UJMR, Volume 4 Number 2, December, 2019, pp 96 - 104 ISSN: 2616 - 0668
https://doi.org/10.47430/ujmr.1942.016

Received: 19/11/2019 Accepted: 4/12/2019

Assessment of Some Potential Plastic Degrading Microbes in Katsina, North Western Nigeria

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Abstract

Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environments. This research was aimed to asses some potential plastic degrading microbes in Katsina. Soil samples from four different locations of about 15,000 meters apart were used for this study. Portions of the soil samples were dried, ground and sieved through a 2mm sieve for Physico-chemical characterization. The soil temperature range from 26.3^{0}c to 29.6^{0}c, pH 5.67 to 6.26, % Nitrate 0.54 to 0.97, % Phosphates 2.03 to 3.83, % organic carbon 1.71 to 3.27, % organic matter 1.97 to 9.43 and % moisture content 7.13 to 16.22. Isolation and the enumeration of bacteria and fungi was done using nutrient agar and potato dextrose agar respectively. The total populations of bacteria ranged from 1.69 x 10^5 to 2.94 x 10^5 CFU/g in the soil while the fungi range from 1.60 x 10^4 to 8.35 x 10^4 CFU/g in the soil. The capability of microbial isolates to biodegrade ground polyethylene bags and plastic bottles was tested weekly. Microbes identified include Staphylococcus aureus, Streptococcus lactis, Bacillus subtilis, Diplococcus sp. and Pseudomonas sp. Eight degrading fungal species identified following fungal colony and color include Alternaria alternate, Aspergillus niger, Aspergillus flavus, Aspergillus ornatus, Aspergillus candidus, Aspergillus nidulans, Aspergillus terreus and Rhizopus stolonifera. It can be concluded that the soil around Kofar Sauri, Kofar Marusa, Kofar Guga and Kofar Kaura of Katsina metropolis contains some plastic degrading microbial species. Hence, further investigation using GC-MS to analyze the microbial enzymes or organic acids in degradation of the polythene and plastics in eco-friendly way is recommended.

***Key words**: Bacteria, Fungi, Soil, Plastic, Polythene bag, Physico-chemical.*

INTRODUCTION

Worldwide utility of polyethylene is expanding at a rate of 12% per annum and approximately 140 million tons of synthetic polymers are produced worldwide each year (Shimao, 2001). Plastics commodities are used in fishing nets, packaging, food industry and agricultural firm (Vatseldutt and Anbuselvi, 2014). Plastics are of great significance in today's world due to their wide use, which has enabled improvement in the quality of human life through ease of packaging of foods and other items, thus lengthening their shelf life (Andrady and Neal, 2009). The plastics used include polyethylene, polypropylene, polystyrene, polyvinyl chloride and polyethylene terephthalate, all of which are high molecular weight polymers whose biodegradability is low. Hence, plastics are persistent in the environment and are one of the sources of environmental pollution.

Plastics are characteristically inert and resistant to microbial attack and therefore survive for years (Kavitha *et al.*, 2014). These disposed plastics are a significant source of environmental pollution, potentially harming delicate life forms. Discarded plastics, besides being highly visible are a rapidly increasing percentage of solid waste in landfills, resistant to biodegradation leading to pollution and harmful to the natural environment.Plastic and polythene contamination of both aquatic and terrestrial environments from waste discharges, industrial raw materials, manufacture pellets or fragments of fishing nets, is becoming a future research priority, since it has been recognize to be an emerging global threat for its multiple (social and environmental) implications.

Plastic can degraded by a variety of mechanisms such as chemical, thermal, photoxidation and biodegradation, all of which take an extremely long time depending on the molecular weight of polymer, it could take up to 1000 years for some types of plastics to degrade (Mahdiyah and Mukti, 2013). Microorganisms can also play a vital role in this process, as over 90 genera of bacteria, fungi and actinomycetes have the ability to degrade plastic (Singh and Gupta, 2014). Generally, the biodegradation of plastic by microorganisms is a very slow process, and some microorganisms can't degrade certain plastics (Singh and Gupta, 2014). However, it would be desirable to have microorganisms capable of biodegradation of plastics and polythene as one solution to the problem of their accumulation in the environment. Some microorganisms, though of low abundance in the environment, mainly soils, have been isolated with ability of attacking plastics because they produce enzymes that enable them to use the plastics as substrate (Tokiwa *et al.*, 2009; Gnanavel *et al.*, 2012). These microorganisms act either directly or indirectly which includes discoloration and deterioration of plastics and serving as carbon or nitrogen source for the growth of the microorganisms. Microorganisms (bacteria, fungi, algae) recognize polymers as a source of organic compounds (e.g., simple monosaccharides, amino acids, etc).Under the influence of intracellular and extracellular enzymes (endo- and exoenzymes) the polymer undergoes chemical reactions and the polymer degrades by the process of cutting of the polymer chain, oxidation, etc (Premraj and Mukesh, 2005). The result of this process is affected by a large number of different enzymes are increasingly smaller molecules, which enter into cellular metabolic processes (such as the Krebs cycle), generating energy in the form of water, carbon dioxide, biomass and other basic products involved in biotic decomposition. The byproducts formed after decomposition are non-toxic in nature and in living organisms. It is considered to be the safest method of breakdown which anticipated producing less toxic side products and having potentials of bio-geo chemical cycling of the substrate (Pramila and Ramesh, 2011). Therefore, there is a need to design biodegradable polymers which degrade upon disposal by the action of living organisms. These polymeric materials are potential sources of carbon and provide energy for microorganisms like bacteria and fungi that are heterotrophic in nature.

MATERIALS AND METHODS

Description of Study Area

Soil samples for isolating microorganisms in order to assess their ability to degrade plastics were collected within the vicinity of the Katsina State North Western Nigeria. It is located between latitude 11.08^{0}N - 13.22°N, longitude 6.52'E - 9.20°E, within a span of 24,1929km^2 (Figure 1). It is bounded in the east by Kano and Jigawa States, in the west by Zamfara State, in the North by Niger Republic.

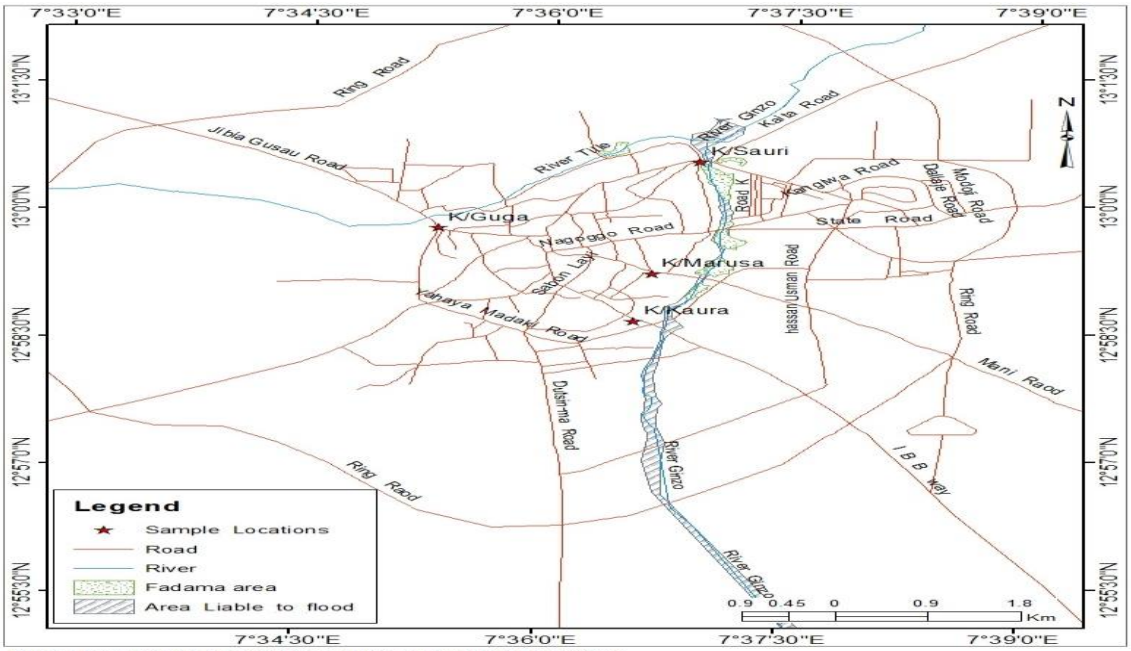

Fig.1: Map of Urban Katsina showing Sample Locations
Source:- National Aeronoutic and Space Administration Spot Image 2018

Soil Sample Collection

Soil samples from four different locations (Figure 1) of about 15,000 meters apart (Kofar Sauri, Kofar Marusa, Kofar Guga and Kofar Kwaya) including those that have harboured waste plastic and polythene for different lengths of time, as well as those that visibly had apparently not encountered any plastic wastes were used for this study. The soils with physical contacts with plastics and polythene were sampled at 5 to10 cm depth. Surface soils exposed to sunlight were avoided because any observation of degradation of waste plastics might be mainly due to UV radiation, rather than by microorganisms (Volke *et al.*, 2002). The soil samples were collected in October 2018 and taken to the laboratory for physicochemical analysis and isolation of bacteria and fungi, which were then subsequently tested for their ability to degrade plastics.

Plastic samples used in the biodegradation test

Plastic samples used in this study were Polythene bags and bottom of plastic bottle cut in a small strips with weight of 0.989g and 1.038g respectively.

Soil Analysis

In the laboratory, Portions of the soil samples were dried, ground and sieved through a 2 mm sieve for Physico-chemical characterization. Soil temperature and pH which were measured at the collection site. The soils were subjected to analysis of some essential parameters including % Nitrate, % Phosphates, % organic carbon, % organic matter and % moisture content.

Determination of Total Nitrogen

Total nitrogen was determined by the micro-Kjedahl digestion-distillation method previously described by Bremner (1996). One gram of soil was digested with concentrated Sulphuric acid (H_2SO_4) in the presence of a catalyst (K_2SO_4 + $CuSO_4$ + selenium powder; mixed in the ration of 10:10:1 by weight). The digest was distilled in the presence of 40% NaOH. The liberated NH_3 was collected in 4% boric acid (with mixed indicator) and titrated against a standard 0.05 MH_2SO_4. Afterwards, the titre was used to calculate the total nitrogen content of the soil sample.

Determination of Total Phosphate

Total phosphate was determined according to the protocol of Bray and Kurtz (1945). Two (2 g) of soil sample was scoop and weighed into a 50 mL Erlenmeyer flask, tapping the scoop on the funnel to remove all of the soil from the scoop. 20 mL of extracting solution to each flask was added and shake at 200 rmp for 5 minutes at 27ºC. 1 cm^3 (~200 mg) of charcoal was added to each flask to obtain a colorless filtrate. Whatman No. 42 filter paper was used to extracts the filtrate. Filtrates was analyse for phosphate in digital colorimeter model S1073 using a blank and standards prepared in the Bray P-1 extracting solution.

Determination of Moisture content

Aluminum dishes were weighed. Aliquot approximately 50 g of moist soil was transferred into each aluminum dish and were reweighed. Hence, the moist weighted of the soil sample was known. The soil samples were dry in an oven at 105 °C for 24 hours. Dishes were removed from the oven, allowed to cool and reweighed. The dry weight of the soil was known. Walkley-Black (1947). Moisture content in the soil was calculated as;

$$\% \text{ Moisture Content (MC)} = \frac{\text{weight of moist soil (M)} - \text{weight of dry soil (D)}}{\text{weight of dry soil (D)}}$$

Determination of Organic carbon

Organic carbon was determined by the wet digestion (oxidation) method of Walkley-Black previously described by Nelson and Sommers (1996). Briefly, 0.5 g soil sample, 10 ml of 1 M $K_2Cr_2O_7$ and 25 ml of concentrated H_2SO_4 were added and allowed to stand for 30 minutes to oxidize organic carbon. Two hundred (200ml) of water was added to cool the mixture followed by of 10 ml of phosphoric acid. The amount of $K_2Cr_2O_7$ reduced was used to estimate the organic carbon content the soil by the titration of excess dichromate against a 0.5 N ferrous sulphate solution using diphenyl amine indicator.

Determination of Soil pH and Temperature

Soil pH was measured using Hanna Direct soil portable meter model HI99121. While soil temperature was measured using Hanna Digital thermometer model HI98501.

Determination of Organic matter

Walkley-Black (1947) method was adopted – one (1 g) gram of soil sample was transferred to 250ml Erlenmeyer and added 10ml of 1N $K_2Cr_2O_7$ and 10ml of concentrated H_2SO_4. After 30 minutes, 50 ml of deionized water, 3ml of concentrated H_3PO_4 and 0.5 ml of 1% defenilamina indicator were added. Then, titrated slowly with 1N $FeSO_4$ solution up to a green color end point.

Media for Microbial Isolation

Medium for isolation of bacteria

Medium used for bacteria isolation was nutrient agar prepared by mixing the following; 5 g of peptone, 3 g of beef extract, 1 g of yeast extract and 15 g of agar in 1 L of distilled water. The mixture was boiled prior to sterilization by autoclaving at 1.05 kg/cm2 (15 lb./sq.in) and 121°C for 15 minutes. The sterile medium was left to cool up to about 50°C and then poured into sterile petri dishes and left to solidify. Cooled petri-dishes containing the medium were aseptically stored at 4°C until used.

Medium for isolation of fungi

The potato dextrose agar (potato glucose agar) medium for isolation of fungi was prepared by adding 200 g of peeled and sliced potatoes into 500 ML of distilled water followed by cooking at 100°C for 1 hour. The mixture was filtered through a cheese cloth. Then, 200 g of glucose and 15 g of agar were added to the filtered potato liquid and the volume adjusted to 1000 ml using distilled water. The medium was then sterilized by autoclaving at 1.05 kg/cm^3 and 121°C for 15 minutes. The medium was left to cool to about 50°C and then poured into petri dishes to solidify.

Biodegradation under field conditions:

The protocol of Kathiresan (2003) was adopted for this study. Sample of plastic bottles strip (1.038g) and polythene bags (0.989g) were buried at a depth of 5 cm in four different soil samples. The plastics were allowed to degrade naturally in the soils and they were sampled at the intervals of 4, 8 and 12 weeks using sterile forceps and transferred to laboratory aseptically. One set of samples was thoroughly washed using distilled water, shade-dried and then weighed for final weight. The degradation was determined in terms of per cent of weight loss of the materials over a period. Another set of sampled materials was washed gently using sterile water to remove soil debris. About one gram of the materials infested with bacteria and fungi was transferred into the conical flask having 99 ml of sterile water. This content, which was shaken vigorously for its equal distribution, was serially diluted. The pour plate method was adopted using the Zobell's agar medium for bacteria and the Martin Rose Bengal medium for fungi. For each dilution, three replicates were made. The plates were then incubated at 30°C for 2-7 days. The bacterial and fungal counts were then made.

Isolation of Bacteria and Fungi from Soils

Ten-fold dilutions of soil samples were prepared prior to microbial isolation as previously described by Usha *et al.*, (2011). 10 g of soil sample was transferred into a bottle containing 90 ml of sterile water and shaken vigorously to suspend the soil particles, thus making the 10^{-1} suspension. One ml of the above suspension was aseptically transferred to a bottle carrying 9 ml of sterile water and shaken to mix well, making a 10^{-2} suspension. Using a fresh sterile pipette, the 10^{-2} was transferred to make a 10^{-3} suspension. Thus, serial ten-fold dilutions were made up to the 10^{-6} dilution. Then, 1 ml aliquots from 10^{-3} to 10^{-6} were poured into different petri dishes in triplicates. To the petri dishes, about 15 ml of nutrient agar for bacteria or potato dextrose agar for fungi were added. The petri dishes were then gently swirled clockwise, then anticlockwise and once forwards and once backwards to mix the soil suspensions and the media. Then, the petri dishes were left to stand for the media to solidify. The plates were then incubated, up-side down, at 30 °C for three to seven days for bacteria and fungi until visible colonies were seen. The developed colonies were sub-cultured respectively on respective agar plates previously prepared to get pure cultures and then preserved in slants of respective media at 4 °C.

Enumeration of Total Microbial Populations

The counting of the microbial populations was done depending on the nature of their growth. The counting for bacteria and fungi was initially done on the third day when the colonies were clearly grown. Then after two days re-counting of all the plates were done for all microorganisms. Colonies were counted on plates that showed a good distribution of colonies estimated at between 30 and 300. The colonies were counted and converted to microbial populations (CFU) per gram of soil sample.

Staining of fungi using lactophenol cotton blue solution

Lactophenol cotton blue solution is a mounting medium and staining agent used in the preparation of slides for microscopic examination of fungi. The reagent was prepared by mixing 20 g of phenol crystals, 20 ml of lactic acid, 40 ml of glycerol, 20 ml distilled water and 0.075 g of methyl blue. The solution was well shaken to mix the contents. A drop of lactophenol cotton blue solution was placed on a slide, using an inoculating needle/loop, followed by careful spreading of fungal culture to obtain a thin preparation on the slide. A coverslip was placed on the drop and lowered to avoid air bubbles under the coverslip and left for about 5 minutes.

The slides were observed under a light microscope with low power for screening in low intensity as previously described by Alfred (2009).

Gram-staining of bacteria

The Gram-staining of bacteria was performed as previously described by Alfred (2009).

The bacteria smears was prepared on microscopic slides. The slides was cleaned with alcohol, followed by drying using paper towels. The prepared slides was labelled by drawing a circle on the underside of the slide using a marker pen to clearly designate the area in which the smear will be prepared. The loop to be used was sterilized by heating it on a burner and cooled. With a sterile cooled loop, a drop of sterile normal saline solution was placed on the slide. The loop was sterilized and left to cool, and a very small sample of a bacterial colony was picked up and gently stirred into the drop of normal saline on the slide to create an emulsion that was subsequently air dried. After the smear was air-dried, the slides was passed through the flame of a bunsen burner two to three times with the smear-side up ready to be stained. The fixed smear was flooded with crystal violet solution and allowed to stand for one minute. The crystal violet was rinsed off with distilled water, and slides were then flooded with iodine solution and allowed to remain for one minute. The iodine solution was rinsed off with distilled water, and the slides were flooded with acetic acid (decolourizer) for five seconds. The decolourizer was rinsed off with distilled water and the slides were flooded with safranin and allowed to stand for 30 seconds, after which safranin was rinsed off with distilled water. The slides were air dried by placing them in an upright position and smears were viewed using a light-microscope under oil-immersion at the 1000x magnification (Alfred, 2009).

Identification of microorganisms

Among the bacterial and fungal colonies, the dominant ones were isolated and sub-cultured repeatedly for getting pure colonies and then preserved in slant tubes for further identification.

The bacterial strains were identified based on the keys detailed by Skerman, (1949), Oliver (l982) and Cheesbrough, (2005). The fungal strains were identified after staining following the keys of Williams, (1785), Raper and Fennell (l987) and Humber, (1996).

Microbial degradation of plastics under Laboratory conditions:

To assess this, the pre-weighed discs of 1-cm diameter prepared from polythene bags and disposable bottom plastic bottles strip were aseptically transferred to the conical flask containing 50 ml of culture broth medium, inoculated with different bacterial and fungal species separately. Nutrient broth medium was used for bacteria and Rose Bengal broth medium for fungi. Control was maintained with plastic discs in the microbe-free medium. Four flasks were maintained for each treatment and left in a shaker. After one month of shaking, the plastic discs were collected, washed thoroughly using distilled water, shade-dried and then weighed for final weight. From the data collected, weight loss of the plastics and polythene bags was calculated.

Statistical Analysis of the Data

Descriptive statistics was used to calculate mean, mean ± standard error (ES), Minimum and maximum values. Percentage was used for both bacteria and fungi, the result obtained was subjected to the analysis of variance to test the level significance at ($p<0.05$) between the four soil samples collected.

RESULTS

Physico-chemical Parameters of Collected Samples

Table 1 shows results of the soil physicochemical characteristics, the temperature range from 26.3^{0}C to 29.6^{0}C, pH, % Nitrate, % Phosphates, % organic carbon, % organic matter and %moisture content range from 5.67 to 6.26, 0.54 to 0.97, 2.03 to 3.83, 1.71 to 3.27, 1.97 to 9.43 and 7.13 to 16.22 respectively. **Degrading microbes identified**

Five bacterial species were identified following biochemical test (Table 2) from degrading polythene bags and plastic; *Staphylococcus aureus*, *Streptococcus lactis*, *Bacillus subtilis* and *Diplococcus* sp. (belong to Gram- positive bacteria);

Table 1: Physico-chemical parameters of the soil samples collected from the four sample sites

Soil sample	Sample location	pH	% Nitrate	% Phosphates	% Organic Carbon	Temperature	% organic matter	% Moisture content
A	Kofar Sauri	5.67±3.05	0.6±0.61	2.03±1.11	2.98±1.08	29.6 ^{0}C±5.57	4.69±3.30	11.82±5.04
B	Kofar Marusa	5.89±4.41	0.84±2.04	2.51±3.38	2.46±1.17	27.4 ^{0}C±5.09	2.85±1.71	7.31±4.81
C	Kofar Guga	6.01±3.33	0.97±0.98	3.83±2.71	3.27±2.84	26. 3^{0}C±5.57	9.43±4.87	16.22±4.72
D	Kofar Kaura	6.28±4.85	0.54±0.57	2.69±1.24	1.71±0.88	27.9^{0}C± 5.75	1.97±1.09	9.38±4.23

Pseudomonas sp. (belong to Gram-negative bacteria). Eight degrading fungal species identified following fungal colony and color include *Alternaria alternate*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ornatus*, *Aspergillus candidus*, *Aspergillus nidulans*, *Aspergillus terreus* and *Rhizopus stolonifera*.

Table 2: Biochemical test of the bacteria in the soil sample collected

Characteristics	B1	B2	B3	B4
Color	white	white waxy	Grayish	White grayish
Gram Staining	Rod +ve	Rod –ve	Rod +ve	Rod –ve
Methyl red	-ve	-ve	-ve	+ve
Catalase	+ve	+ve	+ve	+ve
Indole	-ve	-ve	+ve	-ve

Key:B1=*Bacillus subtilis*,B2=*Streptococcus lactis*,B3=*Pseudomonas* and B4=*Staphylococcus*

Table 3: Microbial population density (CFU/g) of the study soils collected at four different location

Kofar Sauri		Kofar Marusa		Kofar Guga		Kofar Kaura	
Bacteria	Fungi	Bacteria	Fungia	Bacteria	Fungi	Bacteria	Fungi
2.01×10^5	1.60×10^4	1.69×10^5	3.71×10^4	2.94×10^5	8.35×10^4	1.82×10^5	3.19×10^4

Degradation of the polythene and plastics incubated under natural habitat with different soils is shown in Table 4, both polythene bag and plastic strips were weighted (0.989±0.03g and 1.038±0.3g) respectively at 0 week prior to incubation in soil samples. Soil collected from the four different location shows little drops in weight of the plastic strip and polythene bag from week 4 to week 12. Kofar Guga recorded highest drop in polythene bag and plastic strip ranging from 0.989±0.031g to 0.968±1.30g and from 1.038±0.5g to 1.019±1.50g for 0 week to 12 weeks respectively, while least weight drop in the materials was observed from soil sample collected from Kofar Kaura with polythene bag and plastic strip ranging from 0.989±0.5g to 0.980 ±0.51g and 1.038±0.05g to 1.031 ±0.05g from 0 week to 12 weeks respectively.

Table 4: Biodegradation of polythene bags and plastic strip buried for different duration

Weeks of analysis	Biodegradation (% weight loss in grams)							
	Kofar sauri		Kofar marusa		Kofar guga		Kofar kaura	
	Poly	Plas	Poly	Plastic	Poly	Plastic	Poly	Plastic
Week 0	0.989±0.03^{ab}	1.038±0.3^{b}	0.989±0.41^{a}	1.038±0.3^{ab}	0.989±0.031^{a}	1.038±0.5^{b}	0.989±0.5^{a}	1.038±0.5^{a}
Week 4	0.985±0.06^{ab}	1.032±0.03^{b}	0.987±0.04^{a}	1.034±0.04^{ab}	0.974±0.06^{a}	1.029±0.3^{b}	0.989±0.7^{a}	1.038±0.5^{a}
Week 8	0.981±0.06^{ab}	1.030±0.04^{b}	0.985±0.03^{a}	1.032±0.07^{a}	0.970±0.05^{a}	1.025±0.08^{c}	0.985±0.4^{a}	1.033±0.4^{a}
Week 12	0.975±0.04^{ab}	1.028±0.30^{c}	0.972±0.30^{a}	1.029±3.01^{a}	0.968±1.30^{a}	1.019±1.50^{a}	0.980 ±0.5^{b}	1.031 ±0.5^{a}
P value	0.081ns	0.063ns	0.059ns	0.062ns	0.051ns	0.054ns	0.083ns	0.079ns

Note: Means with the same superscripts along the same column are not significantly different (P>0.05), Poly = polythene, Plas = Plastic

DISCUSSION

Soil pH is a measure of hydrogen ion activity in the soil solution. It expresses the acidity and alkalinity of the soil and is a primary factor in plant growth. It is a very important property of soil as it determines the availability of nutrients, microbial activity and physical condition of soil. The mean pH value of soil samples in the study area ranged from 5.67 – 6.28 which indicates that soil samples are acidic in nature. Soil temperature is the factor that drives germination of seeds. Soil temperature directly affects plant growth. Most soil organisms function best at an optimum soil temperature. From this research, the result of all the four soil samples were at an optimum temperature (29.6^{0}C, 27.4^{0}C, 26. 3^{0}C and 27.9^{0}C from Kofar Sauri, Kofar Marusa, Kofar Guga and Kofar Kaura respectively). The Soil temperature impacts the rate of nitrification. It also influences soil moisture

content, aeration and availability of plant nutrients (Kathiresan, 2003).

The % organic matter value ranges from 1.97 to 9.43%, the mean higher value of 9.43±4.87% recorded at Kofar Guga may have been resulted from the decomposition and composting processes of the animal wastes such as animal dung, food wastes, smoke etc. A high content of organic matter in a soil favours increase moisture content, water holding capacity and permeability (Badmus, *et al.*, 2014). The organic matter content depends on number of factors such as level of microbial activity and proportion of organic refuse. The values of percentage organic carbon ranged between 1.7% and 3.27%, these values recorded within the soil collection sites may be as a result of burning of solid wastes dump in the locations.

Some organisms perform anaerobic degradation on some types of substances, while others degrade the same or other substances under aerobic conditions. It is evident that a large number of soil organisms are able to degrade different types of chlorinated compounds. Microorganisms play a significant role in biological decomposition of materials, including synthetic polymers in natural environments (Bollag, 2000; Anonymous, l999). High-density and low-density polyethylenes are the most commonly used synthetic plastics and they are slow in degradability in natural environments, causing serious environmental problems. In this regard, there is a growing interest in non-degradable synthetic polymer biodegradation using effective microorganisms (Priyanka, and Archana, 2011).

The microbial counts on the polythene bags and plastic materials that degraded for different weeks under the four soil samples were recorded. However, there was no statistically significant variation of bacterial counts between the four different locations (Kofar Sauri, Kofar Marusa, Kofar Guga and Kofar Kaura) and or between the weeks of degradation. Moreover, the mean weight reductions per sampling point were lower which is an indication that biodegradation of materials varies by point location depending on the microbial composition of the particular point. Use of weight reduction as a measure of the extent of polyethylene biodegradation has been widely accepted and used by many authors (Ojha, *et al.*, 2017; Sheik, *et al.*, 2015; Deepika and Jaya 2015). These outcomes are in agreement with (Pramilla and Vijaya 2015) who reported the ability of microorganisms to degrade virgin polyethylene.

The counts of total heterotrophic bacteria (THB) ranged from 1.69 x 10^5 CFU/g to 2.94 x 10^5 CFU/g (12 weeks) in the polythene degraded under the soil samples. The fungal counts ranged from 1.60 x 10^4 CFU/g to 8.35 x 10^4 CFU/g (12 weeks) in the polythene degraded, these total count variation may be as a result of some physicochemical factors which include moisture content, heat, organic matter, organic carbon which favours the growth of some microbes thereby increasing the degradation of the plastics.

Various microbial species were isolated in the laboratory for their ability and degrading the polythene and plastics. The species isolated include bacterial; *Staphylococcus aureus*, *Streptococcus lactis*, *Bacillus subtilis*, *Diplococcus* sp. and *Pseudomonas* sp., and fungal species; *Alternaria alternate*, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus ornatus*, *Aspergillus candidus*, *Aspergillus nidulans*, *Aspergillus terreus* and *Rhizopus stolonifera*. The degradation of polythene and plastic strip were compared by the weight loss of the samples on weekly basis and the polythene was observe to be relatively faster and earlier than that of the plastics, this may be due to the thickness of the polythene that is 5-times thinner than the plastics, this agree with the findings of Kathiresan, (2003), in his studies on Polythene and Plastics-degrading microbes from the mangrove soil. Among the microbes observed in this studies, the strains of *Aspergillus flavus*, *Pseudomonas* sp. and *Staphylococcus aureus* are more efficient in biodegradation of both the polythene and plastic, thereby making the surface of plastic strip rough with cracking when view under hand lenses. This may be due to the compounds secreted extracellularly by the microbes that may break the complex molecular structure of plastics, this agree with the finding of Priyanka and Archana, (2011) in their studies on Biodegradability of Polythene and Plastic by the help of microorganism. Polythene and plastic degradation is more active in sample C (Kofar Guga) with degrading capacity in Polythene ranging from 0.989±0.031g to 0.968±1.30g while Plastic strip from 1.038±0.50g to 1.019±1.50g, this could be as a result of high microbial population associated with the soil (Bacterial load 2.94 x 10^5CFU/g and 8.35 x 10^4 CFU/g fungal load) moreover, this could also be due to the higher organic content and moisture which may be favorable for the microorganisms.

CONCLUSION

A total of five bacterial and eight fungi species capable of degrading plastic and polythene have been isolated from collected soil samples. The isolated microbes were native to the collection site of polyethylene disposal and might show some degradability in natural

conditions, yet they also exhibited biodegradation in laboratory conditions on synthetic media. This gives some suggestion that these microbes can be used in both natural and artificial conditions for the purpose of degradation of polymers. Hence, further investigation using GC-MS to analyze the microbial enzymes or organic acids in degradation of the polythene and plastics in eco-friendly way is recommended.

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