# Bioaccumulation of organochlorine pesticides in the parasite *Cosmocerca* sp. (Nematoda: Cosmocercidae) and the amphibian host *Amietophrynus regularis* (Reuss, 1833) within Lagos metropolis, Nigeria

Okechukwu Martin Okeagu [a,*], Bamidele Akinsanya [a], Patrick Omoregie Isibor [b], Josephine Daniel-Rugu [a], Abiodun Benedict Onadeko [a], Babangida Yalwaji [a], Khalid Olajide Adekoya [c]

[a] *Department of Zoology, University of Lagos, Akoka, Lagos State, Nigeria*
[b] *Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria*
[c] *Department of Cell Biology and Genetics, University of Lagos, Akoka, Lagos State, Nigeria*

## ARTICLE INFO

## ABSTRACT

*Background:* Amphibian species are being threatened worldwide and chemical pollution is one of the leading causes of this decline. The use of agrochemicals such as organochlorine pesticides (OCPs) among the several health and ecological challenges it causes, the sharp amphibian population decline is most pressing.
Toad specimens *Amietophrynus regularis* were sampled from three (3) selected areas; each comprising of natural habitat and dumpsites within Lagos metropolis.
*Methods:* The congeners of organochlorine pesticides were tested in the liver, intestine, and parasite (*Cosmocerca* sp.) of the toads and soil samples from the respective locations using gas chromatography-mass spectrometer (GC–MS). Histopathological analyses were conducted on the intestines and liver of the toads using hematoxylin and eosin (H&E) stain and then examined under the binocular dissecting microscope.
*Results:* The concentration of aldrin in the intestine of *A. regularis* sampled at the dumpsites was higher than the concentrations in the intestines of *A. regularis* in the natural habitat. The concentrations of dieldrin in the un-infected *A. regularis* at both dumpsite and natural habitat were higher than the concentrations in the infected *A. regularis* at both environments. This indicated that the parasite *Cosmocerca* sp. may have played a depurative role in sequestering the concentration of dieldrin in the toads irrespective of the location. The parasites exhibited marked sequestration capacity characterized by the notably high total bioaccumulation rate both in the liver and the intestine at the dumpsite. The stunted villi being the common histological alteration in the infected and uninfected toads at the dumpsite but missing in the uninfected counterparts at the natural habitat may be attributed to the differences in the background concentration of the OCP congeners.
*Conclusions:* The parasite- *Cosmocerca* sp. has been shown to be a potential tool in the biomonitoring of these OCP congeners which persists in the environment. Continuous research on these congeners is a searchlight to checkmate the environment to see how compliant industries and the consumers are in terms of regulation of these chemicals.

## 1. Introduction

Frogs and toads constitute about 90 % of the amphibian population [1], therefore they are the main component of the linkage between aquatic and terrestrial ecosystems [2]. Furthermore, most adult frogs and toads feed on insects; hence they constitute a vital energy-efficient trophic link between the invertebrates and the vertebrates [3]. The African common toad (*Amietophrynus regularis*) also known as the African bouncing toad is a source of protein in sub-Saharan Africa [4,5].

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stated that about 787 endangered amphibian species [6], while about 1, 900 threatened (Stuart et al., 2004) underlie the global decline of amphibians. Chemical pollution is one of the leading causes of amphibian population decline [7–9]. Toad's relatively higher skin permeability afford them a distinguished chemical accumulation tendency compared to other aquatic fauna. They are thus suitable bioindicators in ecotoxicology [10,11].

The exponential population increase in the Sub-Sahara region of Africa is the leading factor underlying the rise in the use of agrochemicals for enhanced agricultural yields. The use of agrochemicals such as organochlorine pesticides (OCPs) has however caused several devastating health and ecological challenges; one of such includes the sharp decline in the amphibian population. This may be considered a serious ecotoxicological concern given the indispensable ecological importance of amphibians. Akinsanya et al. [12] earlier reported the ecotoxicological impacts of toad's exposure to trace metals in selected dumpsites within Lagos metropolis. The study emphasized the role enteric parasites may play in protecting the toad from such toxicological effects.

According to Pimentel [13], only about 0.3 % of applied pesticides affect the target organism while about 99.7 % remain as residue in the environment, affecting non-target organisms, particularly the highly vulnerable ones such as amphibians [14]. Organochlorine pesticides (OCPs) persist in the environment, with notable lipophilicity [15,16]. Sofoluwe et al. [17] stated that about 125, 000–130, 000 metric tonnes of pesticides are applied yearly in Nigeria. This underlies the high levels of OCPs (above the 0.01 ppm allowable limit) previously reported within the metropolis of Lagos, Nigeria [18,19]. Residues of OCP have also been reported in water and fish from some rivers in Edo State, Nigeria [20]. Marked concentrations of lindane, aldrin, p,p-DDE, o, p-DDD, p,p-DDD, o,p-DDT, and p,p-DDT reported in environmental media sampled within Lagos have been linked to unregulated use of pesticides within the populous state [20].

Akinsanya, et al. (2015) also reported alpha.-Lindane (a-BHC), beta-Lindane (b-BHC), gamma-Lindane (y-BHC), delta.-Lindane (d-BHC), Heptachlor, Aldrin, Heptachlor epoxide (Isomer B), Endosulfan I, p,p'-DDE (4,4'-DDE), Endrin, Endosulfan II (beta.-Endosulfan), p,p'-DDT (4,4'-DDT), Endrin aldehyde, Endosulfan sulfate, p,p'-DDD, Dieldrin, Endrin ketone and Methoxychlor in the water, bottom sediment and selected fish from Lekki lagoon.

Toxic effects of OCPs on the biotic and abiotic components of Lekki lagoon and the neighboring aquatic ecosystems have been widely reported [16,17,19] and attributed to several organochlorine-based anthropogenic activities abound within the vicinity of the lagoon. Such activities include the predominant use of agrochemicals, glyphosate-based herbicides, synthetic fertilizers in farmlands, and wide application of municipal pesticides [21,22]. Acute toxicity of OCPs may inflict gonadotoxicity on *Amietophrynus regularis*, thereby further threatening the abundance of the animal. This may result in cancer, brain damage, cognitive complications, nephrotoxicity, and deformities [16,17,22] in consumers of the toad.

*Cosmocerca* sp. a nematode parasite belonging to the Cosmocercidae family inhabits moist soil and dry areas where *Amietophrynus regularis* spend most of its time. It has a direct terrestrial life cycle in which the larvae penetrate the skin of the host before migrating to the large intestine [23]. Previous studies have revealed that some nematode parasite of toads has depurative potentials on contaminants [16]. But no study has been done on the depurative potentials of *Cosmocerca* sp. It is therefore hypothesized that *Cosmocerca* sp. may be useful in attenuating the burden of OCPs in its host toad. The study aimed at investigating the concentration of OCPs in the soil, *Amietophrynus regularis,* and its endoparasite *Cosmocerca* sp. will further give information on the depurative potential of *Cosmocerca* sp.

## 2. Methods

### 2.1. The study area

*Amietophrynus regularis* toad specimens and soil samples were collected from three (3) major areas of Lagos namely; Ojota, Badargy, and Ikorodu at natural habitats and dumpsites within these 3 areas. A comparison was made between the accumulation of OCPs in toads from selected natural and contaminated environments within the Lagos metropolis.

Six (6) sampling stations were designated namely; Ojota dumpsite (06° 35' 40"N, 03° 22'39"E), Ojota natural environment (06°34'47"N 03° 23'37" E); Badagry dumpsite (06° 25'42" N, 02° 53'25" E); Badagry natural environment (06° 35'52"E); Ikorodu dumpsite (06° 35.8042'N, 03° 34.8016"E), Ikorodu natural environment (06° 35.766'N, 3° 34.5683'E) (Fig. 1).

### 2.2. Sampling periodicity and replicates

The toad specimens were procured lifeless but fresh from toad hunters within the respective locations. Soil samples were also collected from the locations using a hand-held trowel and preserved in clean foil papers, transported immediately to the laboratory in an ice-laden cooler. Parasite samples were collected from the intestine of the toads by longitudinal excision of the stomach. The parasites were then preserved in a saline solution for further identification (using [14]) and laboratory analysis. The sampling was done repeatedly monthly for 4 months.

#### 2.2.1. Chemicals and reagents
Analar grade reagents and standard reagents obtained with their certificate of analysis were used. Each batch of sample analysis was run with a certified reagent from the same Lot/Batch with Lot number properly documented.

#### 2.2.2. Biological sample extraction with clean up step
Parasite samples were pooled to 15 g, while intestine and liver of *Amietophrynus regularis* were excised and all analyzed using KOH Refluxing/Vortex Extraction [32]. 15 g wet weight of pooled whole parasite (n = 6), toad intestine and liver (n = 20) were weighed into a crucible then macerated and homogenized, then 10 g of each homogenized tissues (parasite, intestine, and liver) the was placed in a 50 mL centrifuge tube, 15 mL of 6 N KOH was added, the tubes were sealed and incubated for 18 h in a 35 °C water bath, shaken vigorously for 30 s and sample was allowed to cool to room temperature. 15 mL of methylene chloride was added to the centrifuge tube, vortexed for 1 min, and then centrifuged at 2000 rpm for 5 min to facilitate phase separation. The upper/aliquot layer was removed using a Pasteur pipette into a 250 mL round-bottom flask. Solvent centrifugation was repeated twice and all aliquots fractions were combined in the round-bottom flask. Sample extracts were concentrated to about 5 ml using a rotary evaporator before fractionation cleanup using silica gel column and GC–MS analysis.

#### 2.2.3. Soil sample extraction with clean up step
Soil samples (n = 6) collected from the sampling locations were each homogenized. 10 g $\pm$ 0.05 g of the soil sample was weighed into a 250 mL Teflon bottle. About 1–3 spatula full of activated Sodium sulfate was added to the samples in the Teflon bottles to eliminate water/ aqueous portions if any. The covered Teflon bottles were then sonicated in an ultrasonic bath at 70 °C for 30 min with 20 mL of 1:1 acetone: hexane was used for extraction procedure (thrice, giving ~60 mL of final extracting solvent). The organic layer was decanted into a clean beaker/ round-bottom flask, further dried with sodium sulfate, and a clean-up procedure using a silica gel column was carried out. The sample extract was then concentrated to ~2 mL using a rotary evaporator before cleanup and GC–MS analysis using an Agilent 7820A gas
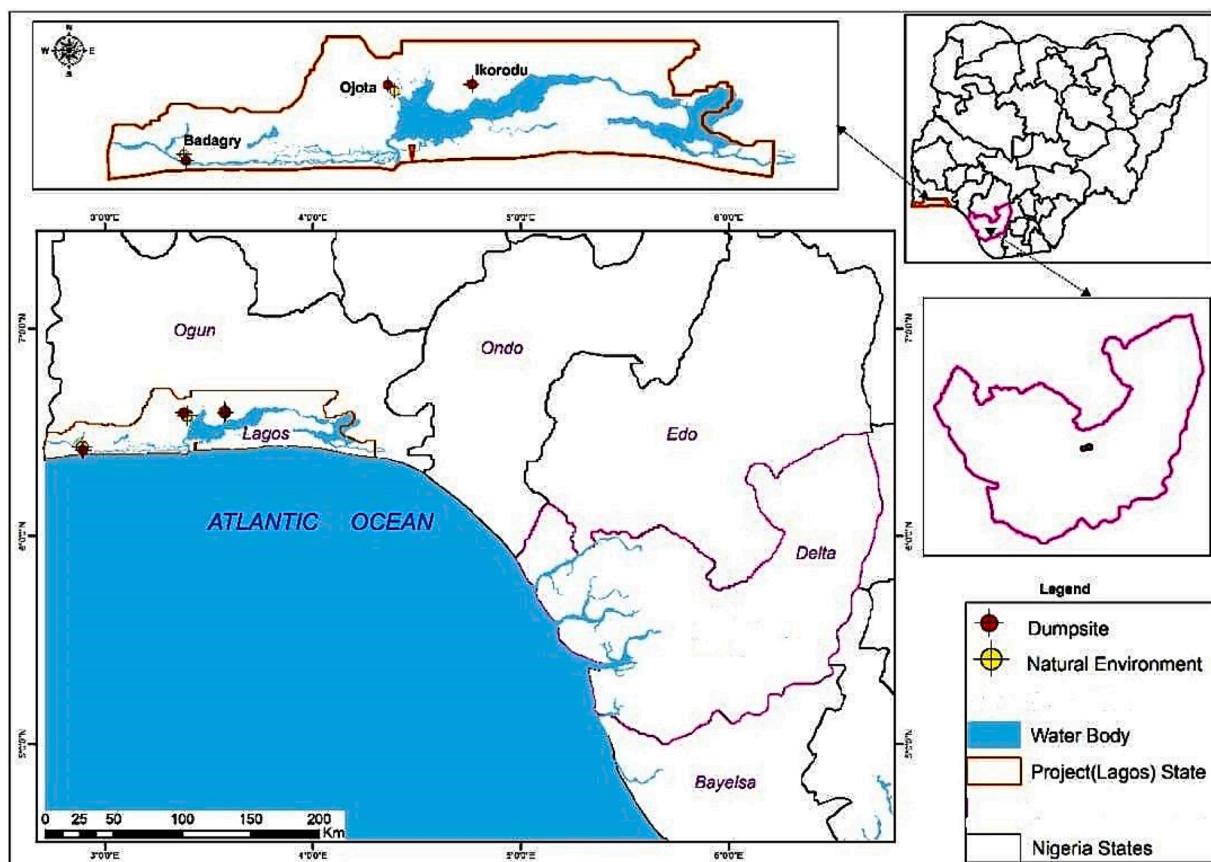
**Fig. 1.** Map showing sampled stations.

chromatography coupled to a 5975C mass spectrometer.

### 2.2.4. Instrumental analysis

Determination of the levels of OCPs in samples was carried out using GC–MS by operating MSD in selective ion monitoring (SIM) and Scan mode to ensure low-level detection of the target constituents. Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple-axis detector) with electron-impact source (Agilent Technologies) was used. The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% Phenyl Methyl Siloxane (30 m length × 0.32 mm diameter x0.25 μm film thickness) (Agilent Technologies). The carrier gas was Helium used at a constant flow of 1.2047 mL/min at an initial nominal pressure of 0.6499 psi and an average velocity of 40.196 cm/sec. 1 μL of the samples were injected in splitless mode at an injection temperature of 250 °C. Purge flow to spilt vent was 30.0 mL/min at 0.35 min with a total flow of 31.278 mL/min; gas saver mode was switched off. The oven was initially programmed at 60 °C (0.5 min) then ramped at 20 °C/min to 140 °C (2 min) and 11 °C/min to 280 °C (10 min). Run time was 29.227 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70 eV with an ion source temperature of 230 °C, a quadrupole temperature of 150 °C, and a transfer line temperature of 300 °C. Acquisition of ion was via Scan mode (scanning from *m/z* 50 to 500amu at 2.0 s/scan rate) and selective ion mode (SIM). After calibration, the samples were analyzed and corresponding OCPs concentration was obtained.

### 2.2.5. Quality control/ quality assurance measures

The GC–MS was calibrated for the analysis before samples were analyzed. Five (5) point serial dilution calibration standards (0.25, 0.50, 1.00, 2.00, 4.00 ppm) were prepared from the stock and used to calibrate the GC–MS. Before calibration, the MS was auto-tuned to

perfluorotributylamine (PFTBA) using already established criteria to check the abundance of *m/z* 69, 219, 502, and other instruments optimal & sensitivity conditions. The limit of detection (LOD) and limit of quantification (LOQ) for the OCPs (ng/mL) were: alpha lindane (0.044, 0.132), beta lindane (0.037, 0.112), delta lindane (0.062, 0.186), gamma lindane (5.4, 16.4), aldrin (0.050, 0.152), dieldrin (0.038, 0.116), endosulfan- I (0.21, 0.62), endosulfan- II (0.18, 0.53), endosulfan sulfate (0.103, 0.312), endrin (0.195, 0.592), heptachlor (0.047, 0.143), methoxychlor (0.099, 0.301), p,p, DDD ($5.7 \times 10$–13, $1.73 \times 10$–12), p,p'-DDE (0.24, 0.71), and p,p'-DDT (0.036, 0.109). Instrument blank (methylene chloride or hexane, initial calibration standards, continuous calibration standard (5 μg/mL or 10 μg/mL), and laboratory reagent blank was run to account for any interferences or contaminant in the solvent, reagent, glassware and other sample processing that may lead to elevated baselines observed by GC/MS detection. Buffalo river sediment (SMR2704) was used to authenticate the data obtained after the initial and final calibrations. The SRM was quantified and the percentage difference did not exceed +/-30 %. A procedural blank was run after every 10 samples which were consisting of all preparation and extraction steps conducted with a sample except when a distilled water sample was used instead of the actual sample.

### 2.3. Histopathological examination

The intestines of the toads from the dumpsite and habitat were categorized into infected and uninfected. The tissues were excised and preserved in Bouin's fluid. The tissues in the fluid were decanted after 6 h and preserved with 10 % phosphate-buffered formalin. Each tissue was routinely dehydrated in an ascending series of alcohol at 30 min intervals. It was then embedded in molten paraffin wax and allowed to solidify. The blocked tissues were sectioned at 4–5 microns, processed, and stained with hematoxylin and eosin (H&E). The stained tissues were

rinsed in clean running water, allowed to dry, and then mounted using DPX mountant. They were then examined under the binocular dissecting microscope (American Optical Corporation, Model 570).

### 2.4. Statistical analysis

The descriptive statistics of the OCP congener concentrations were expressed as mean ± standard deviation. The statistics were subjected to analysis of variance using Graph Pad Prism and Microsoft Excel 2010 to determine the significant differences at probability levels of 0.05 and 0.01 which represented significant differences.

## 3. Results

### 3.1. Levels of OCP concentration in A. regularis intestine and liver

The concentration of aldrin in the intestine of toads sampled at the dumpsites was higher than the concentrations in those in natural habitat although it was not statistically significant ($p > 0.05$). The concentrations of dieldrin in the uninfected toads at both dumpsite and natural habitats were higher than the concentrations in the infected toads in both environments. The concentrations of dieldrin in uninfected intestines of the toads at the dumpsite and natural habitat were significantly higher than the concentrations in the infected intestines (Table 1).

The concentrations of dieldrin and endrin in the intestine of the toads at the dumpsite were at variance with the intestine of the toad at natural habitat (Table 1). No significant differences occurred in the concentrations of other OCP congeners. The concentrations of dieldrin and endrin in the intestines of infected toads at the natural habitat were significantly higher ($p < 0.05$) than the infected toad's concentration at the dumpsite. The concentrations of dieldrin and endrin in the uninfected counterparts were also significantly higher ($p < 0.05$) in the natural habitat than the dumpsite. Moreso, the concentrations of dieldrin and endrin in the infected toads in the natural habitat were significantly higher ($p < 0.05$) than the concentrations in the uninfected ones.

In the liver, same as with the intestine, concentration variability was only detected in the dieldrin and endrin (Table 2). The concentrations of these OCP congeners in the liver of the infected and uninfected toads at the natural habitat were much significantly higher ($p < 0.01$) than the concentrations of the counterparts at the dumpsite. There was no significant difference ($p > 0.05$) in the dieldrin and endrin concentrations between the infected and uninfected toads at the dumpsite. At the

natural habitat, no significant difference occurred in the concentration of dieldrin between the two groups of toads, while the concentration of endrin in the infected toads was much significantly lower ($p < 0.01$) than the concentration in the uninfected ones.

### 3.2. Levels of OCP concentration in parasites

The parasites collected from the intestines of the toads at the natural habitat contained higher concentrations of the majority of the OCP congeners than the parasites from the intestines of the toads at the dumpsites (Fig. 2), except for methoxychlor that had a higher concentration in the parasites from the dumpsite than the natural habitat. Among all OCP congeners analyzed, gamma-lindane had the highest concentration in the parasites from the dumpsite and the natural environment.

The holistic comparison of the concentrations of OCPs in the parasites with the hosts both at the dumpsite and natural habitat depicts the chemical's dynamics in its entirety. The host toad at the habitat had the highest concentrations of endosulfan I, dieldrin, and endrin followed by the hosts at the dumpsite (Fig. 3). Conversely, the host at the dumpsite had a higher concentration of aldrin than the host in the natural habitat. The concentrations of the OCP congeners in the parasites at both environments were low compared to the hosts' concentrations.

### 3.3. Levels of OCP concentration in soil

The soil samples collected from the dumpsite had a much significantly higher concentration of gamma-lindane than the soil samples at the natural habitat ($p < 0.001$). No significant difference occurred in other OCP congeners' concentrations between the two soil samples (Table 3).

### 3.4. Bioaccumulation of OCPs

The parasites collected from *A. regularis* in the natural habitat exhibited higher bioaccumulation of beta-lindane, gamma-lindane, delta-lindane, heptachlor, heptachlor epoxide. Particularly, the bioaccumulation factor (the rate of accumulation of xenobiotics) of p,p'-DDT from the host into the parasites at the natural habitat was outstandingly higher (19.89) than that of parasites at the dumpsite (2.77) (Table 4). While the parasites at the dumpsite as at the time the study was conducted significantly bioaccumulated alpha lindane, heptachlor, beta endosulfan, p,p'-DDD, p,p'-DDT, endosulfan sulfate, and

**Table 1**
Comparative analysis of OCPs in the intestine of toads at the dumpsite and natural habitat (units in ppm).

| | DUMPSITE | | | | HABITAT | | | |
|---|---|---|---|---|---|---|---|---|
| | INFECTED | | UNINFECTED | | INFECTED | | UNINFECTED | |
| | MEAN | SD | MEAN | SD | MEAN | SD | MEAN | SD |
| alpha.-Lindane (a-BHC) | 0.024 | 0.008 | 0.029 | 0.027 | 0.033 | 0.018 | 0.017 | 0.013 |
| beta.-Lindane (b-BHC) | 0.047 | 0.021 | 0.035 | 0.001 | 0.017 | 0.006 | 0.030 | 0.011 |
| gamma.-Lindane (y-BHC) | 1.126 | 0.788 | 1.199 | 1.107 | 0.318 | 0.543 | 0.000 | 0.000 |
| delta.-Lindane (d-BHC) | 0.016 | 0.017 | 0.067 | 0.055 | 0.038 | 0.030 | 0.011 | 0.016 |
| Heptachlor | 0.171 | 0.154 | 0.163 | 0.030 | 0.141 | 0.021 | 0.148 | 0.029 |
| Aldrin | 4.477 | 7.468 | 4.948 | 5.854 | 1.019 | 2.049 | 0.104 | 0.022 |
| Heptachlor epoxide (Isomer B) | 0.071 | 0.183 | 0.386 | 0.335 | 0.035 | 0.029 | 0.408 | 0.688 |
| Endosulfan I (.alpha.-Endosulfan) | 3.363 | 3.544 | 3.516 | 4.718 | 10.804 | 4.801 | 5.205 | 0.175 |
| Dieldrin | **4.379**[d] | **2.056** | **26.959**[b] | **12.488** | **15.185**[c] | **5.326** | **44.234**[a] | **12.327** |
| p,p'-DDE (4,4'-DDE) | 0.114 | 0.056 | 0.153 | 0.022 | 0.092 | 0.070 | 0.094 | 0.044 |
| Endrin | **13.760**[b] | **1.879** | **18.681**[b] | **9.889** | **57.044**[a] | **3.837** | **52.908**[a] | **7.857** |
| Endosulfan II (.beta.-Endosulfan) | 0.231 | 0.070 | 0.091 | 0.072 | 0.906 | 1.033 | 0.191 | 0.050 |
| p,p'-DDD (4,4'-DDD) | 0.010 | 0.007 | 0.013 | 0.008 | 0.013 | 0.011 | 0.007 | 0.007 |
| p,p'-DDT (4,4'-DDT) | 0.033 | 0.019 | 0.016 | 0.009 | 0.019 | 0.006 | 0.009 | 0.001 |
| Endosulfan Sulfate | 0.097 | 0.096 | 0.102 | 0.055 | 0.154 | 0.103 | 0.219 | 0.067 |
| Methoxychlor | 0.106 | 0.022 | 0.160 | 0.036 | 0.108 | 0.030 | 0.111 | 0.067 |
| **TOTAL** | 28.026 | 39.387 | 56.520 | 84.704 | 85.926 | 17.913 | 103.697 | 41.374 |

**Key:** Numbers with different superscripts are significantly different ($p<0.05$).

**Table 2**
Comparative analysis of OCPs in the liver of toads at the dumpsite and natural habitat (units in ppm).

| | DUMPSITE | | | | HABITAT | | | |
|---|---|---|---|---|---|---|---|---|
| | INFECTED | | UNINFECTED | | INFECTED | | UNINFECTED | |
| | MEAD | SD | MEAN | SD | MEAD | SD | MEAN | SD |
| alpha.-Lindane (a-BHC) | 0.028 | 0.014 | 0.054 | 0.054 | 0.030 | 0.012 | 0.022 | 0.009 |
| beta.-Lindane (b-BHC) | 0.018 | 0.012 | 0.039 | 0.011 | 0.021 | 0.011 | 0.034 | 0.010 |
| gamma.-Lindane (y-BHC) | 1.434 | 0.741 | 1.232 | 1.069 | 1.675 | 1.057 | 2.203 | 1.333 |
| delta.-Lindane (d-BHC) | 0.091 | 0.063 | 0.046 | 0.027 | 0.025 | 0.010 | 0.028 | 0.013 |
| Heptachlor | 0.186 | 0.061 | 0.246 | 0.068 | 0.273 | 0.093 | 0.155 | 0.014 |
| Aldrin | 5.933 | 7.087 | 0.259 | 0.150 | 1.364 | 3.001 | 2.495 | 3.839 |
| Heptachlor epoxide (Isomer B) | 0.041 | 0.038 | 0.012 | 0.010 | 0.004 | 0.005 | 0.004 | 0.004 |
| Endosulfan I (.alpha.-Endosulfan) | 0.280 | 0.201 | 2.667 | 1.741 | 3.097 | 5.178 | 2.058 | 2.418 |
| Dieldrin | **1.265[b]** | **0.944** | **1.344[b]** | **0.984** | **9.256[a]** | **10.978** | **7.223[a]** | **4.966** |
| p,p'-DDE (4,4'-DDE) | 0.180 | 0.191 | 0.064 | 0.056 | 0.038 | 0.030 | 0.027 | 0.025 |
| Endrin | **0.681[c]** | **0.183** | **0.994[c]** | **0.548** | **8.778[b]** | **12.869** | **59.364[a]** | **50.847** |
| Endosulfan II (.beta.-Endosulfan) | 0.128 | 0.119 | 0.204 | 0.110 | 0.221 | 0.046 | 0.402 | 0.264 |
| p,p'-DDD (4,4'-DDD) | 0.017 | 0.013 | 0.011 | 0.006 | 0.019 | 0.011 | 0.021 | 0.002 |
| p,p'-DDT (4,4'-DDT) | 0.076 | 0.150 | 0.056 | 0.055 | 0.017 | 0.007 | 0.016 | 0.003 |
| Endosulfan Sulfate | 0.110 | 0.056 | 0.024 | 0.013 | 0.059 | 0.021 | 0.040 | 0.038 |
| Methoxychlor | 0.183 | 0.076 | 0.129 | 0.060 | 0.127 | 0.054 | 0.290 | 0.050 |
| **TOTAL** | 10.651 | 7.996 | 7.381 | 1.835 | 25.004 | 23.426 | 74.382 | 48.489 |

**Key:** The numbers with different superscripts are significantly different ($p<0.01$).
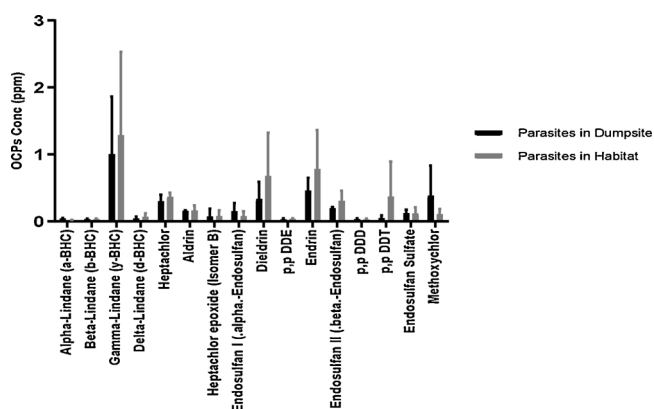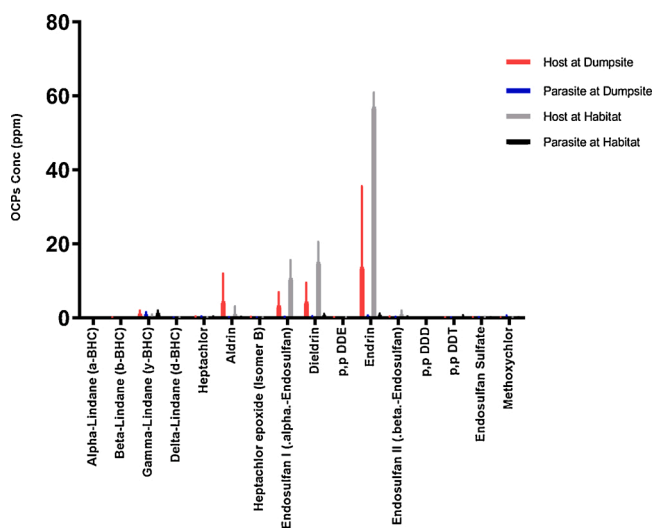


**Fig. 2.** Congeners in the parasites.



**Fig. 3.** Comparison between Congeners in the parasites and toads at the dumpsite and natural habitat.

**Table 3**
Comparison of OCPs in soil samples from the dumpsite and natural habitat.

| | DUMPSITE | | HABITAT | | |
|---|---|---|---|---|---|
| OCP Congeners | MEAN | SD | MEAN | SD | P-value |
| Alpha lindane (a-BHC) | 0.035 | 0.013 | 0.053 | 0.040 | P > 0.05 |
| Beta lindane (b-BHC) | 0.103 | 0.043 | 0.081 | 0.072 | P > 0.05 |
| Gamma lindane (y-BHC) | **5.850[a]** | 5.527 | 1.591[b] | 2.755 | P<0.001 |
| Delta lindane (d-BHC) | 0.105 | 0.078 | 0.040 | 0.027 | P > 0.05 |
| Heptachlor | 0.132 | 0.006 | 0.076 | 0.021 | P > 0.05 |
| Aldrin | 1.767 | 1.857 | 0.702 | 0.985 | P > 0.05 |
| Heptachlor epoxide (Isomer B) | 0.010 | 0.004 | 0.053 | 0.079 | P > 0.05 |
| Endosulfan I (.alpha.-Endosulfan) | 0.791 | 0.269 | 0.353 | 0.166 | P > 0.05 |
| Dieldrin | 0.310 | 0.147 | 0.217 | 0.029 | P > 0.05 |
| p,p'-DDE (4,4'-DDE) | 0.084 | 0.013 | 0.180 | 0.060 | P > 0.05 |
| Endrin | 1.527 | 0.718 | 1.450 | 0.860 | P > 0.05 |
| Endosulfan II (.beta.-Endosulfan) | 0.287 | 0.201 | 0.264 | 0.163 | P > 0.05 |
| p,p'-DDD (4,4'-DDD) | 0.040 | 0.030 | 0.032 | 0.016 | P > 0.05 |
| p,p'-DDT (4,4'-DDT) | 0.054 | 0.047 | 0.017 | 0.011 | P > 0.05 |
| Endosulfan Sulfate | 0.324 | 0.275 | 0.116 | 0.010 | P > 0.05 |
| Methoxychlor | 0.083 | 0.027 | 0.150 | 0.035 | P > 0.05 |

Emboldened figures are significant.

**Table 4**
Bioaccumulation Factors (BAF) of OCP congeners in the parasites at the dumpsite and natural habitat.

| OCP Congener | Dumpsite | Habitat |
|---|---|---|
| alpha.-Lindane (a-BHC) | **1.28** | 0.54 |
| beta.-Lindane (b-BHC) | 0.75 | **1.77** |
| gamma.-Lindane (y-BHC) | 0.83 | **4.03** |
| delta.-Lindane (d-BHC) | 0.64 | **1.74** |
| Heptachlor | **1.81** | **2.58** |
| Aldrin | 0.16 | 0.16 |
| Heptachlor epoxide (Isomer B) | 0.18 | **2.08** |
| Endosulfan I (.alpha.-Endosulfan) | 0.01 | 0.01 |
| Dieldrin | 0.01 | 0.01 |
| p,p'-DDE (4,4'-DDE) | 0.17 | 0.17 |
| Endrin | 0.01 | 0.01 |
| Endosulfan II (.beta.-Endosulfan) | 0.33 | **2.12** |
| p,p'-DDD (4,4'-DDD) | **1.83** | **1.81** |
| p,p'-DDT (4,4'-DDT) | **2.77** | **19.89** |
| Endosulfan Sulfate | 0.74 | 0.68 |
| Methoxychlor | **2.36** | 0.97 |

**Legend:** Emboldened figures indicate significant BAFs (> 1).

methoxychlor, the parasites at the natural habitats significantly bio-accumulated beta-lindane, gamma-lindane, delta-lindane, heptachlor, heptachlor epoxide, p,p'-DDD, and p,p'-DDT.

The entirety of bioaccumulation of OCP congeners from the environment into the liver and intestine of the infected and uninfected toads at the dumpsite and natural habitat showed notable bioaccumulation of the congeners in many instances (Table 5). The liver of the uninfected toad at the dumpsite significantly bioaccumulated (1.6) alpha lindane from the soil. Alpha lindane was also significantly bioaccumulated (1.5) by the uninfected toads at the soil's natural habitat into the intestine. Only the parasites at the dumpsite significantly bioaccumulated (1.5) beta lindane from the host's intestine. As for gamma lindane, the dumpsite parasites significantly bioaccumulated the chemical from the liver (1.4) and the intestine (1.1). Meanwhile, the parasites in the natural habitat significantly bioaccumulated the chemical from the liver (1.2) only. The livers of infected and uninfected toads at the natural habitat significantly bioaccumulated gamma lindane from the soil at BAFs of 1.1 and 1.4 respectively. The infected toads' parasites at the dumpsite significantly bioaccumulated delta lindane (2.3) from the liver. The intestines of the infected and uninfected toads at the natural habitat also significantly bioaccumulated delta lindane at 1.0 and 1.4 respectively. Heptachlor was significantly accumulated in the infected and uninfected toad liver (at 1.4 and 1.9 respectively) and infected and uninfected intestines (at 1.3 and 1.2 respectively) from the soil at the dumpsite. At the natural habitat, on the other hand, heptachlor was bioaccumulated in the liver of the infected (3.6) and uninfected (2.0) toads and infected intestine (1.9) from the soil.

At the dumpsite, aldrin was significantly bioaccumulated in the infected toad liver (3.4), infected intestine (2.5), and uninfected intestine (2.8) from the sediment. Furthermore, aldrin was notably accumulated in the parasites from the liver (38.7 folds) and intestine (29.2 folds) of the toads at the dumpsite. In the natural habitat, on the other hand, Aldrin was bioaccumulated in the infected toad liver (3.6), uninfected toad liver (2.0), and infected intestine (1.9) from the soil. Heptachlor epoxide was bioaccumulated significantly in the infected toad liver (4.2), uninfected toad liver (1.2), and infected intestine (7.3) in the sediment. Heptachlor epoxide was notably accumulated in the uninfected intestine (39.9 folds) from the soil at the dumpsite. The parasite accumulated heptachlor epoxide (1.0) from the intestine of the toad at the dumpsite. The uninfected intestine significantly accumulated heptachlor from the soil in the natural habitat.

From the soil at the dumpsite, endosulfan I was accumulated in the uninfected toad liver (3.4), infected intestine (4.3), and uninfected intestine (4.4). The parasites at the dumpsite significantly accumulated endosulfan I from the liver (1.9) and markedly accumulated from the intestine (22.4 folds). At the natural habitat, endosulfan I was accumulated in the infected toad liver (8.8), uninfected toad liver (5.8), infected intestine (30.6), uninfected intestine (5.3) from the soil, while the parasite at the location significantly accumulated endosulfan I from the liver of the toads.

Dieldrin was accumulated from the soil at the dumpsite in the infected and uninfected toad liver, markedly accumulated in the infected (14.1) and uninfected (87.1 folds) intestine of the toads. The parasites however accumulated the congener from the liver (3.8) and the intestine (13.1). The rate of accumulation of p,p'-DDE from the dumpsite soil into the tissues of the toad was outstripped by the accumulation of the congener into the parasites from the liver (7.7) and the intestine (4.9) of the infected toads at the dumpsite. Accumulation of the congener didn't occur at the natural habitat. Endrin was accumulated from the soil at the dumpsite in the infected intestine (9.0), and uninfected intestine (12.2), while the parasite accumulated the congener from the liver (1.5) and markedly from the intestine (29.9). It was also accumulated from the soil at the natural habitat in the infected toad liver (6.1), uninfected toad liver (40.9 folds), infected intestine (39.3 folds), and uninfected intestine (1.0). The parasites at the habitat accumulated endrin from the liver (5.9) and the intestine (1.9).

Endosulfan II was not accumulated in the tissue of the toad at the dumpsite. It was however accumulated by the parasite from the intestine of the toads at the location. At the habitat, on the other hand, endosulfan II was accumulated from the soil into the uninfected toad liver, infected intestine, and uninfected intestine. The OCP congeners that were accumulated in the tissues of the toad without a further accumulation in the parasites include p,p'-DDT, endosulfan sulfate, and methoxychlor.

### 3.5. Histopathology of toad tissues

The photomicrographs of intestinal tissues of infected toads at the dumpsite (Fig. 4A–D) show mild stunting of villi (thin arrow) and the presence of detritus in the lumen of the intestine (thick arrow). While the intestinal tissues of the uninfected toads (Fig. 4E–H) show a focal area of stunting of villi (thin arrow).

Photomicrographs of infected intestinal tissue of toads at the natural habitat (Fig. 5A–D) show disseminated severe stunting of villi (thin arrow), total loss of intestinal glands, and presence of detritus within the

**Table 5**
Bioaccumulation factors of biological samples at the dumpsite and natural habitat.

| OCP Congeners | Dumpsite | | | | | | Habitat | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ILS | ULS | IIS | UIS | PL | PI | ILS | ULS | IIS | UIS | PL | PI |
| Alpha lindane (a-BHC) | 0.8 | **1.6** | 0.7 | 0.8 | 0.7 | 0.6 | 0.6 | 0.4 | 0.6 | **1.5** | 0.0 | 0.1 |
| Beta lindane (b-BHC) | 0.2 | 0.4 | 0.4 | 0.3 | 0.6 | **1.5** | 0.3 | 0.4 | 0.2 | 0.5 | 0.0 | 0.0 |
| Gamma lindane (y-BHC) | 0.2 | 0.2 | 0.2 | 0.2 | **1.4** | **1.1** | **1.1** | **1.4** | 0.2 | 0.1 | **1.2** | 0.3 |
| Delta lindane (d-BHC) | 0.9 | 0.4 | 0.2 | 0.6 | **2.3** | 0.4 | 0.6 | 0.7 | **1.0** | **1.4** | 0.0 | 0.1 |
| Heptachlor | **1.4** | **1.9** | **1.3** | **1.2** | 0.6 | 0.6 | **3.6** | **2.0** | **1.9** | 0.9 | 0.4 | 0.2 |
| Aldrin | **3.4** | 0.1 | **2.5** | **2.8** | **38.7** | **29.2** | **1.9** | **3.6** | **1.5** | 0.4 | 0.0 | 0.0 |
| Heptachlor epoxide (Isomer B) | **4.2** | **1.2** | **7.3** | **39.9** | 0.6 | **1.0** | 0.1 | 0.1 | 0.7 | **8.4** | 0.0 | 0.0 |
| Endosulfan I (.alpha.-Endosulfan) | 0.4 | **3.4** | **4.3** | **4.4** | **1.9** | **22.4** | **8.8** | **5.8** | **30.6** | **5.3** | **1.7** | 0.5 |
| Dieldrin | **4.1** | **4.3** | **14.1** | **87.1** | **3.8** | **13.1** | **42.7** | **33.3** | **70.0** | **2.1** | **2.4** | **1.2** |
| p,p'-DDE (4,4'-DDE) | **2.1** | 0.8 | **1.4** | **1.8** | **7.7** | **4.9** | 0.2 | 0.2 | 0.5 | 3.4 | 0.0 | 0.0 |
| Endrin | 0.4 | 0.7 | **9.0** | **12.2** | **1.5** | **29.9** | **6.1** | **40.9** | **39.3** | **1.0** | **5.9** | **1.9** |
| Endosulfan II (.beta.-Endosulfan) | 0.4 | 0.7 | 0.8 | 0.3 | 0.7 | **1.2** | 0.8 | **1.5** | **3.4** | **2.3** | 0.3 | 0.8 |
| p,p'-DDD (4,4'-DDD) | 0.4 | 0.3 | 0.3 | 0.3 | 0.6 | 0.4 | 0.6 | 0.7 | 0.4 | 0.6 | 0.0 | 0.0 |
| p,p'-DDT (4,4'-DDT) | **1.4** | **1.1** | 0.6 | 0.3 | **1.6** | 0.7 | **1.0** | 0.9 | **1.1** | **1.1** | 0.0 | 0.0 |
| Endosulfan Sulfate | 0.3 | 0.1 | 0.3 | 0.3 | 0.9 | 0.8 | 0.5 | 0.3 | **1.3** | **3.8** | 0.1 | 0.2 |
| Methoxychlor | **2.2** | **1.6** | **1.3** | **2.0** | 0.5 | 0.3 | 0.8 | **1.9** | 0.7 | 0.4 | 0.3 | 0.4 |
| **TOTAL SIG BAF** | **18.8** | **15.1** | **41.2** | **151.4** | **58.9** | **104.3** | **65.2** | **90.4** | **150.1** | **26.9** | **11.2** | **3.1** |

**Keys:** Emboldened figures represent significant bioaccumulation factors (BAF). ILS = liver/sediment BAF of infected toad, ULS = liver/sediment BAF of uninfected toad, IIS = intestine/sediment BAF of infected toad, UIS = intestine/sediment BAF of uninfected toad, PL = parasite/ liver BAF of infected toad, PI = parasite/ intestine BAF of infected toad.
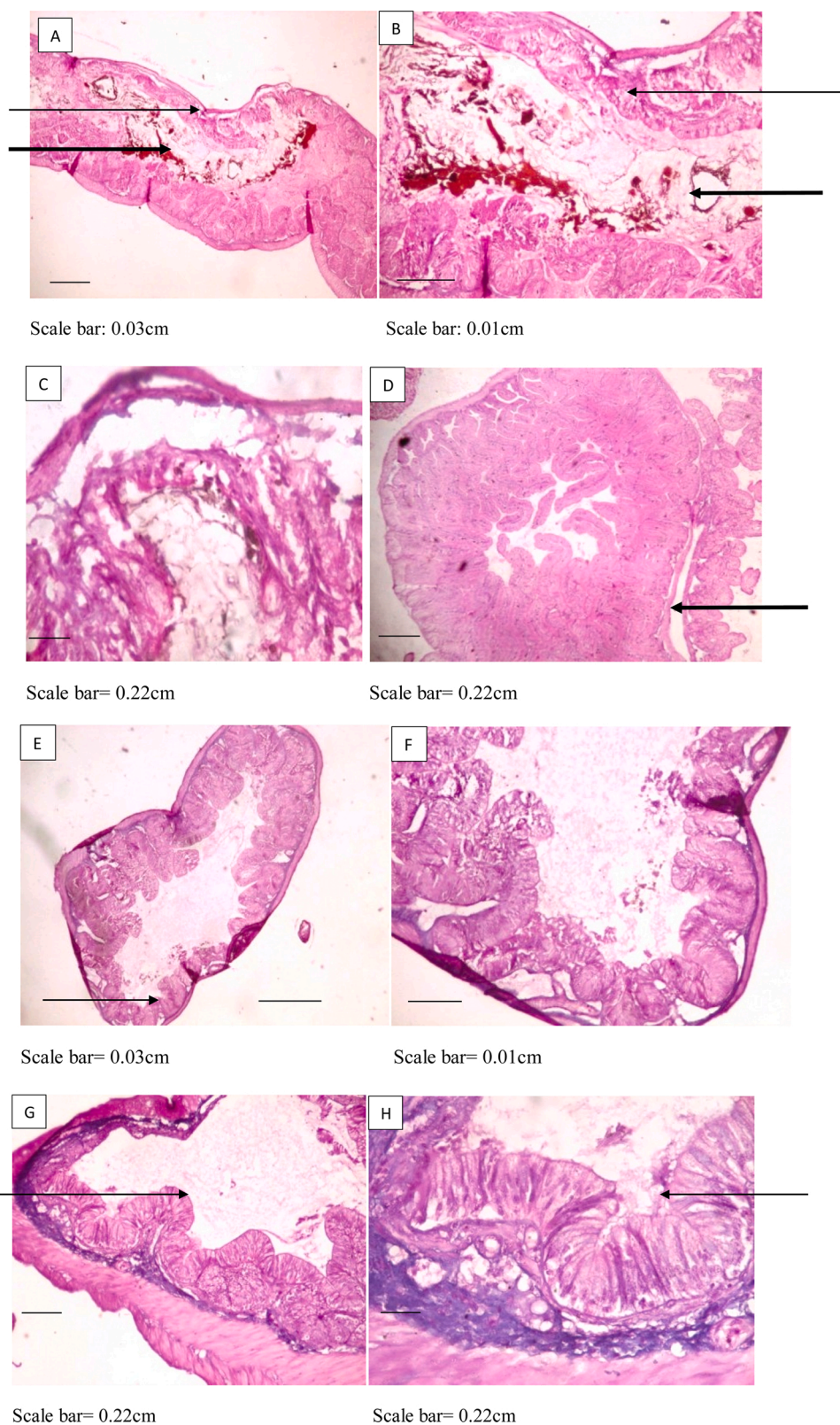
**Fig. 4.** Photomicrographs of intestinal tissues of infected (A-D) and uninfected (E-H) toads at the dumpsite.

lumen (black arrow). While the photomicrographs of the uninfected intestinal tissues at the natural habitat (Fig. 5E–H) show normal villi structure, normal mucosa, submucosa, and muscularis. The normal crypt-villous architecture is well preserved. However, there are lymphoid follicles within the villous structure (thin arrow).

## 4. Discussions

The dumpsites at the locations sampled within Lagos metropolis may have been contaminated with aldrin as the concentrations of the chemical were significantly higher in the toads at the dumpsite than the
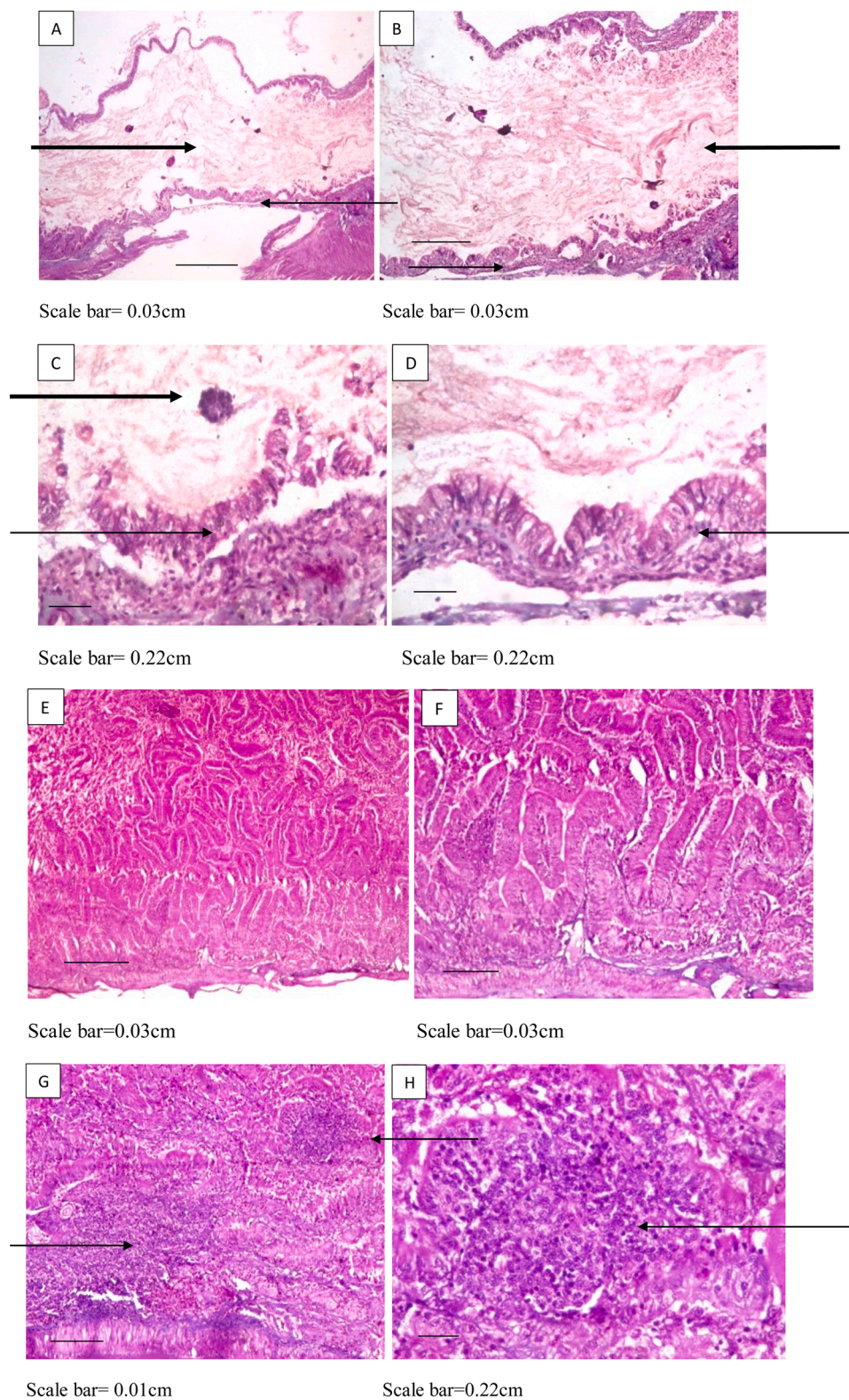
**Fig. 5.** Photomicrographs of intestinal tissues of infected (A-D) and uninfected (E-H) toads at the natural habitat.

natural habitat regardless of the tissues and infection status. Also, the fact that eldrin and dieldrin which are sister congeners were detected at significantly higher concentrations in the natural habitat shows that there might have been a common source to both of them; which is the application of agrochemicals. It is expected that the toads at dumpsites should have a higher concentration of these xenobiotics. But the output of this research exposed the fact that the areas labeled "natural habitat"

had been impacted by anthropogenic activities even greater than the areas labeled "dumpsites." The major sources of these OCPs will be the use of insecticide cans and some other random roundup herbicides and related chemicals. Natural habitat is also exposed to myriads of anthropogenic activities ranging from agricultural through municipal perturbations, cutting across industrial activities. Ojota and Ikorodu, two of the study sites, are areas noted for lots of anthropogenic activities

because Lagos state is densely populated and the larger part of the population is contributed by these study areas. The excess agrochemicals applied within these catchment areas are bound to run off to nearby waterbody depending on the drain of the river basin of each location. It is therefore expected to find concentrations of these agrochemicals which contain eldrin and dieldrin around the locality of our study sites. This is so because many governments do not follow the promulgation of consensus laws that has been established by global environmental bodies. Polices are not enforced down to the grassroots on the use of these agrochemicals and roundup chemicals.

The concentrations of dieldrin in uninfected intestines of the toads at the dumpsite and natural habitat being significantly higher than the concentrations in the infected intestines indicates that the parasite *Cosmocerca* sp. may have played a depurative role in sequestering the concentration of dieldrin in the toads irrespective of the location.

The paucity of information on the bioaccumulation of OCPs by *Cosmocerca* sp. occasioned and informed the design of this study. The outcome of this investigation uniquely unravels the suspicion that *Cosmocerca* sp. might be a good environmental tool for biosequestration of eldrin and dieldrin from the host toad. We hereby suggest further research to investigate the actual capacity of *Cosmocerca* sp. in bioaccumulation of OCP to a significant level that can be of ecotoxicological relevance.

The toads may have been exposed to toxic levels of these chemicals but the parasite demonstrates a novel niche in protecting the host in this regard. This may explain the notably higher concentration of endrin in the liver of the uninfected toads at the natural habitat, as the absence of the parasite may have denied the toad the depurative service of the parasite. Further study is however required to explore the factors that underlie the effectiveness of *Cosmocerca* sp. in the sequestration of the researched chemicals in amphibians. This may contribute effectively towards the amelioration of the chemicals in the environment for the protection of biota and human health.

Gama-lindane, heptachlor, dieldrin, endrin, endosulfan-2, and pp-DDT in the parasite at the habitat were higher than the concentration in the parasite at the dumpsite. The only outstanding trend by parasites at the dumpsite was seen with methoxychlor (Fig. 2). This partly explains the higher bioaccumulation of more OCP congeners by the parasites at the natural habitat than those at the dumpsite. Of notably outstanding bioaccumulation is p,p'-DDT in parasites at the natural habitat. The order of bioaccumulation of the OCP congeners among the biological media was host at habitat > host at dumpsite > parasite at habitat > parasite at the dumpsite.

In the liver of the toads at the dumpsite, the parasites sequestered the OCP congeners in the order of aldrin > pp'-DDE > dieldrin > delta lindane > endosulfan I > pp'-DDT > endrin > gamma lindane. While in the intestine the parasite sequestration was in the order of endrin > aldrin > endosulfan I > dieldrin > pp'-DDE > beta lindane > endosulfan II > gamma lindane > heptachlor epoxide. The sequestration capacity of the parasites on these congeners outstripped the bioaccumulation capacities of the liver and intestine at the dumpsite. At the dumpsite, methoxychlor and pp'-DDT were the only congeners that were significantly bioaccumulated from the soil which were not sequestered by the parasite. The parasites exhibited marked sequestration capacity characterized by the notably high total bioaccumulation rate both in the liver and the intestine at the dumpsite.

In the natural habitat, on the other hand, the parasites sparingly sequestered the OCPs in the liver in the order of dieldrin > endosulfan I > gamma lindane. While in the intestine the order was endrin > dieldrin.

Conversely, in the natural habitat, the depurative capacity of the parasite was surpassed by the bioaccumulation tendencies of the intestine and liver of the toad. This observation corroborates the data presented on the total significant bioaccumulation factors of the parasites at the dumpsite which were 58.9 (liver) and 104.3 (intestine) at the dumpsite, as against 11.2 (liver) and 3.1 (intestine) at the natural

habitat.

A comparison of the soil samples at the dumpsite and the natural habitat showed that only gamma lindane had a significantly higher concentration at the former than the latter. This implies that the contamination levels at both locations are not statistically different for the other OCP congeners.

The comparative histopathology of the toads from the dumpsite and the natural habitat demonstrated the impacts of the parasite *Cosmocerca* sp. on the intestines of the toads. The infected intestine of toads at the dumpsite and natural habitat both showed stunted villi. Furthermore, the infected intestine at the dumpsite also showed the presence of detritus while that of the natural habitat showed total loss of intestinal glands and the presence of detritus within the lumen. As for the intestinal tissues of the uninfected toads in the natural habitat, no alteration occurred. Conversely, at the dumpsite, some evidence of stunted villi was persistent in the uninfected intestine. The stunted villi being the common histological alteration in the infected and uninfected toads at the dumpsite but missing in the uninfected counterparts at the natural habitat may be attributed to the differences in the background concentration of the OCP congeners [24]. The observed stunted villi in the uninfected toads at the dumpsite may have been mediated by gamma lindane which was the only contaminant with a significantly higher concentration in the soil at the dumpsite than the natural habitat. Grabarczyk et al. [25] earlier observed focal degeneration of tissues and cell structures in the liver and kidney of rabbits. Ezemonye and Ogomida [26] demonstrated histopathological changes of the gill, liver, and intestinal tissues of *Clarias gariepinus* treated with sublethal concentrations of gammalin 20 for twelve weeks. The experiment showed gill distortion and fusion of adjacent secondary lamella as a result of hyperplasia. The liver showed swelling of hepatocytes with mild necrosis, pyknosis, and vacuolation, while the intestine showed yellow bodies of the lamina propria at the tip of the mucosal fold. This study shows that gamma lindane may be the most devastating OCP congener to the toads in the sampled habitat.

Studies have investigated the reproductive/gonadotoxicity, embryotoxicity, and teratogenicity of lindane in tests covering all aspects of reproduction using animals such as a mouse, rat, dog, and pig. In a study by IPCS [27], rats were exposed through inhalation to lindane for 6 h/day for over 3 months. At a 100 mg/kg diet, an increase in liver weight, hepatocellular hypertrophy, fatty degeneration, and necrosis were observed. At a 50 mg/kg diet, hepatocellular hypertrophy and fatty degeneration were observed, with a sharp increase in hepatic cytochrome P-450 values occurring at the highest concentration of the chemical. Moreso, fetotoxic and/or maternal toxic effects were observed after administration of lindane at 10 mg/kg body weight and 5 mg/kg body weight was observed as the no-observed-effect level [28].

Scientific knowledge has been established that lindane is not readily decomposed chemically or biologically and is rather persistent [29]. Insecticides cans should therefore be burned in a proper incinerator designed for organochlorine waste disposal. In cases where this is not possible, efforts should be made to bury the materials in an approved dump or landfill [30] where there is no risk of contamination of surface or groundwater [31]. The most important recommended guide to the handling of lindane is total compliance with the local legislation regarding the disposal of toxic wastes.

## 5. Conclusion

The parasite- *Cosmocerca* sp. has been shown in this study as a possible bioindicator and bioaccumulator of eldrin and dieldrin demonstrating a novel niche in protecting the host. Previous studies which have comparatively analyzed the biosequestration potentials of other parasites in lower vertebrates such as fishes have achieved positive observations. But it is expected that going higher the evolutionary trend, stepping up to the amphibians, *Amietophrynus regularis* should be of a greater task to the minute parasite, that is, the biosequestration task on

the parasite should be higher. For *Cosmocerca* sp. to show these significant potentials is noteworthy because the host involved is a bigger and higher vertebrate. This, therefore, deserves further investigation to determine the actual capacity of this parasite in significantly bioaccumulating these xenobiotics. We would also further suggest the determination of the unique gene that codes for the bioaccumulation of these chemicals to investigate the gene in other parasites.

With the high concentration of dieldrin and eldrin recorded in the tissues of *Amietophrynus regularis*, and with lindane in the soil sampled, established regulatory bodies need to be sensitized that developing nations still suffer some unregulated activities that need better regulation in terms of monitoring of these already banned chemicals which are still being traced in the environment. Continuous research is a searchlight to checkmate the environment to see how compliant industries and the consumers are in terms of regulation of these chemicals. These recommendations are promising in safeguarding the high population density of Lagos, Nigeria.

### Ethics approval and consent to participate

Ethical approval for this research was given by the Health Research Ethics committee of the College of Medicine of the University of Lagos. CMULHREC No: CMUL/ACUREC/03/20/729.

### Consent for publication

Not applicable.

### Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of interest

The authors declare no conflict of interest.

### CRediT authorship contribution statement

**Akinsanya Bamidele**, **Patrick Omoregie Isibor**, and **Okeagu Martin Okechukwu:** Conceived and designed the experiments. **Okeagu Martin Okechukwu**, **Daniel-Rugu Josephine**, and **Babangida Yalwaji** performed the experiments. **Patrick Omoregie Isibor:** Analyzed and interpreted the data. **Onadeko A. Benedict** and **Khalid Adekoya:** Contributed reagents, materials, analysis tools, and ideas. All authors have read and approved the manuscripts.

### Declaration of Competing Interest

The authors report no declarations of interest.

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