

Determination of pesticides in the source and drinking waters in Londrina, Paraná, Brazil

Determinação de agrotóxicos em água de manancial e água de abastecimento público em Londrina, Paraná, Brasil

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Abstract

The Brazilian agricultural model is based on the intensive use of pesticides. Once in the environment, these compounds might target hydric sources, mainly through soil lixiviation or during crop application, exposing water biota as well as humans to contaminants. The objective of this research was identify the presence of 14 pesticides (ametrine, atrazine, azoxystrobin, carbendazim, carbofuran, clomazone, chlorpirifos, diuron, hexazinone, imidacloprid, malathion, simazine, tebuconazole and tebutiuron) in source water samples and drinking water samples from the city of Londrina, Paraná State, Brazil. 24 water samples were collected biweekly from December 2014 to October 2015. The sample preparation was done using Solid Phase Extraction (SPE) with 3 mL/500 mg C-18 cartridges. Pesticide determination was performed applying Liquid Chromatography tandem Mass Spectrometry (LC-MS/MS). The frequencies of detection of some compounds in source water were: atrazine (85%), azoxystrobin (95%), carbendazim (83%), diuron (75%), imidacloprid (95%), simazine (12%), hexazinone (4%), tebutiuron (4%) and tebuconazole (33%). In the water supply, the following were detected: atrazine (79%), azoxystrobin (95%), carbendazim (8%), diuron (83%), imidacloprid (95%), simazine (12%), hexazinone (4%), tebutiuron (8%) and tebuconazole (33%). Some of these compounds are included in Brazilian legislation, and in these cases, the concentrations determined in this study varied between 241 and 7 ng L⁻¹, which were lower than the maximum allowable concentrations for each of the compounds.

Key words: Pesticides. Drinking water. LC-MS/MS. Surface water.

Resumo

O modelo de agricultura brasileiro é baseado no uso intensivo de agrotóxicos. Uma vez no ambiente, estes compostos podem atingir recursos hídricos, principalmente através da lixiviação ou durante a aplicação, expondo a biota aquática e até mesmo humanos, aos contaminantes. O objetivo deste trabalho foi identificar a presença dos agrotóxicos ametrina, atrazina, azoxistrobina, carbendazim, carbofurano, clomazona, clorpirifós, diuron, hexazinona, imidacloprido, malation, simazina, tebuconazol e tebutiuron em amostras de água de manancial e de abastecimento público da cidade de Londrina, Paraná, Brasil.

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Foram coletadas 24 amostras, quinzenalmente, de Dezembro de 2014 a Outubro de 2015. Para o preparo das amostras foi utilizado Extração em Fase Sólida (SPE) com cartuchos de 3 mL C-18 de 500 mg. A determinação de agrotóxicos foi realizada utilizando Cromatografia Líquida acoplada à Espectrometria de Massas (LC-MS/MS). Nas amostras do manancial, foram detectados os compostos atrazina (85%), azoxistrobina (95%), carbendazim (83%), diuron (75%), imidacloprido (95%), simazina (12%), hexazinona (4%), tebutiuron (8%) e tebuconazol (33%). Nas amostras de água para abastecimento público, foram detectados atrazina (79%), azoxistrobina (95%), carbendazim (8%), diuron (83%), imidacloprido (95%), simazina (12%), hexazinona (4%), tebutiuron (8%) e tebuconazol (33%). Alguns destes compostos estão inseridos na legislação brasileira e nestes casos, as concentrações determinadas neste estudo variaram entre 241 e 7 ng L⁻¹, as quais foram inferiores às máximas concentrações determinadas para cada um dos compostos.

Palavras-chave: Pesticidas. Contaminação. LC-MS/MS. Águas superficiais.

Introduction

The intensive use of pesticides as a tool to optimize production and facilitate pest control has caused the emergence of adverse effects on flora and fauna (GAMA et al., 2013). Currently, Brazil is the largest consumer of pesticides in the world, and the risks related to the exposure to these compounds are a reality that should be considered relevant in the country (ALBUQUERQUE et al., 2016; BRASIL, 2013, 2011; FAO, 2014). The sources of groundwater and surface water are exposed to pesticides used in agricultural and non-agricultural areas, since water resources act as integrators of the biogeochemical processes of any region (SILVA et al., 2011; RIBEIRO et al., 2007). After application, pesticides suffer various physical, chemical and biological processes that determine their fate in the environment, with leaching being the most important factor related to the contamination of water bodies (SPADOTTO et al., 2004). The pesticides in the water can reach non-target organisms, affecting the endocrine systems of animals and humans through chronic exposure, even in concentrations of nanograms per liter (MONTAGNER et al., 2014; GRANELLA et al., 2013). In Brazil, there are currently a large number of authorized compounds for different crops, needing knowledge about the scenario of contamination of water bodies, especially regarding those that serve for public supply (RIBEIRO et al., 2013). Some studies demonstrate the presence of

pesticides in sewage effluents at levels of concern (MONTAGNER et al., 2017). The objective of this work was to evaluate the presence of pesticides in source and drinking water samples collected at a public Drinking Water Treatment Plant (DWTP) in the city of Londrina, Paraná State, Brazil, using a selective analytical technique with high sensitivity. This approach will deepen the understanding of the scenario of contamination by pesticides in this country and thus support future decision-making in the scope of public policies related to water quality management.

Materials and Methods

Sampling and local area

This study was conducted at a DWTP in the city of Londrina, Paraná State, whose the water source is the Tibagi River, which has an average volume of treated water around 1446 L per second. The treatment plant performs conventional water treatment, through processes such as coagulation, flocculation, decantation, filtration and subsequent disinfection with fluoridation. Samples were collected directly from the captation and final treatment taps, within the treatment plant itself. Twenty-four samples of source and drinking water were collected in polyethylene bottles (1 L), previously washed with ultrapure water and dried at room temperature, transported and kept under refrigeration until extraction. Samples were

extracted on the same day of collection.

The samples were collected fortnightly in the period of December 2014 to October 2015.

Pesticides

The target pesticides were selected based on a survey carried out on the main compounds

in the State of Paraná, Brazil. The insecticides include carbofuran, imidacloprid, chlorpyrifos and malathion; the herbicides include ametryne, atrazine, simazine, hexazinone, clomazone, diuron and tebutiuron; the fungicides include azoxystrobin, carbendazim and tebuconazole. Relevant physical-chemical properties of the selected pesticides are presented in Table 1.

Table 1. Physio-chemical properties of the selected pesticides.

Pesticides	Chemical group	Molecular formula	Log Kow	CAS number	Water solubility (mg L ⁻¹)
Ametryne	Herbicide	C ₉ H ₁₇ N ₅ S	2.98	834-12-8	200
Atrazine	Herbicide	C ₈ H ₁₄ ClN ₅	2.70	1912-24-9	33
Azoxystrobin	Fungicide	C ₂₂ H ₁₇ N ₃ O ₅	2.50	131860-33-8	6
Carbendazim	Fungicide	C ₉ H ₉ N ₃ O ₂	1.48	10605-21-7	8
Carbofuran	Insecticide	C ₁₂ H ₁₅ NO ₃	2.32	1563-66-2	351
Clomazone	Herbicide	C ₁₂ H ₁₄ ClNO ₂	2.50	81777-89-1	1100
Chlorpyrifos	Insecticide	C ₉ H ₁₁ Cl ₃ NO ₃ PS	1.82	2921-88-2	1
Diuron	Herbicide	C ₉ H ₁₀ Cl ₂ N ₂ O	2.68	330-54-1	42
Hexazinone	Herbicide	C ₁₂ H ₂₀ N ₄ O ₂	1.85	51235-04-2	33
Imidacloprid	Insecticide	C ₉ H ₁₀ ClN ₅ O ₂	0.57	138261-41-3	610
Malathion	Insecticide	C ₁₀ H ₁₉ O ₆ PS ₂	2.36	121-75-5	145
Simazine	Herbicide	C ₇ H ₁₂ ClN ₅	2.18	122-34-9	5
Tebuconazole	Fungicide	C ₁₆ H ₂₂ ClN ₃ O	3.70	107534-96-3	36
Tebuthiuron	Herbicide	C ₉ H ₁₆ N ₄ OS	1.79	34014-18-1	2500

Adapted from Montagner et al. (2014).

Reagents and chemicals

High purity standards of pesticides, including ametryne (98.5%), atrazine (98.8%), azoxystrobin (99.7%), carbendazim (97%), carbofuran (99.9%), clomazone (99.7%), chlorpyrifos (99.7%), diuron (99.6%), hexazinone (99.9%), imidacloprid (99.9%), malation (97.5%), simazine (99.9%), tebuconazole (99.7%) and tebutiuron (99.9%), were purchased from Sigma-Aldrich® (Munich, Germany). Individual stock solutions (400 mg L⁻¹) of each pesticide were prepared from the appropriate solid standards in methanol (J.T. Baker, Philipsburg, NJ, EUA) and stored in amber glass bottles (-4°C).

Calibration solutions were prepared at different concentrations (500, 100, 50, 10, 5 e 1 µg L⁻¹) in the 70/30 (v/v) H₂O-MeOH solution.

Solid-phase extraction (SPE)

A 250-mL sample was vacuum filtered using a nylon membrane (0.22 µm, Millipore, USA). The solid phase extraction (SPE) was performed using Strata® C18 Sep-pak (500 mg, Phenomenex, USA) in the manifold system under vacuum. Each cartridge was conditioned with 3 mL methanol and 3 mL ultrapure water, avoiding dry the sorbent.

The sample was percolated at approximately 2 mL min⁻¹. A clean up was performed with 2 mL of purified water and then the cartridge was dried for 20 minutes and stored in a freezer at -4°C until the LC-MS/MS analysis.

The elution of analytes was done with 3 mL of methanol and 3 mL acetonitrile. Solvents were evaporated to dryness with a flow of ultrapure nitrogen gas, and the compounds were redissolved adding 1 mL of the 70/30 (v/v) H₂O-MeOH solution. Final extracts were filtered with a nylon membrane (13 mm, 0.20 µm pore size) and transferred to vials.

LC-MS/MS Determination

The LC-MS/MS analyses were realized using an Agilent 1200 Series LC system coupled to an Agilent 6410 triple quadrupole mass spectrometer with an electrospray ionization source (ESI) and software MassHunter (Agilent, Palo Alto, CA, USA), to evaluate the chromatographic and mass data. The chromatographic separation was realized at 30°C, using a reversed-phase Zorbax SB-C18 (2.1 x 30 mm, particle size 3.5 µm, Agilent, Santa Clara, CA,

USA) and gradient elution using water and methanol and 0.01 % formic acid. Mobile-phase solvents were filtered through 0.2-µm nylon membranes (Sigma-Aldrich®, Munich, Germany). Gradient elution was done at a flow rate of 0.3 mL min⁻¹ and increasing the organic solvent concentrations from 30% to 60% in 1.2 min for 3 minutes, followed by an increase to 70% in 3.5 min, and kept constant for 4 minutes. After being reset to the initial conditions, the system was re-equilibrated for 5 minutes. The injection volume was 10 µL.

After the chromatographic separation, the compounds were ionized using ESI operating in the positive mode for all compounds. The following optimized parameters were used: drying gas temperature of 350°C, drying gas flow rate of 10 L min⁻¹, nebulizing gas pressure of 20 psi and capillary voltage of 4000 V, using nitrogen as the collision gas. The mode of acquisition was multiple reaction monitoring (MRM) transitions between the precursor product ions, which was used for confirmation and quantification of the studied compounds (Table 2).

Table 2. LC-MS/MS quantification parameters used for each pesticide.

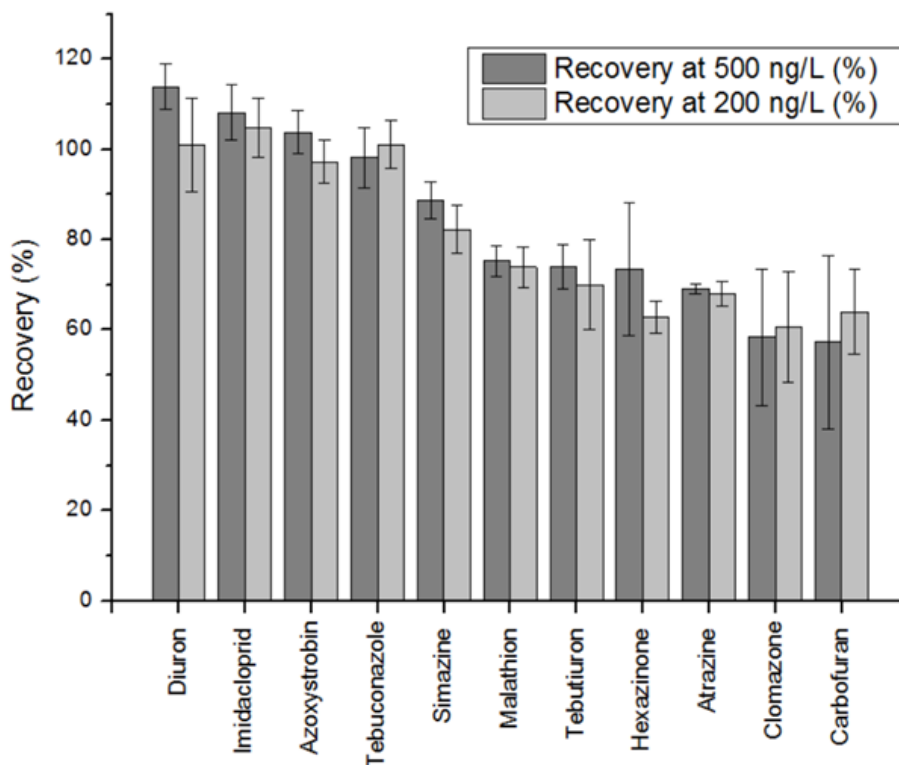
Pesticides	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	Collision energy (eV)	Polarity
Ametrine	5,8	228,2	186,1	15	Positive
Atrazine	5,7	216,2	174,1	15	Positive
Azoxystrobin	7	404,2	344,1	5	Positive
Carbendazim	0,8	192,1	160,1	20	Positive
Carbofurane	4,7	222	123	10	Positive
Clomazone	6,4	240,1	125	1	Positive
Chlorpirifos	7,5	350	198	20	Positive
Diuron	6	233	72,1	20	Positive
Hexazinone	4,7	253,2	171,1	8	Positive
Imidacloprid	1,1	256	175,1	15	Positive
Malathion	7,3	331	99	15	Positive
Simazine	4,3	202	124	15	Positive
Tebuconazole	9,2	308,2	70	10	Positive
Tebuthiuron	5	229,1	172,1	10	Positive

Results and Discussion

The efficiency of SPE extraction was measured by recovery tests using synthetic samples of 200 and 500 ng L⁻¹ of each target pesticide. Figure 1 shows

that the recoveries varied between 60 and 115%, which were considered appropriate for the required chemical analyses.

Figure 1. Average extraction efficiency for each compound at two levels of fortification (500 ng L⁻¹ and 200 ng L⁻¹).



The limit of detection (LOD) and the limit of quantification (LOQ) of the analytical method were determined at ng L⁻¹. Samples were considered positive when detected at concentrations above the LOD, and concentrations were determined only when they exceeded the LOQ of the analytical method. Samples were considered negative (ND) when determined at concentrations below the detection limit of the method. The pesticides detected in the samples at concentrations higher than LOD and lower than LOQ were therefore considered positive but were not quantified and were expressed as values lower than the limit of quantification of the method (<LOQ). During the study period, five of the 14 target pesticides were

quantified in the freshwater and drinking water samples (carbendazim, imidacloprid, atrazine, diuron and azoxystrobin), and another four were detected at concentrations below the limit of quantification of the analytical method (simazine, hexazinone, tebuthiuron and tebuconazole). The compounds carbofuran, amethrin, clomazone, malathion and chlorpyrifos were not detected in any samples at concentrations above the LOD of the analytical method (Table 3).

Most of the target compounds, do not have values stipulated by CONAMA Resolution 357/2005 (BRASIL, 2005) and Brazil's Ministry of Health Ordinance 2914/2011 (BRASIL, 2011).

The pesticide imidacloprid was detected in 95% of the source and water supply, with average concentrations of 27 ng L⁻¹ and 36 ng L⁻¹, respectively. Imidacloprid belongs to the neonicotinoid group and was temporarily banned in the European Union. In Brazil, its use is being re-evaluated by IBAMA due to its toxic effects on non-target organisms, especially bees (CARRILHO et al., 2013; USEPA, 2008). According to Martini et al. (2012), imidacloprid is a compound with high transport potential when dissolved in water. Bortoluzzi et al. (2006) evaluated surface water samples in a microbasin in the region of Agudo (Rio Grande do Sul State) and detected the presence of imidacloprid by HPLC/UV in concentrations between 0.38 to 2.1 µg L⁻¹. Studies developed by Bortoluzzi et al. (2007) evaluated the presence of imidacloprid in surface water samples from the rivers Jacuí, Guaporé and Camaquã (Rio Grande do Sul State) by HPLC/DAD and detected concentrations up to 4.53 µg L⁻¹, which suggests that imidacloprid has a high leaching capacity.

Azoxystrobin, a fungicide of the strobilurin class, was detected in 62% of surface water

samples and 58% of potable water samples, with average concentrations of 28 ng L⁻¹ and 23 ng L⁻¹, respectively. It is currently the most extensively fungicide used in the world (RODRIGUES et al., 2015). According to a report by the USEPA (2006), azoxystrobin is considered highly environmentally hazardous and highly toxic to aquatic organisms. Although it is poorly soluble in water, it can be leached from the source, and therefore can be found in water bodies.

The herbicide atrazine was detected in 58% of surface water samples and 79% of water supply samples. Among the compounds studied, atrazine presented the highest concentrations, 265 ng L⁻¹ in surface water and 241 ng L⁻¹ in drinking water. Atrazine is a common contaminant in water bodies, including groundwater (MOREIRA et al., 2012; BORTOLUZZI et al., 2006; COUTINHO et al., 2006; PESSOA et al., 2003). Studies on fish demonstrate the adverse effects promoted by atrazine, such as the reduction of antioxidant enzymes and biotransformation in hepatic cells of exposed fish, as well as DNA damage in different cell types (SANTOS; MARTINEZ, 2012).

Table 3. Detection of frequency, minimum, maximum and average concentrations and limits of detection (LOD) and quantification (LOQ) for the target pesticides analyzed in 24 wellspring water samples (WW) and 24 drinking water samples (DW) collected in Londrina, PR, from December 2014 to October 2015.

Pesticides	Frequency of positive samples %						Concentrations (ng L ⁻¹)						Brazilian Laws		
	> LD		> LQ		Minimum		Average		Maximum		LD (ng L ⁻¹)	LQ (ng L ⁻¹)	CONAMA 357/2005	PORTARIA 2914/2011	
	WW	PSW	WW	PSW	WW	PSW	WW	PSW	WW	PSW					
Ametrine	0	0	0	0	ND	ND	ND	ND	ND	ND	24	74	-	-	
Atrazine	58	79	8	16	165	101	216	145	265	241	30	90	2 mg L ⁻¹	2 mg L ⁻¹	
Azoxystrobin	95	95	62	58	15	15	27	23	49	38	4	13	-	-	
Carbendazin	83	8	37	4	16	-	18	86	23	-	5	16	-	120 mg L ⁻¹	
Carbofuran	0	0	0	0	ND	ND	ND	ND	ND	ND	6	20	-	7 mg L ⁻¹	
Clomazone	0	0	0	0	ND	ND	ND	ND	ND	ND	33	99	-	-	
Chlorpirifos	0	0	0	0	ND	ND	ND	ND	ND	ND	1	4	-	30 mg L ⁻¹	
Diuron	75	83	4	4	< LOQ	7,1	< LOQ	7,6	< LOQ	8,1	8	24	-	90 mg L ⁻¹	
Hexazinone	4	0	0	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	7	23	-	-	
Imidacloprid	95	95	16	8	11	26	27	36	50	47	6	18	-	-	
Malathion	0	0	0	0	ND	ND	ND	ND	ND	ND	25	76	100 mg L ⁻¹	-	
Simazine	12	12	0	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	8	23	-	2 mg L ⁻¹	
Tebuconazole	33	33	0	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	9	27	-	180 mg L ⁻¹	
Tebuthiuron	4	8	0	0	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	6	19	-	-	

Currently, atrazine is classified as an endocrine disruptor, and studies have demonstrated that atrazine is the most frequent surface water contaminant in the USA, present in 47% of samples analyzed by GC/MS collected at High Mississippi River (USA), with average concentrations of 150 ng L^{-1} (TAGERT et al., 2014), which means almost the same average obtained in this study (216 ng L^{-1}).

The fungicide carbendazim was detected in 83% of surface water samples, with an average concentration of 17 ng L^{-1} . However, in drinking water samples, carbendazim was detected in only 8% of the samples. According to Coutinho et al. (2006), the environmental persistence of carbendazim is variable, depending on the loss of its activity by abiotic or biotic factors, which can vary from 1 to 2 months in peat and up to 25 months in anaerobic conditions, favoring environmental contamination, mainly due to its half-life in the water of approximately 350 days. The presence of carbendazim in the environment may be related to the increase of its solubility at higher temperatures (SILVA et al., 2014). Ribeiro et al. (2013) determined the presence of carbendazim in surface water samples in a degraded source region of the São Lourenço River, using HPLC/DAD, at concentrations between 4 to $28 \text{ } \mu\text{g L}^{-1}$. The toxic effects that carbendazim can promote in animals, such as changes in the reproduction and endocrine systems of rats and other mammals (JIANG et al., 2014). Thus, as well as in aquatic organisms, such as the induction of apoptosis, immunological toxicity and endocrine disruption (JIANG et al., 2015; CUPPEN et al., 2000).

Diuron was detected in 75% of surface water samples and 83% of drinking water samples. According to Garcia et al. (2012), diuron is a compound considered to have low mobility (2,68) and low solubility in water (42 mg L^{-1}), which may explain the low concentrations detected in this study.

Simazine was detected in 12% of both surface and drinking water samples in this study. Previous studies have demonstrated that simazine has a characteristic of medium solubility in water (5 mg L^{-1}), as well as the potential of water contamination and show that water contamination by simazine is a concern, since this compound has a characteristic of high environmental persistence and may cause bioaccumulation (MORAES et al., 2008; DORES; DE-LAMONICA-FREIRE, 2001).

In general, the compounds were detected at low concentrations in the source water and treated water samples. However, the possibility of impact in environmental, plant and animal health cannot be ruled out, as some of these substances have been indicated as likely compounds of mutagenic action and endocrine disruption. In addition, studies demonstrate that chronic exposure to pesticides can promote deleterious effects on non-target organisms and even on human populations (BURILLO-PUTZE et al., 2014; OLIVEIRA et al., 2014; RIGOTTO et al., 2013; COSTA; TEIXEIRA, 2012; CALDAS; SOUZA, 2000). It is also important to highlight that the samplings were punctual, and the treated water samples do not represent the spring water in these cases, once water treatment can cause desorption of the pesticides that could be adhered to the sediment. For compounds having maximum allowable concentrations prescribed in legislation, it is important to note that there are more restrictive laws than those in Brazilian legislation. In the European Union (UE), Regulation (CE) 396/2005 on February 23, 2005 established the maximum value of $0.1 \text{ } \mu\text{g L}^{-1}$ for any pesticide in water for human consumption, and the total value allowed for the total concentration of pesticides of $0.5 \text{ } \mu\text{g L}^{-1}$. The exception occurs only for the compounds aldrin, dieldrin and heptachlor, having a maximum allowable concentration of $0.03 \text{ } \mu\text{g L}^{-1}$ (CE, 2005). In this work, All the agrochemicals surveyed were found in lower limits than those determined by European legislation. However, the low values

detected do not exclude the possibility that these compounds may have harmful effects on exposed ecosystems and organisms, especially in long-term exposures.

Conclusion

Considering the results obtained from the Tibagi River water samples, it is noted that there is contamination by pesticides and that the concentrations obtained were lower than the values stipulated in legislation for the predicted compounds, according to the CONAMA 357/2005 Resolution. Likewise, the concentrations determined in drinking water samples for the expected compounds are within the national water standards provided for in the Ministry of Health Ordinance 2914/2011. This study suggests the use of pesticides has had an impact on water resources of the Tibagi River. The evaluation of the compounds serves as a basis for the knowledge of the main compounds that are being used in agriculture in the region surrounding the Tibagi Basin and its tributaries and provides important parameters for future studies. This work demonstrates the presence of several pesticides in the water that are not recognized by the current legislation, so that studies are still lacking to see if these products, even at low concentrations, can cause adverse effects to exposed organisms, over time.

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Conflict of interests

The authors declare that there is no conflict of interest.

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