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Effects of Plastic Pollution of Soil on the Growth and Survival of Bacteria and Fungi

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ABSTRACT: The study examined the effect of plastic waste on soil bacteria and fungi. The test soil samples were collected from Lokoja international market waste dump site and the control soil sample was collected from non plastic contaminated garden in Salem University, Lokoja. The samples were analysed using Gas chromatography with mass spectrometer. The test soil sample soil sample had high quantity of plastic contaminant which were Methylene chloride 17.45mg/kg, hexane 10.05mg/kg, chloroform 1.56mg/kg, toluene 5.87mg/kg, tetrachloroethylene 1.48mg/kg as compared to the control garden soil sample, which had methylene chloride 0.54mg/kg, hexane 0.26mg/kg, chloroform 0.31mg/kg, toluene 5.87mg/kg and tetrachloroethylene 0.01mg/kg. The result showed the presence of plastic in the soil and it effect on bacteria and fungi. The totals of 11 bacteria were isolated from both soil samples using nutrient agar. The bacteria isolated are; *Corynebacterium* spp. (12%), *Enterobacter* spp. (8%), *Acinetobacter* spp. (6%), *Escherichia coli* (16%), *Epidermis, Bacillus subtilis* (15%), *Serratia* sp. (8%). *Proteus* spp. (4%), *Micrococcus luteus* (7%), *Flavobacterium* spp. (10%), *Pseudomonas aeruginosa* (15%). *Micrococcus luteus, Flavobacterium* spp. *and Pseudomonas aeruginose* were not isolated in plastic composted soil sample due to the presence of plasticizers. The total of 6 fungi were isolated, namely *Penicilliun expansion* (12%), *Sacchromyces* sp. (24%), *Aspergillus niger* (19%), *Fusarium* spp. (20%), *Rhizopus stolonifer* (10%) and *Mucor piriformis* (15%). This study reveals the effect of plastic waste, as it inhibits the growth of microorganism that is important for soil activities, thereby reducing the soil nutrients, fertility and productivity.

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The presence of plastics in the environment has widely been recognized as a global issue. It represents one of the most challenging anthropogenic phenomenon that affects our planet and it's among the major threats to biodiversity due to potential entanglement and ingestion (Shimao, 2001).The issue of plastic pollution in aquatic environments (marine and freshwater) has been gaining increasing attention; the problem of plastic contamination in the terrestrial environment has remained widely unexplored. Plastic pollution may be more dramatically seen in the oceans; however, more than 80% of the plastics found in marine environments has been produced, consumed and disposed of on land (Wiles and Scott,2006). Therefore, plastic pollution on land is a problem both of contamination and damage to terrestrial environments and of transfer to aquatic systems. High levels of plastics contamination on land have been observed – an estimated 4 to 23 times larger than in the oceans (Machado *et al.*, 2017, Horton *et al.*, 2017). Hazards of Plastics each year approximately 140 million tons of synthetic polymers are produced (Shimao, 2001). The synthetic polymers are estimated to be approximately 20% of the municipal solid waste in the United States of America. In Australia, most household waste ends up in municipal landfill sites, estimated to be 25% of total waste by weight (Yayasekara *et al.*, 2005). As per the United States Environmental Protection Agency, in 2011 plastics constituted over 12% of municipal solid waste. In the 1960s, plastics constituted less than 1% of municipal solid waste. The extensive use of plastic usage poses severe environmental threats to terrestrial and marine ecosystem, as they are hardly degradable and voluminously dumped after usage. Due to high production and wide usage of plastics, their disposal is a major problem (Wiles and Scott,2006). Accumulation of plastic products in the environment adversely affects wildlife, wildlife habitat, lands, waterways, and oceans. Chlorinated plastic can release harmful soil, which can affect groundwater ecosystem. Methane gas, a highly powerful greenhouse gas produced during degradation process significantly causes global warming (Hester and Harrison, 2011). In the ocean, plastic pollution can kill marine mammals directly through entanglement in objects, such as fishing ear, but it can also kill through ingestion, by being mistaken for food (Hester and Harrison, 2011). Studies have shown that all kinds of species, including small zooplankton, large cetaceans, most seabirds, and all marine turtles, readily ingest plastics (Kijchavengkul and Auras, 2008). Polyethylene, a form of plastic including shopping bags, disposable bottles and glasses, chewing gums, and toys, is

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believed to be carcinogenic (FAO, 1997). Phthalates, present in emulsions, inks, footwear, and toys among other products, is associated with hormonal disturbances, developmental issues, cancer, reduced sperm count, and infertility and weakened immunity (FAO, 1997). PVC, a form of plastic used in packaging, containers, utility items, and cosmetics has been linked to onset of cancer and birth and genetic conditions. It can also cause bronchitis, skin disease, deafness, and vision problems, and digestion and liver-related problems (Udochukwu *et al*., 2016). Other hazardous poisonous chemicals released during the PVC life cycle, such as mercury, dioxins, and phthalates, may pose irreversible life-long health threats (Atuanya *et al.,* 2016). Some plastic water bottles contain Bisphenol A, a compound that is, believed to cause cancer, impair the immune system, lead to early puberty and trigger development of obesity and diabetes (Hester and Harrison, 2011). This research was carried out to study the effect of plastic waste composted soil on some soil bacteria and fungi.

MATERIALS AND METHODS

*Study Area:*The study area is Lokoja international market waste management landfill site located at Lokoja local government area, Kogi state, Nigeria. Kogi state also known as the confluence state is located in the north central geopolitical zone of Nigeria.

Soil sample collection and analysis: Samples were collected from three different point Lokoja international market waste sites located at Lokoja local government, Kogi state. And also fresh garden soil which has not been exposed to plastic waste was collected from Salem University Lokoja and serves as the control soil sample. The soil samples were collected and analyzed. The samples were analysed for their physicochemical, bacteriological and Bisphenol A composition. The physicochemical parameters analysed were pH, chloride, sodium, electricity conductivity, Iron, total dissolved, biochemical oxygen demand, total suspended solid, calcium, manganese, zinc, copper, sulphide and phosphorus according the method described by (Ho, 2005).

Isolation and Identification of bacteria: Nutrient agar was used in the isolation and enumeration of bacteria using the pour plate method. The pure culture was then transferred into nutrient agar slants for biochemical test. Nutrient and MacConkey agar were used to enumerate the bacteria in the soil samples (Cheesbrough, 2000). Identification of isolates were based on cultural, morphological and biochemical characteristics following standard methods (Garrity *et al*., 2005; Holt *et al.*, 2000).

Analysis of Bisphenol A (BPA) in soil samples: Hewlett Packard 5890 series II gas chromatograph equipped with an Agilent 7683B injector (AgilentTechnologies, Santa Clara, CA, USA), a 30m, 0.25mm i.d. HP-5MS capillary column (Hewlett–Packard, Palo Alto, CA, USA) coated with 5% phenylmethylsiloxane (film thickness 0.25um) and an Agilent 5975 mass selective detector (MSD) was used to separate and qualify the (BPA) compounds. The samples were injected in the split less mode at an injection temperature of 300^{o}C. The transfer line and iron source temperature were 280^{o}Cand 200^{o}C. The column temperature was initially held at 40oC for 1 minute, raised at 120^{o}C at the rate of 25^{o}C/minute, then to 60^{o}C at the rate of 10^{o}C/minute, and finally to 300^{o}C at the rate of 5^{o}C/minute, held at the final temperature for 15 minute. Detector temperature was kept at 280^{o}C. Helium was used as a carrier gas at a constant flow of 1ml/minute. Mass spectrometry was acquired using the electron ionization (EI) and selective ion monitoring (SIM). Fifty ml (50± 0.01 ml) water was measured, and 100ml of dichloromethane (DCM) via separating funnel and shacked for 30mins for BPA
extraction (Kijchavengkul and Auras, 2008). This separating funnel was clamp and a mixture was allowed to separate out. After separation the DCM portion was collected. The process was repeated three times for complete extraction (FAO, 1997). Blanks were prepared following the same procedure without the sample. The standard sample used for quality control was prepared by adding standard solution (BPA) to DCM. All extracts were separated, and activated copper was added to the combine extract for desulphurization. After subsequent filter over anhydrous sodium sulphate, the solution was concentrated to 1.0ml using a rotary evaporator, an internal standard mixture (vinyl chloride) solution was run with the extract for quality control check using Hewlett Packard 5890 series II gas chromatograph with mass selective detection (GC-MS) (Dean and Xiong, 2000).

RESULTS AND DISCUSION

Results obtained in this study showed the effect of plastic polluted soil on the growth and survival of soil bacteria and fungi. It was observed that the plastic contaminants inhibited the growth some of these organisms. This study revealed the presence of plastic contamination in the plastic composted soil. The Gas chromatogram revealed the presence of plasticizers in the soil with methylene chloride 17.45mg/kg having the highest peak in plastic compost soil as against 0.54mg/kg of garden soil sample while benzene was detected at a very low peak in plastic composted soil sample and it was not detected in the garden soil sample (Table 1). Cultural analysis was carried and the following bacteria were isolated from the plastic composted soil sample: *Corynebacterium* spp., *Acinetobacter* spp., *Enterobacter* spp., *Escherichia coli, Bacillus subtilis, Serratia* sp., *Protues* spp.,

Staphylococcus epidermis while the control garden soil sample had *Corynebacterium* spp., *Acinetobacter* spp., *Enterobacter* spp., *Escherichia coli, Bacillus subtilis, Serratia* sp., *Protues* spp., *Staphylococcus epidermis, Micrococcus lutues, Flavobacterium* spp., *Pseudomonas aeroginosa. Micrococcus lutues, Flavobacterium* spp. and *Pseudomonas aeroginosa* were not isolated from the plastic compost soil due to high level of plasticizer concentration in the soil (Table 3). From table 2, the following fungi isolated (*Penicillium expansum, Saccharomyces* sp., *Aspergillus niger, Fusarium* spp., *Rhizopus stolonifer*) were present in both soil samples except *Mucor piriformis* which was only isolated from the control non plastic contaminated soil sample (Table 2).

Table 1: Bisphenol A contingents found in the soil samples

Parameters	Plastic composted soil sample	Control Garden soil sample
Methylene	17.45	0.54
Hexane	10.05	0.26
Chloroform	1.56	0.31
Toluene	5.87	0.07
Tetrachloroethylene	1.48	0.01
Chlorobenzene	0.37	0.00
Dichlorobenzene	0.15	0.00
Benzene	0.11	0.00

Table 2: Fungal isolates from both soil samples

Plastic Composted Soil	Control Garden Soil Sample
Penicillium expansum	*Penicillium expansum*
Saccharomyces sp.	*Saccharomyces* sp.
Aspergillus niger	*Aspergillus niger*
Fusarium spp.	*Fusarium* spp.
Rhizopus stolonifer	*Rhizopus stolonifer*
	Mucor piriformis

Table 3: Bacterial isolates from both soil samples

Plastic Composted Soil	Control Garden Soil Sample
Corynebacterium spp.	*Corynebacterium* spp.
Enterobacter spp.	*Enterobacter* spp.
Acinetobacter spp.	*Acinetobacter* spp.
Escherichia coli	*Escherichia coli*
S. epidermidis	*S. epidermidis*
Bacillus subtilis	*Bacillus subtilis*
Serratia sp.	*Serratia* sp.
Proteus spp.	*Proteus* spp.
	Micrococcus luteus,
	Flavobacterium spp.
	Pseudomonas aeruginosa

Table 4: Frequency of distribution for Fungal isolates

Fungal isolates	Percentage (%)
Penicillium expansum	12
Saccharomyces sp.	24
Aspergillus niger	19
Fusarium spp.	20
Rhizopus stolonifer	10
Mucor piriformis	15

Table 5: Heterotrophic bacterial count for both soil samples

Soil Samples	10^{-2} (cfu/g)	10^{-3} (cfu/g)	10^{-4} (cfu/g)	10^{-5} (cfu/g)	Mean count (cfu/g)
Plastic Composted Soil	TNC	97	42	23	$40x10^{-4}$
Control Garden Soil	TNC	119	78	31	$57x10^{-4}$

The frequency of bacteria isolated from plastics composted soil recorded *Corynebacterium*spp12%, *Acinetobacter* spp. (6%), *Enterobacter* spp. (8%), *Escherichia coli* (16%), *Bacillus subtilis* (14%), *Proteus* spp. (4%), *Micrococcus luteus* (7%), *Staphylococcus epidermis* (10%), *Serratia* sp. (8%), *Pseudomonas aeruginos*a (5%), *Flavobacterium* spp. (10%) (Table 6) while the frequency of fungi isolated are; *Penicllium expansum* (14%), *Saccharomyces* sp. (24%), *Aspergillus niger* (19%), *Fusarium* spp. (20%), *Rhzopus stolnifer* (10%), *Mucor piriformis* (15%) (Table 4). The heterotrophic bacterial count of the plastic composted soil had a mean count of $4.0x10^{-4}$cfu/g while the control garden soil sample had $5.7x10^{-4}$cfu/g (Table 5). The fungi count for the plastic composted soil $1.2x10^{-4}$cfu/g while the control garden soil sample $1.8x10^{-4}$cfu/g (Table 7).

Table 6: Frequency of distribution for Bacterial isolates

Bacterial isolates	Percentage (%)
Corynebacterium spp.	12
Enterobacter spp.	8
Flavobacterium spp.	10
Acinetobacter spp.	6
Escherichia coli	16
Proteus spp.	4
Bacillus subtilis	14
Micrococcus luteus,	7
Staphylococcus epidermidis	10
Serratia sp.	8
Pseudomonas aeruginosa	5

Table 7: Heterotrophic fungal counts for both soil samples

Soil Samples	10^{-2} (cfu/g)	10^{-3} (cfu/g)	10^{-4} (cfu/g)	10^{-5} (cfu/g)	Mean count (cfu/g)
Plastic Composted Soil	TNC	29	12	8	$12x10^{-4}$
Control Garden Soil	TNC	TNC	48	23	$18x10^{-4}$

It is observed that plastics contaminants occupy space on the land fill sites making the land unavailable for agricultural and other numerous purpose (Udochukwu *et al*., 2016). The survival of these bacteria species in the plastic contaminated soil shows that they may possess the ability to degrade plastics present in the soil and therefore may be considered in the bioremediation of plastic polluted soil (Albertsson, 1980; Balasubramanian *et al*., 2014). It was observed that plastics contaminated soil will affect agricultural activities and also the underground water (Gu *et al.*, 2000; Gopferich, 1997), it also affects the organism in the food chain, toxin such as BPA find their way into food chain when ingested (Chandra and Rustgi, 1997). The absent of these microorganism affects soil fertility, soil structure and also increase soil pH (Alexander, 1977). The results obtained in this work agrees with (Hester and Harrison 2011; Udochukwu *et al.*, 2017; Atuanya *et al.*, 2016).

*Conclusion:*This research has shown that soil polluted with plastic contaminants which are called plasticizers is usually acidic due to the constant release of metals through plasticizer biodegradation. This in turn affects the soil microorganisms by making the soil more

acidic thereby altering their optimum pH range. When the growth of these organisms which have special functions in the soil are affected, their major function is lost in the soil environment. This goes further to affect the soil in so many ways which include nitrification process, soil fertility and soil productivity.

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