African Scientist Vol. 20, No. 2 June 30, 2019 1595-6881/2019 $10.00 + 0.00
Printed in Nigeria

http://www.niseb.org/afs

AFS 2019066/20207

Effect of Municipal Dumpsite on Vegetation and Soil

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(Received May 26, 2019; Accepted in revised form June 23, 2019)

ABSTRACT Comparative assessment of the soil and vegetation in a municipal dumpsite at Iguomo, Benin City, Nigeria at different distances from the dumpsite was carried out. An area of $30m^2$ was marked out and divided into three plots of 10 m^2 each and these were designated as plots A, B and C. An area of 10 m^2 was also taken at a distance of 100m away from the dumpsite, it served as the control plot or plot D. Population sampling was done to determine the species diversity across the plots. Soil samples were collected from each plot and microbial and physico-chemical analyses were carried out. Population studies showed that the closest plot to the dumpsite had the highest diversity among the plots. Values of 1.31, 1.04, 0.77 and 0.63 were recorded for species richness for plots A, B, C and D. Diversity indices for the plots were 0.96, 0.82, 0.64 and 0.58 respectively. Similar results were obtained for microbial analyses with bacterial counts of 1.10×10^5 ,7.0×10^4,4.2×10^4 ,6.7×10^4 cfu and fungal counts of 2.8×10^4, 6.0×10^3, 3.0×10^3, 1.4×10^4 cfu recorded for plots A, B, C and D respectively. The result of physico-chemical analyses showed that treatment soil samples contained heavy metals which decreased with distance away from the dumpsites. It was also observed that the high soil nutrient level masked the effect of the heavy metals on the plants.

Keywords: Dumpsite, Heavy metals, Population, Vegetation

Introduction

Nearly all human activities generate waste and the way in which this is handled, stored, collected and disposed of, can pose risks to the environment and to public health (Zhu *et al*., 2008). The earth is very good at recycling waste, but when the amount of wastes generated is far more than the earth can cope with; it poses a big threat to lives, a phenomenon called pollution. Pollution occurs at different levels and affects all lives ranging from plants, animals to man (Skye, 2006). The decay of these solid wastes releases substances that can affect the soil nutrients content, and increase the concentration of heavy metals in the soil, thereby altering the natural balance of nutrients available for plant growth and development while affecting species diversity and agricultural productions. In Nigeria, like in other developing countries, open dump is the only available option for solid waste disposal in the cities. Chopra *et al*. (2009) described waste dumps practices as the disposal of solid waste by infilling depressions on land. The depressions into which solid wastes are often dumped include valleys and excavations. Diversity of vegetation is directly influenced by soil characteristics. Pollution of soil by leachate from surrounding municipal waste dumps has been recognized for a long time (Banar *et al.*, 2006; Tahri *et al*., 2005; Lin *et al*., 2002; Amadi *et al*., 2010). The negligence of the effects of unlined waste dumps on the host soil and underlying shallow aquifers in Nigeria characterized by largely unconfined, porous and high permeable aquiferous system is worrisome. Studies have shown that soil and groundwater system can be polluted due to poorly designed waste disposal facilities, leakage from underground storage tanks and agricultural wastes. Soil and groundwater acidification and nitrification have been linked to waste dumps (Bacud *et al*., 1994) as well as microbial contamination of soil and groundwater system (Awomeso *et al*., 2010). Sia Su (2008) attributed

cancer, heart diseases and teratogenic abnormalities to groundwater contamination via leachate from waste dumps. The contamination of soil by heavy metal can cause adverse effects on human health, animals and soil productivity (Smith *et al.*, 1996). Over the last many years, heavy metals have considerably damaged the soil quality and fertility in consequence of increased environmental pollution from industrial, agricultural and municipal sources (Adriano, 1986). Metals cause physiological disorders in soils as absorption through root system consequently retards plant growth and deprives it of vigour (Moustakas *et al*., 1994). Soil is usually the mostly polluted part of the ecosystem around dumpsites because the seepage of water through the waste dump leaches out undesirable components that pollute it as the main medium of transporting and distributing chemicals elements. Contaminants like heavy metals, cyanides, radioactive substance and industrial chemicals are substance that are not only dangerous in themselves but can greatly react in a way that their total effects can always be greater than the sum of the effects taken independently with other materials. Research has shown that the soil in and around dumpsites is usually nutrient rich as well as heavily contaminated with heavy metals.

Materials and Methods

Study area: Samples were collected from a municipal dumpsite at Iguomo, along Lagos-Benin express way Benin City, Edo State.

Sample collection: Soil samples were collected from an average depth of about 15 cm. the soil samples were stored in plastic bottles and taken to the laboratory for microbial, physic-chemical and heavy metal analysis.

Microbial analysis

Sterilization of materials: The glass wares used for this study were thoroughly washed with detergent and rinsed with distilled water. The glass wares such as beakers, conical flasks, test tubes and pipettes were wrapped with aluminum foil and appropriately sterilized in the hot air oven at 60^{o}C for 1 hour. The culture media were sterilized in an autoclave at 121^{o}C for 15 minutes. Inoculating wire loop was sterilized by dipping in 70% ethanol and then flamed in Bunsen flame.

Preparation of media: Media for microbiological analysis was weighed according to the manufacturer's specifications.

Nutrient agar: 28 g of nutrient agar were dissolved in 1000 ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was then placed in an autoclave to sterilize it for 15 minutes at 121 ^{o}C. After sterilization, the flask was allowed to cool.

Potato dextrose agar: 39 g of potato dextrose agar were dissolved in 1000ml of distilled water in a conical flask corked with cotton wool and foil paper and allowed to dissolve in 1000 ml of distilled water in a conical flask. The medium was then placed in an autoclave to sterilize it for 15 minutes at 121^{o}C. After sterilization, the flask was allowed to cool.

Isolation of microorganisms: 10 g of each soil samples was mixed with 90ml of sterile distilled water in a beaker. Then the samples were serially diluted using tenfold serial dilution and 0.1 ml of the appropriate dilution pour plated onto nutrient agar (NA) and potato dextrose agar (PDA) respectively for bacteria and fungi isolations. The nutrient agar plates were incubated at 37 ^{o}C for 24 hours under aseptic condition. While Potato dextrose agar plates were incubated at 28 ^{o}C for 72 hours.

Identification of microbial isolates: Aseptically purified representative discrete colonies were obtained by streaking on NA and PDA plates. They were stored in agar slants for further characterization. All the bacteria isolated were initially examined microscopically. They were later referred to appropriate genus and species following various physiological and biochemical tests (gram staining, indole catalase, motility, citrate utilization, urea production, oxidase, coagulase and oxidative/fermentative utilization of lactose and glucose). Similarly, the fungal isolates were identified using their cultural and morphological characteristics.

Sub-culturing: The isolates were sub cultured to obtain pure cultures. The pure cultures so obtained were transferred to agar slants by streaking, and from there further biochemical tests were carried out to identify them.

Identification of isolates

Biochemical tests characterization were used to identify the isolates. The biochemical tests performed include: catalase, oxidase, coagulase indole, citrate, urease, glucose and lactose fermentation test.

Gram staining: Smears of the isolates were prepared and heat fixed on clean grease free slides. The smears were stained for one minute with crystal violet. This was washed out with distilled water. The slides were flooded with dilute Grams iodine solution for one minute. This was washed off with distilled water and the smears were decolorized with 95% alcohol for 30 seconds and rinsed off with distilled water. The smears were then counter stained with saffranin solution for one minute. Finally, the slides were washed off with distilled water, air dried and observed under oil immersion objective.

Spore stain: The malachite green staining method was used. Smears of the pure isolates were made on grease free glass slide and heat fixed. The slides were flooded with 5%w/v malachite green solution. The slides were flamed in such a way that the stain steamed but did not boil. The slides were then allowed to stand for 5 minutes. The stains were washed out in running tap water. The smears were counter stained with saffranin for 30 seconds. This was washed off and the slides were blotted, dried and examined under the oil immersion objective. The spores stained green while vegetative cells stained red.

Biochemical tests

Catalase test: This test was used to demonstrate which of the isolates could produce the enzyme catalase that releases oxygen from hydrogen peroxide. This test is usually used as an aid to differentiate staphylococci from streptococci and to differentiate other catalase positive organism from catalase negative. A loopful of the pure colony was transferred on to a plane, clean glass slide. The isolate was then mixed with a drop of 3% (v/v) hydrogen peroxide. The reaction was observed immediately. Gas production indicated by the production of gas bubbles confirmed the presence of catalase.

Coagulase test: The slide method was used. In slide test, clean slide was divided into two sections, to one section of the slide the test organism was smeared on it using a sterile wire loop while a drop of distilled water was added to the other section which serves as control. Then human plasma was added to both sections and the slide was rock gently for some minutes. A clumping/agglutination of the plasma was used to indicate the presence of coagulase.

Oxidase test: This was carried out to identify bacterial species that will produce the cytochrome oxidase enzyme. *Pseudomonas aeruginosa* and *Escherichia coli* were employed as positive and negative controls respectively. A piece of filter paper was placed in a clean Petri dish and 2-3 drops of fresh oxidase reagent was added. A colony of test organism was collected using a wire loop and smeared on the filter paper and observed. Purple colour within a few seconds showed a positive test.

Urease test: This test was used to demonstrate the ability of the isolates to produce the enzyme urease which splits urea forming ammonia. The test is usually used to differentiate organisms like Proteus from other non-urease positive organisms. A loopful of the isolates was used to inoculate a tube of urea-agar. The tubes were incubated at 37 ^{o}C. A change in colour from yellow to red confirmed the presence of urease.

Indole test: This test was used to determine which of the isolates has the ability to split indole from tryptophan present in buffered peptone water. The test is usually used as an aid in the differentiation of gram-negative bacilli especially those of the enterobacteriaceae. Tubes of peptone water were inoculated with young culture of the isolates. The tubes were incubated at 37 ^{o}C for 48 hours about 4 drops of KOVAC reagent were added into 1ml of each of the culture tubes. Positive test was indicated by the red colour that occurs immediately at upper part of the test tube. KOVAC's reagent consists of the following; 150ml of Amyl alcohol, 10 g of 0-Dimethyl amino benzaldelyele and 50 ml of concentrated hydrochloric acid.

Citrate utilization test: This test was used to identify which of the isolates can utilize citrate as the sole source of carbon for metabolism. The test is usually used as an acid in the differentiation of organisms in the enterobacteriaceae and most other genera (Baker, 1976). The medium used for this test is Simon's citrate agar. Slant types of Simon's citrate agar were inoculated with young culture of the isolates. The inoculation was done by stabbing the medium on the tubes using sterile straight inoculation wire containing the culture. The tubes were then incubated at 370 ^{o}C for about 24 hours. A change in colour from green to blue after about 24 hours of incubation indicated positive result.

Sugar fermentation test: Each of the isolates was tested for its ability to ferment a given sugar with the production of acid and gas or acid only. Since most bacteria especially Gram negative bacteria utilize different sugars as source of carbon and energy with the production of either acid and gas or acid only, the test is used as an aid in t heir differentiation. The growth medium used was peptone water and the peptone water was prepared in a conical flask and the indicator phenol red was added. The mixture was dispensed into test tubes containing Durham's tubes. The tubes with their content were sterilized by autoclaving at 121^{o}C for 15 minutes. 1% solution of the sugar was prepared and sterilized separately at 115 ^{o}C for 10 minutes. This was then ascetically dispensed in 5 ml volume into the tubes containing the peptone water and indicator. The tubes were inoculated with young culture of the isolates and incubated at 37 ^{o}C. Acid and gas production or acid only were observed after about 24 hours of incubation. Acid production was indicated by the change of the medium from light green to yellow colour while gas production was indicated by the presence of gas in the Durham's tubes.

Lactophenol cotton blue test: Each fungal isolate was mixed with a drop of lacto phenol cotton blue stain on grease-free glass slide and covered with a cover slip and viewed under x10 and x40 objective of the microscope.

Soil physicochemical analysis

Sample preparation: Soil samples were air dried for 3-4 days at room temperature. These were then ground to pass a 2 mm stainless steel sieve to produce "fine earth" for the physicochemical analysis.

pH and electrical conductivity: 20 g of fine soil was placed in a container and 50 ml of distilled water added. The suspension was shaken for 30 minutes and allowed to settle. Electrical conductivity and pH of the solution

were then measured using a pH meter and conductivity meter. The pH meter was first standardized using a buffer solution.

Nitrogen: 1 g of the soil sample was placed into Kjedahl digestion flask. One tablet of a catalyst and 20ml concentrated tetraoxosulphate acid was added and the mixture was hand shaken to ensure mixing. At completion of digestion, 10 ml distilled water was added and the solution was filtered through a Whatman filter paper. Nitrogen was determined calorimetrically at 625 nm.

Organic carbon: 1g of the soil sample was placed in a 250 ml conical flask. Then 10ml of $K_2Cr_2O_7$ and 20 ml H_2SO_4 were added and the mixture was hand shaken for about 5 minutes. Distilled water was then added to make the volume up to 150 ml. 10ml of phosphoric acid and 8 drops of diphenylamine solution were then added. A blank determination was done by using 10 ml $K_2Cr_2O_7$ and 20 ml H_2SO_4 solution and titrated to a green colour with 0.1 N Ferrous Ammonium sulphate solution.

TOC was calculated as:

$$\% \text{ TOC} = \frac{\text{Titre value of blank-titre value of sample x } 0.3\text{xM}1.334}{\text{Weight of sample}}$$

Available phosphorus: 1 g of soil was shaken for 5 minutes with 10ml of extracting solution containing 0.03 NH4F and 0.1 HCl. The solution was filtered through Whatman filter paper and 3 ml of the filtrate was transferred into a test tube and 3 ml of ammonium molybdate was added. Thereafter, 5 drops of mixture of boric acid, sodium sulphite and sodium sulphate were added. The phosphorus content was determined calorimetrically.

Cation exchange capacity: 5 g of soil placed into sterile conical flask and 20 ml of extracting solution (NH_4OAc) was added into the 250 ml volumetric flask containing the soil samples. Whatman filter paper was then used to filter the solution. Also 0.1 ml of the filtrate was transferred to a test tube and diluted with 10 ml 0.015% strontium chloride solution. The sample was analyzed for Na and K by flame emission and for Ca and Mg by Atomic Absorption Spectrophotometry (AAS).

Particle size analysis: 12 g of air-dried, 2 mm soil sample was pretreated first with 20 ml hydrogen peroxide and dried at 80 ^oC. The soil was further treated with 20 ml hydrogen peroxide and then dried at 100 ^oC. The soil was then cooled and weighed. The mineral soil was shaken overnight with 100 ml water and 10 ml of solution containing sodium metaphosphate and Na_2CO_3. The mixture is quantitatively transferred to a 250 ml graduated cylinder and enough water added to make up the total volume to 200 ml and shaken. 25 ml of the suspension was pipetted out from a depth of 6cm, evaporated to dryness and the weight taken. This weight representing 1/8 of the clay fraction was multiplied by 8 to get the corrected weight. The rest of the slurry was washed through a 50 µm sieve and the fraction retained on the sieve quantitatively recovered, dried and weighed. This represents the sand fraction. Percent clay and %sand are calculated on the basis of the weight of the soil. The silt content (%) was determined by difference.

Sample preparation for analysis of metals: Soil samples were ground into fine powder. 2 g portions of the samples were weighed accurately and 10 ml of concentrated HNO_3 was added to each. The samples were digested on a hot plate for 15 minutes. The digest was cooled and 5ml of concentrated nitric acid was added and heated for additional 30 minutes. The later step was repeated and the solution was reduced to about 5 ml without boiling. The sample was cooled again and 5 ml of concentrated hydrochloric acid and 10ml of distilled water was added and the samples were heated for additional 15 minutes without boiling. The sample was cooled and filtered through a Whatman No. 42 ash less filter paper and diluted to 60ml with distilled water. Metal content in the digested samples were analyzed for Cu, Zn, Cd, Mg, Ca, Pb, Mn and Ni using Atomic Absorption Spectrophotometer.

Results

Table 1 shows the families and species of plant present in the study area. Results show that some species were present in some of the treatment plots but not in the others. *Centrosema pubescens* and *Chasmanthera dependens* were found only in control plot. *Chromolaena odorata* was the only species common in all plots.

Table 2 shows the species richness and diversity indices of the study area. Species richness and diversity was highest in plot A and decreased with increasing distance from the dumpsite. Values of 1.31, 1.04, 0.77 and 0.63 were recorded for species richness for plots A, B, C and D. Diversity indices for the plots were 0.96, 0.82, 0.64 and 0.58 respectively.

Table 3 shows the microbial count of soil samples. Bacterial and fungal counts were higher in plot A as compared to other plots. Control plot also recorded a high microbial count. Bacterial counts of 1.10×10^5, 7.0×10^4,4.2×10^4, 6.7×10^4 cfu and fungal counts of 2.8×10^4, 6.0×10^3, 3.0×10^3, 1.4×10^4 cfu were recorded for plots A, B, C and D respectively

Table 4 shows the occurrence and distribution of microbial isolates. Plot A and D had the highest bacteria and fungi isolates with plot B having the least bacteria isolates. *Aspergillus* sp. was common to all plots.
Table 5 shows the physico-chemical properties of the soil sample. All plots recorded a high pH value with plot B having the highest pH value. Essential nutrients and heavy metal content was higher in Plot A and values decreased with increasing distance from the dumpsite.

Table 1: Families, species and their number present in the study area

Family	Species	Plot A	Plot B	Plot C	Plot D
Amaranthaceae	*Amaranthus spinosus*	75	50	25	-
Asteraceae	*Aspilia africana*	-	100	275	-
Acanthaceae	*Asystasia gigantica*	150	-	-	-
Fabaceae	*Centrosema pubescens*	-	-	-	50
Menispermaceae	*Chasmanthera dependens*	-	-	-	100
Poaceae	*Chloris pilosa*	50	-	-	-
Asteraceae	*Chromolaena odorata*	125	50	25	275
Cleomaceae	*Cleome foetida*	75	-	-	-
Commelinaceae	*Commelina benghalensis*	-	150	-	-
Cucurbitaceae	*Cucumeropsis manni*	125	250	150	-
Convolvulaceae	*Ipomea involucrata*	150	-	-	-
Poaceae	*Panicum maximum*	75	50	50	-
Passifloraceae	*Passiflora foetida*	25	-	150	25
Solanaceae	*Physalis angulate*	100	50	-	150
Portulaceae	*Talinum triangulare*	-	75	-	-
Total number of individuals		950	825	675	600
Total number of species		10	8	6	5

Table 2: Species richness and diversity indices of the study area

Parameter	Plot A	Plot B	Plot C	Plot D
Margalef's Index(d)	1.31	1.04	0.77	0.63
Shannon-wiener Index(H)	0.96	0.82	0.64	0.58
Evenness Index€	0.96	0.9	0.82	0.83

Table 3: Microbial count of soil samples (cfu/g)

Treatment	Bacterial count	Fungal count
Plot A	1.10×10^5	2.8×10^4
Plot B	7.0×10^4	6.0×10^3
Plot C	4.2×10^4	3.0×10^3
Plot D	6.7×10^4	1.4×10^4

Table 4: Occurrence and distribution of microbial isolates

Treatment	Bacterial isolates	Fungal isolates
Plot A	*Flavobacterium sp.* *Pseudomonas sp.* *Bacillus sp.* *Micrococcus sp.* *Clostridium sp.* *Klebsiella sp.*	*Fusarium sp.* *Mucor sp.* *Aspergillus sp.* *Penicillium sp.* *Saccharomyces* *Trichoderma sp.*
Plot B	*Bacillus sp.* *Staphylococcus sp.* *Pseudomonas sp.*	*Aspergillus sp.* *Mucor sp.* *Penicillium sp.*
Plot C	*Pseudomonas sp.* *Klebsiella sp.*	*Aspergillus sp.* *Fusarium sp.*
Plot D	*Enterobacter sp.* *Bacillus sp.* *Escherichia coli* *Klebsiella sp.*	*Aspergillus sp.* *Fusarium sp.* *Penicillium sp.*

Table 5: Physicochemical parameters of soil samples

Parameters	Plot A	Plot B	Plot C	Plot D
pH	7.7	8.2	8.1	7.7
Conductivity	19.4	10.3	8.5	5.7
Carbon (%)	5.32	3.27	1.6	1.34
Nitrogen (%)	0.16	0.11	0.09	0.14
Phosphorus (%)	8.25	5.32	6.96	3.07
Sand (%)	45.7	63.8	56.3	61.4
Silt (%)	4.4	4.9	7.6	5.6
Clay (%)	49.9	31.3	36.1	33
Ca (meq/100g)	2.94	2.8	2.45	1.94
Mg (meq/100g)	1.84	1.53	1.25	0.69
Na (meq/100g)	1.03	0.74	0.96	0.17
K (meq/100g)	0.58	0.51	0.42	0.33
Ni (mg/kg)	6.82	2.23	1.69	0.63
Mn (mg/kg)	72.57	20.6	34.57	8.7
Cr (mg/kg)	1.39	0.97	1.03	0.14
Cd (mg/kg)	0.82	0.64	0.39	0.05
Pb (mg/kg)	6.71	2.82	2.39	0.37
Zn (mg/kg)	80.46	73.11	51.4	3.26
Fe (mg/kg)	913.7	605.4	495.1	41.57
Cu (mg/kg)	6.54	4.86	2.2	1.18

Discussion

In the present study, results from population sampling revealed that species were able to thrive in dumpsites which could be as a result of the presence of organic matter in addition to heavy metals found in dumpsites. Species richness and diversity were favoured by the dumpsite. This could be as a result of high organic matter in the dumpsite. This is however contrary to the observation of Ali *et al.* (2013) who stated that the vegetation status of two sites studied showed that rather than composition, diversity is suffering due to open dumping of

wastes. Also stating that large number of vegetation species were supported by soils of control sites while less number of species was recorded at dumping site.

The result on microbial biomass revealed that the closest plot to the dumpsite had the highest bacterial and fungal counts. This can be attributed to the higher percentage of carbon there in. Microbial biomass increases with increasing organic carbon contents in soil as organic carbon is the main food and energy supply for the microorganisms (Edegbai and Agbo, 2016).

The soil pH was high in all treatment plots. Soil pH generally has a major contribution in metal bioavailability, toxicity and leaching capability into the surrounding areas (Chimuka *et al.*, 2005). The closest plot to the dumpsite recorded the highest amount of essential nutrients and heavy metals. Heavy metal content decreased with increasing distance from the dumpsite. This is in line with the findings of Adefemi and Awokunmi (2009) who stated that high concentrations of Cu, Mn, Fe, Pb and Zn were found in soil samples taken at the centre of the dumpsites while lower concentrations was obtained in soil samples taken at a distance 20 m away from the centre of the dumpsite. This is also in line with the discovery of Magaji (2012) that the concentrations of heavy metals in all the samples cultivated around a dumpsite were higher than those from the control site. The effect of the heavy metals can however be said to have been masked by the essential elements also present in the dumpsite.

Conclusion

The findings in this study corroborate earlier researches which observed that soils in and around dumpsites are usually nutrient rich as well as heavily contaminated with heavy metals. The major reason why we should be worried about open dumpsites is that these heavy metals will remain in the soil and may change location and thereby have an adverse effect on the ecosystem.

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