

Reduction of mercury (II) by humic substances

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ABSTRACT

This study demonstrates that, under abiotic dark condition in aquatic system, humic substances are not only capable of converting Hg(II) to Hg⁰ but also able to bind Hg(II) ion. The degree of Hg(II) reduction is significantly influenced by the ratio of –COOH/-OH groups and the sulfur content in the HS, revealing a strong competition between complexation and reduction of Hg(II). This study suggests that abiotic and dark Hg(II) reduction depends on the pH and salinity of aqueous medium. At lower pH (~4.0) and lower salinity (≤ 5.0 PSU), the reduction of Hg(II) to elemental mercury (Hg⁰) was comparatively rapid. Higher –COOH/-OH ratios in HS, favours dark abiotic reduction of Hg(II) as did a lower sulfur (S) content of HS. . This study provided a rigorously controlled experimental design that showed that dark abiotic Hg(II) reduction by HS can potentially be important in the aquatic environment and is independent of the photochemical reduction observed in both fresh water and sea water.

Keywords: gaseous mercury capture, reduction of mercury; mercury evasion; mercury-humics interaction; elemental mercury

1. INTRODUCTION:

Mercury (Hg) has been classified as a high priority pollutant because of its persistence in environments and toxicity to aquatic and marine organisms. It is considered as a global pollutant because of the long range transport of elemental mercury (Hg^0) in atmosphere (Mason et al 1994; Schroeder and Munthe, 1998). When Hg^0 is oxidized to the mercury ion (Hg(II)) in the atmosphere it is more soluble in water and rates of deposition is more rapid (Costa and Liss, 1999). The fate of Hg(II) in the water column of both fresh water and sea water (after deposition) depends on several factors including the quality and quantity of dissolved organic carbon (DOC). DOC plays an important role in controlling Hg-speciation and its bioavailability in the system. The coupled reactions between Hg redox transformation and complexation with natural DOC are still incomplete (Barkay et al 1997; Barkay et al 2005; Skylberg 2008; Miller et al 2007; Miller et al 2009; Xiao et al 1995; Amyot et al 1997a; O'Driscoll et al 2004; Chakraborty et al 2014a; Chakraborty et al 2014b) due to heterogeneity of DOC (Chakraborty 2007; Chakraborty et al 2007; Chakraborty and Chakrabarti 2008; Chakraborty et al 2014c). Strong complexation of Hg(II) with reduced sulfur ($-\text{S}$) or thiol ($-\text{SH}$) functional groups in DOC at relatively high DOC: Hg ratio decreases Hg bioavailability in aquatic system (Skylberg 2008; Haitzer et al 2002; Hesterberg et al 2001; Skylberg et al 2006). However, reduction of Hg(II) to Hg^0 by DOC also controls mercury flux at water/air interface.

Reduction of Hg(II) can occur both in the light and in dark by DOC and is one of the natural sources of atmospheric Hg (Alberts et al., 1974; Amyot et al., 1997b; Canário and Vale, 2004; Chakraborty et al., 2014; Lin and Pehkonen, 1999; Ravichandran 2004; Skogerboe and Wilson, 1981; Allard and Arsenie, 1991). Surface ocean and lakes contribute a good fraction to the total flux of Hg^0 in the atmosphere (Mason et al., 1994). It has been reported by Morel et al (Morel et al., 1998) that high amount (10 Mmol yr^{-1}) of Hg (Hg^0 and dimethyl mercury) get released from the surface ocean ($<100\text{m}$ depth)(Mason and Fitzgerald, 1991; Mason and Fitzgerald, 1990; Nriagu, and Pacyna, 1988; Vandal et al., 1991) to the atmosphere. Remote lakes have also been reported to release considerable amount of Hg^0 (5Mmol yr^{-1}) (Amyot,et al., 1997a; Nriagu, and Pacyna, 1988). DOC has been considered to initiate reduction of Hg(II) to Hg^0 in aquatic systems. Quinone or semiquinone moieties in DOC has been reported to transfer electrons to reduce Hg(II) (Ravichandran, 2004).

Photochemical reduction and reduction of Hg(II) in dark by dissolved organic matter (DOM) in aquatic system has been reported in the literature(Allard and Arsenie, 1991; Skogerboe and Wilson, 1981; Albert et al., 1974; Rocha et al., 2000; Gu et al., 2011; Zheng et al., 2012; Zheng et al., 2010). It has been observed that photoreduction of Hg(II) is inversely correlated to DOC levels in aquatic systems (Amyot et al., 1997a; Rolffhus and Fitzgerald, 2001). Other studies, however, have shown that the presence of DOC corresponds with significant increase in Hg(II) photoreduction and that reduction rates increase with DOC concentration (Costa and Liss, 1999; O'Driscoll et al., 2004; Xiao et al., 1995). It has also been reported that DOC has an inhibitory effect on Hg(II) reduction (Mauclair et al., 2008; Rocha et al., 2000). These apparently contradictory results suggest an incomplete mechanistic understanding of DOC and Hg interactions and the likely involvement of additional factors.

There is much more information on photochemical driven rates where ultraviolet radiation is the principal driver of the process. However, as shown in the present investigation reduction of mercury can also occur in the dark whereas the photochemical reduction is confined to the upper regions of the water column only. Most of the researches on Hg(II) reduction by DOC have been carried out in the light (Amyot et al., 1997a; Rolffhus and Fitzgerald, 2001 Costa and Liss, 1999; O'Driscoll et al., 2004; Xiao et al., 1995; Mauclair et al., 2008; Rocha et al., 2000) with few papers on Hg(II) reduction by DOC under abiotic dark condition (Gu et al., 2011; Zheng et al. 2012; Zheng et al., 2010). The dark reduction of Hg(II) is potentially quite different from photochemical reduction. Gu et al (2011) reported that reduced dissolved organic matter (DOM) is capable of converting Hg(II) to Hg⁰ in dark anoxic environments. Zheng et al (2012) have suggested that reduced organic matter simultaneously reduces and oxidizes Hg via different reaction mechanisms under dark anoxic condition in aquatic system. It has also been proposed that reduction of Hg(II) is primarily caused by reduced quinones. However, Hg⁰ oxidation is controlled by thiol functional groups via oxidative complexation under dark anoxic condition. Zheng et al (2013) also suggest that thiol-induced oxidation of Hg⁰ is important under anoxic conditions in aquatic systems. This process can affect Hg redox transformation and bioavailability for microbial methylation under dark anoxic conditions. Zheng et al (2010) have further tried to provide the biogeochemical transformation pathways of Hg by tracking isotope fractionation study. However, the reduction mechanism of Hg(II) by DOC under dark abiotic condition is not fully understood.

The current state of knowledge clearly indicates that DOC plays a complex role in the reduction of Hg(II) in aquatic environments under dark abiotic conditions. A major part of the published works has mainly focused on photochemical reduction of Hg(II) by DOC or humic substances (HS) (a major part of DOC) in natural environment. The mechanism of dark abiotic reduction of Hg(II) by natural organic matters, an important process in the natural aquatic environment, is not properly known. Thus, an effort was made in this study to understand dark abiotic reduction process of Hg(II) by HS in aquatic system and recognize the influences of different factors (including pH, salinity, and structural features of HS) on the reduction of Hg(II) in aquatic system.

2. EXPERIMENTAL

2.1. Cleaning Procedures for Containers:

All containers used were made of Teflon (to minimize any kind of Hg adsorption on the wall of the containers). After cleaning with ultrapure water, all the containers were filled to the top with 10% (v/v) nitric acid (HNO₃) and allowed to stand at the room temperature for 1 week. They were then rinsed five times with ultrapure water, filled with ultrapure water. The filling water was renewed daily to ensure continued contact with clean water. Quartz boats were heated to 800°C prior to Hg analysis.

2.2. Reagents

ICP-standard of Hg from Merck, Germany (1000 mg. dm⁻³) was used as stock. Ultrapure HNO₃ (Merck, Darmstadt, Germany) was used to prepare 10% (v/v) HNO₃ to prepare the oxidizing solutions to capture Hg⁰. Analytical Grade Potassium permanganate (KMnO₄) crystal from Merck,

Mumbai, India was used. The reducing agent was prepared from GR grade Tin (II) chloride (SnCl_2); dehydrate crystal from Merck, Mumbai, India.

2.3. Humic substances (HS):

The well characterized Suwannee River Humic Acid (Catalogue No. 2S101H) (SRHA), Suwannee River Fulvic Acid (Catalogue No. 1S101F) (SRFA) and Eliot Soil Humic acid (Catalogue No. 1S102H) (ESHA) were obtained from International Humic Substance Society (IHSS; MN, USA) and were used in this study. The stock solutions of HS were prepared by dissolving approximately 0.1 g of the HS in 100 cm^3 of ultrapure water and NaOH at pH 10. The HS was completely dissolved. The solution was filtered through 0.45 μm cellulose acetate filters and pH was then adjusted to 8.0 with ultrapure HNO_3 . The resulting HS solutions were stored in the dark at 4°C.

2.4. Humic acid solution composition:

The synthetic solutions were prepared (separately) in ultrapure water containing 5.0 $\text{mg}\cdot\text{dm}^{-3}$ of three different types of HS (SRHA, SRFA, ESHA) and 25×10^{-9} M (5.0 $\mu\text{g}\cdot\text{dm}^{-3}$) of Hg. The concentration of Hg used in this study were within relevant ranges (2.0×10^{-8} to 2.5×10^{-6} M) from the polluted coastal waters of India (Balchand and Nambisan, 1986; Krishnakumar and Pillai, 1990; Kureishy et al., 1986; Satpathy et al., 2008; Selvaraj, 1999). The Hg solutions were prepared from the dilution of stock Hg solution.

The pH of the solutions was adjusted using ultrapure NaOH and HNO_3 and measured using a pH meter (Metrohm, 827 pH Lab, Switzerland). The salinity of the synthetic solutions was adjusted by adding suprapur NaCl salt. The reduction of Hg(II) by different HS was studied continuously for 6 days. The process was abiotic and in dark condition. The pH of the solutions was checked again immediately at the end of the experiments and found to be unchanged. The elemental compositions, concentrations of carboxylic and phenolic-OH and carbohydrate in SRHA, SRFA, ESHA are presented in Table 1. Each experiment also included a control. These solutions were composed with everything except the HS.

2.5. Mercury vapour trap

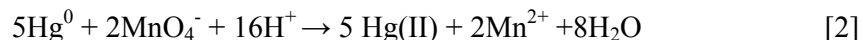
The set up used for capturing Hg^0 is depicted in Figure 1. It consisted of a Teflon reactor. Two Teflon tubes were connected from the top of the reactor. Ultrapure N_2 gas was connected with one of the Teflon tubes. Reduced Hg (Hg^0) produced inside the reactor was carried out by flushing with N_2 gas through the oxidizing solution in such a way that Hg^0 gets oxidized to Hg(II) ion. The N_2 gas flow rate used in this study was $200 \text{ cm}^3 \text{ min}^{-1}$. Elemental Hg^0 , which did not get oxidized in the first trap, was carried to the second chamber (containing the same oxidizing solution) and so on (as shown in Figure 1). The entire system was kept closed in dark (throughout the experiment) and the reduced Hg^0 was determined by measuring the total Hg content in the oxidizing solutions.

2.5.1. Theoretical basis of purge and trap technique:

In order to establish the method and optimize the experimental condition, Hg^0 was produced by allowing Hg (II) to react with SnCl_2 .



The Hg^0 was then oxidized by acidified KMnO_4 solution. The following chemical reaction is involved in this step.



Direct mercury analyzer was used to determine the oxidized Hg(II) concentration in oxidizing solution. Systematic investigations were carried out to obtain the optimal experimental conditions for the purge and trap technique.

2.5.2. Optimization of experimental parameters

Both nitric acid (HNO_3) and sulfuric acid (H_2SO_4) were used (separately) along with potassium permanganate (KMnO_4) (as oxidant) to trap Hg^0 . The total volume of both the oxidizing solutions (including three chambers) was kept 120 cm^3 . Both the acids were expected to be effective in capturing Hg^0 . HNO_3 and KMnO_4 mixture was used as the oxidizing solution for Hg^0 .

The stability of oxidized Hg(II) in the oxidizing solution was also studied. A series of kinetic experiments were performed to understand the stability of Hg(II) in the trapping ($\text{HNO}_3 + \text{KMnO}_4$) solution.

A systematic investigation was performed to determine the influence of KMnO_4 concentration on capturing Hg^0 . A series of solutions, containing Hg(II) ($20.0 \times 10^{-9}\text{M}$) was taken in series of reactors. Stannous chloride (SnCl_2 (0.5M) in HCl of 1.2 M) solution (as a reducing agent) was added in each reactor to generate Hg^0 . All the systems were kept closed. Reduced Hg^0 was carried by N_2 gas through the trapping chambers (as shown in Figure 1). The concentration of HNO_3 was kept constant (0.9M) in the trapping solution. The concentrations of KMnO_4 were varied ($30.0 \times 10^{-6}\text{ M}$ to $400.0 \times 10^{-6}\text{M}$) to find the optimum concentration of KMnO_4 to improve Hg capturing capacity of the trapping solution.

A systematic investigation was carried out to determine the minimum time required for capturing elemental Hg . A synthetic solution of Hg(II) ($20.0 \times 10^{-9}\text{M}$) was prepared. A stannous chloride (SnCl_2 (0.5M) in HCl of 1.2 M) solution was used as a reducing agent. The reactor was closed immediately after the addition of SnCl_2 . Reduced Hg^0 (in gaseous state) inside the reactor was carried out by flushing with N_2 gas to the trapping solutions (with a flow rate of $200\text{ cm}^3/\text{min}$). The concentrations of reduced Hg^0 in trapping solution were determined at different time interval.

2.6. Determination of total Hg by Direct Mercury Analyzer:

Mercury was analyzed by Direct Mercury Analyzer (Tri cell DMA-80) from Milestone, Italy. The tri cell DMA-80 combines the techniques of thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometer. Controlled heating stages are implemented to first dry and then thermally decompose a sample introduced into a quartz boat. A continuous flow of oxygen carries the decomposition products through a catalyst bed where interferences are trapped. All mercury species are reduced to Hg^0 and are then carried along to a gold amalgamator where the Hg is selectively trapped. The system is purged and the amalgamator is subsequently heated which releases all mercury vapours to the single beam, fixed wavelength atomic absorption spectrophotometer. Here, absorbance measured at 253.7 nm is proportional to mercury content in the

sample. The instrument is fully compliant with US EPA method 7473. The Tri-Cell DMA-80 was found to have a dynamic range of 0.0015 ng – 1200 ng.

2.7. QA/QC

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 (sediments) were used to control the quality of Hg determination in water samples. Sediment matrix is quite different from $\text{KMnO}_4/\text{HNO}_3$ solutions. Thus, continuous monitoring of spiked Hg recovery in real samples was done and more than 99% recovery was obtained.

3. RESULTS AND DISCUSSIONS

3.1. Optimization of experimental parameters

It was found that HNO_3 and H_2SO_4 (separately) (with a concentration of 0.9M) along with permanganate ($316 \times 10^{-6}\text{M}$) could capture more than 95% of gaseous Hg^0 ($\sim 20.0 \times 10^{-9}\text{M}$) from the reactor. 96.1% and 95.1% of the total Hg ($\sim 20.0 \times 10^{-9}\text{M}$) could be captured by $\text{H}_2\text{SO}_4 + \text{KMnO}_4$ and $\text{HNO}_3 + \text{KMnO}_4$ mixtures respectively. However, Hg capturing capacity of both the trapping solutions dramatically decreased with the increasing concentrations of gaseous Hg^0 in the reactor.

It was found that only 35.1 and 30.0% of the total Hg was captured by both the mixtures after the reduction of $\sim 5.0 \times 10^{-7}\text{M}$ of Hg in the reactor. This study indicates that permanganate in presence of both the acids are quite good oxidant to trap Hg^0 only at low concentrations. HNO_3 has been used in activating catalyst tube in direct mercury analyzer (DMA-80, Milestone, Italy) and does not have severe impact on the lifetime of the catalyst tube of DMA-80. Thus, KMnO_4 ($316 \times 10^{-6}\text{M}$) in HNO_3 (0.9M) mixture was selected as the oxidizing (trapping) solution to trap elemental Hg^0 in this study. It is important to mention that there was no Hg(II) reduction observed from the control solutions.

3.2. Stability of Hg(II) in trapping ($\text{HNO}_3 + \text{KMnO}_4$) solution

Stability of Hg(II) ion in the trapping solution (containing 0.9M of HNO_3 and $316 \times 10^{-6}\text{M}$ of KMnO_4) was studied. The initial Hg(II) concentration in the trapping solution was kept $\sim 20.0 \times 10^{-9}\text{M}$. The concentration of dissolved Hg in the trapping solution was monitored for 96 h at a particular time interval of 24 h. Figure 2 show that there was no loss of Hg from the trapping solution and trapped Hg was stable in the oxidizing solution. This indicates that $\text{HNO}_3 + \text{KMnO}_4$ solution can be used as a suitable trapping solution for capturing Hg^0 .

3.3 Concentrations of KMnO_4 in HNO_3 solution for Hg^0 trapping

Figure 3 shows that trapping capacity of Hg gradually increased to a maximum with the increasing concentration of KMnO_4 in the trapping solution and reach a plateau. Figure 3 shows that there was no change in concentration of Hg in the trapping solution with the increasing concentrations of

KMnO₄ from $316 \times 10^{-6} \text{M}$ to $400 \times 10^{-6} \text{M}$. Thus, $316 \times 10^{-6} \text{M}$ of KMnO₄ in 0.9M of HNO₃ was used as the trapping solution in this study to capture Hg⁰.

3.4 Time required for capturing mercury

Figure 4 shows that concentrations of Hg in trapping solution gradually increased with time. A complete 100% recovery of Hg (compared to the initial Hg concentration in the reactor) was found at 180 minutes.

The reduction of Hg²⁺ by SnCl₂ in aqueous medium is usually very fast. However, it took 180 minutes to trap all Hg⁰. This is because the Hg⁰ was transferred into the trapping solutions by flushing the headspace of the Teflon reactor with N₂, not by bubbling the Hg solution in Teflon reactor. It is important to mention that this method was successful in pre-concentrating and determining the reduced Hg from a solution (500.0 cm³) with total Hg content of $\sim 0.5 \times 10^{-9} \text{M}$ in the reactor. This method was developed and optimized by reducing Hg(II) ion (by using SnCl₂) at acidic pH and further applied to understand the reduction processes of Hg(II) by the different well characterized HS in aquatic environment. However, the time require for capturing Hg in natural systems (in presence of different heterogeneous natural ligands) is dependent on the nature of DOC, temperature, Hg-DOC complexation etc. Thus, it is recommended to optimize the time for each environmental samples separately. The Hg-DOC solution was also purposely not bubbled with N₂ to avoid any kind of physical forcing during Hg-DOC interactions. It is assumed that the diffusion of Hg⁰ in different humic acid solutions is constant and analysis of Hg⁰ in headspace represents Hg(II) reduction processes by DOC in the aqueous phase. In this experiment, net reduction of Hg(II) (which is the difference between reduction and oxidation in solution) was determined.

3.5. Abiotic Hg(II) reduction by well characterized HS in dark

Aqueous solution of Hg(II) (containing $20 \times 10^{-9} \text{M}$ of Hg(II)) was allowed to react with a definite amount (5 mg. dm^{-3}) of three different well characterized HS (viz. Suwannee River Humic Acid (SRHA), Suwannee River Fulvic Acid (SRFA), and Eliot Soil Humic Acid (ESHA) obtained from the International humic substance society) separately (under dark conditions). The concentrations of Hg(II) used in this study were relatively higher than those of unpolluted environmental water 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 2 (Balchand and Nambisan, 1986; Krishnakumar and Pillai, 1990; Kureishy et al., 1986; Satpathy et al., 2008; Selvaraj, 1999). Thus, the concentrations of Hg used in this study were environmentally relevant.

The reduction of Hg(II) and production of Hg⁰ by the different HS was monitored for 6 days. The reducing capacity of Hg(II) of the three different HS was compared by keeping the [Hg]/HS ratio constant in all the synthetic solutions. Figures 5 a b and c showed that the total concentrations of Hg in the trapping solutions (representative of net reduction of Hg(II)) gradually increased and reached maxima and remained constant (for majority of the samples). The rate of reduction of Hg(II) and production of Hg⁰ by the HS was fast at the beginning of the experiment but gradually decreased with the progress of time. There was slow and linear increase of reduction of Hg(II) particularly at higher pH (9.0). The rate of reduction of Hg(II) was found to vary with different HS. Figure 5 shows

that the rate of reduction of Hg(II) by SRHA was highest in the aquatic environment at pH 7 (production rate of $\text{Hg}^0 = 2.8 \times 10^{-2} \text{ h}^{-1}$) followed by SRFA (reduction rate constant of $\text{Hg}^0 = 2.5 \times 10^{-2} \text{ h}^{-1}$) and ESHA (reduction rate constant of $\text{Hg}^0 = 7.7 \times 10^{-3} \text{ h}^{-1}$). The rates of reduction of Hg(II) to Hg^0 by the SRHA and SRFA (aquatic) were comparable. However, ESHA (soil HA) was found to have slowest Hg(II) reduction rate. This difference of Hg^0 production probably indicates that sources of HS play important role in determining their Hg(II) reducing capacity. The rate of production of Hg^0 by HS (under dark abiotic conditions) are in very good agreement with the Hg^0 production rate (by natural DOC under dark abiotic conditions) reported in literature (Whalin et al. 2007, Gu et al., 2011; Allard and Arsenie, 1991). However, photochemical reduction of Hg(II) has also been found to have very similar Hg^0 production rate (Amyot et al., 1997; Selvendiran et al., 2009). The reasons of these similarities in Hg^0 production rates under experimental conditions are unknown and leave us a scope to do further investigations in future. Influences of several other chemical parameters (pH, salinity) on Hg^0 production by different well characterized HS were further investigated.

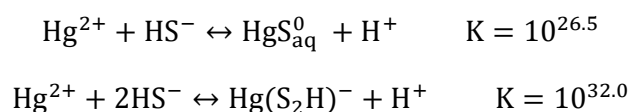
(a) Impact of varying pH and salinity on Hg (II) reduction by HS in aquatic environment

Figures 5 a b and c show the changes in concentrations of Hg^0 (in trapping solution) as a result of reduction of Hg(II) by the different HS at varying pH of the solution. It showed that net reduction of Hg(II) to Hg^0 by the HS was highest at lower pH (4.0). Low Hg volatilization with higher pH level from aqueous medium was due to increase in complexation of Hg(II) with HS in the medium. It is well known that disaggregation of HS predominates at higher pH of the medium. Disaggregation of HS probably opened up new suitable binding sites for Hg(II) ion to form thermodynamically stable complexes. Thus, less and slow reduction of Hg(II) (by HS) took place at higher pH. Comparatively higher net reduction of Hg(II) was observed at lower pH. Weak associations of Hg(II) with hydrophilic groups (-COOH, and -OH) in aggregated HS molecule (at lower pH) might have also increased the net reduction rate of Hg(II) at lower pH. Figures 6 a,b and c show the impact of salinity on the reduction of Hg(II) by different HS in aqueous solution. It shows that increasing salinity in water column decrease the reduction process of Hg(II) by HS in aqueous solution. Figure 6 shows that the reduction of Hg(II) by SRHA gradually decreased with the increasing salinity of the water column. Similar result was found in presence of ESHA (Figure 6 c). This decrease in Hg(II) reduction by HS with increasing salinity in aqueous medium was probably due to the complexation between Hg(II) and Cl^{-1} (at high salinity) ion in solution. At high salinity, HgCl_4^{2-} forms with high $\log K \approx 16.0$. Thus, it is expected that salinity and DOC in water column may determine the competitive complexation of Hg with DOC and Cl^{-1} . More complexation of Hg(II) with Cl^{-1} decrease association of Hg with HS and thus, decreases the reduction process of Hg (II) by HS. However, Hg(II) reduction and complexation by HS is not only dependent on pH and salinity of the medium but also on the (i) percentage (%) of S content (ii) COOH/OH group ratio in HS.

(b) Impact of varying S content (in HS) on Hg (II) reduction in aquatic environment

Based on the preference of a cation for complexation with ligands, Hg is classified as a B-type metal cation, characterized by a “soft sphere” of highly polarisable electrons in its outer shell. Hg shows pronounced preference for ligands of sulfur, the less electronegative halides, and nitrogen over ligands containing oxygen. Thus, binding of Hg to HS under natural conditions is controlled by the reactive sulfur containing functional groups. Dissolved organic-sulfur cycling in aquatic system

plays a significant role in controlling Hg speciation. Hg–organic sulfur complexes have been reported to be thermodynamically quite stable. The stability constants for these complexes are given below.



It has been reported that the high sulphide concentrations in estuaries and seawater make Hg(II) unavailable for methylation in the system (Gårdfeldt et al., 2003). It has also been reported that reduced sulfur in DOM is able to oxidize elemental Hg^0 under dark anoxic condition (Zheng et al., 2012; Zheng et al., 2013). Thus, one could expect that dissolved organic sulfur concentrations (in dissolved HS) may decrease the reduction process of Hg(II) and the production of elemental Hg^0 under the experimental condition.

Figure 7a shows the change in Hg(II) reduction against the total sulfur content of the different HS at different pH (under abiotic and dark condition). It shows that the reduction of Hg(II) gradually decreased with the increasing sulfur content in all the well characterized HS at different pH. ESHA was found to have maximum reducing capacity with lowest amount of total sulfur content. Similarly, SRHA (with highest total sulfur content) was found to reduce lowest amount of Hg with highest amount of total sulfur content.

Figure 7b shows the change in Hg(II) reduction against the total sulfur content of the different HS at different salinity (under abiotic and dark condition). It is evident from Figure 7b that high salinity of medium and high sulfur content of dissolved HS decrease Hg(II) reduction tremendously in aqueous environment. This is probably because of the strong complexation between sulfur and Hg in aquatic environment. It has also been reported that the elemental Hg may adsorb on sulfur containing functional group in HS in aquatic environment (Reddy and Ryan, 1998).

In this study, the concentration of total sulfur in the HS was found to vary from 0.85 to 0.69 μM . The concentration of Hg, used was in the range of $\sim 25\text{nM}$. However, it is known that Hg(II) has strong affinity for reduced sulfur, not the total sulfur in the HS. Only a few percent (1-5%) of the total sulfur in the HS was expected to be in reduced form and was sufficient enough to reflect the effect of Hg(II) binding to reduced sulfur on Hg(II) reduction. Flushing the headspace of the Teflon reactor with N_2 might have increased reduced sulfur concentrations in the solutions.

(c) Impact of varying –COOH/-OH ratio (in HS) on Hg (II) reduction in aquatic environment

Phenolic and carboxylic groups in dissolved organic carbon have been reported to interact with Hg(II). It has been reported that the carboxylic groups of dissolved organic matter favour photo reduction of Hg(II) (Fantozzi et al., 2007). Si and Ariya (2007) have also reported that reduction of oxidized mercury species (Hg(II)) by organic acids to elemental mercury (Hg^0) is significant for understanding the cycling of mercury between the atmosphere and aqueous systems. In this study, the reduction of Hg(II) ions by the three well characterized HS was expected to depend on the concentrations of phenolic and carboxylic groups in the well characterized HS. However, significant

correlation was not obtained between the reductions of Hg(II) and the –COOH or –OH concentrations.

However, the reduction of Hg(II) ions by the three well characterized HS showed significant relation with the ratio of phenolic/carboxylic (–COOH/–OH ratio) groups in the well characterized HS. Figures 8a and b show that the reduction of Hg(II) gradually increased with the increasing –COOH/–OH ratio (calculated from reported value by IHSS; Table 1) in the studied HS at different pH and salinity respectively. This finding probably indicates that the reduction rates of Hg(II) are influenced not only by the substituent functional groups such as –OH, and –COOH on the HS, but also the positioning of these functional groups on the structure. Rocha et al (2000) have also found that degree of Hg(II) reduction is significantly influenced by the ratio of –COOH/–OH.

This study demonstrates that, under abiotic dark condition in aquatic system, humic substances are not only capable of converting Hg(II) to Hg⁰ but also able to bind Hg(II) ion. The degree of Hg(II) reduction is significantly influenced by the ratio of –COOH/–OH groups and the sulfur content in the HS, revealing a strong competition between complexation and reduction of Hg(II). This study suggests that abiotic and dark Hg(II) reduction depends on the pH and salinity of aqueous medium. At lower pH (~4.0), more reduction of Hg(II) by HS takes place. Increasing complexation reaction of Hg(II) ion with increasing total S content of HS in the dissolved phase decreases Hg(II) reduction under abiotic dark condition. However, increasing –COOH/–OH ratio in HS in aqueous medium at different pH and salinity can may alter Hg(II) reduction process in aquatic system.

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CAPTIONS AND LEGENDS

Figure 1. The assembly used for capturing elemental mercury. Reduced Hg (in gaseous state) inside the reactor was carried out by flushing with ultrapure N_2 gas to the oxidizing solutions. The N_2 gas (carrying elemental Hg) was bubbled through the oxidizing solution in such a way that the elemental Hg gets oxidized to Hg (II) ion.

Figure 2. Stability of Hg (II) ion in trapping (HNO_3+KMnO_4) solution as a function of time

Figure 3. Effect of varying concentrations of $KMnO_4$ in trapping solution on capturing elemental Hg

Figure 4. Trapping capacity of Hg (II) ion by trapping solution as a function of time

Figure 5. Hg (II) reduction by well characterized HS at different pH; (a) SRFA, (b) SRHA, (c) ESHA.

Figure 6. Hg (II) reduction by well characterized HS at different salinity; (a) SRFA, (b) SRHA, (c) ESHA.

Figure 7. Change in Hg (II) reduction against the total sulfur content in different HS at different (a) pH and (b) salinity.

Figure 8. Change in Hg (II) reduction against the total $-COOH/-OH$ ratio in the different HS at different (a) pH and (b) salinity.

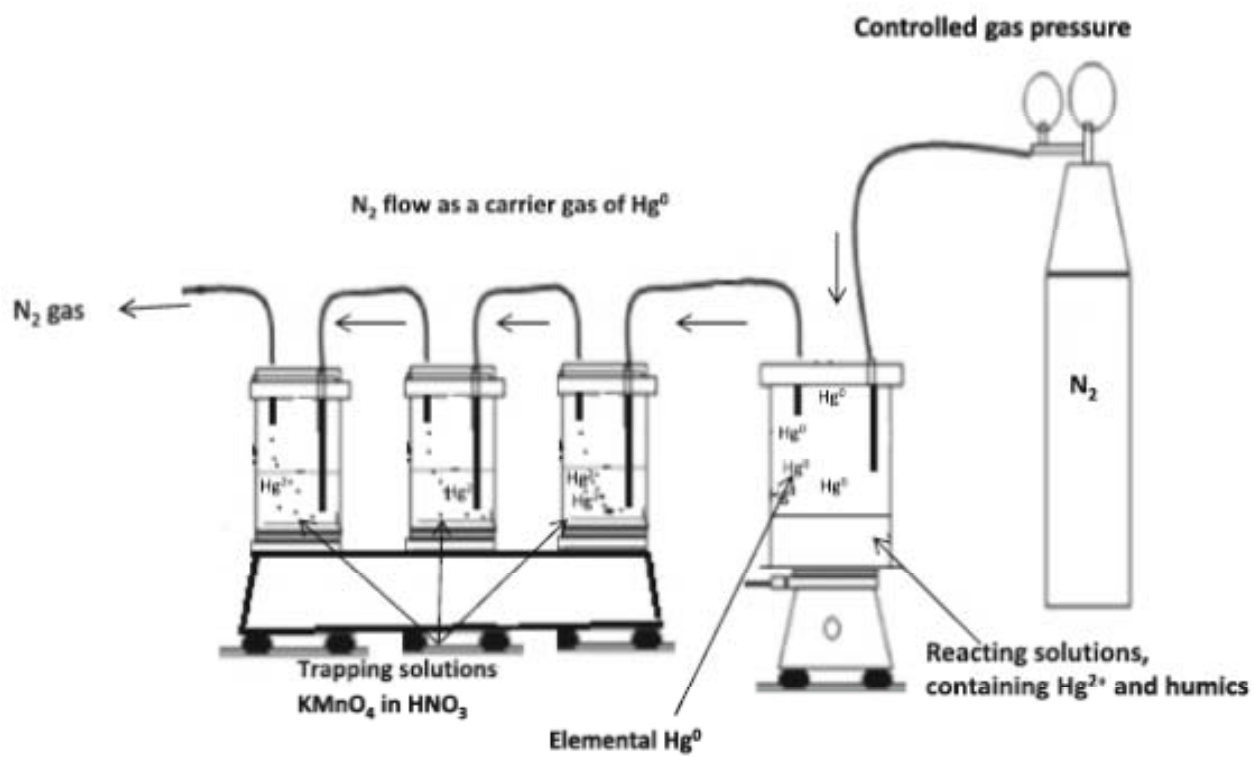


Figure 1

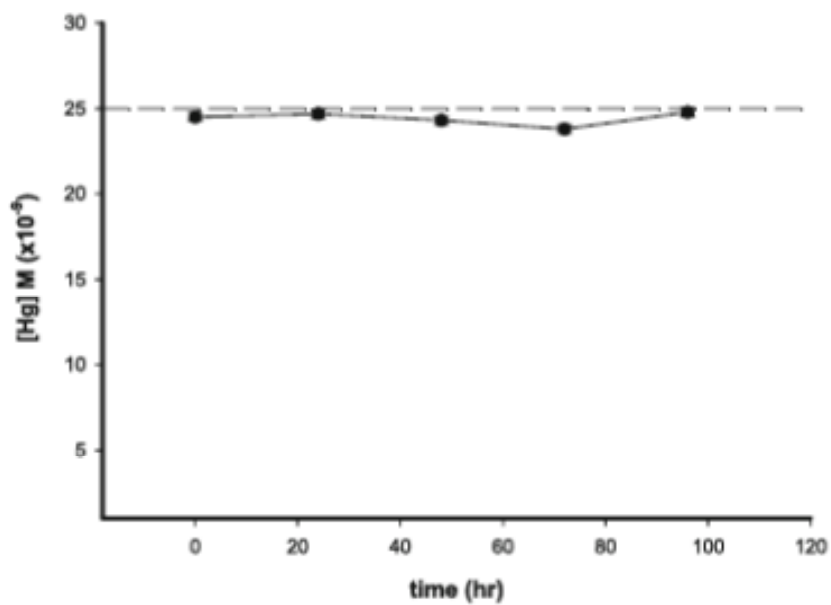


Figure 2

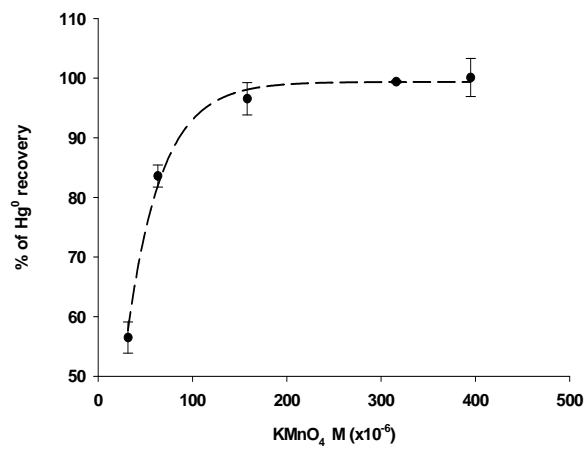


Figure 3

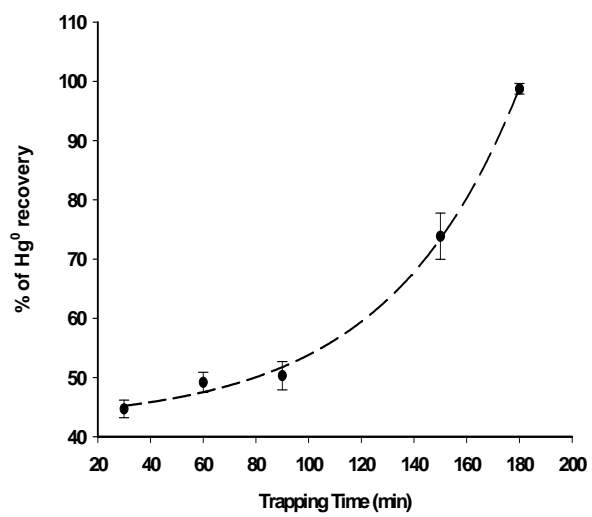


Figure 4

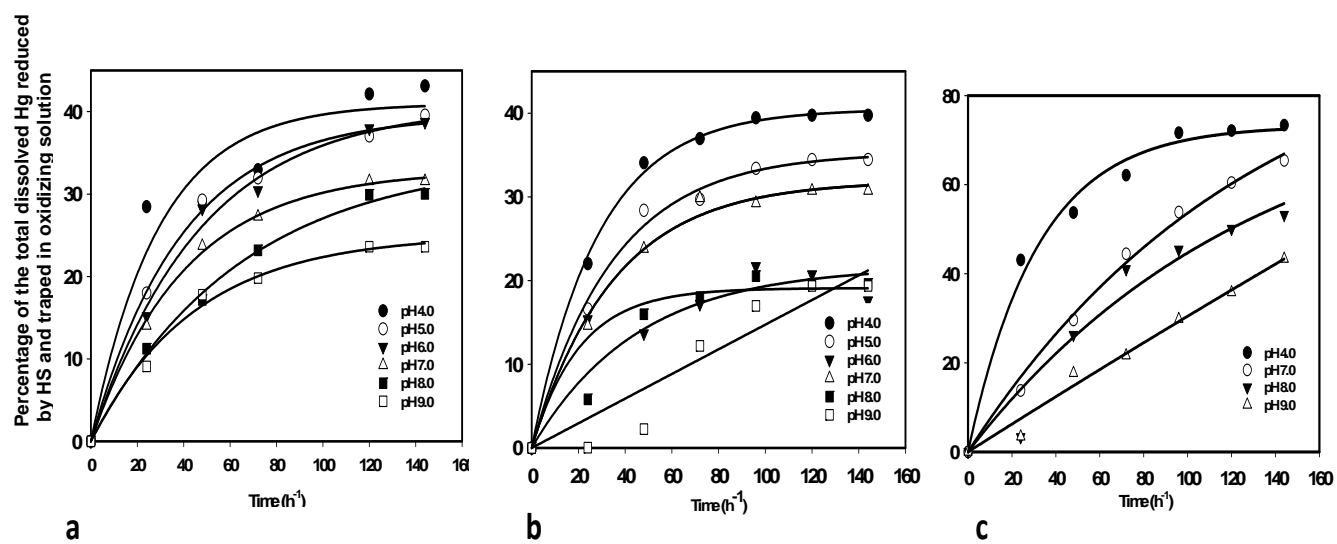


Figure 5

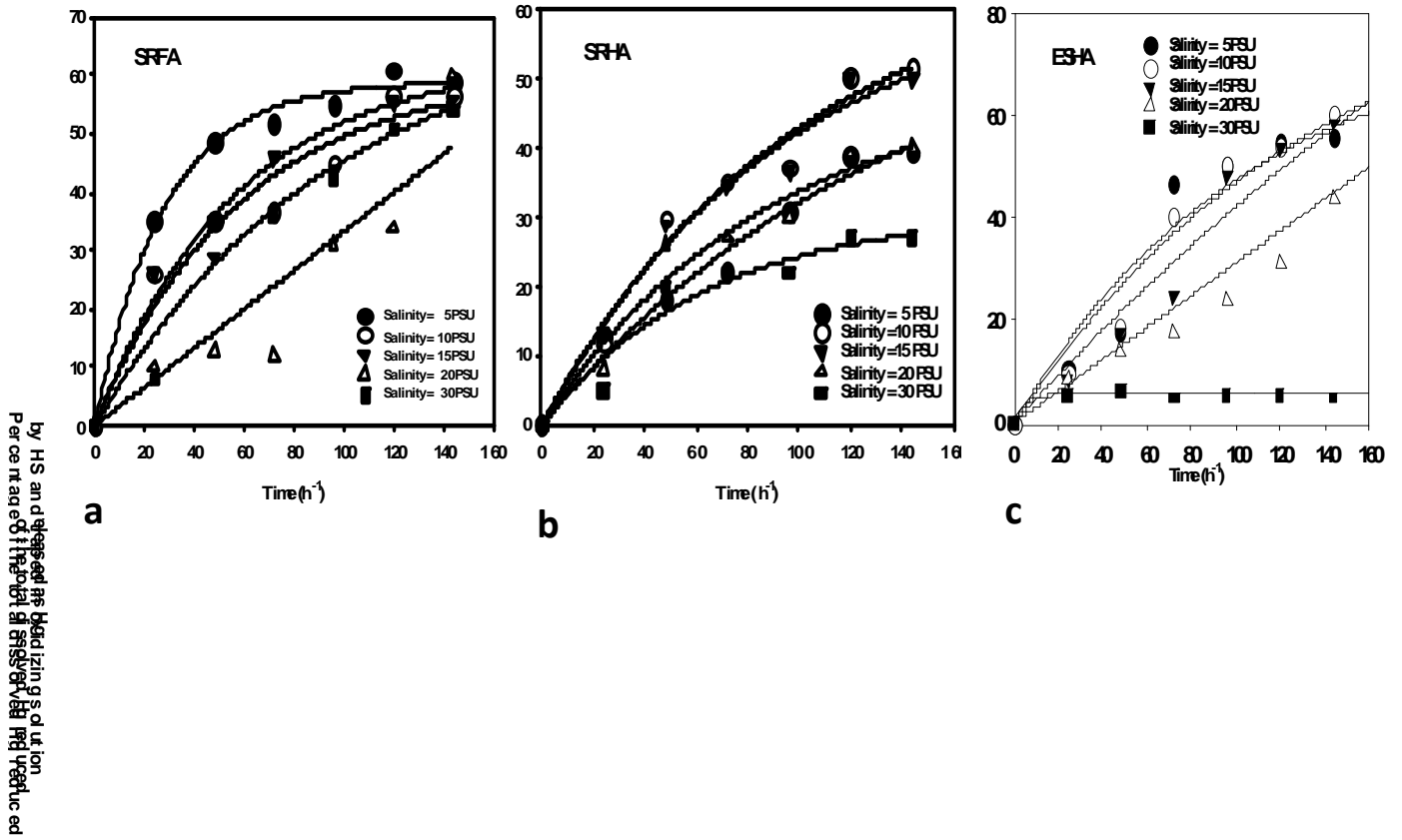


Figure 6

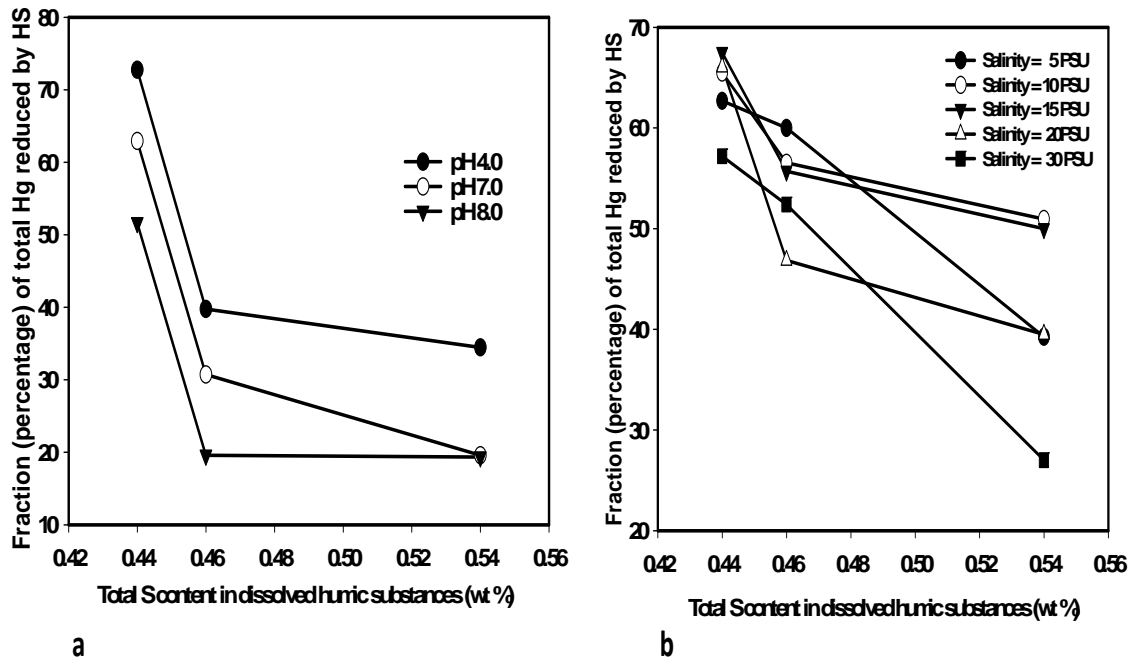


Figure 7

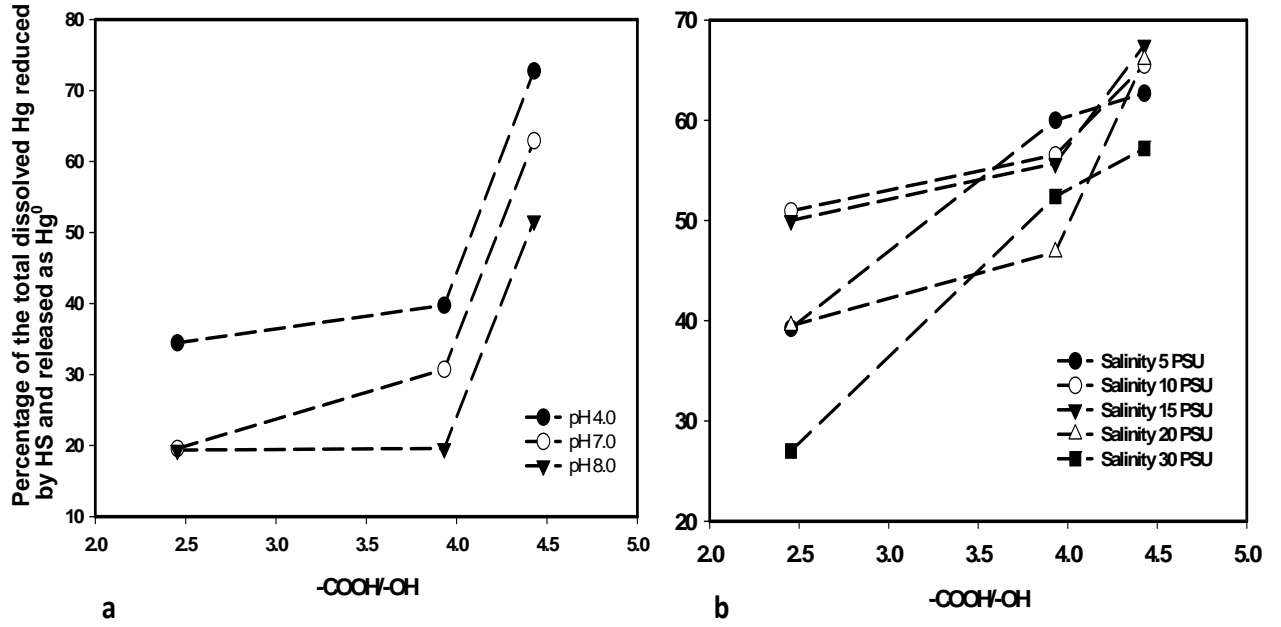


Figure 8

Table 1: Chemical composition of Humic Substances. C, O, N and S are the elemental composition in % (w/w) of a dry, ash free sample. Carboxyl is the charge density (meq/g C) at pH 8.0 and Phenolic is two times the change in charge density (meq/g C) between pH 8.0 and pH 10.0.

Humic Substances	Catalogue no	C	O	N	S	Carboxylic	Phenolic	Carbohydrate
Suwannee River Humic Acid (SRHA)	2S101H	52.63	42.04	1.17	0.54	9.13	3.72	
Suwannee River Fluvic Acid (SRFA)	1S101F	52.44	42.20	0.72	0.44	11.44	2.91	43.2
Elliott Humic Acid (ESHA)	1S102H	58.13	34.08	4.14	0.44	8.28	1.87	206.3

Table 2. Comparative account of mercury levels (M) in the Indian Coastal and estuarine waters

Area	Level of Hg in coastal water	References
Binge Bay, Karwar	$7.5 \times 10^{-10} \text{M} - 1.3 \times 10^{-7} \text{M}$	Kureishy et al 1986
Binge Bay, Karwar	$4.5 \times 10^{-9} \text{M} - 1.3 \times 10^{-8} \text{M}$	Krishnakumar & Pillai 1990
Cochin Backwaters	$6.0 \times 10^{-9} \text{M} - 2.5 \times 10^{-7} \text{M}$	Balchand & Nambisan, 1986
Off Kalapakkam, Bay of Bengal	$3.2 \times 10^{-7} \text{M} - 7.5 \times 10^{-7} \text{M}$	Selvaraj, 1999
Coastal water of Kalpakkam	$1.5 \times 10^{-8} \text{M} - 2.5 \times 10^{-7} \text{M}$	Satpathy et al 2008
Rameshwaram	$7.5 \times 10^{-9} \text{M} - 5.5 \times 10^{-8} \text{M}$	Parthiban, 2012*

* Personnel communication from Dr G. Parthiban, NIO, Dona Paula, Goa