

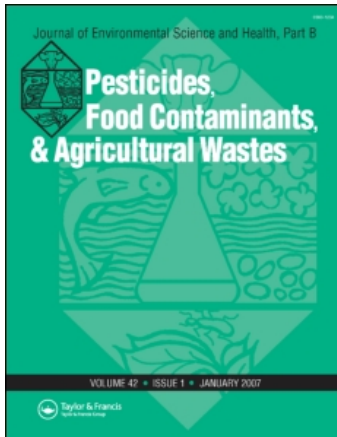
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Pesticide residues in river sediments from the Pantanal Wetland, Brazil

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Sediment samples from 25 sites in 17 rivers of the Pantanal (Brazil) were analyzed with the objective of evaluating pesticide contamination in sediments. Samples were extracted with an acetone, ethylacetate, and water mixture 2:2:1 (v/v/v). The extract was purified by flash chromatography with aluminum oxide and florisil. A multiresidue gas chromatography-mass spectrometry method was applied to monitor 23 pesticides of different chemical classes (organochlorine, organophosphorus, triazines, anilides and pyrethroids) with some of their degradation products. Compounds identified in sediment samples included λ -cyhalothrin (1.0 to 5.0 $\mu\text{g kg}^{-1}$), p,p'-DDT (3.6 $\mu\text{g kg}^{-1}$), deltamethrin (20.0 $\mu\text{g kg}^{-1}$) and permethrin (1.0 to 7.0 $\mu\text{g kg}^{-1}$).

Keywords: Pesticide; sediment; gas chromatography; Pantanal.

Introduction

The Pantanal, located in South America, is the largest wetland in the world that has not been substantially modified by men. The sheer abundance of large birds, reptiles and mammals marks its importance as a huge reservoir of biodiversity.^[1] From January to May, the lowlands are increasingly inundated by flood water, forming the first sedimentation basis for the rivers from the highlands.^[2] This sedimentary basin is an integrating part of the Paraguay River watershed. Twenty eight percent of its territory belongs to Bolivia and Paraguay — the rest is distributed between two Brazilian States: Mato Grosso and Mato Grosso do Sul. These States have been increasingly using a highly mechanized and intensive agriculture during the last two decades with soybean [*Glycine max* (L.) Merr.], corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.) and sugarcane (*Saccharum* spp.) cultures. The Pantanal receives the floodwater of rivers during the rainy season, which drain the internal part of those intense cropped highlands.

Pesticide use can contaminate on-site ground and surface water^[3,4] as well as export pesticides from agricultural regions via riverine or atmospheric transport to other

ecosystems.^[5,6] Especially in tropical countries, agricultural intensification has led to higher pesticide consumption,^[7] and a wide-spread dispersion of pesticides in this environment may also occur.^[8,9] Pesticides are adsorbed onto the organic matter incorporated in sediments^[10–13] and the persistence depends on their physicochemical properties. River sediments are effective supervising tools to appraise pollution episodes, are singular in furnishing historical contamination and reflect the quality of surface water. Since sediments can contain pesticides of different chemical families (organochlorine, organophosphorus, triazines, anilides), it is crucial to use multiresidue methods capable of determining a large number of compounds. Among extraction methods available, Soxhlet extraction has been the most-used technique for the extraction of pollutants in sediments.^[14,15] However, other techniques such as liquid-liquid extraction,^[16] solid phase microextraction,^[17] ultrasonic solvent extraction^[18] and pressurized liquid extraction^[19] had already been used.

Although pesticide application in the States of Mato Grosso and Mato Grosso do Sul has intensified considerably since the introduction of soybean, studies on pesticide occurrence in environmental samples of the Pantanal are scarce and restricted to the northeastern Pantanal basin. The objective of our study was to evaluate if currently used pesticides in the states of Mato Grosso and Mato Grosso do Sul (Brazil) are accumulating in sediments. To this aim, pesticide concentrations were measured in sediment samples collected from 25 sites in 17 rivers.

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Table 1. Studied pesticides and metabolites, monitored ions, recovery, and routine limit of quantification (RLQ) in sediment samples.

Substance	Type ^(a)	Use in the study area	SIM ions ^(b)	Sediment samples	
				Recovery	RLQ
			<i>mz</i> ⁻¹	% of spike ^(c)	$\mu\text{g kg}^{-1(d)}$
Alachlor	H	corn, soybean, cotton	160, 188, 146	109 ± 2	0.8
Ametryn	H	sugarcane	227, 212, 170	95 ± 3	0.4
Atrazine	H	corn	200, 215, 173	92 ± 2	0.4
Aldrin	I	no current use	263, 261, 265	92 ± 5	0.8
Chlorpyrifos	I	corn, cotton, vegetables	197, 199, 314	85 ± 5	0.8
Cyanazine	H	corn, cotton	225, 212, 172	78 ± 8	4
λ -Cyhalothrin	I	soybean, cotton	181, 197, 208	86 ± 4	0.8
Cypermethrin	I	soybean, cotton	163, 181, 165	109 ± 5	4
<i>p,p'</i> -DDE		metabolite of DDT	246, 318, 316	112 ± 3	0.4
<i>p,p'</i> -DDT	I	no current use	235, 237, 165	115 ± 6	0.4
Desethylatrazine		metabolite of atrazine	172, 174, 187	87 ± 12	0.4
Deltamethrin	I	soybean, cotton	181, 253, 251	93 ± 6	4
Dieldrin	I	no current use	263, 277, 279	95 ± 5	0.8
α -Endosulfan	I	cotton, soybean	241, 195, 239	95 ± 1	0.4
β -Endosulfan	I	Cotton, soybean	195, 237, 241	103 ± 2	0.4
Endrin	I	no current use	283, 285, 261	121 ± 3	0.4
Endosulfan-sulfate		ensulfan metabolites (α -, β -)	272, 274, 229	95 ± 2	0.4
Malathion	I	vegetables	173, 125, 127	76 ± 8	2
Methyl-parathion	I	vegetables	263, 109, 125	78 ± 5	4
Methoxichlor	I	no current use	227, 228, 212	122 ± 2	0.8
Metolachlor	H	corn, soybean, cotton	162, 238, 240	89 ± 1	0.8
Metribuzin	H	soybean	198, 199, 144	77 ± 4	0.8
Parathion	I	vegetables	291, 139, 155	83 ± 4	4
Permethrin	I	soybean, cotton	183, 163, 165	98 ± 3	0.8
Simazine	H	corn, sugarcane	201, 186, 173	99 ± 2	0.4
Trifluralin	H	soybean, cotton	306, 264, 290	88 ± 6	0.4

^(a)F, fungicide; H, herbicide; I, insecticide.

^(b)Ions used for substance quantification (first ion) and identification (second and third ions) during the selected ion monitoring (SIM).

^(c)Spike of 0.1 mg kg⁻¹ sediment (n = 3), samples of 25 g sediment dry mass.

^(d)Samples of 25 g of sediment dry mass.

Materials and methods

Chemicals and reagents

Compounds studied were obtained from Dr. Ehrestorfer (Augsburg, Germany), and are listed in Table 1. Every compound had a purity of 98–99%. Alumina, florasil, sodium sulfate and sodium chloride were purchased from Merck (Darmstadt, Germany). All organic solvents used (“picograde” purity) were obtained from Mallinckrodt Baker (Phillipsburg, NJ, USA).

Sampling area

Sampling was performed in 17 rivers from the highlands/lowlands transition area (see details in Fig. 1). The location of the sampling sites and their geographical coordinates can be seen in Table 2. The climate in this area is of the savanna type, with mean annual temperatures of 23°C in the planalto (highlands) and 25°C in the lowlands, and a mean annual precipitation of 1900 mm in the planalto and 1500 mm in the lowlands. Samples were collected during

the rainy period (November to March), when pesticides, nutrients and soil are more easily leached.

Scope of analyzed pesticides

The region studied demonstrates a division between the highlands, intensively used for cash crop production, and the lowlands, used for small-holder vegetable production. Therefore, the scope of pesticides in the highlands is dominated by herbicides and insecticides applied in soybean, cotton, corn and sugarcane (atrazine, endosulfan, metolachlor, trifluralin). Insecticides and fungicides are used in the lowlands for vegetable production (chlorpyrifos, parathion-methyl, tebuconazole). Pesticides that could be measured with gas chromatography–mass spectrometry (GC–MS) of the most frequent use in the study area were selected for the monitoring. Thermally unstable and Ionic pesticides frequently used in the study region could not be included in our study (e.g., dichloride, diuron, glyphosate, methomyl, 2,4-D, teflubenzuron). Persistent organochlorine pesticides completed the scope of analyzed substances (Table 1).

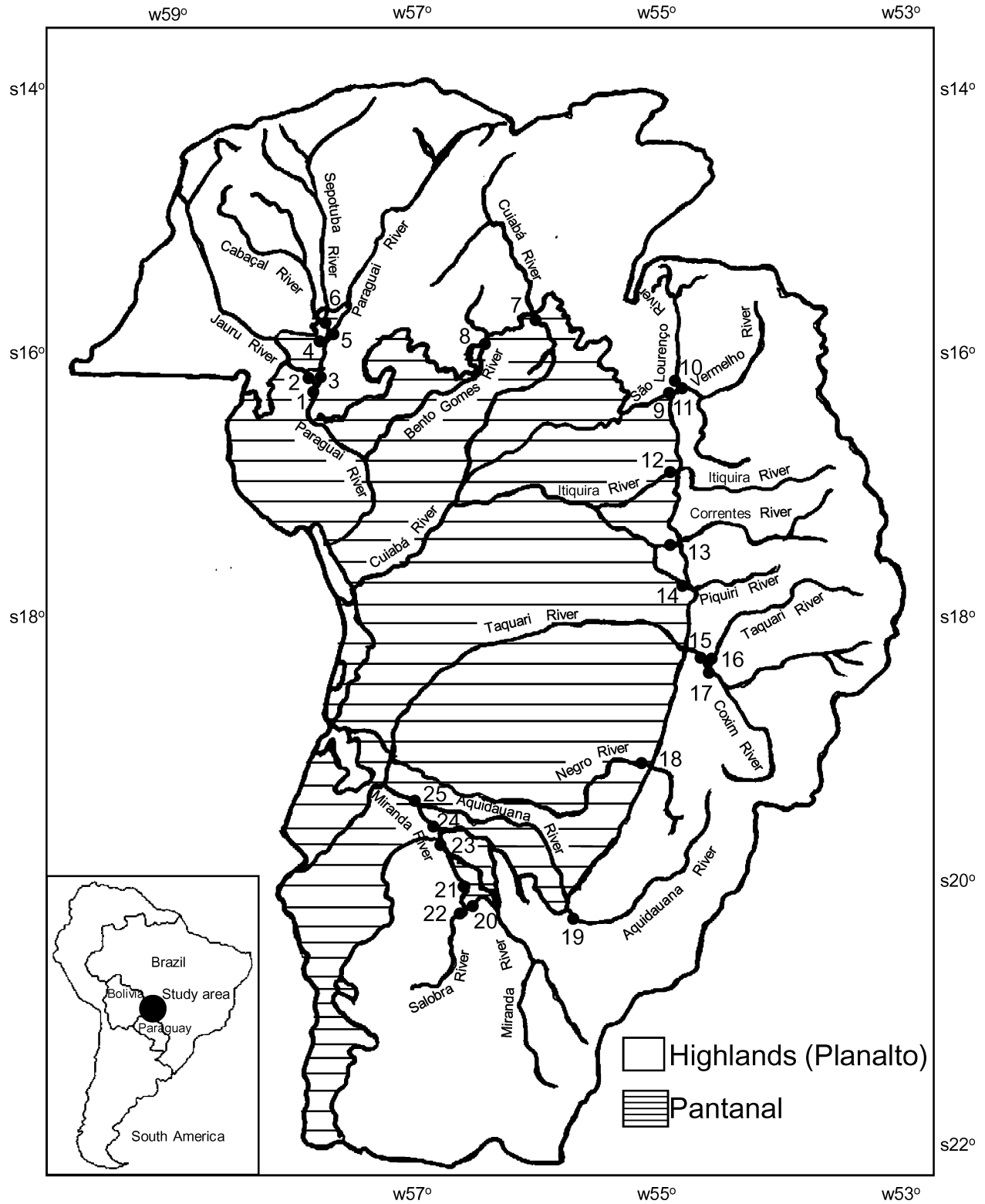


Fig. 1. Location of the study area. Numbers indicate sampling stations in rivers from the Pantanal.

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Table 2. List of sampling sites and corresponding coordinates.

Code	Site	Geographical coordinates
01	Paraguay River	S 16° 20' 37,7'' W 57° 46' 18,7''
02	Jauru River	S 16° 20' 37,2'' W 57° 47' 05,3''
03	Paraguay River	S 16° 21' 02,4'' W 57° 46' 42,8''
04	Cabaçal River	S 15° 59' 05,1'' W 57° 42' 31,2''
05	Paraguay River	S 15° 55' 34,0'' W 57° 38' 29,5''
06	Sepotuba River	S 15° 55' 11,4'' W 57° 38' 54,5''
07	Cuiabá River	S 15° 52' 04,6'' W 56° 04' 59,4''
08	Bento Gomes River	S 16° 18' 56,6'' W 56° 32' 38,6''
09	São Lourenço River	S 16° 27' 48,7'' W 54° 58' 16,7''
10	São Lourenço River	S 16° 28' 40,7'' W 54° 59' 26,4''
11	Vermelho River	S 16° 28' 40,7'' W 54° 59' 26,4''
12	Itiquira River	S 17° 15' 03,5'' W 54° 45' 36,2''
13	Correntes River	S 17° 29' 18,7'' W 54° 37' 16,9''
14	Piquiri River	S 17° 29' 19,5'' W 54° 37' 18,4''
15	Taquari River	S 18° 31' 24,8'' W 54° 44' 53,7''
16	Taquari River	S 18° 31' 39,9'' W 54° 43' 25,4''
17	Coxim River	S 18° 33' 22,0'' W 54° 44' 42,6''
18	Negro River	S 19° 26' 08,5'' W 55° 00' 16,6''
19	Aquidauana River	S 20° 29' 38,1'' W 55° 46' 19,7''
20	Miranda River	S 20° 13' 49,3'' W 56° 29' 45,2''
21	Miranda River	S 20° 12' 40,8'' W 56° 29' 24,8''
22	Salobra River	S 20° 12' 02,6'' W 56° 29' 53,7''
23	Miranda River	S 20° 02' 12,5'' W 56° 39' 10,7''
24	Miranda River	S 20° 02' 12,7'' W 56° 46' 20,3''
25	Miranda River	S 19° 34' 42,4'' W 57° 01' 09,7''

Field sampling

All samples were collected with a Foerst Petersen sampler from river positions at sites where a continuous sedimentation took place, avoiding points consisting of coarse sands, which do not adsorb pesticides in significant amounts.^[20] Composite samples were grouped from five subsamples (0–10 cm) of a 10 m² area, homogenized, and wrapped in aluminum foil. Samples were kept on ice immediately after collection and stored at –20°C in the laboratory until analysis.

Dry weight determination

Dry-to-wet weight ratios of sediment samples were analyzed at the time of sediment sample extractions. Ten g subsamples were weighed into tared aluminum weigh pans and dried in a 105 °C oven for 24 hours. Then the subsamples were removed from the oven and cooled in a desiccator until a constant weight was achieved. The samples were reweighed to determine the dry weight.

Sample extraction

Sediment samples (around 25 g dry weight) were extracted with an acetone, ethylacetate, and water mixture 2:2:1 (v/v/v). After that, the flasks were centrifuged and the extract was filtrated. Following the remotion of the or-

ganic solvents with a rotary evaporator, the water phase was liquid–liquid extracted with dichloromethane (3 × 25 mL). The organic phase was dried with sodium sulfate and concentrated with a rotary evaporator. Then the extract was purified by flash chromatography with an 8 mL glass column packed with 1.0 g of aluminum oxide (deactivated with 0.06 g water g⁻¹ sorbent) on top of 1.0 g florisil (deactivated with 0.1 g water g⁻¹ sorbent). After the sample extract had been transferred to the column in approximately 0.5 mL of toluene, the pesticides were consecutively eluted from the column with 10 mL of n-hexane and 10 mL of n-hexane/ethylacetate 4:1 (v/v). The eluate was concentrated with a rotary evaporator and then analyzed by GC–MS. Glassware used was washed scrupulously using detergent and subsequently with tap water, distilled water and acetone. Then the glassware was baked at 250°C during 4 hours before every use.

Quality control and pesticide analysis

The quantification of pesticides was made with GC–MS (Hewlett-Packard 6890 series gas chromatograph coupled with a Hewlett-Packard 5973 mass selective detector–electron impact ionization), which was operated in the selected ion-monitoring mode at the following conditions: injector block temperature = 250°C; carrier gas = helium; oven temperature program = initial temperature of 92°C held for 2.5 min, heating up to 175°C at 15°C min⁻¹, 175°C held for 13 min, heating up to 280°C at 20°C min⁻¹, 280°C held for 8 min. One target and two additional qualifier ions per substance were measured in order to perform the quantification and identification of the compounds (Table 1). The calibration was made with four-point linear functions, using external and internal standards. One µg of each internal standard (α -HCH, terbuthylazine, and ditalimfos) was added to samples before extraction to control the analytical quality and to compensate for processing losses. The internal standards represented different chemical families during extraction and analysis: α -HCH was banned from agricultural use more than 10 years ago. Terbuthylazine and ditalimfos are not commercialized in Brazil. Traces of internal standards (nanogram range) in samples would not interfere with pesticide quantification, as to every sample 1 µg of each internal standard was added. Internal standard recovery was calculated by relating their concentration to Naphthalene-d8, which was added (1 µg) to concentrated sample extracts prior to GC injection. Samples were injected in toluene (1 µL).

Recovery of internal standards was calculated to check sample processing quality. Retention time, presence and ratios of target and qualifier ions were used to identify the pesticides in chromatograms of samples extracts. Blank concentrations were subtracted from sample concentrations. Routine limits of quantification (RLQ) for all substances are listed in Table 1.

Table 3. Pesticide and metabolite concentrations in sediment samples ($\mu\text{g kg}^{-1}$ of dry weight sediment).

Permethrin (<i>cis,trans</i>)		λ -Cyhalothrin		Deltamethrin		<i>p,p'</i> -DDT	
Site	Conc.	Site	Conc.	Site	Conc.	Site	Conc.
08	7.0 ± 0.6	22	5.0 ± 0.5	17	20.0 ± 1.2	25	3.6 ± 0.4
10	4.2 ± 0.3	18	2.3 ± 0.1	—	—	—	—
03	3.5 ± 0.3	21	1.0 ± 0.1	—	—	—	—
11	2.4 ± 0.1	—	—	—	—	—	—
07	1.0 ± 0.1	—	—	—	—	—	—

Recovery experiments

The analytical method was evaluated by recovery experiments. Uncontaminated sediment from Miranda River was spiked with $2.5 \mu\text{g}$ of each compound in 25 g of sediment dry mass before extraction, processed and analyzed as commented before. Pesticide concentrations measured in sediment samples were adjusted for their respective recovery values (Table 1).

Results and discussion

The sample stations drained regions of agricultural use, where land surface was used for soybean, corn, cotton and sugarcane production. Sediments were extracted to determine a total of 26 compounds. Among them, only 4 contaminants were detected, suggesting that few pesticides are accumulating in sediments. Permethrin (1.0 to $7.0 \mu\text{g kg}^{-1}$) was detected in five samples, followed by λ -Cyhalothrin (1.0 to $5.0 \mu\text{g kg}^{-1}$), found three times. Deltamethrin and *p,p'*-DDT were detected only once (Table 3). Although the agricultural use of DDT was banned in Brazil in 1985, this pesticide was detected in our study. This testifies the persistence of this compound even in tropical environments.^[21] Castilho et al.^[22] detected *p,p'*-DDT in similar concentrations (3.3 to $18.1 \mu\text{g kg}^{-1}$) in sediment samples from Atoya River, Nicaragua, during the rainy season (November-December). Pyrethroids have relatively long persistence and strong affinity for the solid phase.^[23] Formation and transport of chemically enriched fine particles during runoff may offer an explanation for the detection of these compounds.^[24] Lee et al.^[25] observed that the ability of some bacteria in effectively degrading pyrethroids in solution media was greatly inhibited in sediment and the inhibition was attributed to the strong adsorption to the sediment phase.

Concentrations of pesticides detected in our study were generally below $7.0 \mu\text{g kg}^{-1}$ sediment (dry weight), except for one detection of deltamethrin ($20.0 \mu\text{g kg}^{-1}$). The current Brazilian legislature (CONAMA 357/2005) does not define maximum contaminant levels of the studied pesticides in river sediment. Considering a preliminary report in the northeastern Pantanal basin,^[16] contamination of the main rivers of the Pantanal observed in this work presented similar levels.

Conclusion

This research made a preliminary survey to pesticides in rivers sediments from Pantanal. Low concentrations of λ -cyhalothrin, *p,p'*-DDT, deltamethrin and permethrin were found in this survey. On the basis of the above analyses, it is assumed that soil run off and regional flooding might have contributed to the enrichment of pyrethroids in river sediments from Pantanal.

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