

Distribution, Fate, and Effects of ^{14}C -DDT in Model Ecosystems Simulating Tropical Kenyan Freshwater Environments

S. O. Wandiga,¹ B. T. Yebiyo,¹ J. O. Lalah,² G. N. Kamau¹

¹ Department of Chemistry, College of Biological and Physical Sciences, University of Nairobi, Post Office Box 30197, Nairobi, Kenya

² Department of Chemistry, Maseno University, Post Office Box 333, Maseno, Kenya

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The distribution and fate of DDT and other persistent organochlorine pesticides in various tropical marine environments, including the Indian Ocean Coast of Kenya, have been studied by analysis of their residues in field samples of seawater, sediment and biota as well as by analysis of samples taken from simulated laboratory ecosystems (Everaarts et al. 1991, Carvalho et al. 1992, Tanabe et al. 1992, Rajendran et al. 1993, Hong et al. 1995, Everaarts et al. 1996, Wandiga et al. 2002a,b). However, the distribution, fate and effects of DDT in Kenyan fresh water environments have not been studied. Fresh water environments differ from marine environments in terms of salinity, pH, temperature and pollution load. The presence of higher concentrations of organic matter in fresh waters can influence contaminant solubility as well as its bioavailability and toxicity to aquatic organisms. Therefore, this study was designed to investigate the distribution, bioaccumulation, depuration, degradation and acute toxicity of ^{14}C -DDT in model tropical fresh water ecosystems simulating Lakes Naivasha, Baringo and Victoria in Kenya.

MATERIALS AND METHODS

A mixture of uniformly labelled ^{14}C -p,p'-DDT, specific activity 24.95 mCi/mMol and non-labelled p,p'-DDT, 98% pure by TLC obtained from NEN Research products and Aldrich Chemical Company Inc., USA, respectively, was used in the aquaria. Dimethyl POPOP [2,2-p-phenylene bis (4-methyl-5-phenyloxazole)] from Eastman Kodak Co. USA, and PPO (2,5-p-phenyloxazole) from Fisher Scientific as well as Triton X-100 and analytical grade toluene, both purchased from J.T.Baker Inc. USA, were used in the preparation of scintillation cocktail. Radioassay was done using a Packard Tri-carb 1000 TR liquid scintillation counter. A Hewlett Packard model 5890A Gas Chromatograph equipped with a ^{63}Ni -EC detector, SE-30 column and electronic integrator (Hewlett Packard model HP3396 series II) was used for metabolite analysis.

Aquarium glass tanks (dimensions: 30 cm × 15 cm × 15 cm), equipped with air pumps, fluorescent lamps and thermostatic heaters were used to make up tropical freshwater ecosystems consisting of various samples of fish, sediment, lakewater and water lilies taken from three different lakes in Kenya, including experimental and control tanks. The aquarium tanks were filled with approximately 10.6, 10.0 and

13.4 kg of sediment, 42, 30 and 30 litres of water and 15, 15 and 19 (4-5cm long) fish for Lakes Naivasha, Baringo and Victoria, respectively. The fish species in the first two systems (simulating lakes Naivasha and Baringo) were *Tilapia zillii* (6-9 months old) whereas the fish species in the third system, simulating Lake Victoria, were *Tilapia nilotica* (6-9 months old). The temperatures of the lakewaters in the aquaria were maintained at 22 °C using thermostatic heaters while the pH's were kept at 8.6, 8.8 and 7.8, respectively. The stabilized aquaria were then spiked with a mixture of p,p'-DDT (labelled and non-labelled) in a small quantity (0.1 ml) of acetonitrile and samples taken from each compartment for analysis at different time intervals. The control systems comprising exactly the same components as their experimental counterparts were not treated with the pesticide. To study the distribution of residues in sediment, lakewater, fish and water lilies, samples were taken for analysis at various time intervals as shown in Tables 1, 2 and 3. The bioaccumulation factors (BAF) in fish were also determined. The depuration of ¹⁴C-DDT by fish was studied by exposing eleven *Tilapia nilotica* to a 0.1 µg/mL of the pesticide in lakewater for 24 hours and then transferring them to a glass tank containing 7 litres of fresh lakewater and 3.08 kg of sediment (dry basis). The fish were rinsed well with clean lakewater during the transfer and samples from each compartment were taken at time intervals as shown in Table 4 for residue analysis.

Sampling of sediment was done by scooping from different spots in the tank with a stainless steel spoon. The sample was then air-dried, ground in Pestle and Mortar, and thoroughly mixed in a plastic bag and 20 g triplicate samples extracted in a Soxhlet apparatus for 4 hours with 130 mL of methanol. The extractable ¹⁴C-residues were determined by counting the radioactivity in 1 mL of the extract in a liquid scintillation counter. The remaining extract was then concentrated to about 10 ml in rotary-evaporator and partitioned successively into 15/10, 15/5 and 15/10 mL hexane/distilled water mixtures, respectively, in a separatory funnel. Two fish samples were taken at each sampling time, rinsed with distilled water, dried with paper towels, ground with 4-6 g of anhydrous sodium sulfate in Pestle and Mortar until dry and lumpy before extraction with 130 mL hexane for 4 hours. The radioactivity in 1 mL aliquot was counted in the scintillation counter. The remaining extract was concentrated and kept for GC analysis. The determination of the radioactivity in water samples was achieved by taking 1 mL aliquots in triplicates and counting directly in the scintillation counter, after mixing with appropriate amount of counting cocktail. The metabolite (DDT, DDE, DDD) determination in lakewater, was done by taking 100 mL water samples in duplicate and extracting each sample in a separatory funnel with (3×150 mL) of hexane. The extracts were combined, reduced to 10 mL in a rotary-evaporator and retained for GC analysis. Water lilies were harvested from the aquaria, rinsed with distilled water and air-dried over-night in the laboratory. The air-dried samples were then ground with about 10 g of anhydrous sodium sulfate, extracted in a Soxhlet apparatus for 3 hrs with 130 mL of methanol, decolorized with activated charcoal and the extractable residues determined by counting the radioactivity in 1mL aliquot of the extract in the scintillation counter. The remaining extract was concentrated to 10 mL, partitioned into hexane and retained for GC analysis. All the extracts kept for GC analysis were cleaned up by passing through a column packed with 2 g of florisil (magnesium silicate, 60-100 mesh) topped with 2

cm of anhydrous sodium sulfate and a little activated charcoal. The cleaned up extracts were then analysed by GC using the GC conditions reported elsewhere (Wandiga et al. 2002a). Analysis for the various lake sediments gave the following results: Lake Naivasha (sandy loam texture, 65.7% sand, 26% silt, 8.3% clay and 4.75% organic carbon), Lake Baringo (loam texture, 41.2% sand, 36% silt, 22.8% clay and 1.09% organic carbon), Lake Victoria (sandy loam texture, 41% sand, 34.6% silt, 24.5% clay and 2.06% organic carbon).

The acute toxicity of ^{14}C -DDT to fish (*Tilapia zilli*) was studied in a stabilized aquarium containing 30 L of fresh lakewater, 10 kg of sediment, 15 *Tilapia zilli* and 1.3 g of water lilies (dry basis) fetched from Lake Baringo. The DDT concentration in lakewater was 100 ng/mL initially and was then raised to 180 ng/mL after 48 hours. The temperature and pH of the water in the aquarium were maintained at 22°C and 8.8, respectively. Fish samples were harvested by combining all the fish that died within an hour and treating them as one sample. The acute toxicity of ^{14}C -DDT (at a water concentration of 180 ng/mL, pH 7.8, temperature 22 °C) to fish was also studied in a glass tank consisting of 2.5 kg of lake sediment, 30 L of fresh lakewater, and 11 *Tilapia nilotica* obtained from Lake Victoria.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 show the results of the distribution of ^{14}C -DDT in model ecosystems simulating Lakes Naivasha, Baringo and Victoria respectively. The pesticide added to the surface of the fresh water in all the ecosystems distributed rapidly between the various compartments and its concentration in lakewater dropped by about 35% (Lake Naivasha), 46% (Lake Baringo) and 47% (Lake Victoria water), respectively, within the first 2 hrs. The percentages given in the table were calculated based on total ^{14}C -DDT added at the start of the experiment. The DDT residues accumulated in both the sediments and fish during this short period of 2 hours to levels amounting to 1.61%, 1.9%, 1.0 % (in fish) and 27%, 32.1% and 37% (in sediments), respectively, of the total pesticide spiked. Apart from rapid distribution in sediment and biota, DDT residues have been found to volatilize rapidly in water under tropical conditions (Carvalho et al. 1992, Rajendran et al. 1992, Wandiga et al. 2002a). The results of this study also show that whereas the concentration of the residues in fish declined continuously after sharp rise in the first 24 hours, the concentration in the sediments remained almost constant suggesting only little loss due to decomposition and desorption. The concentration decline in the fish was attributed to biodegradation and excretion of the parent compound and its metabolites. Similar rapid distribution of DDT residues in seawater/sediment/biota ecosystem model simulating tropical marine environment has been reported by previous researchers in our laboratory (Wandiga et al. 2002a). Wandiga et al. (2002a) reported a rapid decrease in seawater column due to rapid volatilization and distribution in to sediment as well as uptake by fish in 24 hours after 5.64 ng/mL DDT dosage. They obtained a half-life in the range of 2.07-2.5 hours in seawater in tropical seawater/sediment/fish ecosystems. In our experiments, mass balance assessments of the pesticide injected into the aquaria gave recoveries of 78.7, 78.5% and 82.6% of the total applied pesticide in Lake

Naivasha (after 4 weeks), Baringo (after 2 days) and Victoria (after 4 days), respectively. Losses of residues could be attributed to volatilization, adsorption on glass walls and to complete degradation. The results of our experiments also show that the sediments served as sinks accumulating 62.4-66.5% of the pesticide added to the ecosystems. This distribution of substantial amounts of DDT residues into the sediments was a direct consequence of the organic carbon and high clay contents and also as a result of particulate matter deposition from the water column onto the sediment. Although the sediment uptake rates of the pesticide, especially before the end of 24 hours after dosage were different, a direct relationship between organic carbon content and the accumulated DDT residues was not apparent, and after 48 hours the various sediments did not show significant differences in their adsorption of DDT.

Table 1. The distribution of ^{14}C -DDT residues in different components of the ecosystem simulating Lake Naivasha.

Time	Water		<i>Tilapia zillii</i>		Sediment	
	ng/mL	%	ng/g	%	ng/g	%
0 hr	1.49	99.33	-	-	-	-
2 hrs	0.98	65.30	25.76	1.61	1.63	27.20
18 hrs	0.35	23.33	135.62	9.58	3.68	61.02
24 hrs	0.25	16.67	504.20	31.46	3.00	49.75
48 hrs	0.15	10.00	174.79	10.91	4.18	69.32
1 W	0.17	11.33	218.41	13.60	3.64	60.36
2 W	0.06	4.00	136.49	8.52	4.19	69.49
3W	0.02	1.33	39.26	2.45	4.61	76.45
4W	0.13	8.67	33.82	2.11	4.08	67.83

Note: hr(s) denotes hour(s) and W denotes week(s). % refers to recovered ^{14}C -DDT residues in each compartment expressed as percent of total applied ^{14}C -DDT.

In fish, the relationship between the fat content and the concentration of accumulated DDT residues was direct. The *Tilapia nilotica* (mean fat content 1.79%) had accumulated 5.81 mg/kg within the first 24 hours of exposure to 0.1 $\mu\text{g/mL}$ DDT, giving a peak bioaccumulation factor (BAF) of 447 while the *Tilapia zillii* (mean fat content 0.42%) had accumulated 3.42 mg/kg (a BAF of 311). The results of this study indicate that the two types of fish were able to bioaccumulate the ^{14}C -DDT residues in their tissues rapidly giving a maximum bioaccumulation factor of 2017 for *Tilapia zillii* in Lake Naivasha ecosystem model after 24 hours of exposure to sub-lethal low concentrations of DDT. The peak BAF values for *Tilapia nilotica* and *Tilapia zillii* in Lake Victoria and Lake Baringo ecosystems were 447 and 311, respectively, after 24 hours in low exposure concentrations. Different uptake of DDT residues in fish in tropical marine ecosystems has also been reported (Wandiga et al. 2002a). Peak BAF values of 19,290 in oyster (*Isonomus alatus*) tissue (in seawater, DDT exposure concentration of 0.24-1.18 ng/mL), and BAF of 351 in white shrimp, *Panaeus setiferus*, and BAF of 268 in

fish, *Gobious kinesis*, respectively, after 24 hour static exposure in seawater with DDT exposure concentration of 5.64 ng/mL.

Table 2. The distribution of ^{14}C -DDT residues in the different components of the ecosystem simulating Lake Baringo.

Time	Water		<i>Tilapia zillii</i>		Sediment		Water lily	
	ng/mL	%	ng/g	%	ng/g	%	µg/g	%
0 hr	9.8	98.0	-	-	-	-	-	-
2 hrs	5.4	54.0	176	1.9	93	32.1	-	-
8 hrs	1.9	19.0	233	2.5	168	57.9	1.9	0.08
24 hrs	1.1	11.0	342	3.6	201	69.3	2.8	0.13
48 hrs	0.9	9.0	288	3.1	200	68.9	2.9	0.13

Table 3. The extractable ^{14}C -DDT residues in the different components of the ecosystem simulating Lake Victoria.

Time	Water		<i>Tilapia nilotica</i>		Sediment	
	ng/mL	%	ng/g	(%)	ng/g	(%)
0 hr	9.9	99.0	-	-	-	-
2 hrs	5.3	53.0	36	1.0	83	37.0
6 hrs	4.3	43.0	82	2.4	107	48.0
24 hrs	1.3	13.0	581	17.1	120	53.8
48 hrs	0.6	6.0	458	13.9	134	60.1
72 hrs	0.4	4.0	443	12.8	147	65.9
96 hrs	0.4	4.0	398	11.4	140	62.8

Table 4 shows the depuration of DDT residues from *Tilapia nilotica* after 24 hours exposure to the pesticide, followed by exposure to fresh uncontaminated Lake Victoria water. The elimination of the pesticide from the fish was steady but incomplete, only 59.5% being eliminated after 72 hours in clean lakewater. The initial rate of elimination, however, was very fast and only 2.8 hours was required for the concentration to decline by 50%. Reports in literature indicate that DDT residues are eliminated from fish in the urine or via the bile into the faeces or by diffusion through the gills into the surrounding water (Huckle and Millburn 1990).

The rate of elimination of the pesticide through diffusion decreases as the difference between the concentration of the pesticide in fish and water decreases. This partly explains the decrease in the rate of depuration observed after the first 6 hours. The sediment uptake rate (Table 4), as in the distribution experiments (Tables 1, 2 and 3), was fast (25.6% within the first 2 hours), though the concentration remained constant between 24 and 48 hours due to the equilibrium established between the three components. A 54% (corresponding to a half-life of 25.4 hours) and 97% losses of accumulated ^{14}C -DDT residues were obtained for fish (*Gobious nudiceps*) and white shrimp (*Panaeus setiferus*), respectively, after continuous exposure to clean sea water for 3 days in a previous tropical-marine-ecosystem study (Wandiga et al. 2002a).

Excretion half-lives of DDT in fish (rainbow trout, catfish, sunfish, fathead minnows and gold fish), varying from 31-409 days, have also been reported (Niimi 1978).

Table 4. The depuration of accumulated ^{14}C -DDT residues from fish (*Tilapia nilotica*) after 24 hours of exposure to a sub-lethal concentration.

Time	Water		Fish		Sediment	
	ng/mL	%	ng/g	%	ng/g	%
0 hr	-	-	581	100.0	-	-
2 hrs	2.7	15.9	314	54.0	10.0	25.6
6 hrs	4.2	24.7	274	47.2	10.0	25.6
24 hrs	4.3	25.3	265	45.6	9.0	23.1
48 hrs	4.3	25.3	262	45.1	9.0	23.1
72 hrs	4.4	25.9	235	40.5	11.0	28.2

Note: The total amount of pesticide in the fish after 24 hours exposure was taken as total ^{14}C -DDT residues in the ecosystem in calculating the % age values.

The potential of DDT to evoke toxic effects on fish was demonstrated in the experiment with *Tilapia zillii* and *Tilapia nilotica*, in which the majority of the fish showed poisoning symptoms after 24 and 3 days of exposure respectively. The fish displayed darting, shuddering, and side swimming for some hours after which 40% and 100%, respectively became moribund and died. Some 13%, 40%, 47% and 67% mortalities were recorded after 24, 48, 52 and 60 hours of exposure to 0.1 $\mu\text{g/mL}$ of DDT, respectively, in the Lake Baringo tank. During the experiment concentrations of DDT residues accumulated in the fish were 0.176, 0.233, 0.342, 0.288, 0.574, 1.159 mg/kg after 2, 8, 24, 48, 52 and 60 hours, respectively. In *Tilapia nilotica*, at a slightly higher exposure concentration of 0.18 $\mu\text{g/mL}$, 36%, 82%, and 100% mortality were recorded after 4, 7 and 8 hours, respectively, with accumulated tissue residue concentrations of 0.797, 0.933 and 1.139 ng/g, respectively. The results of the toxicity study with *Tilapia zillii* together with that of the GC analysis show that the rate of uptake and metabolism rather than the extent of exposure determined the lethal and acute toxic effects of DDT on the fish. As was evident, the fish which accumulated 0.342, 0.288 and 0.574 ng/g within 24, 48 and 52 hours of exposure, respectively, died while those which accumulated even up to 0.889 ng/g, in 72 hrs, survived. Moreover, the acute toxic effect of the pesticide on fish was noted to be species dependent. A concentration of 0.1 $\mu\text{g/mL}$ ^{14}C -DDT, which did not have any observable effect on *Tilapia nilotica*, killed 40% of the *Tilapia zillii* after 48 hours. Similar low lethal concentrations, 24 hr LC_{50} of 0.011 mg/kg and 0.116 mg/kg, of DDT in fish (*Gobious nebulos*) and white shrimp (*Panaeus setiferus*), respectively, were obtained recently in tropical marine ecosystems, confirming the known potential toxicity of DDT to non-target organisms (Wandiga et al. 2002a).

The results of the GC analysis of DDT metabolites showed that DDT may have been metabolized by the fish (*Tilapia zillii* and *Tilapia nilotica*) to its primary metabolites, DDD and DDE, with DDE being the major product at all times (Table 5).

Table 5. Metabolite concentration and as percent ratio in different samples.

Sample	L. Naivasha			L. Baringo				L. Victoria		
	Water*	Sed**	Fish ¹ *	Water*	Sed**	Fish ¹ **	Lil*	Water**	Sed**	Fish ² **
DDT:										
ng/g	0.151	2.77	219.22	8.1	0.096	0.134	1.45	8.0	0.036	0.314
%	79.5	58.3	70.1	71.7	80	74	50.3	72.7	52.9	61.2
DDE:										
ng/g	0.022	0.56	48.02	1.8	0.018	0.014	0.32	2.0	0.006	0.167
%	11.6	11.8	15.4	15.9	15	7.5	11.1	18.2	8.8	32.6
DDD:										
ng/g	0.017	1.42	45.36	1.4	0.006	0.034	1.11	1.0	0.026	0.032
%	8.9	29.9	14.5	12.4	5.0	18.6	38.5	9.1	38.2	6.2

Note: *after 24 hours, **after 48 hours, ¹*Tilapia zillii*, ²*Tilapia nilotica*, Sed 'sediment', lil 'water lilies'. The concentrations in water are in ng/mL. After 2 weeks the concentration in L. Naivasha: water (DDT: 0.06 ng/mL, 83.3%, DDE: 0.008 ng/mL, 11.1%, DDD: 0.004, 5.6%), sediment (DDT: 2.6 ng/g, 53%, DDE: 0.6 ng/g, 12.2% and DDD: 1.7 ng/g, 34.7%), fish, *Tilapia zillii* (DDT: 43.11 ng/g, 52.5%, DDE: 24.07 ng/g, 29.3% and DDD: 14.96 ng/g, 18.2%).

It is also evident that some of the metabolites that may have been in the water could have been taken up by the fish during uptake. The GC analysis indicated that the rate of metabolism seemed to be species dependent and of the two species of fish studied *Tilapia nilotica* may have a better mechanism of degrading DDT. This was evident from the tissue concentration levels of DDT, DDE and DDD in *Tilapia zillii* after 48 hours of exposure corresponding to 73.9%, 18.6% and 7.5%, respectively, in comparison to 61.2%, 32.6% and 6.2%, respectively, in *Tilapia nilotica* after only 24 hours. The concentration levels of DDT and its metabolites in *Tilapia zillii* were also noted to decline after 2 weeks of exposure time. The decline was most pronounced for DDT (-80.33%) and least pronounced for DDE (-49.87%). This would be expected since DDE is more lipophilic than DDT and therefore could dissipate to the surrounding water at a lower rate than DDT. Moreover, DDT could continue to degrade to DDE and DDD tending to compensate for the DDE depuration from the fish.

As was found in the fish, the primary metabolites of DDT (i.e. DDE and DDD) were detected in the fresh water sediments as early as 24 hrs after spiking. However, the major degradation product was DDD, with its concentration varying depending on the organic matter content of the sediment and the degree of decomposition of the parent compound. The rate of degradation of DDT was fastest in the sediment sample from Lake Victoria (organic carbon content 2.06%) and slowest in the sediment from Lake Baringo (organic carbon content 1.09%). The much faster rate of decomposition observed in the sediment from Lake Victoria as compared to the sediment from Lake Baringo could be attributed to higher microbial activity usually associated with the higher organic matter content of the sediment (Haque 1975). The rate of transformation of the pesticide in the sediment from Lake Naivasha was slightly lower than that of Lake Victoria despite its higher organic carbon content (4.78%). The activity of microorganisms in the sediment sample from Lake Naivasha seemed to have been counterbalanced by sorption. Sorption, and hence protection against

degradation, increases with organic matter content of the sediment (Haque 1975, Carvalho et al. 1992). Some of the metabolites may have been transferred to the sediment from the water column. In the present study, the concentration of DDT in the sediment decreased by 6.14% between 48 hours and 2 weeks time while those of DDE and DDD increased by 7.14% and 19.72% respectively, in the Lake Nivasha ecosystem, suggesting that DDT may have continued to decompose to DDE and DDD. The total concentration of DDT and metabolite residues on the other hand remained virtually constant (4.8 and 4.9 ng/g at 48 hrs and 2 weeks respectively) suggesting the ineffectiveness of any microorganisms which could break down the pesticide residues completely into CO₂ and water. This is in line with the fact that some sediment microorganisms like *hydrogenomonas* are capable of degrading DDT completely to CO₂ (Khan et al. 1975). The microbial population in sediment which determines the microbial activity in this compartment can be considerably much higher than in the water column. Bacterial concentrations in sediment can be as high as 10²-10⁴ times more than in the overlying water column (Carvalho et al 1992). Microbial degradation to DDD would therefore be expected to be more predominant in fresh water sediment than in the water column. In the lakewaters investigated in this study, DDT degraded only slightly to its primary metabolites, DDE and DDD, respectively. The major break down product was DDE. Some of the detected DDE and DDD may have come from sediment and fish by the processes of desorption and excretion respectively. DDD being the least lipophilic can depurate most from the fish. Similarly, by virtue of its higher water solubility as compared to DDE, DDD tends to enter into the water column from the sediment (Geyer et al. 1987). The amount of metabolites of DDT in water depends on possible photochemical/catalytic and microbial reactions within the water column. Previous reports show that DDT transformation in seawater also occurs and 4.7 % and 3.3 % DDE and DDD, respectively, in relation to DDT, were detected after 2 hours of dosage in a tropical sediment/seawater marine ecosystem (Wandiga et al. 2002a). The percentage of the metabolite ratio rose to 26.8% and 15.5% of DDE and DDD, respectively, in the same ecosystem, after 7 days. In similar laboratory investigations, 6.9% and 11% conversion to DDE and DDD, respectively, occurred in a seawater column after 8 hours of dosage in a tropical seawater/sediment/fish marine ecosystem (Wandiga et al. 2002a). In our experiment, the conversion to DDD in lakewaters seemed to be less prevalent as would have been expected. This could be due to high solubility of DDD in water and possible subsequent rapid loss by evaporation. In earlier studies as found in literature, DDT incubated in freshwater samples was reported to change to DDD significantly, while sea water samples indicated relatively poor metabolic activity (Johnsen 1976). The distribution, bioaccumulation and the observed toxic effects of DDT discussed in this paper may be different from those reported earlier in marine ecosystem models (Wandiga et al. 2002a) since DDT solubility and its distribution in aquatic media as well as its bioavailability to aquatic organisms may be influenced by existing biological factors as well as by the presence of higher concentrations of particulate matter and dissolved organic carbon in freshwater ecosystems.

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