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Binding and Water Solubility Enhancement of ^{14}C-Benzo[a]Pyrene by Dissolved Organic Matter in Freshwater in Kenya

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Dissolved organic matter (DOM) in freshwaters has a number of important ecological and geochemical functions as has been discussed by various researchers (Raber and Koegel-Knaber 1997, Abbt-Braun and Frimmel 1999). DOM functions vary from sample to sample, depending on its characteristic physical and chemical composition, which vary with origin and time. The variability in DOM chemical composition and in its functional properties makes it difficult to directly compare DOM samples from different sources, especially when modelling chemical speciation in freshwaters. It is also expected that DOM content and composition in tropical and temperate freshwaters would be different because of differences in geology and vegetation in the catchments. One of the main functional attributes of DOM in freshwaters, which is reported on in this paper, is its ability to bind hydrophobic organic contaminants. This binding influences contaminant solubility, transport and bioavailability. Despite variations in DOM chemical composition, the similarities and differences in its functional property data such as hydrophobic contaminant binding constants and solubility enhancement factors can provide useful fingerprints for distinguishing various DOM samples and can be used in modelling chemical speciation in freshwaters (Chin et al. 1997, Raber and Koegel-Knaber 1997, Haitzer et al. 1999, Kamiya and Kameyama 2001).

Previous studies indicate that low concentrations of DOM in freshwater can significantly enhance the solubility and stability of hydrophobic contaminants such as PAH's in natural waters. Sorption of DOM to hydrophobic organic contaminants expressed as partition coefficient (K_{DOC}) is found to vary depending on the source of DOM, chemical composition, age and type of isolation used to extract the DOM sample (McCarthy et al. 1989, Kukkonen et al. 1990). The ability to bind to hydrophobic contaminants has also been found to be dependent on DOM molecular size, polarity (expressed in terms of (N+O)/C or H/O ratios) and aromaticity (determined by nuclear magnetic resonance or ultra-violet spectroscopy) (Albert et al. 1994, Chin et al. 1997, Haitzer et al. 1999). These physico-chemical properties have already been studied extensively and have also been used in modelling of chemical speciation in freshwater. Other authors have also shown that DOM origin can be even more significant in explaining variability in hydrophobic contaminant

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binding than DOM concentration as shown by variation in sorption of PAH's to various natural aquatic DOM samples both temporally and spatially. With a given DOM sample, the contaminant partition coefficient increases with decrease in its water solubility. It is therefore expected that solubility enhancement would be largely restricted to those contaminants which are extremely insoluble in water such as PAH's. The relative ability of high MW solutes to enhance solute solubility is attributed to their low polarities and ability to form sizeable intra-molecular non-polar environment (micelles) and the sorption equilibrium constants for the specific contaminant in the aquatic media are also controlled by the pH and ionic strength of the media (Lee and Kuo 1999).

Benzo[a] pyrene (B[a]P) is one of the potent members of PAH family which has been used in studies involved in investigations on contaminant solubility and bioavailability. It has been reported to have a low water-solubility (10^{-4}M) or 3.8 μg/L and, on uptake, will readily interact with biological membranes and effect toxicity/biological activity (Landrum et al. 1996, Jimenez et al. 2002). In previous studies, DOM functional variability has been demonstrated in the extent of binding of benzo[a]pyrene by different DOM samples extracted from different sources i.e. from various forest soils, a wetland and a stream within the same catchment, in which the binding constants were found to vary by a factor of approximately 2.5 (Raber and Koegel-Knaber 1997).

Establishing and quantifying PAH-binding variability of DOM in tropical freshwaters can help in understanding contaminant transport and distribution as well as allow for assessment and prediction of their fate and toxicity in freshwaters. Most of the reported contaminant-binding studies have been done in temperate regions and in South East Asian countries. No such study has been done in Kenya before. With adverse weather conditions and different geological formations one would expect differences in DOC content and composition between temperate and tropical countries in Africa. The objective of our study was to quantify DOC concentrations in various freshwater samples taken from different regions in Kenya and to determine the binding constants (expressed in terms of partition coefficients (Log K_{DOC})) of ^{14}C-B[a]P) and water solubility enhancement factors in the various natural water samples. The use of radioisotopic technique involving ^{14}C-labelled compound provided the right sensitivity and accuracy for determination of these parameters. This study was preliminary but from the results obtained we would recommend that future chemical speciation studies in freshwaters in Kenya should consider the DOM factor and the DOM variations in different freshwaters.

MATERIALS AND METHODS

The radiolabelled compound, 7,10-^{14}C-benzo[a]pyrene (^{14}C-B[a]P) (Figure 1), was supplied in toluene (1 mL) by Aldrich (UK) and had a specific activity of 240 μCi/mg and radiochemical purity of 97.7% by HPLC. B[a]P is very sparingly soluble in water (water solubility of 3.8 μg/L @ 25°C and a Log K_{ow}

of 6.04 [34]. The dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and total dissolved carbon (TDC) concentrations in the freshwater samples were determined in A Shimadzu TOC5050 Analyser operating at 680 °C (Table I). Radioanalysis, to determine the concentration of the test chemical in water samples, was performed on a Packard Tri-Carb Scintillation Analyzer Model 2100 TR. A pH meter was used to determine the pH of the water samples. The ultraviolet-visible (UV-VIS) absorbance was determined in a Hitachi U-2000 Spectrophotometer.

Water samples were taken from various locations in Nyanza, Western, Rift Valley and Nairobi provinces in Kenya in January 2004. This was a period of fairly dry weather throughout Kenya. In total eight sampling sites were covered, including three lake water samples of Lakes Naivasha (sample JA from Naivasha town), Nakuru (sample JH from Nakuru National Park) and Victoria (sample JE from Kisumu city pier), four river water samples of Athi River (sample JB from Athi River town), River Nzoia (sample JC from Imbaya village, Siaya), River Yala (sample JF from Yala town) and Chiromo River (sample JG from Chiromo, Nairobi), and one spring water sample of Kong'wen Spring (sample JD from Nyabeda village, Siaya). Suwannee River Fulvic Acid (SRFA) standard sample obtained from the International Humic Substances Society (IHSS), Minnesota, USA, was also used for comparison (see Table 1).

The radiolabelled compound was diluted by taking a 10μL aliquot into 2 mL ethanol and then a 5μL aliquot (containing 30,000 dpm of radioactivity), equivalent to 13.5 μg, confirmed by liquid scintillation counting (LSC), was taken into a 16 mL-scintillation glass vial, gently placing it at one marked spot at the bottom of the vial. This was done in replicates of five for each water sample. The vials were left under the fume hood for approximately 30 minutes to let all the solvents evaporate completely leaving the ^{14}C-compound stuck at the bottom of the glass vial before addition of water samples. The water samples were first filtered through cellulose nitrate 0.7 μm membrane filters to remove particulates leaving only the dissolved organic matter and were treated before the assay to give a uniform electrolyte concentration of 0.1 M NaCl as follows: In a 250-mL Erlenmeyer flask, 26.7 mL of filtered water sample was mixed with 0.3 mL of 0.001M KH_2PO_4 (phosphate buffer to give a uniform pH 7 for all the samples), 3 mL of 1 M NaCl electrolyte (to give a uniform 0.1 M NaCl electrolyte concentration). The pH of the solution was then precisely adjusted to pH 7 with small μL- aliquots of 2M NaOH/HCl as necessary. After mixing well by gentle shaking, 3mL of sample solution was carefully taken using an Eppendorf pipette (in replicates of five) into each of the glass vials containing the ^{14}C-benzo[a]pyrene compound, leaving them to equilibrate undisturbed at room temperature (20 °C) for 48 hours under the fume hood, with all vials cocked. All the water samples were treated in the same way for the assay. After 48 hours incubation, long enough for equilibrium to be established and maximum amount of the compound to be partitioned into the water, 0.5 mL was taken, in triplicates, from each glass vial carefully with an Eppendorf pipette, taking care to avoid any disturbance of the spot where the compound had been applied at the bottom of the glass vial, into a fresh clean scintillation glass vial and mixed well with 8 mL of liquid scintillation counting cocktail (Ecoscint A®). The radioactivity in each sample vial was then determined. Simultaneously, a control electrolyte solution was also prepared and used as blank water sample (i.e. containing no DOM), using de-ionized water to give an electrolyte solution of 0.1M NaCl ionic strength and pH 7. The control samples were also prepared and placed into glass vials containing the ^{14}C-compound in five replicates and were also left to equilibrate simultaneously at the same temperature and for the same length of time. Radioanalysis of the samples was done as described above. A similar assay as described above was performed on a Suwannee River Fulvic Acid standard reference compound solution of 10 mg/L DOC. The experiments were repeated three times.

The LSC data were recorded in disintegrations per minute (dpm). The mean values were calculated and converted to μg of the compound to get the concentration in water solution (containing DOM) and in the blank electrolyte water. The partition coefficient, K_p was calculated as: K_p (units: L/Kg DOC) = (mg B[a]P bound per kg DOC)/(mg B[a]P free in 1 litre of control electrolyte water). The amount of B[a]P bound (see column 4 of table 2), based on the amount of water, in various DOM, and in various water samples are given. The solubility of the B[a]P in water samples and in electrolyte water, respectively, were calculated (based on the assumption that equilibrium conditions had been attained after 48 hours of equilibration) and the water solubility enhancement factor S_w^*/S_w was calculated as: S_w^*/S_w = (B[a]P solubility in freshwater sample containing DOM (μg/L))/(solubility in water (control electrolyte water) without DOM (μg/L)), where S_w^* = solubility of B[a]P in freshwater sample and S_w = solubility of B[a]P in control electrolyte water solutions.

A UV-VIS absorbance, at 254 nm and 340 nm, analysis of the water samples was performed on the blank (electrolyte) sample and on each water sample using 1 cm path length cell quartz cuvette. At sufficiently low concentration of electrolyte, Lambert-Beer's law [$A = \varepsilon l c$] can be applied, with absorbance A (dimensionless), concentration c (mg L^{-1}), path length l (in cm) and the quantity ε called molar absorptivity (equal to extinction coefficient) (cm^{-1} (g DOC)$^{-1}$). Based on this law, the extinction coefficients (Ext Coeff) were calculated as A_{340}/[DOC] and A_{254}/[DOC] at 340 and 254 nm, respectively (Table 1). The Nakuru water sample with very high DOC content was diluted appropriately before UV-VIS analysis.

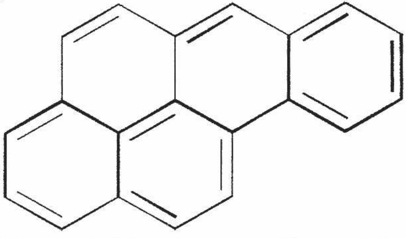

Figure 1. The structure of benzo[a]pyrene.

RESULTS AND DISCUSSION

Table 1 shows the pH of the water samples determined at 22 °C, the dissolved organic carbon (DOC) content, the dissolved inorganic carbon (DIC) content and total dissolved carbon (TC) which is the sum of the DOC and DIC. The DOC of the Suwannnee River Fulvic Acid standard was also determined and is included in the table. The variations in these physical and chemical properties as shown in the table reflect the variations in geological and geographical locations of the sampling sites. 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 68,000 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 big 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.

Table 1. Water samples, sampling sites, pH, DOC and UV-VIS absorbance.

Sample	pH(20°C)	DOC mg/L	DIC mg/L	Ext Coeff (254 nm)	Ext Coeff (340 nm)
JA	7.71±0.13	16.21±0.26	31.97±0.57	16.28	3.45
JB	7.75±0.09	6.71±0.13	18.04±0.32	58.98	17.28
JC	7.51±0.21	4.90±0.24	13.11±0.13	66.90	26.51
JD	6.90±0.05	3.46±0.11	13.93±0.21	13.30	9.25
JE	7.30±0.22	6.97±0.21	17.71±0.11	12.91	5.45
JF	7.40±0.12	2.71±0.12	8.00±0.04	61.99	30.26
JG	7.20±0.14	4.09±0.06	19.08±0.14	32.27	14.67
JH	10.3±0.11	374±4.48	502±10.6	57.22	1.60
SRFA (10 mg/L)	6.90	10	0.41	nd	68.45
Electrolyte	7.00	<1	nd	blank	blank

Note: 'nd' denotes 'not determined'; Sample JA: Lake Naivasha, JB: Athi River, JC: River Nzoia, JD: Kong'wen Spring, JE: Lake Victoria, JF: River Yala, JG: Chiromo River, JH: Lake Nakuru. Ext Coeff expressed as: cm^{-1} $(g\ DOC)^{-1}$.

The pH's of the water samples were within expected normal range and seemed to be quite comparable to those obtained by other researchers in different water samples in temperate regions, which are reported to be ranging from 6.2-7.1 in lake water and 6.6-8.1 in stream and river waters, in central part of UK (Tipping et al. 1988). However, the pH of the water sample taken from Lake Nakuru was very high (pH 10.30) indicating that the part of the lake from where the sample was taken is very alkaline and eutrophic. In fact it is known that most rift valley lakes in Kenya including lakes Nakuru, Naivasha, Elementaita and Bogoria are very alkaline with pH ranges from 9.15-11.25 (Wandiga et al. 1983). The high pH detected in water samples of Lake Nakuru is therefore mainly as result of the geochemical processes in the rift valley region. This lake does not have any outlets and water evaporation losses can also leave high salt concentrations which can consequently influence the pH. The high DOC content (374 mg/L) of this lake can contribute to the water acid-base buffering and therefore also influence the pH. This is an inland lake without any outlets and is a habitat for large population of birds especially flamingos. Part of the lake from where the water sample was taken is also located in Nakuru National Park, removed from human and industrial activities of Nakuru town but subjected to a lot of biomass waste from the wild animals which drink from the lake water and this can influence the water composition and DOM when washed down into the lake by rainfall. The Lake Nakuru water was unusually very brown and this indicated high DOM but it is unlikely that this problem extends all over the entire lake. There is a need to take different samples from various parts of the lake to ascertain the extent of the high alkaline pH and the brownness of the water. The high pH was found to correspond with high DOC concentration (374 mg/L) in Lake Nakuru water compared to other freshwater sources investigated in this study. Apart from Lake Nakuru, all the other sampling sites showed near neutral pH (ranging from 6.9-7.75). The L. Naivasha sample also showed slightly high pH (7.71) which corresponded with a high DOC content (16.21 mg/L) and indicated possible sources as nutrients or algal growth.

Apart from Lake Nakuru and Lake Naivasha, all the rest of the water samples had DOC concentrations <10 mg/L. These two lakes have often received environmental attention from concerns of elevated levels of residual concentrations of heavy metal contaminants in the water. The high DOC content detected in their water samples would support these reported cases. There is also likelihood that organic contaminants levels would also be quite high since both lakes are also located in intensive agricultural regions and near some of the large towns with high volumes of effluent from agrochemical, industrial and domestic waste being discharged into the lakes. An extensive survey needs to be done to determine the extent of chemical pollution in these lakes. 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 and fish 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 agrochemical and industrial activities and the DOC level recorded in this study was also quite high for such a large lake (total surface area of approximately 68,000 km^2). Lake Victoria sample had a pH of 7.3 and a DOC content of 6.97 mg/L. It may be of interest to note the DOC contents in the three tropical lake waters are much higher than some of the DOC contents in reported in lake waters in Europe (Tipping et al. 1988). It would be expected that long hours (an average of 12 sunshine hours daily) of intense solar radiation and constantly high temperatures in these tropical lakes could contribute to greater primary activity and microbial decay compared to temperate lakes and this could contribute to the higher DOC. In addition, Lake Nakuru water sample was taken from an area heavily inhabited by flamingos and therefore bird droppings was assumed to be one of the major contributors to its very high DOC. The other sampling sites also 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 4.903 (River Nzoia), and quite comparable to those reported by others in stream waters (ranging from 0.1-6.2 mg/L) in central part of UK (Tipping et al. 1988). River Nzoia is located in a gentle sloping terrain and could be receiving more DOM than River Yala which passes through a plain topography in the lake basin while 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 low DOC content of 3.46 mg/L. It appeared, in general, that the absorbance increased with DOC content, with the [DOC] showing a linear correlation with UV-VIS absorbance, although this linearity could not be determined with certainty because of the large difference between the Lake Nakuru DOC content (almost 30× higher) and the other freshwater water samples. R^2 value for Abs_{254} and DOC was 0.999 and that for Abs_{340} and DOC was 0.963, considering all the eight samples that were taken. When the Lake Nakuru water sample was not considered, R^2 values were 0.326 (Abs 254) and 0.136 (Abs 340), indicating no relationship between DOC and UV-VIS absorbance.

Table 2. Water solubility enhancement and binding of B[a]P by DOM .

Water sample	Water Solubility (μg/L)	Solubility Enhancement factor	B[a]P Bound (μg/L)	Partition Coefficient (K_p)	Log K_p
JA	4.084±0.44	1.26	0.846	1.6112×10^4	4.21
JB	6.389±0.36	1.97	3.151	1.449×10^5	5.16
JC	4.153±0.24	1.28	0.915	5.763×10^4	4.76
JE	6.30±0.32	1.95	3.066	1.358×10^5	5.13
JG	3.364±0.13	1.04	0.126	9.514×10^3	3.98
JH	6.863±0.32	2.12	3.625	2.993×10^3	3.48
SRFA	5.852±0.21	1.81	2.614	8.073×10^4	4.91
Elect	3.238±0.06				

Note: 'nd' denotes 'not determined'; Elect: Electrolyte.

For surface waters, many studies in temperate regions have obtained linear positive relationships between UV absorbance and DOM concentration as measured by DOC content (Tipping et al. 1988). However, from these few data, correlation appeared between samples from the same type of source e.g. samples from Yala, Athi and Nzoia Rivers which showed linear relationship between Abs_{254} and [DOC] ($R^2 = 0.970$), Abs_{340} and [DOC] ($R^2 = 0.529$), and samples from Lake Naivasha, Nakuru and Lake Victoria which showed linear relationship between Abs_{254} and [DOC] ($R^2 = 0.999$) and Abs_{340} and [DOC] ($R^2 = 1.000$).

One would expect the extent of benzo[a]pyrene binding to be more in water samples containing more hydrophobic DOM. From our results, there was a clear difference in amounts of bound B[a]P in different water samples which indicates that this method would be suitable for testing the binding of hydrophobic contaminants such as B[a]P. The water solubility data in the control electrolyte and in the various water samples as shown in table 2 were precise and comparable to its reported water solubility of 3.8 µg/L (in de-ionized water) (Jimenez et al. 2002). However, the non-linearity shown while attempting to correlate [DOC] with water solubility enhancement indicates that DOM chemical composition influences the binding more that the DOC content. Due to limited data, there were no apparent significant correlations between Log Kp and DOC ($R^2 = 0.328$) and Log Kp and Extinction coefficients ($R^2 = 0.177$ at 254 nm; $R^2 = 0.032$ at 340 nm), with correlation (Log Kp versus Extinction coefficient and Log Kp versus DOC) becoming more significant between samples from the same type of source e.g. lake waters of Lake Naivasha, Nakuru and Lake Victoria which gave some positive linear relationships between Abs_{254} and Log Kp ($R^2 = 0.440$) and DOC and Log Kp ($R^2 = 0.486$). Our results indicate that there was a limit to the maximum amount of B[a]P that could be bound even in presence of high concentrations of DOM as shown for Lake Nakuru water sample whose extremely high DOC concentration was not commensurate with the amount of bound B[a]P. However, these investigations are only preliminary since only a few freshwater samples from a few locations have been analysed. The results of this study show that the radioisotopic method of determining the binding of ^{14}C-B[a]P to natural organic matter in natural water samples is an accurate indicator of the extent of hydrophobic contaminant binding even when applied to pure DOM isolates such as SRFA. The accurate water solubility data obtained by this method also support the accuracy of this functional assay technique in determining the binding of hydrophobic contaminants to natural water DOM and DOM isolates. The results obtained from this study which show that Log K_p values ranged from 3.98 (in DOM in Chiromo River water sample) to 5.48 (in DOM Lake Nakuru water sample) and are comparable to values reported by Raber and Koegel-Knaber for five-ringed PAHs in DOM from soil, sewage sludges, waste water and composts, which ranged from 4.3-4.9 (Raber and Koegel-Knaber 1997). From the results, the water solubility of B[a]P in water samples with very low DOC (i.e. River Yala and Kongwen Spring) could not be determined as the radioactivity data appeared to be almost comparable with those obtained for the blank electrolyte. It was therefore not possible to calculate the water solubility enhancement factor and the partition coefficient for these water samples. A possible explanation would be low hydrophobic acid contents in their DOM although this was not analysed.

It has been reported that some of the physico-chemical properties of DOM, such as absorbance (at 254 nm), H/C and hydrophobic/hydrophilic ratios, aromatic and phenolic group contents and polarity, influence hydrophobic contaminant interactions with DOM and that sorption to DOM is promoted by hydrophobic moieties and impeded by hydrophilic carboxylic acids (Haitzer et al. 1999b, Cho et al. 2002). MW also plays a role, with large molecules tending to be more hydrophobic as this can encourage formation of micelles if there are a number of hydrophilic moieties. Increase in K_{DOC} (partition coefficient) is also expected to decrease with water solubility and decrease in DOM polarity and this was confirmed by our results. Ionic strength and pH effect studies have also shown that K_{DOC} increases with ionic strength and decreases with water pH (Lee at al. 2003). However, in our studies, these effects were eliminated by working at high ionic strength and constant pH.

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