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Biostimulatory Effect of Cassava Peel Waste on the Indigenous Fungi in Atrazine-impacted Soil

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Authors' contributions

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

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ABSTRACT

The biostimulatory effect of cassava peel waste on the indigenous fungi in atrazine-impacted soil was assessed over a period of seven (7) weeks. The study site was at the Teaching and Research Farm of the institution. The herbicide used in this study was atrazine (Atraforce) while the organic waste used was the cassava peel waste (CPW) of *Manihot esculenta*. The fungal and physicochemical characteristics of the soil treated with cassava peel waste (CPW), cassava peel waste and atrazine (CPW+ATZ), atrazine alone (ATZ), and the CONTROL (no treatment) were assessed using culture-dependent and standard analytical techniques respectively. The influence of the treatments on soil organic carbon content, total nitrogen content and available phosphorus was investigated and recorded. The study provided adequate evidence that the study site was naturally endowed with requisite fungi (*Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Saccharomyces* sp., *Fusarium* sp., *Candida* sp., and *Trichophyton* sp.) with potential enzyme repertoire for atrazine

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degradation. All the seven isolated indigenous fungi except *Saccharomyces* sp. showed evidence of atrazine degradation potential. The addition of organic amendments improved the physicochemical status of the impacted soil which culminated to significant ($P<0.05$) increase in soil's fungal population. For example, CPW+ATZ showed a high mean fungal population (week 2 =$30.00x10^3$ CFU/g, week 4 =$35.00x10^3$ CFU/g, week 6 =$81.00x10^3$ CFU/g) which was significantly higher (at $P<0.05$) than impacted but unamended soil, ATZ (week 2 =$9.50x10^3$ CFU/g, week 4 =$12.50x10^3$ CFU/g, week 6=$46.50x10^3$ CFU/g). This study has shown that, although fungi are excellent degraders of herbicides in the soil, some amendments may need to be brought in place in order to stimulate them to degrade pollutants. This work also revealed that the organic waste (CPW) used was a potential stimulatory substrate that enhanced the growth of indigenous atrazine-degrading soil fungi; hence can serve as improved method of waste management and potential soil remediation approach.

Keywords: Atrazine; soil; fungi; cassava peel waste; biostimulation.

1. INTRODUCTION

To minimize the losses caused by weed invasion in farmlands, a novel tool which involves the selective use of herbicides by farmers in the control of weeds is a common practice [1]. Increased productivity became the outcome of the use of herbicides by farmers, but, on the other hand, resulted to some unpleasant consequences, since many of these herbicides are injurious to man and other non-target organisms that come in contact with them in the environment. Therefore, because of the general use of herbicides for a long time, and its abusive and indiscriminate application, experts in public health and other public authorities have shown concern in order to maintain the sustainability of natural resources [2].

Herbicides are directly introduced into the soil during weed control or indirectly through herbicide-contaminated water, plant and animal residues introduced into the soil. After herbicide application, one or more of the following may occur; (i) evaporation of herbicide (volatilization), (ii) leaching of herbicide into deep soil, (iii) inactivation of herbicide by plant, (iv) washing away of herbicide through surface run-off, and (v) adsorption of herbicide residues in soil, in which case they become subject to chemical or microbial degradation [3]. In the soil, the rate of herbicide degradation depends solely on (i) the chemical composition of the herbicides [4], (ii) application dosage [5,6], (iii) physicochemical properties of the soil [7,8], (iv) plant cover, humidity and temperature, and (v) the types of soil microorganisms present [9-11,7,12,6].

Stanley et al. [13] observed that herbicide application did not only affect the target organisms (weeds) but also the population and diversity of indigenous microbial communities present in soils. In animals and humans, the endocrine (hormonal) system is the immediate target of atrazine. Previous studies have revealed that atrazine disrupts the endocrine system of animals by altering their natural hormonal system [14,15]. The effect of this endocrine disruption has been manifested in children's health during sexual development in pregnancy. Exposure of pregnant animals to atrazine has been associated with the danger of increased intrauterine growth retardation and preterm delivery [16,17]. Experimental animals have experienced delays or alterations in pubertal development due to exposure to atrazine [18-22].

Contamination of soils, groundwater, sediments, surface water, and air with hazardous and toxic chemicals is a problem facing the industrialized as well as developing countries today. Hence, necessary remediation plans have led to the advancement in technology where more emphases are on detoxification and removal of pollutants from the environment. One of the approaches is the use of organic materials to stimulate the activities of bacterial degraders. In this study, cassava peel waste (CPW) from *Manihot esculenta* (Crantz) was the choice substrate for biostimulation. The most common by-product in the processing of cassava fermented products in Nigeria is the cassava peels which is dumped indiscriminately in the environment. The selection of this organic waste supports the campaign for green chemistry, reduces environmental pollution and encourages waste management. Based on this work, the organic waste (cassava peel waste) is expected to be used as a potential bio-stimulatory substrate to promote the proliferation of naturally selected fungal degraders for the breakdown of

atrazine in the soil, thereby mitigate the harmful effect of herbicide (atrazine) on man, wild life, plants, and microorganisms.

2. MATERIALS AND METHODS

2.1 Study Site

The study site was located at the Faculty of Agriculture Research farm, University of Port Harcourt, Port Harcourt, Rivers State, Nigeria. The total dimension of the study site was 13.0 m by 9.5 m. The study area was further divided into twelve (12) smaller blocks of 0.25 m^2 area (microcosms) at 3 m apart from each other (3 rows; 4 columns). The geographical coordinate of the site is Latitude 4.908428 (4°54′ 30.34″ N) and Longitude 6.923089 (6°55′ 23.11″ E). The temperature of the soil fluctuated between 26 and 27° C throughout the study period in all the treatments. This study was carried out during the rainy season.

2.2 Herbicide and Organic Waste Used

The herbicide (atrazine) used in this study was purchased from an agricultural dealer store at Rumuokoro, 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 proposed by Pal and Das Gupta (1994) [23] was also adopted. The cassava peel waste (CPW) was collected from harvested cassava (*Manihot esculenta*) in Obinze, Owerri-West, Imo State, Nigeria. The basic proximate mineral element composition of the cassava peel waste was determined using standard laboratory techniques in a research institute, Lagos, Nigeria.

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, shredded, sterilized (autoclaved at 121° C for 20minutes to prevent microbial interference from the organic wastes), cassava peel waste (CPW). After seven (7) days, the herbicide (atrazine) was applied at manufacturer's recommended rate. 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

Plate 1. Dried, shredded cassava peel waste

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 Fungi

Sabouraud dextrose agar (SDA) was used for enumeration and isolation of total culturable fungi using the spread plate technique. Fungal isolates were directly examined and characterized based on cultural morphology and microscopic examination in lacto phenol cotton blue stain with reference to Barnett and Hunter [24] and Larone [25].

2.6 Growth and Degradation Studies

A modified Czapek Dox Agar and Udikovic *et al*. (2003) [26] 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 litre to form Cit-Atz agar. The Cit-Atz medium was used to isolate atrazine degraders. To isolate atrazine-degrading fungi, 75 mL/L antibiotic solution (2.5 g chloramphenicol, 5.0 g streptomycin, and 1.0 L distilled water) [27] was used to inhibit bacterial contamination during the study. 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 min. An equal volume of the 1ml slurry was taken and was added to 250 mL Erlenmeyer flask containing 50 mL of Cit-Atz medium and antibiotic solution. The inoculated flasks were incubated at room temperature (26° C) for 6 days.

2.7 Isolation of Atrazine Degrading Fungal Strain

Atrazine degrading fungal strains were isolated with the Cit-Atz agar using the spread plate method. After a period of six days incubation in Cit-Atz medium, 0.1 mL of each culture was inoculated in Cit-Atz agar appropriately. Isolation of atrazine degrading fungi was achieved by inoculating the cultures on Cit-Atz agar containing antibiotics. The inoculated plates were incubated at room temperature (26° C) and were observed daily for growth for ten days.

3. RESULTS

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 Table1. Table 2 showed the basic mineral element composition contained in the cassava peel waste used in this study.

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.

The effect of the treatments on the mean organic carbon content, total nitrogen content and available phosphorus of the soil was assessed. The observable changes as compared with the control microcosm were as recorded in Table 3.

The total culturable fungal isolates from the various treatments at weeks 0, 2, 4, and 6 were as recorded in Table 4. It also showed the fungal diversity within each treatment across the sampling periods.

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 mean organic Carbon content, total Nitrogen content and available Phosphorus of the soil

		Mean of organic carbon content (%)	Mean of total nitrogen content (%)	Mean of available phosphorus (ppm)
Treatment	CPW	3.02^{a}	0.71^{b}	488.07^{c}
	CPW+ATZ	2.86^{a}	0.66^{b}	$442.56^{b,c,}$
	ATZ	2.92^{a}	0.29^{a}	$406.55^{a,b}$
	CONTROL	3.48^{b}	0.18^{a}	387.85^{a}
Period	Week 0	3.46^{a}	0.16^{a}	373.70^{a}
	Week 2	3.18^{b}	0.44^{b}	431.09^{b}
	Week 4	2.91^{c}	0.53^{b}	446.81^{b}
	Week 6	2.68^{d}	0.62^{b}	461.50^{b}

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

Table 4. Total culturable fungal isolates from the various treatments

Treatment	Week 0 (composite)	Week 2	Week 4	Week 6
CPW	*Rhizopus* sp. *Aspergillus* sp. *Penicillium* sp. *Saccharomyces* sp.	*Rhizopus* sp. *Saccharomyces* sp. *Fusarium* sp.	*Trichophyton* sp. *Saccharomyces* sp. *Penicillium* sp. *Candida* sp. *Fusarium* sp.	*Rhizopus* sp. *Aspergillus* sp. *Penicillium* sp. *Trichophyton* sp.
CPW+ATZ	*Rhizopus* sp. *Aspergillus* sp. *Penicillium* sp. *Saccharomyces* sp.	*Rhizopus* sp., *Aspergillus* sp. *Trichophyton* sp.	*Trichophyton* sp. *Saccharomyces* sp. *Penicillium* sp. *Candida* sp. *Fusarium* sp.	*Rhizopus* sp., *Aspergillus* sp. *Penicillium* sp.
ATZ	*Rhizopus* sp. *Aspergillus* sp. *Penicillium* sp. *Saccharomyces* sp.	*Rhizopus* sp. *Aspergillus* sp. *Trichophyton* sp. *Saccharomyces* sp.	*Trichophyton* sp. *Saccharomyces* sp. *Penicillium* sp. *Candida* sp. *Fusarium* sp.	*Rhizopus* sp. *Aspergillus* sp. *Penicillium* sp.
CONTROL	*Rhizopus* sp. *Aspergillus* sp. *Penicillium* sp. *Saccharomyces* sp.	*Rhizopus* sp. *Aspergillus* sp. *Saccharomyces* sp. *Fusarium* sp.	*Trichophyton* sp. *Saccharomyces* sp. *Candida* sp. *Fusarium* sp. *Aspergillus* sp.	*Rhizopus* sp. *Aspergillus* sp. *Penicillium* sp.

Isolations were also made based on the ability of fungi to grow (utilize atrazine) in the atrazine treated soil, and the fungi that were able to grow in the Cit-Atz medium were as shown in Table 5. It revealed how the treatments spiked with atrazine influenced the indigenous culturable atrazine-degrading fungal diversity (species richness) when compared with the unperturbed (Control) site.

Earlier studies have proved that chemicals like herbicides affect the population of fungi when applied to the soil. Table 5 showed how these microorganisms responded to the various

treatments introduced into the soil. It also considered the population dynamics with time.

4. DISCUSSION

Earlier studies have proved that herbicide (atrazine) application on the soil has a deleterious effect on the biotic and abiotic properties of the soil. This study was aimed at stimulating the catabolic activities of atrazine-degrading indigenous fungi by improving they're *in situ* growth conditions using organic amendment (cassava peel waste). The physicochemical conditions of the study site were determined before amendment with the organic waste and the subsequent spiking with atrazine (Table 1).

Table 5. Culturable Atrazine-degrading fungi

Treatment	Week 2	Week 4	Week 6
CPW+ATZ	*Aspergillus* sp. *Candida* sp. *Rhizopus* sp.	*Candida* sp.	*Trichophyton* sp.
ATZ	*Aspergillus* sp. *Candida* sp. *Trichophyton* sp. *Rhizopus* sp.	*Trichophyton* sp. *Penicillium* sp.	*Trichophyton* sp. *Fusarium* sp.

Plate 2. *Aspergillus* sp.

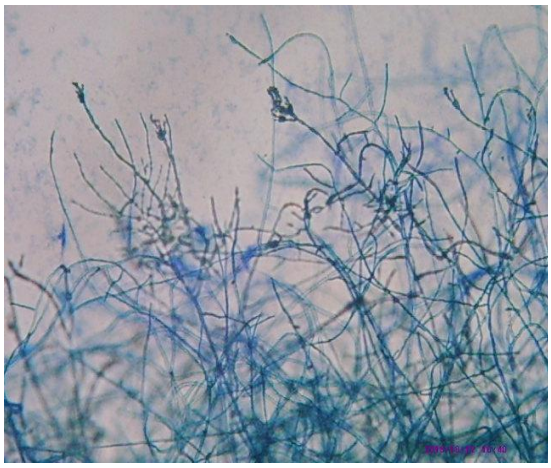

Plate 3. *Penicillium* sp.

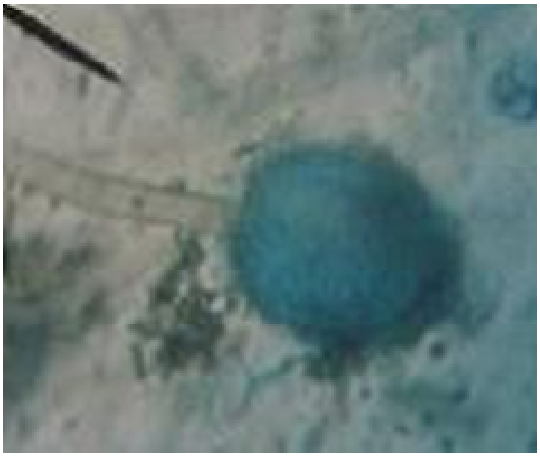

Plate 4. *Rhizopus* sp.

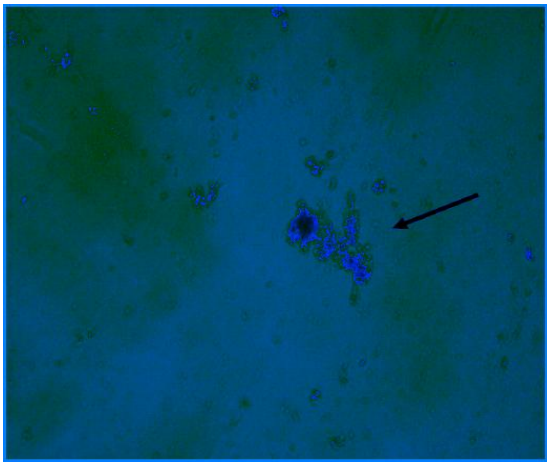

Plate 5. *Saccharomyces* sp. (arrowed)

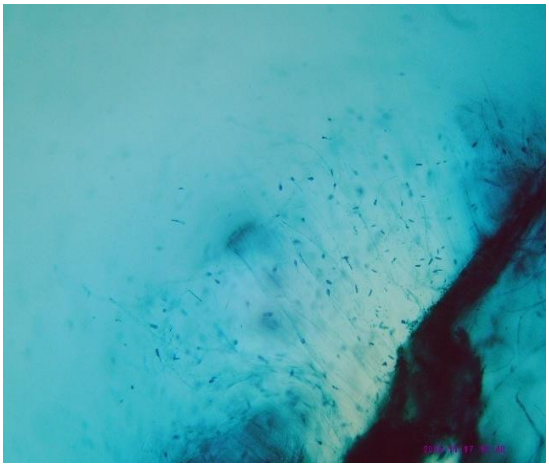

Plate 6. *Fusarium* sp. (with macroconidia)

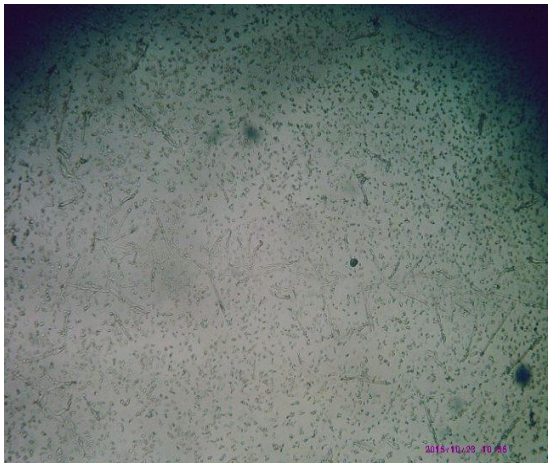

Plate 7. *Candida* sp. (with pseudohyphae)

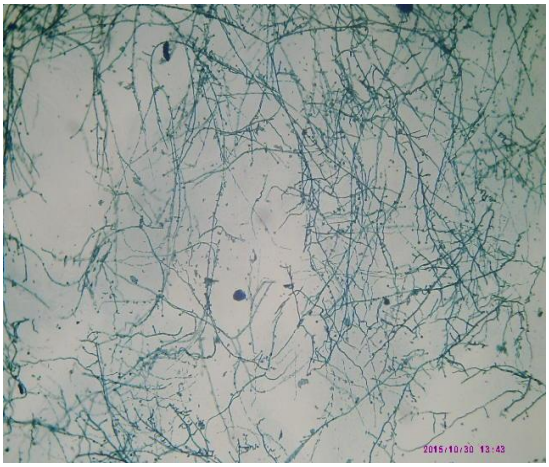

Plate 8. *Trichophyton rubrum* (with microconidia like match-head formed singly all along the sides of the septate hyphae)

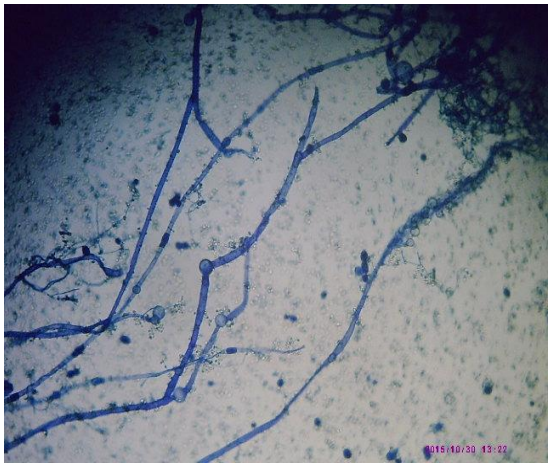

Plate 9. *Trichophyton tonsurans* (having microconidia with various shapes and sizes, and some are like baloon form)

A significant (P<0.05) decrease in the mean total organic carbon (TOC) content of the soil was observed in all the treatments. A clear drop in TOC content across the sampling periods; wk 0 = 3.46%, wk 2 = 3.18%, wk 4 = 2.91%, wk 6 = 2.68% was also reported. Similarly, Sangodoyin and Amori [28] recorded a decrease in carbon content in treatments when compared with the initial carbon content in their study on the degradation process during composting.

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 soil TNC treated with atrazine only (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 within weeks 2, 4, and 6 (Table 3). Karki and Dixit [29] and Sangodoyin and Amori [28] reported that cassava peel waste has high C: N ratio of 48.7:1 and 49:1 respectively. This report corresponds to the C: N ratio (49.8:1) obtained in the cassava peel waste used in this study. Increase in the soil available phosphorus was evident in all the treatments. However, the rise in phosphorus level in the soil treated with atrazine only (ATZ) was not significant at P<0.05, while the increase in other treatments was significant with the soil treated with CPW only having the highest significant value. 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 3). Angelova et al. [30] also recorded a similar effect (increased extractable phosphorus) when soil contaminated with heavy metal was treated with compost.

Table 6. Total culturable fungal population response to organic amendment in atrazine impacted soil

Period	Treatment	Fungi(x10^{3}cfu/g) Mean ± S.D
Week 0	CPW	44.00 ± 0.00^a
	CPW+ATZ	44.00 ± 0.00^a
	ATZ	44.00 ± 0.00^a
	CONTROL	44.00 ± 0.00^a
Week 2	CPW	41.00 ± 2.83^b
	CPW+ATZ	30.00 ± 2.83^c
	ATZ	9.50 ± 4.95^b
	CONTROL	43.50 ± 0.71^b
Week 4	CPW	45.50 ± 3.54^d
	CPW+ATZ	35.00 ± 2.83^e
	ATZ	12.50 ± 7.78^d
	CONTROL	45.00 ± 1.41^d
Week 6	CPW	52.50 ± 31.82^f
	CPW+ATZ	81.00 ± 8.49^g
	ATZ	46.50 ± 4.95^f
	CONTROL	50.50 ± 47.38^f

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

This work revealed the presence of seven (7) culturable fungi in the study site (*Rhizopus* sp., *Aspergillus* sp., *Penicillium* sp., *Saccharomyces* sp., *Fusarium* sp., *Candida* sp., and *Trichophyton* sp.) as shown in Table 4. The table also revealed that the soil treated with CPW, ATZ only, and CONTROL had the same (highest) percentage mean fungal diversity (57.14%; n=7), while CPW+ATZ had 53.57% of mean fungal diversity. This result suggests that the concentration of atrazine applied did not have an observable impact on the soil fungal diversity since there was no difference in the percentage fungal diversity between the CONTROL and ATZ. This observation is similar to Richardson [31], who reported no lethal effect of atrazine application on soil fungi, rather noticed that most of the fungi were retarded to some extent. *Rhizopus* sp. and *Aspergillus* sp. had the highest occurrence (75.00% each), while *Fusarium* sp. (37.50%) and *Candida* sp. (25.00%) were the least isolated fungal genera across the various treatments during the study (Table 4). *Fusarium* sp., *Candida* sp., and *Trichophyton* sp. were not isolated from the composite soil sample before the treatments (week 0), but were evidently present at week(s) 2 and/or 4. This confirms the microbial dynamics of the soil matrix which was also reported by Coleman and Crossley [32]. All the isolated fungi except *Saccharomyces* sp. showed evidence of atrazine degradation potential (Table 5). This finding is in agreement with Kaufman and Blake [33], Mougin et al. [34], and Ojo [35] who reported of the ability of *Aspergillus* sp., *Rhizopus* sp., *Fusarium* sp., and *Penicillium* sp. to degrade atrazine. Other fungi like *Candida* sp. [36], and *Trichophyton* sp. [37] have also been proved to possess atrazine degradation potential.

The isolated fungi from atrazine-treated soil using Cit-Atz agar did not indicate the presence of culturable *Rhizopus* sp. and *Aspergillus* sp. at 4 and 6 weeks after atrazine application. *Penicillium* sp. and *candida* sp. were isolated at week 4 but not at week 6 (Table 5). Based on the findings of Zain et al. [38] and Richardson [31], these fungi whose growths were suppressed or inhibited are believed to reoccur in future time depending on the rate of atrazine dissipation from the impacted soil. The occurrence of *Trichophyton* sp. was outstanding as an excellent atrazine degrader in this study (Table 5). It was observed that CPW+ATZ had the lower fungal diversity of atrazine degraders in this work (Table 5), it is noteworthy that the soil, CPW+ATZ showed a high mean fungal population (week 2=30.00x10^3 CFU/g, week 4=35.00x10^3 CFU/g, week 6=81.00x10^3 CFU/g) which was significantly higher (at P<0.05) than other treatments in each sampling period after atrazine application (Table 6). This implied that cassava peel waste enhanced the proliferation of the naturally selected few atrazine-degrading fungi after atrazine application. This increase in the population of atrazine degraders in the polluted but amended soil will naturally speed-up atrazine degradation rate.

5. CONCLUSION

Results from this *in situ* study are of great importance, as the contamination of soil from herbicide application is continuously increasing and many aftermath effects of the residues have

been frequently reported from the affected areas. The herbicide, atrazine has been classified by regulatory agencies as one of the major target anthropogenic pollutants requiring immediate attention and effective development of methods for decontamination of contaminated matrices. The present study provided adequate evidence that cassava peel waste has great potential in bioremediation of herbicide-contaminated sites. It was observed that the addition of CPW in the impacted soil stimulated the proliferation of the naturally selected atrazine-degrading fungi. Pollutants (atrazine) can improve microbial population (as a result of the proliferation of degraders) but reduce microbial diversity. The addition of organic materials enhanced the resuscitation of suppressed or latent microbes (caused by atrazine application), thereby improving fungal diversity and abundance of atrazine degrading microbes in the impacted soil.. Finally, this work revealed that the organic waste used in this study is a potential bio-stimulatory substrate that enhanced the growth of indigenous atrazine-degrading soil fungi; hence can serve as an improved method of waste management and potential soil remediation approach.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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