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Effects of Dumpsite Leachates on Soil Biological Sentinels in Benin City, Nigeria

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ABSTRACT: In this study, an attempt was made to examine the effects of dumpsite leachate on soil biological sentinels by collecting leachate samples from Ikhueniro and Otofure dumpsites in Benin City Nigeria, and analyzed using standard methods. The parameters include pH, chemical oxygen demand, biochemical oxygen demand, phosphate, sulphate, chloride, nitrate, chromium, cadmium and lead. The mean results showed pH (8.30 ±0.14), BOD (566 mg/l), COD (1713 mg/l), Phosphate (7.73 ± 0.14 mg/l), Sulphate (22.40 ± 0.75 mg/l), Chloride (187.5 ± 1.12 mg/l), Nitrate (1.47 V 0.06 mg/l), Chromium (0.06 ± 0.1 mg/l), Cadmium (0.20 ± 0.1 mg/l), Lead (1.03 ± 0.04) for Ikhueniro and pH (8.0 ± 0.1), BOD (315 mg/l), COD (1095 mg/l), Phosphate (5.85 ± 0.19 mg/l), Sulphate (31.70 ± 0.73 mg/l), Chloride (135.25 ± 1.59 mg/l), Nitrate (0.93 ± 0.09 mg/l), Chromium (0.06 ± 0.1mg/l), Cadmium (0.109 ± 0.1mg/l), Lead (0.015 ± 0.005 mg/l)for Otofure respectively. Different concentrations of leachate ranging between 25% and 100% was used and the growth of *Nitrosomonas* sp reduced from 6.4×10^4(cfu/ml) – 2.9×10^4(cfu/ml), when compared to the control which increased from 6.4×10^4(cfu/ml) – 1.0×10^5(cfu/ml). The percentage survival of *Lumbricus terrestris* was 2.19%, 1.14%, 3.42%, 6.69%, with varying concentrations of 25%, 50%, 75%, and 100% respectively. The method implored for analysis shows the leachate sample was toxic to both test organisms. *Nitrosomonas sp* and *Lumbricus terrestris* may be used as indicator organisms for leachate toxicity in the soil.

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The developing trend of industrialization and modernization is generating tons of solid waste of different categories posing severe environmental threat. The activities of both the developed and developing countries are equally affecting the soil fertility. Though there are many factors affecting the soil characteristics the unscientific disposal and dumping of solid waste has become one of the major concerns due to the irreversible leachate effect (Siva and Prasada, 2016). Dumpsite leachate produced as a result of organic dissolution from dumpsites is generally heavily contaminated and consists of complex waste water that is very difficult to deal with (Longe and Enekwechi, 2007). Most waste landfills and dumpsites in Nigeria have not been designed to protect the environment from pollution. They are unlined and are located in public places surrounded by residential quarters and in wetlands or other areas with seasonally high-water tables. Classically unlined sanitary landfills and open dump are all known to release large amounts of hazardous and otherwise deleterious chemicals into nearby groundwater, surface water and soil as well to the air, via leachate and landfill gas (Allen, 2001). Following the unrelenting urbanization and largely unimpressive performance of the public sector in the provision of infrastructure in many cities in developing countries, the search for alternative strategies for urban environmental services became inevitable. One obvious consequence of rapid urbanization is the growing generation of solid wastes, and many city authorities face unprecedented challenges in managing these, including problems coping with their collection and disposal (Ogu, 2000). This has led to the excessive production of leachates. Earthworms play an important role in the soil by providing suitable aeration which allows for water percolation and plant growth. On the other hand, *Nitrosomonas sp* plays a critical role as an ammonia oxidizing bacteria by converting ammonia to nitrite. This study aims at evaluating the physiochemical properties of leachates and the effect it has on essential biological sentinels of the soil such as *Nitrosomonas sp* and *Lumbricus terrestris*

MATERIALS AND METHODS

Collection of samples: Leachate samples were obtained from both dumpsites using a sterile plastic container. Leachate samples from Ikhueniro dumpsite were collected at different locations, and mixed to form a homogenous sample; same process was repeated for Otofure dumpsite. Leachate from

Ikhueniro dumpsite was labeled as IKH while leachate sample from Otofure was labeled as OTF. Samples were then transported immediately to the lab to test for their physiochemical properties. Surface soil samples (0 -15 cm) depths were collected from soil and placed into sterile polyethene sachets to successfully isolate and identify *Nitrosomonas sp* Juvenile *Lumbricus terrestris* were used for this toxicity test, weighing and average of 2.0 g.

Isolation of Nitrosomonas sp: The methods used for isolation of *Nitrosomonas sp* from soil were adopted by Colwell and Zambruski (1972). *Nitrosomonas sp* was isolated using Winograsky medium for nitrification phase 1. Further identification and characterization of pure culture of *Nitrosomonas sp* were taken under criteria of Krieg and Holt (1994). The broth media used for identification of *Nitrosomonas sp* also served as diluents for producing the various toxicant concentrations.

Lumbricus terrestris culturing: Juvenile species of *Lumbricus terrestris* were used for the toxicity test. Small pieces of fruits (apples) was meshed and used in feeding them. Occasionally, the soil was moistened with water to ensure they were allowed a moist and cool environment to survive. Extremely dry soil is detrimental for the survival of *Lumbricus terrestri*s so this was done every 9 h.

Physiochemical analysis of leachate samples: Leachate samples collected from each dumpsite was analyzed to obtain their physiochemical properties, and most importantly to test for the presence of heavy metals. Each parameter was carried out in triplicates. pH was determined immediately after sample collection using the digital OHAUS portable pH meter. BOD was determined using the incubation method for 5 days in the dark, COD was determined using the titration method. Phosphate was determined using the Ascorbic Acid Method (Orthophosphate phosphorus) with concentration range of 0.2mg/l to 1mg/l. Sulphate was determined using a UV spectrophotometer; five series of standard solution with concentration ranging from 1mg/l to 5mg/l were prepared and analyzed with the leachate samples. Chloride was determined by titration; an aliquot of 50 ml of leachate sample against standard silver nitrate and K_2CrO_4 as indicator. Nitrate was determined using the phenol disulphunic acid method. For heavy metal analysis, leachate sample was digested with 5ml concentrated nitric acid to 125ml distilled water sample. Heavy metals such as Chromium, Cadmium, and Lead were analyzed using the AAS 220GF Spectrophotometer, AA-Buck.

Estimation of the effect of leachate on Nitrosomonas: A fresh dilution and culture was made from the *Nitrosomonas sp* slant. A loopful of the bacteria was collected from the slant and inoculated in 20 ml of peptone water and incubated for 24 h at 30 ^{o}C. The stock culture was prepared by inoculating 180 ml of sterilized peptone water with 20 ml of activated culture. Varying concentrations of the leachate samples all containing 50 ml each was then put into a 250 mL of conical flask and sterilized at 121 ^{o}C for 15 min. On cooling, 10 ml of each cell suspension was then added to each flask containing the different sludge concentration and control (dilution water). The flask were shaken thoroughly to mix, and were incubated at 30 ± 2 ^{o}C to determine the number of viable cells at 0, 8, 16, and 24 h. A 0.1 ml of each test concentration, and the control was collected from the test solution and dispersed onto the surface of an already prepared Winograsky *Nitrosomonas sp* agar plate. The plates were then incubated at 30 ^{o}C for 24 h. The same procedure was repeated for duration of 15 d. The viable cells were counted and recorded.

Estimation of the effect of leachate on Lumbricus terrestris (Earthworm): Varying concentrations of leachates from each dumpsite was used. The leachate samples were diluted to give; 0%, 25%, 50%, 75%, and 100%. A 5 species of *Lumbricus terrestris* were then inoculated into each concentration of leachate samples, making it a total of 50. Before inoculating, the earthworms were placed in a moist filter paper to void their gut contents. Earthworms were not fed during these test periods. For the effect of the pollutants in the leachate on earthworm growth to be evident, earthworm survival and body weight were determined every 12 h for 3 d (Nahmani *et al*., 2007).

RESULTS AND DISCUSSION

The pH value indicates the chemical nature of the soil. pH found in each dumpsites is a pointer that the waste contains less acidic ions, having a value of 8.30 – 8.0 (Table 1). This indicates that waste percolates in each dumpsite and becomes slightly basic (Albert and Osarumwense, 2018). COD is the measurements of the amount of dissolved chemicals that can be oxidized, while BOD measures the amount of organic carbon microbes can oxidize. The values indicates a high value of COD and BOD respectively which means anaerobic microbes will find it difficult to dissolve organic matter. It also serves as an indicator for a high level of pollution. This finding agrees with the report from Magdar and Gaber (2015). They analyzed leachate samples from the landfill in Alexandria Governorate, Egypt phosphate, sulphate, chloride and nitrite are all anionic components. High levels of these components were recorded which is a clear explanation of the high COD value that was recorded. These results are in line with Emenike *et al* (2012). They analyzed leachate from a closed sanitary landfill and the effects its toxicological components has on *Pangasius sutchi* and *Clarias batrachus*. Heavy metals such as chromium, cadmium, and lead were analyzed. Although, the values for cadmium in both dumpsites are in line with National Environmental Standards Regulatory and Enforcement Agency (NESREA) leachate regulatory values, chromium and lead recorded slightly higher values (Albert and Osarumwense, 2018).

Table 1: Physiochemical properties of leachate samples

Parameter	IKHUENIRO	OTOFURE	NESREA
pH	8.30 ±0.14	8.0 ±0.1	6.0-9.0
BOD (mg/L)	566	315	50
COD (mg/L)	1,731	1,095	90
Phosphate (mg/L)	7.73 ± 0.14	5.85 ± 0.19	2.0
Sulphate (mg/L)	22.40 ±0.75	31.70 ± 0.73	250
Chloride (mg/L)	187.50 ±1.12	135.25 ±1.59	250
Nitrate (mg/L)	1.47 ±0.06	0.93 ±0.09	10
Chromium (mg/L)	0.06 ±0.1	0.06 ±0.1	0.05
Cadmium (mg/L)	0.20 ±0.1	0.109 ± 0.1	0.2
Lead	1.03 ± 0.04	0.015 ± 0.005	0.05

All values except BOD and COD are expressed as Mean ± S.D

Table 2: Effect of leachate on survival of *Nitrosomonas sp*

Concentration (%)	*Nitrosomonas sp* Counts (cfu/ml)			
	Day 0	Day 5	Day 10	Day 15
0 (control)	6.4×10^4	7.6×10^4	9.2×10^4	1.07×10^5
25	6.4×10^4	5.9×10^4	5.5×10^4	4.8×10^4
50	6.4×10^4	5.1×10^4	4.7×10^4	4.3×10^4
75	6.4×10^4	4.6×10^4	4.2×10^4	3.7×10^4
100	6.4×10^4	4.2×10^4	3.6×10^4	2.9×10^4

The presence of heavy metals in leachate is responsible for its toxicity as heavy metals displaces important metals such as calcium, zinc, and magnesium (Albert and Osarumwense, 2018). The various level of heavy metals recorded in the leachate is capable of binding to biologically important enzymes essential for biochemical reactions to displace them which is evident by a steady decline in the growth rate of *Nitrosomonas sp* which belongs to a variety of nitrate oxidizing bacteria responsible for the second step of the nitrification process (oxidation of nitrite to nitrate). Mortality increase could also be as a result of the high levels of anionic components in the leachate leading to an increasing COD which is detrimental to microbes (Emenike *et al.,* 2012). A reduction in the growth of *Nitrosomonas sp* indicates that exposure of *Nitrosomonas sp* to highly contaminated leachate leads to the inhibition of biological processes needed for its growth and survival which could eventually become lethal; in conformity with Atuanya and Tudararo-Aherobo, (2015). As shown from the findings from this research, the growth rate of *Nitrosomonas sp* increased from 6.4×10^4 to 1.07×10^5 (Table 2), when *Nitrosomnas sp* was subjected to the test control which contained no trace of leachate. However, the growth rate of *Nitrosomonas sp* reduced from 6.4×10^4 to 4.8×10^4, 4.3×10^4, 3.7×10^4, and 2.9×10^4, using varying concentrations of 25%, 50%, 75%, and 100% respectively (Table 3). This shows that the percentage of mortality increases with a corresponding increase in the concentration of leachate. These findings are in line with Atuanya and Tudararo-Aherobo, (2015).

Table 3: Growth and survival of *Lumbricus terrestris*

Concentration (%)	Initial Weight(g)	Final Weight(g)	Weight Loss (g)	% Survival (g)
0	3.12	3.45	0.00	10
25	2.73	2.67	0.06	2.19
50	2.64	2.61	0.03	1.14
75	2.63	2.54	0.09	3.42
100	2.54	2.37	0.17	6.69

$$\%\mathrm{WT} = \frac{\mathrm{FWB} - \mathrm{IWB}}{\mathrm{IFB}} \mathrm{x}100$$

Where WT = Percentage in weight to be calculated; FWB = Final Weight biomass (g/worm); IWB = Initial Weight biomass (g/worm) and IFB = Initial fresh biomass (g/worm).

Earthworms contribute to soil aeration and are good examples of soil sentinels to test for toxicity as they are sensitive to variety of substances, represents variety of ecosystems, easy to maintain and culture under laboratory conditions. The percentage survival of the earthworms was calculated by measuring the initial weight of the earthworms and their final weight after being subjected to the leachate. The percentage survival of the earthworms decreased with an increase in the concentration of the leachate Atuanya and Tudararo-Aherobo (2015). This could be as a result of the heavy metals contained in the leachate due to the continuous dumping of metallic waste in both dumpsites.

*Conclusion***:** Toxic organic matters dissolved in the leachate samples poses a huge threat to not only the soil and its important sentinels, but also to workers in the dumpsite. Although this study was carried out to determine only the effects of leachate samples in Ikhueniro and Otofure dumpsites on soil biological sentinels which proves to be highly toxic, additional investigation needs to be carried out to explore the potential mutagenic effects on the nitrification process of *Nitrosomonas sp* and in the tissues of *Lumbricus terrestris*.

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