Biochemical and Morphological Response of corn (*Zea may*) to leachate-contaminated soil

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***Abstract*- This study was aimed at investigating the biochemical and morphological response of corn (*Zea may*) to leachate-contaminated soil. The waste is collected from the official dump site at Ebrumede and the leachate was generated by simulation. The experiment was conducted in screen house of the college of science, Federal University of Petroleum Resources Effurun Delta State. Bulk surface soil samples (0-15cm) were collected from an area in the university with a coordinate of; Elevation 33ft, N05, 34', E05, 51', and analyzed using standard methods. Three polythene pots with drainage holes at the bottom, each containing 10kg of surface soil, randomly placed on a table in the screen house in a factorial combination of three treatment levels of watering with 0%, 5%, and 10% v/v simulated leachate and the soil was designated A, B, C respectively. The soil inside pots is homogenized by stirring using a glass rod, wetted with distilled water and allowed to equilibrate for two weeks. Three seeds of maize per pot were planted. Biochemical analysis which includes lipid peroxidation, soluble protein content, and assay of antioxidant enzymes was carried out on the maize plant after 4 Weeks after Planting. From the analysis, it is assay of that the Soluble Protein Content (SPC) and malondialdehyde (MDA) content of leaves of experimental values of leaf of corn planted in PKO leachate-contaminated soil was significantly ($p<0.05$) lower than that of control, while the malondialdehyde (MDA) was significantly higher than the control ($p<0.05$), also activities of enzymes of leaves of corn in B and C were significantly ($p<0.05$) higher than that of control (A), those of A and B were not significantly different from one another ($p>0.05$). The results obtained reported that soil nutrient had been seen to be less mobile in contaminated soil and this condition had also been reported to adversely alter the growth pattern in plants. The Soluble Protein Content of the leaves of corn reduced substantially following soil contamination with leachate.**

***Keywords*- Leachate contaminated soil, Waste, Zea may, Biochemical and morphological response, Toxicity, Tissues.**

I.INTRODUCTION

Waste management in Nigeria is still at its infancy various governments have over the years set up environmental sanitation authorities to tackle waste problems but the problems seems not to have been touched at all [1]. Therefore, the country is fast becoming a dump site for locally generated waste. Water disposal is the final placement where wastes are being dumped or processed which may be chemical deposit, toxic, radioactive household waste, plastic been it and approved place or method [2]. They are several ways for dealing with waste problems, this include open dumps, sanitary land-fill, incineration and the use of facility of processing waste into manure. Open dumps entail the disposal of wastes along corners in market fronts, in burrowed pits or in drainage system. Disposing of waste in open dumps is the most commonly practice in Nigeria. It is an uncovered and hence unsightly and open to scavengers [3]. Sanitary land-fill are parcel of carefully prepared land on which waste is spread in thin layers, compacted and covered with fresh layers of soil on a daily basis. The wastes are placed in land-fill and are allowed to decompose after a while [4]. Also, companies come up with an idea of creating waste management facilities i.e. the idea of "waste to wealth" waste from various places are collected and brought to the facility whereby it undergoes various processes after segregation has taken place [5].

During the cause of managing waste through the above methods mentioned, it is deduced that leachate is being generated [6]. It occurs as a result of heavy precipitation which percolates through the wastes that has been gathered in a dump site, sanitary land-fill and in a wastes management facility and dissolved soluble waste constituents of toxic and non-toxic properties in which after days' various processes such as aerobic, anaerobic degradation take place in the waste site [7]. Even in the process of managing waste through a facility to generate manure it is still detected that leachate is being generated after micro-organisms have acted on the wastes. In the process of leachate generation, leachate is being disposed-off to the soil were plantation of crops takes place, water bodies or stored in a dug pit [8]. Leachate quality varies throughout the operational life of the landfill and long after its closure. During the early stages of waste degradation and leachate generation the composition is acidic and high in volatile fatty acids [9]. The acid leachate may dissolve other components of the wastes, such as heavy metals. The leachate also contains high concentrations of ammoniacal nitrogen and has both a high organic carbon concentration and a biochemical oxygen demand (BOD) [10].

Leachate is formed when the refuse moisture content exceeds its field capacity, which is defined as the maximum moisture that is retained in a porous medium without producing downward percolation [11]. Moisture retention is attributed primarily to the holding forces of surface tension and capillary pressure. Percolation occurs when the magnitude of the gravitational forces exceeds the holding forces [12]. Leachate formation in landfills is influenced by many factors which can be divided into those that contribute directly reedy to landfill moisture, rainfall, snowmelt, ground water intrusion, initial moisture content, irrigation, recirculation, liquid waste co-disposal, and refuse decomposition and those that affect leachate or moisture distribution within the landfill, refuse age, pre-treatment, compaction, permeability, particle size, density, settlement, vegetation, cover, sidewall and liner material, and gas and heat generation and transport [13]. The subsequent migration of leachate through the sides and/or bottom of the landfill into subsurface formations is a serious environmental pollution concern and a threat to public health and safety at both old and new facilities [14].

Leachate from a landfill varies widely in composition depending on the age of the landfill and the type of waste that it contains; it can usually contain both dissolved and suspended material [15]. The generation of leachate is caused principally by precipitation percolating through waste deposited in a landfill. Once in contact with decomposing solid waste, the percolating water becomes contaminated and if it then flows out of the waste material it is termed leachate [16]. Additional leachate volume is produced during this decomposition of carbonaceous material producing a wide range of other materials including methane, carbon dioxide and a complex mixture of organic acids, aldehydes, alcohols and simple sugars [17].

Landfill leachate is the runoff water that occurs from operational or closed landfills. Municipal landfill leachate water is often characterized by four main groups of contaminants: Dissolved organic matter, Inorganic macro components, Heavy metals, Xenobiotic organic compounds. Hazardous waste landfills are characterized by all of the above and potentially more including compounds [18]. Leachate characteristics are determined by the site, design, sol id waste, mode of operation at the landfill, as well as the age of the landfill itself. Young landfills (<5years) tend to have a lower pH as well as higher levels of COD, BOD and heavy metals. Ammonia and salinity can also be a factor no matter the age of the landfill [19].

II.MATERIALS AND METHODS

Solid wastes were collected from the official dump site at Eburumede area, close to DSC round about,Effurun , Delta State with a coordinate of; elevation 3ft, N 05º34¹,E05º49¹, Nigeria. Leachate Simulation was carried out following the ASTM method [20]. The physicochemical properties of simulated-leachate were carried out following standard method [21] and Atomic Absorption Spectrophotometer was used for the determination of heavy metals.

Leachate simulation was carried out following the ASTM method. A sample (0.3kg) of the landfill waste was shredded and packed in a 2l glass flask. A volume of distilled water, four times the sample weight, was added. The resulting sample obtained was thoroughly mixed and allowed to stand for 48hours at room temperature (25ºC). Continuous stirring was done manually at regular intervals of 2hours. After 48hours, the solid and the liquid portions were filtered and the pH of the filtrate was determined. The filtrate which represents the simulated leachate was thereafter stored at 4ºC.

The physicochemical properties of all the water samples were carried out following standard method (APHA, 1985) and Atomic Absorption Spectrophotometer (Buck 210VGP) was used for the determination of heavy metals.

The pH, temperature, moisture content, soil particle size, phosphorus, potassium, sodium, calcium, and magnesium content of the soils were analyzed using the conventional standard methods [22]. The experimental soil is loamy sand (Figure 1) and other characteristics determined at the beginning of the experiment are presented in Table 1.

The experiment was conducted in a screen house of the College of Science, Federal University of Petroleum Resources Effuruns, Delta state Nigeria. The method described by Adewole and Aboye [23] though slightly modified was used. Bulk surface soil samples (0–15 cm) were collected from an area in the University with a coordinate of; Elevation 33ft, N 05º34¹, E 05º51¹S, air-dried for seven days, sieved using 2mm sieve and analyzed using standard methods. Nine polythene pots with drainage holes at the bottom, each containing 10 kg of surface soil, were randomly placed on a table in the screen house in a factorial combination of three treatment levels of watering with (10%, 5% and 0% v/v) simulated leachate and the soil was designated C, B and A respectively. The soil inside the pots, homogenized by stirring using a glass rod, wetted with distilled water and allowed to equilibrate for two weeks. Three seeds of maize (obtained from Effurun market, Effurun, Nigeria) per pot were planted.

The maize stands were regularly watered throughout the growing stage. The maize plants were thinned to two stands per pot at two weeks after planting (WAP). The thinned stands were retained inside the pots from which they were removed so as to put back into the soil what might have been taken up by the plant within the first two weeks of growth. Biochemical analysis of the samples was collected which was carried out at 4 WAP.

Statistical tools were applied to calculate the significance of the differences between control and experimental means. P values of 0.05 or less were considered statistically significant.

Serum biochemical marker: organ tissues homogenates were evaluated for antioxidant enzyme activities; Glutathione reductase (GR) by the method of Kuk et al., (2003), superoxide dismutase (SOD) was assayed by the method of Sarkar et al., (2001), while catalase (CAT) was by the method of Sairam and Srivastava (2001). Lipid peroxidation concentration in the organ tissues homogenate and serum was by peroxidase (POD) by the method of Hernandez et al., (2000), malondialdehyde (MDA) and Ascorbate peroxidase (APX) determination by the methods of Du and Bramlage (1992) and Kuk et al., (2003) respectively. Soluble protein content (SPC) was assayed by the method of Jiang and Huang (2002).

III.RESULTS

The SPC and the MDA content of leaves of experimental corn is shown in Table 2. Generally, it was observed that the SPC values of leaf of corn planted in PKO leachate-contaminated soil was significantly ($p<0.05$) lower than that of control while their MDA was significantly higher than the control ($p<0.05$).

The activities of antioxidant enzymes of the leaf of experimental corn studied followed a definite pattern in which the activities increased as the percentage concentration of simulated leachate increased in the soil (Table 3). Although activities of enzymes of leaf of corn in B and C were significantly ($p <0.05$) higher than that of control (A), those of A and B were not significantly different from one another ($p>0.05$).

Table 1: Physicochemical characteristics of experimental soil

Property	Soil
pH (1:1 soil-water)	6.30±0.27
Organic carbon (g kg–1)	13.4±0.85
Total nitrogen (g kg–1)	91.05±5.8
Available phosphorus (mg kg–1)	23.5±1.25
K (mg kg–1)	80.3±4.2
Na (mg kg–1)	78.3±3.1
Ca (mg kg–1)	15.5±1.03
Mg (mg kg–1)	4.5±1.03
Exchangeable acidity (mg k–1)	0.7±0.02
Fe (mg kg–1)	2.8±0.44
Cu (mg kg–1)	6.4±1.01
Pb (mg kg–1)	1.3±0.01
Bulk density (g cm^{-3})	1.41±0.02
Temperature (°C)	27±0.03
Moisture (%)	13±0.05

Values are means ± SEM of three determinations.

Table 2: Effect of PKO leachate on soluble protein content (SPC) and malondialdehyde (MDA) of maize leaves

GROUPS	SPC (mg g^{-1}FW)	MDA (µmol g^{-1}FW)
A	3.00±0.12^{a}	6.37±0.23^{a}
B	2.64±0.13^{b}	7.25±0.42^{b}
C	2.21±0.11^{c}	8.64±0.36^{c}

Values are means ± SEM of six determinations, $^{a, b, c}$ Column values with different superscripts are significantly different ($p<0.05$).

Table 3: Effect of PKO leachate on activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) activities of maize leaves

GROUPS	SOD (units mg^{-1} protein)	CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	POD (Units mg^{-1} protein)	APX (µ mol ascorbate min^{-1} mg^{-1} protein)	GR (µ mol NADPH min^{-1} mg^{-1} protein)
A	110.56±3.76^{a}	130.90±3.71^{a}	430.03±4.12^{a}	500.45±4.72^{a}	74.09±1.24^{a}
B	121.67±3.31^{b}	139.13±2.89^{b}	452.24±3.78^{b}	533.08±5.01^{b}	83.11±1.35^{b}
C	129.13±2.89^{c}	145.56±3.02^{c}	460.10±3.55^{c}	549.37±4.89^{c}	87.23±1.65^{c}

Values are means ± SEM of six determinations. ^{a,b,c} Column values with different superscripts are significantly different ($p<0.05$).

IV. DISCUSSION

The present study elucidates the mechanism by which leachate affects the biochemistry and physiology underlying the growth of *Zea mays*. The study is the first documented report on the biochemical and morphological response of corn to leachate-contaminated soil. The particle size analysis of the experimental soil indicated that it is loamy sand (Figure 1) while the physicochemical analysis suggested that the soil is good to support the growth of corn (Table 1).

Soil nutrients had been reported to be less mobile in contaminated soils [24] and this condition had also been reported to adversely alter the growth pattern of plants [25]. The SPC of the leave of corn reduced substantially following soil contamination with leachate (Table 2). This could be due to difficulty in getting appropriate nutrient by the root of corn created by the leachate contamination. Reduced soil aeration due to thin film layer formation on the topsoil by the applied leachate could have reduced the air passage through the soil pores, thereby leading to the inadequate air supply to the maize plants [23] and hence, SPC. The artificial drought condition created by the leachate may also be responsible for the increased lipid peroxidation products, MDA, of leaves of experimental corn.

Corn planted in leachate showed a significant increase in SOD, CAT, POD, APX and GR activity (Table 3) in the leaves which resulted in increased lipid peroxidation and oxidative stress in the plant (Table 2). Plant stress may also lead to stomata closure, thereby reducing CO_2 availability in the leaves and inhibiting carbon fixation. This exposes the chloroplast to excessive excitation energy, which could in turn increase the generation of free radicals and induce oxidative stress [26]. The corn plant which is considered moderately drought tolerant [27] might have inadequate ROS scavenging system in addition to other tolerance mechanisms to cope with stress.

PKO leachate-contaminated soil could hinder availability of water, air and nutrients to corn roots, creating a drought condition which could induce oxidative stress in the leaves and consequently limiting growth and yield of corn plant. This is the first report on corn planted in leachate-contaminated soil, most of the previous studies focused on soil contaminated with fuel diesel and spent engine oil.

V. CONCLUSION

Experimental evidence from this study further underscores the importance of caution when channeling run-off from dump sites and industrial wastes to avoid contact with crops. Further research of soil contaminated with leachate should consider superabsorbent polymer (SAP) and various fertilizer regimens.

VI. RECOMMENDATION

From the results, the conclusion part stated that corn (*Zea mays*) responded to leachate – contaminated soil, thereby affecting the biochemical and morphological properties of the plant and also altered the properties of the soil. It is hereby recommended that the federal government should revisit the issue of waste management in our country, and farmers should be enlightened about the use of leachate. Also companies managing waste should treat their generated leachate very well before disposal.

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REFERENCES:

[1] USEPA. “Manual: Groundwater and Leachate Treatment Systems,” Centre for Environmental Research Information, 2(9): 1995, 1-64, 2008.

[2] E. B. Dede. “Realizing effective medical waste management from cradle to grave in Nigeria; being a paper presented at a conference on solid wastes management in the 21st century in Nigeria organized by the senate committee on environment and ecology held in Port Harcourt, in Nigeria”, July 5-7, 2006.

[3] T. Demiral and I. Turkan. "Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance", *J. Environ. Exp. Bot.*, 53: 247-257, 2005.
[4] M. L. Richardson. and S. Gangolli. "The Dictionary of Substances and Their Effects", vol 4. The Royal Society of Chemistry, England, 201, 1994.
[5] V. D. Mocanu. V. D. Mirca. and M. Albu. "Risk assessment of groundwater contamination from the southeastern Bucharest landfill". In: *XXVII IAH Congress: Groundwater in the Urban Environment* (edited by Chilton, P. J.) 1. Balkema, Rotterdam, 1997.
[6] R. L. Bradley. J. W. Fyles and B. Titus. "Interactions between Kalmia humus quality and chronic low C inputs in controlling microbial and soil nutrient dynamics", *J. Soil Biol. and Biochem*., 29: 1275–1283, 1997.
[7] A. J. Cooke. R. K. Rowe. B. E. Rittmann. J. VanGulck. and S. Millward. "Biofilm growth and mineral precipitation in synthetic leachate columns", *J. Geotech. Geoenviron. Eng*. ASCE 127 (10): 849–856, 2001.
[8] I. R. Fleming. R. K. Rowe. and D. R. Cullimore. "Field observations of clogging in a landfill leachate collection system", *J. Can. Geotech*, 36 (4): 289–296, 1999.
[9] J. A. Hernandez. A. Jimenez. P. Mullineaux. and F. Sevilla. "Tolerance of pea (*Pisum sativum L.*) to long-term salt stress is associated with induction of antioxidant defenses", *J. Plant Cell. Environ*., 23: 853-862, 2000.
[10] D. L. Jensen. and T. H. Christensen. "Colloidal and dissolved metals in leachates from four Danish landfills. Water Res", 33 (9): 2139–2147, 1998.
[11] R. D. Cameron. and F. A. Koch. "Toxicity of Landfill Leachates," *J. World Pol. Cont. Fed*, 52, 760-769, 1980.
[12] J. B. Brice. F. B. DeWalle. S. K. Chian. and J. B. Asce. "The Chemical Composition of Leachates Derived from Various Fractions of Domestic Refuse", AERE. R-10938, Harwell, 1984.
[13] H. S. Ehrig and R. Stegmann. "Leachate Production and Quality Results of Landfill Processes and Operation", Proceedings of 2nd International Landfill Symposium, Sardinia, 223-229, 1989.
[14] A. Y. Sangodoyin. "Groundwater and Surface Water Pollution by Open Dump in Ibadan, Nigeria", *J. Disc. and Innov*., 2(1): 37-43, 1991.
[15] G. J. Farquhar. "Leachate Production and Characterization", *Canada J. Civil Eng*,16: 317-325, 1989.
[16] S. C. Alker. C. P. Baccinq. and J. B. Brice. "The Composition of Leachate from Waste Disposal Sites", Green, Bolton, 121-135, 1993.
[17] T. H. Christensen. R. Cossu. and R. Stegmann. "Organic Carbons in Landfill Leachates", Proceedings Sardinia 99, 7th International Waste Management and Landfill Symposium, 4: 117-124, 1999.
[18] FEPA. "National Interim Guidelines and Standards for Industrial Effluents and Water Quality Tests", Federal Environmental Protection Agency, 32-59, 1991.
[19] M. Loizidou. and E. G. Kapetanios. "Effect of leachate from landfills on underground water quality", *J. Sci of the Tot Environ*, 128: 69-81, 1993.
[20] O. Adeyemi. O. B. Oloyede. and A. T. Oladiji "Effect of Leachate-Contaminated Groundwater on the Growth and Blood of Albino Rats", *Intl. J. Haem*, 3(2): 1, 2007.
[21] APHA. "Standard Methods for the Examination of Water and Wastewater," 15th Edition, American Public Health Association, New York, 1998.
[22] APHA/AWWA. "Standard methods for the examination of water and waste water quality", *J. Environ. Toxicol*. Water qual. 11, 72-82, 1985.
[23] M. B. Adewole. and A. O. Aboyeji. "Yield and quality of maize from spent engine oil contaminated soils amended with compost under screen house conditions", *J. Agrobiol* 30(1): 9–19, 2013.
[24] E. J. Atuanya. "Effect of oil pollution on physical and chemical properties of soil: A case study of waste oil contaminated delta soil in Bendel State, Nigeria", *J. Appl Sci* 55: 155–176, 1987.
[25] I. E. Okon. and E. E. Udofot. "Response of Telfairia occidentalis (Hook) to arbuscular mycorrhizal fungi and Gliricidia sepium leaves manure in spent engine oil contaminated soil ", *World J. Agric Sci* 8: 20–25, 2012.
[26] S. Mao. M. R. Ilsam. Y. Hu. X. Qian. F. Chen and X. Xue. "Antioxidant enzyme activities and lipid peroxidation in corn (*Zea mays L.*) following soil application of superabsorbent polymer at different fertilizer regimes", *Afri. J. of Biotech* 10(49): 10000-10008, 2011.
[27] M. R. Islam. Y. Hu. S. Mao. P. Jia. A. E. Eneji and X. Xue. "Effects of water-saving superabsorbent polymer on antioxidant enzyme activities and lipid peroxidation in corn (*Zea mays L.*) under drought stress", *J. Sci. Food. Agric*., 91: 813-819, 2011.