

## INFLUENCE OF AQUATIC ENVIRONMENT ON THE COMPOSITION OF FATTY ACIDS IN ALGAE GROWING IN SINDH, PAKISTAN

Ehsan Elahi Valeem<sup>1</sup> and Mustafa Shameel<sup>2</sup>

<sup>1</sup>Public Private Partnership Unit (PPPU), Planning and Development Department, Government of Sindh, Karachi-74200, Pakistan, and <sup>2</sup>Department of Botany, University of Karachi, Karachi-75270, Pakistan

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**Abstract:** Ninety-seven species of algae were collected from various aquatic habitats of Sindh Province of Pakistan and analysed for their fatty acid (FA) constitution by gas-liquid-chromatographic (GLC) and gas chromatography-mass spectrometric (GC-MS) techniques. Those growing in the marine environment (68 species) were found to differ remarkably in their FA-composition compared to those thriving in freshwater (24 species) as well as in the brackish water environment (5 species). Marine algae were found to have the smallest amounts of SCFAs, PUFAs, CFAs, DCFAs, monoynoic and substituted FAs, medium quantity of VLCFAs, long RCCL, the highest amounts of C16:0 and C18:1 acids and the highest FA-diversity as compared to the other two environments. Freshwater algae differed from them in having more SCFAs, VLCFAs, PUFAs, CFAs, DCFAs, monoynoic and substituted FAs, lesser quantities of C16:0 and C18:1 acids, more unsaturation of C18 and C22 UFAs and slightly larger RCCL than them. They resembled marine algae in the degree of unsaturation of C20 UFAs in the RCCL and FA-diversity, which were almost equal. The brackish water algae differed from both of them in having the highest amounts of SCFAs and monoynoic FAs, medium quantity of PUFAs and substituted FAs, in lacking CFA and DCFA, small proportion of VLCFAs, less amounts of C16:0 and C18:1 acids, and in small RCCL and FA-diversity. They resembled the other two types in the degree of unsaturation of C18, C20 and C22 UFAs. Seaweeds were found to contain higher amounts of EPA and DPA as compared to the algae of other environments. This indicates that Pakistani seaweeds may be good source of human food.

**Keywords:** Algae, fatty acids, freshwater, brackish, marine, Pakistan

### Introduction

On a global scale algae are exceptionally significant for producing about half of the photosynthetic organic material. As inhabitants of freshwater, brackish water and marine environments, algae are the bases of aquatic food webs, supporting an enormous abundance and diversity of animals [1]. Marine algae have always been thought to have curative properties and the Chinese are known to have used various seaweeds in the treatment of throat diseases [2]. Conventionally, marine green algae are good source of lipids [3]. The chief components of all fats are the fatty acids (FAs), which may be saturated (SFA), monounsaturated (MUFA) or polyunsaturated (PUFA) ones. Trans fatty acids (TFAs) can be produced not only through industrial processes, but also through biohydrogenation

in ruminants. Fats containing a high proportion of SFAs or TFAs are solid at room temperature. Saturated fats are usually derived from animal sources *e.g.* lard, suet and butter. Saturated and monounsaturated FAs in the human body, although theoretically could be produced endogenously (*de novo* lipogenesis), are anyway mainly from the diet since most populations consume 20-30 % energy, *i.e.* at least 50 g/day fats, most of which are made up of saturates and monounsaturates.

Two PUFAs, which cannot be made in the body, are linoleic acid (LA) and alpha-linolenic acid (ALA). The PUFAs include two metabolic series of compounds: the n-6 ( $\omega$ -6) and the n-3 ( $\omega$ -3) compound, the LA and AA belong to the n-6 series while ALA refers to both  $\alpha$ -linolenic acid (C18:3, n-3) and to  $\gamma$ linolenic acid (C18:3, n-6). They must be provided by diet and are

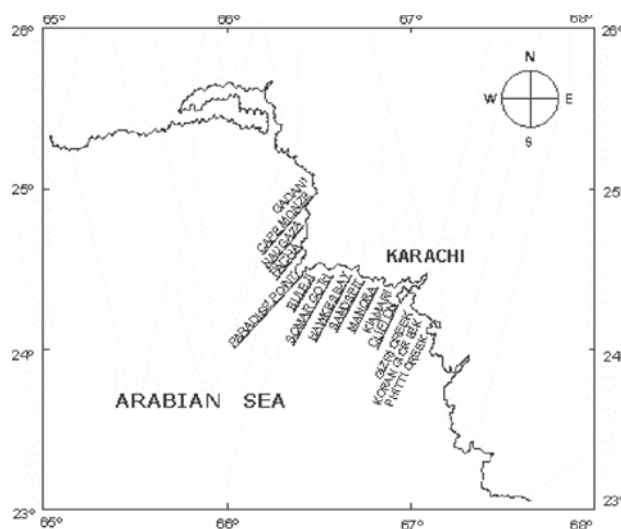
known as essential FAs. Within the body, both can be converted to other PUFAs such as arachidonic acid (AA), or eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). There are two different pathways for for the conversion of C8 PUFAs to the long chain PUFA, as LA is converted to AA in the n-6 series and ALA is converted to EPA and DHA in the n-3 series. Seaweeds contain small amounts of EPA with Wakame being the highest in EPA (186 mg per 100 g raw). Consumed in quantity (100 g is about 3.5 ounces), they can be good sources of EPA [4-6].

From time to time several studies have been undertaken on the FA-composition of seaweeds growing in the coastal waters of Karachi [7-10] as well as algae occurring in the freshwater habitats of Sindh Province of Pakistan [11-13]. But no attempt was made to compare their FA-composition on the basis of their environment. In this regard a comparison of seaweeds with their freshwater and brackish water counterparts was needed. With this idea in mind, the present study was conducted on the freshwater, brackish water and marine algae in order to compare the composition of their fatty acids.

## Materials and Methods

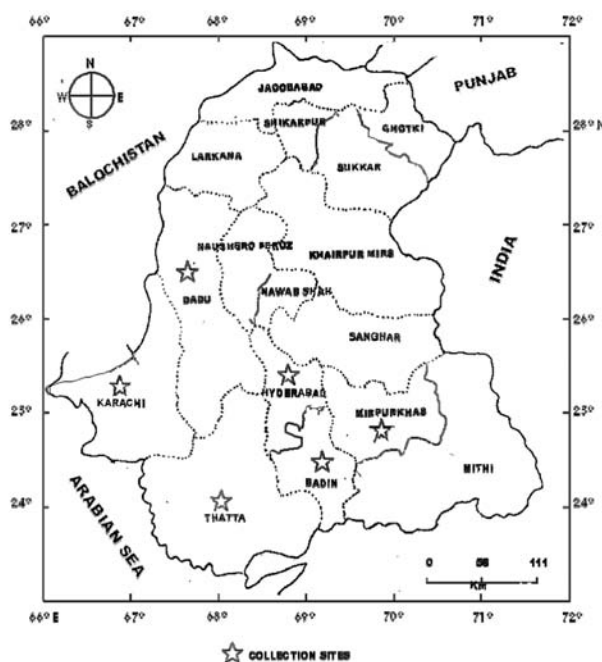
### *Collection of material*

Seaweeds were collected from the coastal areas of Karachi *i.e.* Clifton, Manora, Sandspit, Hawkes Bay, Somar Goth, Buleji, Paradise Point, Pacha, Naugaza Mazar and Cape Monze (Fig. 1) during September 1997 to May 1998. The intertidal algae were detached from the rocks, pebbles and boulders during low tide, when the rocks were emerged. The drift algae were picked up from the tidal pools while wading during rising tides, whereas a large number of sub-littoral organisms were available especially in rocky



**Figure 1.** Part of a coastal area of Sindh (Pakistan) showing localities (underlined) of seaweed collection.

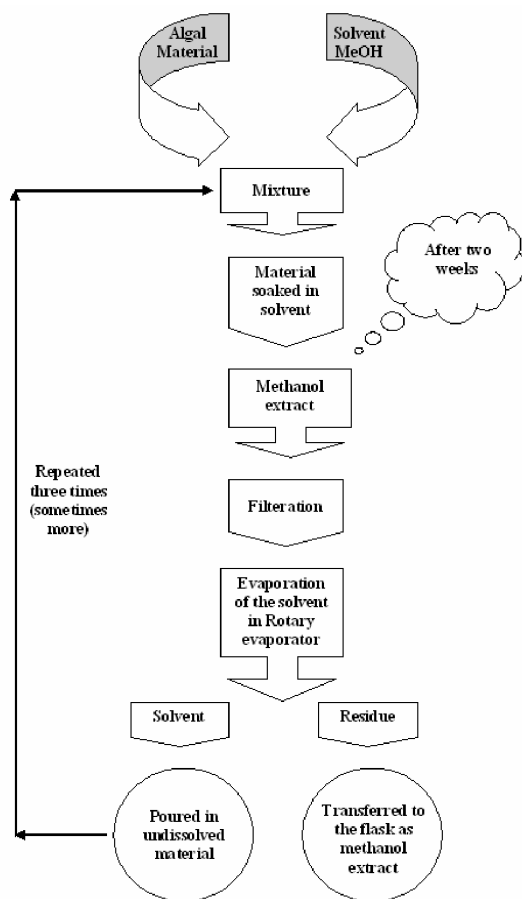
bays as compared to sandy bays. The freshwater and brackish water algae were collected from pools, ditches, lakes, irrigation canals, riverine ponds and other water reservoirs in different areas of the province of Sindh, Pakistan (Fig. 2) during September 1997 to August 1998.



**Figure 2.** Map of Sindh Province of Pakistan showing different localities for the collection of freshwater algae.

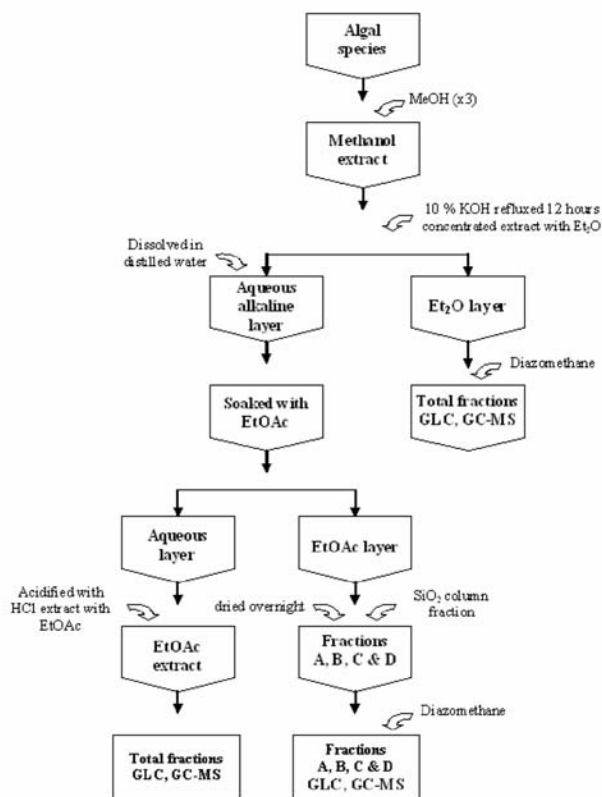
### Isolation of fatty acids

**Extraction:** The algal material in bulk was washed thoroughly with tap water to remove epiphytes, epizoons, animal castings, attached debris and sand particles. The healthy, mature and clean thalli were selected, rinsed with distilled water, 0.5 - 1.5 kg of each species was chopped into small pieces, milled and immediately extracted. The fresh and chopped algal material was kept for cold percolation in methanol (MeOH) to obtain a total soluble extract. This extract was then evaporated under reduced pressure in rotary evaporator and a dark bluish-green, thick residue of 30 to 50 g was obtained depending on the colour and quantity of the algal material. This process was repeated two to three times for each algal species (Fig. 3).



**Figure 3.** Scheme for the extraction of methanol extract.

An aliquot (100 mg) of each extract was saponified with 10% KOH in 50% MeOH and refluxed at 100°C for 6 h. The resulting mixture was evaporated under reduced pressure in rotary evaporator and partitioned between aqueous and ethyl acetate (ETOAC) phases; it was repeated several times. The total combined ETOAC fraction was acidified with 6N HCl (pH 4.5) and re-extracted with ETOAC which was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and then concentrated under vacuum. It was subjected to methylation, 1.5-2.0 mL ethereal diazomethane was added to the FA mixture, which was left in fuming chamber at room temperature overnight until dissolved (Fig. 4). The aliquots were then directly injected into GLC or GC-MS. The methylated FA fractions were analysed first by GLC and finally by GC-MS. The final identification of FAs was carried out with the help of NBS-mass spectral library [14].



**Figure 4.** Scheme for the detection of fatty acids.

**Column chromatography (CC):** The mixture to be analyzed by Column chromatography was applied to the top of the column. The fractions were eluted (100 mL, each) initially in *n*-hexane and then polarity was gradually increased by increasing the amount of Et<sub>2</sub>O. The eluent was passed through the column by gravity or air pressure. The individual components or elutants were collected, as the solvent dripped from the bottom of the column. The initial fractions, which were thick and oily starting from pure *n*-hexane up to about *n*-hexane:ether (70:30), were analysed for FAs after being checked on TLC cards.

**Thin Layer Chromatography (TLC):** The TLC plates (silica gel 254 nM Mikrokarten SIF, 5x10 cm, or 20 x 20 cm, 0.2 mm; Riedel-de-Haen) were used, and for column CC Merck silica gel 60 (230 – 400 mesh size) was employed. It was observed that as the polarity of the solvent system was increased, all the components of the mixture moved faster (and *vice versa* with lowering the polarity). For preparative TLC, the glass plates were first air-dried and then activated in oven at 110° C. The final purity of the compound was checked on TLC (20 x 20 cm) by spraying with 10% solution of Ce (SO<sub>4</sub>)<sub>2</sub> in 2 N H<sub>2</sub>SO<sub>4</sub>.

**Gas Liquid chromatography (GLC):** The unknown FA fractions were analyzed first by GLC along with methyl ester standards on a Shimadzu GC-9A model gas chromatograph, equipped with a Shimadzu C-R6A chromatopac integrator. The column length was 2 mm and outer diameter 5 mm. The column material used was GP 3% SP-2310 / 2% SP-2300 on 100/120 chromosorb WAW. The column initial temperature was 150° C, while final temperature was 250°C with a rate of increase of 8°C, nitrogen flow rate was 30 mL/min.

**Gas Chromatography-Mass Spectrometry (GC-MS):** The GC-MS was performed on a

Hewlett-Packard GC with 11/73 DEC computer data system and a 1.2 m x 4 mm packed glass capillary column coated with gaschrome Q (100-120 mesh 0V 101, 1%). Once the sample solution was introduced into the GC inlet, it was vaporized immediately owing to high temperature, which was programmed between 70-250°C with a rate of increase of 80°C per minute and swept onto the column by the carrier gas (usually Helium). The flow rate of carrier gas was 32 mL/min and the injector temperature 250°C.

## Results and Discussion

Algae are water-loving organisms and thrive luxuriantly being submerged in water. Some of them grow in marine water and others in brackish or freshwater environment. While growing in these three environmental conditions, they differ in their FA-compositions due to varying amounts of illumination in different habitats [15]. Various abbreviations used in the text are explained in the Table 1. When 97 species of algae from various aquatic habitats of Sindh Province of Pakistan were investigated, it was observed that algae (68 species), which were growing in the marine environment possessed small amounts of SCFAs, PUFAs, CFAs, DCFAs, monoynoic and substituted FAs, medium quantity of VLCFAs, long RCCL, the highest amounts of C16:0 and C18:1 acids and the highest FA-diversity as compared to the other two environments (Table 2). They also contained higher amounts (mg/100 g dry wt. of algae) of EPA and DHA as compared to the algae of the other environments. This indicates that Pakistani seaweeds may also be a good source of human food, if digestible.

Freshwater algae (24 species) differed from marine algae in having more SCFAs, VLCFAs, PUFAs, CFAs, DCFAs, monoynoic and substituted FAs, lesser amounts of C16:0 and C18:1 acids, more unsaturation of C18 and C22 UFAs and

**Table 1.** Explanation of the abbreviations or symbols used.

Term	Explanation	Term	Explanation
AA	Arachidonic acid	DUFA	Diunsaturated fatty acid
ALA	$\alpha$ -Linolenic acid	EPA	Eicosapentaenoic acid
C	Carbon	ETOAC	Ethyl Acetate
CC	Column chromatography	Et <sub>2</sub> O	Diethyl ether
*C	Cyclic fatty acid (CFA)	EtOH	Ethanol
CFA	Cyclic fatty acid	FA	Fatty acid
CLA	Conjugatedlinolenic acid	GC-MS	Gas chromatography – mass pectrometry
Cn:0	Saturated Fatty acid (SFA)	GLC	Gas-liquid-chromatography
Cn:1	Monounsaturated fatty acid (MUFA)	h	Hour
Cn:2	Diunsaturated fatty acid (DUFA)	MeOH	Methanol
Cn:3	Triunsaturated fatty acid (TUFA)	MUFA	Monounsaturated fatty acid
Cn:4	Polyunsaturated fatty acid	PUFA	Polyunsaturated fatty acid (with 4-8 DBs)
Cn:5	Polyunsaturated fatty acid	RCCL	Range of C chain length
Cn:6	Polyunsaturated fatty acid	SCFA	Short chain fatty acid (< 9 C)
Cn:8	Polyunsaturated fatty acid	SFA	Saturated fatty acid
Cn:1	Monoynoic fatty acid	*C	Substituted fatty acid
CUFA	Cyclic unsaturated fatty acid	TB	Triple Bond
*C	Dicarboxylic fatty acid (DCFA)	TFA	Trans fatty acid
DB	Double Bond	TUFA	Triunsaturated fatty acid
DCFA	Dicarboxylic fatty acid	TLC	Thin layer chromatography
DHA	Docosahexaenoic acid	UFA	Unsaturated fatty acid
DPA	Docosapentaenoic acid	VLCA	Very long chain fatty acid (>25 C)

**Table 2.** Comparative FA-composition of algae with different environments.

Type of Fatty Acid	Unit	Environments		
		Marine	Freshwater	Brackish water
		1	2	3
SCFAs	%	1.82	3.28	4.55
VLCFAs	%	3.36	4.38	2.73
PUFAs	%	1.73	5.03	4.55
C16:0	%	26.78	11.03	10.98
C18:1	%	7.38	4.10	5.62
C18 UFAs	DBs	1-5	1-6	1-3, 5
C20 UFAs	DBs	1-6	1, 4, 6	1, 6
C22 UFAs	DBs	1, 4	1, 4, 5	1, 4
EPA	Q	131	32	97
DHA	Q	95	11	53
Monoynoic FAs	%	0.19	0.88	0.91
Substituted FAs	%	2.59	6.35	4.55
CFAs	%	0.1	0.22	-
DCFAs	%	0.19	0.22	-
RCCL	C-C	3-32	3-33	7-29
FA-diversity	acids	3-39	4-39	16-28

- = not detected, for abbreviations used see Table 1, Q = mg/100 g dry wt. of algae, DBs = number of double bonds, 1 = average of 68 species, 2 = average of 24 species, 3 = average of 5 species.

slightly longer RCCL than them. They resembled marine algae in the degree of unsaturation of C20 UFAs, in the RCCL and FA-diversity, which were almost equal. Therefore, sharp differences were observed in the FA-composition of algae growing in the freshwater and marine environments.

The brackish water algae (5 species) differed from the other two categories in having the highest amounts of SCFAs and monoynoic FA, medium quantity of PUFAs and substituted FAs, in lacking CFA and DCFA, small proportion of VLCFAs, small quantities of C16:0 and C18:1 acids, small RCCL and FA-diversity (Table 2). However, they resembled the other two types in the degree of unsaturation of C18, C20 and C22 UFAs. The reason for their differences with the other two categories lies in the fact that these algae grow in estuarine environment, where they continuously experience salinity fluctuations and remain under stress. This might cause the saturation of FAs in membrane lipids to decrease membrane lipid fluidity and the accumulation of triacylglycerol, as a storage lipid, to prevent the osmotic imbalance [16]. This indicates that aquatic environment plays a significant role in the constitution of macromolecules composing of the cellular structures of its inhabiting organisms.

Salinity has a great bearing on the amount and type of FAs synthesized. In *Ulva pertusa*, exposure to the light intensity and low salinity combination resulted in huge decrease in the quantity (mg.g<sup>-1</sup> dry wt.) of total FAs. High light intensity increased the quantitative levels of most SFAs e.g. C14:0, C15:0 and C16:0 [17]. In *Grateloupia sparsa*, low and high salinity exposure resulted in increase in the total FAs, total PUFAs and monoenoic FAs, compared to normal salinity 35.5 ‰ S). In *Sargassum piluliferum* a trend of increasing levels of C18:4 (n-3), C20:4 (n-6), C20:5 (n-3), total n-3 and total n-6 FAs were observed with increasing salinity. Exposure to high salinity in

*Ulva* and *Sargassum*, and low and high salinities in *Grateloupia* resulted in higher levels of total FAs [17].

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## References

1. **Campbell, G.S.** 1977. *An Introduction to Environmental Biophysics*. Springer Verlag, Berlin.
2. **Chapman, V.J. and Chapman, D.J.** 1980. *Seaweeds and Their Uses*. 3<sup>rd</sup> ed. Chapman & Hall, London.
3. **El-Din Mohammady, N.G.** 2001. Lipid composition of six marine chlorophytes using infrared technique. *Al-Azhar J. Pharm. Sci.* 28:18-25.
4. **Hong, J.S., Kwon, Y.J., Kim, Y.H., Kim, M.K., Park, I.W. and Kang, K.H.** 1991. Fatty acid composition of miyeok (*Undaria pinnatifida*) and pare (*Enteromorpha compressa*). *Han 'guk Yongyang Silkllyang Hakhoechi* 20:376-380.
5. **Jensen, T.** 2003. *Omega 3 and omega 6 fatty acids*. MS Food News, DFW Vegetarian.
6. **John, W.B., Brent, R.C., Murray, H.G.M., Peter, T.N., and Michèle, R.P.** 2003. Marine natural products. *Nat. Prod. Rep.* 20:1-48.
7. **Qasim, R.** 1986. Studies on fatty acid composition of eighteen species of seaweeds from the Karachi Coast. *J. Chem. Soc. Pak.* 8:223-230.
8. **Shameel, M.** 1987. Studies on the fatty acids from seaweeds of Karachi. In: *Modern Trends of Plant Science Research in Pakistan. Proc. Nat. Conf. Plant Scient.* 3:183-186.
9. **Shameel, M.** 1990. Phycochemical studies on fatty acids from certain seaweeds. *Botanica Marina* 33:429-432.
10. **Shameel, M.** 1993. Phycochemical studies on fatty acid composition of twelve littoral green seaweeds of Karachi Coast. In: *Proceedings of the National Seminar on Study and Management in Coastal Zones in Pakistan*, Pak. Nat. Comm. UNESCO, Karachi, Eds. Tirmizi, N.M. and Kazmi, Q.B. pp.17-25.
11. **Ghazala, B. and Shameel, M.** 2005. Phycochemistry and bioactivity of some freshwater green algae from Pakistan. *Pharmaceut. Biol.* 43:358-369.
12. **Rizvi, M.A. and Shameel, M.** 2005. Phrmaceutical biology of seaweeds from the Karachi Coast of Pakistan. *Pharmaceut. Biol.* 43:97-107.

13. **Shahnaz, L., Ghazala, B. and Shameel, M.** 2006. Phycochemical and bioactivity of *Enteromorpha intestinalis* (Ulvophyceae, Chlorophyta) from Sindh, Pakistan. *International J. Phycol. Phycochem.* 2:59-62.
14. **Helles, S.R. and Milne, G.W.A.** 1978. *EPA/NIH Mass Spectral Data Base*. 4 Vols., NIBS, US Government Printing Office, Washington.
15. **Khotimchenko, S.V.** 2002. Fatty acid composition of algae from habitats with varying amounts of illumination. *Russ. J. Mar. Biol.* 28:218-220.
16. **Tatsuzawa, H., Takizawa, E., Wada, M. and Yamamoto, Y.** 1996. Fatty acid and lipid composition of the acidophilic green alga *Chlamydomonas* sp. *J. Phycol.* 32:598-601.
17. **Floreto, E.A.T. and Teshima, S.** 1998. The fatty acid composition of seaweeds exposed to different levels of light intensity and salinity. *Botanica Marina* 41:467-481.