

Dissolved Folin Phenol Active Substances (Tannin and Lignin) in the Seawater along the West Coast of India

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Information on the distribution of dissolved Folin phenol active substances (FPAS) such as tannin and lignin in the seawater along the west coast of India is provided. Notable amounts of FPAS (surface concentrations: 80 $\mu\text{g/l}$ to 147 $\mu\text{g/l}$ and bottom concentrations: 80 $\mu\text{g/l}$ to 116 $\mu\text{g/l}$) were detected in the seawater along the coast. The distribution pattern brings about a general depth-wise decrease. A seaward decrease was observed in the southern stations whereas reverse was the case in northern stations. A significant negative correlation was observed between FPAS concentration and dissolved oxygen in sub-surface samples. The appreciable amounts of FPAS detected in the coastal waters indicate the presence of organic matter principally originating from terrestrial (upland and coastal marsh) ecosystems in the marine environment. In this context, they may be used as tracers to determine the fate of coastal-born dissolved organic matter in the ocean and to determine directly the relationship between allochthonous and autochthonous organic matter.

Keywords:
· Folin phenol active substances,
· tannins,
· lignins,
· terrestrial origin,
· Arabian Sea.

1. Introduction

With industrial and other wastes, a large number of organic substances find their way into the oceans. Some of these substances are not easily susceptible to biochemical oxidation and may selectively persist in the water and in the bottom sediment. The annual discharges of several million tons of terrigenous organic compounds into the oceans have deteriorated the carbon cycle, with man himself increasingly suffering from the disturbed natural equilibria (Romankevich, 1984).

The use of organic markers in the differentiation of organic inputs to oceanic waters has proved to be a valuable tool in the determination of the contribution of land inputs to the organic fraction of the seawater. Molecular tracers have been used to establish terrestrial inputs to marine waters and to trace pollution in waterways (Readman *et al.*, 1986). The Folin phenol active substances (FPAS), which are mainly hydroxylated aromatic compounds like tannins and lignins, appear to be a valid indicator of terrestrial carbon sources across a wide geographical and climatic area. Tannins and lignins have very special significance from the viewpoint of land-derived

plant constituents. The other substances that will react with Folin phenol reagent include nucleic acid bases, certain amines, sulphites, bisulphites etc. However, their concentrations in the seawater are not sufficient to form a measurable blue colour with the reagent (Richards, 1965; Goldberg, 1965; Duursma, 1965; APHA, 1995).

The tannins and lignins are high molecular weight polycyclic aromatic compounds widely distributed throughout the plant kingdom (Fig. 1) (Schnitzer and Khan, 1972; Finar, 1976; Field and Lettinga, 1987). These compounds are highly resistant to biological degradation and have potential to damage the aquatic environment. The identification and quantification of these compounds can provide information on the presence and quantitative importance of land-derived organic detritus in marine systems. This would enable us to determine directly the relationship between allochthonous and autochthonous organic matter.

2. Materials and Methods

Samples were collected during the 162nd cruise of FORV SAGAR SAMPADA from 27 stations off west coast of India between Cape Comorin and Bombay. The area lies between latitudes 8°3.96'–20°34.05' N and longitudes 77°21.96'–70°44.69' E. Figure 2 shows the location map of the stations and Table 1 gives the list of

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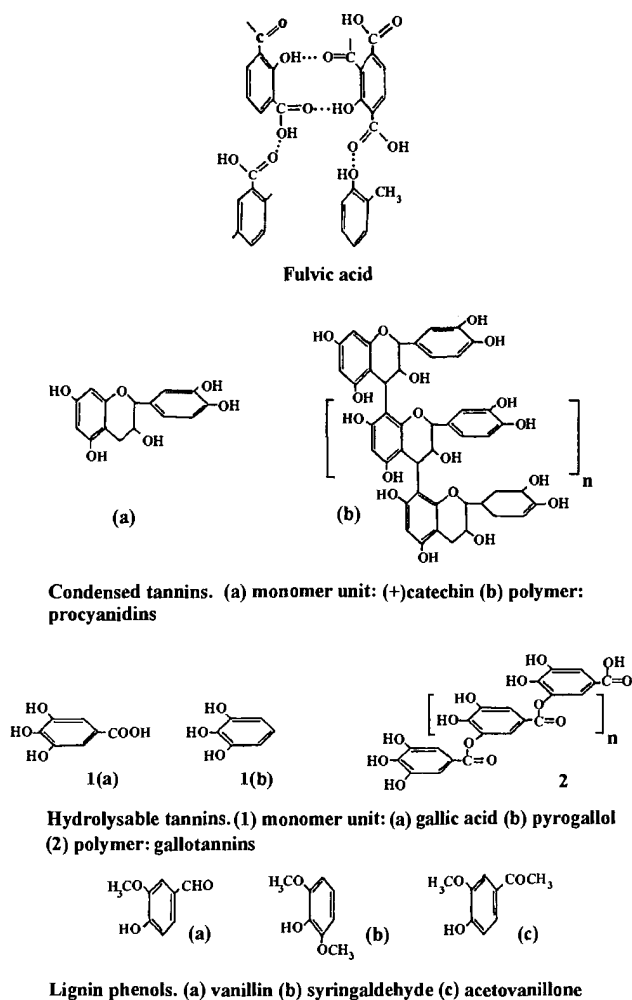


Fig. 1. High molecular weight polycyclic aromatic compounds like tannins, lignins and their derivatives.

stations. The collections were carried out in the premonsoon period (from 18 to 28 February 1998). Seawater samples were collected from the surface and near bottom using Niskin Samplers (Model 1010) of capacity 1.7 liters, connected to a CTD probe. The water samples were filtered through Whatman GF/C (0.45 μm) immediately after the collection and kept shock frozen at -20°C till analysis was performed.

Hydrographical parameters such as salinity and temperature were measured using the CTD probe. Dissolved oxygen in the water was estimated chemically onboard using Winkler's method (Grasshoff, 1983). The estimation of dissolved Folin phenol active substances was performed by the sodium tungstate-phosphomolybdic acid method (APHA, 1995). The principle involved is the development of a blue colour on reduction of Folin phenol reagent by the aromatic hydroxyl groups present in tannins and lignins. The effects of Mg and Ca hydroxides and/or

Table 1. List of stations.

Station No.	Locality	Depth (m)	Distance from shore (km)
1	Cape Comorin	30	4
2	"	50.6	12
3	"	208	55
4	Trivandrum	124	21
5	"	28.7	1.5
6	Kollam (Quilon)	29.7	6.5
7	"	238	33
8	Kochi (Cochin)	30	13
9	"	202	38
10	Kozhikkode (Calicut)	30	9
11	"	200	50
12	Kannur	203	50
13	"	31	6
14	Mangalore	30	6
15	"	205.9	41.5
16	Batkal	204	66
17	"	31	9
18	Goa	32	9
19	"	204	54
20	Bombay	33	14
21	"	51	46
22	"	96	69
23	"	90	104
24	"	86	40
25	"	80	42
26	"	51	17
27	"	32	9

bicarbonates present in the seawater were suppressed by the addition of trisodium citrate solution (Nair *et al.*, 1989). The Folin-phenol reagent was obtained commercially and carbonate-tartrate reagent was prepared according to the prescribed procedure. A solution of 1.6 M trisodium citrate was also prepared. A 1000 ppm stock solution of tannic acid was prepared and stored in freezer. Secondary standards were prepared daily. All reagents used were of Analar grade and solutions were made using milli-Q water. The analytical procedure is as follows. To 10 ml of the sample, added in rapid succession, 5 ml of the citrate solution followed by 1 ml of Folin-phenol reagent and 10 ml of carbonate-tartrate reagent, mixed well and allowed to stand for 30 minutes for colour development. Reagent blanks were similarly prepared omitting the sample. The absorbance was measured at 760 nm using a Hitachi UV-Visible Spectrophotometer (model 150-20). Blank corrections were applied to all sets of readings. The concentrations were measured as tannic acid equivalents with a calibration curve.

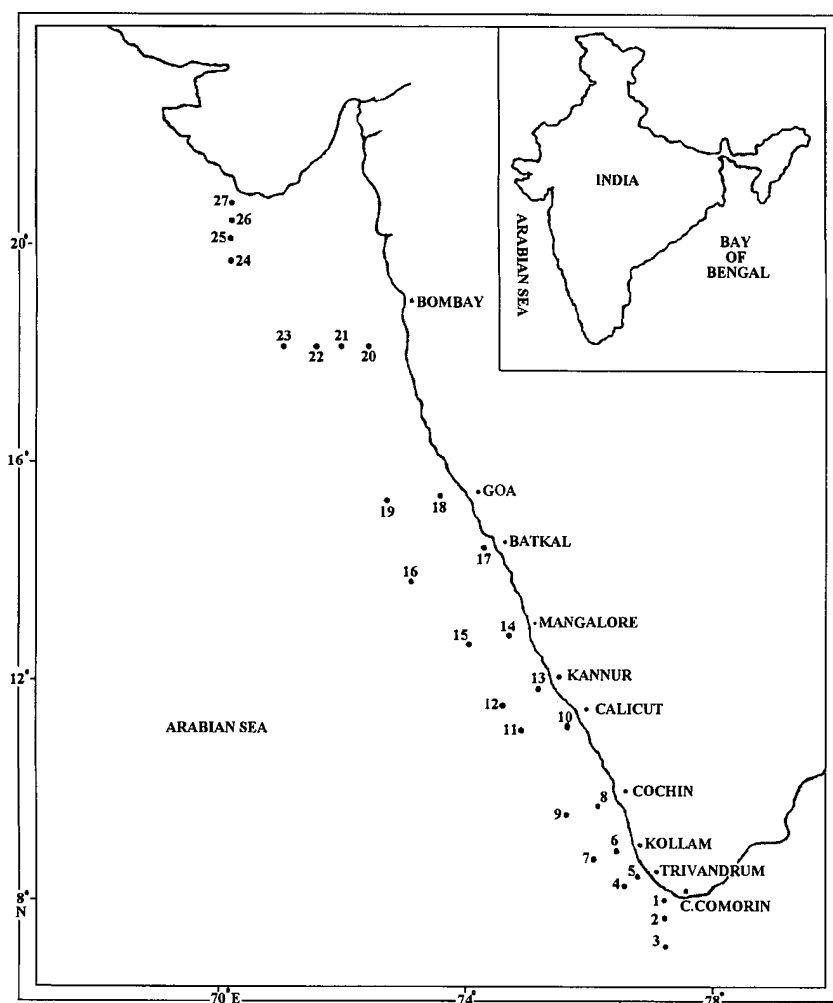


Fig. 2. Location map of stations.

3. Results and Discussion

The variations of hydrographical parameters such as salinity, temperature and dissolved oxygen are given in Fig. 3 and the distribution of FPAS in the surface and bottom waters in Fig. 4.

3.1 Hydrographical parameters

3.1.1 Salinity and temperature

The salinities of both surface and bottom waters were found to increase generally from south (Cape Comorin) to north (Goa). The distribution pattern was similar in both surface and bottom waters (Fig. 3). At stations with water depths of about 200 m, bottom salinity did not vary considerably from south to north, however, surface salinities showed remarkable variation. For example, at stations off Cape Comorin and Goa (each 55 km away from the shore) surface salinities were 33.94 and 35.59‰ while, bottom salinities were 35.06 and 35.42‰, respectively.

At stations of lower depths (about 30 m), the bottom salinities were only slightly higher than surface values whereas, the differences between surface and bottom salinities were considerably higher at stations of higher depths (about 200 m). However, this difference decreased towards north. In the Bombay High region, salinities were found to have the highest values compared to the other parts of the study area. Both surface and bottom salinities ranged from 35.55 to 35.96‰. Here, the difference in surface and bottom salinities was very small. In bottom waters, the salinity was found to increase outward from the coast at all stations whereas in surface waters, a seaward decrease was observed.

From Cape Comorin to Mangalore, the surface waters recorded a temperature above 29°C while the stations north of Goa recorded a temperature below 27°C (Fig. 3). At Bhatkal and Goa, the surface temperatures were in the range 27–29°C. At lower water depths (about 30 m), surface and bottom temperatures were almost the same

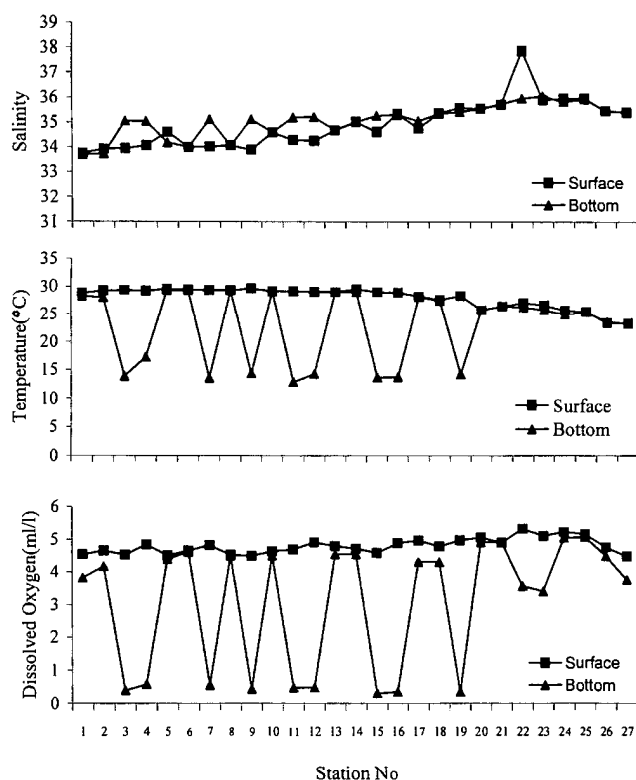


Fig. 3. Stationwise variations of salinity ($\times 10^3$), temperature ($^{\circ}\text{C}$) and dissolved oxygen (ml/l) in the Arabian Sea along the west coast of India.

whereas very low temperatures ($12.9^{\circ}\text{--}14.4^{\circ}\text{C}$) were found at higher depths (about 200 m). An almost isothermal condition found at lower water depth stations may be due to the existence of a surface mixed layer. Pillai (1991) has shown that a surface mixed layer exists in the region off West Coast of India, which extends to a depth greater than 30 m during December–February.

The observed temperature and salinity distribution may be attributed to the existence of different water masses in the study area. Darbyshire (1967) has shown that there are three major water masses present on the shelf *viz.* (1) the Indian Ocean equatorial water (temperature below 17°C with a minimum salinity of 34.9‰), (2) the Arabian Sea water (temperature between 17 to 27°C with a salinity in the range $35.5\text{--}36.3\text{‰}$) and (3) the equatorial surface water (temperature between 27 and 30°C with a salinity range of $30\text{--}34\text{‰}$).

Thus, the salinity and temperature data of the present study shows that, from Cape Comorin to Mangalore, the equatorial surface water occupies the surface (salinity: $33.75\text{--}34.69\text{‰}$ and temperature: $29.1\text{--}29.7^{\circ}\text{C}$) while the Arabian Sea water is found at the surface to the north of Goa (salinity: $35.41\text{--}35.96\text{‰}$ and temperature: $23.4\text{--}27.1^{\circ}\text{C}$). Mixing of the two water masses may be occur-

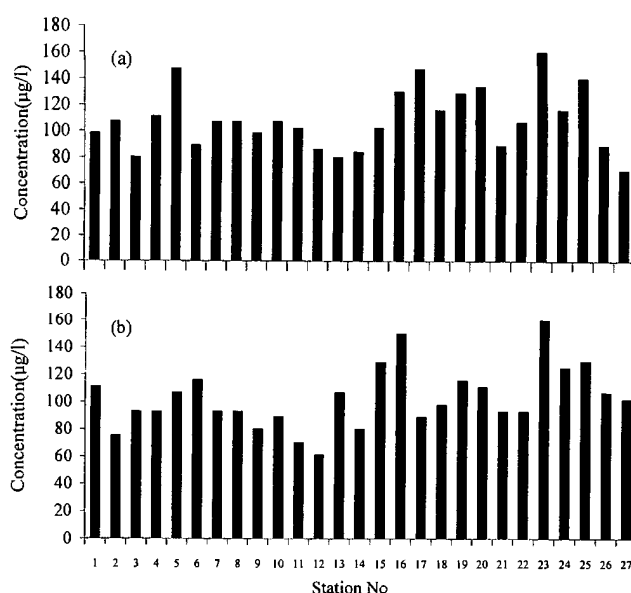


Fig. 4. Stationwise distribution of Folin phenol active substances in the Arabian Sea along the west coast of India. (a) Surface waters, (b) bottom waters.

ring near Goa and Batkal where Arabian Seawater sinks beneath the less dense equatorial surface water resulting in a convergence zone. At lower water depths, the surface and bottom are occupied by the same water mass while at higher water depths (about 200 m) the bottom water is found to be Indian Ocean equatorial water (salinity: $35.04\text{--}35.42\text{‰}$ and temperature: $12.9\text{--}14.4^{\circ}\text{C}$).

From the salinity distribution pattern, it is also found that there is an increase in salinity from south to north, which suggests a gradient current towards south. The increased salinity in the north has possibly resulted out of the influence of high saline north Arabian Sea water reaching the area in the southerly flow during the pre-monsoon period. The decrease in salinity towards south may be due to the slow spreading of the high saline water southwards losing their high salinity characteristics (Pillai, 1991).

The gradient current towards the south suggests a decrease of salinity outwards from the coast, which was also observed in the present study. A seaward increase of salinity found in the bottom waters may be attributed to the existence of low saline equatorial surface water at lower depths and a higher salinity Indian Ocean equatorial water at higher depths.

3.1.2 Dissolved oxygen

In the study area, horizontal distribution of dissolved oxygen in both surface and bottom waters did not show much variation (Fig. 3). The surface dissolved oxygen ranged from 4.51 ml/l to 4.97 ml/l. At lower depths (about 30 m), dissolved oxygen values changed from 3.82 ml/l

to 4.62 ml/l, while at higher depths of 200 m, it ranged from 0.31 ml/l to 0.56 ml/l. At higher depths, bottom values were considerably lower than the surface values. In the Bombay High region, the difference between surface and bottom values were found to be much pronounced at stations 22 and 23 which are at water depths 85 and 75 m, respectively.

The observed vertical and horizontal distributions of oxygen in the seawater result from the interplay between biochemical processes and those by which the oxygen enters and is transported in the water. The competing processes of photosynthesis and respiration are the main causes of in situ changes in the dissolved oxygen in the sea. At lower water depths, there occurs a mixed layer of water column with more or less uniform oxygen content. In this layer photosynthesis may predominate and lead to the liberation of oxygen. A depth of 200 m is below the compensation point where the net production is zero. Below the compensation depth, the dissolved oxygen is consumed by the respiration of plants, animals and bacteria. The ultimate factor causing the depletion of oxygen in the seawater is the oxidation of organic matter (Riley and Chester, 1971).

3.2 Folin phenol active substances (FPAS)

Folin phenol active substances found in the marine environment mainly consist of tannins and lignins, which are major classes of the secondary products of plant metabolism and are ecologically important. By comparison to most biologically synthesized compounds, lignin is highly resistant to biodegradation. The very resistant nature of lignins is suggestive of long term damaging effects on the ecosystems. However, lignin degrades to some extent biologically via oxidative cleavage. Lignins can be readily oxidised by alkaline CuO and the resulting lignin phenols can be used to characterize lignin in a wide variety of natural samples including natural waters (Ertel *et al.*, 1986; Meyers-Schulte and Hedges, 1986). Lignins have been implicated as a major source material for terrestrial humic substances (Flaig *et al.*, 1975).

Tannins may enter the seawater through the process of vegetable matter degradation or through boiler and tannery effluents. Tannins inhibit plant growth (Mahadevan *et al.*, 1984) and significantly decrease the lipid content in the tissues of certain fishes (Beena, 1991). The toxicity of tannins on several enzymes has been established (Tamir and Alumot, 1969; Daiber, 1975). The natural phenolic materials (FPAS) can influence the cycles of metals and other elements in the aquatic environment, and some investigators have explained decreases in primary productivity by deficiency in iron or other metals caused by metal complexation by dissolved humic substances (Guildford *et al.*, 1987). When hydroxylated aromatic compounds react with proteins, these compounds can in-

hibit the performance of many enzymes (Stewart and Wetzel, 1982; Francko, 1986). Terrestrially derived organic matter in the seawater such as tannins and lignins originates mainly from river run-off. The breakdown of terrestrial plant tissue gives rise to a considerable variety of lignin and tannin compounds. These are readily moved about within the environment by processes like run-off. It would, therefore, seem reasonable to expect a proportion of the organic material in the ocean to consist of FPAS like tannins and lignins. Since the west coast of India is characterized by a number of major and minor rivers which flow westward and carry water to the Arabian Sea throughout the year, it is possible to identify land-derived organic matter in the seawater in that region through the identification and quantification of these compounds. The present study provides information on the distribution of FPAS in the coastal waters along the west coast of India, which may enable us to determine the contribution of terrestrial inputs to the organic fraction of the seawater in that region.

It was found through the present study that at lower water depths (about 30 m), surface concentrations of FPAS varied between 80 $\mu\text{g/l}$ and 147 $\mu\text{g/l}$ and bottom concentrations between 80 $\mu\text{g/l}$ and 116 $\mu\text{g/l}$. At higher water depths (about 200 m), the distribution pattern of FPAS in the surface and bottom waters was found to be more or less the same. Surface concentrations varied between 80 $\mu\text{g/l}$ (at station 9) and 132 $\mu\text{g/l}$ (at station 16) while, bottom concentrations between 61 $\mu\text{g/l}$ (at station 12) and 150 $\mu\text{g/l}$ (at station 16). Near Bombay High, water samples were collected from two sections; one normal to the Bombay coast and the other parallel to the Maharashtra coast extending up to the Gujarat coast. The surface concentration varied between 160 $\mu\text{g/l}$ (at station 23) and 70 $\mu\text{g/l}$ (at station 27) while bottom values between 160 $\mu\text{g/l}$ (at station 23) and 93 $\mu\text{g/l}$ (at stations 21 and 22).

At southern stations (south of Kannur), both surface and bottom waters recorded a seaward decrease in FPAS concentration. This may be due to the fact that tannins and lignins are of terrestrial origin and a major fraction of these substances gets removed from seawater by precipitation as it is transported away from the shore. Hedges and Parker (1976) and Moran *et al.* (1991) have reported a decrease in the concentration of terrestrially derived lignin material with distance from the shore. A seaward increase found in the surface waters off Kollam may be due to a local offshore transport of the surface water probably due to wind effect as suggested by an isohaline surface water and a higher concentration of FPAS at 30 m depth compared to the surface water. A random distribution observed at Cape Comorin may be attributed to the presence of two different water masses—equatorial surface water and Indian Ocean equatorial water as suggested by the salinity and temperature data.

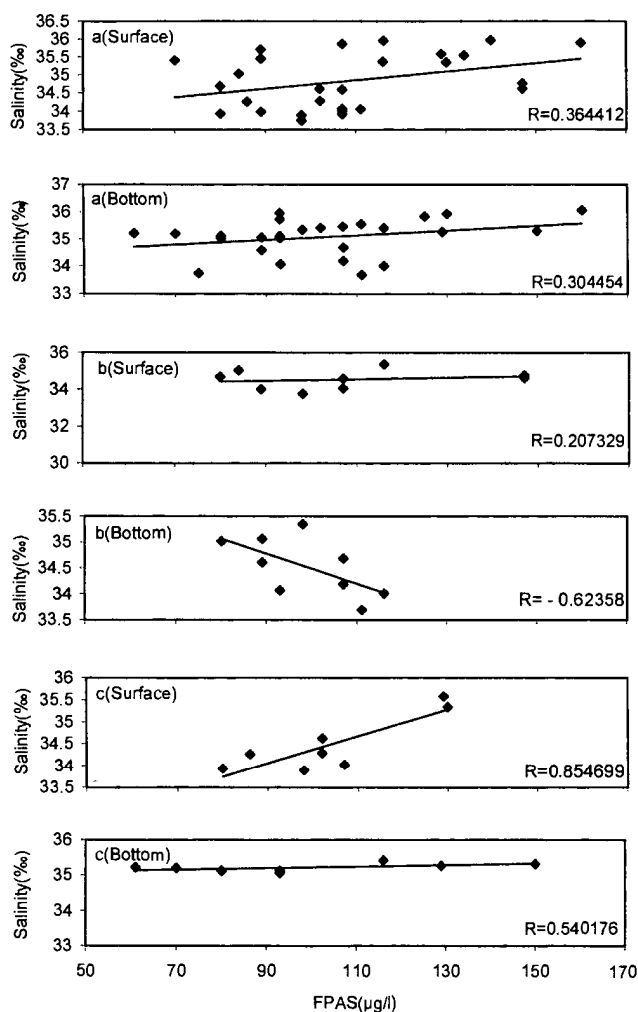


Fig. 5. The relationship between FPAS concentration and salinities at all stations (a) and their variations on depthwise (b) 30 m depth, (c) 200 m depth).

At northern stations, a seaward increase was observed in FPAS content. This may probably be due to the effect of northeast wind prevailing in the west coast of India from November to March. Owing to the combined action of the wind and the effect of the rotation of the earth, the surface waters rich in FPAS tend to be taken away from the coast. As a consequence of these water movements, nearer the coast a certain amount of upwelling from moderate depth will be taking place to replace the water driven seaward. The intensity and the extent of this upwelling are determined by the force and duration of the wind (Jayaraman and Gogate, 1957). Moran *et al.* (1991) have indicated that wind and weather have a significant influence on the concentration and distribution of terrestrially derived lignin phenols in the seawater. Due to the weakening of wind stress in February, an effective wind-driven

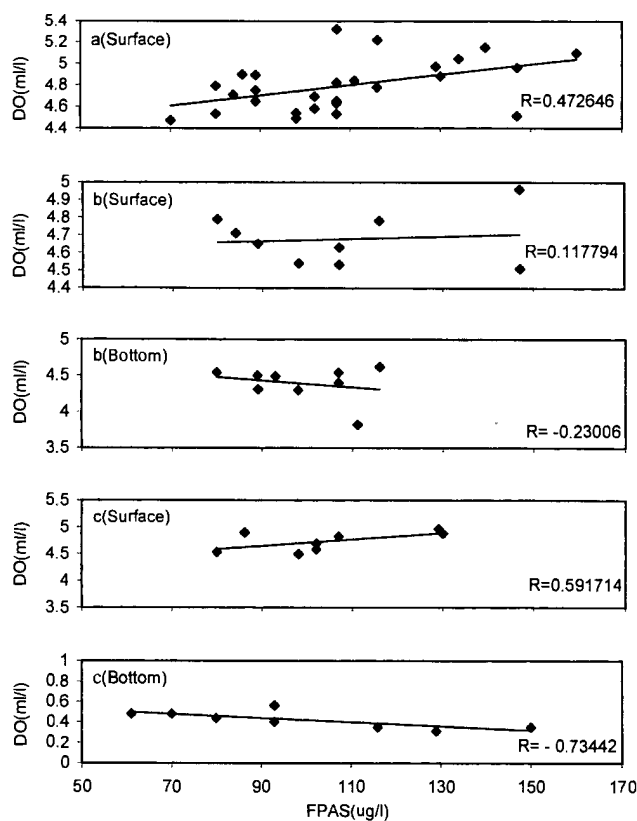


Fig. 6. The relationship between FPAS concentration and dissolved oxygen at all stations (a) and their variations on depthwise (b) 30 m depth, (c) 200 m depth).

offshore movement of surface waters is observed only in the northern stations. Also, the surface currents, off the Indian coast, show an equatorward flow from February. This flow is the eastern limb of the anticyclonic circulation of the Arabian Sea and it has an offshore component (Longhurst and Wooster, 1990).

A general decrease was observed in the FPAS concentration from surface to bottom along the Indian coast. This was relatively well pronounced in the southern stations from Trivandrum to Kannur. This downward decrease may be due to its removal from water media by adsorption onto the microbial cell wall (Day *et al.*, 1953; Woodward *et al.*, 1963) and by processes like coagulation, sorption on particulates and others during its sinking to the bottom. Also, increased concentrations in the surface waters may be due to the fact that bubble-breaking process concentrate organic matter from the bottom to the surface. A downward increase observed at stations 15 and 16 may be due to the existence of the convergence zone. A lower concentration observed in the surface waters off northernmost stations compared to the bottom waters may be due to the influence of Arabian Seawater reaching the area in the equatorward flow.

3.3 Relationship of FPAS concentration with salinity and dissolved oxygen

The relationship between dissolved FPAS concentration and the hydrographical parameters such as salinity (Fig. 5) and dissolved oxygen (Fig. 6) has been determined by finding out the Pearson coefficient, r . Concentrations of FPAS were positively correlated with dissolved oxygen ($r = 0.473$, $P < 0.02$) in the surface waters. Since a high value of dissolved oxygen in surface waters is representative of increased productivity, positive correlation of FPAS with dissolved oxygen may suggest a marine origin for FPAS such as the production of Folin phenol active metabolites by phytoplankton. However, a large spread in FPAS concentrations for a given value of dissolved oxygen suggests that there may not be a direct relationship between FPAS concentrations and dissolved oxygen values in the surface waters. A more detailed study is required before any conclusion is drawn. At lower water depth stations, correlation between FPAS and dissolved oxygen was insignificant. A significant negative correlation was observed at a depth of about 200 m ($r = -0.734$, $P < 0.02$). This is in accordance with the fact that the decomposition of organic matter below the compensation depth causes a decrease in oxygen content along with the accumulation of refractory organic compounds like lignins, tannins etc.

In the bottom waters of lower water depth stations, FPAS concentrations were negatively correlated with salinity ($r = -0.624$, $P < 0.05$) as predicted by the simple mixing of FPAS-rich freshwater and FPAS-poor seawater. A positive correlation observed at the 200 m water depth stations (surface waters: $r = 0.855$, $P < 0.01$; bottom waters: $r = 0.54$, $P < 0.1$) is in accordance with the fact that substances such as lignins behave more conservatively in higher salinity waters once they are resident on the shelf.

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