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Copper Binding by Dissolved Organic Matter in Freshwaters in Kenya

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Abstract The binding of Cu2+ to dissolved organic matter in eight different natural water samples obtained from different locations in Kenya was studied by potentiometric titration method. The DOC contents of the eight natural water samples ranged from 2.71 to 374 mg/L. The water samples were adjusted to a uniform background electrolyte concentration of 0.5 M $NaNO_3$ and pH 6 and the temperature maintained at 20°C during the assay. A Nernst equation E (mV) = –32.175 pCu + 305.5 (R^2=0.9996) obtained for the Cu2+ calibration standards (10^{-4}–10^{-6} M) was used to determine the concentration of free and bound Cu2+, respectively. The bound [Cu2+] varied by a factor of 2, ranging from 2.67×10^{-3} mol/kg DOC (in a high DOC alkaline lake water sample) to 3.2962×10^{-1} mol/kg (in a low DOC river water sample). The binding constants (Log K) varied by a factor of 2 and ranged from 3.43 to 5.57. The percentage binding ranged from 87% to almost 100% in the eight natural water samples. A Suwannee River Fulvic Acid standard sample (40 mg/L DOC) was used for comparison and for validation of the method.

Keywords Copper complexation · Dissolved organic matter · Freshwaters · Kenya

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One of the most important roles of dissolved organic matter (DOM) in natural aquatic ecosystems is its ability to bind to inorganic solid surfaces (e.g. alumino silicate clays), heavy metal ions and organic micro pollutants. DOM therefore plays an important role in the transport and distribution of heavy metals and organic contaminants and influences their bioavailability and toxicity to aquatic organisms. With increasing concern regarding toxic residue contamination in freshwaters in Kenya there is need to understand the fate, transport and toxicity of pollutants within the ecosystems. Since Cu is considered to bind primarily to carboxylic and phenolic groups, Cu-binding studies can reveal compositional characteristics of DOM as well as the influence of DOC concentration and origin on binding and bioavailability of toxic metals in the various freshwaters in Kenya which differ in terms of geological origin, dissolved organic carbon (DOC) content and salt concentration. Heavy metals associate preferentially with hydrophilic DOM fraction and the organic hydrophobic micro-pollutants to hydrophobic DOM and these binding processes increase their solubility and mobility in water, as well as their sorption onto sediments and soils. Ligand exchange is the predominant mechanism of DOM binding to Al oxide surfaces enhanced by hydrophobic effects and the heat of this adsorption has been found to be consistent with ligand exchange mechanisms (Driscoll 1984; Cronan and Aiken 1985). Experiments with $Al(OH)_3$ gel, goethite, to investigate competitive sorption of hydrophobic and hydrophylic dissolved organic matter fractions and the influence of inorganic anions on DOM sorption have shown that inorganic anions such as SO_4^{2-} and $H_2PO_4^{2-}$ cause decrease in sorption of hydrophobic fraction of DOM, leaving hydrophilic fraction therefore as most mobile organic fraction of DOM (Kaiser and Zech 1997).

DOM-induced transport is more pronounced for metals than for hydrophobic organic contaminants because sorption of hydrophobic contaminants to DOM (which is bound to soil and sediment matrix) is relatively stronger resulting in lower mobility (Davis 1989). Potentiometric titration and electrophoresis results indicate that most of the acidic functional groups of the adsorbed organic matter are neutralized by protons from solution and the organic coating is consequently expected to have a great influence on subsequent adsorption of inorganic cations/anions and almost all particles in natural waters are negatively charged due to adsorbed organic matter (Davis 1989; De Witt et al. 1991; Bryan et al. 2002; Jain 2004). Adsorbed organic matter can therefore mask the properties of underlying solid and present a surface with very different physicochemical properties. The adsorption of trace metals by organically coated particles may then be significantly influenced by the presence of carboxylic and phenolic chelating groups at the surface. Adsorption of anions can also have a strong influence on adsorption of trace metals as well, due to competition for surface sites or electrostatic repulsion.

The binding strengths between the metals and DOM are dependent on the intrinsic chemical affinities of the metals for the humates and the electrostatic forces which are dependent upon humic charges and solution strengths (Tipping 1994; Bryan et al. 2002; Jain 2004). The metal solubility in aqueous media is therefore principally controlled by pH, concentration and type of chelating ligands, the oxidation state of the mineral components and the redox status of the environment of the system. However, trace metals never approach solubility limits in natural waters. It is the partitioning of the metals that is affected. The measurement of total metal present in the aquatic environment alone may not suffice to evaluate metal speciation in natural waters as each form of the metal ion or complex may have different bioavailability and toxicity. It is also necessary to understand the binding kinetics of metal species in DOM in natural waters.

In the study reported here, different freshwater samples from various sources in Kenya including Lakes Victoria, Nakuru and Naivasha, which are experiencing increasing pollution loads as a result of rapid growth in population as well as in agricultural and industrial developments, were adjusted to the same ionic strength (0.5 M $NaNO_3$) and pH 6 and used to determine Cu-binding constants in DOM in different natural waters by a Cu ion-selective electrode (ISE) titration method.

Materials and Methods

A Shimadzu TOC5050 Analyser operating at 680°C was used to determine the dissolved organic carbon (DOC), the dissolved total carbon (TC) and dissolved inorganic carbon (DIC) content in water samples. The limit of detection of DOC was 0.5 mg C/L. A Radiometer combination electrode (GK2401C) was used to determine the pH of the water samples. A Cu^{2+}-Ion Selective (ISE) electrode, an Orion Research Microprocessor Ion Analyser 901, was used for titration to determine the concentration of dissolved free Cu^{2+} ($[Cu^{2+}]$) during the experiment.

Freshwater samples, including lake, river and spring waters, were obtained from various locations in Nyanza, Western, Rift Valley and Nairobi provinces in Kenya in January 2004. This month fell in a dry season period with fairly dry weather throughout Kenya. In total, eight sampling sites were covered, including JA: lake water from Lake Naivasha (near Naivasha town); JB: river water from Athi River (near Athi River town); JC: river water from River Nzoia, Siaya (near Imbaya Village); JD: spring water from Kong'wen Spring, Siaya (Nyabeda Village); JE: lake water from Lake Victoria (Kisumu Pier); JF: river water from River Yala (near Yala town); JG: stream water from the small Chiromo River, Nairobi; JH: lake water from Lake Nakuru near Nakuru town (at the Nakuru National Park); SRFA: Suwannee River Fulvic Acid standard obtained from the International Humic Substances Society (IHSS), Minnesota, USA (see Table 1). The water samples were taken in acid-washed polyethylene containers taking care not to disturb the sediment at the bottom. Powder-less gloves were worn throughout the sampling procedure and the containers were rinsed with some sample water prior to collection. In the laboratory, the water samples were first filtered through cellulose nitrate (Whatman GF/F 0.7 μm) membrane filters to remove particulates leaving only the dissolved organic matter and then stored in the fridge in the dark. The dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and total dissolved carbon (TC) concentrations in the water samples were determined in the DOC analyzer and results are in Table 1. The pH's of the water samples were also determined before the assay and are recorded in Table 1.

Table 1 Water samples, sampling sites, pH and DOC contents

Sample	pH (20°C)	DOC (mg/L)	DIC (mg/L)	TC (mg/L)
JA	7.71 ± 0.13	16.21 ± 0.26	31.97 ± 0.57	48.18
JB	7.75 ± 0.09	6.71 ± 0.13	18.04 ± 0.32	24.75
JC	7.51 ± 0.21	4.9 ± 0.24	13.11 ± 0.13	18.01
JD	6.9 ± 0.05	3.46 ± 0.11	13.93 ± 0.21	17.39
JE	7.3 ± 0.22	6.97 ± 0.21	17.71 ± 0.11	24.68
JF	7.4 ± 0.12	2.71 ± 0.12	8.002 ± 0.04	10.71
JG	7.2 ± 0.14	4.09 ± 0.06	19.08 ± 0.14	23.17
JH	10.3 ± 0.11	374 ± 4.48	502 ± 10.6	876
SRFA (10 mg/L)	6.9	10	0.41	10.41

Triplicate 20 mL Cu^{2+} standard solutions of 10^{-6}, 10^{-5}, 10^{-4} M concentrations were prepared in Milli Q water, in HNO_3 acid-washed Milli-Q-water-rinsed 25 mL-volume glass vials by making appropriate dilutions of 0.1 mL $Cu(NO_3)_2$. Aliquots of $NaNO_3$ were added from a 5 M batch to give an ionic strength of 0.5 M $NaNO_3$ for all samples. Appropriate volumes of MES buffer (1 M) to give a final assay buffer concentration of 0.001 M were added. The pH was then adjusted to pH 6 by addition of small aliquots of 2 M $NaOH/HNO_3$ while stirring gently with a magnetic stirrer. The standard solutions were covered with thin plastic films and kept at 4°C in the fridge while waiting for the assay. To determine the Cu^{2+} concentrations, each water sample was transferred into a pre-cleaned glass container connected to circulating cooling water flowing via a water pump. The circulating water was connected to a thermostat-controlled water bath at 20°C to maintain a constant temperature while taking the readings. The readings were taken after 30 min, a time which was decided based on several previously conducted trials to determine the optimum time necessary for maximum binding of the Cu^{2+} by the dissolved organic matter in our experiment. The preparation of the calibration standards was as follows: 10^{-4} M Cu^{2+} solution: mix 0.2 mL 0.01 M $Cu(NO_3)_2$, 0.2 mL 1 M MES buffer, 2 mL 5 M $NaNO_3$, 17.6 mL Milli Q water; 10^{-5} M Cu^{2+} solution: mix 0.2 mL 0.001 M $Cu(NO_3)_2$, 0.2 mL 1 M MES buffer, 2 mL 5 M $NaNO_3$, 17.6 mL Milli Q water; 10^{-6} M Cu^{2+} solution: mix 0.2 mL 0.0001 M $Cu(NO_3)_2$, 0.2 mL 1 M MES buffer, 2 mL 5 M $NaNO_3$, 17.6 mL Milli Q water. The natural water samples containing various concentrations of DOC were all made in the same way to achieve a uniform pH 6 and uniform ionic strength of 0.5 M $NaNO_3$ by replacing equivalent amount of the Milli Q water in each case with same amount of natural water.

The Cu ISE titration of both calibration standards and sample solutions, respectively, was done by recording the Cu-ISE mV readings. The readings were taken while stirring gently until the potential drop (*E*) in millivolts was less than or equal to 0.1 mV in 3 min. In previous trials, we found that this required up to 30 min, depending on the $[Cu^{2+}]$ as the equilibration time is known to influence the stability constant considerably (Ma et al. 1999; Bryan et al. 2002). The determined millivolts readings for the blank calibration standards were used to perform a least squares fitting for a calibration curve [*E*(mV)] versus log [activity (free Cu^{2+})]) giving the Nernst equation. The least squares equation (the Nernst equation) for the calibration standards was used to calculate the pCu ($-\log_{10}[Cu^{2+}]$) values for the water samples using the recorded Cu-ISE millivolts readings at a concentration of added free Cu^{2+} present in the sample during the titration. The bound Cu^{2+} was calculated and the Cu-binding constants obtained as the ratio of the concentration of bound Cu^{2+} (mol/kg DOC)/concentration of free Cu^{2+} remaining in the water (mol/L). The method involved determining the unbound Cu^{2+} (in initial total Cu^{2+} concentrations ranging between 10^{-4} and 10^{-6} M Cu^{2+}) after various additions of different water samples containing different DOC concentrations at 20°C. Similar assays were also performed, simultaneously, on a Suwannee River Fulvic Acid (40 mg/L DOC) standard reference compound (DOM isolate) sample for comparison.

Results and Discussion

Table 1 shows the pH of the water samples determined at 20°C, the dissolved organic carbon (DOC) content, the dissolved inorganic carbon (DIC) content and total dissolved carbon (TC) determined as the sum of the DOC and DIC. The variations in these physical and chemical properties as shown in the table would reflect the variations in geological characteristics of the sampling sites and their locations in Kenya.

Lake Nakuru and Lake Naivasha are both located in the Rift Valley and have no outlets. Both lakes are located adjacent to large industrial towns with high population densities and are recipients of treated industrial and municipal waste effluent. Lake Nakuru is also located in an area of extensive agricultural farming and therefore exposed to a lot of biomass and agrochemical residues. It is also a well-known habitat for hundreds of thousands of birds especially the flamingos and bird droppings are a big component of the biomass that gets into its waters. Lake Naivasha is also close to the fluorspar and diatomite industries. Lake Victoria is the largest lake in Africa (covering an area of approximately 69,000 km^2) and has outlets but is located within an agricultural basin where it receives water from very many rivers and streams which influence its characteristics. The sampling site was in Kisumu City which is a large industrial city and discharges large volumes of industrial and domestic effluent into the lake. The other samples were taken from small rivers, i.e. Yala and Nzoia, both in the Lake Victoria basin, flowing through low-population and small-scale-farming regions and through vegetative swamps. Chiromo River is a small stream passing through the centre of Nairobi and is mainly exposed to domestic waste. The sampling site at Athi River was near a mineral processing factory and a cement factory.

Apart from Lake Nakuru, all the sampling sites showed near neutral pH (ranging from 6.9 to 7.75). However the pH of the water sample taken from Lake Nakuru was very high (pH 10.30) indicating that part of the lake from where the sample was taken is very alkaline and eutrophic. Apart from being a well known habitat for a large population of

Table 2 Free [Cu^{2+}], Bound [Cu^{2+}] (in mol/kg DOC), Cu^{2+}-binding constants (Log *K*) and % Cu^{2+} binding in freshwater samples

Sam	DOC (mg/L)	*E* (mV)	Total [Cu^{2+}] mol/L	Free [Cu^{2+}] pCu	Bound [Cu^{2+}] mol/kg DOC	Log *K* L/kg	% Binding
JA	16.21	89.7	10^{-6}	8.39	6.14×10^{-2}	4.79	99.6
JB	6.71	78.1	10^{-6}	7.85	1.47×10^{-1}	5.17	98.6
JC	4.90	85.4	10^{-6}	7.60	1.99×10^{-1}	5.31	97.5
JD	3.46	101.3	10^{-6}	7.05	2.64×10^{-1}	5.46	91.1
JE	6.97	95.0	10^{-6}	6.89	1.25×10^{-1}	5.16	87.0
JF	2.71	92.4	10^{-6}	6.97	3.30×10^{-1}	5.57	89.9
JG	4.09	93.1	10^{-6}	8.25	2.43×10^{-1}	5.39	99.4
JH1	374	–1.5	10^{-6}	102.57	2.67×10^{-3}	3.43	$\approx$100
JH2	374	101.2	10^{-5}	68.26	2.67×10^{-2}	3.43	$\approx$100
SRF	40	30.5	10^{-6}	8.28	2.49×10^{-2}	4.40	99.5

SAM Sample, *SRF* Suwannee River fulvic acid (SRFA) standard

bird species, part of the Lake Nakuru, from where the water samples were taken, is also located in Nakuru National Park, a little farther from human activities but subjected to a lot of biomass waste from the wild animals which drink from the lake water and this also influences DOM composition. The Lake Nakuru water was unusually very brown and this indicated high DOM. The high pH was found to correspond with high DOC concentration (374 mg/L) in Lake Nakuru water compared with other freshwater sources investigated in this study (Table 2). The Lake Naivasha sample also showed relatively high pH (7.71) and high DOC content (16.21 mg/L) which indicated possible sources as nutrients mainly and partially due to algal growth.

All the water samples had DOC concentrations <10 mg/L, except Lake Nakuru and Lake Naivasha waters. Lake Nakuru and Lake Naivasha have often captured environmental attention due to concerns of elevated levels of residual concentrations of heavy metal contaminants in the water. The high DOC content detected in the water samples from these sites would support these concerns. An extensive survey needs to be done to determine the extent of chemical pollution in these lakes as these high DOC contents can imply distribution of organic contaminants and trace metals within the lake. Lake Naivasha is very important especially for fish, second only to Lake Victoria in production of fish for local consumption and export and existence of trace chemical residues in its water can pose a big threat to the people and the fish industry. Lake Victoria has also been known to experience environmental pollution problems originating from municipal and industrial effluent and the DOC level (6.97 mg/L) recorded in this study was also quite high for such a large lake (total surface area of approximately 68,000 km^2). It is of interest to note that the DOC contents in the three tropical lake waters were higher than some of the DOC contents reported in lake waters in Europe (Tipping et al. 1988). The other sampling sites included fast flowing rivers Nzoia, Yala and Chiromo (stream) and Kong'wen Spring water and the DOC concentrations were lower, ranging from 2.71 (River Yala) to 4.90 mg/L (River Nzoia), and quite comparable to those reported by others in lake and stream waters (ranging from 0.1 to 6.2 mg/L) in central part of UK (Tipping et al. 1988). River Nzoia is located in a gently sloping terrain and could be receiving more organic load than River Yala which passes through a plain topography in the lake basin. Kong'wen Spring is a small spring with water leaching through Savanna type of vegetation but at very fast flow and consequently with very low retention of water and DOM as shown by its relatively low DOC content of 3.46 mg/L.

Cu^{2+}-complexation analysis was done first by manual ISE titration of calibration standards in dilute solutions of $Cu(NO_3)_2$ (in the Cu^{2+}-concentration range of 10^{-4}–10^{-7} M) buffered at pH 6 at 20°C and 0.5 M $NaNO_3$ background electrolyte which gave *E* (electromotive force) in millivolts readings (data not shown) which were used to prepare a standard curve (E vs. pCu) for a linear Nernst equation of the form E (mV) = Eo – spCu, where pCu is equal to –Log[Cu^{2+}], E_o is the *E* intercept on the curve and s is the slope. Our calibration standards gave an equation for the standard curve as: E (mV) = –32.175 pCu + 305.5 ($R^2 = 0.9996$). The optimum linear calibration curve was obtained within the concentration range of 10^{-4}–10^{-7} M Cu^{2+}. Outside this range, best linearity was not obtained. Secondly this was used to calculate the concentration of free Cu^{2+} (pCu) in the water samples, after taking the ISE mV readings for the water samples, all containing initial 10^{-6} M total [Cu^{2+}] concentration before complexation with DOM in the waters (Table 2). The difference between the total [Cu^{2+}] and the free [Cu^{2+}] corresponding to the potential drop after binding had occurred gave the bound [Cu^{2+}]. The partition coefficient (K) for each water sample was calculated as the ratio of the DOM-bound [Cu^{2+}] (mol/kg DOC)/free [Cu^{2+}] (mol/L water) and binding constant Log K (Table 2).

The results obtained from our titrations show the various loads of Cu^{2+} in the water samples ranging from 2.67×10^{-3} mol/kg DOC (L. Nakuru water) to 3.30×10^{-1} mol/kg DOC (R. Yala water) as in Table 2. The bound $[Cu^{2+}]$ was found to vary by a factor of 2, ranging from 2.67×10^{-3} mol/kg DOC (in a high DOC alkaline lake water sample) to 3.30×10^{-1} mol/kg (in a low DOC river water sample). The binding constants (Log *K*) varied by a factor of 2 and ranged from 3.43 to 5.57, respectively. The percentage binding ranged from 87% (Lake Victoria water) to almost 100% (Lake Nakuru water) in the eight natural water samples (Table 2). These binding constants are conditional constants for the binding under experimental conditions employed. However, important factors such as Ca, Fe, and Al concentrations in the organic matter were not controlled and this would make it difficult to compare the values obtained. The bound Cu^{2+} concentrations and the binding constants obtained in our study were similar to those reported by others who have done studies to show heavy metal binding by dissolved organic matter in different waters, mostly in temperate conditions (Buffle et al. 1977; Hering and Morel 1988; McKnight et al. 1983; De Witt et al. 1991; Tipping 1994; Dwaine and Tipping 1998; Bryan 2000; Bryan et al. 2002). The work of Bryan et al. (2002) on Cu binding in unaltered water samples (with DOC concentrations ranging between 4.4 and 26.7 mg/L) demonstrated the variability in metal-binding with DOM origin. They found that substantial amounts of Cu were complexed, especially at low total Cu (10^{-6} M) concentrations, i.e. up to 98% complexation at pH 6. They reported Cu^{2+} loadings ranging from 5×10^{-6} mol/g DOC to 5×10^{-4} mol/g DOC after titrating 15 different water samples taken from lakes, ponds, streams and rivers in North Western part of the UK, using similar ISE titration method with samples buffered at pH 6 and ionic strength of 0.1 M KNO_3 based on an initial total Cu^{2+} concentration of 10^{-6} M. David and Vance (1991) showed that hydrophobic and hydrophilic acids differ in their proton-binding capacities, and that lake water samples differ from stream samples (David and Vance 1991). Iglesias et al. (2003) reported mean Cu^{2+}-binding constant values of 5 (at pH 5.5) and 5.29 (at pH 6.5) while titrating dissolved isolated fulvic acid sample simulating natural water samples, at ionic strength of 0.1 M KNO_3 and DOM concentration of 100 mg/L (fulvic acid). Lu and Allen (2002) reported Cu^{2+}-binding in three different DOM samples extracted by Reverse Osmosis and getting small amounts of strong Cu-binding sites, a total of 4.55 mmol/g DOC, which was much less than the total acidity (carboxylic and phenolic) but very close to the phenolic site content. They suggested that it was the phenolic sites that accounted for the majority of complexation of Cu^{2+} under natural conditions by replacement of H^+ of phenolic moiety by Cu^{2+} at the binding sites.

The results indicate that Cu binding is dependent on the DOC content, although less strongly when very high DOC waters of Lake Nakuru (DOC content of 374 mg l^{-1}) were considered. A plot of Log K versus [DOC] gave a very strong power law relationship [Log $K = 6.2244[DOC]^{-0.0992}$ ($R^2 = 0.9968$)] and if the extreme values for Lake Nakuru and the SRFA are omitted from the relationship, the function coefficients change slightly and the correlation improves further. The variations in log *K* values suggest compositional differences related to source. Despite the strong dependence of binding on DOC concentration, compositional variations in DOM might also be considered as some of the major factors controlling the binding constants as shown by unexpectedly lower Log *K* values for Lake Naivasha (DOC 16.21 mg/L) and Lake Nakuru (DOC: 374 mg/L), respectively. Since Cu^{2+}-binding is expected to be on the carboxylic and phenolic sites of DOM, we found that the binding parameters provide an indication of these functional groups in the different natural waters and ISE titration can therefore provide a very reliable and quick assay method for comparing different water samples and DOM isolates of different chemical composition from different origins. All the samples (except L. Nakuru water with Log *K* of 3.43) that we analysed therefore had DOM with higher carboxylic/phenolic group contents in comparison with the SRFA sample (Log *K* of 4.40). All the water samples taken from R. Yala, R. Nzoia, Kong'wen Spring and Lake Victoria, all found within the Lake Victoria basin, indicated similar high Cu-binding abilities (with Log *K* values ranging from 5.17 to 5.57) which indicated that the waters had DOM with similar carboxylic/phenolic composition. Not all the DOM active sites in the L. Nakuru (JH) water were used up as shown by the fact that when the initial Cu^{2+} concentration was increased to 10^{-5} M, even more binding was found to take place, again giving almost 100% binding. Therefore even though binding constant determines the binding ability and therefore influences bioavailability, DOC concentration indicates ability to mask toxicity of Cu^{2+} at such high concentrations (>16.21 mg/L) as in Lake Nakuru.

Copper is present in pristine natural waters at low concentrations (<1 mg/L total dissolved Cu) in forms of ionic (free) Cu^{2+}, Cu hydroxides and carbonates and Cu-organic complexes and is also a component of several key biomolecules (Verweij et al. 1992; West et al. 2003). It can be toxic at moderately elevated concentrations (as little as 5 mg/L of total dissolved Cu) and the free Cu^{2+} is considered to be largely responsible for Cu toxicity towards aquatic organisms (Verweij et al. 1992; West et al. 2003). As modes of toxicity, Cu can interfere with membrane transport, enzyme functions, respiration, glycolysis and photosystem II electron transport pathway and may also contribute towards formation of damaging oxygen radicals

(Stauber and Florence 1987; West et al. 2003). Cu^{2+} in lake water, especially in soft waters, is moderated by organic ligands within the dissolved organic carbon (DOC) fraction and significantly higher DOC concentrations can provide protection against Cu toxicity (Meador 1991; Winner and Owen 1991). Since DOC varies in concentration and composition with origin, understanding copper binding in natural waters is useful in understanding its speciation and toxicity in these ecosystems.

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