

# Humic Substances from Manila Bay and Bolinao Bay Sediments

Elma C. Llaguno

*Natural Sciences Research Institute & Institute of Chemistry*

*College of Science, University of the Philippines*

*Diliman, Quezon City 1101*

E-mail: ellaguno@chem.upd.edu.ph

## ABSTRACT

The C,H,N composition of sedimentary humic acids (HA) extracted from three sites in Manila Bay and six sites in Bolinao Bay yielded H/C atomic ratios of 1.1-1.4 and N/C atomic ratios of 0.09 - 0.16. The Manila Bay HA's had lower H/C and N/C ratios compared to those from Bolinao Bay. The IR spectra showed prominent aliphatic C-H and amide I and II bands. Manila Bay HA's also had less diverse molecular composition based on the GC-MS analysis of the CuO and alkaline permanganate oxidation products of the humic acids.

## INTRODUCTION

A sediment system has inorganic and organic components. The inorganic components consist of the clay matrix coated with oxides of aluminum, manganese, iron, and other metals. The organic components may be classified into viable organics (biota) and non-viable organics (Leenheer 1991). The latter is a complex mixture of substances with fixed physical and chemical properties such as proteins, carbohydrates, lipids, pesticides, trace organic pollutants, and macromolecules with no fixed properties such as the humic substances (HS).

Humic substances are geopolymers synthesized from products of degradation of plant and animal tissues (Saar and Weber 1982). The HS are mixtures of polymers of wide diversity in terms of molecular weight and molecular composition. The primary factor which influences the structure of humic substances is geographical location or climactic conditions. The secondary factors are type of plant life, microbial activity, and the presence of transport conditions which may result in the assimilation of structural components from distant sources (Wilson 1988).

Humic substances have been characterized as highly colored, polyfunctional, and refractory or highly resistant to degradation. The methods of characterization of humic substances may be classified into non-degradative and degradative techniques. The non-degradative methods include titration and spectroscopic analyses which provide information on functional groups present in the polymer (uv-visible, infrared, nuclear magnetic resonance, electron spin resonance spectroscopy) and those that yield information regarding surface structure, size, shape, molecular weight, and charge (electron microscopy, diffraction analysis such as x-ray and electron diffraction, measurements of viscosity, surface tension, as well as colligative properties) (Schnitzer and Kahn 1978; Hayes et al. 1989).

Degradative methods break down the humic polymer into compounds derived from its molecular components. Degradation can be achieved by hydrolysis, oxidation, reduction, pyrolysis (thermal degradation), and microbial action (biological degradation). A combination of degradative methods is required to fully characterize the molecular composition of humic substances. The molecular components in turn may serve as tracers for various precursor materials (Wilson 1988; Stevenson 1982; Machihara and Ishiwatari 1983; Hatcher et al. 1985).

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Keywords: sedimentary humic acid, Manila Bay, Bolinao Bay, CuO oxidation, alkaline permanganate oxidation, IR, elemental analysis.

This paper summarizes the results of a comparative study of the characteristics of the humic acid fraction of humic substances isolated from Manila Bay and Bolinao Bay sediments. These studies have been reported previously (Llaguno et al. 1993a; Llaguno et al. 1993b). Manila Bay and Bolinao Bay were selected as representatives of different marine environments: Manila Bay as a heavily polluted environment where sediment samples are exposed to a wide variety of industrial and domestic wastes; and Bolinao Bay as a relatively unpolluted environment.

## MATERIALS AND METHODS

### Description of Study Sites and Collection of Samples

#### *Manila Bay*

Manila Bay is enclosed by the provinces of Cavite, Bulacan, Pampanga, Bataan, and Metro Manila. The rivers from these provinces, including the Pasig River that traverses Metro Manila, drain into Manila Bay. The coastal towns along Cavite, Malabon, Navotas, Bulacan, and Pampanga host a mix of industries. Bataan has mainly oil refineries along its coast.

The Manila Bay sediments were collected from the three sites shown in Fig. 1 and Table 1. Site A was located 1.4 nautical miles from the South Harbor; site B was about 3 nautical

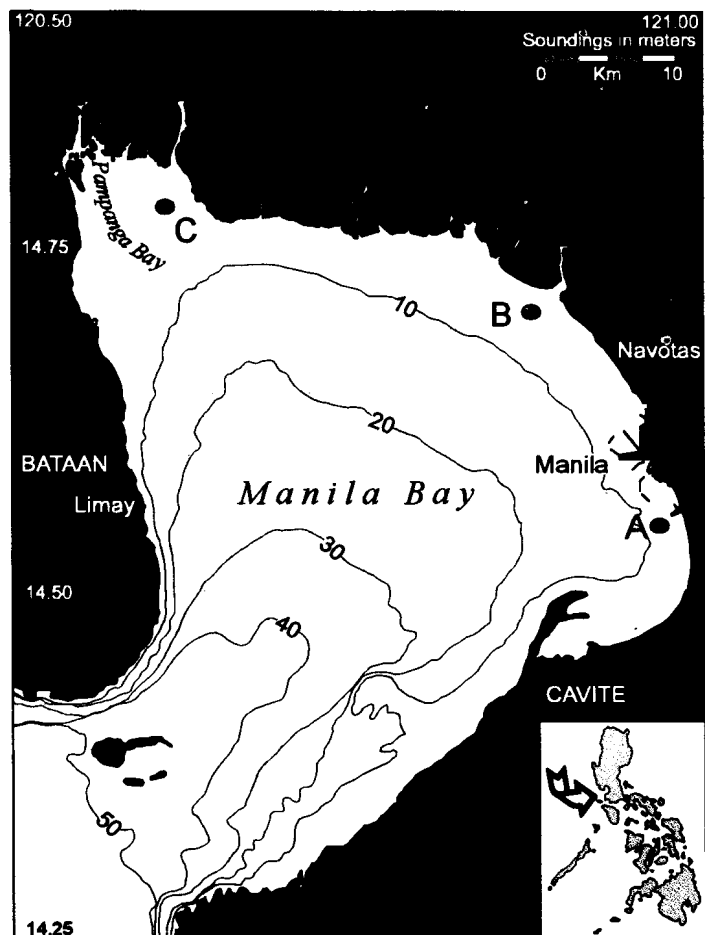


Fig. 1. Sampling sites for Manila Bay sediments.

miles from the mouth of the Bulacan River; site C was close to the mouth of the Pampanga River.

Table 1. Sampling sites for sediment samples

Site	Latitude (N)	Longitude (E)	Depth (m)
Manila Bay (Apr. 1987)			
A	14°33'	120°58'	12
B	14°43'	120°52'	9
C	14°47'	120°37'	9
Bolinao Bay (Dec. 1987)			
A	16°23'24	119°54'48	28
B	16°23'42	119°55'12	15
C	16°23'06	119°55'30	16
D	16°22'48	119°56'02	6
E	16°22'30	119°55'30	5
F	16°21'48	119°55'55	5

#### *Bolinao Bay*

The sediments from Bolinao Bay were collected from six sites (A-F) along the Picocobuan Channel on the northeast coast of Pangasinan near the town of Bolinao (Fig. 2 and Table 1). At the time of sampling, the channel was used mainly by motorized bancas for transporting townspeople from Bolinao to Santiago Island. Coral reefs were found along the coast of Santiago Island. Saltbed and fishpens were also found further south along the Caquipitan Strait.

All sediment samples were collected using an Eckman dredge. The samples were stored in plastic bags, air-dried in the laboratory, then milled to break up the large masses. The Manila Bay sediments contained shells which were manually

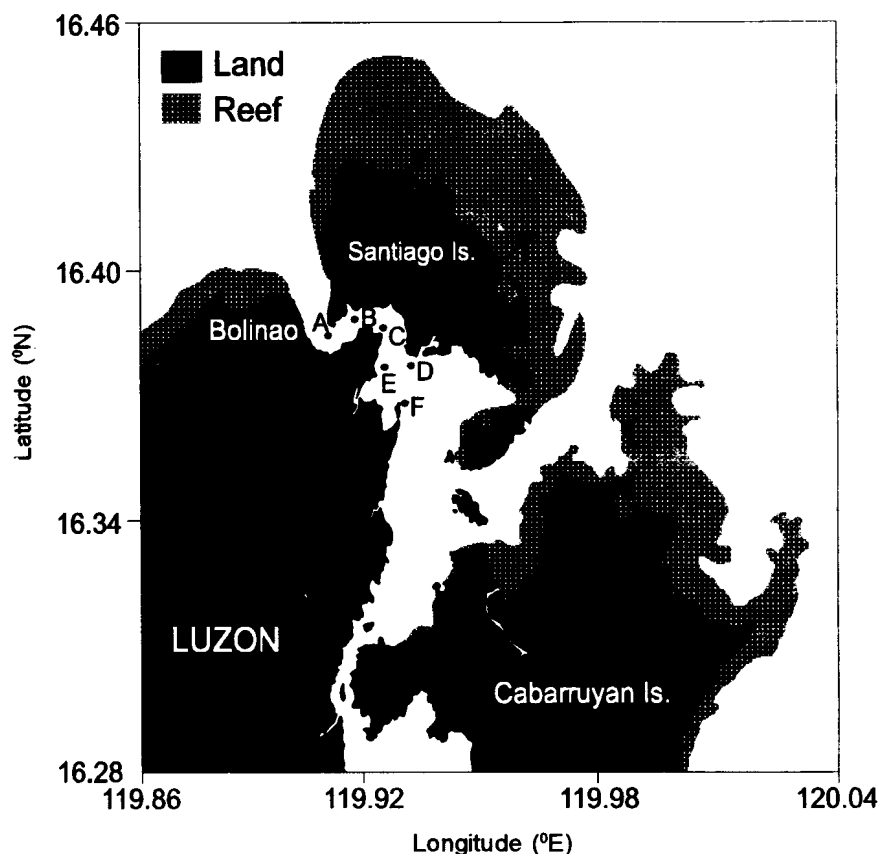


Fig. 2. Sampling sites for Bolinao Bay sediments.

removed. The air-dried samples were passed through a 2 mm sieve. All sediments were pretreated by soxhlet extraction with methanol:benzene (3:2 v/v) to remove lipids prior to the extraction of the HS.

#### Characterization of Sediment Samples

The Manila Bay sediments and the Bolinao Bay sediments from site F were analyzed for mineralogical content by x-ray diffraction using a Rigaku Geigerflex and Rigaku D-max II diffractometer. Other chemical parameters such as percent organic matter, pH, exchangeable acids and bases, and cation exchange capacity (CEC) were measured by the Bureau of Soils using standard procedures (Recel and Labre 1988).

#### Extraction and Fractionation of Humic Substances

The HS from the lipid free sediments were extracted and

fractionated into humic and fulvic acids using the procedure of the International Humic Substances Society (1986). The humic acids (HA) refer to the fraction, which is insoluble in acid, but soluble in base while the fulvic acid (FA) fraction consists of components, which are soluble in both acid and base.

#### Characterization of the Humic Acids: Ash Content, Elemental Composition, and IR Spectra

The ash contents of the freeze dried humic acids were determined using the procedure of Holtzclaw et al. (1976). The elemental analyses were conducted in a CHN Corder 3 YANACO elemental analyzer and by the Australian Microanalytical Service, Melbourne, Australia (Llaguno 1993a).

The IR spectra were taken from KBr pellets of the freeze dried HA prepared by mixing 1–2 mg sample with 200–400 mg dry KBr.

The spectra were recorded on a Perkin Elmer 1330 IR spectrophotometer.

#### Alkaline $\text{KMnO}_4$ and $\text{CuO}$ Oxidative Degradation of the Humic Acids

The HA's from Manila Bay (site A) and Bolinao Bay (site C) sediments were degraded by oxidation with alkaline  $\text{KMnO}_4$  and  $\text{CuO}$  as described in Llaguno et al. (1993b). The degradation products were identified by comparison of their C retention times and mass spectra with those of standards.

## RESULTS AND DISCUSSION

#### The Sediment Samples

The mineralogy and chemical characteristics of the sediment samples are shown in Table 2. The sediments are slightly acidic to neutral (pH 6.8–7.2). The %OM (organic matter) is highly variable among the Manila Bay sediments (0.98–2.06%) with site B having the highest level (2.06%). Site B is close to

Table 2. Physical and chemical characteristics of sediment samples.

Sediment Samples	Physical Properties	Chemical Properties(meq/100g. sediment)							
	Mineral Content	PH (1:1)	% OM	Exchangeable Bases				Exchangeable Acid	CEC Sum (exc. Na)
				Ca	Mg	Na	K		
<b>Manila Bay</b>									
Set A	Set A (kaolinite, muscovite, hematite, biotite) + quartz, glauconite, montmorillonite, orthoclase, plagioclase, aragonite	7.2	1.89	5.4	2.9	9.9	0.1	5.7	14.1
Set B	Set A + quartz, glauconite, chlorite, & vermiculite	6.8	2.06	2.8	2.8	37.8	3.1	3.1	11.8
Set C	Set A + orthoclase, vermiculite, talc, aragonite, & chlorite	6.8	0.98	3.8	3.6	72.8	2.8	2.3	12.5
<b>Bolinao Bay</b>									
Set A	Not available	7.2	2.0	15.4	3.5	72.8	10.0	1.8	30.7
Set B	Not available	7.1	2.15	13.8	13.1	158.8	9.7	1.3	37.9
Set C	Not available	7.1	2.06	2.3	2.3	85.4	8.1	1.6	14.3
Set D	Not available	7.0	2.2	1.8	1.6	62.9	6.3	0.8	10.5
Set E	Not available	7.0	1.62	6.9	6.1	94.5	7.4	2.0	22.4
Set F	Set A + glauconite; montmorillonite; hydroxy apatite; halite	6.9	2.24	7.8	7.1	106.3	7.0	2.8	24.7

the mouth of the Bulacan River, the receiving water for the wastes from the piggeries and chicken farms, which abound in the province. Bolinao Bay sediments, in contrast, have a narrower range of %OM (1.62-2.24%) indicative of the less diverse activity in the area.

Other dissimilarities between the sediment samples lie in the exchangeable bases, which are much higher for Bolinao Bay sediments. The exchangeable acids, however, are higher and more variable for Manila Bay sediments.

The Bolinao and Manila Bay sediments have the following

minerals in common: quartz, plagioclase, kaolinite, and chlorite. Manila Bay sediments exhibited significant variability in mineral composition from site to site with the highest number of minerals observed in site A. Montmorillonite, plagioclase and chlorite are found only in site A sediments; vermiculite is found only in sites B and C; aragonite is found only in sites A and C and talc is found only in site C.

The mineral content of Bolinao Bay sediments from site F differs from that of Manila Bay sediments due to the presence of apatite. It appears to have less diverse mineralogy compared to the Manila Bay sediments.

Table 3. Ash content and elemental composition of humic acids from Manila Bay and Bolinao Bay sediments.

Sample	Humic Acid					
	Elemental Composition (%) (dry ash-free basis)			Atomic Ratio		
	Ash	C	H	N	H/C	N/C
Manila Bay sediment						
A	0.33	52.7	5.0	5.8	1.1	0.09
B	1.01	48.5	5.4	7.3	1.3	0.13
C	1.00	50.6	5.8	7.2	1.4	0.12
Bolinao Bay sediment						
A	Nil	49.0	5.6	8.3	1.4	0.14
B	Nil	50.5	5.7	8.9	1.4	0.15
C	Nil	48.6	5.6	8.1	1.4	0.14
D	Nil	50.7	6.0	9.0	1.4	0.16
E	Nil	52.0	5.9	7.7	1.4	0.13
F	Nil	49.2	5.8	9.2	1.4	0.16

### Ash Content and Elemental Composition

Table 3 shows the ash content and the elemental composition of the humic acids from the sediment samples. Table 4 provides literature values for marine sediment HA's from other sources.

The humic acids from both bays have similar range of values for %C: 48.5-52.7% for Manila Bay and 48.6-52.0% for Bolinao Bay. The percentages for hydrogen and nitrogen are, however, lower in Manila Bay HA.

Although there may have been variations in the methods of extracting the humic acids, the elemental compositions of the Manila Bay and Bolinao Bay HA's are within the range of those from other sources with respect to %H and %C. However, they have rather high %N or N/C atomic ratios. The N/C values of 0.09-0.16 indicate that both sediments are highly enriched with proteinaceous substrates such as phytoplanktons, diatoms, and other biota (Anderson 1989, Filip and Alberts 1993).

Fig. 3. Infrared spectra of humic acids from Manila Bay and Bolinao Bay sediments.

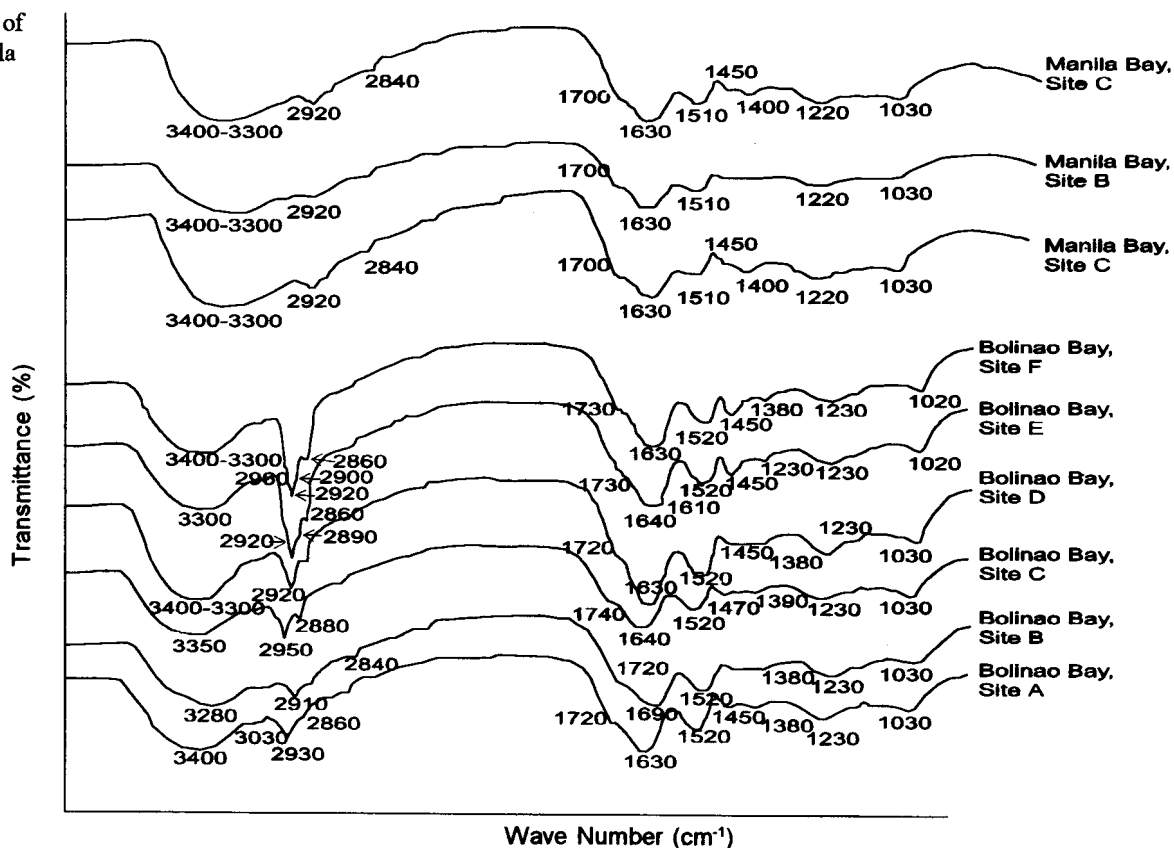


Table 4. Literature values of elemental composition of humic acids from marine sediments.

Sample	Percentage				Atomic Ratio		Reference
	Ash	C	H	N	H/C	N/C	
Washington Slope	2.47	47.22	4.13	4.77	1.21	0.075	Ertel & Hedges (1983)
Gulf of Mexico	1.51	54.94	4.53	5.59	1.22	0.071	
Saanich Inlet	0.08	56.15	5.34	6.18	1.32	0.081	
Pacific Pelagic	0.33	53.73	5.63	5.93	1.32	0.090	
Kerguelen Island (contains marine autochthonous organic matter)					1.4	0.11	Vandenbroucke et. al. (1985)
Mahakam Delta, Indonesia (contains terrestrial organic matter)					1.5	0.09	
Walvis Bay, Namibia		51.9	6.7	5.1			Hatcher et al. (1985)
Mangrove Lake, Bermuda		45.3	6.2	4.3			
Ross Sea (Antarctica), Continental Margin; surface samples fr. 6 sites		51.97-69.75	6.05-9.00	5.98-8.23	1.03-1.51	0.070-0.100	Braguglia, et. al. (1995)
Ligurian Sea (4 sites)		48.18-49.37	4.63-6.05	3.49-5.77	1.24-1.47	0.07-0.100	Petronio et. al. (1994)
Sea of Japan (5-15 cm)		54.39	6.04	4.39	1.32	0.059	Ishiwari (1972)
Humic extract from highly organic marine sediment						0.185	Anderson (1989)

The lower H/C and N/C atomic ratios observed in Manila Bay HA compared to Bolinao Bay HA may be due to differences in the precursor materials and in the metabolic environments in the surface sediments of the two bays.

### IR Spectra of the Humic Acids

The bands in the IR spectra of the humic acids (Fig. 3) may be assigned as follows (Stevenson 1982): 3400-3300  $\text{cm}^{-1}$  (O-H stretching); 2900-2800  $\text{cm}^{-1}$  (aliphatic C-H stretch); 1700-1730  $\text{cm}^{-1}$  (C-O stretch of COOH and ketones); 1630-1640  $\text{cm}^{-1}$  (C-O stretch of amide [amide band I], quinone, and H-bonded ketones); 1510-1530  $\text{cm}^{-1}$  (N-H deformation,

C-N stretch, amide band II); 1440-1470  $\text{cm}^{-1}$  (aliphatic C-H); 1380  $\text{cm}^{-1}$  (C-O stretch of phenolic groups); 1220-1230  $\text{cm}^{-1}$  (C-O stretching and OH deformation of COOH); and 1020-1030  $\text{cm}^{-1}$  (C-O stretching of polyssacharides, Si-O impurities).

The elemental composition of the HA's appears to be supported by the IR spectra. The dominant bands in the humic acids are the amide I and II bands which are consistent with the high N/C atomic ratios obtained. The spectrum of Bolinao Bay HA, which have higher H/C atomic ratios, have sharper and better resolved bands at 2900-2800  $\text{cm}^{-1}$  (aliphatic C-H Stretch) and 1440-1479  $\text{cm}^{-1}$  (C-H deformation of methylene and methyl groups).

Table 5. Summary of  $\text{KMnO}_4$  and  $\text{CuO}$  alkaline degradation products from Manila Bay and Bolinao Bay humic acids.

Degradation Product	Humic Acid		Origin
	Manila Bay	Bolinao Bay	
<b>Monocarboxillic Saturated Acids</b>			
nC <sub>11</sub>	*	*	*
nC <sub>12</sub>	*	*	M
brC <sub>12</sub>	2 isomers	1 isomer	M
nC <sub>13</sub>	*	*	M
brC <sub>13</sub>	2 isomers	1 isomer	*
brC <sub>14</sub>	1 isomer	1 isomer	*
nC <sub>14</sub>	*	*	P(M)
nC <sub>15</sub>	*	*	M
brC <sub>15</sub>	2 isomers	2 isomers	P
nC <sub>16</sub>	*	*	P(M)
brC <sub>16</sub>	1 isomer	1 isomer	M
nC <sub>17</sub>	*	*	M
brC <sub>17</sub>	2 isomers	2 isomers	B
nC <sub>18</sub>	*	*	P(M)
brC <sub>18</sub>	2 isomers	1 isomer	*
nC <sub>19</sub>	*	*	M
nC <sub>20</sub>	*	*	S
nC <sub>22</sub>	*	*	S
nC <sub>23</sub>	*	*	S
nC <sub>24</sub>	*	*	S
nC <sub>25</sub>	*	Nd	S
nC <sub>26</sub>	*	Nd	S
<b>Monocarboxillic mono-unsaturated acids</b>			
NC <sub>16:1</sub>	*	*	P(M)
NC <sub>18:1</sub>	*	*	P(M)
<b>ω-Dicarboxylic Aliphatic Acids</b>			
nC <sub>4</sub>	*	*	*
nC <sub>5</sub>	*	*	M
nC <sub>6</sub>	*	*	M
nC <sub>7</sub>	*	*	M
nC <sub>9</sub>	*	*	M
nC <sub>10</sub>	*	*	M
nC <sub>11</sub>	*	*	*
nC <sub>12</sub>	*	*	M
nC <sub>13</sub>	*	*	M
nC <sub>15</sub>	*	*	M
nC <sub>16</sub>	*	*	S
nC <sub>17</sub>	*	*	S
nC <sub>19</sub>	*	*	S

### Alkaline $\text{CuO}$ and $\text{KMnO}_4$ Oxidative Degradation of the Humic Acids

The humic acids were degraded under alkaline conditions using a strong ( $\text{KMnO}_4$ ) and mild ( $\text{CuO}$ ) oxidant. Oxidation with alkaline  $\text{KMnO}_4$  yields aliphatic and benzene carboxylic acids resulting from the oxidation of unsaturated and hydroxyl containing aliphatic structures, quinones, condensed aromatics, amino acids, proteins, and carbohydrates (Machihara and Ishiwatari 1983). Mild oxidation with  $\text{CuO}$  preserves aromatic structural moieties. This method yields the lignin-derived aromatic constituents in addition to the compounds obtained from alkaline permanganate degradation.

The list of degradation products together with their probable origin have been reported by Llaguno et al. (1993b). A summary of the classes of compounds detected is presented in Table 5.

Both humic acid samples appear to be derived from microbial and terrestrial sources. The terrestrial precursor materials are indicated by the presence of several suberin and lignin derived components. The degradation products from Manila Bay sediment HA, however, are more diverse in terms of their monocarboxylic acid and lignin constituents.

### CONCLUSION

The elemental composition, the IR spectra, and the profile of the degradation products of Manila Bay and Bolinao Bay sediment HA's indicate distinct differences in their composition. Manila Bay HA has a more diverse molecular composition and lower H/C & N/C atomic ratios than Bolinao Bay HA. Sediment HA's of these bays are highly enriched in nitrogen and their levels may be indicative of values for tropical marine sediment HA's.

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