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Effect of Humic Acids on Mercury Toxicity to Marine Algae

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Humic acids extracted from the sediment of Cochin inshore areas exhibited impairment of mercury toxicity to marine algae maintained in Walne's medium under laboratory conditions. Cultures of marine micro alga *Isochrysis galbana* contaminated with sublethal concentration of 10 ppb of inorganic mercury and 10 ppm humic acids registered 18%, 21% and 37% increase in oxygen production for two, four and six hours incubation in light respectively, when compared to the controls. Similarly the daily growth rate of seaweed *Ulva lactuca* registered 26% increase over the control when the seaweed was maintained in medium containing 30 ppb Hg and 30 ppm humic acids. The results are discussed in the light of employing humic acids as growth promoter of marine algae as well as abetting agent of mercury toxicity.

Key words : Humic acids, mercury toxicity, abetting agent, seaweeds

Humified organic matter, existing in coastal waters either as particulate or as dissolved form is collectively known as humic substances. The oceans and seas contain 2.6 x 10^{12} tonnes of organic matter which approximately equal to the world's resources of coal or peat (Skopintsev, 1950) and the predominant form in which organic matter is encountered in natural water is as dissolved matter or water humus. They include humic acid, fulvic acid, humin and hematomelonic acid. They impart yellowish brown colour to coastal water, hence the name yellow substances. Humic acids are considered natural chelators (Saphiro, 1964) and the inhibitor of toxic effects of certain pollutants (Barbera *et. al.*, 1997; Ying *et. al.*, 1996).

Humic acids assume key role in coastal ecosystem as they induce phytoplankton production, nutrient transport and sediment formation (Perumal, 1985). Spatial and temporal variation in humic acid levels have been reported from the mangrove forests of Pichavaram (Perumal, 1985), inshore areas of Cochin (Kaladharan *et. al.*, 1999a) and from the Arabian sea reporting the existence of a north-south gradient of organic carbon and humic acids (Sardessai *et. al.*, 1999). In the present study attempts have been made to estimate the spatial and temporal variation of total humic acids (THA, particulate + dissolved) from the estuarine as well as the inshore areas of Cochin, the levels of certain heavy metals adsorbed to THA of the sediment and the effect of THA on

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mercury toxicity to micro alga *Isochrysis galbana* and to a seaweed *Ulva lactuca*.

Materials and Methods

Sediment samples were collected from inshore areas of Cochin on board R.V. *Cadalmin* –IX from four stations, two in the estuarine and another two in inshore regions. The station particulars are given in Table 1.

Table 1. Details of the sampling sites

Station No.	Position	Depth (m)
1	9° 572 303 N, 76° 172 003 E	6
2	9° 582 133 N, 76° 142 503 E	8
3	9° 582 033 N, 76° 102 213 E	10
4	9° 582 443 N, 76° 062 433 E	20

Total humic acids were analyzed by the method described by Martin & Pierce (1971) and as modified by Hair & Basset (1973). Triplicates of sediment samples and the THA crystals extracted from sediment were digested at 120° c in a sand bath with conc. HNO_3 and H_2O_2 (1: 4, v/v) for 12 h until dissolution (Dalziel & Baker, 1983). All the digests were oxidized with potassium permanganate and potassium persulphate followed by hydroxylamine hydrochloride. Mercury was determined by cold vapour atomic absorption spectrophotometry (Hatch & Ott, 1968) using mercury analyzer (MA 5800 A, ECIL) with a detection limit of 0.02 ìg . Prior to analysis, the instrument was tested for non-specific absorption.

Remaining half of the THA digests were analysed for Cd, Pb, Cu and Zn using a AAS (Perkin Elmer, model 2380) in an air- acetylene flame with an accuracy within 10 % and the percent recovery of 94 %. Cultures of *Isochrysis galbana* and *Ulva lactuca* being maintained at the seaweed biotechnology lab of CMFRI (Kaladharan *et al.*, 1996) were utilized for the present study. LC_{50} values (96 h, Probit analysis) of *Ulva lactuca* and *Isochrysis galbana* for Hg was found to be 35 ppb and 25 ppb respectively. Sublethal levels(5, 10, 15 & 20 ppb for *Isochrysis* and 5, 10, 20 & 30 PPb for *Ulva*) of inorganic mercury ($HgCl_2$) were added to the cultures and incubated in 12 h light regime.

The rate of photosynthetic oxygen released by the mercury treated *Isochrysis* cultures with or without THA was studied for a period of six hours and measured by the Winkler's procedure every two hour. Daily growth rate (DGR) of *Ulva lactuca* was calculated from the weekly gain/loss of wet weight from the initial weight of samples maintained in Walne's medium (Walne, 1974) using the formula

$$DGR = \frac{W^1 - W^0}{T}$$

where,

W^1- Weight (wet) gained or lost after culture

W^0- Initial weight (wet) of samples prior to culture

T- Duration of culture in days

Results and Discussion

Total humic acids in the sediments of estuarine and inshore areas of Cochin showed maximum of 2.313 ppm during the postmonsoon in the estuarine (Stn I & II) and a maximum of 1.396 ppm during the SW monsoon months (Fig. 1) in the inshore area (Stn III & IV). The observed levels agree well with the earlier report

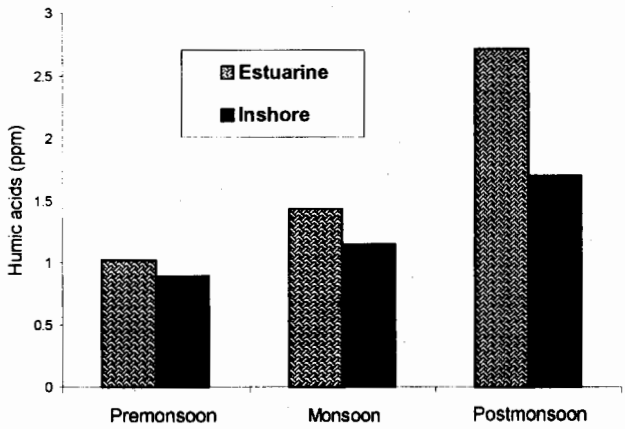
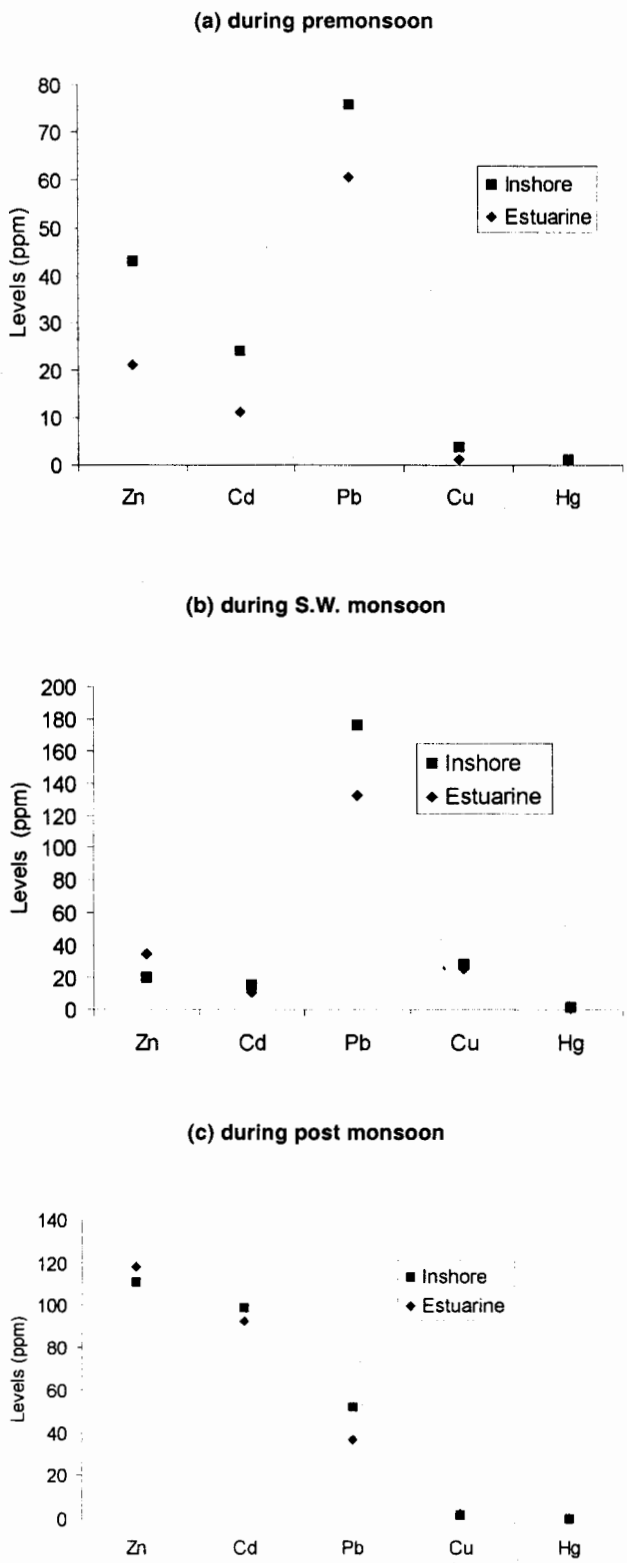


Fig. 1 Humic acids in Cochin sediments

Fig. 2 Metals extracted from the THA of sediments



from Pichavaram mangrove swamps (Perumal, 1985) and from Inshore areas of Cochin (Kaladharan *et al.*, 1999 a). Humic acids occur widely in various natural accumulations of organic matter including soils, peats, brown coals, sedimentary rocks and the range of recent subaqual deposits and the relative content of humic acids in sea sediment range up to 26 % (Bordovsky, 1964).

Temporal variation of metals adsorbed to the THA of Cochin sediments are depicted in Fig.2. Levels of Pb and Zn were much higher than other metals studied. Cd and Cu were at lower levels both in the sediments from estuary as well as from the inshore regions and Hg remained the least. THA of marine sediment was known to contain high levels of Pb and Zn as in the sediment samples. However, levels of Hg were higher in THA extracted from the sediments than in the sediment itself both from the estuary as well as from inshore areas (Fig. 3). Monsoon and Post monsoon seasons registered higher levels of metals in the sediments than in sediment collected during the pre monsoon months.

It is established that humic acids reduce the toxicity of tin (Sn II & Sn IV) towards a cyanobacterium, *Synecocystis aquatilis* in cultures (Barbara *et al.*, 1997). It is demonstrated that both the bioavailability and acute toxicity of Meothrin are decreased significantly by the presence of humic acids at the concentration of 5 and 10 ppm

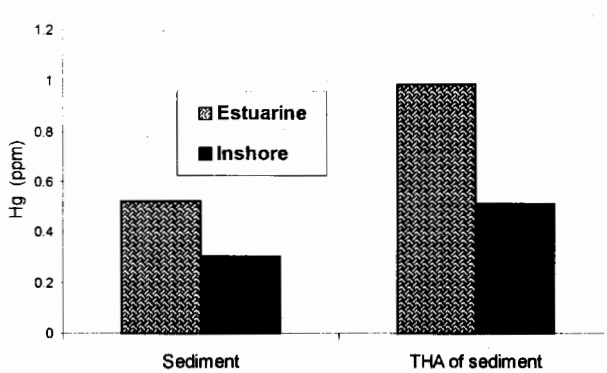


Fig. 3 Levels of Hg in sediment and in THA extracted from the sediment

Table 2. Effect of THA on Mercury toxicity to *Isochrysis galbana*

Treatment	O_2 production (ml/l)					
Hg + THA (ppb) (ppm)	2hr	% increase	4hr	% increase	6hr	%increase
0+0	2.52 ±0.076		2.76±0.023		2.86±0.051	
5+0	2.50±0.029		2.70±0.022		2.73±0.06	
5+5	2.60± 0.018	104	2.91±0.057	108	3.12±0.115	114
10+0	2.30 ± 0.016		2.43±0.052		2.48±0.047	
10+10	2.71±0.052	118	2.95±0.05	121	3.4±0.058	137
15+0	2.25±0.012		2.30±0.045		2.30±0.038	
15+15	2.45±0.067	109	2.53±0.035	110	2.80±0.047	122
20+0	1.57±0.014		2.28±0.032		2.31±0.039	
20+20	1.64±0.038	104	2.48±0.031	109	2.64±0.032	114

(Ying *et al.*, 1996). The rate of oxygen production during photosynthesis by *Isochrysis* in the presence of sublethal levels of mercury at different time intervals influenced by the presence of THA are shown in Table 2. We observed that the mercury toxicity to *Isochrysis galbana* could be impaired effectively (18 %, 21% and 37 % for 2 hr, 4hr and 6 hrs respectively) by the THA up to 10 ppb of mercury; and beyond 10 ppb levels, the inhibitory effect of THA decreased considerably. Addition of THA beyond 20 ppm did not favour the oxygen production in *Isochrysis* in culture (Table 2). However, the mercury toxicity to the seaweed *Ulva lactuca* (Table 3) was inhibited by THA up to 30 ppb (27 % higher than the control). Humic substances are known to form complex substances with metals(Barbera *et al.*, 1997). This complex formation or binding with Hg may be one of the reasons for the inhibitory action of THA on mercury. Greater effect of metal toxicity reduction (135 %) is reported (Rosko and Rachlin, 1975) due to chelation of citric acid and aminoacids on marine diatom *Nitzchia closterium*.

Table 3. Effect of THA on Mercury toxicity to *Ulva lactuca*

Treatment Hg + THA (ppb) (ppm)	Growth rate (mg/day)	% increase
0+0	69.17±0.97	
5+0	62.98±0.968	100
5+5	60.514±0.63	97
10+0	51.02 ±0.37	
10+10	60.04±1.20	117
20+0	43.19±1.81	
20+20	54.47 ±0.56	125
30+0	30.33 ±0.65	
30+30	38.23± 0.96	127

Mercury pollution in India is going to be alarming if unchecked that India's annual mercury import between 1998- 2001 was 170 – 190 tonnes equivalent to 10% of total global consumption. Mercury consumption by the Indian chlor-alkali companies is at least 50 times higher than the average European consumption (Anon, 2002). The total mercury pollution load in India is said to be about 125 tonnes of elemental mercury. Of this 40 % per annum is only due to operational loss of mercury to the environment by the existing mercury cell plants as a result of outdated technology they use. Certain hot spots of mercury contamination in the Arabian sea is reported by Sanzgiry *et. al.*, (1988) and Kaladharan *et. al.*, (1999 b). Mercury pollution would assume lethal proportions if efforts are not made soon to prevent its use. The increasing levels of mercury pollution in the coastal areas can be contained by certain bacteria (Magos *et. al.*, 1964), by phytoplankton (Alavandi *et. al.*, 1990) and also by THA as revealed by our present study.

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