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Earthworm Biomass as Additional Information for Risk Assessment of PCBs: A Case Study of Olusosun Dumpsite, Ojota, Lagos, Nigeria

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Abstract: This study assessed the level of Polychlorinated biphenyls (PCBs) concentrations and the biochemical parameters in earthworms (*E. eugeniae*) as well as histopathological effects in the clitellium of earthworms (*E. eugeniae*) present in Olusosun dumpsite which is the largest dumpsite in Lagos and University of Lagos, a major higher institution located in Lagos, Nigeria. The earthworms were sampled from two different sites in each location and taken to the laboratory for PCBs, biochemical and histopathological analyses. The level of concentration of PCBs in earthworms found in Unilag was significantly higher than the level observed in Olusosun dumpsite. With respect to the biochemical analysis carried out on the clitellum of the earthworm samples collected from Olusosun dumpsite, Malondialdehyde (MDA), Superoxide dismutase activity (SOD) and GST had higher levels when compared with the sample collected from the University of Lagos study site (non-dump site). High levels of Glutathione content (GSH) and Catalase activities (CAT) were only recorded in earthworms from the Unilag sample when also compared with the sample from Olusosun study site. The activities of the enzymes, superoxide dismutase (SOD) and catalase were inhibited in Unilag sample. Histopathological assessments of the clitellium indicated that the major effect observed were increased secretory activity, reduced body mass and disorganized internal organ in the earthworms from Unilag. The implication of the findings in the earthworms from Olusosun dumpsite and University of Lagos are hereby discussed.

Keywords: PCBs, Olusosun Dumpsite, Earthworms, Histopathological Assessment, Glutathione

1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants that have attracted great concern because of their worldwide distribution, persistence in the environment, and possible deleterious effects. PCBs were manufactured in the United States between 1929 and 1978 by Monsanto Corporation, and were marketed under the trade name Aroclor. Their electrical insulating properties and inflammability, combined with unique thermal and chemical stability, led to a wide variety of industrial uses as heat transfer fluids, hydraulic fluids, plasticizers, dielectric fluids, flame retardants, solvent extenders, and organic dieluents [1-4]. The widespread use of PCBs coupled with improper disposal practices resulted in the discharge of large quantities of these environmental pollutants into non-target sites, such as soils, river and lake sediments and land. It is estimated that approximately 1.4 billion pounds of PCBs have been manufactured and several million pounds have been released into the environment (Abramowicz and Olson, 1995; Mousa *et al.,* 1996). PCBs are truly pervasive in the environment

and will remain so for a long period of time. PCBs are reported as contaminants in almost every component of the global ecosystem including air, water, soil, fish, wildlife, plants, domestic animals, human blood, adipose tissue and milk (Crine, 1988; Tanabe, 1988; WHO, 1993). They can bioaccumulate in biological tissues, and their lipophilic behavior poses a serious threat to mammalian systems (Borlakoglu and Haegele, 1991; Brunner *et al.,* 1985). PCBs are known to elicit a spectrum of toxic responses in humans, laboratory animals and wildlife including lethality, reproductive and developmental toxicity, body weight loss, dermal toxicity, liver damage, neurotoxicity, immunosuppressive effects, porphyria, teratogenic effects and carcinogenic effects (Borlakoglu and Haegele, 1991; Mousa *et al.,* 1996).

Earthworms harbour millions of 'nitrogen-fixing' and 'decomposer microbes' in their gut. Their body contains 65% protein (70-80% high quality 'lysine rich protein' on a dry weight basis), 14% fats, 14% carbohydrates and 3% ash (Visvanathan *et al.,* 2005). Earthworms act as an aerator, grinder, crusher, chemical degrader and a biological stimulator wherever they inhabit (Sinha *et al.,* 2002). About 4,400 different species of earthworms have been identified, and quite a few of them are versatile bio-degraders. Earthworms are burrowing animals and form tunnels by literally eating their way through the soil. The distribution of earthworms in soil depends on factors like soil moisture, availability of organic matter and pH of the soil. They occur in diverse habitats specially those which are dark and moist. Earthworms are generally absent or rare in soil with a very coarse texture and high clay content or soil with pH < 4 (Gunathilagraj, 1996). Earthworms are very sensitive to touch, light and dryness. As worms breathe through their skin proper ventilation of air in soil medium is necessary. Water logging in the soil can cause them to come to the surface. Worms can tolerate a temperature range between 5°C to 29°C. A temperature of 20°C to 25°C and moisture of 60-75% is optimum for earthworm function (Hand, 1988). Researches into vermiculture have revealed that worms can feed upon wide variety of materials from earth. Aristotle called them *'intestine of earth'*. Earthworms love to feed upon 'cattle dung' which is preferred food for them. When given a choice between various foods the worms consumed 10 mg dry weight of dung per gram body weight per day together with smaller amount of leaf litter. In about 13 days *Allolobophora caliginosa* consumed 13.1 gram of dung while only 1.3 gram of grass leaves (Barley, 1959). However, firm leaves particularly the grass leaves are not eaten until they had decayed to a moist, brown condition. Worms have 'chemoreceptors' which aid in search of food. The worms secrete enzymes proteases, lipases, amylases, cellulases and chitinases in their gizzard and intestine which bring about rapid biochemical conversion of the organic materials. They ingest the organic materials, pass it through its intestine, adjust the pH of the digested (degraded) materials, cull the unwanted microorganisms, and then deposit the processed cellulosic materials mixed with minerals and microbes as aggregates called 'vermicasts' in the soil. (Dash, 1978). Most earthworms consume, at the best, half their body weight of organic material in a day. *Eisenia fetida* is reported to consume organic matter at the rate equal to their body weight every day (Visvanathan *et al.,* 2005).

Earthworms are representative of soil fauna and may constitute 60–80% of the animal biomass (Vijaya, *et al.,* 2012). They carry out many ecologically important functions, particularly in soil formation, enrichment, aeration and recycling (Edwards and Bohlen, 1996) they break down organic matter and burrow into the soil to mixing material. It is estimated that under favourable conditions, earthworms can move up to 18 tons of soil per acre per year (Evans, 1948). They are also important in the terrestrial food chain. Earthworms are sensitive to environmental influences especially because they are in close contact with soil and continuously exposed to exogenous materials through direct dermal contact and ingestion of soil materials (Double and Brown, 1988). For this reason, earthworms are regarded as reference compartments to observe soil contaminant bioavailability and evaluate the lethal and sub lethal effects of chemical pollutants (Otitoloju, 2009) and are useful to assess the contaminant fractions which may affect other organisms that get in contact with the soil. Earthworm's bioaccumulate chemical contaminants increasing the risk of pollutants transfer to higher trophic levels. It is known that the degree of accumulation by earthworms is species-specific (Nahmani *et al.,* 2007) and influenced by the physicochemical properties of the pollutants and of the environmental scenario (Vijver *et al.,* 2005). Standardized earthworm toxicity tests to evaluate soil contamination have been formulated by the organization for economic corporation and development (OECD, 2004; OECD, 1984). These include a 7 and 14 days acute mortality tests and chronic tests to assess the effects of contaminants on growth, enzyme activity and reproduction (OECD, 2004). These offer the advantage of a more ecologically relevant approach to environmental monitoring and assessment. Most studies on earthworm biomarkers have been conducted using *Eiseniafetida* which is the standard testing organism, this due to its rapid life cycle and simple rearing in the laboratory. However native organisms are used preferably in trying to understand the long-term effects of pollutant that may be emphasized in organisms from natural populations (OECD, 1984). The International Waste Association defines Landfill as "The engineered deposit of waste onto or into land in such a way that pollution or harm to the environment is prevented, and through restoration of land provided which may be used for other purpose" (Bagchi, 1994). Landfills have the potential to create risks to the environment, including negative impacts on air, water and soil quality.

Municipal and industrial solid wastes contain a variety of potentially significant chemical constituents and pathogenic organisms that could negatively affect public health, air, soil and groundwater qualities. These constituents include regulated hazardous priority pollutants such as heavy metals, poly aromatic hydrocarbons (PAHs), polychlorinated

biphenyls (PCBs) and other persistent organic pollutants (POPs) (Osibanjo, 2003).

Disposal of solid wastes in major cities of Nigeria in the last few decades has posed major environmental and public health problems. This has become a source of worry for rural and urban planners in Lagos, the most populous city in Nigeria because of the explosive population growth and urbanization. Most open dumpsites/landfills which were sited at the outskirts, are now within the heart of the mega city. Olusosun landfill, the biggest landfill in Lagos is an example. It has been in existence since 1978 and receives at least 25,000 tons of assorted waste per annum (Lagos Waste Disposal Board, 2006). The objectives of this study therefore are: To investigate the complex links between earthworm biomarkers and polychlorinated biphenyls (PCBs); To study the biochemical parameters (Malondialdehyde (MDA), an index of lipid peroxidation, reduced gluthathione (GSH) and antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) in *E. eugeniae* (whole organism) found in a dumpsite and a non-dumpsite; To assess the environmental effects of polychlorinated biphenyls (PCBs) using Earthworm as a Bio-indicator; To assess the histological effects of the soil on *E. eugeniae* (Clitellum); and to carry out the risk assessment of polychlorinated biphenyls (PCBs) using Earthworm biomarkers as additional information at Olusosun Dumpsite.

2. Methodology

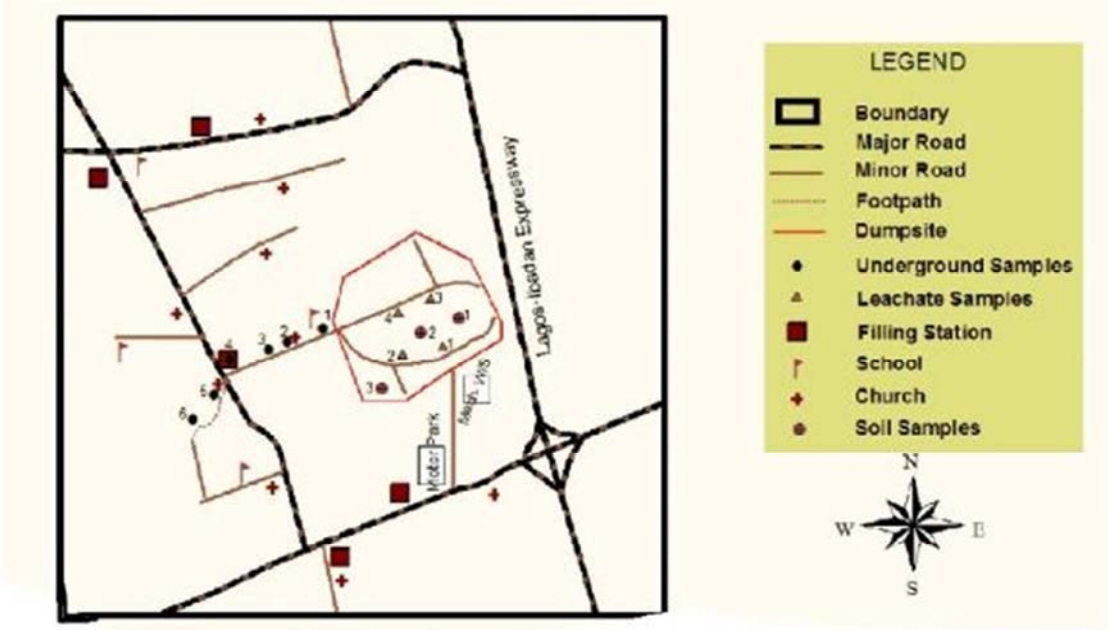

Fig. 1. *Map showing the sampling point at Olusosun landfill.*

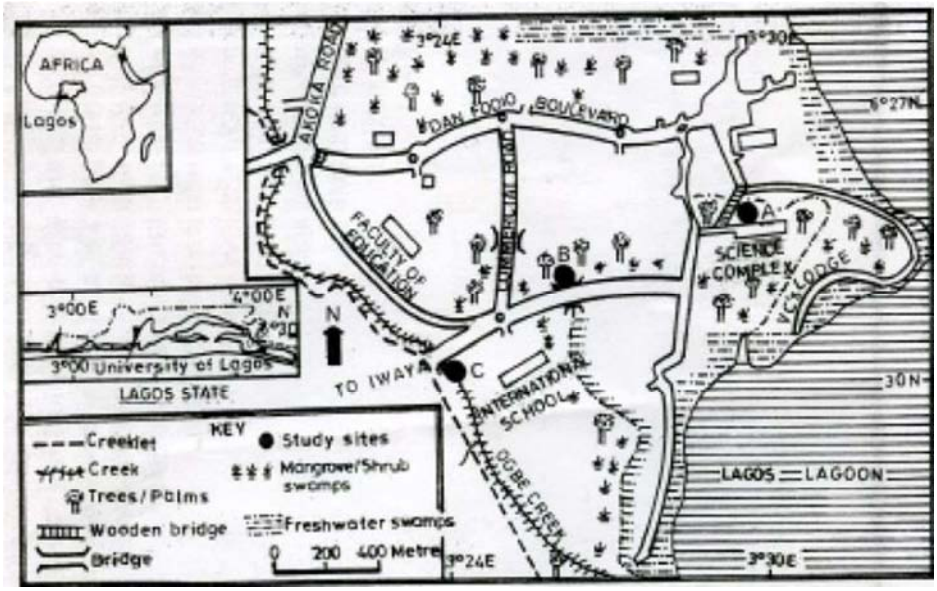

Fig. 2. *University of Lagos (study site) map.*

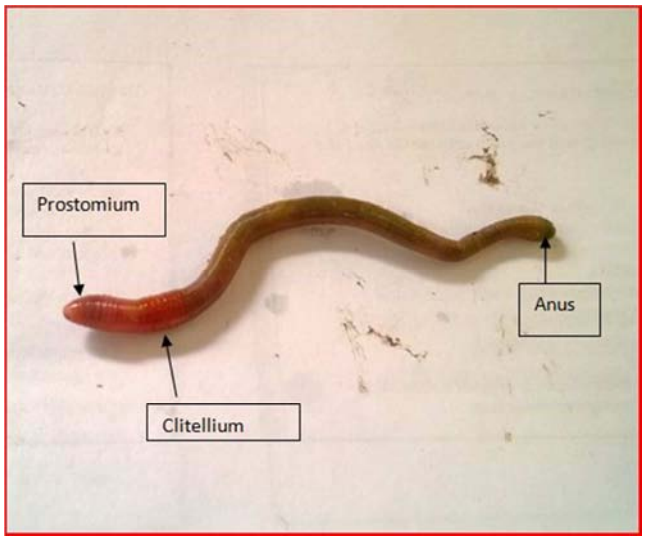

Fig. 3. *Morphology of Eudrilus eugeniae.*

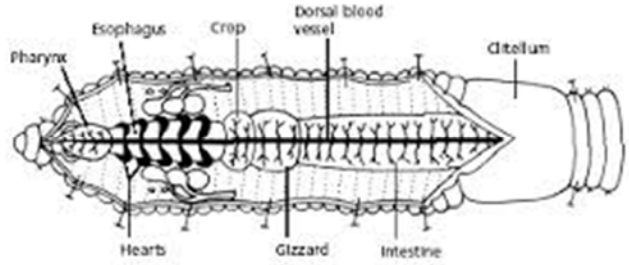

Fig. 4. *Internal anatomy of E. eugeniae.*

Fig. 5. *Collection of the earthworms being carried out.*

2.1. Weight Studies

E. eugeniae were rinsed in bowls containing clean tap water then placed on filter papers to dry before being weighed using a weighing balance in batches of six worms for each site. This was done in line with Hartnic *et al.,* 2008.

2.2. Biochemical Studies

The biochemical studies were carried out in the Department of Biochemistry, Lagos University Teaching Hospital (LUTH). Two Earthworms each were collected from six different sites from the two locations, to assess changes in enzyme activity in the whole samples.

2.3. Homogenization of Samples

The post mitochondria fraction of each animal was prepared according to Habbu *et al*., 2008. Each sample were washed in an iced cold 1.15% KCl solution, blotted and weighed. They were then homogenized with 0.1 M phosphate buffer (pH 7.2), before putting the organs each into the mortar; laboratory sand was added to it (acid washed sand) and it was blended together in the mortar with pestle. The resulting homogenate was centrifuged at a speed of 2500 rpm for 15 mins after which it was removed from the centrifuge. The supernatant was decanted and stored at -21°C until spectrophotometric determination of antioxidant enzymes activity using UV-Visible spectrophotometer.

2.4. Estimation of MDA

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of Yagi (1998). 1.0ml of the super-natant was added to 2ml of (1:1:1 ratio) TCA-TBA HCL reagent (thioarbituric acid 0.37%, 0.24N HCL and 15% TCA) tricarboxylic acid-thioarbituric acid-hydrochloric acid re-agent boiled at 100°C for 15 minutes, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 minutes. The supernatant was removed and the absorbance read at 532nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA- complex of 1.56×105M/Cm.

2.5. Estimation of GSH

The reduced glutathione content as non-protein was estimated according to the method described by Sedlak and Lindsay (1968). To the tissue homogenate, 10% TCA was added, centrifuged. 1.0ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8mg of 5, 5-dithiobisnitro benzoic acid (D TNB) in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm.

2.6. Estimation of SOD

Superoxide dismutase activity was determined by its ability to inhibit auto-oxidation of epinephrine which was estimated by the increase in absorbance at 480 nm as described by Sun and Zigma (1978). The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.02 ml of tissue homogenate and 0.03 ml of epinephrine, 0.005 N HCL was used to initiate the reaction. The reference curvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.02 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 minutes.

2.7. Estimation of CAT

Catalase activity was determined by measuring the

decrease in absorbance at 240 nm due to the decomposition of hydrogen peroxide (H_2O_2) in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of tissue homogenate in phosphate buffer (50mM, pH 7.0) and 2.9 ml of 30 mM H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient for H_2O_2 at 240 nm of 40.0 $M^{-1}cm^{-1}$ was used for the calculation (Aebi, 1984). The specific activity of catalase was expressed as moles of H_2O_2 reduced per minute per mg protein.

2.8. Histological Studies

The histological studies were also carried out at the Lagos University Teaching Hospital (LUTH). The clitellum was cut and placed inside a well labelled universal bottle containing boiuns fluid. The clitellum was then processed using a 24 hours automatic clitellum processor for a time between from 17-19 hours. The processor contains 12 beakers, 10 glass beakers and 2 thermo statistically controlled electric metal beakers containing paraffin wax with the Beaker 1 containing 10% formal saline; for complete fixation, Beaker 2-8 containing different ascending grades of dehydrating fluids (alcohol ranging from 70% alcohol to absolute) which helps to remove water from the tissue samples. Beaker 9-10 containing clearing agents (xylene I and II) which completely clears the dehydrating agent off the tissue sample. Beaker 11-12 containing embedding agents' i.e. molten paraffin wax which provides solid support upon embedding.

The clitellum after being processed is then embedded using an automatic embedding centre, Embedding is a process of submerging a tissue in a metal plastic disposable embedding mould containing molten paraffin wax. The paraffin wax becomes solidified when it is cold. These forms a support medium for the tissue during sectioning.

The sections of the clitellum are then cut using a microtome. The sections are cut and are placed in a clean grease free slide which is then placed on a hot plate for 30 minutes in order for sections to adhere to the slides.

The last method used in the histological studies is known as the staining method; the method used in this research is the H & E staining method. This method is used because it is used to demonstrate the general structure of the clitellum: The clitellum is dewaxed in xylene, sections are taken to water by using descending grades of alcohol ->95% alcohol ->70% alcohol -> water, the tissue is then stained in Haematoxyin for 10 minutes after which its being rinsed in water, differentiation is done in 1% acid alcohol (a dip), with a counter stain done using 1% Eosin for 2-5 minutes and rinsing is done in tap water, the tissue is then dehydrated using ascending grades of alcohol (70% alcohol -> 95% alcohol-> absolute alcohol), the sample is then cleared in xylene, mounted using D.P.X and viewed under the microscope.

2.9. Analysis of Polychlorinated Biphenyl (PCBs) in the Test Organism

Sample preparation and analysis were performed at the analytical laboratory of the Nigeria Institute for Oceaonography and Marine Research (NIOMR) according to the procedures; EPA 3570, Steindwandter and Shutler (1978) with slight modifications. Approximately 10.0 grams of anhydrous sodium sulfate was added to a pre-cleaned mortar and 5grams of fresh earth worm (was added to the mortar and homogenized to a complete mixture with a pestle. The mixture was carefully transferred to a pre-cleaned PTFE extraction tube which has a PTFE screw cap. 5 to 10 pre-cleaned glass beads were added. 25 ml of a mixture of hexane (1:1) was added to the 100 ml PTFE extraction tube; the extraction tube was tightly capped and allowed to stand for minimum of 20 minutes. This allows complete permeation of solvent to the matrix. 20µg/l of the internal standard decaflourobiphenyl in iso-octane directly was added to the sediment and sodium sulphate mixture. The tube was shaken vigorously until the slurry is free-flowing. Any chunks were broken manually with the glass rod, working quickly but gently. The cap was replaced immediately after the breaking of the chunks. More sodium sulfate was added and manually mixed as necessary to produce free-flowing, finely divided slurry. The samples were extracted by rotating end-over-end for at least 30 minutes. Care was taken to release pressure by opening and closing the flasks at intervals.

The solids were allowed to settle for one to two minutes. The solvent layer was filtered through a small glass funnel containing a layer of anhydrous sodium sulfate over a plug of glass wool into a receiving conical flask. The sodium sulfate was thoroughly pre-wetted with hexane before sample filtration. The sodium sulfate was rinsed with 2 to 3 mL of hexane as soon as the surface is exposed. The top of the sodium sulfate layer was not allowed to go dry. The earthworm sample was extracted twice more by adding approximately 15 mL of hexane mixture to the sample, capping the extraction tube tightly, and shaking vigorously by hand for 2 minutes. All the extracts are combined and poured into the round bottom flask of the rotary evaporator.

The round bottom flask of the rotary evaporator is placed in a constant temperature hot water bath so that the concentrator flask is partially, but not completely, immersed. The temperature of the bath was adjusted and the position of the apparatus so that, the solvent heat evenly. The sample volume was reduced to approximately 1.0 mL. The Micro Cold Solvent Extraction and Cleanup for earthworm was according to Steindwandter and Shutler, (1978).The analysis was crried out using gas chromatography/ mass spectrophotometer (GC/MS).

3. Results and Discussion

3.1. Biochemical Analysis

One sample t test was carried out for the biochemical studies and the results revealed the t value for the Olusosun study site as 10.79, 4.208, 16.265, 5.157, 4.181, and 8.169 for the GSH, SOD, CAT, MDA, GST and PRO respectively.

The t value for the University of Lagos study site was analyzed to be 7.958, 67.598, 13.200, 5.212, 75.485 and 11.495 for the GSH, SOD, CAT, MDA, GST and PRO respectively.

3.2. Histological Analysis

The histological analysis revealed that the samples collected showed increased secretory activity in the clitellum glandular for the Olusosun 1a and 1b while the Olusosun sample 2a had a normal secretory activity and the 2b had an increased secretory activity with the Olusosun 3a and 3b had an increased secretory activity all recorded in the clitellum glandular. The Unilag sample 1a, 1b, 2b, 3a and 3b had a normal secretory activity recorded also in the clitellum glandular.

The Olusosun sample 1a and 1b showed evidences of a well-developed muscle mass with the sample 2a showing evidence of a reduced muscle mass and the 2b showing evidence of a well-developed muscle mass. The Olusosun sample 3a, 3b, Unilag sample 1a and 1b also showed evidences of a well-developed muscle mass when their body wall muscle mass is studied. The Unilag sample 2a, 2b and 3b showed signs of a reduced muscle mass while sample 3a showed evidences of a well-developed muscle mass.

Analysis was also carried out on the internal visceral organs with Olusosun samples 1a and 2a having normal internal organs with samples 2a, 3a, and 3b having disorganized internal organs with sample 2b having a reduced internal organs. The Unilag samples 1a, 3a and 3b had normal internal organs with samples 1b, 2a and 2b having disorganized internal organs.

Table 1. *Histological analysis.*

Group	Clitellum Glandular Activity	Body Wall Muscle Mass	Internal Visceral Organs
Olusosun 1a	Increased Secretory Activity	Well Developed Muscle Mass	Normal Internal Organs
Olusosun 1b	Increased Secretory Activity	Well Developed Muscle Mass	Normal Internal Organs
Olusosun 2a	Normal Secretory Activity	Reduced Muscle Mass	Disorganised Internal Organs
Olusosun 2b	Increased Secretory Activity	Well Developed Muscle Mass	Reduced Internal Organ Sizes
Olusosun 3a	Increased Secretory Activity	Well Developed Muscle Mass	Disorganised Internal Organs
Olusosun 3b	Increased Secretory Activity	Well Developed Muscle Mass	Disorganised Internal Organs
Unilag 1a	Normal Secretory Activity	Well Developed Muscle Mass	Normal Internal Organs
Unilag 1b	Normal Secretory Activity	Well Developed Muscle Mass	Disorganised Internal Organs
Unilag 2a	Normal Secretory Activity	Reduced Muscle Mass	Disorganised Internal Organs
Unilag 2b	Normal Secretory Activity	Reduced Muscle Mass	Disorganised Internal Organs
Unilag 3a	Normal Secretory Activity	Well Developed Muscle Mass	Normal Internal Organs
Unilag 3b	Normal Secretory Activity	Reduced Muscle Mass	Normal Internal Organs

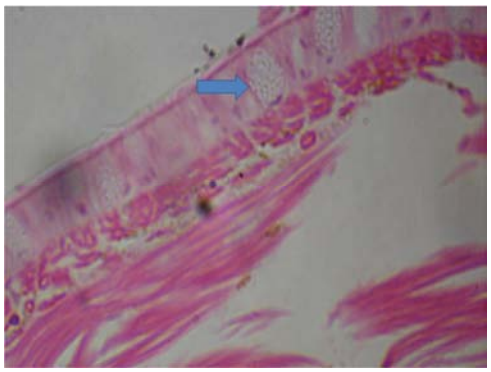

Fig. 6. *Normal mucus cocoon secreting gland activity (see arrow) and albumin secreting gland activity. H&E x400.*

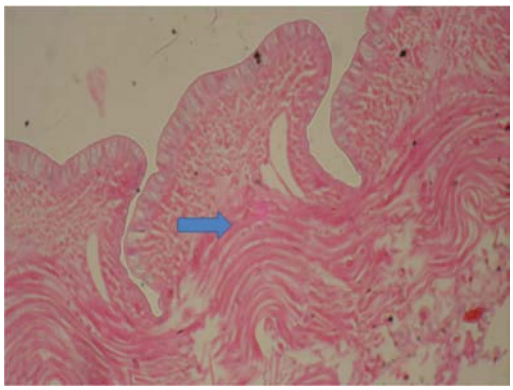

Fig. 7. *Well developed body wall mass (see arrow). H&E x100.*

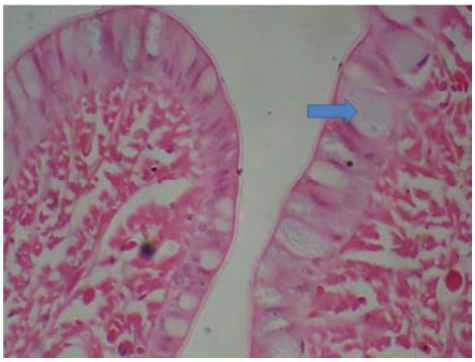

Fig. 8. *Increased mucus cocoon secreting gland activity (see arrow) and albumin secreting gland activity. H&E x400.*

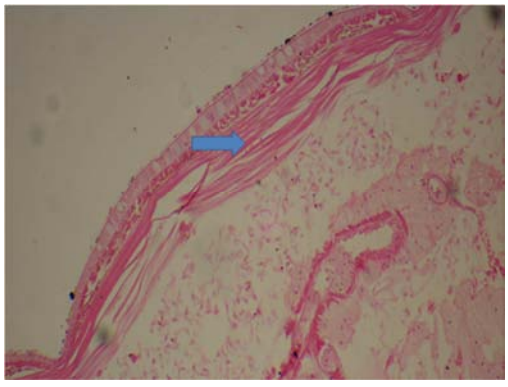

Fig. 9. *Normal body wall mass (see arrow). H&E x100.*

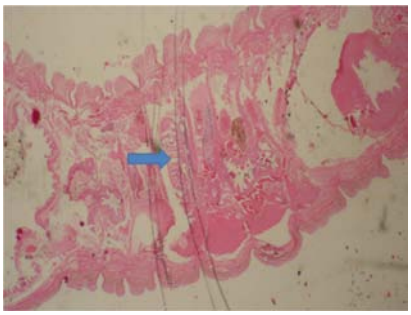

Fig. 10. *Normal internal organs (viscera). H&E x100.*

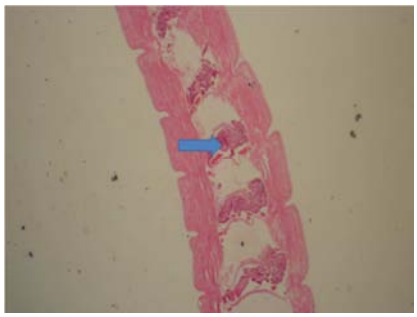

Fig. 11. *Reduction or shrinkage of internal organs (viscera). H&E x40.*

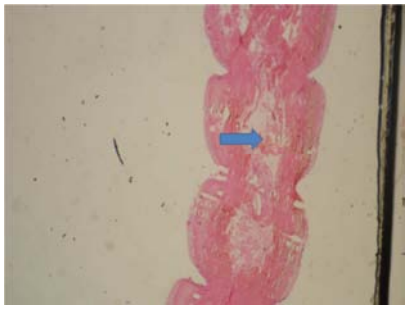

Fig. 12. *Disorganization of internal organs (viscera). H&E x40.*

Table 2. *Analysis of the various PCBs present in the clitellum from the two study sites.*

No	Name	Olusosun 1	Olusosun 2	Olusosun 3	unilag 1	unilag 2
1	PCB 8	ND	ND	ND	ND	ND
2	PCB `18	ND	ND	7.01	ND	ND
3	PCB 28	ND	ND	ND	ND	ND
4	PCB 44	ND	ND	ND	ND	628.75
5	PCB52	ND	ND	ND	ND	ND
6	PCB 66	ND	ND	ND	ND	ND
7	PCB 101	ND	ND	9.35	ND	ND
8	PCB 77	ND	ND	ND	ND	ND
9	PCB 81	ND	25.07	11.21	ND	1.31
10	PCB 105	ND	ND	ND	ND	ND
11	PCB 114	ND	ND	ND	ND	ND
12	PCB118	ND	ND	ND	ND	ND
13	PCB 128	ND	8.15	56.07	ND	ND
14	PCB123	ND	65.55	ND	169.29	ND
15	PCB137	ND	ND	ND	ND	ND
16	PCB126	ND	ND	ND	ND	ND
17	PCB170	ND	ND	ND	ND	ND
18	PCB153	ND	ND	ND	ND	ND
19	PCB156	ND	ND	ND	ND	ND
20	PCB157	ND	ND	ND	ND	ND
21	PCB167	ND	ND	ND	ND	ND
22	PCB180	ND	ND	ND	ND	ND
23	PCB169	61.96	ND	ND	ND	ND
24	PCB187	ND	ND	ND	53.57	ND
25	PCB189	ND	ND	ND	ND	ND
26	PCB195	ND	151.35	ND	895.71	ND
27	PCB206	ND	47.12	ND	337.85	ND
28	PCB209	ND	ND	ND	ND	ND

3.3. Polychlorobiphenyls (PCBs) Analysis

The analysis of the PolyChloroBiphenyls (PCBs) present in the study sites showed that only PCB 169 was detected at 61.95 µg/kg in Olusosun sample 1 with Olusosun sample 2 having 5 PCBs namely PCB 81, PCB 128, PCB 123, PCB 195 and PCB 206 at 25.07 µg/kg, 8.15 µg/kg, 65.55 µg/kg, 151.35 µg/kg and 47.12 µg/kg respectively. The Olusosun sample 3 had 4 PCBs having PCB 18, PCB 101, PCB 81 and PCB 128 at 7.01 µg/kg, 9.35 µg/kg, 11.21 µg/kg, 56.07 µg/kg respectively. The Unilag sample 1 had 4 PCBs; PCB 123, PCB 187, PCB 195 and PCB 206 at 169.29 µg/kg, 53.57 µg/kg, 895.71 µg/kg, 337.85 µg/kg respectively. The Unilag sample site 2 had 2 PCBs; PCB 44 and PCB 81 at 628.75 µg/kg and 1.31 µg/kg respectively. The study site that showed the highest number of PCBs is Olusosun 2 followed by Olusosun 3 with each having 5 and 4 PCBs, this shows that the level of pollution is higher in Olusosun site 2 when compared with other study sites studied.

During the last years, earthworm biomarkers have become increasingly relevant for the evaluation of contaminants effects on soil organisms However, the application of the biomarker approach to soil pollution monitoring, compared to aquatic environment monitoring, is recent and some aspects need to be further evaluated. First, it is necessary to identify and characterize appropriate sentinel earthworm species to be used as field collected organisms, in order to provide a quick assessment of soil pollution. Second, earthworm biomarkers studies have been mostly conducted for heavy metals. Thus, developing biomarkers of exposure/effects to a wider range of chemicals of concern for soil pollution constitutes a major demand. Third, there is a growing interest for increasing the knowledge of biological responses of earthworms to pollutants in order to standardize a suite of sensitive and reliable biomarkers for the detection of the pollutant induced stress syndrome in soil organisms. The clitellum of the earthworm have been identified as a suitable general biomarker of effect that could be included in a multi biomarker strategy (Maria G. L *et al.,* 2012). It provides a sensitive generalized response to pollutants that can integrate the combined effect of multiple contaminants present in the soil. Finally, earthworm biomarkers have been scarcely investigated under field conditions. The most studies on earthworm biomarkers have been carried out in laboratory condition, but only few studies are available which used native earthworm populations for assessment of polluted soils. Hence, there has been a growing interest in field studying earthworm biomarkers and validating their effectiveness in the field conditions as an early warning of adverse ecological effects. This represents an attractive field of research in the light of the growing interest in the use of

earthworm biomarkers as valuable tools for soil pollution monitoring and assessment.

It was found out from the biochemical analysis carried out that the clitellum of the earthworms collected from the Olusosun open dump site had higher levels of Malondialdehyde (MDA), Superoxide dismutase activity (SOD), Catalase activity (CAT), and GST when compared with the sample collected from the University of Lagos study site while high levels of Glutathione content (GSH) were only recorded from the Unilag sample when also compared with the sample from Olusosun.

The histological results showed that the 6 samples from the University of Lagos study site has a normal secretory activity going on in the clitellum glandular and 5 out of 6 samples from the Olusosun study site had an increased secretory activity going on the in the clitellum glandular, this justifies the research of Akolade L.S., 2002 which recorded that the anthropogenic activities going on around the study site have increased the amount of heavy metal contamination on the study site. It also justifies the research carried out by Ogundiran and Afolabi, 2008 which accessed the physicochemical parameters and toxicity of heavy metals on leachates collected from the solid waste open dumpsite-Olusoun in which they recorded high levels of total alakalinity, chemical oxygen demand, soluble and total solids.

The analysis of the body wall muscle mass also showed a well-developed muscle mass five out of the six samples collected from the Olusosun dump site when compared with the results from Unilag which showed reduced muscle mass for 3 out of the 6 samples collected.

The analysis of the internal visceral organs showed that 4/6 of the samples collected from Olusosun had disorganized or reduced internal organs with just two having normal internal organs. The samples collected from Unilag revealed that three samples were disorganized and three were also normal.

The PCB analysis revealed that the dump sites (Olusosun 1, 2 and 3) had 10 PCBs in total when compared with the non-dump site (Unilag 1 and 2) which had 6 PCBs. This shows that the dumpsite is more contaminated with PCBs and it was also observed that PCB 81 was very prevalent across most study sites with its presence in Olusosun 1, Olusosun 3 and Unilag 2 with PCB 206, PCB 195 and PCB 123 all having occurrence in two different sites too.

4. Conclusion

This study shows that samples from the Olusosun dump site were more contaminated and that the analysis of the internal visceral organs is a good tool in biomarker research. It also showed that the dumpsite is contaminated with PCBs than the other site examined.

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