

# The Transfer of Insecticides Used in Cotton Production to Aquatic Ecosystems in the Cotton Basin in Northern Benin

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## Summary

The transfer of two insecticides (acetamiprid and chlorpyrifos) from cotton fields to a nearby water reservoir together with the ecotoxicological risks involved for this receiving environment were assessed by measuring their concentrations in runoff water and sediment samples collected both at the exit of the crop plots and in the reservoir after treatment. The multi-residue method by UPLC-MS/MS was used to determine the concentration of the different active substances in the collected samples. The results obtained indicate that acetamiprid and chlorpyrifos are almost always present regardless of the treatment period. The physico-chemical properties (solubility in water and adsorption to soil particles) explain a difference in behaviour between acetamiprid, present mainly in water, and chlorpyrifos, more concentrated in sediments. Calculations show that 0.005% of the acetamiprid applied in the basin around Gambanè flows to the reservoir, compared to 0.0003% of the chlorpyrifos applied. Depending on the sampling dates, the average runoff quantities for acetamiprid vary from 0.002 to 0.156 g/ha over 96 g/ha and from 0.001 to 0.039 g/ha for chlorpyrifos. The study shows that the transfer by runoff from the fields is relatively fast but is nevertheless influenced by rainfall, the distance of the fields to the reservoir, the slope and the characteristics of the environment. At the concentrations of acetamiprid observed in this receiving environment, the ecotoxicological risk would be negligible (Environmental Risk Index = 4) for aquatic organisms, earthworms and birds. However, concentrations of chlorpyrifos in the environment could be harmful to these organisms (Environmental Risk Index = 272). The aquatic ecosystem in this area is therefore exposed to the harmful effects of these active substances.

**Key words:** acetamiprid; chlorpyrifos; UPLC-MS/MS; cotton

## Introduction

In Benin, the cotton sector accounts for about 80% of exports and 35% of tax revenues [18, 26, 48, 59]. To improve yields and control pests, the cotton sector alone consumes nearly 90% of the insecticide market and 96% of chemical fertilizers sold in the country [33, 60]. However, the use of plant protection products in the control of cotton pests and weeds has consequences for the health of farmers and consumers, as well as for the environment [53]. The vast majority of these chemicals, overused during successive agricultural campaigns, end up polluting the environment by various mechanisms including air drift, direct leaching into the soil or runoff at their surface under the effect of rainfall. Previous research in Benin has shown that local dispersion of plant protection products by wind during treatment

depends, in addition to weather conditions, on the type of spraying equipment used (backpack sprayer or centrifugal cane) and the spraying height [29]. The leaching of active substances in soils has also been extensively documented as well as their transfer by runoff into aquatic ecosystems, which would depend on several factors including climate, crop type, soil condition and agricultural practices [13, 14, 15, 31, 39]. In all cases, the surrounding aquatic ecosystems (both surface and underground) are generally the final recipients of these active substances or their sometimes more toxic derivatives with sometimes harmful effects on indigenous organisms present or benefiting from the resources of these ecosystems [5, 16, 28, 29, 50]. Thus, in the water reservoirs that constitute the receiving aquatic ecosystems in the Beninese cotton basin, organisms could be permanently exposed to these pesticide residues. Some of these residues, may persist

for several years in the environment while others may accumulate in sediments and food chains [2, 12, 23]. The risk of exposure of organisms on land, air and water to pesticide residues has already been assessed locally by several authors [3, 8, 36, 64]. However, the biophysical factors including climate (rain), farming practices and soil type, favouring the runoff transfer of the active substances present in the majority of pesticides currently used in the cotton basin, including acetamiprid and chlorpyrifos, from fields to receiving aquatic ecosystems have not yet been studied. But yet previous studies show that acute toxicity of acetamiprid presents a low risk to fish, amphibians, algae and aquatic plants but presents a high risk to aquatic insects and chlorpyrifos is highly toxic to freshwater fish and aquatic invertebrates [20, 61]. The objective of this study is to assess the influence of these factors transfer into the environment by runoff and the ecotoxicological risks associated with the presence of these insecticides in the environment.

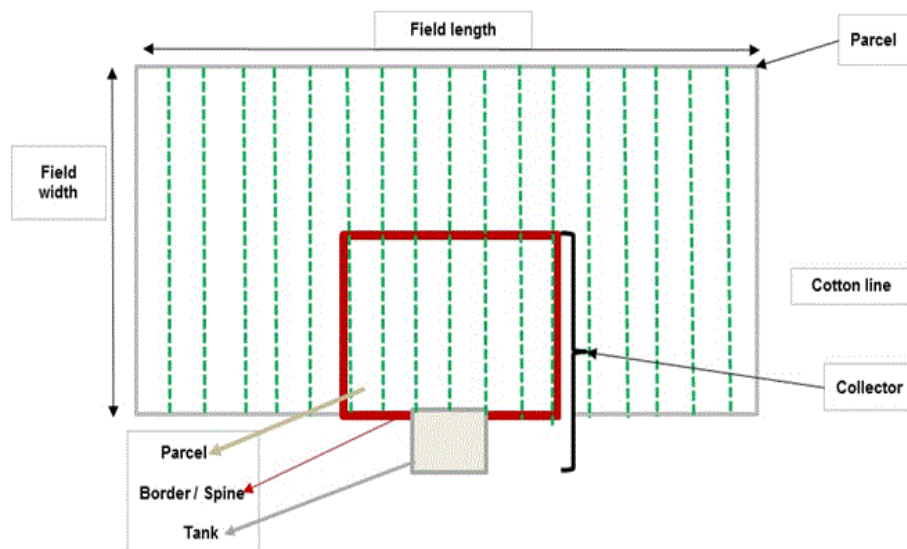
## Materials and Methods

### Location and Characteristics of the Study Site

The study was carried out in the Gambanè water retention sub-watershed located in the commune of Kandi in northern Benin. This region is one of the largest cotton producing regions in Benin with an estimated pesticide consumption of more than 90,000 litres per year [57]. This area is characterized by an average rainfall of 1,210 mm/year, an average temperature ranging from 22°C to 35°C and an average relative humidity of 73% during the rainy season and 38% during the dry season [10]. The use of large quantities of pesticides, proximity to fields, cultural practices, and the lack of natural or artificial development between fields and water retention are all factors that could favour contamination of the environment. Exploratory measurements carried out during previous campaigns have shown high concentrations of pesticide residues in water and sediment samples, as well as in the muscles of tilapia *Oreochromis niloticus* (Linnaeus, 1758) captured in the Gambanè water reservoir.

### Selection of Plots and Installation of Collectors

Six cotton fields, chosen according to their proximity to the water reservoir and the availability and interest of their owners, were the focus of this study. The selected plots were also chosen as they were relatively flat and free of any obstacles (trees, tree stumps, termite mounds, etc.) that could hinder the flow of water to the water reservoir. Where water would flow out of each field, a water collection device was installed with the help and collaboration of the owners. Each collector was thus in the form of a 25 m x 25 m cultivated area, bounded on each side by a raised ridge in the form of 30 cm high bunds, and a tank made of smooth galvanized sheet metal placed in a pit (Figure 1). The 250-litre collection reservoir, comparable to the one recommended by Olivier (2010), was placed below the plot to collect runoff water and sediments carried by rain. Five rain gauges were installed in the Gambanè catchment area to record the quantities of water that fell after each rainfall event.



**Figure 1:** Diagram of the runoff water collection system installed in cotton fields

## Water and Sediment Sample Collection and Storage

The schedule for taking water and sediment samples from the collectors and the water retention was developed based on 1) the cotton treatment schedule divided into three windows (with 2 treatments per window) and 2) the cumulative amounts of rain that could cause runoff during the study period. In total, for each of the 6 fields studied, 16 samples (including 8 water and 8 sediment samples) were taken from the collector reservoir and the water reservoir: one sample from the water reservoir before the first treatment to assess the initial level of contamination, six samples during the treatment phase (one sample after each treatment followed by sufficient precipitation) and one sample 2 weeks after the last treatment. Samples were collected in amber bottles, previously washed and dried, of 1 liter for water and 20 ml for sediment. The bottles were then stored in the freezer at -20°C until they were shipped for analysis. During sampling, the pH of the water ranged from  $8.2 \pm 0.1$  to  $8.9 \pm 0.6$  in the collectors and from  $8.0 \pm 0.4$  to  $8.4 \pm 0.1$  in the water reservoir. The water temperature also varied from  $24.6 \pm 1.3$  to  $30.9 \pm 1.0$ °C in the collectors and from  $23.8 \pm 2.4$  to  $31.6 \pm 3.1$ °C in the water reservoir.

## Analysis of Water and Sediment Samples

Water samples were analysed on a Waters ACQUITY UPLC™ (Ultra Performance Liquid Chromatography), equipped with a quaternary pump and membrane degasser. The separation column, an Acuity UPLC BEH C18, 130Å, 1.7 µm, 2.1 mm x 50 mm, was kept at 40°C. An automatic injector was set to inject 10 µl per sample. The mobile phase components were (A) Mille-Q water with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid. The gradient used was set at a flow rate of 0.4 mL min<sup>-1</sup> of 98% mobile phase A for 0.25 min. From 0.25 min to 7 min, a linear gradient was used to 98% mobile phase B, which was maintained for 1 min. Then, a linear gradient was used to 98% mobile phase A and maintained for 1 min. Sample analyses were performed using a triple quadrupole system with electro spray ionization (Waters Xevo® TQD mass spectrometer detection; Waters, Zellik, Belgium). The capillary needle was maintained at +2 kV. For operation in the MS/MS (Mass Spectrometry) mode, the following parameters were set: curtain gas (N<sub>2</sub>) at 7 bar; temperature 500°C. The active ingredients (AIs) were monitored and quantified using multiple reaction monitoring (MRM). Optimization of the MS/MS conditions, identification of the parent and product ions, as well as the selection of the cone and collision voltages, was performed through direct infusion of their individual standard solutions. After the optimization of the collision cell energy, two different m/z transitions were selected for each analyte, one for quantification and one for confirmation. Chlorinated pesticides were analyzed using an Agilent Technologies 6890N gas chromatograph equipped with an Agilent Technologies 7683 Series auto sampler injector, coupled to an electron capture detector (GC-ECD). Separation was performed on a HP-5MS (5% phenyl methyl siloxane) capillary column (30 m x 0.25 mm, 0.25

µm film thickness). The temperature of the injector and detector was maintained at 200°C and 250°C, respectively. Helium was used as a carrier gas at a flow rate of 1.1 mL min<sup>-1</sup> and the injections were made in the split mode with a split ratio of 52.7:1. Concentrations of 0.004 mg/L, 0.01 mg/L, 0.02 mg/L, 0.04 mg/L and 0.1 mg/L were prepared in hexane from a stock solution for each active ingredient to form the calibration curve. In total, 30 active ingredients were monitored using a multi-residue method. The recovery analysis was conducted for each active ingredient using the spike-placebo recovery method. For this, four blank samples (8 for water sample) were spiked and analyzed under the same conditions with the same extraction procedure. As the concentration and volume of the spiked solution is known, the recovery can be calculated for each active ingredient.

## Estimation of the Quantities of Active Substances Run Off

For each sampling, the quantities of each active substance transferred by runoff from the plot to the water retention were calculated as follows:

- For water samples:  $Q_{\text{WATER}} = C_{\text{ASx}} V_{\text{WC}}$  with:  $Q_{\text{WATER}}$  = Quantity of active substance present in runoff water (mg) per collector; CAS: Concentration in water (mg/L) of active substance at the collector; VWC: Volume of water (in litres) collected by the collector.

- For sediments:  $Q_{\text{SED}} = C_{\text{ASx}} M_{\text{SC}}$  with:  $Q_{\text{SED}}$  = Average amount of active substance (mg) present in the sediment during runoff into the collector; CAS: Concentration in the sediment (mg/Kg) of the active substance at the collector;  $M_{\text{SC}}$ : Mass of sediment in the collector (Kg).

- The total quantity for a treated area of 625 m<sup>2</sup>:  $Q_{\text{TA}}$  (in g) =  $Q_{\text{WATER}} + Q_{\text{SED}}$

Runoff should be expressed as a percentage of the total amount (TQ) of active substance applied per hectare. Runoff was calculated on the basis of the total area of cotton fields in the basin (109 ha) multiplied by the dose/ha of the active substance (16 g/ha acetamiprid and 100 g/ha for chlorpyrifos), multiplied by the number of treatments (6 and 3 respectively). The percentage of runoff of each active substance (P%) over a ha is then determined by the formula:

$$P\% = 100 \times (Q_{\text{TA}} \times 16 / \text{TQ}).$$

In addition, based on the concentrations obtained in the water and sediment samples from the water reservoir, the maximum possible concentration level ( $C_{\text{MAX}}$ ) of the active substances for the entire body of water was estimated to determine whether, in theory and by taking the worst case, the LC<sub>50</sub> or PNEC values (predicted concentration without adverse effect) could be exceeded for the species living in the reservoir. The volume of the reservoir was estimated at 36,300 m<sup>3</sup> (area of 1.23 ha and average depth of 3 m during rainy periods according to the water reservoir management plan).

### Measurement of the Transfer Time from the Fields to the Water Reservoir

To estimate the transfer time of active substances deposited at the plot level to the water reservoir, a fluorescent tracer (sodium fluorescein salt) that colours the water bright yellow was used [34, 56, 58, 65]. Five fluorescein tests were performed to estimate the transfer time per runoff as a function of the amount of rainfall. The method consisted of soaking rags with fluorescein (the greater the distance, the greater the amount deposited due to the expected dilution effect) and placing them on the ground, at a specified distance (100 m; 200 m; 300 m; 400 m and 500 m) from the water reservoir, just before a rainfall. Then, the time when the water in the reservoir began to turn color was measured.

### Risk Assessment

The potential risks associated with the presence of insecticides in the environment were estimated using the method described by Samuel *et al.* (2012), using the Quebec *Pesticide Risk Indicator (PRI) for the Environment (IRPeQ-Environment)* to classify active substances according to their risk level. The Environmental Risk Index (ERI) was calculated on the basis of 6 variables: 3 ecotoxicological variables (impact on terrestrial invertebrates, T; impact on birds, B; impact on aquatic organisms, A) and 3 physico-chemical properties (mobility, M; persistency in soil, P; bioaccumulation, C). The ERI was obtained using the following formula:

$$\text{ERI Active substance} = [1.75 \times (T + B) + A + M + P + C + 1]2$$

(Samuel *et al.*, 2012).

Data on pesticide toxicity and ecotoxicity necessary for the calculation of the ERI were obtained from the *European Pesticides Database*, the *AGRITOX database of the ANSES (France)*, and the *Pesticide Properties DataBase (PPDB, University of Hertfordshire)*.

### Processing and Statistical Analysis of Data

The data collected were entered and processed using Excel spreadsheets (2013). The Kruskal-Wallis non-parametric tests were used to compare the concentrations of acetamiprid and chlorpyrifos obtained in the water and sediment samples from the collectors and reservoir.

### Results

#### Active Substances in Collected Water and Sediment

During the crop year, 12 commercial products (including 8 insecticides and 4 herbicides) were used to control cotton pests and weeds. The insecticides used were only EC formulations and all contain active substances that are toxic to humans and dangerous to the aquatic environment, according to Classification according to Regulation (EC) No 1272/2008 CLP (Classification, Labelling and Packaging of substances and mixtures) Table 1. Acetamiprid, emamectin benzoate and chlorpyrifos are the most commonly used active substances. A total of six active substances contained in the pesticides used were identified in the water and sediment samples collected in the water reservoir and the collectors in the study area. The results of the analyses indicate the almost systematic presence of acetamiprid and chlorpyrifos residues in the samples collected, in water and sediment, in both the collectors and the reservoir. By contrast, residues of cypermethrin, lambda-cyhalothrin, deltamethrin and endosulfan (0.001 mg/L, in a single water sample from the reservoir at the end of the spraying campaign), were found only later, from the second treatment window (3rd to 6th treatment). No trace of lindane or other active substances tested by the multi-residue method were observed.

**Table 1:** List of insecticides used during the 2016 - 2017 Cotton Cropping

Commercial Product names	Active substances	Recommended dose (L/ha)	Application window
Thalis® 112 EC	Emamectin benzoate (48g/L) + Acetamiprid (64g/L)	0.25	1st to 3rd
Sibemac® 112 EC	Emamectin benzoate (48g/L) + Acetamiprid (64g/L)	0.25	
Acer® 35 EC	Acetamiprid (15g/L) + Lambdacyhalothrine (20g/L)	0.5	2nd
Pyrofte+®472 EC	Chlorpyrifos (400g/L) + Cypermethrin (72g/L)	0.5	2nd and 3rd
Vizir®92 EC	Cypermethrin (72g/L) + Emamectin benzoate (20g/L)	0.5	

Lambda super® 2.5 EC	Lambda-cyhalothrin (25g)	0.5	3rd
Dusuban. b super®	Lambda-cyhalothrin (25g)	0.5	
Cotonix® 328 EC	Deltamethrin (12g/L) + Chlorpyrifos (300g/L) + Acetamiprid (16g/L)	1	

**Evolution of Concentrations of Acetamiprid and Chlorpyrifos**

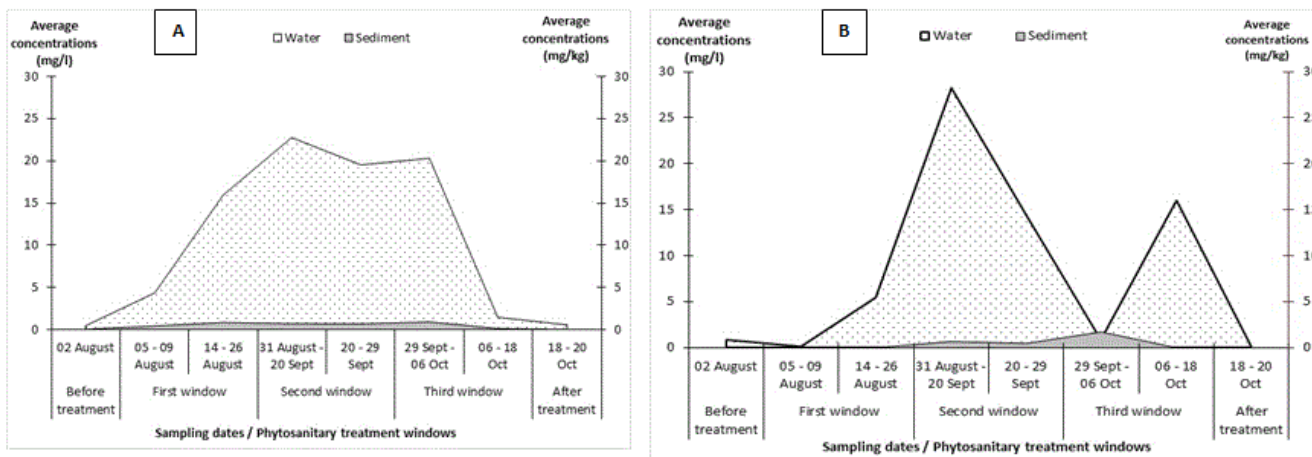
Table 2 shows the minimum, maximum and average concentrations (in mg/L), with their standard deviations, for acetamiprid and chlorpyrifos found in water and sediment samples (12 water and sediment samples per treatment window). Analysis of this table shows that the levels of acetamiprid detected in the water of the collectors vary from 0.002 mg/L to about 50 mg/L, while in the water reservoir they vary from 0.093 mg/L to about 30 mg/L. These concentrations are much lower in sediment in both collectors (< 2.7 mg/kg) and water reservoir (< 1.7 mg/kg). However, for chlorpyrifos, the results indicate significant concentrations in sediment (> 450 mg/kg in the collectors and about 210 mg/kg in the water reservoir), while in water they are relatively low, both in the samples collected in the collectors and in the reservoir. Comparison tests of Kruskal-Wallis averages for the concentrations of each of the two active substances taken separately, in the collectors and in the water reservoir, show that there is a significant difference “(p<0.05)” between the concentrations of acetamiprid in water and those in sediments, depending on the sampling periods. The same is true for chlorpyrifos concentrations measured in water and sediment “(p < 0.05)”.

**Table 2:** Summary of average and extreme concentrations of Acetamiprid and Chlorpyrifos in water (mg/L) and sediment (mg/kg) samples collected from collectors installed around the Gambanè water reservoir during the 2016-2017 cotton cropping (collectors: n = 6 ; water reservoir: n = 1)

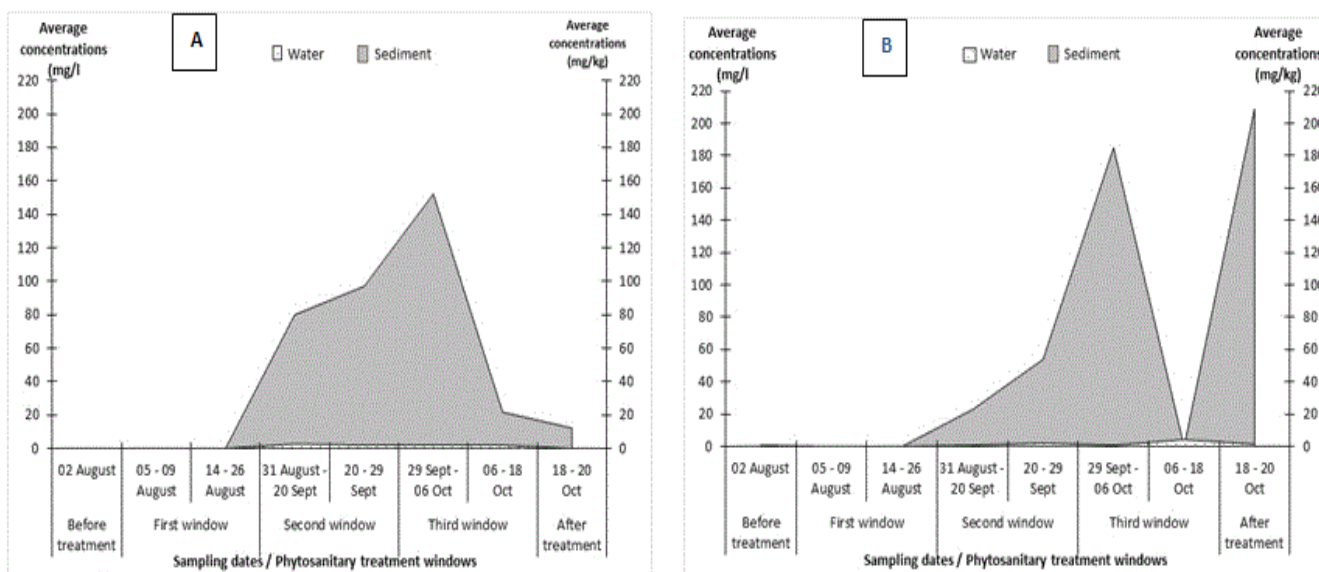
Active substances	Sampling periods	COLLECTORS						WATER RESERVOIR	
		Concentrations of A.S (mg/L) in water			Concentrations of A.S (mg/kg) in sediment			Concentrations of A.S. (mg/L) in water	Concentrations of A.S. (mg/kg) in sediment
		Min	Max	Average	Min	Max	Average		
Acetamiprid	Before treatment	0.011	2.643	0.501 ± 1.052	0.001	0.095	0.048 ± 0.066	0.914	-
	1stwindow	0.005	49.968	10.175 ± 15.023	0.0004	2.12	0.640 ± 0.478	2.776	0.0003
	2ndwindow	0.573	44.143	21.127 ± 9.581	0.026	2.096	0.698 ± 0.619	21.365	0.584
	3rdwindow	1.044	24.046	10.891 ± 5.496	0.071	2.723	0.517 ± 0.560	8.443	0.868
	After treatment	0.002	1.531	0.513 ± 0.881	0.0004	0.137	0.028 ± 0.061	0.093	0.122
Chlorpyrifos	Before treatment	0.002	0.411	0.087 ± 0.162	0.004	0.033	0.014 ± 0.012	0.487	-
	1stwindow	0.0004	0.022	0.008 ± 0.008	0.002	-	0.002	0.008	-
	2ndwindow	0.028	3.719	1.205 ± 0.877	0.198	238.396	88.485 ± 68.925	0.619	38.383
	3rdwindow	0.007	7.019	1.393 ± 1.770	0.006	452.513	86.948 ± 95.431	1.039	92.457
	After treatment	-	-	-	0.02	60.532	12.189 ± 27.025	0.777	208.8



Figures 2 and 3 compare the changes in the average concentrations of acetamiprid and chlorpyrifos in water and sediment recorded in the collectors and reservoir as a function of sampling dates and windows. These figures illustrate the difference in the environmental fate of these two active substances.



**Figure 2:** Average concentrations of acetamiprid (A) and chlorpyrifos (B) in water and collector sediments according to sampling dates and windows



**Figure 3:** Average concentrations of acetamiprid (A) and chlorpyrifos (B) recorded in the water and sediments of the water reservoir as a function of the sampling dates and windows

**Estimate of quantities transferred by runoff**

Tables 3 and 4 show that the quantities of pesticides that run off are relatively small compared to the total mass (TQ) sprayed in cotton fields around the water reservoir. The quantities that run off increase over time then decrease when applications are completed. Maximum concentrations in the water reservoir during the 2016 - 2017 cotton cropping are estimated at 0.0014 mg/L for acetamiprid and 0.0003 mg/L for chlorpyrifos.

**Table 3:** Quantity of Acetamiprid found in the water and sediments of the collectors installed around the Gambanè water reservoir during the 2016-2017 cotton season (collectors: n = 6)

Periods (Sampling dates)	Quantities (mg) found in collectors		Total quantity runoff per collector (QTA,mg)	Quantity of runoff per hectare (mg/ha)	Average amount of runoff per hectare (g/ha)	Median (In g/ha)	Total quantity (g) applied in the basin (TQ)	Runoff (P in %)
	Water (Q Water)	Sediment (QSED)						
Before treatment (02 August)	0.016	-	0.016	0.256	0.002 ± 0.004	0.0003		
	0.025	< 0.0001	0.025	0.397				
	0.555	0.001	0.556	8.896				
	0.018	-	0.018	0.284				
	0.089	-	0.089	1.423				
	0.005	-	0.005	0.086				
1st treatment (05-09 August)	0.047	0.001	0.048	0.759	0.035 ± 0.083	0.001		
	0.003	-	0.003	0.041				
	0.019	0,001	0.019	0.305				
	12.664	0.023	12.686	202.973				
	0.074	< 0.0001	0.074	1.185				
	0.151	< 0.0001	0.151	2.423				
2nd treatment (14 to 26 August)	0.804	-	0.804	12.856	0.106 ± 0.132	0.028	10464	0.01%
	2.165	-	2.165	34.64				
	15.157	0.011	15.169	242.699				
	18.988	0.018	19.006	304.096				
	1.107	0.003	1.11	17.752				
	1.332	0.001	1.332	21.314				

3rd treatment (August 31 - 20 September)	13.372	0.021	13.393	214.28	0.156 ± 0.095	0.1555		
	0.287	0.001	0.287	4.588				
	17.656	-	17.657	282.515				
	8.128	0.005	8.132	130.121				
	11.306	0.004	11.31	180.961				
	7.388	0.006	7.394	118.307				
4th treatment (20 to 29 September)	6.528	0.007	6.535	104.562	0.132 ± 0.053	0.1208		
	8.029	0.003	8.032	128.509				
	11.617	0.006	11.623	185.96				
	7.053	0.015	7.068	113.094				
	3.608	0.01	3.618	57.879				
	12.435	0.003	12.438	198.999				
5th treatment (September 29th - 06 October)	12.023	0.027	12.05	192.795	0.068 ± 0.086	0.024		
	10.19	0.017	10.207	163.308				
	-	0.001	0.001	0.013				
	1.655	0.001	1.656	26.5				
	1.336	0.005	1.341	21.453				
	0.219	0.003	0.223	3.56				
6th treatment (06 to 18 October)	-	0.002	0.002	0.029	0.002 ± 0.005	< 0.0001		
	-	0.001	0.001	0.021				
	-	0.001	0.001	0.012				
	-	-	-	< 0.0001				
	-	0.001	0.001	0.02				
	0.746	0.001	0.747	11.957				
After treatment (18-20 October)		< 0.0001	< 0.0001	< 0.0001	0.001 ± 0.002	< 0.0001		
	0.001	< 0.0001	0.001	0.015				
	-	-	-	< 0.001				
	-	< 0.0001	< 0.0001	< 0.0001				
	0.001	0.001	0.003	0.039				
	0.306	< 0.0001	0.306	4.899				
As an indication, the percentage of runoff (P%) calculated on the basis of the median value is 0.003%.								



**Table 4:** Quantity of Chlorpyrifos calculated in the water and sediments of the collectors installed around the Gambanè water reservoir during the 2016 - 2017 cotton season (collectors: n = 6; reservoir: n = 1)

Periods (Sampling dates)	Quantities (mg) found in collectors		Total quantity runoff per collector (QTA, mg)	Quantity of runoff per hectare (mg/ha)	Average amount of runoff per hectare (g/ha)	Total quantity runoff per collector	Total quantity (g) applied in the basin (TQ)	Runoff (p in %)
	Water (Q water)	Sediment (QSED)						
Before treatment (02 August)	0.0038	0.0003	0.0042	0.067	0.001 ± 0.001	0.0001		
	0.0024	0.0002	0.0026	0.042				
	0.0176	0.0001	0.0177	0.283				
	0.0008	0.0001	0.0009	0.015				
	0.0041	< 0.0001	0.0042	0.067				
	0.2056	0.0001	0.2056	3.29				
1st treatment (05 - 09 August)	0.001	-	0.001	0.016	< 0.001	< 0.0001		
	0.0002	-	0.0002	0.003				
	0.0023	-	0.0023	0.037				
	-	-	0	< 0.0001				
	0.0002	0	0.0002	0.003				
	0.0081	-	0.0081	0.13				
2nd treatment (14 to 26 August)	0.0043	-	0.0043	0.069	< 0.001	< 0.0001	32700	0.00%
	0.0067	-	0.0067	0.108				
	-	-	0	< 0.0001				
	0.0032	-	0.0032	0.051				
	0.0009	-	0.0009	0.014				
	0.0008	-	0.0008	0.012				
3rd treatment (August 31-20 September)	0.0156		0.0156	0.249	0.020 ± 0.017	0.0238		
	0.9595	1.6008	2.5603	40.965				
	0.011	0.0018	0.0128	0.206				
	0.8265	0.5497	1.3762	22.02				
	1.3388	0.7173	2.0561	32.898				
4th treatment (20 to 29 September)	0.5379	1.0658	1.6037	25.659	0.023 ± 0.012	0.02		
	0.2524	0.726	0.9784	15.654				
	0.3699	0.4121	0.7821	12.513				
	0.6951	0.7916	1.4867	23.787				
	0.3344	2.5017	2.8361	45.378				
	0.1443	1.285	1.4293	22.868				
	0.7261	0.3486	1.0747	17.196				

5th treatment (September 29th - 06 October)	3.5095	4.432	7.9415	127.065	0.039 ± 0.048	0.0147	0.039 ± 0.048	0.0147
	1.4357	2.5974	4.033	64.529				
	0.3141	0.1588	0.4728	7.565				
	0.2749	0.1446	0.4195	6.712				
	0.086	1.0122	1.0982	17.572				
	0.0756	0.6682	0.7438	11.901				
6th treatment (06 to 18 October)	0.535	0.1668	0.7017	11.228	0.007 ± 0.003	0.077	0.007 ± 0.003	0.077
	0.0024	0.2405	0.2428	3.885				
	0.0037	0.153	0.1566	2.506				
	0.4339	0.0001	0.4339	6.943				
	0.3029	0.2578	0.5607	8.97				
	-	0.5281	0.5281	8.45				
After treatment 18-20 October)	-	0.0003	0.0003	0.005	0.004 ± 0.004	< 0.0001	0.004 ± 0.004	< 0.0001
	-	0.0018	0.0018	0.029				
	-	0.0002	0.0002	0.004				
	-		0	< 0.0001				
	-	0.6221	0.6221	9.953				
	-	0.0021	0.0021	0.033				
As an indication, the percentage of runoff (P %) calculated on the basis of the median value is 0.0002%.								

### Estimation of the transfer time from the fields to the water reservoir

The transfer time per runoff between the emission site and the reservoir is relatively short regardless of the distance between the emission point and the water reservoir Table 5. The results show that the transfer of fluorescein takes only a few minutes, even for distances of 500 m (about 10-11 min), despite a relatively small average slope (1 to 2% on the plots and maximum 3% between the highest point of the catchment area and the water retention) and despite an average rainfall.

**Table 5:** Results of fluorescein tests for the estimation of transfer time by runoff from the plot to the water reservoir

Trial number	Distance to water retention (m)	Recorded rainfall (mm)	Transfer time (min)	Flow velocity (in m/s)
Test n°1	100	8.6	2 min 03 s	0.81
Test n°2	200	9.2	3 min 57 s	0.84
Test n°3	300	10.6	5 min 34 s	0.9
Test n°4	400	9.4	5 min 58 s	1.11
Test n°5	500	17	10 min 47 s	0.77

### Environmental Risk Indexes (ERI)

The calculated ERIs for the six active ingredients identified in the water and sediment samples analyzed are presented in Table 6. The analysis of this table shows that all identified active substances present a high risk of toxicity to aquatic organisms. In terrestrial invertebrates, only acetamiprid does not present an ecotoxicological risk (both in earthworms and bees), unlike others, which present a risk, particularly in bees. Regarding birds, only endosulfan and chlorpyrifos are potentially toxic. In all cases, endosulfan presents the highest risk to the environment, with an ERI of 380, followed by chlorpyrifos (ERI = 272) and deltamethrin (ERI = 196).

**Table 6:** Environmental risk indices (ERIs) for the identified active substances (n = 6) in the waters of the Gambanè basin

Parameters (values)									
Active substances	Ecotoxicological				Physicochemicals				
	Chemical groups	Impact on terrestrial Invertebrates(T)		Impact on birds(B)	Impact on aquatic organisms (A)	Mobility (M)	Persistency in soil (P)	Cumulative (C)	Environmental Risk Index (ERI)
		Earthworms	Bees						
Acetamiprid	Neonicotinoid	0	0	0	1	0	0	0	4
Chlorpyrifos	Organophosphate	0	4	2	3	0	0	2	272
Endosulfan	Organochlorine	4	4	2	4	0	2	2	380
Cypermethrin	Pyrethroid	0	2	0	3	0	2	2	123
Lambda-cyhalothrin		0	2	0	4	0	0	2	110
Deltamethrin		0	4	0	4	0	0	2	196

### Discussion

#### Variability of concentrations of acetamiprid and chlorpyrifos in water and sediment

Analysis of the samples indicates that acetamiprid levels are higher in water than in sediment, unlike chlorpyrifos. Thus, the concentrations of acetamiprid detected in the water of the collectors vary from 0.002 mg/l to almost 50 mg/l, and from 0.093 mg/l to about 30 mg/l in the water samples from the reservoir. In sediments, chlorpyrifos levels of more than 450 mg/kg were detected in the collectors and about 210 mg/kg in the water reservoir. These differences are explained by the physicochemical properties of these two compounds, particularly solubility, persistence, volatility, mobility and adsorbability [15]. Transfer, residual concentrations and persistence of these two active substances, which are mainly found in the environment in Gambanè during the cotton growing season, depend mainly on their intrinsic properties which are fundamentally different. The Sw value of acetamiprid shows that it is highly soluble in water, and according to its Koc it will have little tendency to adsorb to sediments, which explains why this substance is also mobile and concentrated in water samples [22]. Table 7. According to PMRA (2002), biodegradation will be the main route of elimination of acetamiprid because it is not hydrolyzed at room temperature

and pH values between 4 and 9 (which is the case here). Under aerobic conditions, acetamiprid will be weakly persistent in soil and moderately persistent in water. Under anaerobic conditions, the rate of degradation will be slower. In contrast, chlorpyrifos will be moderately mobile or even immobile in soils. Its solubility in water is very low and its adsorption constant on organic carbon (Koc) is high. These parameters explain the high concentrations recorded in the sediments of the collectors and the reservoir, and its low concentration in the water samples. Especially since chlorpyrifos is weakly persistent in water under aerobic conditions (half-life of 5 days) and its hydrolysis rate varies with the pH of the medium. For pH values between 5 and 7, its half-life is 72 days, but at pH 8 it is only 16 days.

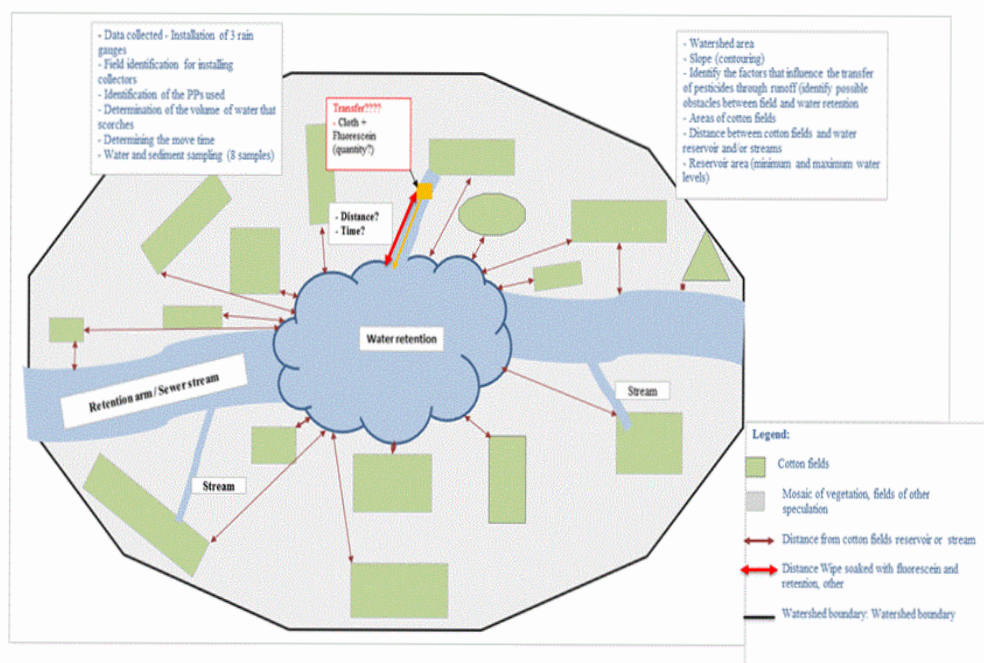
#### Runoff: the main route of transfer of active substances

When the pesticide is released, the deposition obtained in the crop is much lower than the theoretical deposition due to plant interception, drift, volatilization and runoff [54]. Tests conducted on drift under practical conditions [29] have shown that drift can lead to contamination of adjacent crops, but that transfer by this route to the reservoir will be limited to fine droplets that volatilize into the air and are carried off. On the other hand, the concentrations of active substances measured in the water and sediments of the collectors installed in the cotton plots allow us

to affirm that runoff is the most important route for the transfer of pesticides to aquatic environments. These results confirm further work on runoff [11, 13, 31, 39, 62, 63]. According to these authors, the extent of runoff depends on climate, crop type, soil conditions and agricultural practices. In the Gambanè basin, apart from the influence of farming practices, two factors explain the importance of the quantities of active substances transferred to the water body by runoff: rainfall and poor phyto sanitary practices (i.e. repeated over-doses). When rainfall exceeds a few tenths of a millimetre, surface runoff begins and carries the active substances. This observation accords with other authors who have shown that, under similar conditions, high concentrations are observed within a few hours of rainfall events [46]. For Labreuchee et al. (2005), transfers of plant protection products by runoff were very strongly linked to the volumes of water runoff. Chocat et al. (2007) found that water runoff was often accompanied by erosion and entrainment of particles previously deposited on the ground in dry weather (treatment fallout in particular) while Moilleronet et al. (2002) and Saget (1994) conclude that runoff is one of the main processes of pollutant input into non-target environmental compartments. In the current study, the total quantities that have been transferred by runoff to the water reservoirs were relatively small (respectively 0.005% for acetamiprid and 0.0003% for chlorpyrifos), consistent with the physico-chemical properties of the substances and comparable to the values found in the literature.

**Table 7:** Adsorption, solubility and persistency parameters of Acetamiprid and Chlorpyrifos (Source: Pesticide Properties Database, University of Hertfordshire)

Active substances	Parameters				
	Koc (ml/g)	Kd	Kow	Sw (mg/L)	DT50 (days)
Acetamiprid	200	1.1	6.31	2950 - 4250 (according to pH)	3
Chlorpyrifos	5509	126.6	5.01.104	1.05	5



Schematic representation of cotton fields in the catchment area. Runoff water collectors are installed in 6 cotton fields. After each rainfall event, preceded by phytosanitary treatment in cotton fields, water and sediment samples are taken from these collectors and sent to the laboratory for analysis

## Environmental risks related to the presence of acetamiprid and chlorpyrifos residues

Classification according to CLP regulation (Classification, Labelling and Packaging of substances and mixtures) No 1272/2008 (CE) shows that acetamiprid and chlorpyrifos are highly toxic to aquatic organisms. Chlorpyrifos is harmful and causes long-term adverse effects to aquatic organisms. Fish are particularly at risk whenever chlorpyrifos is used near surface waters (FAO 1997), and acetamiprid poses a risk to aquatic invertebrates (PMRA, 2002). Mamadouet *al.* (2005) have shown that the use of chlorpyrifos in the Niger River valley has had environmental risks with deleterious effects on insects. According to Emanset *al.* (1992), the environmental impact of a plant protection product will depend on the degree of exposure, resulting from its dispersion and concentration in the environment, and its toxicological properties. Based on the ERI calculation, chlorpyrifos presents a higher risk to aquatic organisms (ERI = 272) than acetamiprid (ERI = 4). Tests conducted with chlorpyrifos by Giesy *et al.* (1999) have shown that in fresh water invertebrates, such as crustaceans and insect larvae (essential for the food chain), are more sensitive to chlorpyrifos than molluscs, rotifers or even vertebrates. Although its ERI is low, the recurrent presence of acetamiprid in various environmental compartments poses a risk to biodiversity because several authors have shown that neonicotinoids generally act at very low doses on the central nervous system, affecting insects and particularly bees. They affect their mobility through symptoms such as tremors, uncoordinated movements and hyperactivity [19, 37]. In mammals, acetamiprid also causes lymphocyte depletion and bleeding in exposed rats (Mondalet *al.*, 2009). In addition, growth inhibition was found in Australian catfish (*Tandanus tandanus*) exposed to 2 or 10 µg/L chlorpyrifos [32]. Unfortunately, the period of intense cotton production (characterized by heavy rains that run off into aquatic ecosystems) corresponds to the period of high use of plant protection products and coincides with the breeding season of several fish species living in these rivers, such as clarias, tilapias, Chrysichthys, Heterobranchus, etc. [38]. By comparing the estimated concentration of acetamiprid with the LC50 values of 264.50 mg/L for *Clarias gariepinus* fingerlings and 182.90 mg/L for *Oreochromis niloticus* (Linnaeus, 1758) fingerlings (values obtained in the AquaTox project), we can roughly estimate that the risk would be zero for this active substance for fish [2]. This confirms the results of Zoumenouet *al.* (2018), who found low concentrations of acetamiprid, in the range of the ppb, in 3 water reservoirs in the cotton basin (in Benin), including Gambanè, which should not have negative impacts on aquatic species present in these environments.

## Conclusion

This study has highlighted the importance of the phenomenon of transfer by runoff of plant protection products. During heavy rainfall and in the absence of any obstacles, the transfer of active substances from the point of emission to the water reservoir is

possible in a few minutes. Almost all the active substances used against cotton pests in the cotton basin in Benin are found in water reservoirs with an almost systematic presence of acetamiprid and chlorpyrifos found even after phytosanitary treatment periods. All these substances are known to be toxic to the environment and present risks to aquatic organisms according to the ERI values. The least soluble compounds attach themselves to soil particles and organic matter. They are then carried away by erosion during major runoffs, along with soil particles. In addition, this study showed us that the aquatic environment of this high pesticide use area is therefore exposed to the harmful effects of these active substances, of which cotton producers are unaware.

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