Asian Journal of Biotechnology and Bioresource Technology

3(1): 1-12, 2018; Article no.AJB2T.40086
ISSN: 2457-0125

Biostimulation of Atrazine-impacted Soil Using Cassava Peel Waste Considering the Associated Soil Bacteria

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Authors' contributions

This work was carried out in collaboration between both authors. Author EMM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author EMM managed the analyses of the study. Author HOS managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2018/40086
Editor(s):
(1) Pasquale Russo, Professor, Department of Science of Agriculture, Food and Environment, University of Foggia, Italy.
Reviewers:
(1) Luis G. Torres Bustillos, UPIBI-IPN, Mexico.
(2) Monda Vincenzo, University of Study of Campania "Luigi Vanvitelli", Italy.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/23720

Original Research Article

Received 5th January 2018
Accepted 10th March 2018
Published 20th March 2018

ABSTRACT

The biostimulation of atrazine-impacted soil using cassava peel waste (CPW) considering the associated soil bacteria was assessed over a period of seven (7) weeks. The study was carried out at the Teaching and Research Farm of the Faculty of Agriculture, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. The aim of this work was to enhance indigenous soil bacteria in the biodegradation of atrazine through biostimulation using organic wastes. To achieve this, the physicochemical properties of the soil (before and after treatments) and the basic proximate mineral elements of the organic waste was determined before application using standard analytical methods. The bacterial characteristic and pH of the soil treated with CPW, CPW+ATZ, ATZ, and CONTROL (no treatment) were assessed using culture-dependent and standard analytical technique respectively. The study provided adequate evidence that the study site was naturally

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endowed with requisite bacteria (*Acinetobacter* sp., *Enterobacter* sp., *Pseudomonas* sp., and *Bacillus* sp.) with potential enzyme repertoire for atrazine degradation. The addition of organic amendment improved the physicochemical status of the impacted soil which culminated to significant ($P<0.05$) increase in soil microbial population and diversity. The study also showed that pollutants (atrazine) at a tolerable level can improve bacterial population (as a result of the proliferation of naturally selected degraders) but reduce bacterial diversity. This was observed in the treatment, ATZ which had the least bacterial diversity but with the highest bacterial density ($35.00\pm4.24\times10^8$cfu/g) at week 6. Although some bacteria are good degraders of herbicides in the soil, however, it may require some amendments in order to stimulate them to degrade pollutants. This work showed that the organic waste used in this study was a potential stimulatory agent that enhanced the growth of indigenous atrazine-degrading soil bacteria; hence can serve as an improved method of waste management and potential soil remediation approach.

Keywords: Soil; atrazine; bacteria; cassava peel waste; biostimulation.

1. INTRODUCTION

Basically, food is being made available to man through agricultural activities which involve the planting of crops and rearing of livestock. Among the plant species that threaten agricultural yield of crops are the weeds that, when invading crops, has the potential to significantly reduce quality and yield of the harvest. Therefore, in order to improve the quality and amount of crop yield, the removal of these unwanted plants from agricultural farmlands becomes imperative [1]. When weeds are controlled by the use of a particular herbicide, sometimes many of the compounds enter into the environment, which could be in the atmosphere, water, soil, and or in the harvested products [1]. As a result of the general use of these herbicides over a long period of time, their effects have been observed as a result of the accumulation of the residues in the environment, where they cause severe pollution in the ecosystems and harmful disruption of the biota [2]. Mada et al. made a survey on the effect of the continuous application of herbicides on soil and environment in Adamawa State, Nigeria and discovered that the exposure of farmers to herbicide from 2001 – 2011 has led to problems such as acute poisoning and death of farm workers in southern Adamawa State. The survey also revealed some ill health farmers complained of which include heart pain, skin rash, eyes problems, nervous disorder and respiratory condition [3]. More so, Atrazine at concentrations of 5, 8.5, 17, 51 µM increased the frequency of chromosome aberrations in human lymphocytes exposed to the herbicide [4]. Also, increase in the DNA fragmentation in the erythrocytes of Nile tilapia exposed to atrazine at 6.25, 12.5, 25 µg/L has been observed [5]. As a result of the toxicological features of atrazine and its high incidence of contamination, great efforts are being made by researchers in seeking for potential bioremediation techniques towards the restoration of atrazine polluted sites [6]. Many bacteria have the potential to metabolize the *N*-alkyl group of the herbicide as a source of carbon [7]. Rather than individual species, bacterial consortia have been confirmed to be more efficient for atrazine degradation in the soil [8,9]. Although the rapid clean-up of herbicides from polluted sites has been proved to be achievable through bioaugmentation, however, there are reports of its ineffectiveness in full-scale field clean-up exercise [10]. Kadian et al. suggested that the stimulation of indigenous microbial degraders using organic amendments is a more cost-effective, sustainable and environmentally friendly approach towards the remediation of herbicide-contaminated soil [11]. In this study, the organic waste (cassava peel waste) is being used as a potential bio-stimulatory substrate to promote the growth of naturally selected bacterial degraders for the breakdown of atrazine in the soil, thereby alleviating the harmful effect of herbicide (atrazine) on man, wild life, plants and soil biota.

2. MATERIAL AND METHODS

2.1 Study Site

The research was carried out at the Faculty of Agriculture Research farm, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. The complete size of the study site was 13.0m by 9.5m. The study area was further divided into twelve (12) smaller blocks of $0.25m^2$ area (microcosms) at 3m apart from each other (3 rows; 4 columns). The geographical coordinate of the site is Latitude 4º54′ 30.34″ N and Longitude 6º55′ 23.11″ E. The research was done during the rainy season.

2.2 Organic Waste and Herbicide Used

The cassava peel waste (CPW) used in this study was collected from harvested cassava (*Manihot esculenta*) in Obinze, Owerri-West, Imo State, Nigeria. The herbicide (atrazine) was purchased from an agricultural dealer store in Port Harcourt, Rivers State, Nigeria. It contains 50% SC atrazine as the active ingredient. The herbicide was prepared according to manufacturer's recommendation and the model described by Pal and Das Gupta [12] was also adopted.

2.3 Experimental Design and Soil Treatment

Plants/weed was completely removed from each of the microcosms to avoid phytoremediation. The microcosms (area of 0.25 m^2 each) were first treated with the dried, sterilized (autoclaved at 121°C for 20minutes to prevent microbial interference from the organic wastes), organic waste (CPW). After seven (7) days, 1L of the herbicide (100 $mg^{-1}L$ atrazine) was applied once on the appropriate microcosms. The treatments applied in this study were: atrazine only (ATZ); cassava peel waste (380 g) and atrazine (CPW+ ATZ); cassava peel waste (380 g) only (CPW). There was "CONTROL" in which no treatment was applied. Each treatment and the control were made in three replicates. The experimental design used in this study was the completely randomized block design (CRBD).

2.4 Samples Collection

A composite soil sample (10 cm depth) was first taken to determine the fungal and physicochemical condition of the soil within the sampling area before treatment. Samples from each treatment and control were collected fortnightly for 6 weeks. Samples collected were properly mixed and characterized to determine the soil physicochemical properties and fungal population/diversity response to atrazine treatment.

2.5 Isolation and Characterization of Total Culturable Bacteria

Nutrient agar (NA) was used for enumeration and isolation of total culturable bacteria. Bacterial isolates were characterized based on cultural characteristics, staining, and biochemical reactions. Identification of bacterial isolates thereafter was made with reference to the Bergey's Manual of Systematic Bacteriology (1984).

2.6 Growth and Degradation Studies

A modified Czapek Dox Agar and Udikovic et al. [13] method were employed using Sodium citrate as the sole carbon source and atrazine as the only nitrogen source. The Citrate-Atrazine (Cit-Atz) medium contained sodium citrate (1.0 g), $MgSO_4$ (0.5 g), $CaCl_2$ (0.5 g), K_2HPO_4 (1.0 g), $MnSO_4$ (1.0 g), $CuSO_4.5H_2O$ (0.4 g), atrazine (100 mg/L), and distilled water (1 L). The agar medium of the above composition was made by adding 16 g agar per liter to form Cit-Atz agar. The Cit-Atz medium was used to isolate atrazine degraders. To isolate atrazine-degrading bacteria, 0.1 g of nystatin was used per liter of media prepared to knock-off fungal contamination [14]. One gram of soil sample from each microcosm treated with atrazine was added to 10ml of mineral salt medium (MSM) (with no atrazine, and antibiotics) in sterile test tubes and vortexed for 1 minute. An equal volume of the 1ml slurry was taken and was added to 250 ml Erlenmeyer flask containing 50ml of Cit-Atz medium and nystatin. The inoculated flasks were incubated at 30ºC and isolation of degraders were made at 2 days interval for 10 days.

2.7 Isolation of Atrazine-degrading Bacteria

Atrazine degrading bacteria were isolated with the Cit-Atz agar using the spread plate method. After a period of six days incubation in Cit-Atz medium, 0.1m of each culture was inoculated in Cit-Atz agar appropriately. Isolation of atrazine-degrading bacteria was realized by inoculating the cultures on Cit-Atz agar containing nystatin. The inoculated plates were incubated at 30ºC and were observed daily for growth for ten days.

2.8 Characterization of Soil Samples and Organic Waste

Characterization of soil samples and the cassava peel waste were carried out using various standard analytical processes at a research institute in Lagos, Nigeria.

2.9 Soil pH

The pH of the treated and untreated soil samples were determined using Equip-Tronics digital pH

meter model EQ-610. The technique described by Onyeike and Osuji (2003) was used.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Properties of Soil before Treatment and Mineral Composition of the Organic Waste Used

The physicochemical status of the study site was carried out first in order to ascertain the prevailing soil condition before the experiments. The various soil parameters determined were as shown in Tables1, 2 showed the basic mineral element composition contained in the cassava peel waste used in this study.

3.2 Effect of Treatments on the Nutritional Status of the Soil

Tables 3, 4, and 5 revealed the changes that occurred in soil carbon, nitrogen and phosphorus contents of the soil respectively after the treatments.

3.3 Bacterial Identification and Degree of Occurrence

The indigenous culturable heterotrophic bacteria in the soil were isolated before and during the study using standard microscopic, cultural and biochemical techniques. Table 6 revealed the identification processes used for the characterization of bacterial isolates.

The total culturable heterotrophic bacterial isolates from the various treatments at weeks 0, 2, 4, and 6 were as recorded in Table 7.

Isolations were also made based on the ability of bacteria to utilize atrazine as a nitrogen source in the atrazine treated soil. The bacteria that have this capability were those that were able to grow in the Cit-Atz medium as shown in Table 8.

Table 1. Physicochemical properties of soil before treatment

Parameters	Quantity
pH	6.05
Soil type	Sandy Loam
Moisture content (%)	69.46
Organic carbon (%)	3.46
Total nitrogen (%)	0.16
Available phosphate (ppm)	373.70
Exchangeable cations (cmol/kg)	
Ca	14.44
K	6.07
Mg	5.07
Na	5.80
The particle size of soil	
Clay (%)	27.20
Sand (%)	46.80
Silt (%)	26.00

The values of the parameters were taken from composite soil sample and average of three replications

Table 2. Basic proximate mineral element composition of the cassava peel waste used

N (%)	C (%)	P (ppm)	K (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	Na (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mn (mg/kg)
0.87	43.33	19.19	51.88	31.09	96.70	19.00	25.36	3.88	388.49	21.46

Table 3. Effect of the treatments on the organic carbon content of the soil

		Mean of organic carbon content (%)	95% confidence Interval	
			Lower bound	Upper bound
Treatment	CPW	3.02[a]	2.82	3.22
	CPW+ATZ	2.86[a]	2.66	3.05
	ATZ	2.92[a]	2.72	3.12
	CONTROL	3.48[b]	3.28	3.68
Period	Week 0	3.46[a]	3.31	3.61
	Week 2	3.18[b]	3.03	3.33
	Week 4	2.91[c]	2.76	3.06
	Week 6	2.68[d]	2.53	2.83

ANOVA: Mean with the same superscript in the same column are not significantly different from one another at P<0.05.

Table 4. Effect of the treatments on the total nitrogen content of the soil

		Mean of total nitrogen content (%)	95% Confidence interval	
			Lower bound	Upper bound
Treatment	CPW	0.71[b]	0.54	0.88
	CPW+ATZ	0.66[b]	0.49	0.83
	ATZ	0.29[a]	0.13	0.46
	CONTROL	0.18[a]	0.01	0.34
Period	Week 0	0.16[a]	0.03	0.29
	Week 2	0.44[b]	0.32	0.57
	Week 4	0.53[b]	0.41	0.66
	Week 6	0.62[b]	0.49	0.74

ANOVA: Mean with the same superscript in the same column are not significantly different from one another at P<0.05.

Table 5. Effect of the treatments on the available phosphorus content of the soil

		Mean of available phosphorus (ppm)	95% confidence interval	
			Lower bound	Upper bound
Treatment	CPW	488.07[c]	454.57	521.57
	CPW+ATZ	442.56[b,c,]	409.05	476.06
	ATZ	406.55[a,b]	373.05	440.05
	CONTROL	387.85[a]	354.35	421.35
Period	Week 0	373.70[a]	348.38	399.03
	Week 2	431.09[b]	405.76	456.41
	Week 4	446.81[b]	421.49	472.14
	Week 6	461.50[b]	436.18	486.83

ANONA: Mean with the same superscript in the same column are not significantly different from one another at P<0.05.

Tables 9 and 10 above showed the effect of the treatments on the total culturable heterotrophic bacterial population and the soil pH respectively.

3.4 DISCUSSION

This research is aimed at reducing the deleterious effect of atrazine application on living organisms in the environment through biostimulation using organic waste. The addition of the cassava peel waste is to promote soil nutritional condition and stimulate soil bacteria for the degradation of atrazine, thus reducing the 'leaching window' available for the herbicide. The mean of the total organic carbon (TOC) content of the soil decreased significantly (P<0.05) in all the treatments. There was also a steady significant drop in the mean of the soil TOC content across the sampling periods; wk 0 = 3.46%, wk 2 = 3.18%, wk 4 = 2.91%, wk 6 = 2.68% (Table 3). Similarly, a degradation process during composting as observed by Sangodoyin and Amori (2013), affected the total organic carbon, causing a decrease in carbon content in treatments when compared with the initial carbon content [15]. Contrariwise, there are other reports where an increase in soil carbon content was observed via biostimulation [9,16,17].

The ANOVA result on the mean of the total nitrogen content (TNC) of soil revealed that soil treated with CPW and CPW+ATZ had high significant (P<0.05) total nitrogen content, while the increase in TNC in soil treated with ATZ was statistically insignificant. Generally, the treatments increased the soil nitrogen content significantly, but there was no significant change in the mean of the TNC of the soil at weeks 2, 4, and 6 (Table 4).

The rise in Phosphorus level in the soil treated with ATZ only, was not significant at P<0.05, while the increase in other treatments containing CPW was significantly high with the soil treated with CPW only having the highest significant value. Based on the sampling period, there was significant (P<0.05) increase in soil Phosphorus level at weeks 2, 4, and 6 when compared with week 0 (before treatment), but the values at weeks 2, 4, and 6 were not significantly different from one another (Table 5). Angelova *et al*. (2013) also recorded a similar effect (increased extractable phosphorus) when soil contaminated with heavy metal was treated with compost [18].

Table 6. Morphological and biochemical characteristics of bacterial isolates from the study

Isolate code	Gram reaction	Cell morphology	Spore	Motility test	Catalase test	Indole test	Citrate utilization test	Methyl red test	Voges-Proskauer test	H_2S production	Glucose fermentation	Lactose fermentation	Sucrose fermentation	Mannitol fermentation	Xylose fermentation	Probable generic identity
A	-	Short rods	-	-	+	-	+	-	-	-	+	-	-	-	+	*Acinetobacter* sp.
B	+	Rods	+	+	+	-	+	-	+	-	+^{g}	+	+^{g}	+	-	*Bacillus* sp.
C	-	Rods	-	+	+	-	+	-	+	-	+^{g}	+	+^{g}	+^{g}	+	*Enterobacter* sp.
D	+	Rods in chain	-	-	-	-	+	+	-	-	+	-	+^{g}	-	-	*Lactobacillus* sp.
E	+	Tiny cocci	-	-	+	-	-	-	-	+	-	-	-	+	+	*Micrococcus* sp.
F	-	Rod	-	+	+	+	-	+	-	+	+^{g}	-	+	-	-	*Proteus* sp.
G	-	Rods	-	+	+	-	+	+	-	-	+^{g}	-	-	+	-	*Pseudomonas* sp.

Key: + = Positive result; - = Negative result; g = Gas production

Table 7. Total culturable heterotrophic bacterial isolates from the various treatments

Treatment	Week 0 (composite)	Week 2	Week 4	Week 6
CPW	*Bacillus sp.* *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp.	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp.	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp.	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp.
CPW+ATZ	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp.	*Pseudomonas* sp. *Bacillus* sp. *Proteus* sp. *Micrococcus* sp.	*Acinetobacter* sp *Bacillus* sp. *Pseudomonas* sp. *Micrococcus* sp.	*Acinetobacter* sp *Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp
ATZ	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp.	*Acinetobacter* Sp. *Pseudomonas* sp.	*Acinetobacter* Sp. *Bacillus* sp. *Pseudomonas* sp.	*Acinetobacter* Sp. *Bacillus* sp. *Pseudomonas* sp. *Micrococcus* sp
CONTROL	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp.	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp.	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp	*Bacillus* sp. *Pseudomonas* sp. *Proteus* sp. *Enterobacter* sp. *Micrococcus* sp. *Lactobacillus* sp

Table 8. Isolated culturable Atrazine-degrading bacteria

Treatment	Week 2	Week 4	Week 6
CPW+ATZ	*Bacillus* sp. *Pseudomonas* sp.	*Acinetobacter* sp *Bacillus* sp. *Pseudomonas* sp.	*Acinetobacter* sp *Bacillus* sp. *Pseudomonas* sp. *Enterobacter* sp.
ATZ	*Acinetobacter* Sp. *Pseudomonas* sp.	*Acinetobacter* Sp. *Pseudomonas* sp.	*Acinetobacter* Sp. *Bacillus* sp. *Pseudomonas* sp.

The study revealed the presence of four-gram negative (*Acinetobacter* sp., *Enterobacter* sp., *Proteus* sp., *Pseudomonas* sp.) and three gram-positive (*Bacillus* sp., *Lactobacillus* sp., *Micrococcus* sp.) indigenous cultural heterotrophic bacteria present in the study site (Table 6). These bacteria responded differently to the sampling period and various treatments (atrazine application and organic amendment) applied in this study. Soil treated with organic wastes only gave the highest occurrence of these bacteria (CPW only = 85.71%). The organic amendment improved the soil condition which necessitated the increase in the culturable heterotrophic bacterial diversity when compared with the control (82.14%) (Table 7). It was observed that the total culturable heterotrophic bacterial diversity was reduced by 28.57% in the soil treated with atrazine only (ATZ), while biostimulation using organic materials: CPW+ATZ improved the diversity by 17.86%. Zhen *et al.* (2014) reported similar scenario on the excessive application of chemical fertilizers on croplands which resulted in the degeneration of soil microbial biomass, communities, and nutrient content. To mitigate this deplorable soil condition, they found out that adding manure compost significantly increased the number of cultivable microorganisms and microbial biomass. Thus, concluded that the application of manure compost can immediately improve the microbial community structure and diversity of degraded cropland soils. A decline in the culturable bacterial diversity of the impacted soil

was observed at week 2 (after atrazine application). This could be as a result of the direct impact of the herbicide on the organisms, because it has been proved by earlier studies [19,20,21] that pollutants (like herbicides) have the capability to kill microorganisms, make them dormant, or become viable but nonculturable (VBNC). A gradual recovery of the culturable heterotrophic bacterial community after atrazine application was observed. It is believed that this gradual recovery in bacterial diversity was hinged to a reduction in pollutant's concentration in the soil and gradual stabilization of the perturbed soil. *Pseudomonas* sp. was found to be outstanding among all the isolates because it occurred in all the treatments and at all the sampling periods, having 100% occurrence. *Bacillus* sp. was the next commonly isolated bacteria (96.43%). *Acinetobacter* sp. was the least isolated bacteria (32.14%) but having an exceptional feature in the sense that it was isolated only in atrazine-containing treatments (Table 7). This suggests that the herbicide (atrazine) has the potential to stimulate the culturability of certain latent bacteria in a perturbed environment. As earlier reported [22,19], atrazine application impacted negatively on the soil bacterial diversity. This was clearly observed in the soil treated with atrazine only (ATZ) in which organisms like *Enterobacter* sp., *Lactobacillus* sp., *Micrococcus* sp., and *Proteus* sp. were completely inhibited (were not culturable hence not isolated) two weeks after atrazine application till the end of the study except *Micrococcus* sp. which recurred at the sixth week of post herbicide application.

Table 9. Total culturable heterotrophic bacterial population response to organic amendment in atrazine impacted soil

Period	Treatment	Bacteria($x10^8$cfu/g) mean ± S.D
Week 0	CPW	30.00 ± 0.00^a
	CPW+ATZ	30.00 ± 0.00^a
	ATZ	30.00 ± 0.00^a
	CONTROL	30.00 ± 0.00^a
Week 2	CPW	18.05 ± 2.14^b
	CPW+ATZ	14.00 ± 4.80^b
	ATZ	11.90 ± 2.83^b
	CONTROL	28.40 ± 3.39^a
Week 4	CPW	20.50 ± 3.33^c
	CPW+ATZ	25.00 ± 2.21^a
	ATZ	20.50 ± 2.99^c
	CONTROL	28.50 ± 2.73^a
Week 6	CPW	25.25 ± 2.47^c
	CPW+ATZ	32.25 ± 4.60^d
	ATZ	35.00 ± 4.24^d
	CONTROL	31.60 ± 0.57^d

ANOVA: Mean with the same superscript in the same column are not significantly different from one another at P<0.05 S.D = Standard Deviation

Table 10. Effect of the treatments on soil pH

		Mean pH	95% confidence Interval	
			Lower bound	Upper bound
Treatment	CPW	5.245^a	5.034	5.456
	CPW+ATZ	5.553^a	5.342	5.763
	ATZ	5.420^a	5.209	5.631
	CONTROL	6.030^b	5.819	6.241
Period	Week 0	6.050^a	5.891	6.209
	Week 2	5.351^b	5.192	5.511
	Week 4	5.137^b	4.978	5.297
	Week 6	5.224^b	5.065	5.384

ANOVA: Mean with the same superscript in the same column are not significantly different from one another at P<0.05

This study also revealed that not all the isolated bacteria from atrazine-spiked soil were actually degrading/utilizing atrazine as a source of nutrient in the various treatments containing atrazine. This was manifested by *Proteus* sp. and *Lactobacillus* sp. which were isolated in atrazine-treated soils but were absent when atrazine degradation study was carried out (Table 8). The bacteria that indicated atrazine degradation potential as isolated using Cit-Atz agar were *Acinetobacter* sp., *Enterobacter* sp. *Pseudomonas* sp. and *Bacillus* sp. (Table 8). It was observed that *Enterobacter* sp. was never isolated as atrazine degrader in the soil treated with ATZ only but manifested as atrazine degraders in CPW+ATZ (*Enterobacter* sp. at week 6) (Table 8). This finding corresponds to the report of Tejada *et al*. (2010) who established that when organic materials are added to the soil, they work through many mechanisms that are beyond activation of soil microbial activity and promotion of the activities of important soil enzymes, which would ultimately culminate to increased microbial populations [23]. Soil amendment using organic wastes as further buttressed by Coleres, (2005), has the capability to restrict contaminant availability to microorganisms leading to the evolution of microbial population that specialize in accessing bound contaminants [24]. The study showed that CPW+ATZ had more diverse atrazine-degrading bacteria than ATZ. With this, it is established that the addition of organic materials to atrazine-polluted soil enhances its recovery (microbiologically) after perturbation, and the recovery is directly proportional to time (week 6 having the highest diversity of atrazine degraders) and the kind of amendment added. The information on Table 8 showed that *Pseudomonas* sp. was the most predominant atrazine degrader across all the treatments and at all sampling period. This confirms the report of Yanze-Kontchou and Gschwind (1994), Mandelbaum et al., (1995), Ojo (2007), and Hernandez *et al*. (2008) in which *Pseudomonas* spp. were declared potential atrazine degraders [25,26,27,28]. Also, *Bacillus* sp. [29] *Acinetobacter* sp. [30] and *Enterobacter* sp. were all affirmed atrazine degraders.

The result as shown in Table 9 revealed that the various treatments had a significant effect on the soil bacterial population. At week two, a significant ($P<0.05$) drop in mean bacterial population in all the treatments (CPW=$18.05\pm2.14\times10^8$cfu/g; CPW+ATZ=$14.00\pm4.80\times10^8$cfu/g; ATZ=$11.90\pm2.83\times10^8$cfu/g) was observed when compared with the Control ($28.40\pm3.39\times10^8$cfu/g). The treatments, CPW+ ATZ and Control were statistically higher in bacterial population than CPW and ATZ treatments at week four. Table 9 also showed that CPW maintained the least in the bacterial count at week six. This result revealed a gradual recovery of the bacterial status of the soil to its original state based on the prevailing environmental condition. The result in Table 9 also showed that the bacterial population in ATZ at week six ($35.00\pm4.24\times10^8$cfu/g) was higher than the Control ($31.60\pm0.57\times10^8$cfu/g) and the other treatments (CPW=$25.25\pm2.47\times10^8$cfu/g; CPW+ ATZ=$32.25\pm4.60\times10^8$cfu/g) though lower in diversity (Tables 7 and 8). This result is in agreement with Richardson (1970), who observed that the growth of some microorganisms was stimulated by the lower doses of atrazine [31]. This phenomenon of enrichment by polluting compounds as observed by Zrafi-Nouira et al. (2009) in the swing between *Gamma* and *Alpha Proteobacteria* in terms of abundance and diversity, which latter led to the proliferation of *Gamma Proteobacteria* was called “Gamma-shift” [32]. Therefore, it can be deduced from the result obtained that the increase in bacterial population in the polluted microcosms was as a result of this shift.

It was also observed that the mean of the soil pH was significantly reduced at $P<0.05$ after the treatments. Results of the ANOVA showed no significant ($P<0.05$) reduction in pH between soil treated with CPW (5.25), CPW+ATZ (5.55) and ATZ (5.42). However, all the treatments showed reduced soil pH, which was significantly ($P<0.05$) lower than the value obtained in the Control (6.03). Weeks 2, 4, and 6 showed no significant difference within the mean of their pH values but were significantly more acidic than the pH at week 0 (Table 10). This is in agreement with the results obtained by Maduike et al. (2013), Stanley et al. (2013), and Ayansina and Oso (2006) which reported a decline in soil pH after atrazine application [33,19,34]. Also, this pH results concurred with Angelova et al. (2013) who reported a significantly reduced soil pH after treatment with organic material (compost) [18].

4. CONCLUSION

The nutrient composition analysis of the organic waste used in the study (cassava peel waste), showed that it has the capability of improving organic nutrient level in the atrazine-impacted

soil, thus a great potential in bioremediation of atrazine-contaminated soil. It was observed that the addition of CPW in the impacted soil stimulated the proliferation of the naturally selected atrazine-degrading bacteria which is believed will improve the rate of atrazine degradation in the polluted soil. From the observations made in the study, pollutants (atrazine) at a tolerable level can improve bacterial population (as a result of the proliferation of naturally selected degraders) but reduce bacterial diversity. It is also believed that the addition of organic materials enhanced the resuscitation of suppressed or latent microbes (caused by atrazine application), thereby improving microbial (bacterial) diversity and abundance of atrazine degrading microbes in the impacted soil. This showed that the addition of organic wastes on atrazine-impacted soil helps to reduce the effect of the pollutant on soil microorganisms (bacteria). From the study, it can be deduced that not all microorganisms isolated in a polluted environment are actually degrading or utilizing the pollutant. It is evident that some microorganisms may have the ability to thrive in the presence of a pollutant and derive their nourishment from other sources within the polluted environment other than the pollutant itself. This was proved by the presence of *Proteus* sp. and *Micrococcus* sp. in atrazine-polluted sites and their absence when atrazine degradation study by bacteria was carried out.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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