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Contamination of Water, Sediment and Fish with Residues of Pesticides Used in Cotton Production in Northern Benin

Caroline Douny[1] · Y. M. Berny's G. Zoumenou[1,2] · Martin Aïna[2] · Ibrahim Imorou Toko[3] · Ahmed Igout[4] · Léa Guedegba[6] · Sika K. Chabi[5] · Patrick Kestemont[6] · Marie-Louise Scippo[1]

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Abstract

In Northern Benin, insecticides are used for cotton production. These insecticides can be easily transferred to water ponds close to cotton fields. To monitor insecticides levels in water, sediments and fish samples from water ponds, a GC–MS analytical method was developed to detect residues of endosulfan, DDT and its parent compounds, isomers of HCH, pyrethroids and chlorpyrifos. In addition, the influence of storage conditions of water sample on pesticides determination performance has been studied. The limits of quantification were between 0.16 and 0.32 µg/L in water, 0.5 and 1 µg/kg in sediment and 1 and 2 µg/kg in fish. Twenty samples of water, twenty of sediments and forty of fish were taken in four different water reservoirs at five different times. Alpha-endosulfan, lambda-cyhalothrin and permethrin were identified in sediment while p,p′-DDE, α- and β-HCH, chlorpyrifos, lambda-cyhalothrin and permethrin were detected in fish. Only organochlorines were determined in water because of the lack of recovery of pyrethroids from water stored in glass. Concentrations of insecticide residues in sediment for all water ponds ranged from non-detected to 101 µg/kg and from non-detected to 36 µg/kg in fish. Preliminary risk assessment for consumers of the North of Benin showed that the Estimated Daily Intakes were lower than the Acceptable Daily Intakes and Acute Reference Doses for all consumers. However, as one fish can be contaminated by five pesticide residues at the same time, it is not possible to exclude a risk for the consumer due to his exposure to mixtures of pesticides.

The northern zone of Benin is recognized for its cotton production. Cotton is the most exported raw material and is the base of the Beninese economy. But many pesticides are used to fight cotton pests and to improve productivity. These chemicals are mostly used against insects that have become resistant to many treatments, which leads to increased spraying of pesticides (Okoumassoun et al. 2002). In order to reduce the resistance issue, Benin has opted for the use of pyrethroids because of their wide spectrum of action and because they have the advantage of being easily degraded and less persistent in the environment. But they are not free of risks. Indeed, studies have revealed that pyrethroids were extremely toxic to aquatic organisms, including fish such as *Clarias gariepinus* for example, for which the LC_{50} of lambda-cyhalothrin is 0.103 mg/L after a 21-day exposure (TDC Environmental 2003; Oluah and Chineke 2014).

In addition to pyrethroids, Beninese producers illegally use prohibited organochlorine insecticides such as endosulfan or DDT, which they can acquire in the neighboring countries thanks to the lack of control at the Beninese borders. Organochlorine insecticides are persistent organic pollutants containing chlorine. In 2011, endosulfan was very frequently

Caroline Douny and Y. M. Berny's G. Zoumenou have contributed equally to this work as first authors.

✉ Caroline Douny
cdouny@uliege.be

1 Laboratory of Food Analysis, Department of Food Science, FARAH-Veterinary Public Health, University of Liège, Liège, Belgium

2 Laboratoire des Sciences et Techniques de l'Eau, Ecole Polytechnique d'Abomey-Calavi, Université d'Abomey-Calavi, Godomey, Benin

3 Laboratoire de Recherche en Aquaculture et Écotoxicologie Aquatique (LaRAEAq), Faculté d'Agronomie, Université de Parakou, Parakou, Benin

4 Department of Biomedical and Preclinical Sciences, Faculty of Medicine, University of Liège, Liège, Belgium

5 Laboratoire de Contrôle et de Sécurité Sanitaire Des Aliments (LCSSA), Cotonou, Benin

6 Unit of Research in Environmental and Evolutionary Biology, Laboratory of Ecophysiology and Ecotoxicology, University of Namur, Namur, Belgium

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used in the North of Benin (commune of Banikoara) and represented 75% of the total amount of insecticides used (Agbohessi et al. 2011). This situation may be due to a lack of supervision of the cotton producers. Indeed, Adam and co-workers showed that 42% of farmers did not benefit from any technical supervision for the use of fertilizers and pesticides in the communes of Gogounou, Kandi and Banikoara, in the North of Benin (Adam et al. 2010).

The toxicity of organochlorine insecticides is not to be demonstrated anymore. Indeed, in *Oreochromis niloticus*, acute exposure to endosulfan causes imbalance metabolism and damage to vital organs such as the liver and gills (Kumar et al. 2016). In humans, organochlorine insecticides can have a toxic effect following acute or chronic exposure, by oral, dermal or inhalation route (Patočka et al. 2016; Menezes et al. 2017). Since 1970, the use of organophosphate insecticides has grown following the ban on the use of organochlorine insecticides. Although less persistent than organochlorine insecticides, organophosphates, such as chlorpyrifos, have a detrimental effect on the health of fish (Nwani et al. 2015; Brandt et al. 2015) and humans (Timoroğlu et al. 2014; Ismail et al. 2017).

In some localities, cotton fields are very close to water reservoirs, increasing the probability of their contamination with pesticides. Also, practices such as using pesticides to catch fish or washing empty bottles of pesticides in reservoirs are other sources of water pollution, with direct consequences on the ecosystem, mostly on water, sediment and fish species (Osibanjo et al. 1994; Pazou et al. 2006). Recently, many studies have focused on the issue of pesticide pollution due to the cotton production (Dognon et al. 2018; Gouda 2018; Gouda et al. 2018). Some of these studies revealed the bioaccumulation of pesticides in several aquatic species (Adam et al. 2010; Agbohessi et al. 2015). Their accumulation in low concentration in the body fat of fish (through the food chain) may pose health problems (Agbohessi et al. 2015; Molina-Ruiz et al. 2015).

Concerning the determination of the metabolites of organochlorine pesticides in water, several authors have proposed the development of analytical methods but rare are those who determined the concentrations of metabolites in real water samples (Reemtsmaa et al. 2013; Tiwari and Guha 2013; Zhou et al. 2013; Ramandeep et al. 2014; Mohammadnejad et al. 2017). In Northern Benin, no pesticide exposure assessment exists up to now for the population living close to cotton field and eating fish caught in water reservoirs. Consequently, the aim of this paper was to develop an analytical method based on gas chromatography coupled to mass spectrometry (GC–MS) to investigate the distribution of organochlorine, organophosphate and pyrethroid insecticide residues in water, sediment, fish of water ponds close to cotton fields of the North Benin and to provide more information about the possible risk for the local population consuming fish from these water ponds. From previous experiments, we observed that pyrethroids became undetectable in plastic or glass containers. Thus, we have also monitored the target pesticides of this study in water samples stored in glass or plastic containers, at various temperatures and for different times.

Materials and Methods

Study Area

The study was conducted in four municipalities in Northern Benin. Three municipalities (Banikoara, Kandi and Gogounou) (Fig. 1) were chosen in the cotton basin among the most productive communes of cotton with a high use of pesticides (Agbohessi et al. 2015). They are crossed by the Alibori River and display several water reservoirs that never dry, where fishing is allowed. The water reservoirs sampled in these three municipalities were, respectively, the Batran, Gambane and Sori reservoirs (Fig. 1). A recent survey carried out with 150 cotton producers of the study area showed that for the Sori reservoir, 12%, 32% and 56% of the cotton fields were located, respectively, at less than 100 m, between 100 and 500 m and at more than 500 m from the water reservoir, respectively. A fourth reservoir, the Songhaï reservoir located in the municipality of Parakou (Fig. 1), where cotton is produced on a lower scale relative to Batran, Gambane and Sori reservoirs, was chosen as a "reference site," because it is expected to be less contaminated with pesticides than the three other reservoirs.

Sample Collection

Water, sediment and fish were collected from June 2016 to November 2016 which coincides with annual periods of cotton production, and an additional sampling was performed in March 2017, prior to annual cotton production.

For each reservoir, the number of samples to be analyzed was adapted to the constraints of transport and refrigeration of the samples from the sampling sites to the laboratory in Benin and a total a total of five water samples were collected (one sample before and after the application periods of pesticides, and three samples during the application period). For each sampling, three sub-samples of 200 mL were collected at 25 cm below the surface of water at three different locations in the same reservoir. The sub-samples were pooled to form one sample representative of the reservoir. They were directly stored in brown glass bottles at 4 °C and frozen at − 20 °C at the laboratory until the analysis was performed.

Samples of sediment were collected in triplicate using a tipper in each water reservoir. The subsamples from each site were pooled, homogenized and transported in glass

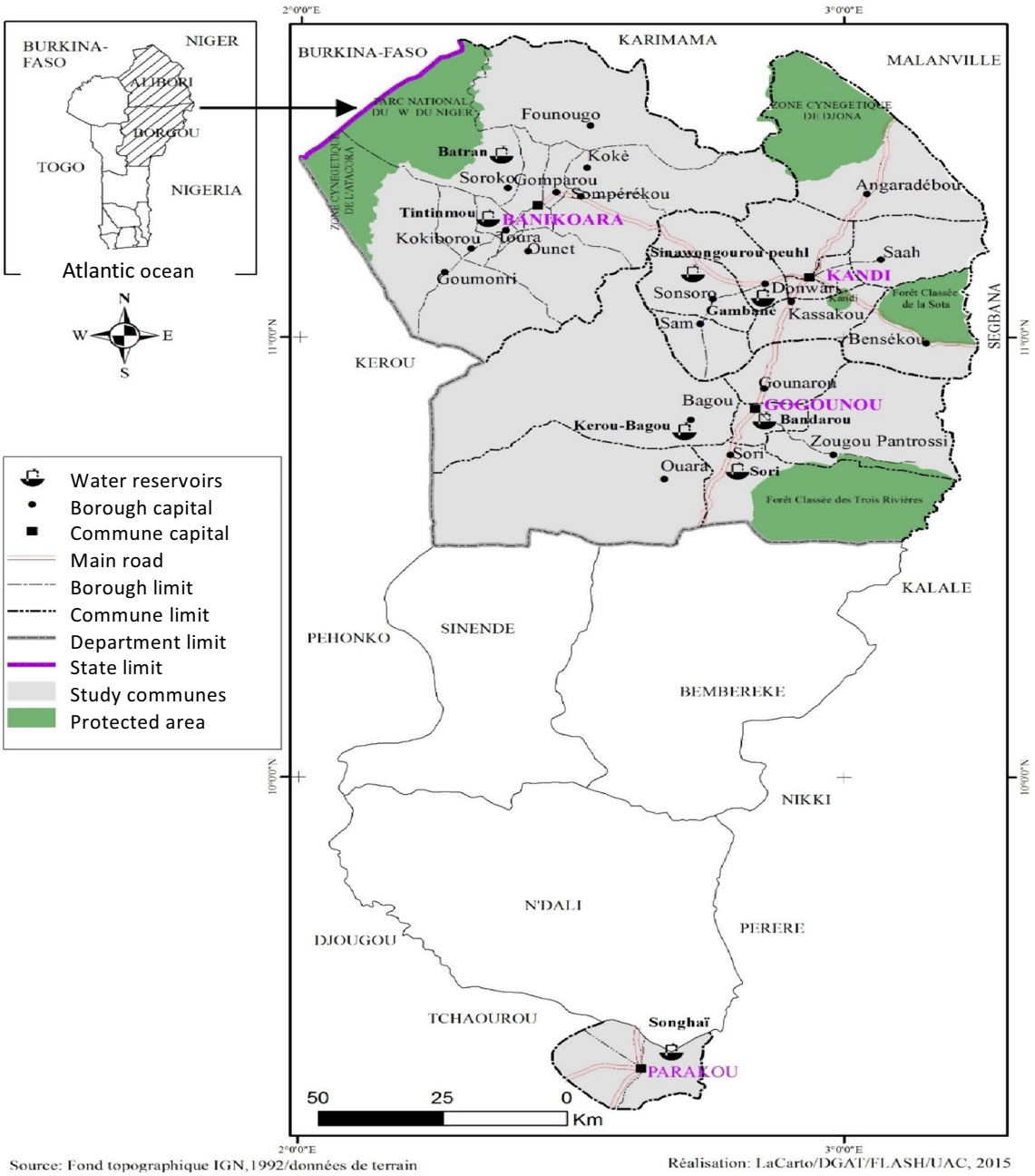

Fig. 1 Geographical location of water reservoirs in the communes of Banikoara, Kandi, Gogounou and Parakou, in Northern Benin

containers with Teflon lids to the laboratory. The sediment samples were dried at 37 °C for 48 h and were sieved to remove gravel, shells and other particles larger than 2 mm. The samples were frozen at − 20 °C until their analysis. All the materials used for sample collection, transport and preparation were free from all types of pesticides.

Two fish species, namely *Clarias gariepinus* and *Oreochromis niloticus*, were sampled in this study. Both species of fish are the most common in the Beninese ecosystems, the most used in fish farming in Benin, often consumed by the population of Northern Benin and also belonging to two different trophic levels. When possible, three fish of the same species were collected at each sampling time, in each sampling location, at the same time than the sediment samples. The fishing was performed by using conventional gill nets, hawk nets with the help of the local fishermen. The size of the sampled fish corresponded to those commonly consumed in the study area. The morphometric measurements of fish were performed on the spot. The average weight of the fish *O. niloticus* was 69 ± 23 g and that of the *C. gariepinus* was

300 ± 343 g. The total and averaged length of *O. niloticus* were of 18 ± 7 and 33 ± 13 cm, respectively. The selected fish samples were transported to the laboratory where each fish flesh, including skin, was homogenized. The fish samples were stored at − 20 °C prior to analysis.

Chemical Reagents and Materials

Standards of lambda-cyhalothrin, permethrin, cypermethrin, beta-cyfluthrin, alpha-HCH, beta-HCH, gamma-HCH, delta-HCH, p,p′-DDD, o,p′-DDT, p,p′-DDT, p,p′-DDE, alpha- and beta-endosulfan, trifluralin, chlorpyrifos, chlorpyrifos D10 were purchased from Dr Ehrenstorfer (Augsburg, Germany) with a purity of at least 99%. Florisil, anhydrous sodium sulfate Na_2SO_4 and anhydrous $MgSO_4$ were from Sigma-Aldrich (St. Louis, MO, USA). The C18 SPE cartridges (Chromabond, 6 mL, 1000 mg) were purchased from Macherey–Nagel (Düren, Germany). Hexane was of picograde quality and provided by Promochem (Wesel, Germany). Dichloromethane (DCM), diethyl ether, chloroform and ethyl acetate were all provided by VWR International (West Chester, Pennsylvania, USA). Falcon polypropylene graduated conical tubes (50 mL) with cap were commercially available from Greiner Bio-One (Kremsmünster, Austria). Acrodisc® 25-mm syringe filters (with 5 µm Versapor® membrane) were purchased from Pall Life Sciences (NY, USA) and hydrophilic single-use syringe filters (0.2 µm pore size, Minisart) were from Sartorius (Goettingen, Germany). One hundred-milliliter ASE glass tubes with cap were from Greiner Bio-One (Kremsmünster, Austria).

Extraction Procedures

The pesticides were extracted from the water samples by liquid–liquid extraction based on the modified protocol of Nguyen Quoc et al. (2018). The pH of the samples was adjusted to 7 by adding a few drops of hydrochloric acid (0.1 M). The test portion was 100 mL of water sample, to which 10 µL of internal standard trifluralin D14 (1000 pg/µL in acetone) was added. Extraction was carried out in a separatory funnel using 40 mL of an ethyl acetate/chloroform mixture (50/50, v/v). After two stirrings of a few seconds and sedimentation, the ethyl acetate/chloroform phase was transferred in a clean glass tube. Extraction was repeated twice.

Pesticides were extracted from the sediment samples using a solid–liquid extraction, followed by a solid phase extraction (SPE). Extraction was carried out from 15 g of dried sediment supplemented with 10 µL of a solution of internal standard trifluralin D14 (1000 pg/µL acetone), using 40 mL of a dichloromethane/hexane (50:50, v/v) mixture, in an ultrasonic bath during 30 min. After decantation and removal of the liquid phase, the extraction was repeated a second time. The dichloromethane/hexane extracts were combined and evaporated to 5 mL using a Turbovap. The SPE was performed using a column containing 6 g of florisil and 4 g of sodium sulfate and conditioned with 20 mL of hexane. Pesticides were eluted using 30 mL of a mixture of hexane/diethyl ether (7:3, v/v).

Pesticide residues in edible portions of fish samples (*Clarias gariepinus* and *Oreochromis niloticus)* were extracted according to the modified method described by You and Lydy (2004). Briefly, 2 g of ground fish tissue was mixed with 10 µL trifluralin D14 (1000 pg/µL acetone), 1 g anhydrous $MgSO_4$ and 25 mL of acetonitrile containing 10% methanol. The mixture was sonicated for 10 min. The extract was centrifuged for 10 min at 2000 rpm. The supernatant was concentrated to a volume of 5 mL and diluted with water to a final volume of 15 mL, before to be loaded on a C18 SPE cartridge, previously conditioned with 3 mL of hexane, 3 mL of acetone, 3 mL of methanol and 4 mL of water. The C18-cartridge was dried for 10 min under vacuum. A cartridge containing anhydrous Na_2SO_4 was connected on top of a normal phase adsorbent cartridge (florisil) and both columns were conditioned with 3 mL of hexane. These cartridges were then attached below the dried C18-cartridge. Twelve milliliters of a 3% toluene in hexane solution were added to the three cartridges and they were eluted at a rate of approximately 1 drop/s under vacuum. The eluent was collected. The C18-cartridge was removed and the two remaining cartridges were eluted with 15 mL of a 6% ethyl ether in hexane solution. The eluent was collected. After the solvent was eluted from both bottom cartridges, the C18-cartridge was reconnected on top of the two other cartridges and 10 mL of a hexane/toluene/ethyl ether (5/3/2, v/v/v) solution was used to elute the more polar pesticides from the cartridges. The eluent was collected, combined with earlier eluents and evaporated to less than 1 mL using a gentle flow of nitrogen.

GC–MS Conditions

The extracts were evaporated to about 500 µL in a Turbovap (Zymark SA) and filtered through Chromafil® (Macherey–Nagel). After evaporation to dryness under a stream of nitrogen, each extract was dissolved using 90 µL of hexane and 10 µL of chlorpyrifos D10 (1000 pg/µL in acetone) was added, as injection standard. The mixture was vortexed and then transferred to a vial with an insert, sealed and stored at − 20 °C prior to GC–MS analysis.

The quantitative analysis of organochlorines, organophosphates and pyrethroids was performed by GC–MS. Pesticides were separated on a Focus GC gas chromatographer (Thermo Fisher Scientific) using an Optima 5MS column (30 m × 0.25 mm × 0.25 µm) (Sulpelco, Bellefonte, PA, USA) and analyzed with an ion trap PolarisQ mass

spectrometer (Thermo Fisher Scientific). The GC conditions were as follows: inlet: 250 °C; splitless injection; helium as the carrier gas at 1 mL/min; temperature program: 110 °C for 1 min, followed by an increase of 15 °C per min to 200 °C and hold for 2 min, then 5 °C per min to 280 °C and hold for 4 min, then an increase of 20 °C per min to 315 °C and hold for 2 min; total run time was 32.75 min. Injection volume was 2 µL. The peaks were identified by following specific ions and by comparing retention times with those of the corresponding standards.

Pesticides were considered as positively identified in samples if the ratio between the chromatographic retention time of the pesticide and that of chlorpyrifos D10, i.e., the relative retention time (RRT) of the pesticide, corresponded to that of the average retention time of the calibration solutions within a ± 0.5% tolerance as set by the legislation (EC 2002).

For MS, transfer line temperature was 320 °C; the ion source temperature was 220 °C and collision energy was 70 eV; positive ionization mode was used. In each chromatographic run, two or three ions were monitored for each pesticide analyzed, which allowed to perform detection and quantitative analysis. Results were calculated using Xcalibur Software, version 3.0.63 (Thermo Fisher Scientific).

Calibration Curves and Validation

To evaluate the performances of the developed method, a «in house» validation was realized, according to European (Commission Decision 2002/657/EC; Directive 2009/90/CE; SANTE 2015) and international (ICH 2005) guidelines.

For calibration curves and the validation of the method, "blank" water was taken from a private pond in Belgium. The pH of the water samples was adjusted to 7. The soil of a private backyard in Belgium was used as "blank" sediment and "blank" fish was purchased from a local supermarket (Delhaize, Belgium). Using the developed method, no pesticide residue was detected in these "blank" samples, which were used to validate the developed method and for the calibration curves.

For matrix-matched calibration curves, six samples of sediment or fish were spiked with 10 µL of a solution of trifluralin D14, used as internal standard, at a concentration of 1000 pg/µL and with all the pesticides to reach final concentrations of 0.5, 1, 2, 4, 8 and 16 µg/kg for sediment and 1, 2, 4, 6, 8 and 10 µg/kg for fish. For water, eight aliquots of 100 mL of "blank" water were supplemented, directly in the separatory funnel, with all pesticide standards dissolved in acetone to reach final concentrations in water of 0, 0.16, 0.31, 0.63, 1.25, 2.5, 5 and 10 µg/L, respectively.

The samples were then extracted and analyzed as described above. These extracts were used to construct the calibration curves: the responses (ratio between peak areas of each pesticide and of the trifluralin D14) were plotted versus standard concentrations. A quadratic regression was used and "no fit weighting" was applied.

For the validation, "blank" samples were spiked with trifluralin D14 and two different concentrations of pesticides (1.5 and 4 µg/kg for sediment, 2 and 6 µg/kg for fish and 0.5 and 2.5 µg/L for water), before to be subjected to the extraction and the GC–MS analysis as described above. The levels of pesticides in the samples were analyzed in triplicate each day over three different days and were used to evaluate the precision (repeatability and within laboratory reproducibility) and the trueness of the method. Repeatability and within laboratory reproducibility were expressed as relative standard deviations. The recovery (the proportion of the amount of analyte added to fish, sediment and water sample, which is extracted and presented for measurement) was measured as an average recovery from the analysis in triplicate per concentration used in the calibration curve (see above). For each concentration, the recovery was calculated from the ratio between the response of the spiked "blank" fish or soil sample and the response of a standard solution, at the equivalent concentration, the response being calculated using the injection standard (chlorpyrifos D10) instead of trifluralin D14.

Study of the Impact of the Storage Conditions of the Water Samples on the Analytical Result

Several conditions of temperature (22 °C, 4 °C and − 20 °C), containers (plastic and brown glass bottles) and time of storage (from 1 to 28 days) were used to determine the influence of storage conditions on the recovery of the pesticides using the developed analytical method. To perform this study, "blank" water supplemented with pesticides at a final concentration in water of 1 µg/L of each pesticide was used. In all cases, water was stored in dark conditions, for maximum 28 days. Water stored at room temperature in the dark, in both plastic (Falcon tubes) and glass containers was sampled after 24 h and 48 h and then, every 2 days. Water stored at 4 °C and − 20 °C was sampled every week and every two weeks, respectively. At each sampling time, the stored sample was analyzed in triplicate.

Risk Assessment for Water and Fish Consumers

Pesticide residue levels in water and fish were combined with a water and fish daily intake of 2 L and 200 g, respectively (arbitrarily chosen as no consumption data are available for the Beninese population) to calculate the exposure (estimated daily intake or EDI) of the local population for each pesticide found in water and in fish. The EDI was then compared to the acceptable daily intake (ADI) and the Acute Reference Dose (ARfD) of each pesticide, to assess the risk for the Beninese consumer. The EDI was calculated for

adults considering a body weight of 70 kg and for children considering a body weight of 20 kg.

Statistical Analysis

The mean values ± standard deviations were calculated. Analysis of variance was performed on the basis of mean values to determine the significant difference between values at $p \leq 0.05$. Statistical analysis was undertaken using Excel software version 16.16.27.

Results and Discussion

Chromatographic Separation and Mass Spectrometric Determination

The optimization of the separation by gas chromatography (GC) and the detection by mass spectrometry (MS) of the 16 compounds was carried out by injecting several solutions of different pesticide standards. The separation of the 16 compounds including the two internal or injection standards was achieved within 32.75 min. The RTx® 5MS column (30 m × 0.25 mm ID, 0.5 µm thick), due to its performance, was finally adopted and made it possible to separate the compounds of interest according to the chromatogram shown in Fig. 2. It was not possible, however, to completely separate β- and γ-HCH, as well as p,p′-DDD and o,p′-DDT. These pesticides were thus quantified together. The selection of ions to be monitored in single ion monitoring (SIM) mode was based on the literature (Francesc et al. 2006; Barbini et al. 2007; Restek 2014). The targeted ions and the retention time of each detected compound are shown in Table 1.

Table 1 Monitored ions (*m/z*) in single ion monitoring (SIM) mode and retention times (minutes) of the 16 compounds of interest and 2 internal and injection standards analyzed by GC–MS in this study

Compounds	Ions (SIM)	RT (min)
Trifluralin D14 (internal standard)	267–315	9.16
Trifluralin	264–306	9.24
α-hexachlorocyclohexane (α-HCH)	181–183	9.88
β-HCH*	181–183	10.56
γ-HCH*	181–183	10.71
δ-HCH	181–183	11.34
Chlorpyrifos D10 (injection standard)	324–326–260	13.73
Chlorpyrifos	314–316	13.73
α-endosulfan	195–193	16.11
p,p′-DDE	246–248	16.77
β-endosulfan	241–195	17.99
p,p′-DDD$^{\Delta}$	235–237	18.19
o,p′-DDT$^{\Delta}$	235–237	18.39
p,p′-DDT	235–237	19.42
Lambda-cyhalothrin	181–197	22.93
Permethrin	182–165	24.32–24.56
β-cyfluthrin	206–127	25.86
Cypermethrin	181–127	26.09–26.19–26.48

*Compounds quantified together; Δ: compounds quantified together

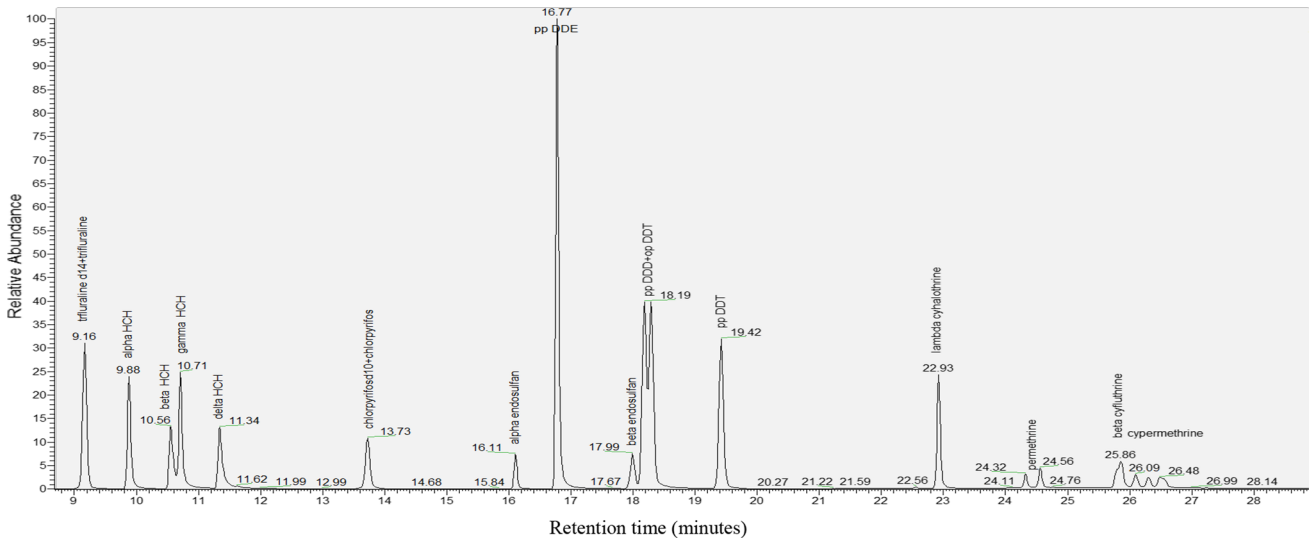

Fig. 2 Chromatographic separation obtained after injection in GC–MS of 2 µL of a solution containing all the pesticides at a concentration of 10 ng/µL

Validation of the GC–MS Method for the Determination of Pesticides in Water

For water samples, when water was spiked directly in the separation funnel used for extraction and extracted immediately after spiking, the method showed good performance, as shown in Table 2 and as described as follows.

Matrix-matched calibration curves for each pesticide showed a good linearity with typical correlation coefficients of 0.998 (data not shown). The precision of the method was determined by repeatability and within laboratory reproducibility studies, expressed by the relative standard deviation. The RSD of repeatability (or intra-assay precision) (data not shown) ranged between 1 and 24%, depending on the day of analysis of triplicate samples for organochlorine pesticides, between 1 and 17% for chlorpyrifos, and between 1 and 26% for pyrethroids (data not shown). The RSD of within laboratory reproducibility (or inter-day precision) ranged between 5 and 12%, 8 and 10%, 4 and 13% for organochlorine pesticides, chlorpyrifos and pyrethroids, respectively, according to the spiking level, while the trueness of the method ranged between 91 and 130%, 104 and 108% and 72 and 94%, respectively (Table 2).

The limit of quantification (LOQ) for all compounds was arbitrarily fixed as the first point of the calibration curve (i.e., 0.16 µg/L for all pesticides, except for the sum of beta- and gamma-HCH and p,p′-DDD and o,p′-DDT for which it was 0.32 µg/L). The detection limit (LOD) was arbitrarily fixed as half of the LOQ, which was similar to what was reported by Han et al. (2018) for the four pyrethroids lambda-cyhalothrin, permethrin, cyfluthrin and cypermethrin in water showing LOD ranging between 0.012 and 0.11 µg/L, using a solid phase extraction before GC–MS analysis. Pastor-Belda et al. (2018) showed an LOQ between 0.4 and 0.8 µg/L, which is much higher than our LOQ values for these four molecules.

Recovery Yield of Pesticide Residues from Water Samples After Storage in Various Conditions

For the analysis of pesticide residues in water, glass bottles are generally preferred to plastic bottles to sample and store the water (Mekebri et al. 2008; Akan 2013; De Perre et al. 2015) to avoid adsorption of compounds on the plastic and the subsequent underestimation of the residue concentration in the water. But, according to Lee and Gan (2002), adsorption of pesticides on glass can occur too. The aim of this part of this study was to check if the recovery yield shown above, calculated from analytical results obtained using water samples directly analyzed after spiking with pesticides were still true for samples stored in various conditions. In order to mimic the field situation (i.e., on site water sampling and storage in Northern Benin), pesticide spiked water (at a final concentration of 1 µg/L) was stored up to 28 days, at 22 °C, 4 °C or − 20 °C in plastic or glass bottles, before GC–MS analysis.

The evolution of the measured pyrethroid concentrations in water samples stored in glass or plastic bottles as a function of storage time is presented in Figs. 3 and 4, respectively. In these graphs, the pesticide concentrations measured at day 0 correspond to the result of the analysis performed immediately after spiking the water in the glass or plastic bottle. For the four pyrethroids (lambda-cyhalothrin, beta-cyfluthrin, permethrin and cypermethrin) of this study,

Table 2 Within laboratory reproducibility, trueness and recovery yield of the GC–MS method for the quantification of sixteen pesticides in water samples spiked at two levels of concentration, directly before extraction

Pesticides	Introduced concentrations (µg/L)	Measured levels, respectively (µg/L)	Within laboratory reproducibility, respectively (RSD, %)	Trueness, respectively (% of introduced concentration)	Recovery yield (%)
α-endosulfan	0.5–2.5	0.6–2.9	8–9	120–116	87
α-HCH	0.5–2.5	0.6–3.5	7–7	120–140	93
β-cyfluthrin	0.5–2.5	0.4–1.8	10–13	80–72	91
β-endosulfan	0.5–2.5	0.6–2.7	5–9	120–108	85
β + γ-HCH	1–5	1.1–6.2	7–7	110–124	93
δ-HCH	0.5–2.5	0.5–2.7	6–8	100–108	94
Chlorpyrifos	0.5–2.5	0.5–2.7	8–10	100–108	94
Cypermethrin	0.5–2.5	0.4–2.1	10–10	80–84	92
λ-cyhalothrin	0.5–2.5	0.4–2.0	4–13	80–80	91
Permethrin	0.5–2.5	0.5–2.1	9–11	100–84	93
p,p′-DDD + o,p′-DDT	1–5	1.1–4.8	6–11	110–96	92
p,p′-DDE	0.5–2.5	0.5–2.2	8–10	100–88	94
p,p′-DDT	0.5–2.5	0.5–2.3	8–12	100–92	93
Trifluralin	0.5–2.5	0.5–2.6	12–14	100–104	93

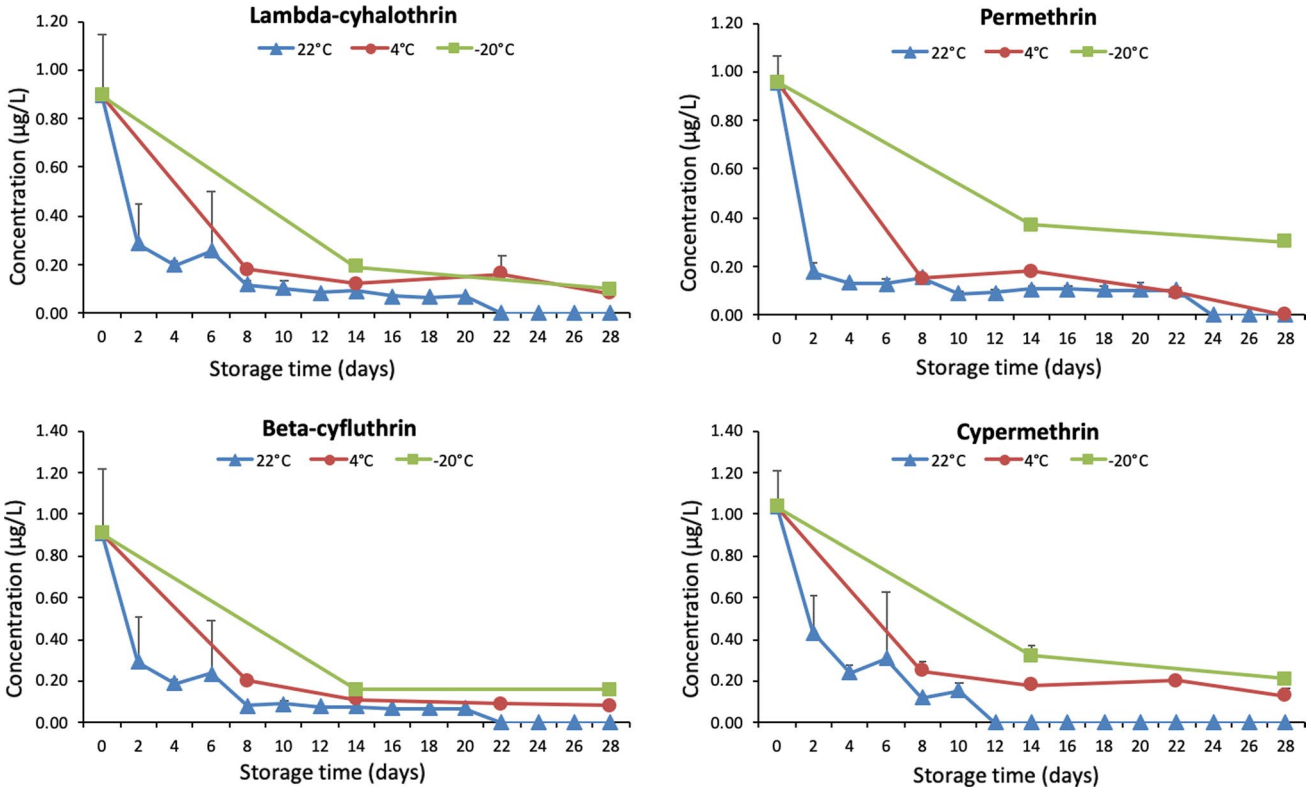

Fig. 3 Evolution of the concentration of pyrethroid residues concentration in spiked water samples (1 µg/L of each pesticide) stored in glass bottles with different temperatures (22 °C, 4 °C, − 20 °C) and time of storage (up to 28 days)

a dramatic decrease in recovery was observed at all temperatures of storage as shown in Figs. 3 and 4. After two days of storage at 22 °C in glass bottles, the loss was already around 60 to 80%. It dropped below 10% after 4 weeks of storage at 4 °C. After 2 weeks of water storage in glass bottles at − 20 °C, the recovery of pyrethroids ranged between 20 and 40%; however after 10 weeks, beta-cyfluthrin and lambda-cyhalothrin became undetectable, and about 20% of permethrin and cypermethrin were still detected. For the other pesticides, no dramatic decrease in the recovery was observed, after storage for several weeks at − 20 °C in glass bottles, except for alpha-endosulfan in water stored at 22 °C (Table 3), for which the recovery dropped to zero after 10 days of storage. However, as expected, the recovery was the lowest when the water was stored at 22 °C in plastic bottles (Table 4).

To avoid an underestimation of the pesticide residues concentration, water should be sampled directly in glass bottles and stored as soon as possible at − 20 °C before analysis. The analyses should be performed as soon as possible after sampling. However, on the field, these conditions were not always respected because, for instance, of the distance between the sampling site and the location of sample storage. Most of the time, the sampling was first made in plastic container before to be transferred into a glass bottle, sometimes one or two days after sampling. According to this study, after two days of storage in plastic bottle, most of the pesticide residues were below the limit of detection. However, even if the aim of this study was to mimic field conditions of sampling and storage, it did not consider the water content of organic matter for example, that probably influenced the level of adsorption of the pesticide on the glass or the plastic walls of the bottle.

Concentration of Pesticides in Water

Sixteen different compounds (alpha-, beta-, gamma- and delta-HCH, p,p′-DDD, o,p′-DDT, p,p′-DDT, p,p′-DDE, alpha- and beta-endosulfan, trifluralin, lambda-cyhalothrin, permethrin, cypermethrin, beta-cyfluthrin and chlorpyrifos) were determined in water samples coming from the reservoirs of Batran, Gambane, Sori and Songhaï, in Northern Benin. Results for pyrethroids (lambda-cyhalothrin, permethrin, cypermethrin and beta-cyfluthrin) in water are not presented because of the very poor recovery shown here above for these compounds when the water sample was stored for several days, even at − 20 °C, in glass bottles.

Table 5 shows the levels of the pesticides of interest in water samples according to the sampling period (before, during and after the period of cotton treatment with

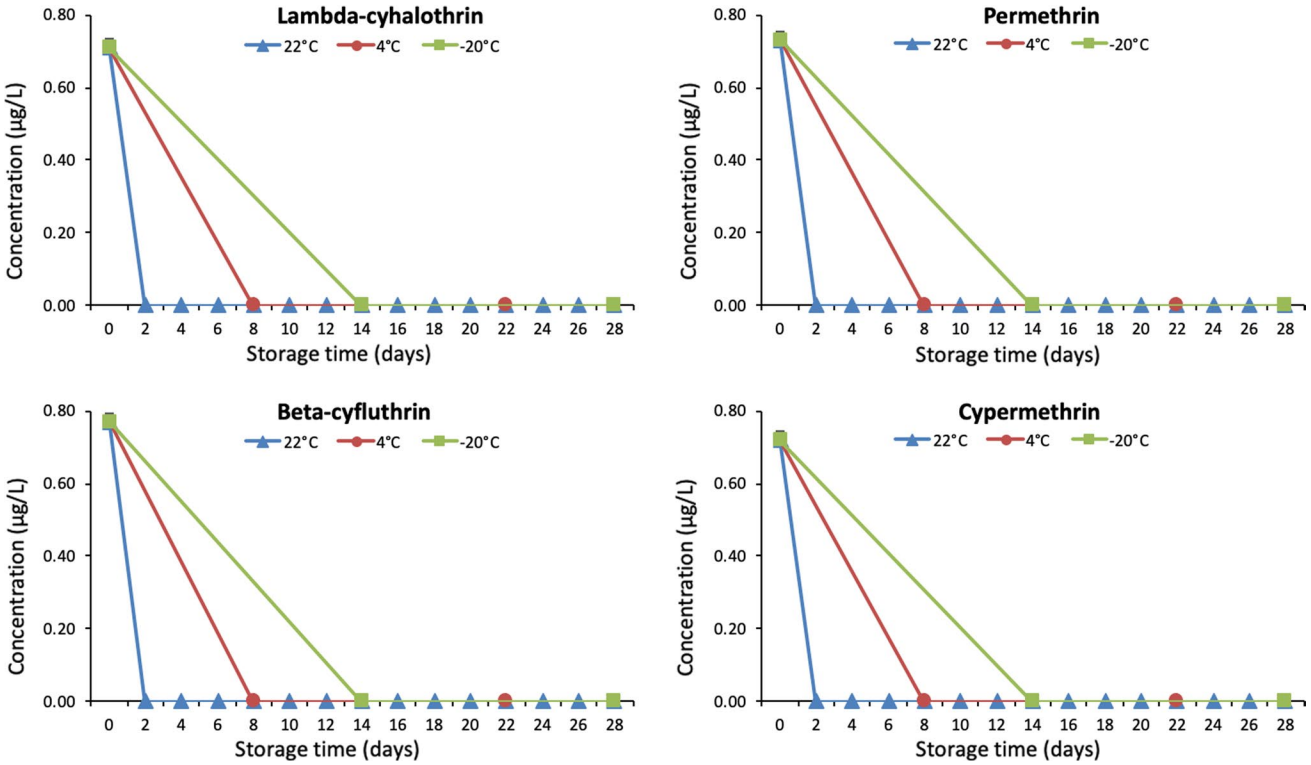

Fig. 4 Evolution of the concentration of pyrethroid residues concentration in spiked water samples (1 µg/L of each pesticide) stored in plastic bottles with different temperatures (22 °C, 4 °C, − 20 °C) and time of storage (up to 28 days)

insecticides). No trifluralin residue was found in water, which is in accordance with surveys about the use of pesticide for cotton treatment that did not mention the use of trifluralin (Gouda et al. 2018). Three organochlorine insecticides (α-HCH, α-endosulfan and p,p′-DDE) were found in water, at low levels (the higher level found was 2.1 µg/L for α-HCH in the reservoir of Sori during the 3rd application period). In the reservoir of Sori, α-HCH was found at each sampling time, while both α-HCH and α-endosulfan were found after the first and second applications. In the reservoirs of Gambane and Songhaï, insecticides were found at 3 sampling times, and only at one sampling time for the reservoir of Batran. Several factors may explain the low presence of these organochlorine insecticides in water. They could be related to their low water-soluble nature (in case of current use) or residual contamination of the pond by organochlorines used in the past. The concentration of organochlorines found in the water reservoirs was consistent with the results found by Fosu-Mensah et al. (2015) in Ghana's drinking water where organochlorine residues were below 1 µg/L. The work of Montory et al. (2017) revealed the presence of organochlorines in Chile's Ñuble River ranging from 0.12 to 26.28 ng/L. These concentrations found were lower than 1 µg/L which was consistent with the results presented here. However, Isworo et al. (2015) found organochlorines at concentrations greater than 1 µg/L (8–16 µg/L) in Lake Rawa Pening water in Indonesia.

Unyimadu et al. (2018) have showed the presence of α- and beta-endosulfan and their metabolite endosulfan sulfate in the Niger River. The concentrations of these compounds found in the water of the Niger River were in descending order: beta-endosulfan > endosulfan sulfate > α-endosulfan. α-endosulfan was detected in 100% of samples, while beta-endosulfan and endosulfan sulfate were detected in 70 and 80% of the samples, respectively. In this study, α- and beta-endosulfan were investigated, but only α-endosulfan was detected in water samples from reservoirs. The concentrations of endosulfan found in the water reservoirs (0.2–0.9 µg/L) were also similar to those found by Mawussi (2008) in the waters of the Mono River in Benin (0.69 µg/L), those found by Agagbé (2008) in the Agbado river (Savalou, Benin) (0.29–0.47 µg/L) and those found by Gouda (2018) in the Gambanè water reservoir (1 µg/L). In contrast, Soclo (2003) found higher concentrations of endosulfan in the waters of the cynegetic areas of Pendjari, Atacora and Djona (35–46 µg/L) in Benin, compared to those found in this study. This high concentration could be explained by the fact that in 2003, organochlorines such as endosulfan were allowed in Benin for the protection of cotton crops (Agbohessi et al. 2012). The concentrations of endosulfan

Table 3 Recoveries (expressed as a percentage of the concentration measured at day 0) from spiked (1 µg/L of each pesticide) water samples stored in glass bottles at different temperatures

Pesticide/temperature of water storage (°C)	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 22	Day 24	Day 26	Day 28
Alpha-endosulfan														
22	35[a]	21[b]	14[b]	13[b]	0	0	0	0	0	0	0	0	0	0
4				61[a]			58[a]				42[b]			31[b]
− 20							80[a]							59[b]
Alpha-HCH														
22	94[a]	95[a]	95[a]	93[a]	76[a]	75[b]	75[a]	78[b]	72[b]	65[c]	64[d]	65[c]	59[c]	57[d]
4				93[a]			91[a]				84[b]			80[a]
− 20							92[a]							85[a]
Chlorpyrifos														
22	83[a]	91[a]	85[a]	78[a]	62[b]	64[b]	55[c]	53[b]	48[d]	47[e]	33[f]	35[e]	27[e]	27[g]
4				75[a]			80[a]				69[a]			63[a]
− 20							85[a]							78[a]
p,p′-DDT														
22	66[a]	67[a]	67[a]	67[a]	66[a]	67[a]	68[a]	68[a]	64[a]	62[a]	60[a]	60[b]	61[a]	60[b]
4				69[a]			63[a]				58[a]			57[b]
− 20							80[a]							79[a]
Trifluralin														
22	65[a]	57[a]	45[a]	38[b]	29[b]	24[b]	20[c]	18[c]	16[c]	16[c]	10[c]	12[c]	9[c]	11[c]
4				58[a]			61[a]				52[a]			52[a]
− 20							70[a]							60[a]

Each value is a mean of three replicates; different letters within one row mean significant difference at $p \leq 0.05$

(0.2–0.9 µg/L) found in the water of the reservoirs are lower than the concentrations (3.85–7.7 µg/L) which could modify the histology of the gonads in males, or lead to a feminization of fish (Agbohessi et al. 2015).

DDT, DDE and DDD were detected in 78, 80 and 82% of water samples of the Niger River, respectively, with concentrations of the different compounds in the order descending next: DDD > DDT > DDE (Unyimadu et al. 2018). These authors indicate that after DDT applications, much of the DDT is slowly converted to DDE and DDD under aerobic and anaerobic conditions. In the context of this study, only the DDE was detected in water samples.

Validation of the GC–MS Method for the Determination of Pesticides in Water Sediment and Fish

For the sediment, matrix-matched calibration curves for each pesticide showed a good linearity with typical correlation coefficients of 0.98 (data not shown). The RSD of repeatability (or intra-assay precision) (data not shown) ranged between 1 and 5% on day 1 of analysis, 3 and 21% on day 2, 3 and 11% on day 3, 1 and 14% on day 4 and 2 and 11% on day 5 for organochlorine insecticides. For chlorpyrifos, it ranged between 1 and 6% on day 1, 2 and 12% on day 2, 2 and 6% on day 3, 1 and 4% on day 4 and 5 and 16% on day 5. For pyrethroid insecticides, it ranged between 11 and 25% on day 1, 5 and 26% on day 2, 3 and 15% on day 3 and 10 and 26% on day 4 and 18 and 26% on day 5. The RSD of within laboratory reproducibility (or inter-day precision) ranged between 10 and 18%, 16 and 20%, 9 and 14% for organochlorines, chlorpyrifos and pyrethroids, respectively, according to the spiking level (Table 6). The trueness of the method ranged between 82 and 99%, 84 and 91% and 72 and 90% for organochlorines, chlorpyrifos and pyrethroids, respectively (Table 6). The recovery rate of each pesticide from sediment is also presented in Table 6. It ranged between 65 and 107%, depending on the compound. The limit of quantification (LOQ) in sediment was arbitrarily fixed as the first point of the calibration curve (i.e., 0.5 µg/kg) except for the sum of beta- and gamma-HCH and the sum of p,p′-DDD and o,p′-DDT, for which it was 1 µg/kg. The detection limit (LOD) was arbitrarily fixed as half of the LOQ (i.e., 0.25 and 0.5 µg/kg, respectively).

For fish, matrix-matched calibration curves for each pesticide showed a good linearity with typical correlation coefficients of 0.97 (data not shown). The repeatability (or intra-assay precision) (data not shown) for organochlorine insecticides ranged between 3 and 25% on day 1 of analysis and 7 and 19% on day 2, between 9 and 13% on day 1

Table 4 Recoveries (expressed as a percentage of the concentration measured at day 0) from spiked (1 µg/L of each pesticide) water samples stored in plastic bottles at different temperatures

Pesticide/temperature of water storage (°C)	Day 2	Day 4	Day 6	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 22	Day 24	Day 26	Day 28
Alpha-endosulfan														
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4				25[a]			22[a]				25[a]			20[a]
−20							43[a]							38[a]
Alpha-HCH														
22	43[a]	28[a]	17[a]	17[a]	15[a]	13[a]	13[a]	0	0	0	0	0	0	0
4				73[a]			77[a]				71[a]			68[a]
−20							89[a]							80[a]
Chlorpyrifos														
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4				0			0				0			0
−20							34[a]							31[a]
p,p′-DDT														
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4				32[a]			0				0			0
−20							33[a]							0
Trifluralin														
22	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4				0			0				0			0
−20							37[a]							34[a]

Each value is a mean of three replicates; different letters within one row mean significant difference at $p \leq 0.05$

Table 5 Pesticide levels found in water samples (µg/L) of four water reservoirs of Northern Benin

	Before pesticide application (29/06/16)	1st application period (22/07/16)	2nd application period (19/08/16)	3rd application period (13/11/16)	After application (period without cotton) (15/03/17)
Batran	–	–	–	α-endo: 0.2	–
Gambane	α-HCH: 0.2 α-endo: 0.4	–	α-HCH: 0.7	–	α-HCH: 0.6
Sori	α-HCH: 0.9	α-HCH: 0.3 α-endo: 0.3	α-HCH: 0.3 α-endo: 0.5	α-HCH: 2.1	α-HCH: 0.6
Songhaï	α-HCH: 0.6 α-endo: 0.2	–	α-endo: 0.9 p,p′-DDE: 0.2	–	α-HCH: 0.7

α-endo = Alpha-Endosulfan; α-HCH = Alpha-Hexacyclohexane; p,p′-DDE = 1,1-Dichloro-2,2-bis(*p*-chlorophenyl) ethylene

and 19 and 25% on day 2, for chlorpyrifos and between 8 and 24% on day 1 and 6 and 23% on day 2 for pyrethroids. The within laboratory reproducibility (or inter-day precision) ranged between 8 and 24%, 9 and 15%, 6 and 25% for organochlorines, chlorpyrifos and pyrethroids, respectively, according to the spiking level, while the trueness of the method ranged between 71 and 116%, 78 and 115% and 76 and 97%, respectively (Table 7). The recovery rate of each pesticide from fish is presented in Table 7. It ranged between 83 and 104%, depending on the compound.

The limit of quantification (LOQ) in fish was arbitrarily fixed as the first point of the calibration curve (i.e., 1 µg/kg) except for the sum of beta- and gamma-HCH and the sum of p,p′-DDD and o,p′-DDT, for which it was 2 µg/kg. The detection limit (LOD) was arbitrarily fixed as half of the LOQ (i.e., 0.5 and 1 µg/kg, respectively). The study made by Choi et al. (2016) reported LODs between 0.23 and 0.51 µg/kg for determination of organochlorines in fish by GC–MS. Another study, conducted by Mekebri et al. (2008), reported LODs between 1 and 3 µg/kg for

Table 6 Validation parameters of the GC–MS method for the quantification of thirteen pesticides in sediment

Pesticides	Introduced concentrations (µg/kg)	Measured levels, respectively (µg/kg)	Within laboratory reproducibility, respectively (RSD, %)	Trueness, respectively (% of introduced concentration)	Recovery (%)
α-endosulfan	1.5–4	1.5–3.5	15–18	100–88	85
α-HCH	1.5–4	1.4–3.6	10–15	93–90	84
β + γ-HCH	3–8	2.8–6.8	16–17	93–85	84
Chlorpyrifos	1.5–4	1.4–3.4	16–20	93–85	65
δ-HCH	1.5–4	1.4–3.5	14–17	93–88	103
Lambda-cyhalothrin	1.5–4	1.2–2.9	14–28	80–73	63
p,p′-DDD + o,p′-DDT	3–8	2.9–7.0	12–15	97–88	77
p,p′-DDE	1.5–4	1.1–3.3	15–16	73–83	80
p,p′-DDT	1.5–4	1.5–3.7	17–18	100–93	71
Permethrin	1.5–4	1.2–3.6	9–11	80–90	107
Trifluralin	1.5–4	1.4–3.4	13–14	93–85	65

Table 7 Validation parameters of the GC–MS method for the quantification of twelve pesticides in fish

Pesticides	Introduced concentrations (µg/kg)	Measured levels, respectively (µg/kg)	Within laboratory reproducibility, respectively (RSD, %)	Trueness, respectively (% of introduced concentration)	Recovery (%)
α-endosulfan	2–6	2.0–5.1	16–24	100–85	104
α-HCH	2–6	1.4–4.8	28–34	70–80	94
β + γ-HCH	4–12	4.4–11.9	13–26	110–99	103
beta-cyfluthrin	2–6	2.5–5.4	12–30	125–90	81
Chlorpyrifos	2–6	2.2–4.7	9–15	110–78	83
δ-HCH	2–6	1.7–5.2	8–10	85–87	98
Lambda-cyhalothrin	2–6	1.5–5.2	6–25	75–87	98
p,p′-DDD + o,p′-DDT	4–12	4.6–11.0	12–15	115–92	95
p,p′-DDE	2–6	1.5–4.7	10–13	75–78	95
Permethrin	2–6	2.7–5.8	10–33	135–97	99
Trifluralin	2–6	2.2–5.6	7–8	110–93	97

determination of pyrethroids (bifenthrin, cyfluthrin, cypermethrin, esfenvalerate/fenvalerate, lambda-cyhalothrin, permethrin) in fish by GC–MS/MS. For Corcellas et al. the limit of quantification for the GC–MS determination of pyrethroids (permethrin, cyhalothrin, cyfluthrin and cypermethrin) in fish ranged from 0.1 to 1.54 µg/kg lipid weight (Corcellas et al. 2015).

Some pesticides were not well recovered from all the matrices analyzed in this study. It was the case for beta-endosulfan, which was well extracted from water, but not from sediment and fish, while p,p′-DDT was well extracted from water and sediment, but not from fish. Among pyrethroids, some compounds were not determined in sediments or fish because of poor recovery: it was the case of beta-cyfluthrin and cypermethrin in sediments and cypermethrin in fish.

Concentration of Pesticides in Sediment

Thirteen different compounds (alpha-, beta-, gamma- and delta-HCH, p,p′-DDD, o,p′-DDT, p,p′-DDT, p,p′-DDE, alpha- and beta-endosulfan, chlorpyrifos, trifluralin, lambda-cyhalothrin and permethrin) were determined in sediment samples coming from the water reservoirs mentioned above.

Table 8 shows the levels found in the sediments according to the cotton treatment calendar. Much more insecticide residues were found in sediment than in water samples. Interestingly, α-endosulfan was found in 18 out of 20 sediment samples (it was not found in only 2 sediment samples coming from the reservoir of Sori, after the end of the cotton season and, surprisingly, the reservoir of Songhaï during the second period of cotton treatment). The level of α-endosulfan found in these sediment samples ranged between 0.6 and

Table 8 Pesticide levels found in sediments samples (µg/kg) of four water reservoirs of Northern Benin

Before pesticide application (29/06/16)	1st application period (22/07/16)	2nd application period (19/08/16)	3rd application period (13/11/16)	After application (period without cotton) (15/03/17)
Batran				
α-endo: 0.6 p,p′-DDE: 2.7 permet.: 1.8	α-endo: 8.3 permet.: 0.8	α-endo: 1.0 p,p′-DDT: 1.8 λ-cyhalot.: 1.3	α-endo: 4.3 p,p′-DDT: 1.7 p,p′-DDE: 0.9 λ-cyhalot.: 1.4 permet.: 1.1	δ-HCH: 1.1 α-endo: 3.5 chlorp.: 1.0 p,p′-DDT: 1.2 p,p′-DDE: 1.5 λ-cyhalot.: 1.7 permet.: 1.8
Gambane				
α-endo: 24 p,p′-DDE: 0.9 λ-cyhalot.: 1.1	α-endo: 1.1	δ-HCH: 0.9 α-endo: 7.8 chlorp.: 1.5 p,p′-DDE: 2.8 λ-cyhalot.: 0.8	α-endo: 1.1 p,p′-DDE: 1.1 λ-cyhalot.: 1.5	δ-HCH: 0.7 α-endo: 101 p,p′-DDE: 1.1 λ-cyhalot.: 8.8
Sori				
α-endo: 18 p,p′-DDE: 0.7	α-endo: 1.0 permet.: 0.8	α-endo: 1.4 p,p′-DDT: 1.0 λ-cyhalot.: 1.1 permet.: 2.1	α-endo: 20 p,p′-DDT: 1.2 p,p′-DDE: 0.7 λ-cyhalot.: 0.9 permet.: 2.6	α-endo: 0.8 p,p′-DDT: 1.5 p,p′-DDE: 2.1 λ-cyhalot.: 2.7 permet.: 13
Songhaï				
α-endo: 3.0 p,p′-DDT: 1.1 p,p′-DDE: 0.8 λ-cyhalot.: 1.9 permet.: 4.6	α-endo: 1.5	λ-cyhalot.: 0.8	α-endo: 33 p,p′-DDT: 1.7	β + γ-HCH: 3.6 δ-HCH: 1.0 α-endo: 2.7 p,p′-DDD + o,p′-DDT: 1.6 p,p′-DDT: 1.2 p,p′-DDE: 0.8 λ-cyhalot.: 7.5 permet.: 11

8.3 µg/kg in the reservoir of Batran, 1.1 and 24 µg/kg in the reservoir of Gambane, 1.0 and 20 µg/kg in the reservoir of Sori and 1.5 and 33 µg/kg in the reservoir of Songhaï. DDT and parent compounds are also present in 15 out 20 samples, at low levels (below 3 µg/kg). Low levels of isomers of HCH (from 0.8 to 3.6 µg/kg) were found in 5 samples (but in each reservoir). The presence of organochlorine insecticides in the aquatic environment in Benin was already reported by other authors, such as Pazou et al. (2006) who found DDT, endosulfan and HCH (at concentrations ranging between 0.1 and 809 µg/kg) in the sediments of the Ouémé River. Adam et al. (2010) showed the presence of endosulfan (120–150 µg/kg) in the sediments of the water reservoirs of Northern Benin. In Nigeria, a neighbor country, Ezemonye et al. (2015) reported the presence of organochlorines (alpha-, beta- and gamma-HCH, DDT, endosulfan) in the sediments of the Ogbesse River, at concentrations ranging from 810 to 2140 µg/kg and Ogbeide et al. (2015) found DDT and endosulfan (between 810 and 1060 µg/kg) in the sediments of the Owan River.

Chlorpyrifos was found in only two samples, one from Batran and one from Gambane reservoir, at a level of 1.0 and 1.5 µg/kg, respectively. The presence of chlorpyrifos in Gambane reservoir is corroborated by the results of Gouda et al. (2018) who also found chlorpyrifos in the Gambane water reservoir. In water samples, they found concentrations ranging between 8 and 1700 µg/L and much higher concentrations in sediments (between 58 and 208 800 µg/kg).

Pyrethroids (permethrin and/or λ-cyhalothrin) were present in 16 samples out of 20 (with levels ranging from 0.8 to 13 µg/kg). Similarly, in 2010, Adam and co-workers showed the presence of pyrethroids (lambda-cyhalothrin and cyfluthrin) in the sediments of the reservoirs of the communes of Gogounou, Kandi and Banikoara (i.e., the same communes of the present study) at concentrations ranging between 36 and 205 µg/kg. Gouda (2018) found cypermethrin in the sediments of the Gambane reservoir at a concentration of 2099 µg/kg.

Concentration of Pesticides in Fish

Twelve different compounds (alpha-, beta-, gamma- and delta-HCH, p,p′-DDD, o,p′-DDT, p,p′-DDE, alpha- and beta-endosulfan, chlorpyrifos, trifluralin, lambda-cyhalothrin and permethrin) were determined in *C. gariepinus* and/

or *O. niloticus* sampled in the water reservoirs mentioned above.

Table 9 shows the levels found in the fish according to the cotton treatment calendar. Unfortunately, it was not possible to sample both fish species in all water reservoirs, at all sampling times. One to three specimens were sampled at each sampling time, for each species. When more than one fish was analyzed, the pesticide content indicated in Table 9 corresponds to the higher level measured.

Interestingly, and in contrast to what was found in sediment samples, α-endosulfan (at a low level of 2.4 µg/kg) was found in only one fish sample (*C. gariepinus* sampled in the Batran reservoir during the second pesticide application period). No HCH isomers were found in any fish sample while DDT and parent compounds were found in 15 out of 26 fish samples (the maximum level being 36 µg/kg of p,p′-DDE in *C. gariepinus* sampled in the Gambane reservoir during the 3rd pesticide application period). In Nigeria, in contrast, very high levels of organochlorine insecticides (1.86 mg/kg of α-HCH and 0.13 mg/kg of endosulfan) have been found in *C. gariepinus* fish, in the Ogbesse River (Ezemonye et al. 2015). In another study, the same team (Ogbeide et al. 2015) showed the presence of DDT and endosulfan between 40 and 380 µg/kg in *C. gariepinus* fish, in the Owan River in Nigeria.

Chlorpyrifos was found in 7 fish samples out of 26 (with levels ranging from 2.5 to 4.5 µg/kg), in all reservoirs except the reservoir of Songhaï. Pyrethroids (permethrin and/or λ-cyhalothrin and/or beta-cyfluthrin) were present in only 5 fish samples (but in each reservoir) out of 26 (with levels ranging from 4.5 to 11 µg/kg).

As C. gariepinus lives in sediments, a relationship between pesticide concentrations in sediments and fish may be expected but this was not observed in this study. This absence of correlation between sediments and fish was also shown in the study of Pazou et al. (2006) who showed that various parameters influence the bioaccumulation of pesticides in fish, including water solubility, degree of ionization, stability and size of the chemical, as well as the lipid content of the fish species (Pazou et al. 2006). The fact that α-endosulfan was almost not found in fish while it was present in high levels in sediments could be explained by the fact that this pollutant was recently used and had no time to accumulate yet in fish. Furthermore, several studies have shown that endosulfan residues were rapidly eliminated from aquatic invertebrates and fish. Toledo and Jonsson (1992)

Table 9 Pesticide levels found in fish samples (µg/kg) of four water reservoirs of Northern Benin

	Before pesticide application (29/06/16)	1st application period (22/07/16)	2nd application period (19/08/16)	3rd application period (13/11/16)	After application (period without cotton) (15/03/17)
Batran					
Oreochromis N	Chlorp.: 1.9	Chlorp.: 3.3	p,p′-DDD + o,p′-DDT: 3.2	–	No sample
	p,p′-DDE: 5.3	p,p′-DDE: 4.5	p,p′-DDE: 4.6		
	λ-cyhalot.: 8.4				
	Permet.: 4.5				
	β-cyflut.: 11				
Clarias G					
	p,p′-DDE: 8.0	Chlorp.: 3.3	α-endo: 2.4		
		p,p′-DDD + o,p′-DDT: 8.7	p,p′-DDE: 6.8	No sample	No sample
Gambane					
Oreochromis N	p,p′-DDE: 1.0	–	β-cyflut.: 6.6	β-cyflut.: 7.5	–
Clarias G	No sample	Chlorp.: 4.5	No sample	Chlorp.: 2.5	p,p′-DDE: 1.7
		p,p′-DDE: 4.5		p,p′-DDE: 36	
Sori					
Oreochromis N	p,p′-DDE: 1.3	λ-cyhalot.: 7.4	p,p′-DDD + o,p′-DDT: 2.1 p,p′-DDE: 3.2	Chlorp.: 3.1 p,p′-DDD + o,p′-DDT: 2.8 p,p′-DDE: 6.0	–
Clarias G	No sample	Chlorp.: 1.8 p,p′-DDE: 2.1	No sample	No sample	p,p′-DDE: 2.7
Songhaï					
Oreochromis N	–	β-cyflut.: 9.0	–	–	No sample
Clarias G	No sample	No sample	No sample	No sample	No sample

reported half-lives of 2.9 and 5.1 days for the elimination of α- and β-isomers of endosulfan in zebra fish (*Brachydanio rerio*). Ernst (1977) reported a half-life of 34 h for the elimination of the α-isomer of endosulfan in blue mussels (*Mytilus edulis*). In contrast, the concentrations of p,p′-DDE found in fish are much higher than those found in sediments.

Pesticides have a negative impact on the health of fish (Agbohessi et al. 2015). Also, Kumari et al. (2017) demonstrated that chronic exposure to endosulfan at a concentration of 2.884 ppb (1/10 of its LC_{50}) for 30 days alters the immune response, making *C. gariepinus* susceptible to microbial infection. In the present study, the highest concentration found for endosulfan was 2.4 µg/kg. Similarly, *O. niloticus* was exposed for 96 h to endosulfan at a concentration of 7 ppb (1/2 of its LC_{50}) and there was evidence of impaired immune response in fish by stimulation of macrophage activity (Tellez-Bañuelos et al. 2009).

About chlorpyrifos, studies indicate that chlorpyrifos is estrogenic and modifies embryonic hatching, cell proliferation and apoptosis in zebra fish (Yu et al. 2015). In addition, it has been shown that chlorpyrifos (0.01—1 mg/L) is highly toxic to zebra fish (Jun et al. 2014).

Pyrethroids have also been shown to be toxic for fish. In a study conducted by Gadhave et al. (2014), the fish (*Labeo rohita*) exposed during 96 h to lambda-cyhalothrin at a concentration of 2.72 µg/L showed irregular swimming, loss of balance, surfacing and convulsions. Lambda-cyhalothrin has also been shown to have the potential to disrupt the thyroid endocrine system in exposed zebra fish embryos (Tu et al. 2016).

Evaluation of the Risks Related to the Consumption of Water and Fish (*O. niloticus* and *C. gariepinus*) Contaminated with Insecticides in Northern Benin

A quick risk assessment, based on the maximum level of endosulfan and HCH found in the reservoirs (i.e., 0.9 and 2.1 µg/L) and a hypothetical water consumption of 2 L of water per day, shows that endosulfan and HCH estimated daily intake (EDI) from water consumption will be 1.8 and 4.2 µg/day. This corresponds to EDIs of 0.026 and 0.06 µg/kg body weight/day for a 70 kg adult for endosulfan and HCH, respectively. These EDIs correspond to 0.43 and 20% of ADI for a 70 kg adult, for endosulfan and HCH, respectively, meaning no specific risk for humans due to the consumption of this endosulfan and HCH contaminated water. These data as well as the data obtained for 20 kg children are presented in Table 10. The work of Zoumenou et al. (2019) showed that there was no risk related to the water consumption of water reservoirs contaminated with acetamiprid. Taking the total concentrations of these three pesticides (acetamiprid, endosulfan and HCH) in water into account, the sum of concentration is greater than 0.5 µg/L, which is the reference value of European legislation for drinking water, thus the consumption of the water of the reservoirs must be forbidden.

The exposure of fish consumers has been estimated for DDT, endosulfan, chlorpyrifos, lambda-cyhalothrin and beta-cyfluthrin by considering the maximum concentration found in fish and a daily consumption of fish arbitrarily fixed at 200 g, as no specific data for Benin are available. This may be a very high level of daily consumption, but this can be considered as a "worst case" scenario for people eating a lot of fish from these water reservoirs.

To estimate the risk, the pesticide intake has been compared to the acceptable daily intake (ADI) and the acute reference dose (ARfD) for both adults (body weight = 70 kg) and children (body weight = 20 kg). The results are presented in Table 11.

For DDT, for example, considering a consumption of 0.2 kg of *O. niloticus* or *C. gariepinus* in the population (either adult or child), the chronic risk characterization shows an EDI of 0.3% of the ADI and thus much lower than the ADI. The same kind of results have been found for the other pesticides: the intake for both adults and children is much lower than the ADI or the ARfD. It appears thus that no chronic or acute risk seems to be present for adult or children fish consumers from the study area. In contrast, in

Table 10 Pesticide intake of adult (body weight = 70 kg) and children (body weight = 20 kg) consumers of water taken in the water reservoirs from Northern Benin and comparison with toxicological references values

Pesticide	ADI (µg/kg bw/day)	ARfD (µg/kg bw)	Maximum conc. found in water (µg/L)	EDI Adult (µg/kg bw/day)	% ADI/% ARfD adult	EDI Children (µg/kg bw/day)	% ADI/% ARfD children
Endosulfan	6[a]	20[a]	0.9	0.026	0.43/0.13	0.09	1.50/0.45
HCH	0.3[b]	na[b]	2.1	0.060	20.00/na	0.21	70.00/na

A daily consumption of 2 L of water has been considered

na not applicable

[a]JMPR (2006)

[b]EFSA (2005)

Table 11 Pesticide intake of adult (body weight = 70 kg) and children (body weight = 20 kg) consumers of fish caught in water reservoirs from Northern Benin and comparison with toxicological references values

Pesticide	ADI (µg/kg bw/day)	ARfD (µg/kg bw)	Maximum conc. found in fish (µg/kg)	EDI Adult (µg/kg bw/day)	% ADI/% ARfD adult	EDI Children (µg/kg bw/day)	% ADI/% ARfD children
Chlorpyrifos	1[a]	5[a]	4.5	0.013	1.29/0.26	0.045	4.50/0.90
Sum of DDT and parent compounds	10[b]	na	36	0.103	1.03/na	0.360	3.60/na
Endosulfan	6[c]	20[c]	2.4	0.007	0.11/0.03	0.024	0.40/0.12
λ-Cyhalothrin	2.5[d]	5[d]	8.4	0.024	0.96/0.48	0.084	3.36/1.68

A daily consumption of 200 g of fish has been considered

na not applicable, *ADI* acceptable daily intake, *ARfD* acute reference dose, *EDI* estimated daily intake

[a]EFSA (2014)

[b]JMPR (2000)

[c]JMPR (2006)

[d]EFSA (2015)

Nigeria, the work of Ezemonye et al. (2015) revealed that there was a risk for the population consuming *Clarias gariepinus* from the Ogbesse River because of the presence of 16 different residues of pesticides.

It should be noted that, in the present study, the combined action of all these insecticides was not considered in this assessment, as well as the risks associated with the consumption of other food besides fish from water reservoirs. In addition, there are other active ingredients used in the cotton that have not been included in this preliminary risk assessment. Assuming the contribution of other active ingredients, it cannot be excluded, after calculation, that the overall daily ingestion of pesticides is higher than the ADI and the ARfD.

Conclusion

In this study, a new multi-residue analytical method has been developed for the extraction of some pesticides from water, sediment and fish samples prior to GC–MS measurement. The optimized method provided good accuracy and within laboratory reproducibility for the simultaneous extraction of pyrethroids, organochlorine insecticides and chlorpyrifos.

After storage even for a limited period of time, in both glass and plastic bottles, the recovery of pyrethroid pesticides from water samples seems very low. For organochlorines, trifluralin and chlorpyrifos, a storage at − 20 °C for several weeks does not seem to decrease this recovery. For the analysis of pyrethroids in water samples, it should be recommended to treat the glass bottles with an aqueous solution containing 5% of polyethyleneglycol (PEG) (which, according to Lee et al. (1998) makes it possible to reduce the adsorption phenomenon).

The various results obtained allow us to confirm that the water, sediment and fish collected in the water reservoirs of Northern Benin are contaminated with these insecticides. The presence of insecticides in these different compartments of the environment is not only observed during the period of cotton processing, but throughout the year. This is explained by the fact that outside cotton, these same insecticides are used in other crops. This was confirmed by Dognon et al. (2018) which states that cotton insecticides are used for crops cereals (maize, sorghum, etc.), food crops and market garden products. The assessment risk of each insecticide (taken individually) shows that the human exposure to these compounds appears to be very low and below both the respective acceptable daily intake and acute reference dose of these compounds. This is a theoretical exercise showing the potential risk associated with residues of pesticides in food, especially for children. To get a more realistic idea of the situation, a comprehensive risk assessment should be carried out, including all pesticides used in the cotton basin as well as all food consumed locally, likely to be contaminated by the pesticides of interest. Data on food consumption for the local population should also be carried out in order to have all the data necessary to realize this overall risk assessment for the Beninese population involving all pesticides used in the cotton basin.

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