Humic acids from the sediments of three ecologically different estuarine systems - a comparison
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ABSTRACT
Humic acids from the sediments of mangrove, estuarine and barmouth regions were isolated and characterized on the basis of elemental and functional group analyses. From the empirical formula deduced, it could be seen that humic acids segregated from the sediments of barmouth region is much different than those from estuarine and mangrove sediments. Humic acids from mangrove regions are proved to be much developed. The interactive effects of these developed humic acids with toxic metals like lead and cadmium were studied. The sorptional studies at varying metal concentrations and pH showed that lead binds on to humic acid easily than cadmium. Characterization with isothermal models revealed that the adsorption is more prominent at neutral pH. adsorption of lead fits well in to both Langmuir and Freundlich isotherms, but of cadmium only to Freundlich isotherm. At higher pH humic acid facilitates the dissolution of metal to the water column there by enhancing the toxicity and bioavailability.

Key words: Barmouth, characterization, estuary, humic acids, mangrove, metal interaction.

1. Introduction
Sediments have been the principal depositories of organic debris. Organic materials embedded in the sediments are classified into Non-humic substances, Compounds that are survivors of diagenisis and humic substances, i.e., product of diagenisis. Humic substances comprise a general class of biogenic refractory yellow black organic substances that are ubiquitous occurring in all terrestrial and aquatic environments. Humic substances are produced as the result of the chemical and biological transformations of animal and plant residues and they constitute major organic fraction in soil. The main task of humic substances in environmental chemistry is to remove toxic metals, anthropogenic organic chemicals and other pollutants from water. These materials are of interest because of their geological significance, possible nutritive properties, involvement in biological processes and challenge afforded by their chemical and structural complexities.

There are three major fractions of humic substances, which are operationally defined in terms of their solubility. Humic acids that are soluble under high pH conditions, fulvic acids that are soluble under all conditions and humin insoluble under all pH conditions. Humic acids are operationally defined as those which comprises of base soluble and acid insoluble fraction of the soil. Humic acids differ in quantity and chemical composition depending on many variables like climate, parent material (Gracia et al., 1985), altitude, vegetation type (Singbal and Sharma, 1983)and soil management(Milglierina and Rosell, 1995). Their elemental
composition as well as functional group content varies depending on their origin and preparation procedure. The main functional groups are carboxylic and phenolic, but minor amounts of other groups such as amino acids are always present (Cezikova et al., 2001). The ability of these highly functionalized bio polymers to form complexes with polyvalent cations are well established. It is usually assumed that oxygen containing functional groups act as metal binding sites. However the involvement of other groups cannot be discounted. These interactions play an important role ion metal mobility and bio availability in the environment. This paper is dealt with isolation and characterization of humic acids from the sediments of three ecologically distinct environments of a tropical estuarine system, viz, mangrove, estuarine and barmouth regions. The study is also addressed to the interactive effect of humic acids with two environmentally significant toxic trace metals (lead and cadmium) under varying simulated conditions.

2. Materials and methods

Cochin estuarine system is the largest tropical estuarine system along the west coast of India, extending between 9° 40' and 10° 12' N and 76° 10' and 76° 30' E. The three different ecological settings of the Cochin estuarine system were selected. The area of investigation and sampling stations are depicted in Fig 1. Station 1, Mangalavanam, the ‘Green lung’ of Ernakulam city is a patchy mangrove area. It is almost an enclosed, protected system of estuarine waters with a single narrow opening to the Cochin estuary. Thick forest and water adjacent attract to it a number of exotic and rare varieties of migratory birds of different species, which periodically find shelter during their breeding seasons and enrich the system with lot of organic inputs. Station 2, Cochin estuary, received huge quantities of municipal sewage and was hence typical of urban influence. Station 3, The bar mouth of cochin estuary - a channel of 450m wide in Cochin gut, through which estuary makes permanent connection with Arabian sea. This area represents the confluence region of estuary and Arabian Sea. The surface sediment samples were collected from the three stations using Van - Veen grab. Samples were homogenized and were stored at a temperature of -5°C in polythene covers, till the analyses were performed.

Figure 1: Map showing the study area

Sediment organic carbon was estimated by chromic acid oxidation method (ElWakeel and Riley, 1957) and sediment texture analysis was done using particle analyser. Humic acids were extracted according to the method of IHSS (Swift, 1996). Dried sediment samples were...
sieved using a 2mm sieve. Samples were equilibrated to a pH of 1-2 with 1M HCl at room temperature. The volume of the solution was adjusted with 0.1M HCl, to provide a final concentration that had a ratio of 1g dried sample/10ml liquid. Suspension was shaken for one hour. Then the supernatant was separated from the residue by decantation. The sediment residue was neutralized to pH 7 with 1M NaOH. Then 0.1M NaOH was added under the atmosphere of nitrogen. The final extractant to the soil ratio was 10:1. Extract the suspension under the atmosphere of nitrogen with intermittent shaking for a minimum of 4 hours. Allowed the alkaline suspension to settle overnight and the supernatant were collected by filtration. The supernatant was acidified with 6M HCl with constant stirring to pH 1 and then allowed the suspension to stand for 12-16 hours. The precipitated humic acids were separated by centrifugation.

Humic acid fraction was redissolved in a minimum volume of 0.1M NaOH under nitrogen atmosphere, reprecipitated the humic acid using 6M HCl with constant stirring to pH 1. The suspension was allowed to stand for 12-16 hours. Centrifuged and supernatant was discarded. This was repeated for 5 times. Humic acid thus obtained was suspended in weak solution of HF and HCl (0.5ml conc.HCl and 0.5ml conc.HF diluted to 100 ml with distilled water.) as suggested by Khan(1971). Humic acid was then dialysed against distilled water until dialysis water gave a negative chloride test with silver nitrate. Humic acid were dried in an oven below 60°C and kept in a dessicator. The total acidity was estimated by the baryta absorption method originally suggested by Brooks and Sternhell (1957) and modified by Schnitzer and Gupta (1965). The method suggested by Blom et al. (1957) and modified by Schnitzer and Gupta (1965) was followed for the determination of carboxyl groups. Total acidity of organic matter is a function of the activity of carboxyl and phenolic hydroxyl groups. Therefore, the content of phenolic hydroxyl groups was determined by difference. Total carbohydrate content of humic acid was determined colourimetrically by phenol-sulphuric acid method (Dubois et al., 1956). Total phosphorous content of humic acids were determined by Molybdenum-Blue method (Nissenbaum, 1979). Elemental compositions of humic acid samples were determined using Vario EL III CHN analyzer.

For sorptional studies, 10 mg of HA was equilibrated with 10ml of metal (Cd and Pb) solutions of known concentration in a 100ml beaker. The concentrations of solutions used were 1, 3 and 5ppm. The desired initial pH value of the solution was obtained by adjusting with 0.05N HNO₃ and 1:1 NH₃. After the equilibration time of 24 hours, the phases were separated by filtration. The metal content of the filtrate was determined using graphite furnace AAS (Perkin Elmer 3110). The concentrations of the metal sorbed onto the HA were calculated from the change in metal concentration of the solution before and after equilibrium sorption.

3. Results and discussion

The textural analyses of the sediment samples collected from the ecologically distinct estuarine environments have shown that mangrove sediments were clayey and barmouth sediments were predominantly sandy (Table 1). Organic carbon contents of these sediments were also in accordance with the grain size. Clayey mangrove sediments recorded 49 mg/g of organic carbon in it, while 1.66 mg/g was found in estuarine sediments and 0.8mg/g in sandy barmouth sediments. The percentages of humic acids in the sediments of three different environments are also given in Table 1.

Table 1: Characteristics of sediments collected from distinct estuarine regions
Humic acids from the sediments of three ecologically different estuarine systems - a comparison

Parameters | Stations
---|---
SOC mg/g | 1 | 2 | 3
Sand% | 18.47 | 56.10 | 98.20
Silt% | 32.83 | 31.15 | 1.80
Clay% | 48.70 | 12.74 | 0.00
Humic acids mg/g | 1.43 | 0.52 | 0.025

The abundance of humic acids in sediments depends on the productivity of the environment, sediment texture and microbial activity, also the rate of accumulation and bioturbation effect on surface sediments (Aiken et al., 1985). Mangrove sediments characterized by larger clay content and rich vegetation have the highest humic acid content in it and the sandy barmouth sediments were impoverished in humic acid level. The concentrations of organic compounds in sediments are highly influenced by surface area of the sediments (Brian et al., 1997) and it is well established fine textured clay particles absorb higher amounts of organic matter than coarse textured soil when supplied with similar input of organic material (Harsink, 1994).

Table 2: Elemental composition of humic acids collected from distinct estuarine regions

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>%C</td>
<td>56.18</td>
</tr>
<tr>
<td>%H</td>
<td>6.12</td>
</tr>
<tr>
<td>%N</td>
<td>3.67</td>
</tr>
<tr>
<td>%O</td>
<td>34.03</td>
</tr>
<tr>
<td>C/H</td>
<td>9.18</td>
</tr>
<tr>
<td>C/N</td>
<td>15.30</td>
</tr>
<tr>
<td>C/O</td>
<td>1.65</td>
</tr>
<tr>
<td>O/H</td>
<td>5.56</td>
</tr>
</tbody>
</table>

Elemental compositions and atomic ratios of elements in humic acids are reported in Table 2. Bird and May (1981) have described, using Null hypothesis method that polluted humic acids contain greater percentage of nitrogen than unpolluted ones, while carbon values are statistically identical, hence nitrogen can be considered as a good index of pollution. It is seen that percentage of carbon is almost similar in all the three sediments, but slightly more in mangrove humic acids and less in barmouth; Now considering the percentage of nitrogen, the humic acids of virgin mangalavanam mangrove areas have lesser percentage, while estuarine and barmouth areas with more anthropogenic input showed greater percentage of nitrogen. So also organic nitrogen rather than organic carbon is lost preferentially during decomposition of organic matter, during the early diagenesis in sediments (Hedges and...
Oades, 1997). Transformation of certain organic molecules like carbohydrates, proteins, lipids to randomly polymerized heterogeneous macromolecules i.e., (humic substances), is influenced by organic matter input, grain size composition, redox condition and bioturbation (Calace et al., 2006).

Considering the atomic ratios, C/N ratio is found to be 15.30 in mangrove humic acids and 14.61 in estuarine, while 10.47 in barmouth. This shows that the humification process has not happened much in barmouth sediments, instead accelerated mineralization of polysaccharides is being indicated. These differences in the rate of humification can again be stated as one of the reasons for difference in abundance of humic acids in sediments, while other factors are texture and organic matter contents of the sediments. Humification process is much disturbed in the barmouth region with much tidal actions but is prominent in static mangrove sediments where the above said organic molecules are in plenty from leaf litter and other organic wastes like bird excreta as the mangrove station considered here is Mangalavanam, a tiny forest which is a shelter of a number of exotic and rare varieties of migratory birds of different species. The rate of humification in estuarine sediment is the intermediate of the other two extremes. Interpreting the C/H values, barmouth recorded lesser values, and higher by mangrove humic acids. The lower values of C/H of humic acids in barmouth region shows that the lignin mineralization rather than humification is predominant in the in the confluence region. These inferences are again evidenced by O/H ratios, in which the increased value of O/H ratios shows accelerated humification. Detrital organic phosphorous is accumulated along with the stratified build up of partially decomposed plant remains, with relatively little humification (Ramesh and Ronald, 2008). This could certainly be the cause of lesser phosphorous content in the highly humified humic acids of mangrove region. The concentration of carbohydrates were found to be much higher in mangrove humic acids (Table 3) as the process of carbohydrate mineralization is seemed to be not focused in anaerobic mangrove sediments and that the carbohydrate moieties have actively taken part in humification.

Earlier study by Janet (2002) had reported that the humic acids of mangalavanam region has the structure given in figure 2.

![Figure 2: Structure of humic acid](image)

The values of total acidity, carboxylate acidity and phenolic acidity are presented in Table 3. High COOH acidity was found in mangrove humic acids. The content of carboxylic groups represents a valuable index of humification (Rossell et al., 1989). This again strengthens the fact that the mangrove humic acids have the highest degree of humification. Higher COOH and lower phenolic contents indicate a well humified humic acid (Yong and Mourato, 1988). High phenolic OH content indicates the presence of poorly decomposed or not well humified humic acids (Lavti and Paliwal, 1981).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Stations</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
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</tbody>
</table>

Table 3: Analytical characteristics of humic acids from mangrove and estuarine sediments
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The estuarine humic acids contained less developed organic matter, which represented recently added organic wastes. All the discussion converges to a single conclusion that the humic acids of mangroves are well developed in comparison to the humic acids of estuarine and barmouth environments.

3.1 Characteristics of metal sorption

Higher humified humic acids with high degree of aromaticity and high content of carboxylic groups are expected to participate in binding and interaction with organic and in organic pollutants in sediments and therefore affect their residual toxicity (Barancikova et al., 1997).

Table 4: Concentration and pH dependence of sorption of lead and cadmium by humic acids

<table>
<thead>
<tr>
<th>Metal conc (ppm)</th>
<th>pH 6</th>
<th>pH 7</th>
<th>pH 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pb</td>
<td>Cd</td>
<td>Pb</td>
</tr>
<tr>
<td>1</td>
<td>0.39</td>
<td>0.18</td>
<td>0.27</td>
</tr>
<tr>
<td>3</td>
<td>1.26</td>
<td>0.596</td>
<td>0.48</td>
</tr>
<tr>
<td>5</td>
<td>2.45</td>
<td>1.92</td>
<td>0.65</td>
</tr>
</tbody>
</table>

The fate of heavy metals in the aquatic environment is of extreme importance because of their impact on the ecosystem. Sediment organic matter, humic substances in particular has much influence on the fate of toxicity of metals, as they play a predominant role in partitioning of metals between solid and liquid phases. The interaction of two environmentally significant toxic metals lead and cadmium, with highly humified mangrove humic acids was studied under varying simulated conditions of pH and concentrations.

The results of sorptional analyses are presented in Table 4. The sorption percentage is always greater for lead than cadmium. Earlier works have revealed that binding capacity on humic acid for lead is greater than for Cadmium. (Coles et al., 2006; Adenkule et al., 2007). The greater uptake of Pb$^{2+}$ as compared to Cd$^{2+}$ ions (hydrated radius = 0.45 vs0.50 nm) for metal retention by ion exchange may also be due to their relative sizes (Dean, 1985).
It is believed that small materials can get easily trapped inside voids within the humic acid molecules (Schulten and Schnitzer, 1995). It is seen that sorption capacities increased with increase in metal concentration of the solution, provided no dissolution of humic acid into the solution. It could be seen from the results that as pH increased to 8 sorption decreased and that maximum sorption was obtained at a slightly lower pH. Calace et al (2006) had studied the sorptional capacity of humic acids at varying pH from 2 to 6 and had established that the adsorption increased as the pH increased from 2 to 6. Comparing these two works, it could be seen that maximum adsorption is at almost neutral pH.

The pH dependent surfaces of Humic acids cause flocculation at low pH and dispersion at high pH (Spark, 1997). Humic acids may remove metals from solution at lower pH but at higher pH metal bonding with dissolved humic acids results in the formation of soluble metal humate species (Hatton and Pickering, 1980). Significant distinctiveness of the adsorption process by humic acids were tried to be analyzed with Langmuir and Freundlich adsorption isotherms and the constants derived from them. (Fig. 3). The Langmuir adsorption model, with the assumption of monolayer limited coverage is the representative of wide range of equilibrium sorption isotherm data. This
isotherm expresses the relation between the equilibrium concentration of the metal ion and
the amount of metal adsorbed by unit mass of the adsorbent. Both capacity and intensity
factors can hence be calculated.

\[ \frac{X_e}{X} = \frac{X}{K_1} + \frac{1}{K_1 K_2} \] 1

Here, \( X \): amount of metal adsorbed per gram of the humic acid
\( X_e \): equilibrium metal concentration in the solution

A plot of \( \frac{X_e}{X} \) against \( X_e \) gives a straight line if data were fitted in the equation.

\( K_1 \) and \( K_2 \) are constants calculated from the slope and y-intercepts respectively.

Here \( K_1 \) represents the adsorption maximum which in turn is the measure of the effective
adsorption sites that will take part in adsorption process. \( K_2 \) the equilibrium coefficient is a
function of energy, by Arrhenius equation \( K = Ae^{-Ea/RT} \), and increase in \( K_2 \) represents decrease
in energy involved in adsorption process. Freundlich model is used when more than
monolayer coverage of surface is expected. An unlimited supply of unreacted sites is
assumed to be available and have different binding energies. Freundlich isotherm is
expressed by the equation

\[ X = K X_e^{1/n} \] 2

Linearly expressed as, \( \log X = \log K + \frac{1}{n} \log X_e \). Here, \( K \) and \( N \) are empirical parameters
representing relative indicators of sorption capacity and sorption intensity respectively.

**Table 5**: Langmuir and freundlich constants of metals in humic acids

<table>
<thead>
<tr>
<th>Metal</th>
<th>pH</th>
<th>( K_1 )</th>
<th>( K_2 )</th>
<th>( \log K )</th>
<th>( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lead</td>
<td>6</td>
<td>-3.80373</td>
<td>-0.14954</td>
<td>0.702749</td>
<td>0.800256</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.908513</td>
<td>0.52572</td>
<td>0.182096</td>
<td>5.24109</td>
</tr>
<tr>
<td>Cadmium</td>
<td>6</td>
<td>*</td>
<td>*</td>
<td>0.227562</td>
<td>0.630438</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>*</td>
<td>*</td>
<td>0.182096</td>
<td>5.24109</td>
</tr>
</tbody>
</table>

* does not fit in to the model

Only the adsorption of lead fits into langmuir adsorption isotherm, which is obeyed only at
pH 6 and 7. The constant in langmuir relation, \( K_1 \), represents adsorption maximum, i.e., the
measure of all possible adsorption sites. The value of \( K_1 \) is found to be more at pH 7. The
number of ‘effective’ adsorption sites is more at pH 7. The constant \( K_2 \), function of energy is
also found to be more at pH 7. By description, an increase in \( K_2 \) represents a decrease in the
energy involved in the adsorption process. The Freundlich constant \( K \) gives the adsorption
capacity which is found to be more at ph 6, but the value constant \( n \) shows that sorption
intensity is more at pH 6.
The sediments studied differ in their quantity and quality of humic acid content. Organic rich sediments of mangrove regions found to be copious in much developed humic acids. The empirical formula deduced confirms that the structures of humic acids are highly reliant on their environment. The studies of interaction with metals revealed that the sorption is most at neutral pH. Freundlich and langmuir adsorption constants authenticate that the adsorption intensity is maximum at pH 6. The implications of these studies in contemporary environmental problems are obvious. Because of its increased solubility at greater pH, the chances of accretion of toxic metals in alkaline water enriched in organic matter will enhance considerably.

4. References


