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## Seasonal variations of sediment phenolics and aerobic heterotrophs in mangrove swamps

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In the sediments of the mangrove swamps of Cochin, phenolic compounds and total plate count (TPC) ranged from 0.018 ppm to 16.75 ppm, and  $25 \times 10^4/g$  to  $110 \times 10^4/g$  respectively and showed distinct seasonal variations. Phenol concentration was highest (16.75 ppm) during monsoon month, when the bacterial abundance was the lowest ( $24.5 \times 10^4$ ). The diversity of bacteria and their numbers were higher when phenol concentration was less in the sediment (<2 ppm). Both the parameters showed inverse correlation with each other ( $r = -0.58496$ ;  $P \leq 0.05$ ). The qualitative study of aerobic heterotrophs showed the presence of *Aeromonas* sp., *Alcaligenes* sp., *Bacillus* sp., *Enterobacteriaceae*, *Flavobacterium* sp., *Micrococcus* sp., *Pseudomonas* sp. and *Vibrio* sp. Various hydrological and sediment parameters showed significant correlations among each other and are presented in detail in the present paper.

Mangroves are one of the most productive natural ecosystems and form an important part of the coastal and estuarine ecosystem and are nursery ground for many organisms<sup>1</sup>. Phenols are one of the major groups of secondary metabolites in plants. Phenolic acids in soil are naturally formed during humic acid breakdown. Other sources in sediment are flavanoids leached from plant debris, those formed during lignin decomposition and those synthesized by soil micro-organisms<sup>2</sup>. Presence of phenolics and bacteria in mangrove sediments have been reported earlier<sup>3-8</sup>. In nature phenol will form complexes with nitrogenous compounds and makes them less susceptible for microbial degradation as compared to free proteins and amino acids. This reduces mineralization and release of nutrients. Therefore, the abundance of phenolics in sediment plays an important role in nutrient cycling<sup>2</sup>. Present paper reports on the abundance and seasonal variation of phenolics and aerobic heterotrophs in the mangrove sediments of Cochin along with other environmental parameters.

### Materials and Methods

Surface water and sediment samples were collected monthly (March 1990-August 1991) from "Mangalavana", a patchy mangrove area in Cochin, Kerala during low tides. Water samples were analyzed for temperature, pH, salinity, dissolved

oxygen, nitrate-nitrogen, phosphate-phosphorous and silicate-silicon<sup>9-12</sup>. Sediment samples were analyzed for temperature, pH, organic carbon and organic matter, sediment phenolics and total plate count (TPC) of aerobic heterotrophic bacteria<sup>13-16</sup>. Qualitative analysis of microbial isolates was done up to generic level using the scheme of Simidu & Aiso<sup>17</sup>. Duplicate samples were analyzed for precision of results.

Sample mean and standard deviation were calculated for the data of each season. Correlation analysis was carried out to find out inter-relation among different parameters. Multiple regression analysis was also done to arrive at a regression equation<sup>18</sup>.

### Results

Variations in physico chemical parameters for a period of 18 months are given in Fig. 1. The seasonal mean and standard deviation are given in Table 1. The seasons are classified as premonsoon (February-May), monsoon (June-September) and postmonsoon (October- January). Water and sediment temperatures ranged between 25.7°C and 31.5°C. The pH varied from 5.7 to 8.3. Water salinity recorded a low of 0.26‰ (June) and a high of 23.3‰ (March). Dissolved oxygen level ranged from 0.4ml/l to 8.5ml/l. Maximum concentration of nitrate-nitrogen (0.62 ppm) and phosphate-phosphorous (1.3 ppm) were encountered in monsoon months. Lowest level

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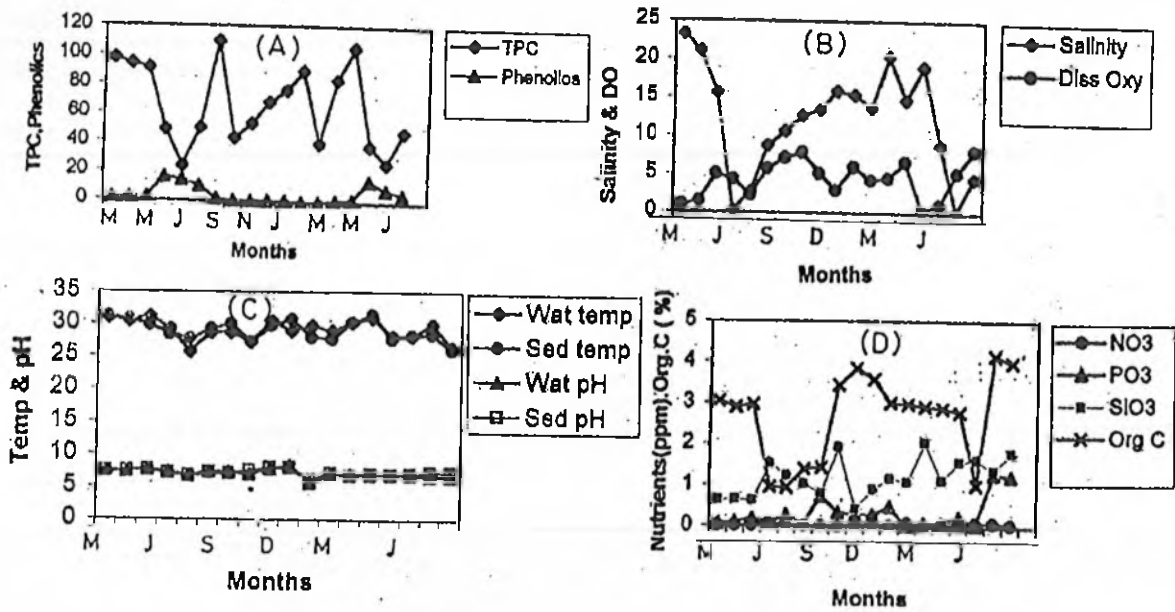


Fig. 1—Monthly variation of different physicochemical parameters in mangrove swamp. A) Sediment phenolics and total plate count (TPC) of bacteria, B) water salinity and dissolved oxygen, C) temperature and pH of water and sediment, and D) nitrate, phosphate, silicate and organic carbon.

Table 1—Seasonal variation of physico-chemical parameters in mangrove swamp

Parameters	Premonsoon	Monsoon	Postmonsoon
<b>Water</b>			
Temp (°C)	30.17±1.27	28.16±1.51	29.28±1.47
Salinity(‰)	18.38±3.54	5.30±4.10	4.45±1.57
Oxygen (mg/l)	3.45±2.36	5.00±2.60	5.63±1.90
pH	7.25±0.35	7.40±0.32	7.25±1.07
PO <sub>4</sub> -P (ppm)	0.11±0.08	0.54±0.56	0.37±0.09
NO <sub>3</sub> -N (ppm)	0.04±0.03	0.08±0.06	0.04±0.02
SiO <sub>3</sub> -Si (ppm)	0.11±0.59	1.35±0.44	1.13±0.0
<b>Sediment</b>			
Temp (°C)	29.88±1.55	28.30±1.22	29.00±1.65
pH	7.20±0.36	7.14±0.24	7.40±0.90
Org C (%)	2.93±0.16	2.00±1.40	3.58±0.53
Organic matter (%)	5.38±1.07	3.85±3.36	5.99±0.01
Phenolics (ppm)	1.61±1.03	10.78±5.19	1.55±0.35
Total plate count (no ×10 <sup>4</sup> /g)	86.35±20.97	49.78±28.09	60.75±14.25

of nitrate-nitrogen (0.004 ppm) and silicate-silicon (0.42 ppm) were observed during postmonsoon months. Highest value of silicate-silicon (2.04 ppm) and lowest value of phosphate-phosphorous (0.01 ppm) were recorded in premonsoon months. Phenolics, bacterial numbers and organic carbon content in the sediments varied from 0.018 ppm to 16.75 ppm, 0.95 to 4.18% and 24.5×10<sup>4</sup>/g to 110×10<sup>4</sup>/g, respectively.

The correlation matrix for the 13 parameters studied are given in Table 2 (n=18 and P=0.05).

Sediment phenolics showed inverse correlation with water salinity (r=-0.76103) and TPC (r=-0.58496) and positive correlation with sediment temperature (r=0.34244), organic carbon (r=0.6452) and nitrate-nitrogen (r=0.5183). Bacterial TPC was significantly correlated with water temperature (r=0.38741), silicate-silicon (r=0.45813) and sediment temperature (r=0.35437). While nitrate-nitrogen showed negative correlation (r=-0.33586).

The physico-chemical parameters, which had significant influence with phenolics, were further

Table 2—Correlation matrix of various parameters

	1	2	3	4	5	6	7	8	9	10	11	12	13
1	1.0000												
2	0.4732	1.0000											
3	-0.1052	-0.3363*	1.0000										
4	-0.2305	0.2248	-0.2586	1.0000									
5	-0.2815	-0.4655*	0.5192*	-0.2907	1.0000								
6	-0.3773	-0.5656*	0.0387	0.0586	0.1361	1.0000							
7	-0.4827*	-0.2410	0.2147	-0.3897*	0.2288	0.1980	1.0000						
8	0.7503*	0.5066*	-0.2307	0.4943*	0.4081*	0.2047	0.5352	1.0000					
9	0.2845	0.4512	0.3285	0.0498	0.2218	0.1589	0.1985	0.3345	1.0000				
10	0.2869	0.2960	0.2970	0.0291	0.4508	-0.1367	0.0065	0.0257	0.3368*	1.0000			
11	0.2881	0.2969	0.2970	0.0292	0.0450	0.1369	0.0067	0.0259	0.3371*	0.9999*	1.0000		
12	-0.4099*	-0.7610	-0.1861	-0.1003	0.0304	0.5185*	0.2116	0.3424	0.1536	0.6475	0.6481	1.0000	
13	0.3874*	0.6404*	-0.1768	0.1714	0.2228	-0.3359*	0.4581	0.3544	0.0478	0.0786	0.0792	-0.5870*	1.0000

Critical value (2-tail, 0.05) = +/- 0.32860\* Significant correlation P=0.05; N=18

1	Water temperature	8	Sediment temperature
2	Salinity	9	Sediment pH
3	Dissolved oxygen	10	Organic carbon
4	pH	11	Organic matter
5	Phosphate-phosphorous	12	Sediment phenolics
6	Nitrate-nitrogen	13	Total aerobic heterotrophs
7	Silicate-silicon		

statistically analyzed and a multiple regression equation was obtained to find out which parameter is having maximum influence on it (Table 3). Organic carbon appears to control phenol distribution as evident from the highly significant correlation with sediment phenolics ( $r^2=0.4522$ ) followed by salinity ( $r^2=0.3638$ ) and nitrate-nitrogen ( $r^2=0.6260$ ). The coefficient of determinant ( $R^2$ ) of the variables was significant at level 79.12%. Likewise, in the case of TPC also, multiple regression analysis was done (Table 4). Salinity had maximum

influence ( $r^2=0.3165$ ) followed by silicate-silicon ( $r^2=0.1955$ ), sediment temperature ( $r^2=0.3638$ ) and nitrate-nitrogen ( $r^2=0.0104$ ). The coefficient of determinant ( $R^2$ ) of the above variables was 53.42% and is significant (>50% is significant).

Bacterial colonies were randomly selected from the highest dilution showing growth on culture plates, sub-cultured, purified and maintained in seawater agar (SWA). The colonies were examined for motility, pigmentation, reaction to Gram stain, and various biochemical and physiological tests. Using

these tests the bacteria were identified up to generic level. *Aeromonas* sp., *Alcaligenes* sp., *Bacillus* sp., *Enterobacteriaceae*, *Flavobacterium*, *Micrococcus* sp., *Pseudomonas* sp. (Group II, III and IV) and *Vibrio* sp. were the most abundant bacteria during the period of study.

### Discussion

The present study indicates that mangrove sediments contain phenolics at different concentrations with seasonal variations. These sediments are rich in organic debris derived from plants and the decomposition of it by bacteria, fungi and actinomycetes results in the release of phenolics<sup>2,19</sup>. Mangrove sediments of Goa were reported to contain 0.26 to 1.01 ppm of phenolics<sup>4</sup>. In the present observation, 0.018 ppm to 16.75 ppm concentrations of phenolics were recorded. The higher concentration can be attributed to effluent discharge from terrestrial sources or degradation of plant detritus<sup>2,3</sup>. Sardesai reported that during monsoon, terrestrial run-off would result in high levels of organic matter and inorganic nutrients in the mangrove swamps<sup>1</sup>. A

Table 3—Regression analysis between sediment phenolics and other variables

Variables	Regr. coeff	SE	T(DE=32)	Prob	Partial R <sup>2</sup>
Wat temp	0.0416	0.505	0.082	0.93490	2.264E-04
Sal	-0.4241	0.1024	-4.142	0.00026	0.3638
NO <sub>3</sub> -N <sub>2</sub>	19.0617	13.4727	1.415	0.16741	0.0626
Org matter	-0.4376	0.2889	-4.976	0.00002	0.4522
Sed. temp.	-0.1685	0.5115	-0.329	0.74410	0.0036
Constant	19.6134				
Std. Error of Est	= 2.873				
Adjusted R Squared	= 0.7564				
R squared	= 0.7912				
Multiple R	= 0.8895				

Table 4—Regression analysis between total aerobic heterotrophs and other variables

Variables	Regr. coeff	SE	T(DE=32)	Prob	Partial R <sup>2</sup>
Water Temp	0.5041	3.5281	0.143	0.88734	6.70986
Salinity	2.8070	0.7531	3.727	0.00080	6.70986
NO <sub>3</sub> -N <sub>2</sub>	57.1137	101.5205	0.563	0.57790	0.0104
Silicate	-21.2962	7.8881	-2.700	0.01129	0.1955
Sed Temp	-3.9020	3.6429	-1.071	0.29265	0.0368
Constant	152.7506				
Std. Error of Est.	=20.5110				
Adjusted R squared	=0.4565				
R squared	=0.5342				
Multiple R	=0.7309				

similar trend was observed in the present investigation also. The total plate count was the minimum during monsoon ( $25 \times 10^4/g$ ) and maximum in postmonsoon ( $110 \times 10^4/g$ ). During this season mangroves consist largely of fungal populations and most of the bacteria increase with summer temperature only<sup>10,20</sup>. Salinity was also lowest during monsoon. This was the time when maximum concentration of phenol was obtained in the sediment samples. The effect of temperature and salinity on bacterial distribution is well documented<sup>21</sup>. It is also reported that changes in bacterial population was a function of salinity<sup>16</sup>. Organic matter and nitrate-nitrogen were also found to influence the distribution of phenolics and aerobic heterotrophs in mangrove swamps at Cochin. At higher temperature and salinity, degradation of phenol is reported to be more with more generic diversity of phenol degrading bacteria. During this season mangroves consist largely of fungal populations and most of the bacteria increase with summer temperature only<sup>7,17</sup>.

As in the present study, occurrence of *Bacillus* sp., *Micrococcus* sp., and *Pseudomonas* sp. has been observed in mangroves of Goa<sup>4</sup>. The negative correlation between plate count and sediment phenolics may be due to the antimicrobial activity of phenols and reports in this regard are very sparse.

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#### References

- Sardessai S, *Indian J Mar Sci*, 22 (1993) 54.
- Burges A, *Soil biology*, (Academic Press, New York) 1967, 35.
- Karanth N G, Lokbharathy P A & Nair S, *Indian J Mar Sci*, 4 (1975) 215.
- Gomes H R & Mavinkurve S, *Mahasagar-Bull Natnl Inst Oceanogr*, 15 (1982) 111.
- Odum W E & Heald E H, *Estuarine research*, (Academic Press, New York) 1975, 265.
- Venkatesan V & Ramamurthy V D, *J Oceanogr Soc Japan*, 27 (1971) 1.
- Matondkar S G P, Mohanthy S & Mavinkurve S, *Indian J Mar Sci*, 9 (1980) 119.
- Matondkar S G P, *Mahasagar- Bull Natl Inst Oceanogr*, 14 (1981) 325.
- Strickland J D H & Parsons T R, *Bull Fish Res Bd Canada*, 167 (1968) 2.
- Parson T, Yoshiak R & Lalli C M, *A manual of chemical and biological methods for seawater analysis*, (Pergamon Press, Oxford) 1984, 283.
- Murphy J & Riley J P, *Anal Chim Acta*, 27 (1962) 31.
- Mullin J B & Riley J P, *Anal Chim Acta*, 17 (1955) 162.
- FAO, *Manual of methods in aquatic environment research, Part I Methods for detection, measurement and monitoring of water pollution*, FAO Fisheries Technology Paper 137 (1975) 15.
- APHA, *Standard methods for the examination of water and waste water* (American Public Health Association Inc, New York) 1975, 574.
- Zobell C E, *Bacteriol Rev*, 10 (1946) 3.
- Rodina A G, *Methods in aquatic microbiology*, (University Park Press, Baltimore) 1972, 185.
- Simidu U & Aiso K, *Bull Jap Soc Sci Fish*, 28 (1962) 1135.
- Snedecor G W & Cochran W G, *Statistical methods*, (Oxford & IBH Publications Co, London) 1967, 593.
- Joseph, I, *Biodegradation of phenolic compounds in different ecosystems in Cochin*, Ph D thesis, Cochin University of Science and Technology, India, 1997.
- Haeckel A M & Rheinheimer G, *Aquilo Series Zool*, 22 (1983) 51.
- Bent G E & Goulder R, *Mar Biol*, 62 (1981) 35.
- Bodungen B V, Brockel K V, Smetaceck V & Zeitschel B, *Skr*, 239 (1975) 179.