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# Interactions of mercury with different molecular weight fractions of humic substances in aquatic systems

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### ABSTRACT

Interactions of mercury (Hg) with different molecular weight fractions of humic substances (HS) play an important role in controlling distribution, diffusion, speciation and bioavailability of Hg in natural system. This study suggests that Hg prefers to associate with higher molecular weight fractions of HS and this kind of association particularly predominate at low pH and high ionic strengths of the medium. The concentrations of aggregated HS (with higher molecular weight) become high at lower pH (acidic condition) and high ionic strength and the molecular weight of HS gradually decreases with the increasing pH (basic condition) and low ionic strength of the medium. The disaggregation property of HS which involves the release of monomers from the surface of the aggregates produces HSs of different intermediate molecular weight fractions of HS is dependent on aggregational and disaggregational properties of HS in aquatic medium. Association of Hg with high molecular weight fraction of HS may alter distribution and bioavailability of Hg in a system as the bioreactivity of organic matter decrease along a continuum of size in aquatic medium.

Keywords: Ultrafiltration technique; Molecular weight distribution of humic substances; fate of Hg-DOC complexes, Hg pollution; Hg speciation

#### 1. Introduction

The chemical forms of mercury (Hg) in water column and sediments are closely related to its overall impacts on living organisms. Hg in natural water is predominantly found in complexes with ligands including humic substances (HS), polysaccharides, and proteins. Interactions between Hg and HS play an important role in defining the chemical forms of Hg and controlling their transport and fate in water column (Cai et al. 1999; Adriano 2001; Yu et al. 2008). HS are physically and chemically heterogeneous and thus, Hg-HS complexes are polydisperse and different in thermodynamic stability. Little is known, however, about the mechanism and strength of Hg interactions with HS. Strong correlations have been reported between the total dissolved concentrations of Hg and HS (a major component of dissolved organic carbon (DOC)) in natural water (Lindberg and Harriss 1974; Driscoll et al. 1995; Ravichandran 2004). The difficulty and complexity in understanding of Hg-HS interactions is mainly due to the coupled reactions between Hg redox transformation and complexation with natural DOC(Gasper et al. 2007). These processes critically control the bioavailability and lifetime of Hg in aquatic environments. Diffusion coefficients of Hg-HS complexes are dependent on the molecular weight of the HS. Due to the unknown nature of HS and their aggregational and disaggregational property, understanding of distribution of Hg in different molecular weight fractions of HS in aquatic system is poor.

Cai et al (1999) has reported the distribution of Hg and MeHg (methylmercury) in different molecular weight fractions of DOC in natural waters. However, DOC was fractionated only in three different molecular weight fractions (30kDa, 10kDa and 1kDa) to understand the distribution of Hg. They have reported that Hg prefers to associate with bigger molecules of DOC and MeHg prefers to associate with smaller molecules of DOC. Similar experiments has also been performed by Guentzel et al (1996) to understand the distribution of particle reactive Hg in colloidal particles in estuarine systems. They have classified and separated DOC into four categories by ultrafiltration technique (i) particulate matter (>0.4 $\mu$ M) (ii) high molecular weight (>10kDA) (iii) Medium molecular weight (1-10kDa) (iv)low molecular weight (<1kDa). They have identified the association of Hg with different molecular weight fractions of DOC. Han et al (2008) has also reported the distribution of Hg in water column and particulate matter. They have also reported the influence of sulphide concentrations in partitioning of Hg in isolated DOM from Florida, Everglades.

It has been reported that HS may form relatively large aggregates by H-bonding and van der Waal's attraction. These aggregates are able to disaggregate/reaggregate, depending on the nature and concentration of HS, pH, and ionic strength of the medium (Swift 1999; Marcelo and Wilkinson 2002; Šmejkalová and Piccolo 2007). The molecular weights of HS may vary from few kDa to thousands of kDa, and may exhibit a continuum of any given chemical property (Leenheer 1981). It is extremely

difficult to separate and understand the interactions of Hg with different molecular weight fractions of HS. It was, thus, necessary to understand the interactions of Hg with different molecular weight fractions of HS under different experimental conditions. The aim of this investigation was to understand the impacts of 1) varying Hg loading; 2) changing pH and (3) ionic strengths of the medium, on Hg distribution in different molecular weight fractions of HS in model solutions and freshwater. This study is the first attempt to understand the impact of aggregational and disaggregational properties of HS on Hg distribution in eight different molecular weight fractions of HS in aquatic environments.

#### 2. Material and methods

#### 2.1 Chemicals and Reagents.

A stock solution (1000 mg. dm<sup>-3</sup>, NIST Hg(NO<sub>3</sub>)<sub>2</sub> in 10% HNO<sub>3</sub>CertiPUR) of Hg was purchased from Merck Chemicals, Germany. Standard solutions of Hg were prepared daily by dilution of the stock solutions with ultrapure water (of resistivity 18.2 M $\Omega$  cm<sup>-1</sup>) acidified to contain 1%(v/v) ultrapure HNO<sub>3</sub> (Merck, Germany). Stock solutions of humic acids (HA) were prepared by using HA from Fluka (commercialized by Sigma-Aldrich, cat. No. 53680, Sigma Aldrich, Inc, Milwaukee, WI, USA). This HA was used as the representative of HS.

The elemental composition of the HA has been reported to contain 48.36% of C, 26.91% of O, 4.24% of H, 0.78% of N and 0.78% of S (Rodrigues et al. 2009). The atomic ratios reported in the literature are 1.04 (H/C), 0.42 (O/C), and 0.012 (N/C) which are within the values reported in the literature for soil HAs, with the exception of the N /C ratio. This indicates that the HA is soil humic acid. Thus, Fluka HA may not be a good representative of aquatic humic substances. The molar ratio of protons on saturated and unsaturated carbon atoms is approximately 2.1 : 1 in the studied HA (Rodrigues et al. 2009). It is well known that soil HSs that leach out from soil usually become an integral part of dissolved organic carbon in river or coastal waters via runoff and drainage. The purpose of using Fluka HA in this study was to understand the distribution of Hg in soil HA in aqueous medium. It is also necessary to mention that Fluka HA was cheap and easily available. However, further investigation was also performed to understand the distribution of Hg in humic-like compounds in the Godavari River.

#### 2.2 Model Solutions and Freshwater Samples

All the model solutions were prepared in Teflon container to minimize Hg adsorption on the wall of the containers. A series of model solutions were prepared to understand the impact of Hg loading on Hg distributions in different molecular weight fractions of HA. The model solutions, containing HA (5.0 mg.dm<sup>-3</sup>) and varying concentrations of Hg (ranging from ~8nM to 434 nM), were prepared in ultrapure water. The concentrations of Hg used in this study were relatively higher than those of environmental

samples. However, the Hg concentrations in the coastal surface water, near the industrial cities in India have been reported to be high. A comparative account of Hg levels in the Indian coastal and estuarine water is presented in Table 1(Kureishy et al. 1986; Balchand and Nambisan 1986; Krishnakumar and Pillai 1990; Selvaraj 1999; Satpathy et al. 2008). Thus, the concentrations of Hg used in this study were not environmentally irrelevant. It was also extremely difficult to understand the distributions and affinity of Hg with different molecular weight fractions of HS at environmentally relevant concentrations of Hg due to heterogeneity and complexity of HS. Thus, the purpose of using relatively higher concentrations of Hg was also to clearly understand the distribution of Hg in different molecular weight fractions of Hg.

A series of model solutions were prepared to understand the impact of pH on Hg distribution. The pHs of the solutions were varied from pH 2.5 to 7.5. The pH was adjusted by using 2M ultrapure HNO 3 and/or 2 M purified NaOH. The concentrations of Hg and HA (5.0 mg.dm<sup>-3</sup>) were kept constant. WTW series inoLab pH/ Conductivity meter 720 (WTW, Germany) was used to check and adjust the pH of the solutions. The range of pH of the model solutions were varied from 2.5 to 7.5 to understand the aggregational/disaggregational properties and its impact on the distribution of Hg in different molecular weight fractions of HA. The ionic strength of the test solutions (containing Hg (12.3nM) and HA (5.0 mg.dm<sup>-3</sup>)) were adjusted (from 10.0 to 0.1 mM) by using ultrapure NaNO<sub>3</sub> (99.995% trace metals basis, Aldrich, US) solution. It is important to mention that the experiments were carried out with a background electrolyte in solution, (NaNO<sub>3</sub>). Indeed, in ultra pure water, loading of  $Hg^{2+}$  can increase the ionic strength and likely modify the colloidal stability of the solution. A condition was created where addition of Hg<sup>2+</sup> did not change the ionic strength of the solutions, in order to avoid combining electrostatic effects with chemical complexation processes. The lower pH values (2.5 and 3.4) were environmentally unrealistic but the study was performed to understand the changes in aggregational/disaggregational properties of HS and its impact on Hg distribution in different molecular weight fractions of HS.

The distribution of Hg in different molecular weight fractions of humic-likes compound in freshwater was also investigated. The freshwater sample was collected from Godavari River (the third largest river in India). Sampling station, Kotipalli, was located far upstream of Yanam estuary (Figure 1). The samples collected (in the month of April 2012) represent typical freshwater. The dissolved concentration of Hg in freshwater was not detectable. Different concentrations of Hg were spiked to understand the distribution of Hg with different molecular weight fractions of humic-like compound in the freshwater. A calibration curve was prepared with different concentrations of HA and the absorbance of the HA was measured at 254nm (supporting documents Figure 1SD). The same calibration curve was used to

determine the concentrations of humic-like compound in the freshwater at 254nm. In order to understand the distribution of Hg in the different molecular weight fractions of humic-like compounds in freshwater, a definite amount of Hg was added and equilibrated for 72 h in the dark. Hg associated with different molecular weight fractions of humic-like compounds were separated by ultrafiltration technique in eight different molecular weight fractions: > 300 kDa, 300-100 kDa, 100-50 kDa, 50-30kDa, 30-10kDa, 10-5kDa, 5-1 kDa, < 1kDa.

Detailed descriptions of 1) ultrafiltration technique 2) Direct mercury analyzer-DMA-80 (for the determination of total Hg associated with different molecular weight fractions of HA) and 3) UV/Visible spectroscopy (for the determination of humic-like compounds) are provided as supporting documents.

#### 2.3 QA/QC

Experimental procedure is achieved with blank analyses. The quality control for Hg analyses included repeated and periodic analysis of the certified reference sediment standards (from National Research Council Canada), MESS-3 and PACS-2 in every 2-h operation of the equipment. One reagent blank and two certified reference materials were included for every 9 samples analyzed. A prior set of samples were re-analyzed if the measured value differed from the certificate value by > 5%. The use of DMA-80 (from Milestone, Italy) enables us to analyze for Hg in virtually any sample matrix – liquids, solids, and even gases - without any sample preparation and thus, certified reference sediment standards were used to control the quality of Hg determination in water samples.

#### 3. Results and discussion:

Interactions of Hg with HS play key roles in controlling speciation of Hg in natural systems. The nature of interactions between Hg and HS depends on [Hg]/[HS] ratio in a system. At low Hg loading, Hg prefers to undergo strong complexation reactions with strong binding sites (mainly reduced sulphur sites, less than 1.0% of the total binding sites) within HS (Qian 2001; Satchell 1977). However, Hg undergoes complexation reaction with the major functional groups (-COOH, -OH etc.) after saturating all the strong sites of the HS. The distributions of Hg in different molecular weight fractions of HS were determined at varying Hg loading by using ultrafiltration technique. HA was used in this study as a representative of HS. The fractionation of HA was performed in absence of Hg (at different pH and ionic strengths) to understand the impact of Hg (if any) on the aggregation/disaggregation processes of the HA. The variation in concentration of Hg (used in this study) had no impact on the aggregation/disaggregation processes of the HA (under the experimental conditions).

#### 3.1 Distribution of Hg at different molecular weight of HA at varying Hg loading:

The distributions of Hg (as percentage of total Hg concentrations) in different molecular weight fractions of HA, at varying Hg loading are presented in Figure 2, shows that major fractions of the total Hg was associated with higher molecular weight fraction of HA (molecular weight higher than 300kDa and in between 300-100kDa) at varying Hg loading. The corresponding data are presented as supporting documents (Table 1SD).

The concentrations of Hg associated with the colloidal phase ( $0.4\mu$ m-10kDa) of HA were found to be ~90-99% of the total Hg concentrations ([Hg]<sub>T</sub> concentrations ranging from  $8.3 \times 10^{-9}$  M to  $4.3 \times 10^{-7}$  M). It has been reported in the literature that the association of Hg with colloidal phase ( $0.4\mu$ m-10kDa) in freshwaters usually predominates (Schuster 1991; Wang and Driscoll 1995; Rytuba 2003). It has been reported in literature that 72% of the total Hg concentration can be associated with colloidal phase (in natural freshwaters) even when the total Hg concentration is very low ( $1.4 \times 10^{-10}$  M)(Babiarz et al. 2001). This study also demonstrate that colloidal phase in aquatic system plays an important role in regulating the partitioning of Hg.

Figure 2 shows that the association of Hg with higher molecular weight fractions of HA (higher than 300kDa and 300-100kDa) increases with the increasing Hg loading. Hg is more uniformly distributed among the different molecular weight fractions of HA at low Hg loading.

It is well known that soft metals like Hg shows a pronounced preference for ligands of sulphur, the less electronegative halides and nitrogen over ligands containing oxygen. It has been reported that the studied HA contained 0.78% of N and 0.78% of S(Rodrigues et al. 2009). Thus, it can be assumed that these minor elements are distributed among the different molecular weight fractions of the HA. Thus, one could infer that Hg prefer to associate with different molecular weight fractions of HA to form thermodynamically stable complexes (with these minor sites) at low Hg loading. The increasing concentrations of the total Hg in the system saturate these minor sites and Hg moves towards the weaker binding sites associate with colloidal phase of HA. An amount of 35-82% of the total Hg has been reported to associate with macromolecular organic matter (>1 kDa) in the Ochlockonee estuary (Guentzel et al. 1996).

However, the behaviour of Hg-bound colloidal HA is still not well known. The major metal binding sites present in colloidal particles are mainly carboxylic or phenolic in nature. However, Guentzel et al( 1996) has reported that the presence of thiol type group in colloidal HA involves in Hg binding. The association of Hg (at low loading) with the macromolecular HA indicates that Hg interacts with surficial reduced sulphur groups to form strong complexes. However, Hg starts to occupy the surficial

weaker compexing sites of the macromolecular HA with the increasing Hg loading and probably forms weak complexes.

Variation in the structure (size) of humic substances (aggregational/disaggregational properties) has been reported by (Chakraborty 2010) using Cd as a probe at varying pH. Further study was performed to understand the effects of varying pH and ionic strengths of the medium on the distributions of Hg in different molecular weight fraction of HA.

#### 3.2 Influence of pH on the distribution of Hg

The concentrations of HA with molecular weight in between 300-30 kDa was found to be highest (18.7%) at low pH (pH =5) and the concentrations of HA of lower molecular weight gradually increased with the increasing pH from 2.5 to 7.5. This probably indicates that the increasing pH of the medium increases the charge density within the structure of the HA and the process of disaggregation of the HA predominates with the formation of HA of lower molecular weights.

Figures 3a and 3b show the distributions of Hg in different molecular weight fractions at varying pH of the medium. It shows that Hg always prefers to associate with HA of higher molecular weight at different pHs (pH 2.5 to 7.5). However, the concentrations of Hg associated with HA fraction of molecular weight >300kDa, was comparatively low and the highest concentration of Hg was found to associate with HA of molecular weight of <1kDa at pH 2.4.

The decrease in association of Hg with colloidal HA molecule and increase in concentration of Hg in dissolved phase (<1kDa) at pH 2.4, as a result of high protonation of HA which reduced its ability to bind  $Hg^{2+}$ . Similar observation was reported by Lu and Jaffe (2001). They have reported that decreasing pH from 8 to 3.5 causes increase competition between protons and  $Hg^{2+}$  for the binding sites in dissolved organic matter (DOM). They have also reported (by measuring fluorescence emission intensity of Hg-DOM) that Hg does not bind to humic substances at pH 2.5 or below. An increasing concentration of  $Hg^{2+}$  (<1kDa) was found with the decreasing pH. The concentrations of Hg associated with HA of molecular weight >300kDa was highest at pH 6.4 but decreased at pH 7.5. The concentration of Hg associates with HA of intermediate molecular weight (50-5kDa) increases with increasing pH. This variation in association of Hg with different molecular weight fractions of HA may be attributed to the physical heterogeneity and dynamic behaviour of HA at varying pH. The corresponding data are presented as supporting documents (Table 2SD).

#### 3.3 Effects of ionic strength on the distribution of Hg

Figure 4a shows the variation in concentrations of Hg associated with different molecular weight fractions of HA at different ionic strengths. It was found that Hg was distributed in different molecular

weight fractions of HA at lower ionic strength of the medium. However, the association of Hg with HA of higher molecular weight fractions predominates at higher ionic strengths of the medium.

HA prefers to aggregate into spherical particles through van der Waals forces at high ionic strength (Chakraborty 2010) and thus, one could expect that the concentrations of Hg associated with HA of bigger molecules would be high at high ionic strength (as Hg prefer to associate with bigger molecules). A similar result was obtained as shown in Figure 3a. The variation in concentrations of Hg associated with HA of molecular weight >300kDa and <1kDa are shown in Figure 4b. This study suggest that association of Hg (presented as % of the total Hg concentrations) with higher molecular weight fraction of HA gradually increases with the ionic strength of the medium. These results again indicate that Hg prefer to associate with HA of higher molecular weight plays an important role in regulating Hg speciation in aquatic system nevertheless, these data unable to address the fate of Hg-HA complexes under different conditions. The corresponding data are presented as supporting documents (Table 3SD). Figure 5 represents a schematic diagram to understand the impact of pH and ionic strength on aggregation/disaggregation processes of HS and their effects on Hg distribution.

#### 3.4 Fate of Hg-humate complexes

Hg usually forms very strong complexes (at low loading of Hg) with HA in natural waters. If, however, the formation of stable Hg-HA complexes also would lead to a subsequent reduction to  $Hg^0$  by an intra molecular electron transfer, the dissolved HA would be particularly important for the speciation and mobility of Hg in the aqueous environment, acting both as a strong complexing agents as well as reducing agents even when the biological transformation of Hg is slow. The transformation of  $Hg^{2+}$  (from Hg-HA complexes) to  $Hg^0$  in test solution (i.e. decrease in concentrations of dissolved Hg in solutions) was used to understand the fate of the Hg-HA complexes at different pH.

This study indicates (Figure 6a) that the total concentrations of Hg in solution remain same at pH 2.5. However,  $Hg^{2+}$  starts to get reduced with the increasing pH from 2.5 to 4.5 (with the decrease in total Hg concentration in solution). The reduction of  $Hg^{2+}$  ion in the solution was found to decrease with further increase in pH. This observation can be justified by the following way.

The reducing capacity of HS (HA and fulvic acid (FA)) has been demonstrated in a number of systems. It is assumed that intermediates like semiquinones which are present in HA are involved in these redox processes. The following redox reactions has been reported in the literature for HA and FA

$$HA(ox) + e^{-} + nH^{+} \Leftrightarrow HA(red) \qquad E^{0} = 0.7 V - ....[1]$$

$$FA(ox) + e^{-} + nH^{+} \Leftrightarrow FA(red) \qquad E^{0} = 0.5 V - ....[2]$$

$$Hg^{2+} + 2e^{-} \Leftrightarrow Hg^{0} \qquad E^{0} = 0.659 V - ....[3]$$

The above redox reactions suggest that  $Hg^{2+}$  ion can be reduced by humic substances except at very low pH. It was observed that the reduction rate of  $Hg^{2+}$  is negligible at pH 2.5. However, the reduction of  $Hg^{2+}$  gradually increased with the increase in pH and reached maxima at pH 4.5 Further increase in pH of the medium (pH 4.5 to 6.4) start to decrease the reduction of  $Hg^{2+}$  ion in the solution. This decrease in the reduction of  $Hg^{2+}$  ion is probably because of the formation of Hg-HA complexes at higher pH.

These results (presented as supporting documents, Table 4SD) suggest that Hg associated with colloidal HA at lower pH are stable and will not be reduced by HA. However, the association of  $Hg^{2+}$  with HA will be low at low pH.  $Hg^{2+}$  ion associated with colloidal HA at higher pH may form strong complexes with HA but the reduction still may happen, assuming an intra molecular process that is possible only when the complex has been formed.

# 3.5 Distribution of Hg in different molecular weight fractions of humic-like compounds in natural freshwater

The pH of the freshwater sample was measured immediately after collecting the sample by WTW series inolab pH/Conductivity meter, 720 (WTW, Germany) and found to be  $8.05 \pm 0.05$ . The concentrations of humics-like compounds in freshwater was found to be  $4.2 \pm 0.6$  mg dm<sup>-3</sup>. The concentrations of major anions such as F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, NO<sub>2</sub><sup>-</sup>, SO<sub>4</sub><sup>2-</sup>, PO<sub>4</sub><sup>3-</sup> and the total concentrations of Na<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> are presented as supporting document (Table 5 SD). The total concentration of Hg in the freshwater was not detectable by DMA-80. A definite concentration of Hg was spiked in freshwater, equilibrated and ultrafiltered to understand the distribution of Hg in the eight different molecular weight fractions of natural humic-like compound.

Hg speciation in aquatic environments is affected by inorganic and organic ligands present in water. The relative importance of each ligand for Hg complexation is dependent on the concentrations of Hg and the ligand and the binding strength for the Hg-ligand complexes. Among the inorganic ligands, hydroxide, chloride and sulphide are considered important in controlling the speciation of Hg in aquatic environments.

The distributions of mercury in the different molecular weight fractions of humic-like compounds present in freshwater sample at different Hg loading are shown in Figure 6b. Figure 6b shows the similar

trend as found in the synthetic solutions (presented before). A major part of the total Hg was associated with the higher molecular weight fractions of humic-like compounds. The concentrations of Hg in the colloidal phase ( $0.4\mu$ m-10kDa) comprised up to ~70-90% of the respective unfiltered total Hg concentrations. However, it is interesting to note that the concentrations of Hg associated with humic-like compounds of molecular weight less than 1kDa were higher than the Hg concentrations associated with HA of molecular weight <1kDa found in model solutions.

The effects of pH/ionic strength on Hg distribution in the humic-like compounds are shown in Figure 6 c and d. Figures 6b and c show that the distribution of Hg was highest with bigger molecules of HA at lower pH and higher ionic strengths. It has been reported by Miretzky et al(2005) that Hg undergoes maximum adsorption in the pH range 3.0-5.0(>90%). It is interesting to note that the distribution of Hg at lower molecular weight of humic-like compound was more prominent in freshwater than in model solutions. This is probably due to the presence of smaller natural complexing ligand in the freshwater.

#### 4. Conclusions

This is the first study that rigorously estimated the distributions of Hg in eight different molecular weight fractions of humic substances and showed the effects of varying pH/ionic strength of the medium on the aggregational/disaggregational properties of HS and its impact on Hg distributions. This study suggests that Hg prefers to associate with HS of higher molecular weight. It is found that aggregational property of HS predominates at low pH and high ionic strength of the medium and Hg prefers to associate with HS of higher molecular weight (under the same condition). Secondly, disaggregational property of HS predominates at high pH and low ionic strengths of the medium) and Hg starts to associate with HS of low molecular weight. Association of Hg with high molecular weight fraction of HS may alter distribution and bioavailability of Hg in a system as the bioreactivity of organic matter decrease along a continuum of size in aquatic medium. The dynamic behaviours of HS make Hg–HS interaction study more interesting and challenging. Further experiments are needed for the better understanding of Hg-DOC interactions in aquatic system.

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## References

Adriano DC (2001) Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. 2<sup>nd</sup> ed. Springer-Verlag, New York

Babiarz CL, Hurley JP, Hoffmann SR, Andren AW, Shafer MM, and Armstrong DE (2001) Partitioning of total mercury and methylmercury to the colloidal phase in freshwaters. Environ Sci Technol *35*: 4773–4782

Balchand, A. N. and Nambisan, P. N. K., (1986) Effect of pulp paper effluents on the water quality of Muvattupuzha River emptying into Cochin backwaters. *Indian Journal of Marine Sciences* 15, 253 - 259

Cai Y, Jaff R, and Jones RD (1999) Interactions between dissolved organic carbon and mercury species in surface waters of the Florida Everglades. Appl Geochem *14*: 395–407.

Chakraborty P (2010) Study of cadmium–humic interactions and determination of stability constants of cadmium–humate complexes from their diffusion coefficients obtained by scanned stripping voltammetry and dynamic light scattering techniques. Anal Chim Acta 659: 137–143.

Driscoll CT, Blette V, Yan C, Schofield CL, Munson R, and Holsapple J (1995) The role of dissolved organic carbon in the chemistry and bioavailability of mercury in remote Adirondack lakes. Water Air Soil Poll *80*: 499–508

Gasper JD, Aiken GR, and Ryan JN (2007) A critical review of three methods used for the measurement of mercury (Hg<sup>2+</sup>)-dissolved organic matter stability constants. Appl Geochem 22: 1583–1597

Guentzel JL, Powell RT, Landing WM, and Mason RP (1996) Mercury associated with colloidal material in an estuarine and an open-ocean environment. Mar Chem 55: 177–188

Han S, Obraztsova A, Pretto P, Deheyn DD., Gieskes J, Tebo BM (2008) Sulfide and iron control on mercury speciation in anoxic estuarine sediment slurries. Mar Chem. 111: 214–220

Krishnakumar P K and V K Pillai (1990) Mercury near a caustic soda plant at Karwar, India. *Mar.Pollut. Bull.*, 21:304-307

Kureishy TW, Mesquita A, and Sengupta R (1986) Mercury concentration in and around Binge Bay, Karwar, India. In: Contributions in Marine Science. Dr. S.Z. Qasim Sastyabdapurti , Felicitations Volume, 247-353

Leenheer JA (1981) Comprehensive approach to preparative isolation and fractionation of dissolved organic carbon from natural waters and wastewaters. Environ Sci Technol. *15*: 578-587

Lindberg SE, and Harriss RC (1974) Mercury-organic matter associations in estuarine sediments and interstitial water. Environ Sci Technol 8: 459–462

Lu X, Jaffe R (2001) Interaction between Hg(II) and natural dissolved organic matter: a fluorescence spectroscopy based study. Water Res 35: 1793-1803

Marcelo J, and Wilkinson KJ (2002) Disaggregation kinetics of a peat humic acid: mechanism and pH effects. Environ Sci Technol *36*: 5100–5105.

Miretzky P, Bisinoti MC, Jardim WF, and Rocha JC (2005) Factors affecting Hg (II) adsorption in soils from the Rio Negro basin (Amazon). Quím Nova 28: 438–443

Qian J (2001) Mercury species in environmental samples studied by spectroscopic methods, PhD Thesis, University Umeå, 202: 34

Ravichandran M (2004) Interactions between mercury and dissolved organic matter—-a review. Chemosphere 55: 319–331

Rodrigues A, Brito A, Janknecht P, Proença MF, and Nogueira R (2009) Quantification of humic acids in surface water: effects of divalent cations, pH, and filtration. J Environ Monitor 11: 377–382

Rytuba JJ (2003) Mercury from mineral deposits and potential environmental impact. Environ Geol 43: 326–338

Satchell DPN (1977) Metal-ion-promoted reactions of organo-sulphur compounds. Chem Soc Rev 6: 345–371

Satpathy KK, Natesan U, Sarguru S, Mohanty AK, Prasad MVR and Sarkar SK (2008)Seasonal variations in mercury concentrations in the coastal waters of Kalpakkam, Southest coast of India. Curr Sci 95:374-381

Schuster E (1991) The behavior of mercury in the soil with special emphasis on complexation and adsorption processes-a review of the literature. Water Air Soil Poll *56*: 667–680

Selvaraj K (1999) Total dissolvable copper and mercury concentrations in innershelf waters, off Kalpakkam, Bay of Bengal. Curr Sci 77: 494-497

Šmejkalová D, and Piccolo A (2007) Aggregation and disaggregation of humic supramolecular assemblies by NMR diffusion ordered spectroscopy (DOSY-NMR). Environ Sci Technol *42*: 699–706.

Swift RS (1999) Macromolecular properties of soil humic substances: fact, fiction, and opinion. Soil Sci 164: 790-802

Wang W, and Driscoll CT (1995) Patterns of total mercury concentrations in Onondaga Lake, New York. Environ Sci Technol 29: 2261–2266.

Yu R, Yuan X, Zhao Y, HU G, and Tu X (2008) Heavy metal pollution in intertidal sediments from Quanzhou Bay, China. J Environ Sci 20: 664–669

# **Captions and Legends for figures**

Figure1: Map of the sampling site along the Godavari estuary (Andhra Pradesh region) of India. Kotipalli is the locations of the sampling sites.

**Figure 2:** Effects of Hg loading in its association with different molecular weight fraction of HA **Figure 3:** a) Effects of pH on Hg distributions with different molecular weight fraction of HA; b) Effects of pH on Hg association with largest and smallest molecular weight fraction of HA

**Figure 4:** a) Effects of ionic strengths on Hg distributions with different molecular weight fraction of HA; b) Effects of ionic strengths on Hg association with largest and smallest molecular weight fraction of HA

**Figure 5:** Schematic diagrams of humic substances at three different conditions. A) At low pH (highly acidic) and high ionic strength; humic acid molecules are aggregated due to neutralization of negative charges by H+. Hg<sup>2+</sup> remains mainly as free in the solution and hence an increase the concentrations of Hg<sup>2+</sup> ion in the solution of < 1kDA.

B) At an intermediate pH, humic acid disaggregates to smaller molecules. With the availability of complexing sites for  $Hg^{2+}$  ions to undergo complexation, reduction reactions and resulting in the lowered  $Hg^{2+}$ ion and association of Hg with humic acid of intermediate molecular weight increases. C) At higher pH, humic acid do not disaggregate further. However, Hg undergoes strong complexation. There is a formation of  $Hg(OH)_2$ 

**Figure 6:** a) Reduction of  $Hg^{2+}$  in presence of HA at varying pH; b) Effects of Hg loading; c) pH and d) ionic strengths on distributions of Hg with different molecular weight fractions of humic-like compounds from Godavari River.



Figure 1



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Figure 3



#### Figure 4



Hg-Humics interactions at low pH and high ionic strength: aggregation of HS predominates; free Hg<sup>2+</sup> ion in solution

 
 Hg-Humics interactions at intermediate pH and ionic strength: disintegration of HS begins; less Hg<sup>2+</sup> in solution and more complexation
 Hg-Humics interactions at high pH and low ionic strength: disaggregation of HS predominates; complexation of Hg<sup>2+</sup> ion and precipitation of Hg(OH)<sub>2</sub>

Figure 5



Table 1. Comparative account of mercury levels (µg.dm<sup>-3</sup>) in the Indian Coastal and estuarine waters

Area	Level of Hg (µg.dm <sup>-3</sup> ) in coastal water	References
Binge Bay, Karwar	0.15-26.68	Kureishy et al 1986
Binge Bay, Karwar	0.91-2.62	Krishnakumar& Pillai 1990
Cochin Backwaters	1.2-50.0	Balchand &Nambisan,1986
Off Kalapakkam, Bay of Bengal	64–1513	Selvaraj, 1999
Coastal water of Kalpakkam	3.0-50.0	Satpathy et al 2008
Rameshwaram	1.5-11	Parthiban, 2013*

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