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The Effect of E-waste Dumpsite Soil on Cultured Tilapia Fish and Associated Microorganisms

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Authors' contributions

This work was carried out in collaboration between both authors. Authors ADV and SAI designed the study. Author SAI performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed literature searches. Author ADV managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the toxicological effect of e-waste on cultured tilapia and identifying microorganisms associated with the tilapia aquaria polluted with soil from e-waste dumpsite.
Study Design: The soil samples, water from the polluted aquaria and the harvested fish were then subjected to physicochemical, microbiological, proximate and toxicological analyses.
Place and Duration of Study: Lagos State and the study were undertaken for five weeks.
Methodology: The organic contents were determined using gravimetric techniques, nitrogen content was determined using kjeldhal method, exchangeable bases were determined using flame emission spectrometry and EDTA classical methods titration, heavy metals determination were estimated using atomic absorption spectrometer, the toxicological study and the microbiological analyses were carried out using standard methods.
Results: The physiochemical parameters of the soil samples differs with higher heavy metals values in e-waste soil sample. Some of microorganisms isolated were of the genera; *Bacillus, Proteus, Listeria, Pseudomonas, Lactobacillus*, *Aspergillus, Articolospora, Penicillium, Rhizopus, Mucor,* and *Zoopage*. The types and number of microorganisms from the e-waste dumpsite soil,

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aquaria polluted with soil from e-waste dumpsite differs from those from controls. The ash content (9.68^{a}±0.08 - 14.29^{e}±0.51) showed improvement over the control (9.49^{a}±0.20). Rise in fibre content of the tissue (0.86^{b}±0.02 - 0.98^{c}±0.01) was also observed over the control. Deformities of the gills and livers of the fish were observed while the packed cell volume, haemoglobin, oxygen carrying capacity and the red blood cell of fish from polluted aquaria were lower than that of fish from unpolluted aquaria. Immunological responses were also observed.

Conclusion: The histopathological and the haematological effects alongside the effect on the proximate composition and the microbial isolate variation in the soil samples and aquaria showed the adverse effect of the e-waste on the fish and its environment.

Keywords: E-waste; toxicology; microorganisms; proximate composition; tilapia.

1. INTRODUCTION

Global consumption of fish continues to climb, both amongst the wealthy as well as the poor sections of any population [1]. There are many health benefits from seafood consumption; these include cardio-vascular benefits, anti-inflammatory properties, childhood brain and sight development [2]. Fish and fish products constitute up to 60% of total protein intake in adults of rural habitats in Nigeria, and are used as medications (fish oils), in recreations and vital inclusions of livestock feeds [3]. Nigeria has 860 km of coastline on a major gulf of the South Atlantic, abundant water resources with major rivers of the Niger and the Benue traversing its territory in addition to numerous smaller rivers and streams crisscrossing its vast terrains [4]. It has large fishing grounds of lakes, swamps, lagoons, deltas and estuaries. Fish supplies in Nigeria come from three main activities, which include artisans, commercial trawlers and fish farming [3]. Fishing then can be maximized if these water bodies are kept from pollution. However, increasing human activities in the vicinity of lakes and rivers, particularly due to urbanization, industrialization, technological development, growing human population, indiscriminate sewage and waste disposal, agricultural activities, oil exploration and exploitation may lead to an increase in man-made pollutants in aquatic environment [3]. The gap between fish demand and supply is unfortunately widening due to increasing population, drop in meat and fish supply, thus prompting the search for methods of improving fish quantity and quality. Consequently, many methods have been used, including the application of herbicides for the control of Hyacinth, observed to have a profound effect on fish production attributed to the upsurge of available food for fish and increased nymphal proliferation at the post-application period [5]. The application of chemical poison in fishing and during handling of fish may contribute to contamination of both the aquatic environment and fish and fish products with heavy metals among other contaminants [5]. Water quality parameters are essential for the survival, growth and reproduction of fish and other aquatic animals. Both terrestrial and aquatic food chains are capable of accumulating certain environmental contaminants up to toxic concentrations [6].

Electrical and electronic equipment contain different hazardous materials which are harmful to living things, human health and the environment if not disposed of carefully. While some naturally occurring substances are harmless in nature, their use in the manufacture of electronic equipment often results in compounds which are hazardous (e.g. chromium becomes chromium VI). E-waste is any household or office appliance consuming electricity and reaching the end of its life cycle [7]. E-waste comprises discarded electronic appliances, of which computers and mobile telephones are disproportionately abundant because of their short lifespan. E-Waste accounts for 40 percent of the lead and 75 percent of the heavy metals found in landfills [8]. Although safe when used, once electronics are discarded in a landfill, the acidic conditions provide an environment in which lead and other heavy metals may leak out. If the landfill's liner fails, the groundwater supply may become contaminated. E-waste contaminants can enter aquatic systems via leaching from dumpsites where processed or unprocessed e-waste may have been deposited [9]. Similarly, the disposal of acid following hydrometallurgical processes into waters or onto soils, as well as the dissolution or settling of airborne contaminants, can also result in the contamination of aquatic systems [9]. The knowledge of the levels of contaminants in aquatic environment and fish is of considerable importance because of its

potential effects on the fish on one hand, and on the top-level predators that consume them, including humans, on the other hand. Although, the possibility of contamination of lakes, ponds and rivers and its effect on fisheries exists in Nigeria, the literature is still limited on electronic waste (e-waste) contamination of water bodies and the effect on fishes. This study was therefore designed to investigate the toxicological and proximate compositional effect of soil from e-waste dumpsite; Alaba International Market, Lagos State, Nigeria on cultured tilapia fish and associated microorganisms.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Soil samples were collected using sterile plastic containers from e-waste dumpsite Alaba International Market, Lagos, Lagos State (Coordinates: 6°35′N 3°45′E/ 6°583′N 3°750′E), Nigeria. The plastic containers were appropriately labeled and were immediately transported to the laboratory for analysis and those that could not be analyzed immediately were stored at 4℃ in a refrigerator for subsequent analyses [10]. Soil samples from six different spots were collected at a depth of 0–6 cm from the e-waste dumpsite. The samples were then mixed together to give a general view of the dumpsite soil. This was repeated for soil without e-waste.

2.2 Set up and Pollution of Aquaria

Seven aquaria in triplicates each containing six juvenile tilapia (*Oreochromis niloticus*), were polluted with three different quantities of the e-waste soil sample and soil without e-waste (soil from the same environment but 50 m away from e-waste dumpsite) (25 g, 50 g and 75 g for both soil samples) in the ratio of 1:1, 1:2, 1:3 of water to soil samples after acclimatization and feeding (2 mm **copen** fish feed) of the fishes for six weeks and the seventh aquarium is the second control (not polluted with any soil sample). The aquaria were monitored weekly for five weeks for physiochemical parameters; pH, dissolved oxygen, biochemical oxygen demand, while the microbial analyses include monitoring of microbial loads, isolation and identification of microorganisms in the polluted fish aquaria.

2.3 Physiochemical Parameters

The physiochemical parameters measured were; pH: The hydrogen ion concentration (pH) of each sample and temperature were measured using a digital pH meter. The electrode probe was inserted into a glass beaker containing about 20 ml of the sample and the result was read from the screen and recorded. The pH meter was calibrated before and after each reading using freshly prepared pH buffers (7.00), (4.00) and (9.00) [11]. Biochemical oxygen demand: BOD was determined by measuring the amount of dissolved oxygen present in the given water samples before and after incubation in the dark at 20℃ for five days (BOD_5). Dissolved oxygen at first day (day zero) was measured and recorded. The water samples from each fish aquaria were put into BOD bottles and incubated in the dark for five days after which the dissolved oxygen in the sample was again measured. The difference in the dissolved oxygen at day zero and at day five gave the BOD_5 [12]. Organic carbon determination, Organic matter, total phosphate determination and nitrogen determination: Soil samples sieved, weighted and treated with appropriate reagent for each parameter then the actual values were calculated using appropriate formula [13,14] and the heavy metals in the soil and the fish samples were determined using flame atomic absorption spectrophotometer (AAS) after homogeneity and digestion of samples [15].

2.4 Biochemical and Morphological Identification of Bacteria Isolates

Individual colonies from the soil samples and the aquaria (water from the aquaria) were identified by morphological and biochemical techniques using Holt et al. [16] and Fawole and Oso [17]. The medium used for the culturing of the bacteria was nutrient agar and the following biochemical tests; gram staining, catalase test, spore staining, motility test, starch hydrolysis, coagulase test, sugar fermentation test were carried out to identify each bacterium.

2.5 Identification of Fungi

This was done based on the cultural, morphological and microscopic examination of the colonies grown on potato dextrose agar [18]. The morphological examination was done using visible observation and microscope at low power magnification (x40), the parameters such as colony color, characteristics of the submerged hyphae rhizoid, spiral or regular and characteristic shape of mature fruiting bodies were all observed. The microscopy examination involved transferring a small piece of mycelium

free of medium using a sterile inoculating loop unto a clean glass slide containing a drop of cotton blue-in-lactophenol and the mycelium was spread properly. The preparation was covered with a clean grease free cover slip and observed under medium power (x100). The observations made were used in identifying the fungi organism.

2.6 Toxicological Analyses

Histopathological, haematological and the proximate analyses were done using methods described by Silva et al. [19], Cheesbrough [20] and AOAC [21] respectively. Data obtained were subjected to one way analysis of variance (ANOVA) and Duncan's New Multiple Range Test at 95% confidence level using SPSS 16.0 version. Differences were considered significant at $P \leq 0.05$.

3. RESULTS

3.1 Physiochemical Parameters of Soils from E-waste Dumpsite and Soil without E-Waste

The soil physiochemical parameters are shown in Table 1. Soil from e-waste dumpsite is black in colour, sandy-loamy in texture, had higher moisture content (3.86%), lead (64.90 mg/kg), cadmium (0.32 mg/kg), zinc (35.50 mg/kg), cobalt (0.83 mg/kg), chromium (0.54 mg/kg), manganese (18.60 mg/kg) and nickel (2.82 mg/kg) while soil without e-waste is brown in colour, sandy in texture, had higher pH value of 8.70, organic phosphorus of 160.00 mg/kg and calcium of 245.00 mg/kg.

3.2 Microbial Isolates from Soil Samples

There are eight different genera and nine species from both soil samples (Tables 2 and 3). The genera of the isolates were *Bacillus, Proteus, Enterobacter, Staphylococcus, Candida, Zoopage, Articulospora* and *Varicosporium*. *Bacillus subtilis* and *Proteus vulgaris* were the bacteria present in both soil samples while *Candida* sp and *Articulospora inflata* were the fungi present in the two soil samples.

3.3 Bacterial Isolates in Tilapia Aquaria

The bacteria isolates from tilapia aquaria were; *Staphylococcus aureus, Proteus vulgaris, Bacillus cereus, Bacillus subtilis, Listeria monocytogenes, Salmonella* sp, *Enterobacter* sp, *Pseudomonas aeruginosa, Lactobacillus bulgaricus* and *Corynebacterium fascians. Lactobacillus bulgaricus* was only found in aquaria polluted with soil from e-waste dumpsite. These are shown in Table 4.

Table 1. Soil physiochemical parameters

Parameter	A	B
pH	7.90	8.70
Moisture content (%)	3.86	2.24
Organic matter (%)	17.60	5.00
Organic carbon (%)	10.17	2.89
Organic nitrogen (%)	0.35	0.21
Organic phosphorus (mg/kg)	146.65	160.00
Lead (mg/kg)	64.90	3.06
Cadmium (mg/kg)	0.32	0.02
Zinc (mg/kg)	35.50	3.34
Cobalt (mg/kg)	0.83	0.05
Chromium (mg/kg)	0.54	0.26
Manganese (mg/kg)	18.60	2.99
Nickel (mg/kg)	2.82	0.08
Sodium (mg/kg)	24.40	31.40
Potassium (mg/kg)	33.30	32.90
Calcium (mg/kg)	182.00	245.00
Magnesium (mg/kg)	34.00	29.70

Source: Adegunloye and Sanusi, 2015 [22];
Key: A- Soil from e-waste dumpsite, B- Soil without e-waste

Table 2. Isolated bacteria from e-waste soil and soil without e-waste

Bacteria isolates	E-waste soil	Soil without e-waste
Bacillus subtilis	+	+
Bacillus cereus	+	-
Proteus vulgaris	+	+
Enterobacter sp	-	+
Staphylococcus aureus	+	-

Table 3. Isolated fungi from e-waste soil and soil without e-waste

Fungi isolates	E-waste soil	Soil without e-waste
Candida sp	+	+
Zoopage nitospora	+	-
Articulospora inflata	+	+
Varicosporium elodeae	+	-

3.4 Fungal Isolates in Tilapia Aquaria

Table 5 shows the probable fungi isolates in tilapia fish aquaria. The numbers of fungi isolates from the control aquaria was lower than the

polluted aquaria. *Aspergillus repens* was the only fungi isolated from the polluted aquaria and not in the control aquarium.

3.5 Proximate Composition of Harvested Tilapia Fish Tissue

Table 6 shows the proximate composition of harvested tilapia fish. The percentages of the moisture content of harvested tilapia fish ranged from 4.21^{a}±0.02 (from aquaria polluted with 25 g of soil without e-waste) - 12.97^{e}±0.22 (from aquaria polluted with 75 g of soil without e-waste), with 6.74^{c}±0.10, 11.57^{d}±0.19 and 12.97^{e}±0.22 from aquaria polluted with 75 g of e-waste soil, aquaria polluted with 50 g of soil without e-waste and aquaria polluted with 75 g of soil without e-waste respectively higher than the control (6.38^{c}±0.04). The samples had higher percentages of fibre than the control while the protein percentage compositions were only higher than the control in the harvested fish from e-waste soil treated aquaria.

3.6 Haematological Parameters of Harvested Tilapia Fish Blood

Tables 7 and 8 show the haematological parameters of harvested tilapia fish blood and the differential count of harvested tilapia fish blood respectively. The blood samples from harvested fish from the control aquaria had higher values of packed cell volume, haemoglobin and red blood cell with the exception of packed cell volume (26.50^{bc}±0.50), haemoglobin (8.75^{e}±0.25) and red blood cell (2.89^{e}±0.12) of tilapia from aquaria polluted with 75 g of soil without e-waste that were higher than that of the control. Leucocyte and neutrophil are the most prominent of the differential count measured with neutrophil values increasing with increased pollution except that of tilapia from aquaria polluted with 25 g of soil without e-waste with 56.50^{a}±1.50 neutrophil counts which is lesser than the control (59.00^{ab}±1.00).

Table 4. Probable bacterial isolates from tilapia aquaria

Isolates	Control	Soil without e-waste	E-waste soil
Staphylococcus aureus	+	+	+
Proteus vulgaris	+	+	+
Bacillus cereus	+	+	+
Bacillus subtilis	-	+	+
Listeria monocytogenes	+	+	+
Salmonella sp	+	+	+
Enterobacter sp	+	+	+
Pseudomonas aeruginosa	-	+	+
Lactobacillus bulgaricus	-	-	+
Corynebacterium fascians	+	+	+

Table 5. Probable fungal isolates from tilapia aquaria

Isolate	Control	Soil without e-waste	E-waste soil
Penicillium italicum	+	+	+
Candida sp	+	+	+
Articulospora inflata	+	+	+
Aspergillus niger	+	+	+
Rhizopus stolonifer	+	+	+
Aspergillus flavus	+	+	+
Mucor mucedo	+	+	+
Zoopage nitospora	+	+	+
Varicosporium elodeae	+	+	+
Aspergillus repens	-	+	+

Table 6. Proximate composition of harvested tilapia fish

Sample	MC	Ash (%)	Fat (%)	Fibre (%)	CHO (%)	Protein (%)
Control	6.38^{c}±0.04	9.49^{a}±0.20	14.46^{bc}±0.46	0.43^{a}±0.01	16.67^{c}±0.23	52.55^{d}±0.10
ES1	4.30^{a}±0.11	9.68^{a}±0.08	17.61^{d}±0.30	0.86^{b}±0.02	10.54^{a}±0.18	57.33^{f}±0.19
ES2	5.38^{b}±0.23	11.32^{b}±0.07	12.97^{a}±0.49	0.88^{b}±0.03	10.61^{a}±0.29	60.08^{g}±0.13
ES3	6.74^{c}±0.10	10.24^{a}±0.06	15.39^{c}±0.04	0.95^{bc}±0,04	11.87^{b}±0.18	54.69^{e}±0.21
SWE1	4.21^{a}±0.02	13.43^{d}±0.20	14.47^{bc}±0.10	0.92^{bc}±0.04	16.31^{c}±0.12	50.85^{c}±0.02
SWE2	11.57^{d}±0.19	12.58^{c}±0.07	13.99^{b}±0.02	0.98^{c}±0.01	12.37^{b}±0.07	48.50^{b}±0.11
SWE3	12.97^{e}±0.22	14.29^{e}±0.51	17.71^{d}±0.15	0.92^{bc}±0.03	10.25^{a}±0.27	42.87^{a}±0.06

Values are presented as Mean ±S.E (n=3). Means with the same superscript letter(s) along the same column are not significantly different (P>0.05)

Key: *ES1 – Polluted with 25 g of e-waste soil, ES2 – Polluted with 50 g of e-waste soil, ES3 - Polluted with 75 g of e-waste soil; SWE1 – Polluted with 25 g of soil without e-waste, SWE2 – Polluted with 50 g of soil without e-waste, SWE3 - Polluted with 75 g of soil without e-waste, MC- Moisture content and CHO- Carbohydrate*

Table 7. Haematological parameters of harvested tilapia fish blood

Sample	PCV	Hb	OCC	WBC	RBC
Control	22.50^{a}±0.50	7.85^{d}±0.15	9.61^{e}±0.06	4810.00^{d}±10.00	2.55^{d}±0.05
ES1	20.00^{b}±1.00	7.05^{bc}±0.05	8.88^{c}±0.01	5450.00^{bc}±50.00	2.26^{bc}±0.04
ES2	21.50^{a}±0.50	7.14^{bc}±0.26	9.27^{d}±0.04	4400.00^{d}±100.00	2.53^{d}±0.08
ES3	18.50^{d}±0.50	6.16^{a}±0.06	7.64^{a}±0.03	7900.00^{a}±100.00	1.95^{a}±0.05
SWE1	22.00^{e}±1.00	7.52^{cd}±0.08	9.63^{e}±0.09	8750.00^{cd}±250.00	2.48^{cd}±0.07
SWE2	20.50^{c}±0.50	6.60^{ab}±0.10	8.37^{b}±0.02	6050.00^{b}±50.00	2.23^{b}±0.03
SWE3	26.50^{bc}±0.50	8.75^{e}±0.25	11.22^{f}±0.12	5650.00^{e}±150.00	2.89^{e}±0.12

Values are presented as Mean ±S.E (n=3). Means with the same superscript letter(s) along the same column are not significantly different (P>0.05)

Key: *ES1 – Polluted with 25 g of e-waste soil, ES2 – Polluted with 50 g of e-waste soil, ES3 - Polluted with 75 g of e-waste soil; SWE1 – Polluted with 25 g of soil without e-waste, SWE2 – Polluted with 50 g of soil without e-waste, SWE3 - Polluted with 75 g of soil without e-waste, PCV- Packed cell volume, HB- Haemoglobin, WBC- White blood cell, RBC- Red blood cell and OCC – Oxygen carrying capacity*

Table 8. Differential count of harvested tilapia fish blood

Sample code	Eosinophils	Leucocyte	Monocyte	Neutrophils
Control	0	41.00^{e}±0.58	0	59.00^{ab}±1.00
ES1	0	30.00^{b}±0.58	2.50^{c}±0.50	66.00^{d}±1.00
ES2	2.50^{b}±0.50	39.00^{d}±0.58	0	62.50^{bcd}±2.50
ES3	2.50^{b}±0.50	24.00^{a}±0.58	1.50^{b}±0.50	73.00^{e}±1.00
SWE1	0	44.00^{f}±0.58	0	56.50^{a}±1.50
SWE2	0	32.00^{c}±0.58	2.00^{bc}±0.00	64.00^{cd}±1.00
SWE3	0	39.00^{d}±0.58	0	61.00^{abc}±1.00

Values are presented as Mean ±S.E (n=3). Means with the same superscript letter(s) along the same column are not significantly different (P>0.05)

Key: *ES1 – Polluted with 25 g of e-waste soil, ES2 – Polluted with 50 g of e-waste soil, ES3 - Polluted with 75 g of e-waste soil; SWE1 – Polluted with 25 g of soil without e-waste, SWE2 – Polluted with 50 g of soil without e-waste and SWE3 - Polluted with 75 g of soil without e-waste*

3.7 Histopathology of the Gills and Livers of the Harvested Fishes

Normal structure of gill and gill rakes of tilapia fishes are shown on Plates 1 and 5, without pathological damage while Plates 2–4 and Plates 6–8 show the different histopathological damages on the gills of tilapia fish (*Oreochromis niloticus*) from aquaria polluted with soil from e-waste dumpsite and from soil without e-waste polluted aquaria respectively.

Plates 9 and 13 show livers of harvested tilapia fish with normal liver cells without histopathological damages while Plates 10-12 and Plates 14–16 show various histopathological damages on liver cells of *Oreochromis niloticus* from aquaria polluted with soil from e-waste dumpsite and from soil without e-waste polluted aquaria respectively.

4. DISCUSSION

The soils (soil from e-waste dumpsite or e-waste soil and soil without e-waste) analyzed vary in their microbiological and physicochemical properties. E-waste soil and e-waste soil polluted tilapia fish aquaria had higher number of isolates compared to soil without e-waste and soil without e-waste polluted tilapia fish aquaria (Tables 2 - 4). These can be attributed to high percentage of organic contents (carbon, organic matter and nitrogen) and moisture content of the e-waste soil (Table 1), which might have encouraged and supported the growth of those microbes. This is in conformity with the findings of Margesin and Schnner [23] about microbial needs for growth. Bacteria isolated are of the genera; *Staphylococcus, Proteus, Bacillus, Listeria, Salmonella, Enterobacter, Pseudomonas, Lactobacillus* and *Corynebacterium* while the genera of fungi isolated were *Candida, Zoopage, Articulospora*, *Varicosporium*, *Aspergillus, Penicillium, Rhizopus* and *Mucor.*

Bacillus cereus, Bacillus subtilis, Listeria monocytogenes and *Lactobacillus bulgaricus* are normal floral of the fishes which are dependent on the environment in which the fish lives, cultured, fish feed [24]. *Salmonella* sp, *Pseudomonas aeruginosa*, *Aspergillus flavus* and *Mucor mucedo* have been associated with fish spoilage [24-27]. *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa*, *Articulospora*

inflata, Varicosporium elodeae, Penicillium sp*, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer* and *Mucor mucedo* had been isolated from many environments (such as crude oil polluted environment, gastrointestinal tract, agricultural soil). Their presence also in these environments (e-waste soil, soil without e-waste, polluted fish aquaria and harvested fish parts) could have been as a result of their ability to adapt to different environmental conditions and

Plate 1 **Plate 2** **Plate 3** **Plate 4**

Plate 1. Normal gill architectural structure (fish from control aquaria)
Plate 2. Total loss of gill structure and distortion of the gill filaments (fish from ES1 aquaria)
Plate 3. Distortion and gradual loss in the gill filaments (fish from ES2 aquaria)
Plate 4. Total loss of gill architectural structure (fish from ES3 aquaria)
ES1- Polluted with 25 g of e-waste soil; ES2 -Polluted with 50 g of e-waste soil; ES3 - Polluted with 75 g of e-waste soil

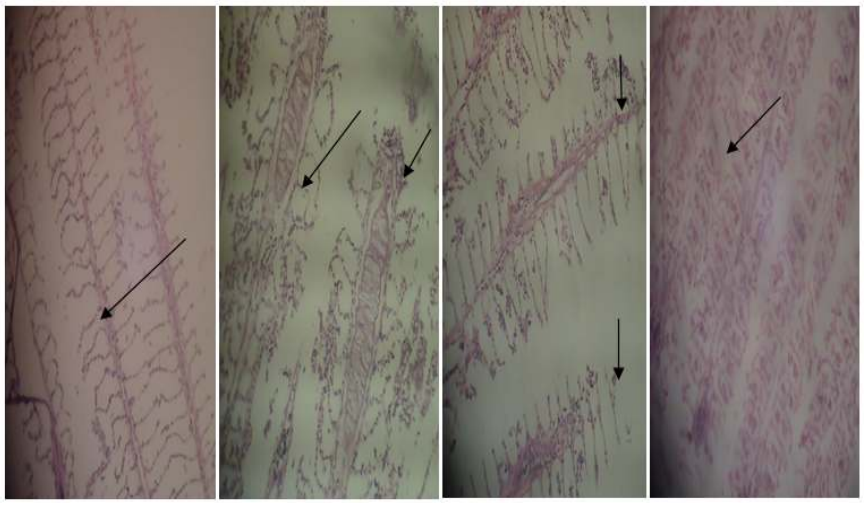

Plate 5 **Plate 6** **Plate 7** **Plate 8**

Plate 5. Normal gill architectural structure (fish from control aquaria)
Plate 6. Detaching filaments and loss of gill structure (fish from SWE1 aquaria)
Plate 7. Distorted gill filament and loss of gill structure (fish from SWE2 aquaria)
Plate 8. Fused gills (fish from SWE3 aquaria)
SWE1- Polluted with 25 g of soil without e-waste; SWE2 -Polluted with 50 g of soil without e-waste; SWE3 - Polluted with 75 g of soil without e-waste

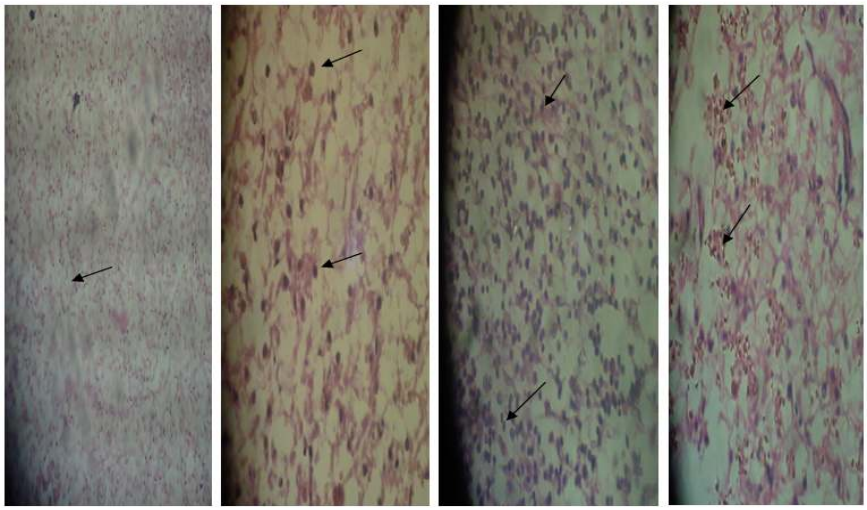

Plate 9 **Plate 10** **Plate 11** **Plate 12**

Plate 9. Liver with normal cells (fish from control aquaria)
Plate 10. Mild infiltration of liver by kuppfer cells (fish from ES1aquaria)
Plate 11. Liver with high infiltration of kuppfer cells (fish from ES2 aquaria)
Plate 12. Liver cells with necrotic effects (fish from ES3 aquaria)
ES1- Polluted with 25 g of e-waste soil; ES2 -Polluted with 50 g of e-waste soil; ES3 - Polluted with 75 g of e-waste soil

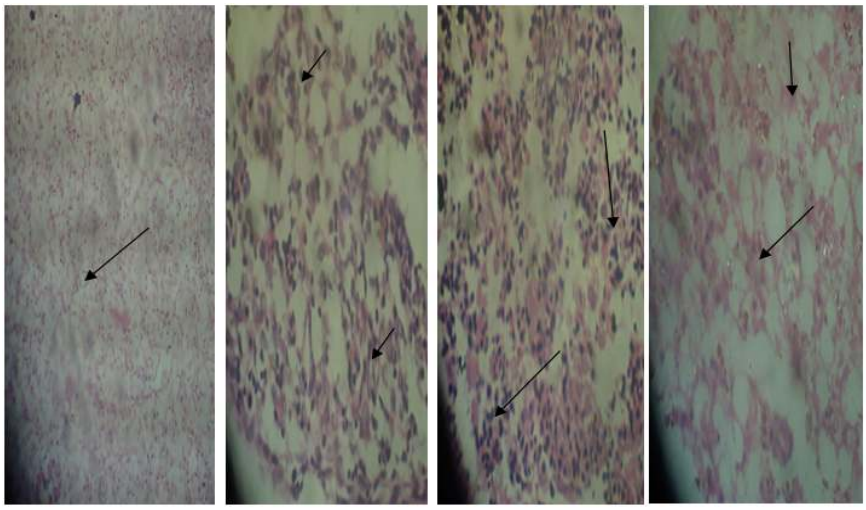

Plate 13 **Plate 14** **Plate 15** **Plate 16**

Plate 13. Liver with normal cells (fish from control aquaria)
Plate 14. Liver cells with mild infiltration of melanocytes (fish from SWE1aquaria)
Plate 15. Liver cells with high infiltration of melanocytes (fish from SWE2 aquaria)
Plate 16. Deshaped/distorted hepatocytes (fish from SWE3 aquaria)
SWE1- Polluted with 25 g of soil without e-waste; SWE2 -Polluted with 50 g of soil without e-waste; SWE3 - Polluted with 75 g of soil without e-waste

use wide range of food substances as nutrient source [28,27]. It was observed from this study that, the dominant bacteria species were gram positive, catalase positive, coagualase negative, rod bacteria (Tables 2). *Bacillus* spp. which is the most prominent bacteria species in the these research (found in the soil samples and the polluted aquaria) has also been known to be

related to carbon mineralization to crude oil, some have been isolated from soil polluted by crude oil or petroleum product and also known as one of the commonly found rod bacteria in the soil [29,30]. Its adaptive versatility may be responsible for their prominence in this research.

Most of the fungi species isolated in this research are moulds. It is could be that moulds are better adapted to e-waste polluted environments than yeast. And possibly they can be of remediative purposes (in biosorption and bioleaching processes) in are polluted with e-waste. This is in line with the findings of Iqbal et al. [31], who documented that fungi of metal contaminated soil have high level of metal tolerance and biosorption properties. Needhidasan et al. [32] documented that Autotrophic bacteria (*Thiobacilli* sp.), heterotrophic bacteria (such as *Pseudomonas* sp., *Bacillus* sp.) and heterotrophic fungi (like *Aspergillus* sp., *Penicillium* sp.) are the three major groups of microbes involved in bioleaching of metals**.** Bioleaching is the transformation of solid metallic compounds to its solubility and extractable form by microbes [33]. Brandl et al. [34] showed how *Thiobacilus* bacteria and fungi (*Aspergillus niger, Penicillium simplicissimum*) could facilitate metal leaching from electronic scrap. Creamer et al. [35] employed *Desulfovibrio desulfuricans* to recover gold, platinum and copper from e-waste.

The isolation of human pathogenic bacteria genera *Proteus* and *Staphylococcus* from the soil samples suggests recent human activities (possibly discharge of fecal matters and urine). Human interaction with such soil could pose health risk.

Proximate composition of harvested tilapia reveals (Table 6) that the percentages of fibre and ash contents were higher than the control, while the percentages of carbohydrate were lower than the control. However the percentages of protein content observed in the tissue of harvested tilapia in the soil without e-waste polluted aquaria were found to be lower than those from e-waste soil polluted aquaria. It was observed that the soil types pollutions (e- waste soil and soil without e-waste pollution) leads to increase in the composition of the ash, fibre and protein (harvested tilapia from e-waste polluted aquaria only) parameters measured, probably the building materials for these parameters can be found readily from the pollutant and utilized by the fish.

The haematological results revealed that blood samples from the randomly selected tilapia fish from each treatment were affected by the soil-type pollutions (Table 7). The haemoglobin, oxygen carrying capacity, packed cell volume and red blood cell of fishes in polluted aquaria were lower than fishes from unpolluted aquaria (except fish from aquaria polluted with 75 g of soil without e-waste that have values above the unpolluted). The lower oxygen carrying capacity in harvested tilapia fish from polluted aquaria confirms the observation of other researchers who also reported the decrease of oxygen carrying capacity in fishes such as *Heteropneustes fossilis* exposed to mixture of copper and NH_3 [36], *Oreochromis mossambicus* exposed to copper and zinc [37]. This decline could be attributed to the fact that heavy metals damage the structure of red blood cell consequently instead of four, less molecules of oxygen binds to the haemoglobin [38]. This loss of haemoglobin and consequent reduction in the oxygen carrying capacity of the blood is a feature of anemia in tilapia fishes from polluted aquaria. Similar haematological response by Mehjbeen and Nazura [38] was also observed in *Channa punctatus* from polluted water.

The white blood cell (WBC) and the neutrophils (immune response parameters) values of both fishes were higher in fishes from polluted aquaria than fishes from the unpolluted (Tables 7 and 8). Shaheen and Akhtar [39] also reported significant increase in WBC count of *Cyprinus carpio* when exposed to Cr (VI). This alterations in the immune parameters showed the fish body immune system probably stimulated immune responses to the environmental stress brought about by the soil pollution. Since neutrophils are one of the first set of white blood cell differential respond to inflammation thus their progressive increase with the pollution. Inflammation can be caused by bacteria infection, environmental condition, cancer which will result in chemical signals such as interleukin-8, leukotriene B4, interferon gamma which the body response to by recruiting immune cells such as neutrophils [40]. Similar neutrophil response to environmental pollution exposure by human has been documented by Jacobs and colleagues [41] in “subclinical responses in healthy cyclist briefly exposed to traffic-related air pollution.”

Several histopathological alterations were observed in the gills of the harvested tilapia fish (Plates 1-8). These pathological alterations include; complete fusion of lamellae, hypertrophy

and epithelial lifting, distortion and loss of architectural structure of the filaments. The deformities observed in the gill of the fishes is probably due to exposure to the pollutant probably the heavy metals present in the pollutant, similar results have been reported in fishes such as *Tilapia mossambica* exposed to copper, nickel, chromium [42], *Cyprinus carpio* exposed to chromium [43].

The livers tilapia exhibited histopathological lesions (Plates 9-16) such as deshaped hepatocytes, infiltrations of kuppfer cells. Degeneration or damages of liver tissue could be due to the infiltration of leucocytes which were induced by the presence of this pollutant. Similar results have been reported in liver of different fishes, *Oreochromis mossambicus* exposed to cadmium and zinc [44], *Clarias batrachus* to $ZnSO_4$ [45] and *Tilapia zilli* to Aluminum [46].

5. CONCLUSION

The introduction of soil from e-waste dumpsite and soil without e-waste into cultured tilapia fish aquaria influenced the microbial types in the water and the health of the fish under study. The numbers of microorganisms isolated were higher in the polluted aquaria than the unpolluted aquaria. The health of tilapia fish, a major protein source, has also been affected which lead to deformity to its gills and liver. Hence, some scientific method of detoxification or removal (biosorption) is essential should such pollution occur in order to improve a healthy environment for the microbial floral and to prevent or reduce damages to the health of this economic fish. Government should also intact laws for proper disposal of e-waste to prevent the pollution. Further studies may be necessary on the reproductive aspects of the fish in order to check its reproductive potential which will help to conserve the species.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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