves. The seasonal variation pattern was identical for green and yellow leaves with differences in the magnitude of values from each location. The pattern of change in water content in black leaves from two sites was not comparable. Samples from back waters, however, showed highly fluctuating seasonal values.

According to the annual average value, total ash content in green and yellow leaves was almost similar in respective samples from both sites. Black leaves showed higher ash values. Seasonal changes in total inorganic content of green and yellow leaf types varied within 4 - 6 % through the year.

Average values for crude protein were comparatively higher in back water samples. Highest average values were recoreded for green leaves and the lowest were in yellow leaves. Yellow leaves when decompose and turn black showed an increase in the crude protein. Crude protein values in green leaves remained low during November and June from back waters and during November and July from Bakran creek.. Values than increased and stayed high during rest of the year. Total protein in yellow and black leaves varied within a narrow range through the year and does not present a distinct seasonal pattern of protein variation.

Total lipid as dipicted by annual averages from both locations, was highest in the green leaves and lowest in the yellow leaves. However, Bakran creek samples had more lipid content compared to back water samples. Seasonal fluctuations showed a highly variable pattern for all leaf types from both locations.

Total carbohydrate values, including curde fiber, was highest in yellow leaves and lowest in green leaves from both locations. However, Bakran creek samples showed higher carbohydrate content compared to back water samples. Green leaves from both locations had low values during July to August. The highest values were attained in May and June in leaves from back water and Bakran creek, respectively. Maximum value of carbohydrate in yellow leaves was in October from back waters and in November from Bakran creek. Variation pattern for both yellow and black leaves was almost similar.

Annual average of orgánic carbon showed lowest concentration in green leaves from both locations followed by yellow and green leaf type. Bakran creek samples, however, contained more carbon in all leaf types compared to leaves from back waters. Seasonal pattern of fluctuation in organic carbon

TABLE 1. YEARLY AVERAGE OF BIOCHEMICAL COMPONENTS FOR THREE LEAF TYPES. VALUES ARE IN GRAM PERCENT + STANDARD ERROR. CALORIES ARE SHOWN IN kcal/gm.

	Back water			Bakran creek		
	Geeen leaves	Senescent leaves	Decomposing leaves	Geeen leaves	Senescent leaves	Decomposing leaves
Water	65.59+0.59	61.84+0.65	71.26+1.34	63.81+1.16	58.07+0.69	69.75+0.75
Ash	12.87+0.34	12.96+0.52	15.24+0.99	12.02+0.34	11.78+0.45	14.92+1.17
Organic matter	87.88+0.96	87.08+0.55	84.76+0.99	87.99+0.34	88.22+0.45	85.10+1.18
Crude protein	12.57+0.66	4.79+0.34	7.86+0.43	9.81+0.89	3.64+0.20	6.46+0.28
Total lipid	9.49+0.50	6.87+0.49	5.56+0.30	10.82+0.56	8.47+0.89	6.03+0.59
Carbohydrate	65.25+0.87	75.40+0.94	71.33+1.14	67.38+1.35	76.10+0.63	73.20+1.12
Organic carbon	37.38+0.82	40.29+0.46	41.53+0.59	39.13+0.76	41.47+0.26	42.39+0.48
Total nitrogen	2.01+0.10	0.79+0.07	1.26+0.07	1.59+0.14	0.58+0.03	1.03+0.05
C: N ratio	19.30+1.18	59.06+7.06	33.77+1.30	27.21+2.35	76.01+7.74	42.15+2.02
Calories(calculated from % carbon)	5.45+0.12	5.89+0.07	6.09+0.09	5.72+0.12	6.07+0.04	6.23+0.07
Calories (calculated from matabolite equivalent)	4.32+0.03	4.05+0.03	3.39+0.04	4.42+0.05	4.17+0.06	3.96+0.06

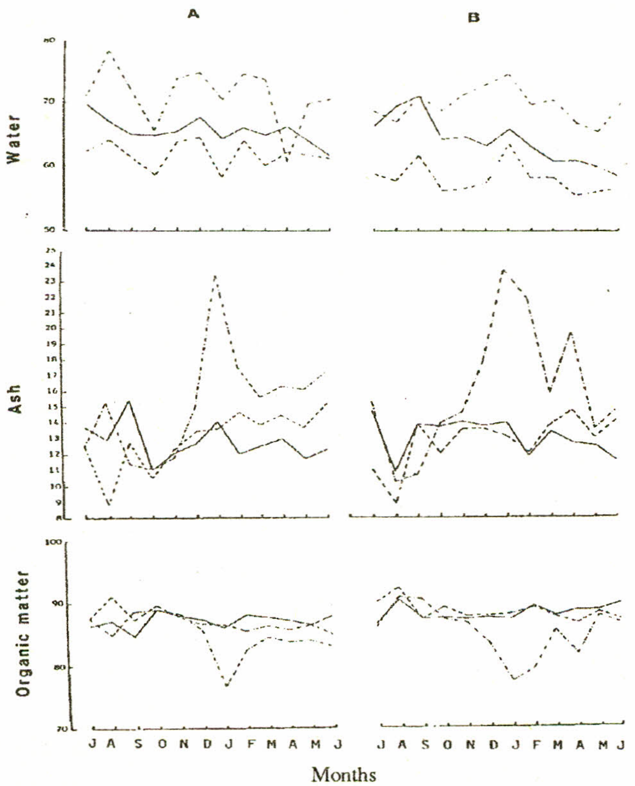

Fig. 1. Monthly variation in water, ash and organic matter (g%) in mangrove leaves from back waters (A) and Bakran creek (B).
___ green leaf; ---- yellow leaf; -·-·-·- decomposing leaf.

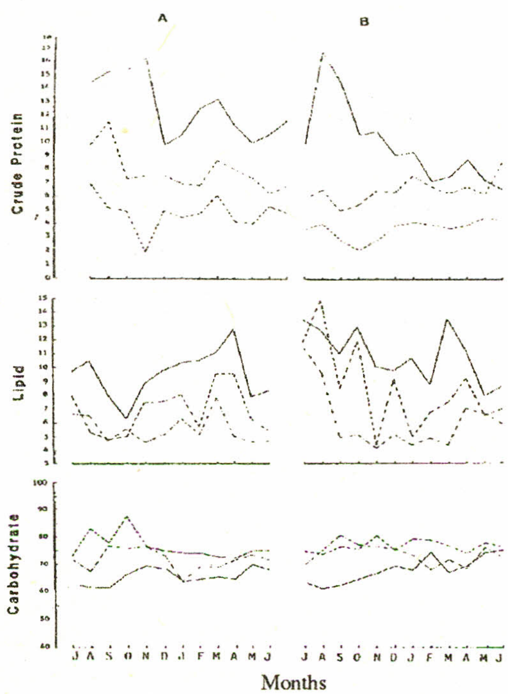

Fig. 2. Monthly variation in crude protein, lipid, and carbohydrate contents in mangrove leaves from back waters and Bakran creek (B). All values are in g%.
___ green leaf; ---- yellow leaf; -·-·-·- decomposing leaf.

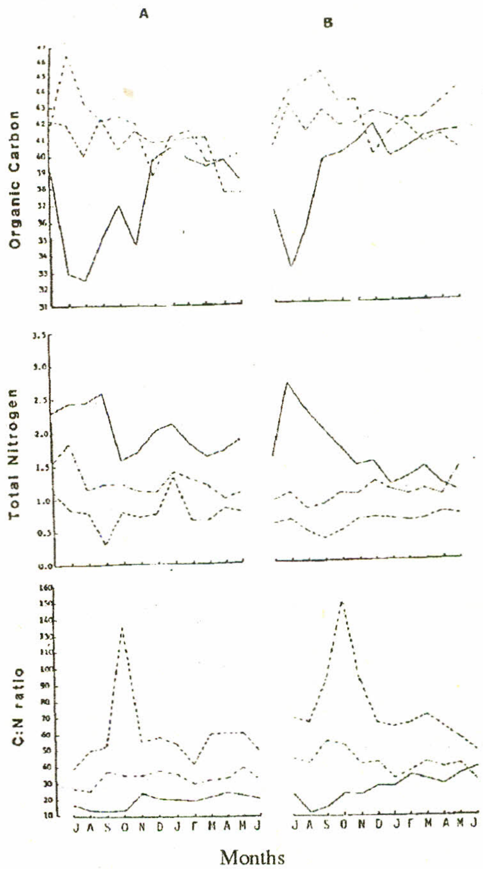

Fig. 3. Monthly variation in organic carbon, total nitrogen (values are in g%) and carbon to nitrogen ratio (C:N) in mangrove leaves from back water (A) and Bakran creek.
___ green leaf; ---- yellow leaf, -·-·-·- decomposing leaf.

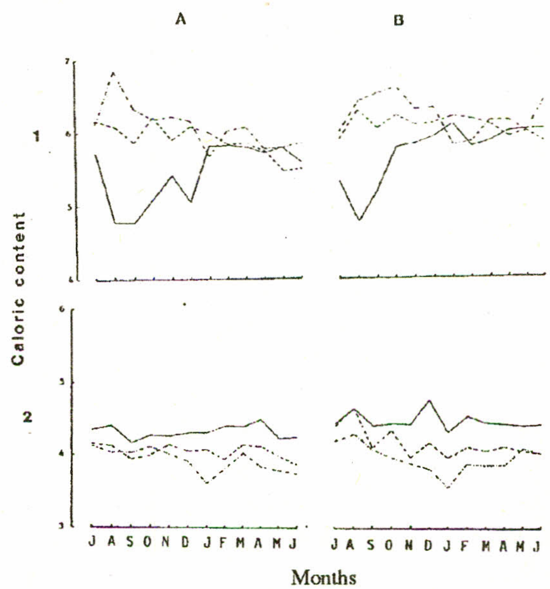

Fig. 4. Monthly variation in caloric content (Kcal/g) as calculated from organic carbon (1) and major metabolites (2) in mangrove leaves from back water (A) and Bakran creek (B).
___ green leaf; ---- yellow leaf; -·-·-·- decomposing leaf.

of green leaf showed lowest values during August (back waters) and September. (Bakran creek). Later, values started to increase and reached at a maximum during January and February from Bakran creek and Back waters, respectively. Yellow and black leaves had organic carbon values varying within a narrow range and showed no distinct pattern.

Carbon to nitrogen values for green leaves were low during July to October which corresponds to high nitrogen and low carbon levels in leaves in these months. Yellow leaves had higher values compared to leaves. Increase in the nitrogen content in decomposing black leaves enhanced the carbon to nitrogen ratio.

Average calorific values calculated from organic carbon dipicted highest values in black leaves and lowest in green leaves from both locations. Interestingly, the calorific values when calculated on the basis of major metabolites showed highest values in green leaves and lowest in black leaves. Seasonal pattern of calorific content calculated using carbon content calculated using carbon content was similar to the pattern of carbon variation in leaves. The seasonal values for calorific content calculated on the basis of major metabolites varied within a narrow range.

Macro-elements (sodium, potassium, calcium, magnesium and phosphorus) and microelements (manganese, copper and iron) were analysed and the results are summerised in Table 2 and Figs 5-6. Among macroelements sodium was recorded in highest concentration in all leaf types. Average concentration of sodium was higher in green leaves from back waters. Black leaves had lowest sodium content. Similar potassium concentrations were obtained. The highest concentration was in green leave and the lowest was recorded for black leaves. Calcium and phosphorus concentrations were almost similar in green and yellow leaves from both locations. However, black leaves and less calcium and more phosphorus compared to other leaf types. Magnesium content showed the lowest values in all leaf types compared to all other macroelements. It is interesting to note that yellow leaves from both locations and high magnesium content compared to green and black leaves, and that green and black leaves had almost identical values. Seasonally all macroelements varied only within a narrow range and showed a highly variable trend of fluctuation through the year.

Among all microelements measured, iron showed highest concentration in all leaf types from both locations. Average values were higher in samples from Bakran creek compared to the other location. Black leaves had highest concentration of iron followed by yellow and green leaves. The highest manganese content was in yellow leaves from both locations. Black leaves from back waters and green leaves from Bakran creek exhibited lowest concentration of magnanese. Similarly, highest copper content was in yellow leaves and the lowest concentration in green leaves from both location. The average values of respective leaf type from two locations were almost identical. Seasonal pattern of fluctuation of microelements showed that the highest variablility was in black leaves. Green and yellow leaves varied within narrow range and the fluctuation in the content of both leaf types followed each other closely. No distinct seasonal pattern was observed for microelements.

Seasonal variation in biochemical constituent of leaf is very complex process. Our data on the leaves from two sites showed different trends. Differences in chemical composition of eight different mangrove species have been observed [14, 15], and attributed to the magnitude of deviation in environmental conditions or species. On the average, organic cons-

TABLE 2. YEARLY AVERAGE OF INORGANIC COMPONENTS FOR THREE LEAF TYPES. VALUES FOR MACRO ELEMENTS ARE IN gm % + STANDARD ERROR, AND FOR MICRO ELEMENTS ARE IN mg % + STANDARD ERROR.

Heavy metals	Back water			Bakran creek		
	Geeen leaves	Senescent leaves	Decomposing leaves	Geeen leaves	Senescent leaves	Decomposing leaves
MACROELEMENTS						
Sodium	4.58+0.11	4.21+0.28	2.88+0.15	3.87+0.15	3.15+0.15	2.74+0.12
Potassium	1.29+0.03	1.13+0.08	0.31+0.02	1.28+0.04	1.06+0.03	0.30+0.02
Calcium	1.40+0.40	1.37+0.11	0.94+0.08	1.30+0.03	1.20+0.04	1.35+0.08
Phosphorus	0.65+0.02	0.54+0.06	1.36+0.06	0.67+0.04	0.68+0.07	0.91+0.04
Magnesium	0.30+0.02	0.65+0.06	0.38+0.01	0.38+0.03	0.61+0.03	0.40+0.01
MICROELEMENTS						
Manganese	9.09+1.03	11.84+0.69	7.28+0.50	15.48+1.49	21.28+1.14	18.59+2.71
Copper	4.71+0.50	6.23+0.42	5.22+0.42	4.19+0.23	5.53+0.17	4.66+0.39
Iron	22.51+2.69	31.47+2.34	152.73+25.15	35.75+2.69	53.01+5.25	224.01+27.11

tituent of green leaves was in conformity with data reported previously [2-9, 14-16]. Average water, ash and organic contents from two sites were similar as were the average organic carbon, total lipid and carbohydrate contents of leaves. Total nitrogen and protein contents, however, were higher in leaves from back waters. High nitrogen values also renders lowed C:N ratio in all leaf types from back waters. Low C:N ratio is regarded as high nutritive index [9, 12]. Black decomposing leaves showed much better nutritive value compared to yellow leaves. Increase in total nitrogen and crude protein during decomposition is due to the bacterial colonization of decaying material [18]. It may be interesting to note that calorific content obtained through percent carbon is consistantly higher compared to those calculated using metabolite equivalent. This may suggest the presence of extra carbon source in mangrove leaves other than major metabolites [5].

Decrease in water, protein, lipid and nitrogen contents of yellow leaves were observed in the present study. The lowering of these components upon senescence are regulated processes in plants [25]. Senescence of green leaves occur with the degeneration of defined cells, break down of adjoining cell walls and the formation of abscission zone [26]. During senescence many organnic components transfer to the growing parts of the plant [26]. This may be the reason for the higher carbohydrates and organic carbon, and lower nitrogen contents in yellow leaveas compared to the average values of green leaves. A marked decrease in the crude protein and total nitrogen value and an increase in the organic carbon may be noted during October and November, the autumn period. A high production of leaf litter has been noted from mangrove stand in Karachi during these months indicating high rate of leaf senescence [17] and hence the transferance of nitrogen

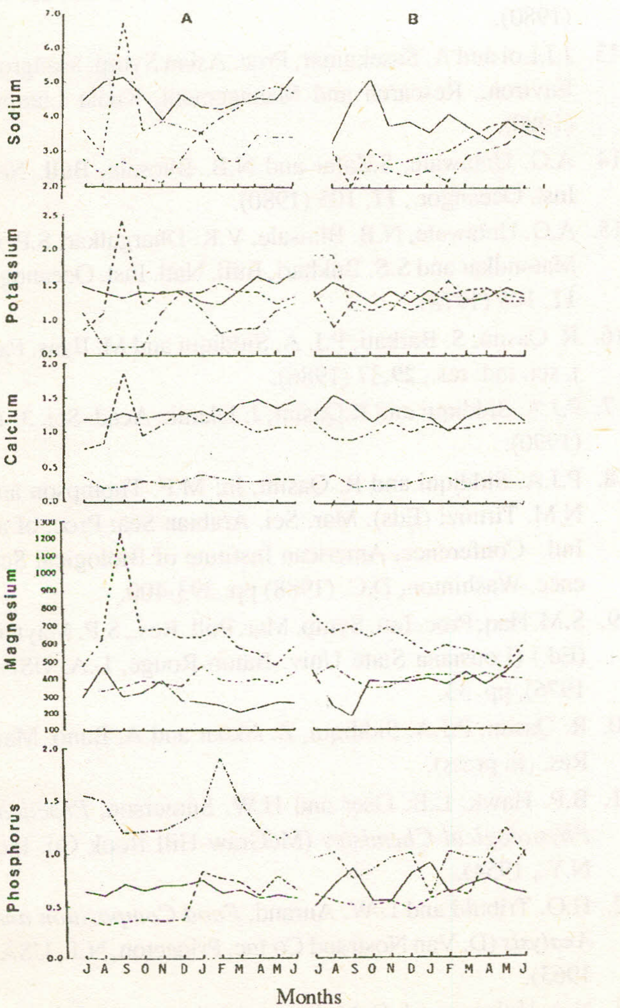

Fig. 5. Monthly variation in macroelements in mangrove leaves from back water (A) and Bakran creek (B). Values are in g% except for mangnesium which are in mg%.
___ green leaf; ---- yellow leaf; -·-·-·- decomposing leaf.

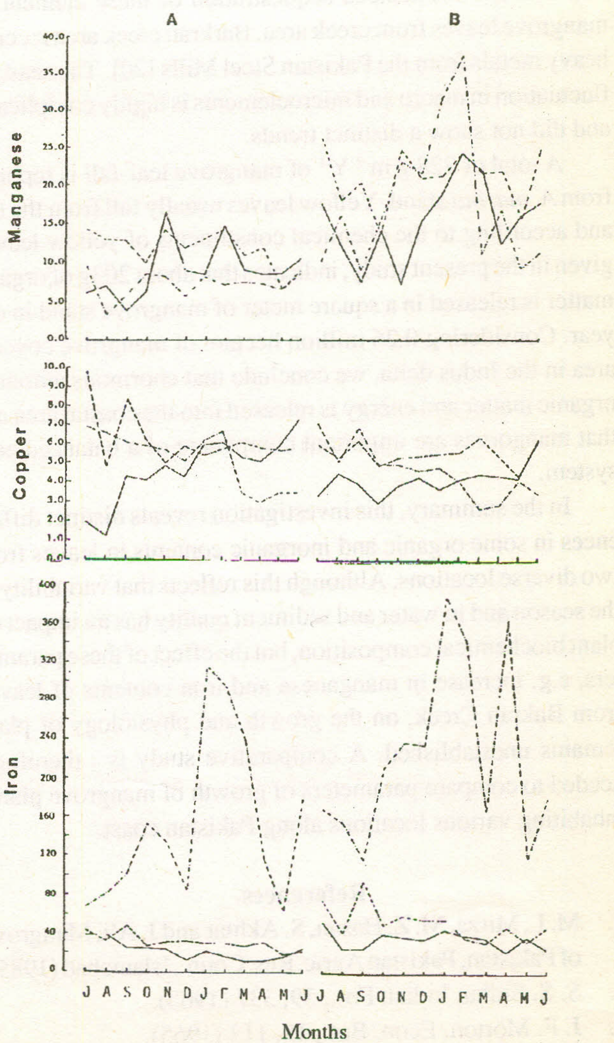

Fig. 6. Monthly variation in microelements contents (mg%) in mangrove leaves from back water (A) and Bakran creek (B).
___ green leaf; ---- yellow leaf; -·-·-·- decomposing leaf.

components to the young green parts of the plant.

Total inorganic content of black leaves is highest among all leaf types. This probably is due to remaining soil particles in leaves even after washing. Average values of sodium, potassium and calcium in green foliage from this study are similar to the mangrove leaves from India [2,7]. Magnesium and phosphorus concentrations in green leaves from this study are higher compared to previous reports [8, 27, 28]. Average values and seasonal pattern of macroelements from two locations were in conformity. On the other hand, microelements showed variable values in leaves from two sites. Manganese content from this study is lower than the recoreded by Untawale [14], whereas, the values were higher than reported by Kotmire and Bhosale [7]. Bhosale [27] reported a wide range of manganese concentration and our result fits well within this range. Iron and copper values are considerally higher than that of values reported from India [27]. The values for manganese and iron show enhanced sequestration of these elements in mangrove leaves from creek area. Barkran creek area receives heavy metals from the Pakistan Steel Mills [20]. The seasonal fluctuation in macro and microelements is highly complicated and did not show a distinct trends.

A total of 228 g m^{-2} Y^{-1} of mangrove leaf-fall is reported from *A. marina* stand. Yellow leaves usually fall from the tree and according to the chemical constituents of yellow leaves, given in the present study, indicates that about 203g of organic matter is released in a square meter of mangrove stand in one year. Considering 0.26 million hectare of mangrove covered area in the Indus delta, we conclude that enormous amout of organic matter and energy is released into the coastal area and that mangorves are important component of a balanced ecosystem.

In the summary, this investigation reveals distinct differences in some organic and inorganic contents in leaves from two diverse locations. Although this reflects that variability in the season and in water and sediment quality has an impact on plant biochemical composition, but the effect of these parameters, e.g. increase in manganese and iron contents of leaves from Bakran Creek, on the growth and physiology of plant remains unestablished. A comparative study is , therefore, needed to compare parameters of growth of mangrove plants inhabiting various locations along Pakistan coast.

References

1. M. I., Mirza, M. Z. Hasan, S. Akhtar and J. Ali, Mangrove of Pakistan, Pakistan Agric. Res. Coun., Islamabad (1985).
2. S. S. Sidhu, Indian For., **39**, 337 (1963).
3. J. F. Morton, Econ. Bot., **19**, 113 (1965).
4. M.R. Atkinson, G.P. Findlay, A.B. Hope, M.G. Pitman, H.D. W. Saddler and K. R. West, Aust. J. Biol. Sci., **20**, 589 (1967).
5. N. V. Bhosale, V. K. Dhargalkar, S. P. G. Matondkar and S. S. Bukhari, Indian J. Mar. Sci., **5**, 239 (1976).
6. A. G. Untawale, N. B. Bhosale, V. K. Dhargalkar, S. P. G. Matondkar and S. S. Bukhari, Indian J. Mar. Sci., **6**, 104 (1977).
7. S. V. Kotmire and L. J. Bhosale, Indian J. Mar. Sci., **9**, 299 (1980).
8. A. P. Waghmode and G. V. Joshi, Indian J. Mar. Sci., **11**, 104 (1982).
9. H. Parnas, Soil Biol. Biochem., 161 (1975).
10. P. F. E. Goulter and W. G. Allaway, Second Intl. Symposium on Biology and Management of Mangroves and Tropical Shallow Water Communities, Papua New Guinae (1980).
11. J. W. Fell and I. M. Master, Can. J. Bot., **53**, 2908 (1975).
12. J. W. Fell and I. M. Master, Botanica Marina, **23**, 257 (1980).
13. J.J.Loi and A. Sasekumar, Proc. Asian Symp. Mangrove Environ., Research and Management, Kuala Lumpur, (1980).
14. A.G. Untawale, S.Zafar and N.B. Bhosale, Bull. Natl. Inst. Oceangor., **11**, 105 (1980).
15. A.G. Untawale, N.B. Bhosale, V.K. Dhargalkar, S.P. G. Matondkar and S.S. Bukhari, Bull. Natl. Inst. Oceanogr., **11**, 105 (1978).
16. R. Qasim, S. Barkati, P.J. A. Siddiqui and M. Ilyas, Pak. j. sci. ind. res., **29**,37 (1986).
17. P.J.A. Siddiqui and R.Qasim, J. Islamic Acad. Sci. **3**, 15 (1990).
18. P.J.A. Siddiqui and R. Qasim, In: M.F. Thompson and N.M. Tirmizi (Eds), Mar. Sci. Arabian Sea: Proc. of an Intl. Conference, American Institute of Biological Science. Washinton, D.C. (1988) pp. 393-400.
19. S.M. Haq, Proc. Intl. Symp. Mar. Poll. Res., S.P. Mayters (Ed.) (Lousiana State Univ. Baton Rouge, L.A. USA., 1976), pp. 33.
20. R. Qasim, P.J.A. Siddiqui, Z. Akbar and A. Bano, Mar. Res. (In press).
21. B.P. Hawk, L.B. Oser and H.W. Sumerson, *Practical Physiological Chemistry* (McGraw Hill Book Co. Inc. N.Y., 1954).
22. H.O. Tribold and L.W. Aurand, *Food Composition and Analysis* (D. Van Nostrand Co Inc. Princeton, N.J., USA, 1963).
23. N.A. Holme and A.D. McIntyre, *Methods for the Study of Marine Banthose* (IBP Handbook No. 16, Blackwell Scientific Publication, U.K. 1971).
24. O.H. Lowry, N.R. Roberts K.Y. Liener, M.L. Wu and

A.L. Far, J.Biol. Chem., **207**, 1 (1954).
25. J. Borner and J.E. Varner (Eds), *Plant Biochemistry* (Academic Press Inc., NY, USA, 1976), 3rd edn.
26. F.T. Addicott, Biological Review. Cambridge Phil. Soc., **45**, 485 (1970).
27. L.T. Bhosale, Indian J.Mar. Sci., **3**, 58 (1979).
28. K.L.Gulati, K.K. Nagpaul and S.S. Bukhari, Mahasagar, Bull. Natl. Inst. Oceanogr., **12**, 183 (1979).