Bioaccumulation of Organochlorine Pesticides, *Procamallanus sp.* (Baylis, 1923) infections, and Microbial Colonization in African Snakehead fish Sampled from Lekki Lagoon, Lagos, Nigeria

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Abstract

The exponential rise in the Nigerian population has necessitated the use of agrochemicals for enhanced agricultural yields to meet the ever-rising demand for food. However, agrochemicals such as organochlorine pesticides (OCPs) have caused several devastating health and ecological challenges. The study was therefore aimed at assessing the bioaccumulation of OCPs and the associated parasitological and microbial susceptibility in *P. obscura* to determine the possible ecological impacts of the chemical. A total of 106 specimens of *Parachanna obscura* fish species were sampled between July and November 2019 from Lekki Lagoon in Lagos, Nigeria. Four culture media, namely nutrient agar (NA), MacConkay agar (MCA), eosin methylene blue (EMB), and sabouraud dextrose agar (SDA) were employed in microbial culture. These microbes were subjected to ceftazidime, ceftriaxone, cefuroxime, gentamicin, ofloxacin, augmentin, nitrofurantoin, ciprofloxacin, and erythromycin to test for resistance, susceptibility and intermediate statuses before and after curing. OCPs were tested in the water, sediment, and tissues of *P. obscura* using gas chromatography flame ionization detector (GC-FID). *P. obscura* sampled in the lagoon had poor growth exponent which was characterized by negative allometry (slenderness) in the sampled fish. Although the incidence of parasitic infection in the fish was not alarming, the situation might be aggravated if the prevalent anthropogenic activities persist, resulting in immunosuppression. Regulation of anthropogenic activities in the catchment area is recommended to forestall the prognosis of health and environmental hazards associated with the agricultural, industrial, pharmaceutical, and municipal activities around the lagoon. Bacteria that conferred the most resistance to the majority of the antibiotics were *Staphylococcus* sp*., Micrococcus* sp. *Escherichia coli* and *Klebsiella* sp., testing positive to plasmid profile. They conferred high resistance to the antibiotics before plasmid curing but became highly susceptible post- plasmid curing. This implies that the gene for resistance in the bacteria isolates was plasmid-mediated, that is, they were obtained from the environment. In the event of an outbreak of waterborne diseases such as cholera, typhoid, dysentery, and diarrhea, there may be non-response to treatment among the infected inhabitants. The incidence of antibiotic resistance in bacteria colonies recorded in this study is of great public health concern, given the possibility of the antibiotic-resistant bacteria strains being passed to humans through fish consumption, resulting in increased multi-drug resistance in humans. Regulation of anthropogenic activities around the lagoon is recommended to forestall prognosis of health and environmental hazards associated with OCPs from agricultural, industrial, pharmaceutical, and municipal sources.

Keywords: bioaccumulation, parasite intensity, antibiotic susceptibility, plasmid, growth exponent.

Bioacumulação de pesticidas organoclorados, infecções por Procamallanus sp. (Baylis, 1923) e colonização microbiana em peixes africanos Snakehead coletados na lagoa Lekki, Lagos, Nigéria

Resumo

O aumento exponencial da população nigeriana exigiu o uso de agroquímicos para aumentar a produção agrícola e, assim, atender à crescente demanda por alimentos. No entanto, agroquímicos como pesticidas organoclorados (OCPs) causaram vários problemas de saúde e ecológicos. Portanto, o estudo teve como objetivo avaliar a bioacumulação de OCPs e a suscetibilidade parasitológica e microbiana associada em Parachanna obscura, a fim de determinar os possíveis impactos ecológicos desse produto químico. Foi amostrado um total de 106 espécimes de P. obscura entre

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julho e novembro de 2019 da lagoa Lekki, em Lagos, Nigéria. Quatro meios de cultura, como o ágar nutritivo (NA), o ágar MacConkay (MCA), o ágar eosina azul de metileno (EMB) e o ágar sabouraud dextrose (SDA), foram empregados na cultura microbiana. Esses micróbios foram submetidos a ceftazidima, ceftriaxona, cefuroxima, gentamicina, ofloxacina, augmentin, nitrofurantoína, ciprofloxacina e eritromicina para testar resistência, suscetibilidade e status intermediário antes e depois da cura. Os OCPs foram testados na água, sedimentos e tecidos de P. obscura usando um detector de ionização de chama por cromatografia em fase gasosa (GC-FID). Os peixes amostrados de P. obscura da lagoa apresentaram um expoente de crescimento ruim, caracterizado por alometria negativa (esbelteza). Embora a incidência de infecção parasitária nos peixes não tenha sido alarmante, a situação pode ser agravada se as atividades antropogênicas prevalecentes persistirem, resultando em imunossupressão. Recomenda-se a regulamentação de atividades antropogênicas na área de captação para prevenir o prognóstico de riscos à saúde e ecológicos associados a atividades agrícolas, industriais, farmacêuticas e municipais ao redor da lagoa. As bactérias que conferiram maior resistência à maioria dos antibióticos foram Staphylococcus sp., Micrococcus sp., Escherichia coli e Klebsiella sp., com teste positivo para o perfil plasmidial. Elas conferiram alta resistência aos antibióticos antes da cura do plasmídeo, mas se tornaram altamente suscetíveis após a cura dele. Isso implica que o gene de resistência nos isolados de bactérias foi mediado por plasmídeo, ou seja, foi obtido do ambiente. No caso de surtos de doenças transmitidas pela água, como cólera, febre tifoide, disenteria e diarreia, pode haver não resposta ao tratamento entre os habitantes infectados. A incidência de resistência a antibióticos nas colônias de bactérias registradas neste estudo é de grande preocupação para a saúde pública, dada a possibilidade de que as cepas de bactérias resistentes a antibióticos sejam transmitidas aos seres humanos por meio do consumo de peixes, resultando em maior resistência a múltiplas drogas em seres humanos. Recomenda-se a regulamentação de atividades antropogênicas ao redor da lagoa para impedir o prognóstico de riscos à saúde e ecológicos associados aos OCPs de fontes agrícolas, industriais, farmacêuticas e municipais.

Palavras-chave: bioacumulação, intensidade do parasita, suscetibilidade a antibióticos, plasmídeo, expoente de crescimento.

1. Introduction

The exponential increase in the Nigerian population has necessitated the use of agrochemicals for enhanced agricultural yields to meet the ever-rising demand for food. However, agrochemicals such as organochlorine pesticides (OCPs) have caused several devastating health and ecological challenges. The industries that produce the chemicals also contribute immensely to its release into the environment.

According to Pimentel (1995) only a small percentage about 0.3% of applied pesticides affect the target organism while about 99.7% remains as a residue in the environment, affecting non-target organisms, especially aquatic organisms as the aquatic ecosystem serves as a repository for contaminants. Organochlorine pesticides (OCPs) and polychlorinated biphenyl (PCBs) are stable chemicals, with a notable affinity for soil, particulates, and lipid tissues. These properties underlie their persistence in the environment (Ravindran et al., 2016). Studies have shown that these chemicals either stimulate or suppress the immune system of fish (Akinsanya et al., 2019) and all components of the immune system may be affected, thereby increasing their susceptibility to parasitic infections and microbial load.

According to Sofoluwe et al. (2013), an estimated 125, 000–130, 000 metric tonnes of pesticides are applied yearly in Nigeria. Alani et al. (2013) reported high levels of organochlorine pesticides (OCPs) and other persistent, bio-accumulative, and toxic (PBT) organic micro-pollutants in the soil samples of Iddo and Okobaba areas of Lagos, Nigeria. Iyamu et al. (2007) studied the concentrations of OCP residues in water and fish from some rivers in Edo State, Nigeria. They detected some notable residues of lindane, aldrin, p,p-DDE, o,p-DDD, p,p-DDD, o,p-DDT, and p,p-DDT in the water, and even higher concentrations were detected in the fish of most rivers investigated. Iyamu et al. (2007) attributed the marked bioaccumulation of OCPs in the fatty fish to the lipophilicity of the compound.

Impacts of OCPs on the biotic and abiotic components of Lekki lagoon and other linking aquatic ecosystems have been previously reported (Alani et al., 2013; Sofoluwe et al., 2013; Akinsanya et al., 2019). Several organochlorine-based anthropogenic activities abound within the vicinity of the lagoon. Such activities include the predominant use of agrochemicals, round-up herbicides, synthetic fertilizers in farmlands, and wide application of pesticides within the catchment areas (Akinsanya et al., 2007; Ayejuyo et al., 2008). Akinsanya, et al. (2015) reported 18 organochlorine congeners, namely: 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.

The characteristics of pesticides, which include high lipophilicity, bioaccumulation, the long half-life, and the potential of long-range transport, have increased the chances of air, water, and soil contamination for extended periods. Acute toxicity of OCPs may result in death depending on the immune vulnerability of the exposed biota. On the other hand, chronic toxicity may result in cancer, brain damage in children, compromised cognitive performance, nephrotoxicity, and congenital deformities (Ayejuyo et al.,

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2008; Sofoluwe et al., 2013; Akinsanya et al., 2019). It may adversely affect aquatic lives, especially fishes, resulting in reduced population and species diversity.

Parachanna obscura (Gunther, 1861), commonly referred to as the African snakehead fish, is highly sought for its hardness, large size, and unique taste. Its high commercial value may be a key driver in the over-exploitation of the wild stock in Lekki lagoon (Akinsanya et al., 2010; Kpogue et al., 2013). The fish is a benthopelagic freshwater Channidae (Riede 2004; Bamidele et al., 2018), hence interacts with the bottom sediment which is a potential reservoir for the OCPs. *P. obscura* is however a notable voracious carnivore, which feeds on other lower fishes, aquatic insects, and benthic invertebrate fauna (Reed et al., 1967; Bonou and Teugels, 1985; Idodo-Umeh, 2003; Paugy and Leveque, 2006). Its piscivorous and insectivorous feeding habits puts it in the vulnerable ecotoxicological position in the food web as the ultimate receptor of the OCP residues in the various linked food chains. Being linked to multi-bioaccumulation channels (Kalfakakour and Akrida-Demertzi, 2000), the fish may have accumulated impressible amount of OCPs that may be unhealthy for the consumers.

Furthermore, as a result of exposure to OCPs in the environment, fish may undergo immunosuppression which may foster susceptibility to parasites, and extreme cases of parasite prevalence may in turn influence internal and external microbiota. Hence, the parasite and microbial load on the fish may serve as a reliable supportive tool with bioaccumulation indices in the determination of the ecotoxicological implications of OCPs in the lagoon. There may be possible synergistic, antagonistic or supra-addictive interactions among these key playing factors which may ultimately determine the health and palatability of the fish (Akinsanya et al., 2010; Odo, 2012; Kuton et al., 2015; Oden et al., 2015; Ravindran et al., 2016; Olanrewaju et al., 2017; Oyeyiola et al., 2017).

The current study was aimed at assessing the bioaccumulation of OCPs and the associated parasitological and microbial susceptibility in *P. obscura* to determine the possible ecological impacts of the chemical. This study is significant for informed policy on the management of the aquatic habitat and the protection of the consumers.

2. Materials and Methods

2.1. The study area

The study was carried out on Lekki Lagoon (4°00ᴵ 4°15ᴵ E and latitude 6°25ᴵ and 6°37ᴵ N) in Lagos, Nigeria (Figure 1). The lagoon has a surface area of 247 km² and an average depth of 64m (Akinsanya et al., 2019). It is a part of interconnected wetlands that consists creeks and lagoon along the Southwestern coast of Nigeria from the Dahomey border to the Niger Deltas, stretching over a distance of about 200 km, it is bordered by the Epe lagoon (freshwater) on the East and the Lagos Lagoon
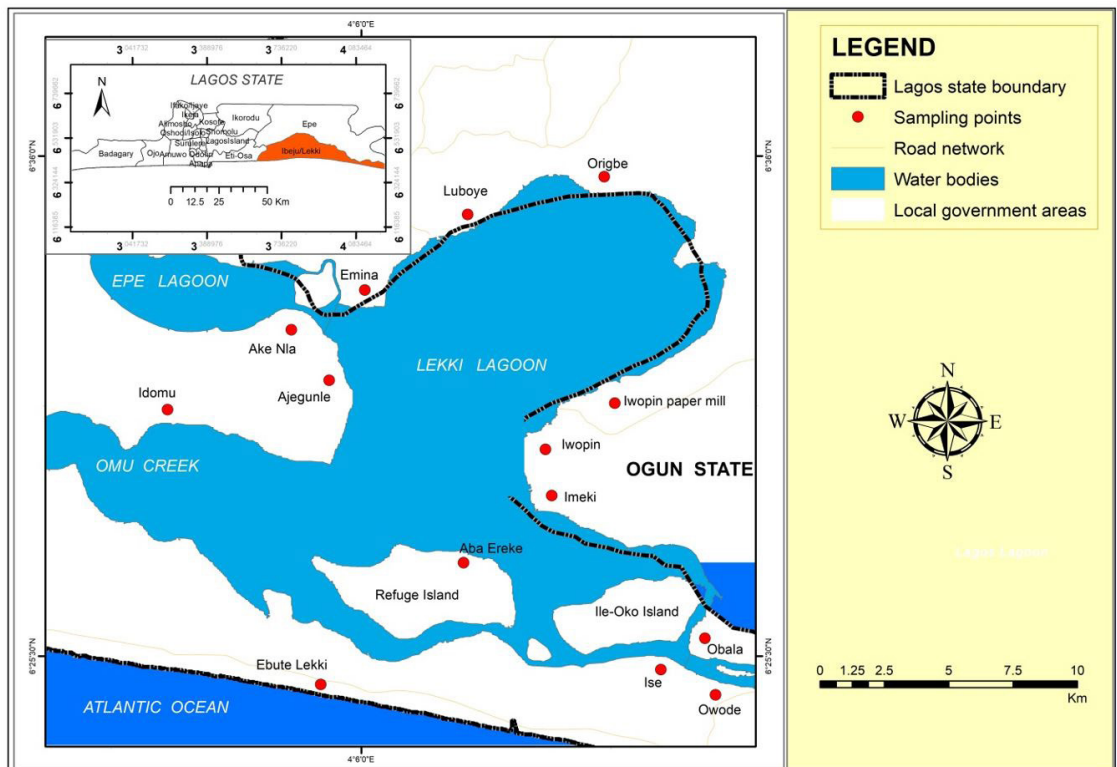

Figure 1. Map showing the study area.

(brackish water) on the West (Akinsanya et al., 2019). It is surrounded by rural settlements inhabited by farmers, fishermen, miners, and transporters of people and goods using motorized boats.

The seasons are dry and rainy seasons, typical of the Southern part of Nigeria. The predominant vegetation is characterized by shrubs and raphia palms e.g. *Raphia sudanica* and oil palms *Elaeis guineensis,* coconut palms *Cocos nucifera*, and mainly water hyacinth (*Eichhornia crassipes*) which is a great indicator of pollution (Nwankwo and Onitiri, 1992; Akinsanya et al., 2007; Lawal et al., 2010).

The lagoon supports major fishery in Nigeria and traverses from the River Oni, Northeast, and Rivers Oshun and Saga, Northwest.

2.2. Water and sediment sampling

The surface water sample of the study area was collected in sterile amber bottles, preserved in an iced chest cooler, and transportation to the laboratory for analysis to determine the concentration of OCPs. Sediment samples were with the aid of a Van Veen grab sampler and stored in sterile aluminum foil paper, labeled accordingly, and transported to the laboratory for analysis.

2.3. Collection of fish and parasite samples

A total of 106 specimens of *Parachanna obscura* fish species were sourced from the lagoon between July and November 2019.

The sex of the fish was determined by visual identification upon the dissection of the fish. Sexual identification was conducted based on the presence of eggs in females and the presence of testes in the males.

The fish specimens were carefully dissected and the intestine and the liver were excised. The intestines were divided into two parts; one part was preserved and stored in labeled EDTA (Ethylenediaminetetraacetic acid) sample bottles with saline while the other part was preserved and stored in Bouin's fluid before further analysis.

The liver the fish was aseptically extracted with the aid of scissors and forceps, ensuring the liver is not crushed in the process and then stored in a labeled EDTA bottle for analysis.

The intestines were placed in labeled Petri dishes filled with normal saline for parasite recovery. They were carefully split open longitudinally from the anterior to the posterior end using a sterile blade to aid gastrointestinal helminth parasites emergence using sterile blades. The parasites collected were identified as *Procamallanus sp.* (Baylis, 1923).

2.3.1. Parasite collection and histopathology of fish intestine

The recovered parasites were counted, recorded, and released in sampling bottles containing saline solution. Identification of specimens to species level was undertaken at the pathology laboratory of the department of veterinary pathology, University of Ibadan, Nigeria.

The number of parasites recovered from each fish was recorded and preserved in saline solution for analysis.

Parasite prevalence (Equation 1), abundance (Equation 2), and mean intensity (Equation 3) were calculated as follows;

$$Parasite\ Prevalence = \frac{Number\ Infected}{Number\ Examined} \times 100 \tag{1}$$

(Saliu et al., 2014)

$$Parasite\ Abundance = \frac{Number\ of\ Parasites}{Number\ Examined} \tag{2}$$

(Ezewanji et al., 2005)

$$Mean\ Intensity = \frac{Number\ of\ Parasites}{Number\ of\ Infested\ Host} \tag{3}$$

(Ezewanji et al., 2005)

The intestinal tissues were placed in bottles containing Bruins fluid for 6 h, after when it was decanted and 10% buffered formalin was added to preserve the tissue (Akinsanya et al., 2020). Then, the tissues were dehydrated in an ascending percentage of alcohol in the order of 1%, 3%, 5%, 8%, 11%, and 13% at 30 min interval. The dehydrated tissue was then embedded in molten paraffin wax and left to solidify. The compact tissue was sectioned at 4-5 microns processed and stained with hematoxylin and eosin (H&E). Afterward, washed off in distilled water and the overstained was destained in 1% alcohol (Akinsanya. et al., 2015). The tissue was then dried and mounted on a glass slide using DPX mountant. Coverslip was mounted over the sectioned tissue. The photomicrograph was taken with the aid of a Camera (INFINITY, 3-3URC 4.54x 4.54 μm) using a binocular dissecting microscope (American Optical Corporation, Model 570).

2.3.2. Analysis of OCPs in environmental matrices

100 mL water sample was measured into 250 mL separatory funnel and extracted 3 times with 20 mL of methylene chloride, approx. 60 mL of final extracting solvent. The mixture was centrifuged at 2500 rpm for 2 min, periodically venting the funnel to release excess pressure. The organic layer was allowed to precipitate from the water phase for 10 min. The organic layer was then decanted into a clean beaker in which it was concentrated to 2 mL using a rotatory evaporator.

Sediment samples were dried at room condition and grinned with mortar. Sediment, liver/ intestine/ pooled parasite samples (2 g) were weighed in an amber glass bottle with Teflon cap and 100μL of 1.0μg/L surrogate standards (2, 4, 5, 6 Tetrachloro-m-xylene and Decachlorobiphenyl) were added. 60 mL of hexane: acetone mixture in ratio 3:1 was added to the bottle and closed for ultrasonic bath extraction for 2 h. At this stage, the analysis of water samples joined the subsequent procedures. The extracts were firstly eluted through Na_2SO_4 column to eliminate the water moisture and subsequently cleaned with the florist column to eliminate the polar compounds that interfere with the analytes. The mixture was evaporated with a rotary evaporator and 100 μL internal standard (pentachloronitrobenzene) having a concentration of 0.5μg/L was added. The final

solution was pre-concentrated to 1 mL with a minivan evaporator under pure nitrogen gas and taken into a 2 mL amber glass vial and kept in a refrigerator at 4 °C before the analysis using Agilent 7890B gas chromatography coupled to flame ionization detector (GC-FID).

Surrogate standards, internal standards, and standard reference materials were used for quality assurance/quality control studies. An HP (Hewlett Packard) 6890 series gas chromatography coupled with HP 5973 mass spectrometer was used for the analysis. A 30m, 0.32mm id., 0.25mm film thickness, cross-linked 5% Phenylmethylsiloxane, HP 5MS, capillary column (Agilent Tech.) was applied for the separation of OCPs throughout the study.

2.3.3. Fish morphometrics

Morphological parameters of the fish which include the total standard length (cm) and weight (g) were read with the use of a meter rule (cm) and a digital electronic weighing balance (Camry EK5055) respectively.

The standard length was obtained from the tip of the snout to the point where the caudal fin begins. The length-weight relationship was presented as Le Cren's cube logarithmic equation and was calculated thus:

$$W = a + L^b \qquad (4)$$

The logarithmic conversion of Equation 4 above was represented thus:

$$Log\ W = loga\ +\ blog\ L \qquad (5)$$

In Equation 5 above, W= weight (g), L= standard length in centimetres (cm), a = regression constant/intercept and b = regression coefficient/ growth exponent (Ayo-Olalusi, 2014).

2.3.4. Microbial analysis

Four culture media, namely nutrient agar (NA), MacConkay agar (MCA), eosin methylene blue (EMB), and sabouraud dextrose agar (SDA) were employed. The NA was used to enumerate the total bacteria count (TBC), the colonies were counted and expressed as $x10^4$ colony forming unit per milliliter ($x10^4$ cfu/mL). The MCA showed the presence of the coliform group in the fish samples, EMB indicated the presence of fecal *Escherichia coli,* and SDA was used to isolate fungi present in the fish samples.

The media were prepared according to the manufacturer's specifications. NA, MCA, and EMB after inoculation with the sample were all incubated at 37°C for between 24 to 48h, while samples inoculated on SDA were all incubated at 27°C for up to 7days. Bacterial colonies were identified by individual morphological characteristics on the culture media.

2.3.4.1. Antimicrobial susceptibility test (AST)

This was carried out on bacteria isolates to determine sensitivity and resistance to a wide range of antibiotics. The antibiotic Susceptibility Test was performed by the agar disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2018). Bacteria were spread to a nutrient agar plate and the antibiotics were aseptically placed on the surface of the medium using a sterile forceps.

The plates were incubated for 18 h at 37 °C, and then the diameters of complete growth inhibition zones were measured. Susceptibility to the following nine (9) antimicrobials was determined following CLIS regulation (2018).

CAZ - Ceftazidime (30 μg), CRX - Ceftriaxone (30 μg), CXM – Cefuroxime. (30 μg), GEN - Gentamicin (10 μg), OFL -Ofloxacin (5 μg), AUG - Augmentin (30 μg), NIT - Nitrofurantin (30 μg), CPR - Ciprofloxacin (5 μg) and ERY – Erythromycin (5 μg)

2.3.4.2. Plasmid profiling and curing

Plasmid analysis was performed by the TENS method (Zhao et al., 2016). Bacterial cells were grown in Nutrient Broth (LAMB) for 18 h; it included at least two steps of treatment with phenol. After DNA resuspension at room temperature, samples were subjected to electrophoresis on 0.7% (w/v) agarose gels (Roche, molecular grade) for 30 min at 80 V. Marker used for the profiling was 10,000 kilobase pairs (10kb).

Nutrient broth culture of 1.5 mL was dispensed into a microfuge tube and left to settle overnight. The deposit was spun for 1 min at 10 rpm to pellet the cells. The supernatant was discarded, and 150ul of media was left in the tube, it was vortexed to re-suspend the cells and TENS buffer of 300μl was added to the vortexed cells. The tube was inverted 3 to 4 times gently to lyse the cells completely (the liquid turned from turbid to clear). Sodium acetate 3M (pH 5.6) of 150ul was added and invert 3 to 4 times gently to allow for a white precipitate formation, and then the white precipitates were spurned for 5mins to pellet. The clear supernatant was pipetted to a clean tube, ethanol (95%) of 900 μL was added and tubes were inverted to mix. The DNA pellets were recovered by spinning at maximum speed for 2 min, the solution was discarded and ethanol (70%) of 500 μL was added to wash the pellet by vortexing and was spurned for 1 minute, the ethanol (70%) was discarded and the DNA pellets were dried to pellet. DNA was then dissolved in 50μL 10mM Tris (pH 8). TENS buffer: Tris-Hydrogen Chloride (HCL) 10 mM (pH 8.0) EDTA 1 mM Sodium hydroxide (NaOH) 0.1 N Sodium Dodecyl sulfate (SDS) 0.5% (w/v).

2.3.4.3. Quality assurance and quality control

Extra pure analytical grade solvents and standards were utilized in the experiments. The organochlorine pesticide standards (EPA Method 508-Chlorinated Pesticide Mix 1, 1000μg/mL), internal standards (AccuStandard, Pentachloronitrobenzene, 1.0mg/mL) and surrogate standards (2, 4, 5, 6-Tetrachloro-m-xylene, 10 ng/μl and Decachlorobiphenyl, 0.5mg/mL). All materials used for the extraction were sterilized. The sodium sulfate, florist were pre-cleaned with hexane and acetone solvents by passing through a glass column and then conditioned in an oven at 400 °C for 4h. For column packing glass wool was used, it was pre-cleaned with hexane and acetone then

were conditioned overnight in an oven at 200 °C. All of the glassware used in the laboratory were cleaned with alconox detergent (supelco) in hot and deionized water. They were allowed to dry in an oven after rinsing with hexane and acetone at 100 °C.

2.4. Statistical analysis

The OCP concentrations in the water, sediment, parasite, and fish tissues (liver and intestines) were subjected to Analysis of Variance (ANOVA) using IBM SPSS (version 20.0) to determine any significant difference which was ascertained by Duncan multiple range test. All statistical analyses were conducted at a significant level of 5% ($p < 0.05$).

The bioaccumulation factor was calculated as given in Equation 6

$$BAF = \frac{OCP \; concentration \; in \; intestine}{OCP \; concentration \; in \; sediment} \qquad (6)$$

(Kalfakakour and Akrida-Demertzi, 2000; Isibor et al., 2020)

The biota-sediment accumulation factor (BSAF) was calculated as stated in Equation 7

$$BSAF = \frac{OCP \; concentration \; in \; intestine}{OCP \; concentration \; in \; sediment} \qquad (7)$$

(Rashed, 2001)

3. Results

3.1. Prevalence of Procamallanus sp. in P. obscura

Among the male fish, the highest parasite intensity (6) occurred among the standard length (SL) cohorts 22.0 – 27.9 cm, while the lowest (1) occurred among the SL cohorts 10 – 15.9 cm (Table 1). Among the female counterparts on the other hand, the higher parasite intensity (3) occurred among the SL cohorts of 21.0 – 31.9 cm, while the lower intensity (1) occurred among the cohorts of 10.0 – 20.9 cm.

3.2. Bioaccumulation of OCPs

No variability occurred in the accumulation of OCPs in the liver compared to the intestine (Table 2). There was no significant difference between the concentrations of OCP congeners in the liver and the intestine (p>0.05). Adsorptions to the bottom sediment were recorded in alpha.-Lindane (a-BHC), beta.-Lindane (b-BHC), gamma.-Lindane (y-BHC), delta-Lindane (d-BHC), Heptachlor, Aldrin, Endosulfan I,

p,p'-DDE (4,4'-DDE), Endrinmarked, Endrin Aldehyde, Endosulfan sulfate p,p'-DDD, Dieldrin, characterized by their adsorption coefficient ($K_{s/w}$) > 1 (Isibor et al., 2020).

No significant bioaccumulation of any OCP congener occurred in the fish intestine and liver, from the surface water or bottom sediment. The parasite however exhibited significant bioaccumulation of Endosulfan II (beta.-Endosulfan), p,p'-DDD, and notable bioaccumulation of p,p'-DDE (4,4' DDE), all marked by intestine-parasite bioaccumulation factors ($BAF_{p/i}$) > 1 (Isibor et al., 2020).

3.3. Length-weight relationship in fish

The length-weight relationship of P. obscura indicates that the fish population comprises slender individuals marked by negative allometry which is characterized by growth exponent 0.959 being < 3 (Figure 2). The regression value indicates significant reliability of the data, characterized by the length accounting for 98% of the variations that occurred in the weight.

3.4. Microbial analysis

3.4.1. Microbial colonization in the gastrointestinal tract and epidermis of P. obscura

The microbiological analysis of the gastrointestinal tract (GIT) and skin of P. obscura showed the presence of a total of 7 bacteria and 2 fungi. The bacteria Escherichia coli and Staphylococcus sp. were observed on the GIT and the skin respectively, while the fungus Aspergillus niger and Mucor sp. was isolated in the GIT and skin respectively (Table 3). Bacillus sp. had the highest prevalence (55%) in the GIT, while enteric bacteria Salmonella sp. and Klebsiella sp. had the highest prevalence (45%) in the skin of P. obscura procured.

3.4.2. Antibiotics susceptibility test

An antimicrobial susceptibility test (AST) was carried out on the bacteria isolated from the gastrointestinal tract and skin of P. obscura. Staphylococcus sp., Micrococcus sp., and Klebsiella sp. conferred the highest resistance to all, being resistant to four different antibiotics, but uniformLy to Ceftriaxone (CRX), followed by E. coli that conferred resistance to three antibiotics (Table 4). All bacteria isolates conferred either susceptible or intermediate reaction to ciprofloxacin while Salmonella sp. conferred susceptibility, resistant and intermediate reaction to the antibiotics in the ratio 7: 1: 1 respectively.

Table 1. *Prevalence of Procamallanus sp.* in *P. obscura* in relative to standard length (SL).

SL (cm)	Number Examined	Number Infected	Prevalence (%)	Worm load	Mean intensity
Male					
10 – 15.9	54	1	2	1	1
16.0 – 21.9	20	5	25	11	2
22.0 – 27.9	5	1	20	6	6
28.0 – 33.9	5	2	40	3	2
Female					
10.0 – 20.9	9	3	33	3	1
21.0 – 31.9	13	3	23	9	3

Table 2. Concentrations of OCP (ppm) congeners Lekki lagoon and bioaccumulation indices in enteric parasite and tissues of *P. obscura*.

OCP Congeners	Water	Sediment	Intestine	Liver	Parasite	K_{SW}	BAFi/w	BSAFi/s	BAFl/w	BSAFl/s	BAFp/i
alpha.-Lindane (a-BHC)	8.157	2.784	0.851	0.688	0.393	0.3	0.1	0.3	0.1	0.2	0.5
beta.-Lindane (b-BHC)	17.998	18.318	0.771	0.877	0.412	**1.0**	0.0	0.0	0.0	0.0	0.5
gamma.-Lindane (y-BHC)	14.439	28.234	0.914	1.231	0.754	**2.0**	0.1	0.0	0.1	0.0	0.8
delta.-Lindane (d-BHC)	10.122	39.626	2.138	1.669	1.812	**3.9**	0.2	0.1	0.2	0.0	0.8
Heptachlor	51.851	67.7	2.036	1.195	0.000	**1.3**	0.0	0.0	0.0	0.0	0.0
Aldrin	5.859	23.364	0.025	0.067	0.000	**4.0**	0.0	0.0	0.0	0.0	0.0
Heptachlor epoxide (Isomer B)	1.455	1.265	0.000	0.000	0.000	0.9	0.0	0.0	0.0	0.0	
Endosulfan I	60.223	78.547	2.678	3.032	0.915	**1.3**	0.0	0.0	0.1	0.0	0.3
p,p'-DDE (4,4'-DDE)	4.712	7.077	0.025	0.057	0.252	**1.5**	0.0	0.0	0.0	0.0	**10.2**
Endrin	10.078	47.479	0.000	0.000	0.000	**4.7**	0.0	0.0	0.0	0.0	
Endosulfan II (.beta.-Endosulfan)	77.097	44.122	5.370	5.765	5.261	0.6	0.1	0.1	0.1	0.1	**1.0**
p,p'-DDT (4,4'-DDT)	10.51	9.536	0.637	1.179	0.433	0.9	0.1	0.1	0.1	0.1	0.7
Endrin Aldehyde	20.852	142	14.565	18.178	9.000	**6.8**	0.7	0.1	0.9	0.1	0.6
Endosulfan sulfate	11.453	26.254	0.000	0.016	0.000	**2.3**	0.0	0.0	0.0	0.0	
p,p'-DDD	63.247	98.056	2.841	3.533	2.867	**1.6**	0.0	0.0	0.1	0.0	**1.0**
Dieldrin	24.529	32.469	1.663	2.034	0.231	**1.3**	0.1	0.1	0.1	0.1	0.1
Endrin Ketone	127.424	55.939	0.000	5.085	0.000	0.4	0.0	0.0	0.0	0.1	
Methoxychlor	26.472	13.697	2.482	1.169	0.887	0.5	0.1	0.2	0.0	0.1	0.4

Emboldened values are significant indices. KS/W= soil-water adsorption coefficient, BAFi/w= intestine-water bioaccumulation factor, BSAFi/s= intestine-sediment accumulation factor, BAFl/w= liver-water bioaccumulation factor, BSAFl/s= liver-sediment accumulation factor, BAFp/i = parasite-intestine bioaccumulation factor.

Table 3. Microbial colonization in the Gastrointestinal Tract and epidermal of *P. obscura*.

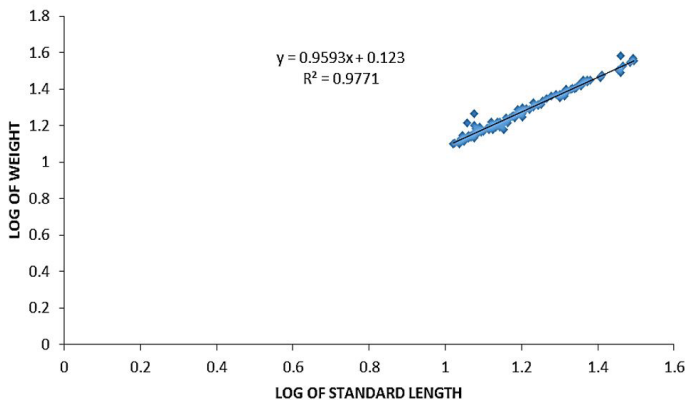| MICROBIAL ISOLATES	NUMBER OF OCCURRENCES		PREVALENCE (%)	
	GIT	Skin	GIT	Skin
Bacillus sp.	6	3	55	27
Pseudomonas sp.	2	4	18	36
Salmonella sp.	1	5	9	45
Micrococcus sp.	3	1	27	9
Klebsiella sp.	1	5	9	45
Escherichia coli	1	0	9	0
Staphylococcus sp	0	4	0	36
Mucor sp.	1	0	9	0
Aspergillus niger	0	1	0	9

y = 0.9593x + 0.123
R² = 0.9771

Figure 2. Length-weight relationship of *P. obscura*.

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Table 4. Antibiotics susceptibility test of *P. obscura*.

ISOLATES	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	ERY
Staphylococcus sp.	R	R	R	I	I	I	R	I	S
Bacillus sp	I	S	R	S	S	I	S	I	R
Pseudomonas sp.	R	R	I	I	I	S	S	S	S
Escherichia coli	R	R	I	I	S	I	S	I	R
*Micrococcu*s sp.	I	R	S	R	R	R	S	I	I
Salmonella sp	S	S	R	I	S	S	S	S	S
Klebsiella sp.	R	R	R	I	S	I	R	S	S

Key: CAZ - Ceftazidime, CRX - Ceftriaxone, CXM – Cefuroxime, GEN - Gentamicin, OFL Ofloxacin, AUG - Augmentin, NIT - Nitrofurantin, CPR – Ciprofloxacin, ERY – Erythromycin, R – Resistant, S – Susceptible and I- Intermediate.

Table 5. Antimicrobial susceptibility pattern in bacteria isolates after plasmid curing.

ISOLATES	CAZ	CRX	GEN	CXM	OFL	AUG	NIT	CPR	ERY
Staphylococcus sp.	I	R	I	I	S	S	S	S	S
Bacillus sp.	I	S	I	S	S	S	S	S	I
Pseudomonas sp.	I	I	S	S	S	S	S	S	S
Escherichia coli	S	S	S	S	S	S	S	S	S
Micrococcus sp.	S	I	S	I	S	S	S	S	S
Salmonella sp	S	S	I	S	S	S	S	S	S
Klebsiella sp.	S	S	S	I	S	S	S	S	S

Key: CAZ - Ceftazidime, CRX - Ceftriaxone, CXM – Cefuroxime, GEN - Gentamicin, OFL Ofloxacin, AUG - Augmentin, NIT - Nitrofurantin, CPR – Ciprofloxacin, ERY – Erythromycin, R – Resistant, S – Susceptible and I- Intermediate.
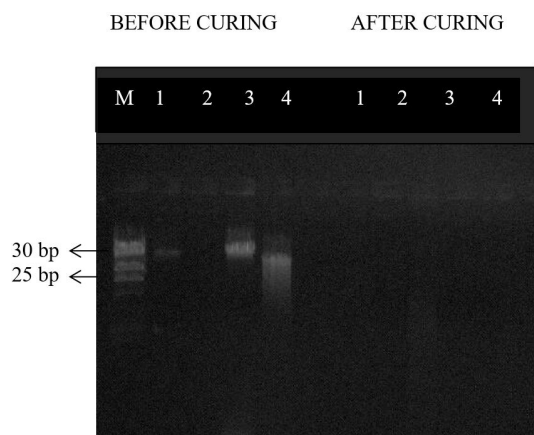

Figure 3. Gel Electrophoresis Showing Absence of Resistant Gene after Plasmid Curing.
Key: M- DNA marker, *1 – Staphylococcus* **sp.,** 2 - *Micrococcus* sp., 3 – *Klebsiella*, sp. and 4 -*E. coli* (Bacteria profiled for plasmid).

Plasmid profiling for antibiotic resistance showed that the bacteria *Staphylococcus* sp., *Micrococcus* sp., *Klebsiella* sp. and *E. coli* that conferred the highest rate of resistance to the antibiotics were profiled for plasmid and it was detected in the four bacteria. All bacteria isolates conferred either susceptible or intermediate reaction post-plasmid curing (Table 5), except *Staphylococcus* sp., which was still resistant to ceftriaxone.

The plasmid in the bacteria S*taphylococcus* sp., *Micrococcus* sp., *Klebsiella* sp., and *Escherichia coli* were cured to determine whether resistance was plasmid-mediated. The gel electrophoresis (Figure 3) showed the presence resistance before curing, which was absent/ lost upon plasmid curing as shown by the visible bands/lines before curing and absence of bands after curing. Hence, resistance conferred by the bacteria was plasmid-mediated and not innate.

4. Discussions

The observed emptiness of the fish gut when excised for parasite collection explains why an appreciable number of the OCP congeners were adsorbed in the bottom sediment without corresponding accumulation in the tissues of the fish. This may be possibly due to the reduced biodiversity of the aquatic insects and other organisms that constitute the diet of the fish following the impact of contaminants in the repository bottom sediment. The shortage of food may have also been responsible for the slenderness characterized by the negative allometry recorded in the sampled fish population.

The parasite intensity appeared to be directly proportional to the standard length. This may be due to the larger surface area available for parasitic infections in the lengthier fish cohorts. The results of the current study contradict the observation of Oden et al. (2015) who reported that nematode infection in the *P. obscura* population from the lower Cross River system of Nigeria was independent of the fish morphometrics.

The study showed that male fish were more infected than females. This result agreed with that of Biu et al. (2014)

who reported a lower incidence rate of parasitism in females than the males among *Oreochromis niloticus* from Lake Alau, Maiduguri, Nigeria. Osho (2017) had the same observation on the parasitic helminth fauna of *Parachanna obscura* in River Ogun, Southwest Nigeria. The current study also corroborates the observations and Akinsanya and Adekogbe (2017), and Isibor et al. (2020).

Endrin ketone was the congener with the highest concentration in the surface water was bioaccumulated in the liver from the bottom sediment but was not detected in the intestine of the fish. This result corroborates the work of Iyamu et al. (2007) which demonstrated the presence of lindane, aldrin, p,p-DDE,o,p-DDD, p,pDDD, o,p-DDT, and p,p-DDT in some rivers in Edo state. The presence of DDT, endosulfan I, heptachlor, dieldrin, and aldrin in Lagos lagoon, Nigeria. Conversely, Iyamu et al. (2007) however detected higher concentrations of OCPs in the fish than the surface water. Yehouenou A Pazou et al. (2013) also reported higher concentrations of OCPs in *P. obscura* than what was obtained in the water and sediment. I this study, OCP congeners were poorly accumulated in the tissues of the fish possibly because the parasite's depurative tendency may have surpassed the accumulation rates in the fish owing to hampered ingestion. Yehouenou A Pazou et al. (2013) however attributed the variability in bioaccumulation capacities among fish species to species-specificity in accumulation capacity and the niche, coupled with behavioral differences.

Among the 18 organochlorine congeners sampled in the Lekki Lagoon, 11 showed significant sediment adsorption (adsorption coefficient, $K_{s/w} \geq 1$) in the order; Endrin aldehyde > Endrin > Aldrin > delta.-Lindane (d-BHC) > Endosulfan sulfate > gamma.-Lindane (y-BHC) > p, p'-DDD > p, p'DDE > Dieldrin > Heptachlor > Endosulfan I > beta.-Lindane (b-BHC) while the remaining 7 congeners p,p'-DDT (4,4'-DDT), Heptachlor epoxide (Isomer B), Endosulfan II (.beta.Endosulfan), Methoxychlor, Endrin Ketone, and alpha.-Lindane (a-BHC) had no significant adsorption ($K_{s/w} < 1$). Akinsanya et al. (2015) earlier reported that Lindane and Heptachlor epoxide had the highest concentration in the sediment of the lagoon. The sediment is a repository for most chemical contaminants and as such, benthic organisms exhibit the contamination levels in the aquatic system, being sentinel bio-accumulator species, they are used in environmental monitoring to assess and discern the dynamics of the pollutants in the environment. Overall, there was no significant biota-sediment accumulation factor among the OCP congeners in the fish population sampled. Although the parasite intensities in infected fish were low, the depurative potential may have played significant role sequestration of OCPs in the fish, particularly in the intestine where they collected.

The epidermal and GIT microbial flora isolated from *P. obscura* suggested high fecal contamination in the lagoon, especially with the high occurrence on the skin compared to the gut. Shewan (2000) stated that the microbial flora associated with freshly harvested fish is more of a function of the ecological condition than species-

specificity. According to Adelaja et al. (2013), the normal bacteria flora in fish include *Bacillus* spp., *Staphylococcus saprophiticus*, *Micrococcus* spp., *Eschericia coli,* and *Staphylococcus aureus*, hence presence of *E. coli* in the gut of *P. obscura* is not far-fetched (Andreas et al., 2003). However, the combined presence of other enteric bacteria *Pseudomonas* sp*., Salmonella* sp., and *Klebsiella* sp. with a relatively high prevalence of 36%, 45%, and 45% respectively compared to that obtained in the GIT is of great concern. The result of this study conforms to that of Al-Bahry et al. (2009) on the impact of coastal sewage on fish concerning antibiotic resistance of *Pseudomonas* sp., *E. coli*, and *Klebsiella* sp.

The bacteria species isolated conferred an overall high resistance when antibiotic susceptibility test, with bacteria, isolates conferring either susceptible or intermediate reaction to ciprofloxacin. Resistance in microorganisms may be a natural endowment that affords them better coping strategies with unfavorable conditions. Increased occurrence of antibiotics in agricultural run-offs, pharmaceutical effluents, and ultimately water bodies may have increased the incidence of antimicrobial genes in microorganisms.

In this study, the bacteria that conferred the most resistance to the majority of the antibiotics were *Staphylococcus* sp*., Micrococcus* sp. *Escherichia coli* and *Klebsiella* sp., testing positive to plasmid profile. They conferred high resistance to the antibiotics before plasmid curing but became highly susceptible post- plasmid curing. This implies that the gene for resistance in the bacteria isolates was plasmid-mediated, that is, they were obtained from the environment, as against being innate. This observation corroborates the work of Alonso et al. (2001) which demonstrated that human and animal pathogenic bacteria constantly released with wastewater into the water environment, mostly harbor antibiotic-resistance genes, which are eventually inserted into genetic mobile platforms (plasmids, transposons, integrons) and able to spread among water and soil bacterial communities. Hence the crucial need for stringent regulations and management of all spheres of effluents into water bodies, as there is usually a direct or indirect, significant or insignificant effect on the inhabitants.

In the event of an outbreak of waterborne diseases such as cholera, typhoid, dysentery, and diarrhea, there may be non-response to treatment among the infected inhabitants. Although the incidence of parasitic infection in the fish was not alarming, the situation might be aggravated if the prevalent anthropogenic activities persist (Otitoloju, 2018), through immunosuppression (Omar et al., 2018).

5. Conclusion

Regulation of anthropogenic activities in the catchment area of the lagoon is recommended to forestall the prognosis of health and environmental hazards associated with agricultural, industrial, pharmaceutical, and municipal perturbations from the catchment area around the lagoon. The incidence of antibiotic resistance in bacteria colonies

recorded in this study is of great public health concern, given the possibility of the antibiotic resistant bacteria strains being passed to humans through fish consumption, resulting in increased multi-drug resistance in humans.

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