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A COMPARATIVE ANALYTICAL STUDY OF THE CADMIUM AND HUMIC ACIDS CONTENTS OF TWO LENTIC WATER BODIES IN TAMIL NADU, INDIA

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ABSTRACT

Humic acids, which are derived mainly from decaying organic matter, are considered as complex macromolecules having various functional groups and are very effective in the binding and removal of different cations including heavy metals. Two lentic water bodies selected for the comparative analysis of humic acids and cadmium content in this study are the Devarajan lake (polluted lake) and Srinivasapuram lake (reference lake) located in the Denkanikottai taluk of Krishnagiri district, Tamil Nadu, India. Water and soil samples were collected on every alternate month for one year from Feb. 2008 to Feb. 2009 from five fixed sites in both the lakes. While water samples were collected in polyethylene bottles, sediment samples were collected using a core sampler. In addition to the determination of cadmium in the water and sediment samples using atomic absorption spectrophotometer, the humic acids contents in the sediment samples were also quantified on a bimonthly interval. The dissolved oxygen (DO) content and biochemical oxygen demand (BOD) of the two water bodies were also monitored. BOD and DO showed considerable variations between the two lakes indicating the pollution load of the Devarajan lake. Both the lakes exhibited significant differences between their humic acids contents as well as cadmium contents. In both the lakes, the amount of cadmium in the sediments was positively correlated to the respective humic acids contents indicating the possible interaction and complex formation between humic acids and cadmium. Such interactions may be responsible for the reduction of the bioavailability of toxicants including cadmium especially in the polluted lake leading to the survival of the aquatic fauna in it.

Key words: Humic acids, Heavy metal, Cadmium, Lentic water bodies

INTRODUCTION

Aquatic ecosystems are among the most precious, dynamic and diverse ecosystems of the world. The biodiversity of the aquatic ecosystems are so diverse and these species have evolved and adapted to the aquatic habitats over millions of years. However the indiscriminate mixing of effluents, sewage and other contaminants with the water bodies not only impair the physicochemical composition of the receiving water but also bring in adverse impacts on the inhabitant organisms with far reaching consequences. The issue becomes more compounding as many of the aquatic organisms such as crustaceans, molluscs and fish are preferred food sources for human beings. Recently Jayakumar *et al.* (2009) have reported the ecotoxicological status of the water body of Devarajan lake, Tamil Nadu, India after assessing the physicochemical indices. According to them the lake is chronically contaminated with pollutants of various types including heavy metals and organic contaminants.

The natural organic matter, which is derived from the degradation of organic substances and is present in the natural water bodies, may be broadly divided into humic and non-humic substances (Zazouli *et al.,* 2007). According to Mahvi *et al.*

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(2009) humic substances are the predominant type of natural organic matter present in ground and surface waters. Humic substances are generally classified into humic acids, (molecular weight 50,000 to 100,000 Daltons), fulvic acids and humin (Uygrener and Bekbolet, 2005). Even though the increased quantity of humic substances in the sediments is an indication of the organic pollution load of the water body; it could also ameliorate the deleterious effects of many toxicants including heavy metals. According to Öztürk *et al.* (2009), sediments are important sinks for pollutants like pesticides and heavy metals. Further, humic acids with hydroxyl, phenoxyl and carboxyl reactive groups form coordination compounds with metals (Pandey *et al.,* 2000) and have excellent complexing and metal chelating abilities. The capacity of humic substances to form complexes with heavy metals is significant in view of the observation (Jayakumar, 2009) that even though heavy metals and organic contaminants pose environmental concerns for the Devarajan lake, various life forms including fishes, molluscan forms and other aquatic fauna still thrives in this lake ecosystem.

In this context, in order to assess the possible influence of humic acids on ameliorating cadmium toxicity, efforts have been made in the present study to estimate the amount of humic acids present in the bottom sediments of Devarajan lake (hereafter referred to as polluted lake) in comparison to that of the relatively less polluted Srinivasapuram lake (Jayakumar, 2009) (hereafter referred to as reference lake) located in the same taluk. Efforts have also been made to determine the quantity of the common heavy metal pollutant cadmium present in both the lakes and to associate the presence of humic acids in the polluted lake bed to the survival of aquatic organisms in it through the complex formations between the humic acids and the pollutants including cadmium. Both the lakes are located in the Denkanikottai taluk of Krishnagiri district, Tamil Nadu, India.

MATERIALS AND METHODS

Sample collection

Water and sediment samples from both the lakes were collected from five fixed locations on every alternate month for one year from February 2008 to February 2009. Since the two lakes are 8 km apart, samples collection from the reference lake was carried out on 15th and that from the polluted lake was done on 17th of every sampling month. All sample collections were made between 8.30 a.m. to 10.00 a.m. While the water samples for the determination of dissolved oxygen (DO) and biochemical oxygen demand (BOD) were collected directly as specified by Murugesan and Rajkumari (2005), sediment samples and water samples for the quantification of humic acids and/or cadmium content were collected as detailed in the subsequent paragraphs. All the glass wares used were acid washed and profusely rinsed with de-ionized water.

BOD and DO

BOD and DO were determined by following the standard methods prescribed by APHA *et al.* (2005) and Murugesan and Rajakumari (2005). At each sampling interval, the averages of all the five samples were taken into account separately for the reference and polluted lake.

Sampling of sediments

For the quantification of humic acids and cadmium, bottom sediments were collected from both the lakes using a core sampler of 5 cm diameter up to a depth of 3 cm. Sampling was done from the five fixed sites in the reference and polluted lake in each sampling interval. All the five sediment samples collected from each of the lakes in each sampling interval were separately pooled together and mixed well (Ovaiasogie and Aghimien, 2003) to make it homogeneous. Five sub samples of 5 g each were taken from the bulked samples for the extraction and quantification of humic acids. Similarly, five sub samples of 1 g each were taken from the bulked samples of the two lakes separately and were freeze-dried for the determination of cadmium.

Sampling of water for cadmium analysis

Samples were collected in 2 L polyethylene bottles washed with nitric acid and rinsed thoroughly with triple distilled de-ionized water. For sampling, the pre-conditioned sampling bottle was immersed into the water about 10 cm below the water surface and about 1.5 L sample was collected. The samples were immediately acidified with HNO_3 and brought to the laboratory on ice.

Extraction and quantification of humic acids

Humic acids content from the sediment samples were extracted and quantified basically by following Gu *et al.* (2001) and Oviasogie and Unuigbe (2006). The sediment sample was mixed with 0.5 N NaOH (1:5), incubated at room temperature in a shaker for 24 hours and centrifuged at 5000 rpm for 30 minutes. The supernatant was decanted, filtered and pH was brought to 2 using 6 N HCl to precipitate humic acids. The brownish deposit of humic acids was collected by centrifugation. SiO_2 was removed from the deposit using a mixture of 0.1 N HCl and 3N HF with intermittent changing of the solution and the precipitate was dialyzed against distilled water to pH=5, dried in an oven at 37°C and the weight was calculated in mg/g soil.

Determination of cadmium in the sediment and water samples

Determination of cadmium in the sediment samples was done basically by following Abrahim *et al.* (2007). Each of the 1 g freeze-dried sub samples was digested separately by boiling gently in 20 mL of nitric acid for 45 minutes in a 100 mL pyrex beaker. The digest was cooled to atmospheric temperature and filtered through an acid washed Whatman No. 540 filter paper and made up to 50 mL using triple distilled de-ionized water and proceeded for atomic absorption spectrophotometric determination of cadmium using a Perkin Elmer A Analyst atomic absorption spectrophotometer.

The cadmium content of the water samples was determined basically by following Das and Jana (2004). Each of the water samples was filtered through acid washed membrane filter and 1 L of the filtrate was concentrated to 10 mL by slow evaporation and 20 mL of HNO_3, H_2SO_4 (1:3) mixture was added. The mixture was evaporated to near dryness. The residue was extracted and made up to 50 mL using triple distilled de-ionized water and preceded for atomic absorption spectrophotometric determination of cadmium using a Perkin Elmer A Analyst atomic absorption spectrophotometer.

Table 1: Variations in the DO and BOD ($\bar{X} \pm$ SD) of the reference and polluted lakes during the study period

Month and year	Reference lake		Polluted lake	
	DO	BOD	DO	BOD
February 2008	6.39 ± 0.02	0.53 ± 0.13	5.03 ± 0.12	8.21 ± 0.03
April 2008	6.40 ± 0.04	1.61 ± 0.42	4.59 ± 0.06	10.46 ± 0.03
June 2008	6.50 ± 0.02	3.72 ± 1.23	3.96 ± 0.13	15.01 ± 0.08
August 2008	6.51 ± 0.05	2.67 ± 1.03	4.21 ± 0.09	14.31 ± 0.04
October 2008	6.58 ± 0.10	0.89 ± 0.05	4.96 ± 0.03	9.32 ± 0.05
December 2008	6.60 ± 0.05	0.34 ± 0.04	5.56 ± 0.06	6.82 ± 0.02
February 2009	6.40 ± 0.02	0.48 ± 0.16	5.12 ± 0.04	10.23 ± 0.02

Note: BOD = Biochemical oxygen demand, DO = dissolved oxygen (all measurements in mg/L)

Statistical analysis

The data generated from the various experiments were subjected to standard statistical processing including student's 't' test and Pearson correlation.

RESULTS

BOD and DO

While the polluted lake showed marked decreases DO; its BOD, showed marked increases in comparison to the reference lake (Table 1).

Humic acids contents

The humic acids contents (Table 2) also exhibited significant variations between the two lakes ($p<0.01$). While the humic acids contents of the reference lake varied from 0.030 ± 0.001 to 0.052 ± 0.002 mg/g sediment, that of the polluted lake varied from 0.080 ± 0.001 to 0.108 ± 0.001 mg/g sediment. In both the lakes, the humic acids contents showed an increasing trend during the monsoon and post-monsoon months (October

Table 2: Student's 't' test showing significant variations in the humic acids and cadmium contents of the bottom sediments of the reference and polluted lakes at various sampling intervals

Lake	**February 2008**	**April 2008**	**June 2008**	**August 2008**	**October 2008**	**December 2009**	**February 2009**
			Humic acids content (mg/g)				
Reference ($\bar{X}$ ± SD)	0.049 ± 0.002	0.043 ± 0.002	0.032 ± 0.002	0.030 ± 0.001	0.037 ± 0.001	0.046 ± 0.001	0.052 ± 0.002
Polluted ($\bar{X}$ ± SD)	0.107 ± 0.001 a*	0.086 ± 0.001 a*	0.082 ± 0.001 a*	0.080 ± 0.001 a*	0.083 ± 0.001 a*	0.104 ± 0.002 a*	0.108 ± 0.001 a*
			Cadmium content (ppm)				
Reference ($\bar{X}$ ± SD)	0.006 ± 0.001	0.006 ± 0.001	0.004 ± 0.001	0.003 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
Polluted ($\bar{X}$ ± SD)	2.916 ± 0.075 b*	2.798 ± 0.122 b*	1.491 ± 0.043 b*	1.279 ± 0.031 b*	2.311 ± 0.091 b*	3.071 ± 0.144 b*	3.016 ± 0.141 b*

Note: a = between humic acids contents of reference and polluted lakes; b = between cadmium contents of reference and polluted lakes; * = $p<0.01$; (comparison is made between the of reference and polluted lakes of the respective sampling month)

to February) and were gradually reduced during the summer and pre-monsoon period (March to September). However at each month the rate of increase was significantly higher in the case of polluted lake (Table 2).

Table 3: Correlation matrix showing the relationships between the means of parameters of reference and polluted lakes

Parameters	**Annual mean ± SD**	**Reference lake humic acids content**	**Polluted lake humic acids content**	**Reference lake sediment cadmium**	**Polluted lake sediment cadmium**
Reference lake humic acids content	0.041 ± 0.008	1			
Polluted lake humic acids content	0.093 ± 0.012	0.915**	1		
Reference lake sediment cadmium	0.005 ± 0.001	0.762**	0.642**	1	
Polluted lake sediment cadmium	2.411 ± 0.707	0.0931**	0.802**	0.756**	1

Note: ** = correlation is significant at the 0.01 level (2-tailed)

Cadmium content

While the water and sediments compartments of the polluted lake showed the presence of the heavy metal cadmium, it was not detected in the water body of the reference lake (Table 2; Fig. 1). However its presence was detected in trace amounts in the bottom sediments of the reference lake. In general, the increasing quantities of humic acids in both the lakes were accompanied by a corresponding increase in the cadmium contents of the bottom sediments (Table 3) indicating a significant positive correlation.

DISCUSSION

The marked variations observed between the chemical indices of the two lakes may be attributed to their contamination status. Devarajan lake being a polluted one (Jayakumar *et al.,* 2009), it is quite natural to have decreased DO.

Similarly the increased BOD in the polluted lake indicate the presence of higher concentration of organic matter because according to Singh *et al.* (1999), BOD is an indicator of the quantity of biodegradable organic matter present in the water. Murugesan and Rajakumari (2005) are of the opinion that in turbid waters, the increased organic matter induces proliferation of microbes. As the polluted lake remains turbid, the proliferating microbes may be utilizing greater amounts of oxygen to oxidize the organic matter and this could be one of the probable reasons for increased BOD in the polluted lake.

In contrast to the observations made by Jayakumar (2009), the present study has revealed the presence of traces of cadmium in the reference lake also. This observation is significant because according to Censi *et al.* (2006), among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic ecosystems. However

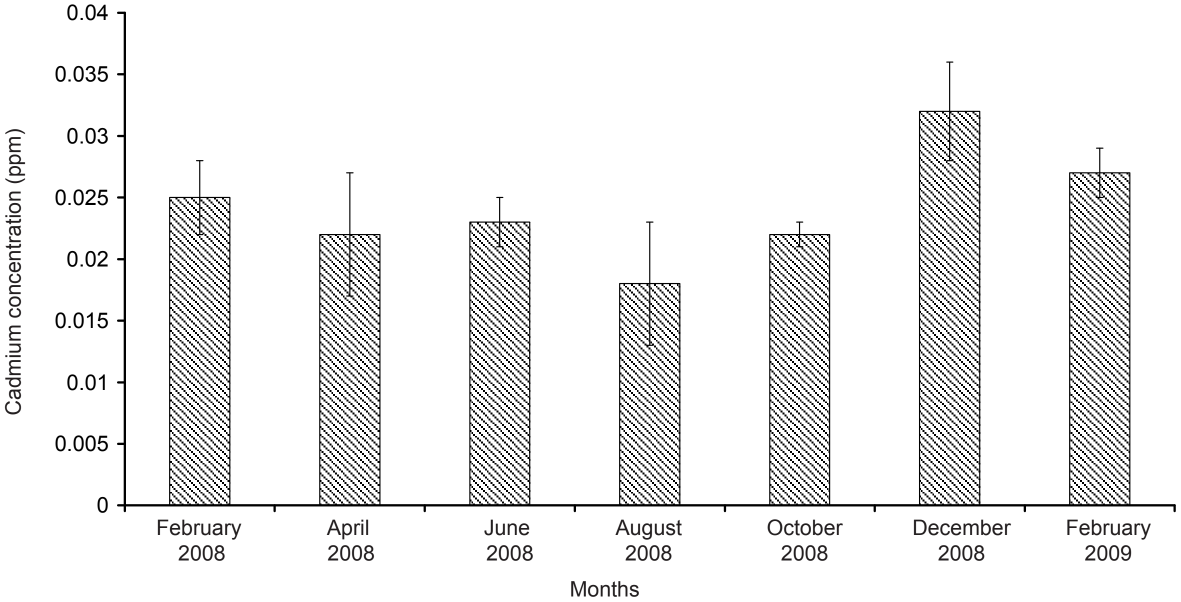

Fig. 1: Distribution of the heavy metal cadmium (in ppm) in the water body of the polluted lake at various sampling intervals

it is worth mentioning that no detectable amount of cadmium is present in its water body and whatever quantity is available is adsorbed into the bottom sediments. The increased quantity of cadmium present in the bottom sediments of the polluted lake in comparison to its water column suggests the increased probability of cadmium getting adsorbed into the bottom sediments. In this context, the role of humic acids in the formation of complexes with the xenobiotics including heavy metals needs special mention as the bottom sediments of the polluted lake contain more quantities of humic acids in comparison to the reference lake. Since humic acids behave as weak acid polyelectrolytes containing a variety of oxygen containing functional groups such as carboxylic, phenolic, carbonyl and hydroxyl (Naddeo *et al.,* 2007); sediments with the humic substances could trap pollutants of various types. Further increase in the quantities of humic acids in both the lakes during the monsoon and post-monsoon seasons could be primarily attributed to the fresh loads of organic contaminants brought in by the monsoon floodwater because according to Zazouli *et al.* (2008), humic substance are derived both from natural degradation of organic substances within the ecosystems and from human activities. Similarly the source of the significant surge in the cadmium content of the polluted lake during the monsoon seasons may also be traced back to the rain runoff from the industrial town of Denkanikottai, which ultimately finds its way to the polluted lake. On the other hand the reference lake is located in the forest area having no direct connection with any industrial area.

Since the disintegration and decay of various organic materials yield humic substances, the presence of comparatively higher amounts of humic acids in the bottom sediments of the polluted lake may be attributed to the high organic load of the lake. This assessment is in line with the fact that the reference lake contained only lesser quantities of decaying organic matter and humic acids, indicating the reduced quantity of decaying organic matter in it. Further, the increased quantity of humic acids in the polluted lake might be modulating the toxicity of the various toxicants because humic acids are able to form complexes with a variety of contaminants as they possess both aromatic and aliphatic characteristics with the dominant functional groups being phenolic and carboxylic groups (Pandey *et al.,* 2000). The observed positive correlation between the humic acids content and cadmium concentration in both the lakes also underscores the possibility of complex formations between them. Further, Schaumann (2006) is of the view that humic substances make up a large portion of the dark matter in the humus and consist of heterogeneous mixtures of biomolecules that exhibit both macromolecular and supramolecular characteristics. Due to all these molecular characteristics, humic acids could bind and chelate toxicants such as cadmium and thereby rendering them unavailable for bioaccumulation or at least limit their bioavailability to various fauna, leading to their survival in the contaminated aquatic ecosystems as exemplified by the polluted lake of the present study. This opinion is again substantiated by the reports that depending on the structure of the molecules, humic acids bind and chelate variety of contaminants such as chlorinated disinfectants, heavy metals, pesticides and a number of other positively charged multivalent ions (Wu *et al.,* 1999; Christl *et al.,* 2001; Gustavino *et al.,* 2005).

According to Sanjay *et al.* (2000), an aggregate of the various functional groups and molecules in humic acids constitute elongated bundles of fibres at very low pH. As pH increases they become open flexible structures perforated by voids and these voids can trap and adsorb both organic and inorganic particles if the charges are complementary. Therefore, as humic acids are highly reactive towards positively charged ions (*e.g.,* heavy metal ions), they may be binding divalent cations in the same way as that of EDTA. Hence the increased humic acids contents of the polluted lake might be responsible for ameliorating the toxicity of contaminants in the polluted lake of the present study and thereby facilitating the survival of aquatic fauna in it irrespective of the fact that it is chronically contaminated by various xenobiotics including heavy metals.

It may be concluded that binding of organic and inorganic pollutants including heavy metals to humic acids is of great importance to the lake ecosystems studied. Such bindings can have impacts on the free metal ion concentration, metal ion mobility in the ecosystem and metal bioavailability to various fauna. Therefore the increased presence of humic substances in the polluted lake of the present study may be responsible for retaining many contaminants including heavy metal such as cadmium in the bottom sediments by adsorption, complex formation and/or chelation. This could be one of the major reasons for the survival of the various aquatic fauna in the polluted Devarajan lake.

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