

but as the depth increases, respiration and decomposition become more important leading to the production of  $\text{CO}_2$  and the utilization of oxygen. Hence the degree of saturation decreases. At station 1 (off the central west coast of India;  $16^\circ 00' \text{N}$ ,  $77^\circ 27' \text{E}$ ; date of sampling, 10 June 1973), a crossover from supersaturation to undersaturation was observed at approximately 1000 m (Fig. 1A) after which the degree of saturation became almost constant down to a depth of about 2100 m.  $\text{pH}$  increased after passing through a minimum at about 1000 m depth (Fig. 1B). Increase in carbonate concentration due to increase in  $\text{pH}$  is balanced by increase in solubility product of calcite. At station 2 (along the equator  $0^\circ 07' \text{N}$ ,  $59^\circ 43' \text{E}$ ; date of sampling, 2 June 1973), the water was near-saturated at about 1000 m. Degree of saturation at this depth reached a minimum corresponding to a minimum in  $\text{pH}$ . Beyond this depth, degree of saturation gradually increased down to a depth of about 1800 m to a maximum, associated with an increase in  $\text{pH}$ . At greater depths it again tended to decrease. This decrease in the degree of saturation with depth is largely due to the increase in the solubility product. A crossover from supersaturation to undersaturation was observed around 2600 m. This pattern is similar to the one reported by Ben-Yaakov and Kaplan<sup>5</sup> for the Pacific Ocean with the exception of maximum in the subsurface layer. However, at the depth where a minimum occurs, the water was not found to be undersaturated as reported by these authors in the Pacific waters. At station 3 (off the Somali coast, along the equator;  $0^\circ 0' \text{N}$ ,  $48^\circ 0' \text{E}$ ; date of sampling 19 June 1973), no crossover was observed down to a depth of approximately 2400 m. However, the water showed only slight supersaturation at about 1000 m and continued to be so down to 2400 m. Since the calcium concentration at all stations particularly below 1000 m was found to be nearly constant ( $\pm 1\%$ ), the difference in depths at which the crossover from supersaturation to undersaturation occurs, may be attributed largely to the differences in the total carbon dioxide (Fig. 1C) and the associated equilibria of the carbon dioxide system in the sea water determining the concentration of carbonate ion.

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### Distribution of Phenolic Acids in Soils from Two Mangrove Areas of Goa

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Mangrove soil samples from Agacaim (6 km from Zuari river mouth) contained *p*-hydroxy benzoic, *p*-coumaric, vanillic, phloretic, ferulic and syringic acids while samples from Banastarim (15 km from Zuari river mouth) contained all others except the last two. The origin and chemistry of these phenolics in the mangrove soil appears to be important and may provide a clue to the processes involved in the degradation of organic matter of the mangrove swamps.

SOME phenolic compounds which occur in soil as degradation products of lignins, humus and other polymeric materials are known to be toxic to plants<sup>1,2</sup>. Although there are reports on the pattern of phenolics present in the orchards and agricultural soils<sup>3-5</sup> very little information is available on the types of these compounds in the specialized environments such as mangroves. Therefore, it is desirable to look into the pattern of these phenolics in mangrove soils with a view to studying their microbial metabolism and the consequent effect on the plant growth. The present paper deals with the isolation and identification of phenolic acids in soils from 2 mangrove areas of Goa.

Standard phenolic acids were obtained from Sigma Chemical Co., St Louis, USA, and also from Koch-Light Laboratories Ltd, UK. Other chemicals were of AnalaR grade. Distilled peroxide-free ether was used throughout the studies.

Soil samples were collected from 2 mangrove areas, (i) Agacaim about 6 km from the Zuari river mouth, and (ii) Banastarim in the Cambarjua canal, about 15 km from the Zuari river mouth. The samples were collected in January and March 1975 from the top 15 cm of the bottom soil. Undecomposed plant residues were removed and the soil analysed in duplicates.

*Isolation and identification of phenolic acids* — To approximately 2 kg of soil sample, ethanol (95%) was added immediately after the collection to suppress any alteration of phenolics by microbial activity. The suspension was adjusted to  $\text{pH}$  11 by the addition of 10N NaOH. The sample was then mixed well and left for 24 hr with periodic stirring. At the end of this period,  $\text{pH}$  was checked again and readjusted to 11. The clear supernatant solution

was filtered through Whatman No. 1 filter paper. Ethanol was again added, stirred for 1 or 2 hr and filtered as before. The procedure was repeated several times till the pooled filtrate was about 2 l. This solution was evaporated to near dryness at 50°C. The residue was taken up with the smallest possible amount of water, acidified to pH 2 with 1N HCl and extracted 5 times, each with 50 ml aliquots of peroxide-free ether. The ether layer was pooled and dried over anhydrous sodium sulphate and evaporated under vacuo.

The soil phenolics thus extracted were analysed by two dimensional ascending paper chromatography on Whatman No. 3 filter paper using the following solvent systems:

(A) Isopropanol-ammonia-water (20: 1: 2, vol./vol.)

(B) Benzene-acetic acid-water (10: 7: 3, vol./vol, organic phase)

(C) Formic acid-water (2: 98, vol./vol.)

After separating the phenolic acids employing solvent systems A/B and B/C, they were located under UV light and detected by spraying the chromatograms with diazotized *p*-nitroaniline followed by 1N NaOH. The compounds were identified by comparing their colour and position on the chromatogram with those of standard samples.

Samples from area (i) showed the presence of 9 compounds (Fig. 1A), 2 of them had the fluorescence and the rest quenching under UV light. Of these, 6 were phenolic acids. They were identified (colour reaction with diazotized *p*-nitroaniline is indicated in parenthesis) to be *p*-hydroxy benzoic acid (rose), *p*-coumaric acid (blue; *p*-hydroxy cinnamic acid, both *cis* and *trans* isomers), vanillic acid (purple), phloretic acid (light rose; *p*-hydroxy phenyl propionic acid), ferulic acid (sky blue) and syringic acid (blue). All these 6 phenolic acids were present in the samples collected in January as well as in March.

Samples from area (ii) contained only 5 compounds, of which 4 were quenching under UV light and were identified as *p*-hydroxy benzoic acid, *p*-coumaric acid (both *cis* and *trans*), vanillic acid and phloretic acid. The other spot which gave a light yellowish green fluorescence under UV light was not a phenolic compound and hence was not identified. Interestingly, syringic and ferulic acids were absent in this station both in January and in March. Both *cis* and *trans* isomers of *p*-coumaric acid did not resolve in the solvent systems A/B (Fig. 1A), while they got separated well in B/C systems (Fig. 1B).

The mangroves are specialized ecological niches found widely along the Indian coasts. These swamps generally support a high primary productivity<sup>6</sup> and are being exploited for a variety of fish, shell fish and crustaceans<sup>7</sup>. In Goa, dense mangrove vegetation is seen bordering both Mandovi and Zuari estuaries. While most of these regions have high productivity, some pockets are low in their benthic and phytoplankton productivity in spite of a substantial carbon content in the substratum<sup>8</sup>. This low productivity is attributed to the depository type of environment, presence of

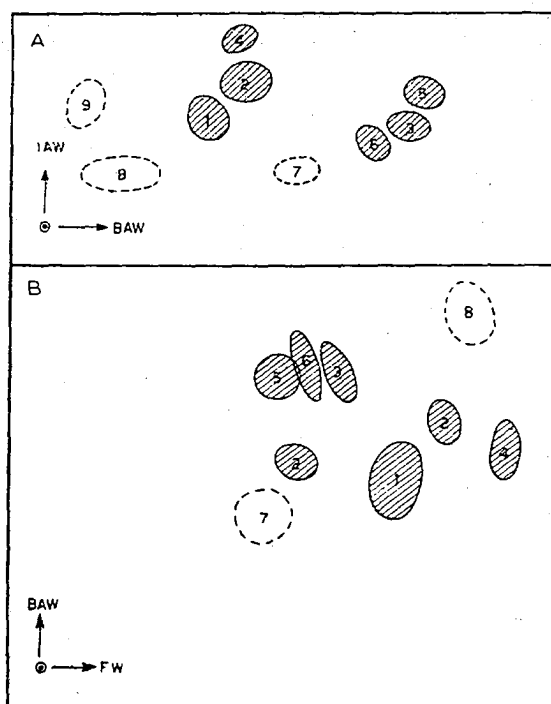


Fig. 1 — Distribution pattern of phenolic acids of mangrove soils [Solvent systems employed: (A) isopropanol-ammonia-water (IAW); benzene-acetic acid-water (BAW) and (B) formic acid-water (FW); BAW. 1, *p*-hydroxy benzoic acid; 2, *p*-coumaric acid; 3, vanillic acid; 4, phloretic acid; 5, ferulic acid; 6, syringic acid; 7 & 8, fluorescent non-phenolics; and 9, quenching non-phenol *Cis* and *trans* isomers of coumaric acid (2) are resolved in (B)]

hydrogen sulphide, anaerobic conditions, weak tidal force, etc. However, the presence of phenolic substances in these regions and their role in the productivity is not well understood.

It is known that complex organic matter of soil has to be degraded before the soil becomes productive<sup>9</sup>. The phenolics are formed as intermediates in the microbial degradation of organic matter which is derived from plants and humus<sup>10</sup>. Therefore, microbial metabolism of these phenolics is essential for the cycling of nutrients in the environment<sup>9,11-13</sup>. The detection of different phenolics in mangrove soils could help in understanding the productivity of these regions. Therefore, a study has been undertaken to ascertain the pattern of phenolics present in the mangroves to assess their effect, if any, on the productivity of these areas.

The mangrove soils analysed in the present study show the presence of *p*-hydroxy benzoic, *p*-coumaric, vanillic, phloretic, ferulic and syringic acids and it is the first report of its kind from mangroves. The absence of ferulic and syringic acids in area (ii) suggests that in this region the humus is degraded probably following a different pathway without the intermediate formation of these two acids, or the formation occurs in such a low concentration as might have escaped detection. However, the latter view appears untenable as the soil

taken each time for the analysis is nearly 2 kg and in all the replicate analyses ferulic and syringic acids were uniformly absent.

Wang *et al.*<sup>1</sup> have reported that syringic and ferulic acids occurred only in a few agricultural soil samples analysed by them. The present work supports this observation that in mangrove soils also these two acids are not present universally.

*p*-Coumaric, ferulic, *p*-hydroxy benzoic, vanillic and syringic acids have been reported to reduce the yield of sugarcane in Taiwan<sup>3</sup>. Some of these phenolics suppress the root elongation of wheat and rye<sup>4</sup>, and at the same time they promote root elongation of barley. Chandramohan *et al.*<sup>15</sup> have reported that cinnamic acid, even at low concentration (0.0001M) was detrimental to the growth of rice seedlings. Therefore, it is clear that the effect of these phenolics on seed germination and root elongation is inhibitory or stimulatory, depending on the concerned plant species and the concentration of phenolic acids as well.

Reports on the presence of phloretic acid in the soil are very scanty<sup>16-18</sup>. Soil samples analysed from both the areas of mangrove swamps in the present investigation always show its presence and it is absent in other soils. These phenolics generally inhibit the activity of phenyl ammonia lyase—an important enzyme involved in seed germination<sup>19</sup>. Also, it is interesting that some phenolic acids are associated with indole acetic acid activity in plants<sup>20,21</sup>.

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