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Assessment of the Level of Pesticide Contamination in Amphibians from Cocoa Plantations at Ojo Camp-Ugboke, Southern Nigeria

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ABSTRACT: Amphibian skin is extremely permeable to environmental contaminants and their health is inextricably linked to environmental health. Hence the objective of this study was to evaluate the level of pesticide contamination in amphibian tissues and their environment (soil and sediment) at Ojo Camp-Ugboke cocoa plantations in Edo State, southern Nigeria using HP 5890 series gas chromatography system coupled with an Electron Capture Detector (ECD). Data obtained showed that thirteen organochlorine pesticides, one triazine herbicide, carbamate, and organophosphate were found in high concentrations. All pesticides components were detected in amphibian tissues, most with higher concentrations than other samples. The high permeability of amphibian skin is responsible for the detected in-tissue concentrations. Pesticide residues in amphibian tissues are not only harmful to their health, but also pose a health risk to those who consume amphibian meat as a delicacy. Local farmers' unabated/unregulated use of pesticides may contribute in amphibian population decline. For a healthier environment, it is best to increase public awareness while also supporting routine monitoring and regulation by relevant agencies.

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Amphibians are cold-blooded vertebrates with hairless, scaleless, and furless skin. They are extremely sensitive to environmental contaminants because their skin is permeable and functions as an organ of respiration and water uptake regulation in both aquatic and terrestrial forms (Van Meter *et al*., 2015). They live a life that includes both land and water, exposing them to contaminants (Bruhl *et al*., 2013) and parasites in either environment. Modern orders of Amphibia are Caudata (salamanders and newts), Gymnphiona (caecilians), and Anurans (frogs and toads). Most Anura species provide a substantial and affordable source of animal protein to the tropics' growing human populations (Onadeko *et al*., 2011). Some frog species are edible and considered a delicacy by some ethnic communities in Nigeria. All *Rana* spp. are harvested as wild bush meat in the south-south and south-western states of Nigeria from waterlogged areas of rural and urban centers. Frogs are also used in traditional medicine (Mtewa *et al*., 2018). Cocoa plantations have become a significant source of pesticide exposure in the environment (Okoya *et al*., 2013; Idowu *et al*., 2013; Fosu-Mensah *et al*., 2016). Cocoa plantations are frequently associated with pest and disease infestations, which have cost farmers huge economic losses of up to 30% or more, depending on the case (Adeniyi, 2019). These pest and disease infestations force farmers to spray pesticides on cocoa trees on a regular basis, especially during the wet season, when pest infestation on cocoa pods is high. Farmers' uncontrolled pesticide use has sparked widespread concern (Hamadamin and Hassan, 2020) due to the residual effects of pesticides in the environment and the organisms that inhabit it (Jayaraj

et al., 2016). Furthermore, farmers mismanage pesticides due to lack of awareness and appropriate spraying equipment. Nonetheless, most of these pesticides have been banned or restricted in developed countries (European Union, 2021) due to their negative effects on human and animal health (Ali *et al*., 2020) as well as their environmental impact. Because pesticides suppress their immune systems, amphibians have become more vulnerable to disease and parasitic infections. Pesticide exposure could also be a factor in amphibian decline (Brühl *et al*., 2013). Although amphibian ecotoxicology has recently gained attention (Van Meter *et al*., 2015; Jayawardena *et al*., 2016), majority of these studies are laboratory-based. Field studies are therefore required to assess pesticide contamination in this group of organisms that are extremely useful in environmental monitoring. Due to their ability to forage between terrestrial and aquatic ecosystems, as well as the permeability of their skin, amphibians have become useful for pesticide monitoring in the environment. Because amphibian health is inextricably linked to environmental health (Brühl *et al*., 2013), they are a good indicator of environmental stress (Strong *et al*., 2017). In order to improve the environmental health of amphibians and humans, this study was undertaken to assess the level of pesticide contamination in amphibians as well as soil and sediment samples from cocoa plantations at Ojo Camp-Ugboke, Edo State, Southern Nigeria.

MATERIALS AND METHODS

Study Area and samples collection: Samples were collected in cocoa plantations at Ojo Camp-Ugboke, Edo State, Nigeria (latitude 6º32'20" and 6º 45'35"N; longitude 5º15'20" and 5º17'46"E) for 12 months (May, 2014 to April, 2015). Sampling was carried out in both wet (April to October) and dry (November to March) seasons. A total of 54 amphibians were collected for this study. Collection of sediment and soil was achieved with the aid of soil auger which was prewashed with absolute alcohol. Sediment samples were taken from a depth of 0 – 12cm at three different spots along the stretches of randomly selected water courses/puddles inside the plantations to make a single composite. This was replicated in triplicate to give three composites of sediment on each visit (n=36). Three replicates of soil samples (n=36) were also collected at random from the plantation floor at depths ranging from 0 to 5cm. All samples were wrapped in aluminum foil and kept cool in an icebox in the field. In the laboratory, samples were kept at 4ºC (<2 days) before extraction.

Pesticides residues extraction in samples: Extraction, clean up and analysis of samples were carried out at Earthquest International Limited, Delta State. The procedures by Steinwandter (1992) and Mahugija (2012) were adopted with modifications. A homogeneous mixture of amphibian tissues was obtained from pounding the whole organism and 10g was measured into a solvent rinsed beaker, to which adequate amount of anhydrous sodium sulfate was added and stirred thoroughly. Thereafter, 20ml of acetone/n-hexane mixture (1:1v/v) was added, stirred and spiked with 1ml of the surrogate mix (tetrachloro-m-xylene) and then sonicated for a maximum of 1h at about 50ºC. 2ml of acetone/hexane extract (containing 1g of sample) was transferred into a 20ml bottle and concentrated to 1ml using rotary evaporator in water bath at 35-40°C. 20g each of soil/sediment was weighed and 3-10g of anhydrous sodium sulphate (drying agent) was added, stirred and 40ml of acetone/n-hexane mixture (1:1v/v) was added. This was stirred again and sonicated for a maximum of 1h. The solvent was then separated from the solute and 10ml of the supernatant was pipettes into a 20ml bottle and concentrated to 1ml using rotary evaporator. All samples were now ready to be cleaned up using florisil packed cartridges. A florisil column was conditioned with n-hexane and the concentrated 1ml of each sample was applied to the column and the pesticides eluted with 10ml of n-hexane/acetone mixture (99%:1%). The eluate was concentrated to 1ml and then used for gas chromatography (GC) analysis/quantification.

Quantification of pesticide residues in samples: The concentrated clean extract from the soil, sediment and tissues were respectively analyzed for pesticides with HP 5890 series gas chromatography system coupled with an Electron Capture Detector (ECD). The stationary phase for compounds separation was HP-5 column (crosslinked PHME siloxane) 19091J-413 with film thickness, 0.25µm; length, 30m; phase ratio, 320 and ID, 0.32mm. The carrier gas was nitrogen with linear velocity of 30cm/sec. The extract injection (1µl) was split-less. The injector and detector temperatures were set at 225ºC and 300ºC, respectively, with an equilibration time of 3.0 minutes. Initial oven temperature was 150ºC with initial hold time of 2mins increasing at a rate of 2.5ºC/min to an intermediate value of 290ºC with a hold time of 1min. The method validation parameters for the GC analysis included accuracy (78.1%), precision (79.5%), spiking and percentage recovery. Quantification of organochlorine pesticides was accomplished using a nine – point external standard calibration (between 0.002mcg/kg and 10000mcg/kg) purchased from Accustandard – USA. The standard curves were linear with correlation coefficients between 0.993 and 0.997.

The limit of detection (LOD) ranged from 0.002 to 0.005mcg/kg while the limit of quantification (LOQ) was from 0.004 to 0.018mcg/kg. The injection syringe was cleaned 2-3 times with n-hexane before being used to withdraw 1µl of the extracted sample which was injected into the GC column and was run for 45 minutes. The data generated by the GCECD was interpreted using the chemstation version A10 software.

Data analyses: The resultant data were tested for normality and homogeneity of variance using Shapiro-Wilk's test and Levene's test respectively. The data did not satisfy the assumptions of parametric tests; hence they were subjected to the non-parametric Kruskal-Wallis *H* test with alpha (α) at 5%. Upon detection of a significant variation within variables Dunn's tests of multiple (rank-sums) comparisons were adopted as an appropriate post-*hoc* test using R version 4.1.1 (R Core Team, 2021).

RESULTS AND DISCUSSION

Thirteen organochlorine pesticides (OCPs), one triazine herbicide (atrazine) and one organophosphate (phosphono methyl glycine) component were detected in amphibian tissues, soil and sediment samples at varying concentrations (mcg/kg). One carbamate (carbofuran) was detected in all the samples with the exception of soil. All pesticides components reported in this study were detected above the limit of detection (LOD) and limit of quantification (LOQ). Lindane (γ-BHC) was the most prominent component occurring in almost all the samples analyzed. It was detected in 95.8%, 86.1% and 50.0% of amphibian tissues, sediment and soil samples, respectively (Fig. 1). Endosulfan aldehyde was the least detected occurring in 2.8% of sediment and soil, and in 8.3% of amphibian tissues. Carbofuran was not detected in soil samples but it was detected in sediment and amphibian tissues.

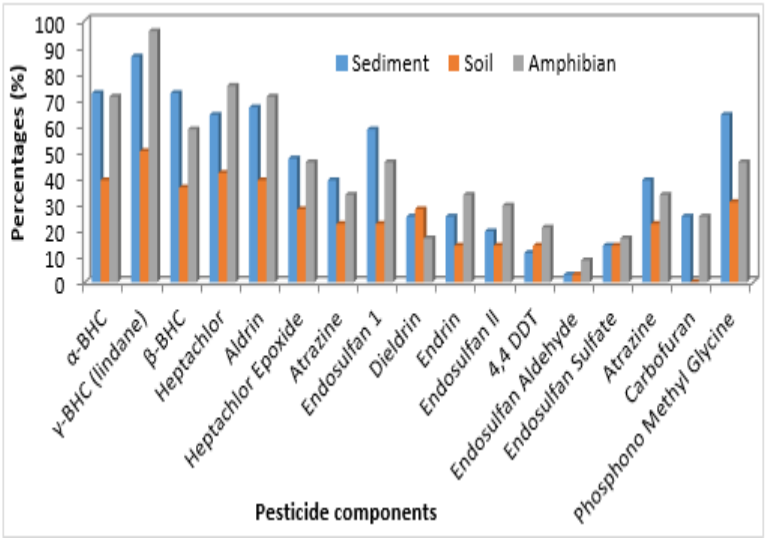

Fig. 1: Percentage of various samples from cocoa plantations at Ojo Camp, Ugboke with pesticides components

All pesticides components were detected in amphibian tissues, ten of these components had higher concentrations in the tissues than other samples (Table 1). Lindane had the highest mean concentration of 0.875±0.246 and 0.639±0.150mcg/kg in amphibian tissues and sediment, respectively; followed by heptachlor and atrazine with 0.492±0.172mcg/kg and 0.394±0.257mcg/kg in amphibian tissues and sediment, respectively. Phosphono methyl glycine (0.308±0.127), aldrin (0.283±0.090) and α–BHC (0.213±0.057) were other components with significant concentrations in the tissues. The concentration of Dieldrin, Endosulfan aldehyde and Endosulfan sulphate were 0.042±0.026, 0.058±0.054 and 0.050±0.034, respectively but this difference was not significant ($p>0.05$). In soil, the most detected pesticide component was β-BHC with a mean concentration of 0.169±0.055 followed by α-BHC (0.147±0.046), γ-BHC (0.125±0.028) and aldrin (0.125±0.036).

Other components with significant concentrations observed in soil were Atrazine, Heptachlor epoxide, Dieldrin and Endosulfan 1 with concentrations of 0.111±0.043, 0.100±0.047, 0.089±0.028 and 0.081±0.032mcg/kg, respectively. Irrespective of these variations, there was no significant differences among most of them ($p>0.05$) as shown in Table 1.

Carbofuran was below detectable limit in soil. In sediment, lindane had the highest concentration (0.639±0.150mg/kg), followed by atrazine (0.394±0.257), β–BHC (0.317±0.092), heptachlor (0.311±0.112) and aldrin (0.306±0.089).

Other major components present in sediment were phosphono methyl glycine, endosulfan 1, heptachlor epoxide, α–BHC, endrin and dieldrin with mean concentrations of 0.236±0.062, 0.233±0.064, 0.222±0.069, 0.203±0.037, 0.142 ±0.053 and 0.111±0.040mcg/kg, respectively.

Table 1: Mean concentration (mcg/kg) of pesticides in samples from cocoa plantations

Component	Amphibian tissue ($\overline{X}$±S.E)	Soil ($\overline{X}$±S.E)	Sediment ($\overline{X}$±S.E)
α–BHC	0.213±0.057^{cd}	0.147±0.046^{de}	0.203±0.037^{c}
γ-BHC (lindane)	0.875±0.246^{f}	0.125±0.028^{e}	0.639±0.150^{g}
β–BHC	0.167±0.042^{cde}	0.169±0.055^{de}	0.317±0.092^{cg}
Heptachlor	0.492±0.172^{df}	0.106±0.028^{de}	0.311±0.112^{cf}
Aldrin	0.283±0.090^{cd}	0.125±0.036^{de}	0.306±0.089^{c}
Heptachlor epoxide	0.179±0.065^{bce}	0.100±0.047^{ad}	0.222±0.069^{cef}
Endosulfan I	0.171±0.051^{bce}	0.081±0.032^{acd}	0.233±0.064^{cf}
Dieldrin	0.042±0.026^{a}	0.089±0.028^{ade}	0.111±0.040^{ade}
Endrin	0.187±0.102^{abe}	0.044±0.020^{abc}	0.142 ±0.053^{ade}
Endosulfan II	0.108±0.056^{abe}	0.056±0.027^{abc}	0.092±0.034^{abd}
4,4 DDT	0.092±0.057^{ab}	0.061±0.027^{abc}	0.039±0.020^{ab}
Endosulfan aldehyde	0.058±0.054^{a}	0.006±0.006^{bc}	0.011±0.011^{b}
Endosulfan sulfate	0.050±0.034^{a}	0.036±0.017^{abc}	0.017±0.012^{ab}
Atrazine	0.150±0.076^{abe}	0.111±0.043^{ad}	0.394±0.257^{def}
Carbofuran	0.163±0.090^{abe}	0.000±0.000^{b}	0.083±0.027^{abde}
Phosphono methyl glycine	0.308±0.127^{bce}	0.075±0.023^{ade}	0.236±0.062^{cf}

Note: Values represented by similar letters as superscripts were not significantly different from each other (p>0.05)

Table 2: Seasonal comparison of pesticide components in Amphibian tissues

Pesticide components	Wet ($\overline{X}$±S.E)	Dry ($\overline{X}$±S.E)	p-value
α–BHC	0.214±0.095	0.210±0.038	0.231
γ-BHC (lindane)	1.329±0.378	0.240±0.062	0.004213
β–BHC	0.164±0.054	0.170±0.068	0.8065
Heptachlor	0.707±0.282	0.190±0.067	0.1541
Aldrin	0.357±0.150	0.180±0.042	0.7187
Heptachlor epoxide	0.264±0.106	0.060±0.027	0.3533
Endosulfan I	0.214±0.078	0.110±0.055	0.4816
Dieldrin	0.050±0.043	0.030±0.021	0.7867
Endrin	0.257±0.172	0.090±0.041	0.7009
Endosulfan II	0.121±0.092	0.090±0.048	0.8268
4,4 DDT	0.143±0.096	0.020±0.013	0.7728
Endosulfan aldehyde	0.100±0.093	0.000±0.000	0.2461
Endosulfan sulfate	0.057±0.057	0.040±0.022	0.2066
Atrazine	0.221±0.128	0.050±0.022	1
Carbofuran	0.279±0.149	0.000±0.000	0.02312
Phosphono methyl glycine	0.443±0.211	0.120±0.051	0.587

Table 3: Seasonal comparison of pesticide components in soil

Components	Wet ($\overline{X}$±S.E)	Dry ($\overline{X}$±S.E)	p-value
α–BHC	0.148±0.043	0.147±0.095	0.2881
γ-BHC (lindane)	0.105±0.031	0.153±0.052	0.5931
β–BHC	0.210±0.082	0.113±0.065	0.3602
Heptachlor	0.133±0.044	0.067±0.023	0.5889
Aldrin	0.148±0.054	0.093±0.041	0.9713
Heptachlor epoxide	0.148±0.079	0.033±0.016	0.6544
Endosulfan I	0.062±0.042	0.107±0.051	0.2168
Dieldrin	0.095±0.041	0.080±0.034	0.7759
Endrin	0.024±0.024	0.073±0.036	0.08769
Endosulfan II	0.019±0.015	0.107±0.061	0.3106
4,4 DDT	0.038±0.026	0.093±0.052	0.3643
Endosulfan aldehyde	0.000±0.000	0.013±0.013	0.2598
Endosulfan sulfate	0.014±0.010	0.067±0.039	0.3235
Atrazine	0.129±0.060	0.087±0.061	0.7913
Carbofuran	0.000±0.000	0.000±0.000	NA
Phosphono methyl glycine	0.090±0.036	0.053±0.026	0.6224

Seasonal Variation of Pesticides Residue Levels in Amphibian Tissues, Soil and Sediment: The concentration of pesticide components in amphibian tissues, soil and sediment were generally higher during the wet season compared to the dry. The concentration in amphibian tissues ranged between 0.050±0.043 and 1.329±0.378mcg/kg in wet season and 0 to 0.240±0.062mcg/kg during the dry season. The concentration in sediment samples ranged from 0.019±0.019 to 0.724±0.218mcg/kg during the wet season and 0 to 0.520±0.196mcg/kg in the dry season, while in soil, the concentration ranged from 0 to 0.210±0.082mcg/kg (wet season) and 0 to 0.153±0.052mcg/kg (dry season). All pesticide components analyzed were present in amphibian tissues during the wet season with concentration higher than that observed during the dry season with the exception of β–BHC which had higher concentration during the dry season (Table 2). The variations in pesticide residue levels observed between the wet and dry seasons were not significant (P>0.05) except for γ-BHC (lindane) and carbofuran. Endosulfan aldehyde and carbofuran were not detected in amphibian tissues during the dry season. There was a positive correlation between γ-BHC (lindane), heptachlor, aldrin and dieldrin in amphibian tissue and sediment (Fig. 2 - 5). In soil seven pesticide components (lindane, Endosulfan I and II, Endrin, 4,4, DDT, Endosulfan aldehyde and Endosulfan sulfate) had higher concentrations during dry season; other components were higher during the wet season (Table 3). Endosulfan aldehyde and carbofuran were not detected during the wet season.

Carbofuran was also below detectable limit during the dry season. In sediment, variations in all pesticide mean concentrations were observed between wet and dry season but these differences were not significant (P>0.05) except for phosphono methyl glycine whose mean concentration was very high during the dry season (Table 4). Endosulfan aldehyde and Endosulfan sulfate were below detectable limit during the dry season.

Pesticide residues in amphibian tissues, soil and sediment samples from cocoa plantations at Ojo camp, Ugboke: The high number and concentration of pesticide residues detected in this study attest to pesticides' long-term use. Pesticides degrade quite faster when exposed to sunlight that even the most persistent pesticides will degrade partially when exposed to radiation (Fernandez *et al*., 2001). However, the dense forest cover in the plantations, which prevents direct sunlight from penetrating the plantation environment, could have slowed the degradation process significantly. It is not surprising that more OCPs components were found in the various samples than organophosphate and carbamate groups. Because of their affinity for fatty tissues in plants, animals, and humans, organochlorine pesticides have the potential to be more persistent in the environment (Jayaraj *et al*., 2016). Furthermore, they are highly preferred by farmers because they are cheap, very effective, and accessible (Fosu-Mensah *et al*., 2016).

Table 4: Seasonal comparison of Pesticide components in sediment

Pesticide components	Wet ($\overline{X}$±S.E)	Dry ($\overline{X}$±S.E)	p-value
α–BHC	0.190±0.038	0.220±0.072	0.9606
γ-BHC (lindane)	0.724±0.218	0.520±0.196	0.1745
β–BHC	0.395±0.150	0.207±0.065	0.3959
Heptachlor	0.390±0.183	0.200±0.077	0.7907
Aldrin	0.376±0.141	0.207±0.084	0.2991
Heptachlor epoxide	0.276±0.109	0.147±0.066	0.4544
Endosulfan I	0.252±0.105	0.207±0.051	0.277
Dieldrin	0.095±0.036	0.133±0.085	0.7354
Endrin	0.110±0.060	0.187±0.098	0.7515
Endosulfan II	0.067±0.042	0.127±0.057	0.3293
4,4 DDT	0.024±0.024	0.060±0.036	0.1957
Endosulfan aldehyde	0.019±0.019	0.000±0.000	0.4302
Endosulfan sulfate	0.029±0.021	0.000±0.000	0.2413
Atrazine	0.643±0.436	0.047±0.019	0.3053
Carbofuran	0.052±0.032	0.127±0.045	0.09957
Phosphono methyl glycine	0.138±0.048	0.373±0.126	0.04189

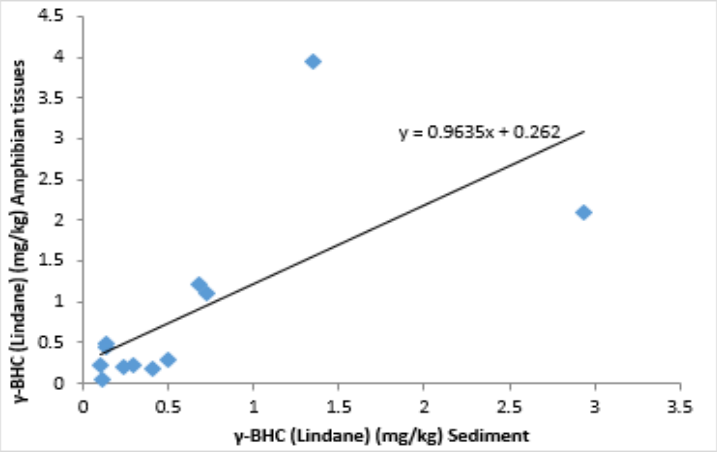

Fig. 2: Regression of γ–BHC (lindane) in amphibian tissues on γ–BHC (lindane) in sediment

Despite the fact that many OCPs have been banned or their use restricted due to their negative effects, they are still used to control agricultural pests in most developing countries around the world, including Nigeria (European Union, 2021). The presence of significant concentrations of Benzene hexachlorides isomers (α-BHC, β-BHC, γ-BHC) in the various samples analyzed reflects the heavy use of these pesticides in cocoa plantations. Lindane (γ-BHC) was the most common pesticide because it was more prevalent and persistent than other pesticides (Fig. 1 and Table 1). The presence of heptachlor epoxide (a metabolite of heptachlor) in various samples from cocoa plantations indicates that the pesticide heptachlor was used on the farm, as heptachlor epoxide is not available for direct use. In all samples tested, the concentration of aldrin was greater than that of dieldrin. This contradicts the findings of Akan *et al*. (2014), who discovered a significantly higher concentration of dieldrin in plant material and attributed this to photolysis of aldrin to dieldrin. Mahugija (2012) also reported the rapid conversion of aldrin to dieldrin in soil sample. It is possible that the canopy cover in the plantations reduced solar penetration and thus inhibited aldrin photolysis to dieldrin. The presence of four endosulfan isomers (endosulfan I and II, endosulfan aldehyde, and endosulfan sulfate) in the various samples indicates that the endosulfan compound was widely used in cocoa plantations. Endosulfan I was detected in more samples than endosulfan II (Fig. 1). Idowu *et al*. (2013) found much higher levels of endosulfan I in water and sediment samples. Endosulfan I predominates over endosulfan II because, while endosulfan I contributes approximately 67% by weight of total endosulfan content in manufactured endosulfan compound, endosulfan II contributes approximately 32% (WHO, 1990).

As a consequence of the pesticide application, it is obvious that more endosulfan I will be present in the plantations. Furthermore, because endosulfan I has greater thermal stability than endosulfan II, it is easily converted to endosulfan I in the environment (Hapeman *et al*., 1997).

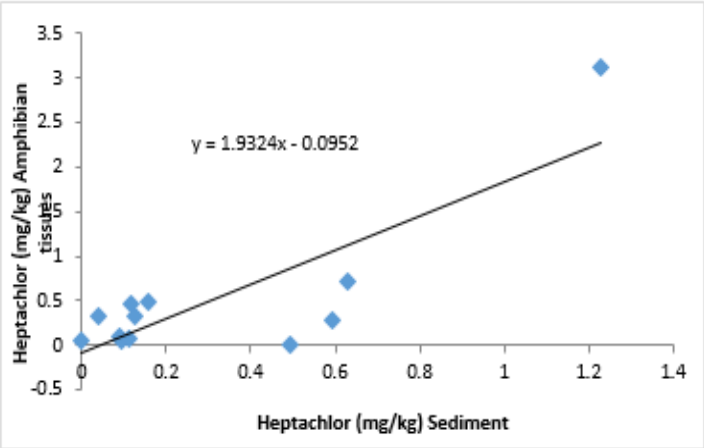

Fig. 3: Regression of Heptachlor in amphibian tissues on Heptachlor in sediment

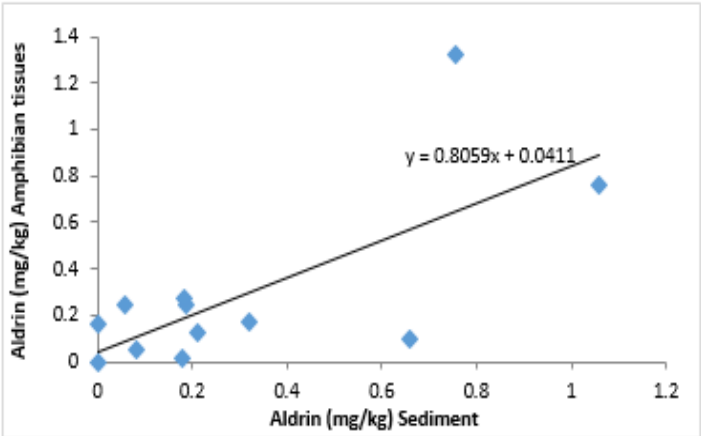

Fig. 4: Regression of Aldrin in amphibian tissues on Aldrin in sediment

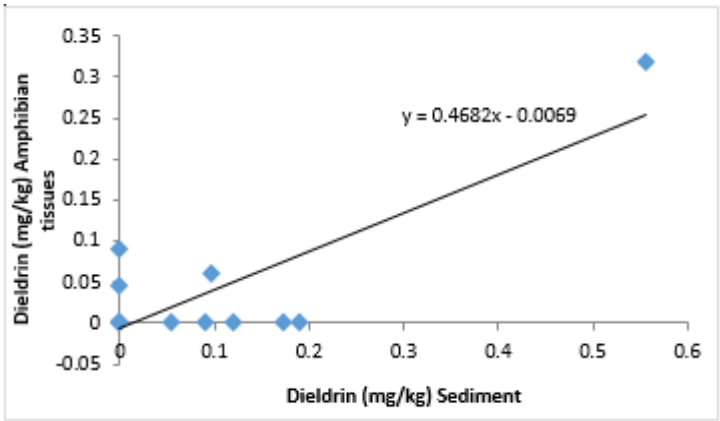

Fig. 5: Regression of Dieldrin in amphibian tissues on Dieldrin in sediment

Endosulfan compounds are highly degradable and do not persist in the environment like other organochlorine compounds (Jayaraj *et al*., 2016). These factors could have contributed to the low concentrations or absence of their compounds in some samples (Table 2, 3 and 4).

There is currently no information available in Nigeria about the maximum residue limit for organochlorine pesticides in soil, sediment, and amphibian tissues (Egbe *et al*., 2021).

Amphibian Tissues: In this study, all 16 pesticide residues were found in significant concentrations in amphibian tissues (Table 1). This could be ascribed to the dual lifestyle of amphibians which predispose them to contamination both on land and in water. The high permeability of amphibian skin to environmental contaminants undoubtedly contributes to this (Van Meter *et al*., 2014; 2015). Pesticides can also be absorbed by amphibians via their legs (Brühl *et al*., 2011), oral membranes, nasal inhalation, and the gastrointestinal tract (Jayaraj *et al*., 2016).

Frogs frequently shed their skin, which they then consume for nutrition and likely absorb any pesticide residues on the skin (Cramp *et al*., 2014). Compared to the 13 OCPs found in amphibians in this study, Yehouenou *et al*. (2014) reported fewer OCPs [α-endosulfan (endosulfan l) and five DDT metabolites] in *Bufo regularis* and *Xenopus muelleri* from a cotton plantation in the Republic of Bénin. This confirmed that pesticides were used more frequently on the cocoa farms in our investigation.

Soil: Soil is commonly thought to be a depository for organochlorine pesticides (Mahugija, 2012; Sánchez-Palencia *et al*., 2017). However, when compared to other samples in this study, the soil had the lowest concentration of pesticide residues (Table 1). This is due to a number of factors that may impact pesticide persistence in soil. These include leaching, evaporation, surface run-off, uptake by plants, migration of animals with residues on their bodies, and the tendency of pesticides to be retained in deeper soil depths (Akan *et al*., 2014). Furthermore, the unique environment of cocoa plantations prevents pesticides from settling directly on the soil surface.

The plantation floor is usually covered with deep leaf litter, and pesticides settle on this leaf litter during application, protecting the soil from direct impact and accumulation. In this study, however, thirteen OCPs components were observed in soil samples. In contrast, Fosu-Mensah *et al*. (2016) reported only four OCPs (lindane, beta HCB, dieldrin, and p,p'-DDT) but at higher mean concentrations in soil in Ghanaian cocoa farms. For example, in their study, lindane was detected in 31.3% of soil samples at a concentration of 0.04±0.01mg/kg, whereas in our study, lindane was detected in more (50.0%) soil samples but at a lower concentration (0.125±0.028mcg/kg). The lindane concentration in our study was also lower than the 0.002mg/kg and 0.257mg/kg reported by Agyen (2011) and Aiyesanmi and Idowu (2012) from soil in cocoa farms in Ghana and Ondo State, Nigeria, respectively, but higher than that of Olayinka (2013) who recorded 0.0001mg/kg from soil in cocoa farms in Ekiti State. In a similar investigation, Egbe *et al*. (2021) reported 15 OCPs components in cocoa farms soil at Owena, Ondo State, Nigeria. Of these 10 (α-HCH, β-HCH, ϒ-HCH (lindane), Aldrin, Dieldrin, Heptachlor, Heptachlor epoxide, α-endosulfan, β-endosulfan and Endosulfan sulphate) were detected in our study albeit with lower concentrations.

Sediment: High pesticide concentrations were found in sediment samples, and it is likely that the terrain of the cocoa plantations played a role in the pesticide accumulation. The plantations are developed on sloppy terrain that drains into nearby bodies of water. When it rains, farm run-offs wash pesticides into the water bodies, and because pesticides are not soluble in water, organic matter in sediment becomes the preferential site for the adsorption of these hydrophobic pollutants, including OCPs (Kouakou *et al*., 2015). As a result, the sediment serves as a sink for persistent pesticides (Kafilzadeh *et al*., 2012).

Most of the pesticide residues detected with significant concentrations in sediment samples from Ugboke were absent in the report of Idowu *et al*. (2013). Even those found were at low concentrations. These variations could be attributed to the sampling site. These authors collected samples from rivers near cocoa farms, whereas our study collected samples from water bodies within cocoa plantations. In contrast to our findings, Yehouenou *et al*. (2014) found fewer OCPs (α-endosulfan, pp'DDE' and pp'-DDT) in sediment from a pesticide-contaminated cotton plantation in the Bénin Republic.

Seasonal Comparison: In amphibian tissues and sediment, more and higher pesticide components/concentrations were detected during the wet season compared to the dry season. This could be due to their intense use during this period to combat heavy infestations of insect pests and diseases on cocoa pods. Okoya *et al*. (2013), on the other hand, found lower OCP concentrations in sediments during the wet season. A lower concentration was recorded in soil because what would have accumulated there could have been trapped in the plantation floor's leaf litter.
There was a positive correlation between lindane, heptachlor, aldrin, and dieldrin in sediment and amphibian tissues, indicating that amphibians most likely acquired these pesticides from sediment. On the other hand, amphibians may have absorbed these pesticides transdermally. These compounds are so toxic to most aquatic organisms that even at low concentrations their effects can become evident after a short or long period of time (Smalling *et al*., 2012). They can also alter reproductive ability, subdue immune systems, and influence amphibian health and populations (Deknock *et al*., 2020).

Conclusion: It is evident from this study that the mixture of pesticides sprayed by farmers in the cocoa plantations accumulates in the environment and in amphibian tissues. The general trend of pesticides concentration in the different samples were amphibian tissues>sediment>soil. There is an indication that amphibians acquired most of these pesticides from the sediment. It is therefore needful to educate farmers about the dangers/health implications of pesticides while encouraging continuous monitoring and regulation by relevant government agencies.

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