PRINT ISSN 1119-8362
Electronic ISSN 2659-1502

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 27 (4) 673-679 April 2023

Proximate, Phytochemical and Antibacterial Properties of Ethanol Extracts of *Zeamays* and *Teiferia occidentalis* Plant Leaves from Plastic Enriched Compost Soil in Edo State, Nigeria

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ABSTRACT: This study examined proximate, phytochemical and antibacterial properties of ethanol extracts of maize (*Zea mays*) and pumpkin (*Terferia occidentalis*) plant leaves from plastics enriched compost soil in Edo State, Nigeria using standard techniques. In this study, the highest phytochemicals constituents were found in control plants with saponin and flavonoid being the most abundant. However, observable decrease and absence of some photochemical constituent were observed in plastic enriched plants. Thus, the presence of microplastic in the soil may have played a part in the reduction of plant phytochemical constituent and possibly reduce the antibacterial properties of our medicinal plant. In proximate analyses, it was observed that phytochemical parameters such as protein, fat, fiber, and carbohydrate, decreased in percentage as the microplastic concentration increases in the soil.

DOI: https://dx.doi.org/10.4314/jasem.v27i4.5

Cite this paper as: ATUANYA, E. I; IGUNBOR, N; EMEJULU, M. I (2023). Proximate, Phytochemical and Antibacterial Properties of Ethanol Extracts of *Zeamays* and *Teiferia occidentalis* Plant Leaves from Plastic Enriched Compost Soil in Edo State, Nigeria. *J. Appl. Sci. Environ. Manage.* 27 (4) 673-679

Dates: Received: 17 February 2023; Revised: 08 April 2023; Accepted: 16 April 2023
Published: 18 April 2023

Keywords: soil microplastics; phytochemical; antimicrobial; *zea mays; teifeira occidentalis*

Over the past decade there has been a steady increase in plastic products resulting in a proportionate rise in plastic waste in the environment (Kaithiresan, 2003). The wide use of plastic can be attributed to their properties which include low-cost, light weight, durability, relatively unbreakable and aesthetic values (Kumar *et al*., 2007). In traditional African society, with lower population figures, the native leaves were used in wrapping most of needed materials (Jimoh, 2002). But the challenge of ever-increasing population has made Nigerian to learn how to use fairly improved means of wrapping such as polyethylene bags. Also, other factors including population growth rate, increasing urbanization, industrialization and economic growth has brought about the phenomenal increase in the volume of wastes generated daily in the country. Polyethylene bags are used in virtually all shopping centres, markets, restaurants and farms in Nigeria (Aziegbe, 2007). In Nigeria, drinking water is sold in plastic bottles and sachets. The public has developed a strong taste for such water since they are portable and can easily be carried from one place to another. There is also a perception that such bottled or sachet water is cleaner and more mineralized than tap water. After gulping down the liquid content, these bags are discarded indiscriminately thereby littering the environment. They then collect around the city, choking gutters, threatening small animals, polluting beaches and damaging the soil for long periods. It reduces soil fertility and prevents the growth of plant life this posing environmental problems (Vijaya and Reddy, 2007). Phytochemical are chemical compounds that occur naturally in plants (Phyto means "plant") in Greek. some are responsible for the colour and other organoleptic properties such as deep purple of blueberries and the smell of garlic(Nawza *et al*.,

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2018).The term is generally used to refer to those chemicals that may have biological significance(antioxidant properties) but are not established as essential nutrients (Brown *et a*l., 2013).Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties.They are nonessential nutrients meaning that they are not required by the human body for sustaining life. There are many phytochemicals and each works differently. Some of the possible actions are via antioxidants, hormonal actions and stimulation of enzymes with DNA replication, antibacterial effects and physical action. An antimicrobial is an agent that kills microorganisms or steps their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against for example, antibiotics are used against bacteria, and antifungal are used against fungi (Rajini *et al* 2010). The use of antimicrobial medicines to treat infections is known as antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection is known as antimicrobial prophylaxis (Seth *et al*., 2014). Vegetables plays an important role in human nutrition and has been part of human diet from time immemorial. *Telfaria occidentalis* (Fluted pumpkin) is a tropical vine grown in West Africa as a leaf vegetable and for its edible seeds. It is used primarily in soups and herbal medicines (Bharath *et al*., 2003). *Zea mays* (maize) is a cereal grass plant that produce kernel (seeds) which provides many nutritious edible grain food products. Based on the foregoing, this study examined the proximate, phytochemical and antibacterial properties of ethanol extracts of maize and pumpkin plant leaves from plastic enriched compost soil in Benin City, Edo State, Nigeria.

MATERIALS AND METHODS

Sample collection: Soil samples were collected at a depth of (0.15cm) using a sterile shovel from two major dumpsites in Benin City, Edo state, Nigeria. The first was dumpsite located at Aduwawa express way in Ikpoba Okha Local Government area of Benin City, Edo state, while the second dumpsite location was Benin-Ondo express way by-pass dumpsite which is located in Benin City, Edo state. The soil samples were transferred into sterile polyethylene bags and taken to our laboratory. The control soil samples were taken from a fertile farmland with no presence of waste dump around it and placed in a sterile labelled polyethylene bag and transported to the laboratory.

*Experimental Design:*This research was conducted with two plants samples of Maize and pumpkin. The plastic types used were finely ground plastic polyethylene bags particles collected from ILOJIE plastic mill located at around Ramat Park in Ikpoba Okha local government area of Benin City, Edo state. The plastic particles were grounded into fine granules to reduce the particles size and worked into the soil using farm tools according to the direction of the research. The experimental design consists of ten treatments containing an aggregate of five experimental buckets per plant sample. Each bucket, containing 1kg of soil with perforated base and set out on the field. The treatment were as follows;

"A"; Control soil with maize seed.
"B"; Control soil with pumpkin seed.
"C"; 10g of control soil with 25g of plastic granules with maize seeds.
" D"; 10g control with 25g of plastic Granules with pumpkins seeds.
"F"; 10g of control soil with 50g of plastic granules with pumpkin seeds.
" G"; Aduwawa dumpsite soil with maize seeds.
"H"; Aduwawa dumpsite soil with maize seeds.
" I"; Ondo by-pass dumpsite soil with maize seeds.
"J"; Ondo by-pass dumpsite soil with maize seeds.

Each treatment was mixed in its allocated bucket and left for 24 hours before maize and pumpkin seeds were planted in them and watered at regular intervals. The plants were left for two or three weeks before harvesting the leaves for further analyses. The soils samples collected from various dumpsites meant for soil analysis were placed on plastic trays and kept to air dry on a laboratory bench. The air-dried soils samples were then ground with mortar and pestle and passed through a 2mm sieve and kept for further analysis.

Preparation of plant extracts: The harvested plant leaves were air dried at room temperature (28-3°C) and pulverised, crushed into fine powder using manual blender according to method of Erdogan and Rao (2015). The aqueous extract of the plant's samples were prepared by soaking 1000g of the dry powdered plant material in 5 liters of double distilled water and kept at room temperature for 48 hours. At the end of water extraction period, the extracts were filtered first through a whatmann filter paper No 42 (125mm) and then through the cotton wool. The filtrate was concentrated using a rotary evaporator with a water bath set at 40°C to one tenth of its original volume and then finally with freeze drier. The dried residue (crude extract) was then stored at 40°C. Aliquot portion of the

crude extract residue were weighed and dissolved in distilled water for used on each day of the experiment.

Physicochemical Analyses of Soil Samples: The following physicochemical of soil parameters were carried out; Soil particle size analysis was determined by hydrometer method as described by Furhan (1962). Soil pH and conductivity were determined using Jenway pH and conductivity meter (450 a model). Total nitrogen was determined using Kjeidel method according to APHA (1999). Available phosphorus was determined using the Bray method for acid soils and the concentrated solution was measured using a 490nm spectrophotometer according to APHA (1999). Heavy metals (Cd, Cu, Pb and Fe) were determined using atomic absorption spectrophotometer according to APHA (1999).

Seed Viability Test: The seed viability test was carried out prior to the planting of maize and pumpkin seeds by flotation method as described by Atuanya *et al*., (2012). Growth was monitored for the first six weeks of germination.

Screening For Antimicrobial Activities: Using a glass sterile spreader, 100ûl of standardized fungal spore suspension were spread on sabour and dextrose agar (SDA). (Standardized inoculum were first adjusted to an OD 600nm of 0.1ml before use). Wells were then bored into agar media using a sterile 5.mm cork borer and then wells were filled with 50ml extract. The inoculated agar plates were allowed to stand on the bench for 1hr to allow for proper extract diffusion into the media. Plates were incubated at 25°C for 7 days and later observed zones of inhibition were measured and recorded in mm. Culture media with streptomycin at concentration 10mg/gl were used to control the growth of bacterial contaminants. Then the effects of the extracts on fungal isolates were compared. For antibacterial activity, the bacterial strains were grown on nutrient broth and incubated at 37°C for 24 hours. Similarly, yeast strain was grown on PDA and incubated for 24-48 hours at 28°C. The bacterial and fungal cultures were grown on sterile Petri-plates (90mm dia) containing nutrient agar/PDA medium. Preparation of agar disc and sterilization procedure was the same as described previously. The 5mm disc dipped in different concentrations (10.0, 5.0, 2.5 and 1.25 mg/ml) of the plant extracts prepared in ethanol which were placed on the medium and incubated at 37°C for 24 hours. Controls plates were also maintained wherein streptomycin (10mg/ml) dipped disc were used. All the treatment were replicated thrice in completely randomised design. After incubation, the microbial growth was recorded.

Minimum Inhibitory Concentration (Mic): Determination of minimum inhibitory concentration (MIC) of all extracts were carried out by two-fold serials dilution as described by Blasing and Ameling (2018) and MIC were read in ug/ml after being incubated at 37°C for 24 hours and at 25°C for up to 72 hours for bacteria and fungi respectively.

Extraction And Determination of Phytochemicals: The powdered (100g) of the plant was soaked in 500ml absolute ethanol in a corked conical flask and heated in a water bath (40-60°C) for 24 hours to extract the phytochemicals. The content was filtered and the filtrate collected for qualitative phytochemical analysis. The presence of alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides and phenolics compounds in the leaf extract were determined using analytical methods stipulated by the Association of Official Analytical chemist (AOAC, 1984).

Extraction of Plant Samples and Proximate Analysis: Determination of moisture content, total solids, ash content, crude fat/lipids, and crude protein content using analytical methods stipulated by association of Official Analytical Chemists (AOAC, 1984).

Statistical Analysis: The data obtained from the different parameters were subjected to Statical package for social sciences (SPSS), Using Analysis of variance. The probability level user for statistical significance was $p<0.05$ for the test.

RESULTS AND DISCUSSION

Table 1 shows the physicochemical properties of the test soil sample. While Table 2 shows the the growth measurement of *Zea mays* plant across the various soil samples. Table 3 shows the growth measurement of *Teifeira occidentalis* (pumpkin) plant across the various soil samples. The morphological assessment of test plants revealed shoot height of plant samples with the 25g concentration. *Zea mays* treatment growing to a height of 154cm which was significantly higher than the control plant which grew to a height of 121.5cm which in turn grew longer than the 50g concentration which at the time of this study grew to a height of 51 cm (Table 2.0). The calculated number of leaves on the *Telfaria occidentalis* Peaked on the control plants produced significantly lesser number of leaves (139,53) respectively (Table 3).

Table 1: Physicochemical Properties of the Test Soil Samples

Parameters	By-Pass Dumpsite Soil	Aduwawa Dumpsite Soil	Farmland Soil
pH	5.83	6.23	6.93
Moisture content (%)	4.69 + 0.71	9.30 ± 0.24	8.40 ± 0.74
Conductivity (uS/cm)	48.20 ± 0.05	55.50 ± 0.41	31.10±0.00
Total organic Carbon (%)	2.94± 1.16	1.10±0.04	0.91 ± 0.00
Phosphorus (%)	3.58± 1.81	6.30 ± 2.72	2.50 + 0.00
Particle size			
Sand	64.22 ± 0.06	61.25 ± 0.50	61.40 ± 0.00
Silt	12.59 4.45	18.91 ± 1.55	18.40+0.21
Clay	24.06 ± 2.23	19.84 ± 0.64	20.20 + 0.1 1
Heavy metal (ppm)			
Cadmium (mg/kg)	0.07 + 0.02	0.05 ± 0.03	ND
Copper (mg/kg)	0.80±0.10	0.55±0.15	0 03 + 0.00
Lead (mg/kg)	0 97±0.32	1.43± 1.01	<0.01
Iron(mg/kg)	0 97±0.42	1.22±0.17	0.76±0.00

Table 4 and Table 5 showed the antimicrobial activities of microplastic contaminated maize and pumpkin leaves extracts respectively. The antibiotic sensitivity pattern of microbial isolates varied across the different antibiotics used. However, bacterial isolates were most sensitive to ketoconazole and Ciprofloxacin and more resistant to Ofloxacin and Nitrofurantoin respectively (Table 4 and 5). Table 6 shows the proximate composition of maize and pumpkin leaves grown on plastic enriched compost. Carbohydrates and protein percent reduced significantly as the concentration of soil microplastics increased across the treatment plants. Tables 7 and 8 showed the phytoconstituents of *Zea mays* in *Teifeira occidentalis* on the various soil samples respectively. The phytochemical analysis of the test plants revealed phytochemical such as flavonoids, phenol, Saponin, alkaloid and tannin in varying quantities and their significant decrease in phytochemical percentage found in the microplastic contaminated plants.

Table 2: Growth Measurement of *Zea mays* plants across the various soil samples

W	R	A	B	C	D	E	F	ASH	ANL	BSH	BNL
1	1	10	2	9	3	6.1	4	2.1	2	3.3	2
	2	9.2	2	15	3	12	5	1.9	2	3.7	2
	3	12	3	5.7	2	5.7	4	2.2	3	1.5	1
2	1	25	5	32	5	11.5	11	5.1	4	4	3
	2	15.8	6	20	4	17	10	5.7	4	4.7	4
	3	19.2	6	113	5	10.5	12	4.4	3	3.2	3
3	1	34	10	40	8	15	15	10.2	7	5.1	4
	2	30	11	31	9	26	19	11	6	6	5
	3	29.2	9	21	8	17	18	12.2	6	6.9	5
4	1	53	12	76	15	28	22	17.3	11	11	6
	2	41.3	15	39	12	33	26	15.5	9	14.2	6
	3	40.2	12	39	12	28	22	18	9	15.2	5
5	1	81	17	91	17	36	28	22	15	17	9
	2	42.2	18	50	14	44	31	20	12	19	9
	3	69.2	15	48	14	38	27	21	11	21	9
6	1	121.3	20	154	19	41	30	34	17	27	11
	2	77.4	24	61	19	48	39	38	15	29	13
	3	86	20	60	18	50	31	30	12	34	12

Key: *W = week; R=replicate; A= Control shoot height (cm); B=Control No of leaves; C=25g conc. Shoot height; D=25g conc. No of leaves; E=50g conc. shoot; height; F=50g. conc. No of leaves; ASH= Aduwawa shoot height; ANL=Aduwawa No. of leaves; BSH=By-pass shoot height (cm); BNL = By-pass number of leaves.*

The results from the physicochemical analysis shows various degree of variations some of the physiochemical parameters such as pH (5.83±1.50), Ec (31.10±0.00 us/cm), TMC (8.40±0.74%) Phosphate (2.50±0.00 mg/l), Pb (0.97±0.32 mg/l), Fe (0.76±0.00 mg/l), Cu (0.03±0.00).

The moisture content of the control plant samples *Zea mays* and *Telfairia occidentalis* (2.88%, 7.16%) were slightly higher when compared to that of the 25g and 50g with the 50g conc having the lowest moisture level of (6.36% 2.53%). Thus, it can be inferred that the introduction of microplastics into the soil has slightly significant effects on the physiochemical parameters of the soil. The morphological assessment of test plants revealed shoot height of plant samples with the 25g conc.

Zea mays treatment growing to a height of 154cm which was significantly higher than the control plant which grew to a height of 121.5cm which in-turn grew longer than the 50g conc. which at the time of this study grew to a height of 5 lcm. The calculated number of leaves on the *Telfairia occidentalis* peaked on the control plant (197) leaves, the 25g and 50g conc. plants produced significantly lesser number of leaves (1 39, 53) respectively.

Table 3: Growth Measurement of *Teiferia occidentalis* plants across the various soil samples

W	R	A	B	C	D	E	F	ASH	ANL	BSH	BNL
1	1	18	10	8	4	15	4	0	0	0	0
	2	15.2	9	6	4	12	8	3.2	4	0	0
	3	8.1	9	7.2	7	10.3	8	0	0	0	0
2	1	31	18	22	12	27	11	4.8	4	5.1	3
	2	35.3	19	17	14	30.3	10	6.1	4	4.2	4
	3	20.2	17	18	18	23.2	12	3.2	3	3.8	4
3	1	51.5	31	44	25	53.1	20	25	7	20	9
	2	44.3	36	38.2	20	47	21	28	7	19	12
	3	39.2	28	38.6	31	40.2	36	14	8	22	8
4	1	71.3	58	61	39	77	39	36	15	29	16
	2	68.2	50	53	33	69	40	41	19	35	23
	3	59	54	49	46	70	41	39	17	44	30
5	1	112	92	70	51	89	54	57	28	44.3	24
	2	94	83	69	49	82	62	48	30	51.4	41
	3	80	135	76	58	93	61	60	38	63.8	60
6	1	144	127	92	70	121	84	34	17	63.5	54
	2	158	132	88	64	147	82	38	15	82.2	63
	3	162	197	103	139	138	53	30	12	91.4	81

Key: *W = week; R=replicate; A= Control shoot height (cm); B=Control No of leaves; C=25g conc. Shoot height; D=25g conc. No of leaves; E=50g conc. shoot; height; F=50g. conc. No of leaves; ASH= Aduwawa shoot height; ANL=Aduwawa No. of leaves; BSH=By-pass shoot height (cm); BNL = By-pass number of leaves.*

Table 4: Antimicrobial activity of microplastic contaminated maize extract

Strain	Micro-organism	Zone of inhibition (nm)		
	Ciprofloxacin(10mg/ml)		25g conc extract (10mg/ml)	50g conc. Extract(10mg/ml)
Gram Positive	*Bacillus cereus*	15.00+0.64	08.17+0.67	13.06+0.81
	Bacillus subtillis	12.88+0.51	12.00+0.95	1 2.27+0.93
	Staphylococcus aereus	13.02+0.67	9.45+0.84	10.68+0.81
Gram Negative	*Escherichia coli*	12.11+0.01	9.22+0.01	5.21+0.0
	Pseudomonas aeruginosa	12.23+0.80	9.45+0.81	1 1.63+0.80
	KETOCONAZOLE			
Fungi	*Aspergillus fumigatus*	29.0+0.8	10.8+0. I	11.3+0.40
	Aspergillus niger	28.01+0. I	13.8+0. I	16.5+0. I
$CD_{0.05}$	Micro-organism -6.31;	Organic solvent ethanol 80% Conc		

Table 5: Antimicrobial Activity of Microplastic Contaminated Pumpkin Extract.

Strain	Micro-organism	Zone of inhibition(nm)		
	Ciprofloxacin(10mg/ml)		25g conc extract (10mg/ml)	50g conc. Extract(10mg/ml)
Gram Positive	*Bacillus cereus*	21.0+0.1	20.0+0.3	13.1+0.2
	Bacillus subtillis	20.0+0.1	15.0+0.2	12.0+0.2
	Staphylococcus aureus	22.0+0.6	9.5+0.1	14.04+0.5
Gram Negative	*Escherichia coli*	21.0+0.1	20.0+0.3	13.1+0.2
	Pseudomonas aeruginosa	20.5+0.80	20.5+0.7	18.2+0.3
Fungi	*Aspergillus fumigatus*	21.0+0.10	25.1+0.50	19.3+0.40
	Aspergillus niger	20.01+0.10	19.2+0.40	16.54+0.10
$CD_{0.05}$	Micro-organism -6.31;	Organic solvent ethanol 80% Conc		

This suggests that the number of leaves produced decreased drastically as the level of microplastic concentration increased. This is finding agrees with Bintao *et al.,* (2021) who also experienced significant difference between the heights of Soy beans planted in clear soil against those planted in plastic residue contaminated soil. The fat and fiber percent of the control plant (2.40%, 2.40%) was significantly lower than that of the 50g and 25g conc. (3.10%, 3.01%) and (2.86%, 2.87%) respectively for the maize plant. *Telfairia occidentalis* control recorded slightly higher percentage of fat and fiber than the microplastic conc. plant. Carbohydrate level of maize and pumpkin control was slightly higher when compared to the Percentage Of carbohydrate derived from the concentrated treatment plants. Carbohydrate percent reduced significantly as the concentration of soil microplastics increased across the treatment plants.

The phytochemical analysis of the plants revealed phytochemicals such as, flavonoid, phenol, saponin, alkaloid and tannins in varying quantities. There was observable difference in the proximate composition of the control plants when compared to the plastic enriched plants.

Table 6: Proximate composition of maize and pumpkin leaves grown on plastic enriched compost

Proximate content	Sample		Plastic Enriched Compost Soil (%)			
		control	50g conc	25g conc	By-pass	Aduwawa
	maize	8.26	6.36	6.42	6.22	6.01
Mositure	pumpkin	2.88	2.53	2.42	2.41	2.88
Ash	maize	2.49	2.07	2.09	1.88	1.76
	pumpkin	13.90	13.90	13.29	12.54	12.77
Fat	maize	2.40	3.10	3.01	3.05	3.06
	pumpkin	13.90	12.85	12.88	13.88	13.82
fibre	maize	2.81	2.66	2.67	2.55	2.61
	pumpkin	3.20	2.32	2.36	3.11	2.41
Carbohydrate	maize	81.46	76.51	75.88	74.22	76.21
	pumpkin	41.21	43.02	42.75	42.11	42.22
Protein	maize	9.15	9.10	9.13	9.46	9.23
	pumpkin	25.66	25.40	25.11	25.31	25.22
$CD_{0.05}$	Micro-organism -6.31;	Organic solvent ethanol 80% Conc				

Table 7: Phytoconstituents of *Zea mays* on the various soil samples

Samples	%Phenol	%Flavonoid	%Tannin	%Saponin	%Alkaloid	%Terpenoid
Control	2.18	5.23	1.19	6.86	1.14	2.22
25g Concentration	--	--	--	5.22	1.09	--
50g concentration	--	4.41	1.01	---		1.76
Aduwawa	1.87	4.98	1.54	---	1.12	
By-pass	1.88	3.56	---	4.98		

Each valuer is a mean determinant of triplicates

Table 8: Phytoconstituents of *Teriferia occidentalis* on The Various Soil Samples

Samples	%Phenol	%Flavonoid	%Tannin	%Saponin	%Alkaloid	%Terpenoid
Control	3.12	7.22	2.22	6.55	1.11	---
25g Concentration	4.22	3.72	1.02	6.19	1.09	--
50g concentration	4.11	2.42	---	1.01	1.76	---
Aduwawa	2.0p	4.33	2.00	3.01	1.45	---
By-pass	2.67	4.22	---		4.54	

Each value is a mean derivative of the triplicate.

The significant decrease in phytochemical percentages found in the microplastic contaminated plants indicates the interference of plant phytochemicals by soil microplastics however, the presence of these phytochemicals indicates the usages of the sample plants as having medicinal properties which shows various biological activities particularly the antioxidant, antimicrobial, antidiabetic, anti-obesity, anti-proliferative, hepatoprotective, cardio-protective, and renal-protective activities. On the account of its high antioxidant potential, all parts of corn plant can be used for the management of oxidative stress and the treatment of various diseases (Nawaz *et al*., 2018). Proximate analysis carried out on the test plants revealed proximate parameters such as moisture content, carbohydrate, dry matter, fiber, ash, protein and crude fat in varying percentages. The bacterial count varied with the soil samples and there was significant difference between the higher concentrations of microplastic contaminated soil over time. The bacteria and fungi count peaked at 1 1.67±0.87x106cfu/g and 6.43±0.01x106cfu/g respectively. The inhibitory effect of the ethanol extract of the tested plants against *Bacillus cereus* (13.17±0.67mm), *Bacillus subtilis* (12.16±0.95mm), *Staphylococcus aureus* (11.45±0.84mm), *Escherichia coli* (25.2±0.7mm), Pseudomonas aeruginosa (12.23±0.5 mm) and *Aspergillus niger* (19.80±0.1 mm). The antibiotics sensitivity pattern of bacterial isolates such as *Bacillus cereus*, *Bacillus subtilis*, *Bacillus sp*. *Staphylococcus aureus*, Escherichia coli, *Pseudomonas aeruginosa* and *Aspergillus fungiatus* varied across the different antibiotics used. However bacterial isolates were most sensitive to ketoconazole and Ciprofloxacin; and more resistant to Ofloxacin and Nitrofurantoin respectively.

*Conclusion***:** It is evident from this study that microplastics impact on receiving soil and it's organisms in several ways. Microplastics in the soil may be able to affect the phytochemical properties of plants thus rendering them less efficient in their medicinal use. Disposal of plastic waste without proper recycling could cause long lasting damage to the environment. May contribute to the invasion of pest and rodents, destroy the aesthetics of the environment and possibly reduce the antimicrobial properties of our medicinal plants which is a major

national resource for fighting against diseases and infections especially in the rural parts of Nigeria.

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