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Effect of pH on transport and transformation of Cu-sediment complexes in mangrove systems

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Abstract

Impact of pH variation of overlying water column on transport and transformation of Cu-sediment complexes in mangrove sediments was investigated by using different metal extraction studies. The total Cu concentration in the studied sediments varied from $\sim 64 \pm 1$ to 78 ± 2 mg.kg⁻¹. The sequential extraction study showed that a major part of the sedimentary Cu (85-90% of the total sedimentary Cu) was present within the structure of the sediments with minimum mobility and bioavailability. The redistribution of non-residual Cu among the different binding phases of the sediments was observed at different pH. It was observed that Cu moved from other non-residual binding phases to organic binding phase of the sediments at higher pH. Partial leaching of sedimentary Cu-SOM complexes (with increasing stability) was observed at higher pH (kinetic extraction study). This study infers that increase in pH of overlying water column increases the mobility of Cu- complexes in the system.

Key words: Copper speciation; fractionation of Cu; pH variation; Cu-sediment complex; mangrove sediment.

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1. Introduction

Copper (Cu) is a micronutrient but may become toxic to aquatic organisms at an elevated level in coastal and estuarine systems (Li.,1991; Hall et al., 1997, Chakraborty et al., 2010, Chakraborty et al., 2015). Residence time of Cu in water column depends mainly on the concentration of dissolved organic carbon (Widerlund., 1996) and Cu/DOC ratio (Hue et al., 1996) in the system. Dissolved Cu may be removed from water column by different adsorption processes on particulate matters (Hart et al., 1982). These particulate matters (associated with Cu) settle down and eventually become an integral part of sediments in coastal and marine environment (Bruland., 1983; Chester., 2003). The hosting phases of sedimentary Cu are broadly classified into two categories: (i) Cu in residual phase (where Cu present within the structure of the sediment and is considered as an inert, immobile phase of Cu. Mostly this fraction is not subjected to any alteration in normal conditions.) and (ii) Cu in non-residual phase (which includes, water soluble Cu-complexes, Cu in carbonate/bicarbonate forms, reducing binding phase and oxidizing binding phase) (Tessier et al., 1979, Chakraborty et al., 2015a).

Major fractions of non-residual Cu has been reported to associate with sedimentary organic matter (oxidizing binding phase) (Chakraborty et al., 2015a, 2016, 2014b, 2008; Flemming and Trevors., 1989; Hanson and Quinn 1983). Complexation of different Cu-binding ligands in sedimentary organic matter reduces mobility and bioavailability of Cu in marine systems (Moffett and Brand., 1996; Moffett et al., 1997). Reducible phase (Fe/Mn-oxyhydroxide binding phase) has also been reported as another hosting phase for Cu in marine sediment. Mobility and bioavailability of Cu complexes in these binding phases depend on several factors, few of them are, redox condition, trace metal competition, pH and ionic strength of the system.

The influences of changing redox condition on geochemical fractionation of Cu in sediment has been widely reported in the literature (Mortimaer and Rae. 2000; Chakraborty et al., 2016). Mortimaer and Rae (2000) have found that changing redox conditions control Fe cycling, and controls Cu fractionation and its mobility in sediments. Chakraborty et al (2016) have reported that Cu forms strong complexes with sedimentary organic matter under low oxygen environment. Cu has been reported to undergo ligand-ligand exchange reaction from Fe/Mn oxyhydroxide to sedimentary organic matter under hypoxic condition (Chakraborty et al., 2016). Different diagenetic processes occurring in mangrove sediments have also been identified to release Cu from sediment (Teasdale et al., 2003). Besides the effects of changing redox condition of a system, an increase in concentrations of other cations (such as Fe^{3+} , Al^{3+} etc.,) has been reported to outcompete Cu from undergoing complexation reaction with different sorption sites in marine sediments (Avendano, L., 2016). There are several studies have been reported on the effect of trace metal competition on Cu-fractionation in

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marine sediments. Chakraborty et al., (2016b) have shown that water exchange rate, out-sphere stability constant, ligand field stabilization energy and Jahn-Teller effect play important role in Cu-sediment interaction in marine system.

The pH of overlying water column has been reported as important factor to control chemical speciation, mobility, and bioavailability of Cu at the bottom sediment (Millero et al., 2009; Gledhill et al., 2015). A decrease in pH has been reported to reduce Cu association with sedimentary organic ligands. Increase in Cu mobility and bioavailability in sediment system has been reported under acidic condition (Avendano et al., 2016). Atkinson et al., (2007) have reported that release and sequestration of Cu by estuarine/coastal sediment is affected by changing pH (5.5 to 8) of the overlying water column. More Cu release from the bottom sediment has been reported at lower pH (5.5). Gledhill et al., (2015) have also reported that complexation of Cu with sedimentary organic matter decreases and concentrations of inorganic Cu complexes increases with decreasing pH. The free ion concentration of Cu (Cu^{2+}) has been predicted to increase by 115% in coastal waters in the next 100 years due to ocean acidification (from 8.23 to 7.80) (Pascal et al., 2010; Richard et al., 2011). Millero et al., (2009) have also predicted that approximately 30% increase in the concentration of free form of Cu (Cu^{2+}) under a similar change of pH.

In this study, an effort was made to understand the fate and speciation of Cu in the mangrove sediments from the Zuari estuary, Goa, India. It is well known that mangrove forests act as useful filters and control the fluxes of trace metals in the nearby coastal marine waters (Marchand et al., 2012, Sadiq 1992). Mangrove forest efficiently traps suspended particulate matters from the water column (Furukawa et al., 1997). The high concentration of sedimentary organic matter in mangrove sediments helps them to bind trace metals from the water column (Chakraborty and Babu 2015, Nissenbaum and Swaine, 1976). Saenger and McConchiefine, (2004) have reported that fine textured mangrove sediments are highly efficient and effective in complexing trace metals from water column. Nevertheless, the fate of trace metal complexes in mangrove sediments is not well known. The cycling of trace/heavy metals in mangrove ecosystems is thus, a serious concern addressed by many authors during the last few decades (Harbison, 1986, Lacerda et al., 1988, Clark et al., 1998 and Tam and Wong, 2000). However, there are no information available on the distribution, speciation and fate of trace metal complexes in tropical mangrove sediments from India. The aim of this study was to provide a better understanding on the impact of pH variation of overlying water column on fate, mobility and transformation of Cu-sediment complexes in tropical mangrove sediments.

2. Materials and methods

2.1 Description of study sites

The sediment samples were collected from the four different sampling locations (Sancoale station) in Zuari estuary Goa, central west coast of India (**Figure 1**). The Zuari estuary is 63 kms in length. The Zuari estuary, a tropical estuary influenced by monsoonal climate, receives abundant fresh water discharge during monsoon (Vijith et al.2009). The river drains through rocks of the Goa group belonging to the Dharwar Super Group of the Archaean–Proterozoic age (Gokul et al., 1985). The Sanvordem formations occupy a large area in the downstream of Zuari river basin which includes metagreywack, conglomerate (tilloid) and argillite (Mascarenhas and Kalavampara, 2009).

A luxuriant growth of mangroves and associated swamps can be observed along most of the water bodies within the estuarine reaches. About 900 ha of mangroves are found along the Zuari estuary (ENVIS Goa Centre, GSCST, 2005). It has been reported that *Avicennia marina* accounted for more than 50% of the total mangroves present. The other important species in the area has been reported to be *Sonneratia alba*, *Bruguiera gymnorrhiza* and *Rhizophora mucronata*. Other mangrove species, which are present in relatively smaller number, are *Avicennia officinalis*, *Excoecaria agallocha*, *Bruguiera cylindrica*, and *Aegiceras corniculatum* (<http://www.forest.goa.gov.in/mang/>).

The surface sediments samples from the study area were collected on August 2015 during the monsoon season from the four stations (ST-1, ST-2, ST-3 and ST-4) as depicted in **Figure 1**. The stations were within a radius of about 200 m from Sancoale (a small town in South Goa district). The surface sample was collected during low tide 3-5 meters away from the mangrove forest by using a pre-cleaned PVC pipe of diameter 100 mm. The sediment samples were collected in triplicate from each station. The top layer (0-3 cm) of each core was considered as the surface layer. The overlying water was also collected by using a Niskin water sampler (5L) in each station. Environmental parameters such as pH and salinity were determined immediately after the sample collection.

2.2 pH and salinity measurement

The pHs of the overlying water samples were determined by using a pH meter (Metrohm, 827 pH Lab, Switzerland). The salinity of the water samples were determined by using Eutech instruments Cyberscan series-600 water proof portable meter.

2.3. Elemental analysis

The sedimentary total carbon (TC) and total nitrogen (TN) content were determined by using Flash 2000 CHN elemental analyzer (Thermo Fisher Scientific Incorporation, USA) with a precision of $\pm 5\%$. Soil NC was used as certified reference material (CRM: Soil NC, UK). Total inorganic carbon

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(TIC) was determined by UIC coulometer (UIC Inc CM 140, Country name). The accuracy of the data was checked by using anhydrous calcium carbonate (Merck, 99.5% pro analytical grade, Germany) as certified reference material. Relative standard deviation of the analysis was $\pm 2\%$. Total sedimentary organic carbon (SOM) was derived by deducting TIC from TC.

2.4 Electrothermal atomic absorption spectrometer (ETAAS)

Electrothermal atomic absorption spectrometer (Perkin Elmer, PinAAcle 900T, Waltham, Massachusetts, USA) was used to determine sedimentary total metal concentrations. This instrument fitted with an AS900 auto sampler. The transversely heated graphite tube (Perkin-Elmer) was pyrolytically coated and equipped with integrated L'vov platforms. Internal argon gas passed through the furnace at a rate of $300 \text{ cm}^3 \text{ min}^{-1}$ during the drying, ashing and clean-up cycles. The gas flow stopped during the atomization step. Every fifth sample analyzed was a blank. Replicate extractions of three independent sub-samples analyzed during each experiment. All measurements were carried out in triplicate.

2.5 Acid digestion of mangrove sediment for the determination of total Cu in mangrove sediment:

Sedimentary Cu concentrations determined by digesting 0.05 g of sediment samples with 15.0 cm^3 of acid mixtures. The acid mixture contained HF (Merck, Suprapure 40%, Darmstadt, Germany), HNO_3 (Merck, Suprapure 65%, Darmstadt, Germany) and HClO_4 (Fluka analytical 70%, Sigma Aldrich, Canada) in 7:3:1 ratio. The sediment and the acid mixture were heated on a hot plate at $180\text{-}200^\circ\text{C}$ for complete digestion. The slurry was evaporated to dryness. The residue was re-dissolved in 2% HNO_3 (Merck, Suprapure 65%, Darmstadt, Germany) and analyzed by ETAAS. MAG-1, a certified reference material (obtained from United States Geological Survey, Virginia, USA) was used for checking the recovery of the digestion procedure. The reported certified reference concentration of Cu in MAG-1 was $30.0 \pm 3.0 \text{ mg.kg}^{-1}$, and the recovery of the certified value was more than 98.5 %.

2.6 Determination of water soluble sedimentary organic matter (SOM)

Quantification of leached SOM from sediment to water soluble fraction at different pH was determined by using Spectroquant UV/VIS Spectrophotometer (Model: Pharo 300, Merck Germany). Standard humic acid was obtained from Fluka Sigma (Fluka Sigma Humic Acid, Cat. No. 53680, Sigma-Aldrich, Inc, Milwaukee, WI, USA). The stock solution of humic acid (HA) was prepared by dissolving 0.1 g of the HA in 100 cm^3 solution of NaOH at pH 10. The solution was then filtered through $0.45\text{-}\mu\text{m}$ cellulose acetate filter papers and pH was then adjusted to different pH with ultrapure HNO_3 . A series of standard solutions of humic acid were prepared and used as standard for

external calibration. The absorbances were determined for the calibration at a wavelength of 311 nm. A strong linear response was observed with an R^2 value of 0.99 (**Figure SM 1**).

2.4 Sequential extraction protocol

The community Bureau of Reference of the European Commission recommended BCR sequential extraction protocol, further modified by Rauret et al. (1999), was used to determine the geochemical fractionation of Cu in the studied sediments. Detailed description of the modified protocols has been vividly described in Chakraborty et al., (2015).

The different Cu fractions were: water soluble Cu complexes (Fr-1); ion-exchangeable, and carbonate/bicarbonate forms of Cu (Fr- 2); Cu bound to Fe–Mn oxyhydroxides i.e., reducible fraction of Cu (Fr-3); concentrations of Cu associated with organic phases, i.e., oxidizable fraction of Cu (Fr- 4) and residual Cu fraction (Fr-5) in the sediment.

In order to find out the effect of changing pH on geochemical fractionation of sedimentary Cu, 1g of sediment sample was taken separately in 4 different containers; added 40 ml of ultrapure water with resistance of 18.2 Ω m (Merck Millipore synergy system, SYNS50001, USA) and then adjusted the pH of the water to 5.00 \pm 0.05, 6.00 \pm 0.05, 7.00 \pm 0.05 and 8.00 \pm 0.05. The pHs of the slurries were monitored and adjusted till the desired pH of the slurries attained. The sediment samples were kept at different pH by using a vertical shaker for 8 hours. The slurries were centrifuged and the water parts were separated. The sediment samples (treated at different pH) were further subjected to geochemical fractionation study by using BCR protocol.

All the extraction processes were performed in Teflon containers. The reagents used in this study were of suprapure grade or better (ultrapure). The concentrations of Cu in each extracted solution and in residual fraction were determined by electrothermal atomic absorption spectrometer (ETAAS). The BCR-701 was used as certified sediment reference material (CRM) (produced by IRMM - Institute Reference material and Measurements, Geel, Belgium and marketed by Sigma Aldrich). The recovery of Cu in each fraction was more than 98%. Validation of the sequential extraction method was also done by comparing the total metal concentrations (determined by ETAAS) with the sum of the concentrations of metal fractions obtained by following the modified sequential extraction protocols.

2.7 Robustness of the straight line fits

A simple mass balancing was done to quantify the change in Cu distribution in different binding phases of sediments under varying pH. The mass balance equation is given below. The total receives

and losses of Cu concentration in four different binding phases of mangrove sediments was assumed to be zero.

$$\sum_{i=1}^4 Fr_i = \text{constant} \quad (1)$$

Here Fr_i , $i=1, 4$ concentration of Cu in the four different fractions.

Therefore, the derivative of Cu concentration with respect to pH is zero. i.e.

$$\frac{d(\sum_{i=1}^4 Fr_i)}{dpH} = 0 \quad (2)$$

Furthermore, we fit the variation of each of the fractions of Cu (Fr_i) with pH using straight lines in Figure 3.

$$\text{i.e., } Fr_i = m_i pH + c_i \quad (3)$$

Here m_i and c_i are the slope and intercept of each line for fraction i of the Summing over the four fractions we get,

$$\sum_{i=1}^4 Fr_i = (\sum_{i=1}^4 m_i) pH + \sum_{i=1}^4 c_i \quad (4)$$

To satisfy equations (1) and (2), $\sum_{i=1}^4 m_i$ has to be equal to zero.

$$\text{i.e., } \sum_{i=1}^4 m_i = 0 \quad (5)$$

2.8 Kinetic Extraction experiment.

Kinetic extraction experiments were carried out to understand the stability of water soluble Cu-SOM complexes at different pH by using Chelex-100 cation exchange resin (Analytical grade, 50-100 mesh, Sodium form, Sigma Aldrich).

2.8.1. Purification of Chelex-100.

Purification of cation exchange, *Chelex-100*, resin was done by following the standard protocol described in the literature (Sunda et al., 2005). This cation exchange resin is a styrene divinylbenzene copolymer containing iminodiacetate ions which act as chelating groups in binding polyvalent metal ions. High selectivity of chelex-100 resin for transition metals in contrast to the alkali earth elements makes it ideal for separation of trace metals from environmental water matrices. A brief description of the procedure is as follows: 40g of Chelex-100 was soaked in 200 cm³ of methanol for 3-4 hours at room temperature and rinsed with 750 cm³ of Mili-Q water using a

scintered glass filter funnel. Later the chelex from the first step was soaked overnight in 1M HCL at room temperature and was then rinsed with 1 dm³ Milli-Q water. After the rinsing, the chelex was again soaked in 3M NH₄OH for 1 week at room temperature. This was followed by rinsing with 1dm³ Milli-Q water. Finally the chelex was soaked in 0.1 M HCl for 10 minutes and then rinsed with 2 dm³ Milli-Q water. Later, the colloidal Chelex-100 was divided into four equal parts and titrated slowly to desire pH levels (5, 6, 7, and 8) by using 1M NaOH.

2.8.2. Extraction of water soluble SOM, Cu complexes and determine the lability of Cu-SOM complexes by kinetic experiment.

In order to extract water soluble Cu-SOM complexes, 10 g of sediment was added to 100 cm³ of MQ water in Teflon beakers. Four sets of slurries were made. The pHs of the slurries were adjusted to 5.00 ±0.05, 6.00 ±0.05, 7.00 ±0.05 and 8.00 ±0.05 (by using 1M ultra-pure HCl and 1M NaOH). The mixture was continually stirred with Teflon- coated magnetic stirring bars for 24 h. The pHs of the solutions were checked periodically to maintain the pH as required. The solutions were transferred to centrifuge tubes, centrifuged (rpm 4500) and collected in to four Teflon beakers (at four different pH). Approximately 1.0 g of purified *Chelex-100* resin bead was added to 100 cm³ of the above solutions containing water soluble Cu-SOM complexes. The mixtures were continuously stirred with magnetic stirrer bars throughout the experiment. At set time intervals, ~1.5 cm³ of the solution was filtered through 0.2 µm syringe filter (Millex syringe filter with mixed cellulose ester membrane, company name, country). The sample aliquots were collected for a period of 3h. The initial time for the kinetic measurements (t=0) was taken as the time just before adding *Chelex-100*. The changes in water soluble Cu concentrations (at different pH) in presence of *Chelex-100* with respect to time were determined by ETAAS (Perkin Elmer, PinAAcle 900T, and Waltham, Massachusetts, USA). Amount of the sample needed for this experiment was high (~10g each pH). Thus, ST-1 was taken as a representative station for this experiment.

3. Theory

3.1 The Kinetic Model

The Kinetic Model developed by Olson and Shuman, (1985) was adapted (Chakraborty and Chakrabarti, 2006; Chakraborty et al., 2006, 2009, 2011) to investigate the kinetically labile forms of Cu complexes. The detailed description of the model has been given in literature (Chakraborty et al., 2006, 2009, 2011).

The dissociation reaction of Cu-sediment complexes is presented by the following pseudo first order rate law.

$$\frac{-dc_{Cu-SOM_i}}{dt} = k_i c_{Cu-SOM_i} \quad (1)$$

The integrated rate law derived from Eq. (1), expressed in terms of Cu-Chelex, the concentration of Cu associated with chelex, $C_{Cu-Chelex}$. the Cu concentration in water soluble fraction after adding Chelex, which is decreasing exponentially with time to a limiting value as follows,

$$c_{Cu-WL(t)} = \sum_{i=1}^n c_{Cu-WL_i} (e^{-k_i t}) \quad (2)$$

Where, WL is the water soluble ligand.

The above system can be estimated by a two component first order reaction model.

$$c_{Cu-WL}(t) = c_1 (e^{-k_1 t}) + c_2 (e^{-k_2 t}) \quad (3)$$

Where $c_{Cu-WL}(t)$ is the concentration of Cu present in the water soluble fraction after adding Chelex at time t , c_1 , c_2 are the concentrations of Cu with the ligand present in the water soluble fraction as first (labile with respect to chelex) and second (inert with respect to chelex, within the time scale of measurement) component respectively. k_1 and k_2 are the corresponding dissociation rate constants.

3. Results and discussion

3.1 Variation of biogeochemical parameters

The general chemical parameters of the overlying bottom water column (pH and salinity) and the sediment samples (such as, total sedimentary nitrogen (TN) and total sedimentary organic matter (SOM) at the four stations is presented in Table 1. Not much variation of pH and salinity of the overlying water column was observed in all the four stations. The pH and salinity ranged from 7.2 - 7.6 and 13.8 - 14.6 PSU respectively.

The sedimentary TN varied between 0.14 - 0.22% with a mean of $0.18 \pm 0.03\%$ (in weight percentage). The concentration of SOM ranged from 1.96 - 4.02% with a mean of $3.19 \pm 0.69\%$. A strong positive correlation between the SOM and TN (as shown in **Figure SM 2**; $r^2 = 0.84$, $p < 0.05$) suggests that the sedimentary TN was mainly associated with the SOM. A very similar observation has been reported in the literature (Gireeshkumar et al., 2013; Kurian et al., 2013; Paropkari et al., 1987; Chakraborty et al., 2015; Yuan Li et al., 2016) from different estuarine/ marine sediments. They have also reported that strong positive correlation between the SOM and TN can be used as an indicator of the nature of sedimentary N (organic or inorganic). The C: N ratio in the studied sediments varied from 16.7 to 18.3. Sedimentary organic matter has been widely classified based on

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their C/N ratio (Meyers and Ishiwatari 1993; Meyers 1997; Zhang et al. 2009, Chakraborty et al. 2016b). Chakraborty et al. (2016) have reported that SOM with C: N ratios ~11-21 are non-labile and SOM with low C: N ratios (C: N=4-10) are labile in nature. The high C: N ratio (ranging from ~16 to 18, Table 1) in the studied sediments suggests that the SOM were non-labile in nature and probably derived from the nearby mangroves and terrestrial regions.

The total sedimentary Cu concentration varied from 63.7 ± 0.5 to 77.9 ± 0.9 mg. kg⁻¹ (Table 1). A very similar concentration of sedimentary Cu has been reported in the literature (Cheryl and Nayak, 2016; Heidi and Nayak, 2016). The average range of sedimentary Cu concentration reported in Zuari estuary varies from ~20-112 mg.kg⁻¹.

The Zuari River originates from the Western Ghats and mostly drains through the geological formations such as, Quartz-sericite schist, phyllite with banded iron formations, Granite gneiss, metabasalts, metasediments and Quartz-clorite-biotite schists (Dessai et al. 2009). Therefore, the sediments in the Zuari river are mainly formed due to weathering of both mafic and felsic composition. However, total Cu content is not a useful key to understand the speciation and bioavailability of Cu in sediment. Thus, further investigation was carried out to know the distribution and speciation of Cu in different binding phases of the estuarine sediments.

3.2 Geochemical fractionation of Cu in mangrove estuarine sediments

The distributions of Cu in different binding phases of the sediment are shown in Figure 2 (the absolute concentrations of Cu associated with different binding phases are shown in Table 2).

The lowest fraction of the sedimentary Cu was present in the form of water soluble Cu- complexes (Fr-1) (the percentage varied from 0.23 to 0.32% of the total Cu). The fraction of exchangeable and CO₃²⁻/HCO₃⁻ forms of Cu (Fr-2) varied from ~0.4 to 0.1.3 % of the total Cu. The fraction of Cu associated with reducible phase (Fe-Mn oxyhydroxide binding phase) (Fr-3) varied from 0.5 to 1.1 % of the total Cu. The oxidizable binding phase (sedimentary organic matter and sulphide) was the primary hosting phase for non-residual Cu. A major part of the total Cu (5-8% of the total Cu) was associated with the oxidizable phase (Fr.4). The geochemical fractionation study suggested that major part of the total sedimentary Cu was present within the structure of the sediment (i.e., in the residual phase, Fr. 5) in all the four stations ranging from ~55-70 mg kg⁻¹(85-90% of the total sedimentary Cu). The high concentration of Cu in the residual fraction of the sediment was from the contributing source rock (Badri and Aston.,1983). The residual Cu concentration in this study was well fit with the average Cu concentration in basalt and gabbro (common mafic rocks) is 40–60 mg kg⁻¹(Ure and Berrow, 1982).Cu has been reported to form thermodynamically stable complexes with

sedimentary organic matter in the sediment (Chakraborty et al., 2007; 2016a). Many studies have reported that the conditional stability constants of Cu-SOM complexes are high and ranging from 10^8 to 10^{14} (Van der berg et al.1984, Coale and Bruland., 1990; Moffet et al., 1990; Chakraborty et al 2016b). The high SOM content in the studied sediments and higher affinity of Cu towards organic binding phase probably increased the concentration of Cu associated with sedimentary organic matter (oxidizing binding phase).

It is important to note that SOM are usually polyfunctional, polyelectrolyte and polydisperse. Therefore, pH is expected to have tremendous impact on Cu binding capacity of oxidizable phase of the sediments. It has also been reported that (Vanderberg et al., 1982) the stability of Cu complexes changes with changing pH of the system. Thus, further study was carried out to recognize the impact of varying pH of overlying water on mobility and transformation of Cu-sediment complexes in a mangrove system.

3.3. Impact of changing pH on Cu distribution in different binding phases of the estuarine sediments.

Table 3 shows the variation of Cu content in different geochemical phases of the studied sediment as a function of pH. The concentrations of Cu in residual phases did not vary with changing pH of the overlying water in all the stations. This result indicates that mobility and lability of Cu complexes in residual phase does not get affected with changing pH of the overlying water column.

However, there was redistribution of Cu among the non-residual binding phases of the sediments with changing pH. The concentration of water soluble Cu complexes (Fr-1) in the sediments was found to increase approximately 0.04-0.12 mg.kg⁻¹ with increasing pH (pH 5.00±0.05 to 8.00±0.05) of the system (**Figure 3a**).

The correlations of the changing concentrations of Cu in Fr-1 with varying pH (5.00±0.05 to 8.00±0.05) were found to be positive and strong with r^2 values 0.96, 0.79, 0.99 and 0.93 at ST-1, ST-2, ST-3 and ST-4 respectively. This is typically not the case that is reported in the literature. Decrease in water soluble sedimentary Cu is expected at higher pH. (Li et al.,2013; Tills and Alloway., 1983; Ram and Verloo., 1985; Chuan et al., 1996; Thornton., 1996).

However, with the increase in pH, the concentration of Cu in Fr-2 and Fr-3 decreased (Figure 3b shows negative correlation with r^2 values 0.87, 0.84, 0.93 and 0.93 and Figure 3c with r^2 values 0.96, 0.88, 0.68 and 0.59 at ST-1, ST-2, ST-3 and ST-4 respectively). At higher pH, higher association of Cu with Fr.2 and Fr.3 was expected due to increasing availability of binding sites in

carbonate/bicarbonate and Fe/Mn oxyhydroxide phases of the sediments but the opposite trend was observed.

It has been reported that organic matter opens up new binding sites at higher pH values for metal complexation. Disaggregation of organic matter at high pH probably helps to open up new binding sites for Cu complexation (Chakraborty et al., 2010). Faster water exchange rate of Cu(II) enhance the complexation rate of Cu with the organic ligands. In addition this Cu-ligand complexes gain extra stability by the Jahn-Teller effect. The combined effect of these two factors and high ionic potential leads to the high affinity of Cu towards organic binding sites which shifts more Cu association with sedimentary organic matter than any other binding phases in the sediments. (Chakraborty et al., 2016b). Thus, in spite of having more of carbonate/bicarbonate and Fe/Mn oxyhydroxide phase at higher pH, Cu preferred to associate with the organic phase to form thermodynamically more stable complexes.

To understand the unexpected observation of increasing water soluble Cu complexes, further investigation on water soluble Cu-complexes were carried out. The concentration of dissolved SOM in Fr.1 of the sediments is presented in Table 4. Increasing concentration of dissolved SOM was observed with increasing pH of the system (Figure 4). The high concentration of water soluble SOM at high pH was due to the disintegration and dissolution of SOM at higher pH. Therefore, it was assumed that Cu-SOM complexes might have leached out at higher pH and increased Cu concentration in water soluble fraction (Fr-1) of the studied sediments. This study showed that there was redistribution of Cu among Fr-1, Fr-2, Fr-3 and Fr.4 sedimentary phases with varying pH.

3.4 Mass balance of Cu in different binding phases of the mangrove sediments

The out flow and inflow of Cu from one binding phase to another is pictorially represented in **Figure 5**. It shows that there were outflows of Cu from Fr-1 (Water soluble Cu-complexes), Fr-2 (ion-exchangeable, and carbonate/bicarbonate forms of Cu) and Fr-3 (Cu bound with Fe–Mn oxyhydroxides) towards Fr-4 (Cu associated with organic phases) of the sediments.

Outflow of Cu was probably there from Fr-4 to Fr-1 of the sediments. The exact values of mass balancing clearly indicate that there was exchange of Cu from one binding phase to another (**Table 5**). The decrease in Cu concentrations (with increasing pH of the overlying water column) in Fr-2 and Fr-3 were found to be equal to the increase in Cu concentrations in Fr-1 and Fr-4 (i.e. mass losses was equal to the mass gain in the system). Previously, it was shown that there was increase in concentration of SOM and Cu in Fr1. Thus, one could conclude that Cu-SOM complexes probably leached out to water column at higher pH.

At ST-1, as the pH increased, there was an increase of approximately 0.29 mg.kg⁻¹ of Cu, together in Fr-1 and Fr-4, and a decrease of Cu, together in Fr-2 and Fr-3, by a similar magnitude. Sediment samples at ST-2, ST-3 and ST-4 also showed similar mass balancing (**Table 5**). This study showed that the concentration of water soluble Cu complexes increased with increasing pH of the overlying water column. By extension, the mobility of Cu complexes is expected to increase in estuarine mangrove sediments at higher pH.

For the sake of simplicity we assume that the concentration of water soluble Cu complexes is directly proportional to the pH of the solution, and fit the values in the equation of a straight line, $y=mx+c$. Positive slope value (m) clearly indicated an inflow of Cu- complexes and negative m value indicated an outflow of Cu- complexes from the other binding sites.

The slopes and intercepts of the straight line fits in Figures 3 is given in Table 6. It may be noted that the sum of slopes for a particular station is near zero, as required by Equation (5). This implies that, the straight lines fitted in **Figure 3** are robust. An extension of this is that the inferences drawn, in this section, based on mass balance is well made.

3.5. Stability of water soluble Cu complexes in estuarine sediments-

The stability of water soluble Cu complexes in estuarine sediment at different pH was determined by using competing ligand exchange method in conjunction with ETAAS. *Chelex-100* cation exchange resin was used as a competing ligand. Figure 6 shows the changes in concentrations of kinetically labile (water soluble) Cu complexes as a function of time. Each curve in Figure 6 shows an exponential decay (in presence of *Chelex-100*) in dissolved Cu concentration with time. The curves in Figure 6 were fitted to two component first order reaction model. Each curve shows two different features, a fast descending part and a slow descending part. The fast descending part represents a rapid dissociation of Cu complexes from water soluble fraction (c_1 %) with a rapid dissociation rate constant kd_1 (i.e rapid association of Cu with *Chelex-100*). The slowly descending part represents more strong water soluble Cu species (c_2 %) with a dissociation rate constant kd_2 , which is relatively reluctant to dissociate to form chelate with *Chelex-100*. The figure clearly shows stability of water soluble Cu complexes was different at different pH. This study showed that the water soluble Cu complexes at pH 8 in ST-1 had the smallest percentage of labile Cu- complexes (37.3 %) with a dissociation rate constant $K_{d1} = 37.2 \times 10^{-3}(s^{-1})$. The water soluble fraction at pH 7 had significantly higher amount of labile Cu-complexes than that of pH 8 (41.5%) with a dissociation rate constant ($K_{d1} = 0.67 \times 10^{-3} s^{-1}$). About 52.1 % of labile water soluble Cu-complexes were found at pH 5 with a dissociation rate constant of $K_{d1} = 2.7 \times 10^{-3} s^{-1}$. Experimental data are presented in Table 7. The

kinetic study showed that there was an increase in the concentration of water soluble Cu with increasing pH. It was interesting to note that there was an increase in the stability of water soluble Cu-SOM complexes with increase in pH. It is widely reported in the literatures (Tills and Alloway., 1983; Ram and Verloo., 1985; Chuan et al., 1996; Thornton., 1996) that metal solubility tends to increase at lower pH and decrease at higher pH values. However, the first time we present that disaggregation of sedimentary organic matter (complexed with Cu) may increase mobility of sedimentary Cu at higher pH in a mangrove system.

Conclusion

This study shows the impact of pH variation of overlying water on transport and transformation of Cu complexes in mangrove sediment system. The major part of the sedimentary Cu was largely present within the structure of the sediments (residual phase). The concentration Cu in the residual phase remains unchanged under varying pH of the overlying water column.

The fraction of Cu associated with different non-residual binding phases was found to undergo redistribution among the different binding phases with changing pH of the surrounding environment. The strong complexation of Cu with sedimentary organic matter (SOM) increased the association of Cu with SOM at higher pH. Disaggregation of SOM and subsequent opening up of new Cu-binding sites in sediment probably facilitated Cu to move towards the sedimentary organic binding phase of the sediments with increasing pH. This study proved that a part of sedimentary Cu-SOM complexes disaggregated and leached into the water soluble fraction at higher pH. However, the stability of water soluble Cu complexes (mainly Cu-SOM) increased at higher pH. This study proposes that increasing pH of overlying water column may decrease lability of Cu- complexes in sediments but may increase mobility of Cu complexes in mangrove sediment system.

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CAPTIONS AND LEGENDS FOR FIGURES

Figure 1: Study area, Sancoale, Zuari estuary, Goa, India.

Figure: 2 Geochemical fractionation of Cu in different binding phases of the sediments

Figure: 3 Variation in concentration of Cu (mg.kg^{-1}) at different pHs in the studied sediments.

Figure: 4 Variation in concentration of water soluble sedimentary organic matter (SOM) (mg.kg^{-1}) at different pH in the studied sediments.

Figure: 5 Graphical representation of rearrangement of Cu concentration within the different binding phases of the estuarine sediments (mass balancing) during pH variation from 5 to 8.

Figure 6: Kinetically distinguishable water soluble Cu complexes at four different pH, using Chelex-100 as a competing ligand. The Cu values are presented as percentage of the total water soluble Cu concentration.

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Table 1: Geographical locations of sediment sampling sites, pH and salinity of the overlying bottom water, and percentage of total nitrogen (TN), percentage of sedimentary organic matter (SOM), molar C/N ratio, and total Cu concentration (mg.kg^{-1}) in the studied sediments.

Station	Lat ($^{\circ}\text{N}$)	Long ($^{\circ}\text{E}$)	pH	Salinity (psu)	TN (%)	SOM (%)	Molar C/N	Total Cu mg.kg^{-1}
ST-1	15 $^{\circ}$ 24'23.16	73 $^{\circ}$ 53'18.55	7.3 \pm 0.1	14.2	0.16	2.89	18.3	64.0 \pm 1.0
ST-2	15 $^{\circ}$ 24'21.78	73 $^{\circ}$ 53'19.14	7.5 \pm 0.1	13.8	0.14	1.96	17.2	63.7 \pm 1.0
ST-3	15 $^{\circ}$ 24'21.04	73 $^{\circ}$ 53'16.22	7.3 \pm 0.1	14.1	0.22	3.46	16.7	77.9 \pm 2.0
ST-4	15 $^{\circ}$ 24'20.88	73 $^{\circ}$ 53'12.84	7.6 \pm 0.1	14.6	0.18	2.93	17.4	71.2 \pm 1.0

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Table:2 Concentration of Cu associated with different binding phases of the studied estuarine sediments.

Station	Fr-1 (Water soluble Cu) (mg.kg ⁻¹)	Fr-2 (Exchangeable, Cu associated with CO ₃ ²⁻ /HCO ₃ ⁻), (mg.kg ⁻¹)	Fr-3 (Cu associated with Fe-Mn oxyhydroxides) (mg.kg ⁻¹)	Fr-4 (Cu in organic fraction) (mg.kg ⁻¹)	Fr-5 (Residual Cu) (mg.kg ⁻¹)
ST-1	0.20±0.0	0.84±0.1	0.43±0.0	7.01±0.1	55.5±0.7
ST-2	0.15±0.0	0.28±0.1	0.35±0.1	3.15±0.1	59.8±2.1
ST-3	0.23±0.0	0.63±0.0	0.51±0.1	6.27±0.1	70.3±0.6
ST-4	0.18±0.0	0.77±0.1	0.75±0.1	5.72±0.1	63.8±1.1

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Table: 3 Concentration of Cu associated with different binding phases of the studied sediments at different pH.

Station	pH	Fr-1 (mg.kg ⁻¹)	Fr-2 (mg.kg ⁻¹)	Fr-3 (mg.kg ⁻¹)	Fr-4 (mg.kg ⁻¹)	Fr-5 (mg.kg ⁻¹)
ST-1	5.00±0.05	0.07±0.0	0.33±0.0	0.33±0.1	7.27±0.1	55.5±0.7
	6.00±0.05	0.08±0.0	0.68±0.1	0.28±0.0	7.44±0.1	
	7.00±0.05	0.11±0.0	0.67±0.0	0.25±0.0	7.44±0.1	
	8.00±0.05	0.13±0.0	0.62±0.1	0.23±0.0	7.50±0.0	
ST-2	5.00±0.05	0.08±0.0	0.52±0.1	0.42±0.1	2.93±0.1	59.8±2.1
	6.00±0.05	0.08±0.0	0.35±0.1	0.33±0.1	3.18±0.1	
	7.00±0.05	0.09±0.0	0.37±0.1	0.30±0.0	3.19±0.1	
	8.00±0.05	0.12±0.0	0.26±0.0	0.28±0.0	3.28±0.1	
ST-3	5.00±0.05	0.04±0.0	0.61±0.0	0.37±0.1	6.62±0.1	70.3±0.6
	6.00±0.05	0.08±0.0	0.60±0.1	0.34±0.0	6.63±0.1	
	7.00±0.05	0.11±0.0	0.54±0.1	0.31±0.0	6.68±0.1	
	8.00±0.05	0.16±0.0	0.49±0.1	0.32±0.0	6.67±0.1	
ST-4	5.00±0.05	0.14±0.0	1.62±0.1	0.29±0.0	5.36±0.1	63.8±1.1
	6.00±0.05	0.19±0.0	1.47±0.1	0.27±0.0	5.48±0.1	
	7.00±0.05	0.20±0.0	1.44±0.1	0.29±0.0	5.50±0.1	
	8.00±0.05	0.26±0.0	1.37±0.1	0.24±0.0	5.54±0.1	

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Table:4 Concentration of sedimentary organic matter in water soluble fraction (SOM) (mg.kg^{-1}) at different pH in the studied estuarine sediments.

pH	Concentration of sedimentary organic matter in water soluble fraction (SOM) (mg.kg^{-1})		
	ST-1	ST-2	ST-3
5.00 \pm 0.05	3.9 \pm 0.1	3.4 \pm 0.1	3.9 \pm 0.1
6.00 \pm 0.05	4.8 \pm 0.1	4.2 \pm 0.1	4.6 \pm 0.1
7.00 \pm 0.05	6.2 \pm 0.1	4.7 \pm 0.1	6.0 \pm 0.1
8.00 \pm 0.05	8.6 \pm 0.2	6.5 \pm 0.1	8.2 \pm 0.2

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Table: 5 Concentration difference (inflow and out flow) of Cu (mg.kg⁻¹) when pH varies from 5 to 8 in each fraction of the studied sediments. (Negative sign indicates the outflow/ decrease in concentration)

Concentration of Cu (mg.kg ⁻¹) pH varies from 5-8				
Stations	Inflow in Fr-1	Out flow from Fr-2	Out flow from Fr-3	Inflow in Fr-4
ST-1	0.07	-0.20	-0.09	0.22
ST-2	0.04	-0.26	-0.14	0.35
ST-3	0.11	-0.12	-0.05	0.05
ST-4	0.11	-0.25	-0.04	0.18

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Table 6: The slopes and intercepts of the straight line (Figure 3)

STATIONS	Constants for fractions m ($\times 10^{-2}$)				Sum Of m values
	Fr-1	Fr-2	Fr-3	Fr-4	
ST-1	2.3	-6.1	-3.1	6.9	0.001
ST-2	1.3	-7.6	-4.4	10.7	-0.001
ST-3	3.8	-4.1	-1.7	2.1	0
ST-4	3.7	-7.9	-1.3	5.6	0.001

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Table 7: Kinetically distinguishable water soluble Cu species and their corresponding dissociation rate constants at different pH of estuarine sediment, ST-1.

pH	c ₁ (%)	k _{d1} $\times 10^{-3}$ (s ⁻¹)	c ₂ (%)	k _{d2} (s ⁻¹)
5	52.1 \pm 1.3	2.7 \pm 0.1	50.4 \pm 1.3	2.7 \pm 0.1 $\times 10^{-3}$
6	82.3 \pm 2.3	14.0 \pm 0.4	23.0 \pm 0.6	< 1 $\times 10^{-4}$
7	41.5 \pm 1.4	0.67 \pm 0.0	53.5 \pm 1.8	< 1 $\times 10^{-4}$
8	37.3 \pm 0.8	37.2 \pm 0.8	81.4 \pm 1.7	< 1 $\times 10^{-4}$

Formatted Table

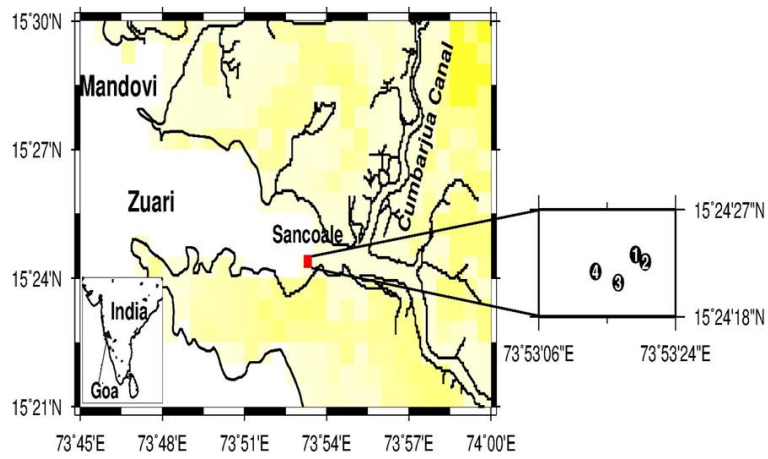


Figure 1

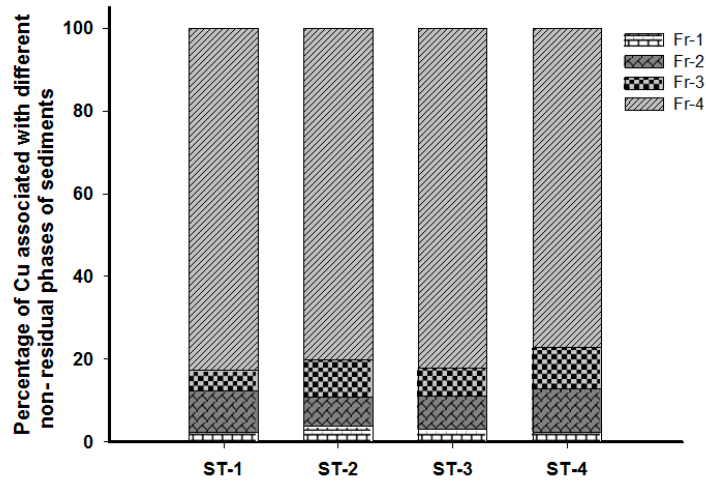


Figure 2

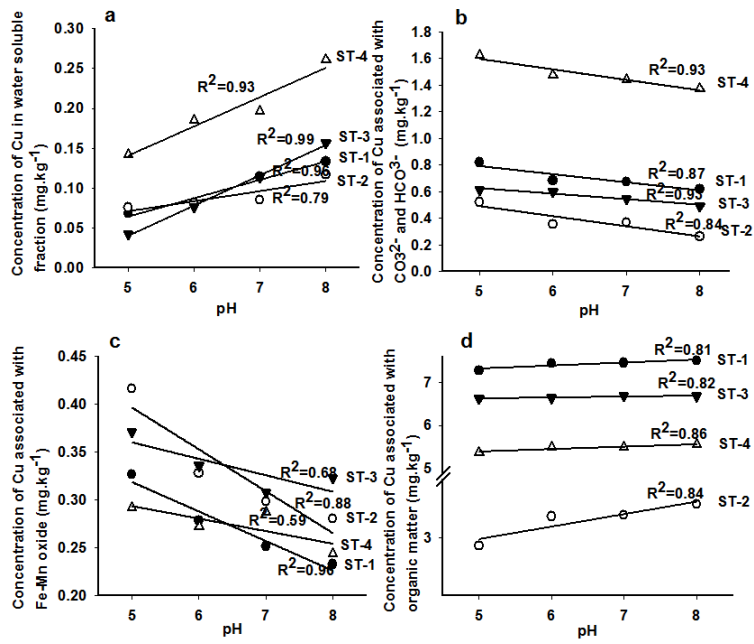


Figure 3

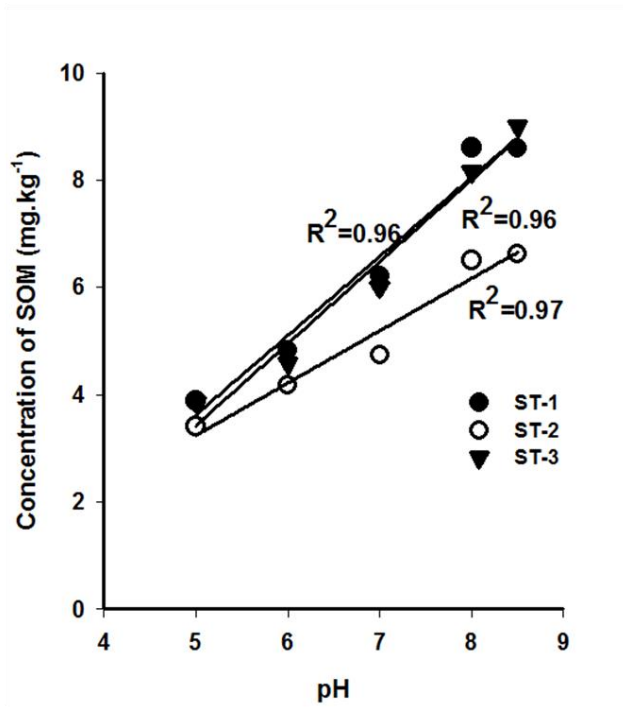


Figure 4

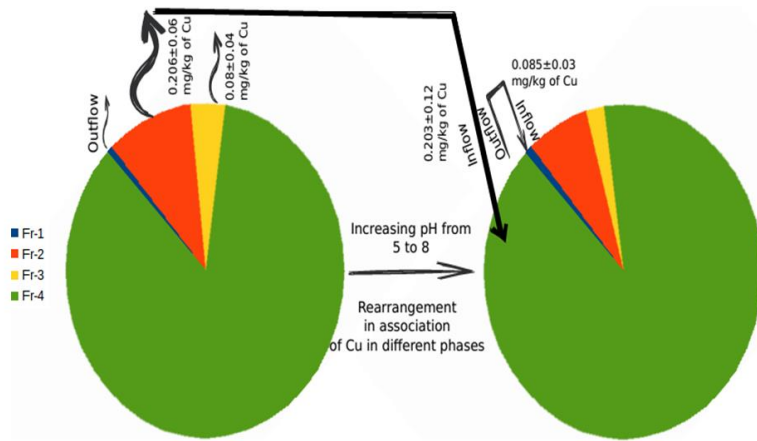


Figure 5

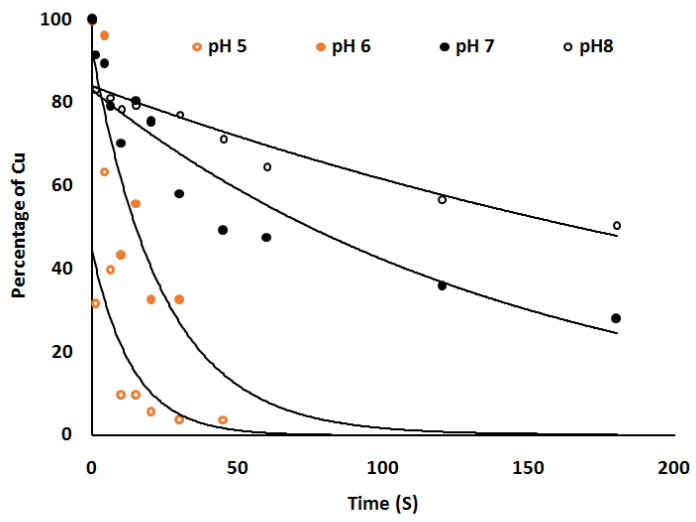


Figure 6

Supporting documents

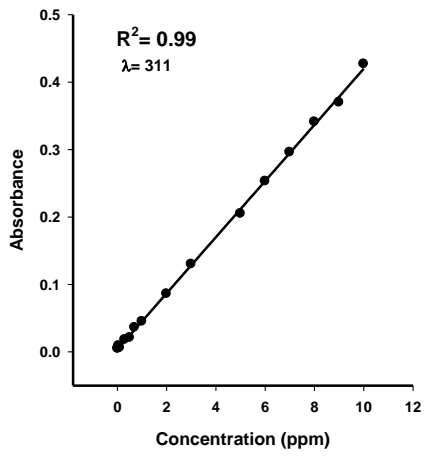


Figure SM 1 Calibration curve of Fluca sigma humic acid at wavelengths 311.

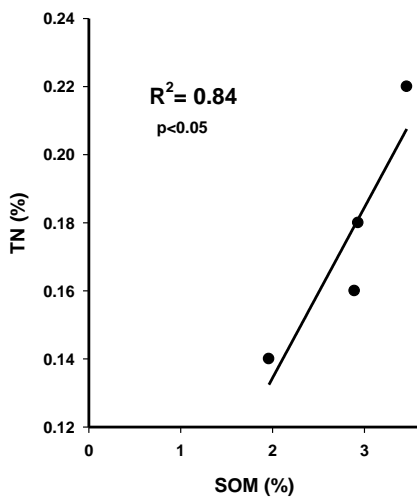


Figure SM 2: Variation of TN with SOM.