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Assessment of the Biodegradation of Herbicides by Bacteria Isolated from the Soil

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Authors' contributions

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

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Short Research Article

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ABSTRACT

Aim: The aim of this research is to assess the biodegradation of herbicides by bacteria isolated from the soil.
Study Design: This study is designed to isolate, identify and characterize bacteria from the soil, to determine the degradation of herbicides by the bacterial isolates.
Place and Duration of Study: Department of Microbiology, Nasarawa State University Keffi, Nasarawa State, Nigeria, between January 2017 and December 2017.
Methodology: Bacteria were isolated from two different soil samples using pour plate method. The isolated bacteria include the species of *Bacillus, Pseudomonas* and *Staphylococcus aureus* which were grown on three different herbicides, containing mineral salt medium to determine their biodegradation potential. And degradation was measured by optical density checked using a spectrophotometer at 3 days interval.
Results: The result revealed degradation of the herbicides by the isolates. All isolates were able to utilize the glyphosphate in the medium and as such growth was noticed. Out of the three isolates used (*Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus*), the best degradation

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observed was by *B. subtilis* at 82.61% on glyphosphate, followed by *P.aeruginosa* at 69.67% on Glyphosphate and finally *S. aureus* at 47.83% also on glyphosphate. The result also revealed low percentage degradation of the other herbicides by these organisms. Microorganisms were able to degrade glyphosate due to their ability to utilize the organophosphorus as the sole phosphorus source.

Conclusion: From this study it can be deduced that the isolated bacteria can be used for bioremediation of herbicide contaminated and polluted soil although further studies may be required to standardize the procedure.

Keywords: Biodegradation; herbicides; Bacillus sp.; Pseudomonas sp.; Staphylococcus sp.; glyphosate; organophosphorus.

1. INTODUCTION

Soil is top layer of the Earth's surface, consisting of rock and mineral particles mixed with decayed organic matter (humus), and capable of retaining water, providing nutrients for plants, and supporting a wide range of living and non-living communities. Soil is a mixture of minerals, organic matter, gases, liquids, and countless organisms that together support life on Earth. It is called the "Skin of the Earth" and interfaces with its lithosphere (rocks), hydrosphere (water), atmosphere (gases) and biosphere [1].

Biodegradable matter is generally organic material that serves as a nutrient for microorganisms. Microorganisms are so numerous and diverse that a huge range of compounds are biodegraded, including hydrocarbons (e.g. oil), polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances. The degradation rate of many organic compounds is limited by their bioavailability. Compounds must be released into solution before organisms can degrade them [2].

Bacteria constitute a large domain of prokaryotic microorganisms typically a few micrometres in length, with a number of shapes, ranging from spheres to rods and spirals. Once regarded as plants constituting the class *Schizomycetes*, bacteria are now are classified as prokaryotes. Unlike cells of animals and other eukaryotes, bacterial cells do not contain a nucleus and rarely harbour membrane-bound organelles. Bacteria inhabit soil, water, acidic hot springs, radioactive waste [3,4] and the deep portions of Earth's crust. Bacteria also live in symbiotic and parasitic relationships with plants and animals. The soil typically contains 40 million bacterial cells in a gram and approximately 5×10^{30} bacteria on Earth [5] forming a biomass which exceeds that of all plants and animals. Most bacteria have not been characterized, and only about half of the bacterial phyla have species that can be grown in the laboratory [6].

Herbicides persist over decades in groundwater, although bacteria are in principle abundant and potentially able to degrade them for unknown reasons. This may be related to the observation that microbial degradation appears to stall at low herbicides concentrations in low-nutrient environments such as groundwater. As yet, very little is known about herbicides biodegradation under such conditions. Methods have been lacking to follow biodegradation in groundwater over the relevant long time scales and to isolate relevant degraders from such environment [7]. Glyphosphate (GP) bioaccessibility for degrading microorganisms may be reduced because of adsorption on the soil matrix [8,9]. Most herbicide accumulates in the upper horizon at the depths up to 10–15 cm, where it is readily accessible for degrading microorganisms; at the same time, its migration to the underlying horizons may result in herbicide accumulation due to the absence or a strong reduction in the microbial population [9].

Weed resistance to herbicides has become a major concern in crop production worldwide. Resistance to herbicides is often attributed to lack of rotational programs of herbicides and to continuous applications of herbicides with the same sites of action [10]. Thus, a true understanding of the sites of action of herbicides is essential for strategic planning of herbicide-based weed control [11]. It was not until recently that researchers became interested in applying glyphosphate-degrading bacteria for bioremediation of polluted soils. The first attempt to apply the laboratory strain *Pseudomonas* sp. 4ASW, which is capable of cleaving glyphosphate with the production of sarcosine, was unsuccessful, because its C–P lyase was completely inactivated under field conditions [8].

2. MATERIALS AND METHODS

2.1 Sample and Sample Collection

Soil sample for this study was collected from a Cassava farm in BCG area of Keffi, Nasarawa state and Soil from the school botanical garden Nasarawa State University Keffi. Soil sample had no herbicides treated on them and the top soil of (5-10centimeters) was collected using a spade and transferred into separate polythene bags which were transported to the laboratory for further analysis.

2.2 Microbial Counts and Isolation

One (1.0) gram of the soil sample was weighed using a weighing balance and was suspended in 9ml of sterile water. It was properly mixed and a 10 fold serial dilution was carried out into seven dilutions ($10^{-1,}$ $10^{-2,}$ 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}). The diluted samples from 10^{-3} to $^{10-7}$were plated on sterile prepared and gelled Nutrient agar and Herbicide agar by pour plate method and incubated at 37°C for 48 hours for enumeration of total aerobic heterotrophic bacteria. Colonies were observed on the plates and were counted and recorded as colony forming units per gram of soil (cfu/g). The isolates were sub-cultured on Nutrient agar and Herbicide agar to obtain a pure culture, which were grown and stored on agar slants for further characterization and identification. The pure cultures were identified based on basis of their cultural, morphological and a physiological characteristic in accordance to the method described by Damales & Eleftherohorinos (2011) [12] and was stored at 4℃ as stock culture.

2.3 Characterizations and Identification of Bacteria Isolates

The bacteria isolates were gram stained and characterized based on their cell morphology, colony shape, color, grams reaction and biochemical test.

2.4 Utilization of Herbicides by Bacteria Isolates

Nutrient broth was prepared, 7 mL was dispensed into tests tubes after which 3 mL of herbicides was introduced into the nutrient broth and sterilized. To each test tube bacteria isolated was inoculated in them and incubated for 48 hours. Mineral salt medium was prepared according to Zajic and Supplisson (1972) [13], it was sterilized and 18 mL was dispensed into boiling tubes. To each tube, 2 mL of nutrient broth grown culture of herbicides bacteria isolated were added and incubated for 21 days. Degradation was measured by checking cell growth through optical density at 3 days interval. Turbidity in liquid medium is an indication of cell growth and can be measured using a spectrophotometer. The cell culture was placed in a transparent cuvette and the absorbance was recorded at a wavelength of 600 nm along with a control to check for biodegradation of herbicides by the bacteria isolates using the spectrophotometer.

3. RESULTS AND DISCUSSION

3.1 Microbial Counts

All the plates for microbial count yielded growth. At dilution factor of 10^3 there was 17.2, at 10^4 there were 11.6, at 10^5 there were 5.2 colonies, at 10^6 there were 2.8 and 1.2 colonies at $10^{7.}$

3.2 Degradation Studies

All three isolates were promising in the degradation of glyphosphate. As for Weed off and Atrazine there was little degradation observed.

4. DISCUSSION OF RESULTS

In the present study, bacteria were isolated from the soil. Table 1 shows the total bacteria count of the soil sample collected and plated, the plate counts ranging from 1.2×10^7 to 17.2×10^3. Table 2 shows the total viable bacterial counts on herbicide agar, no growth was observed on the herbicide agar infused with the herbicides Athrazine and Weed off. This is an indication that the organisms are unable to utilize the herbicides as a source of nutrient for growth [9,14]. However, there was growth on the herbicide agar infused with glyphosphates which points to the fact that the organisms in the medium have been able to utilize the glyphosphate as a source of nutrient for growth. Table 3 shows the results of the biochemical tests carried out in characterization and identification of the bacterial isolates. The bacteria isolated were *Bacillus subtilis, Pseudomonas aeruginosa* and *Staphylococcus aureus.* These organisms were inoculated on three different herbicides containing mineral salt medium to determine their ability to degrade herbicides. Mineral salt medium serves as a carbon source in supporting the growth of the organisms in the herbicides. Tables 4,5 and 6 show the results of the

Table 1. Total viable bacteria counts in the soil sample when cultured on nutrient agar

Bacterial count (cfu/g) in soil					
Dilution factor	10^3	10^4	10^5	10^6	10^7
Bacteria	17.2	11.6	5.2	2.8	1.2

Table 2. Total viable bacterial counts on herbicide agar

Herbicide utilizing bacteria count (cfu/g) in soil			
Herbicides	10^3	10^4	10^5
Weed off	NG	NG	NG
Glyphosphate	23.0	12.0	5.0
Atrazine	NG	NG	NG

NG= No Growth

Table 3. Morphology and biochemical characterization of pure isolates

Biochemical test	*S. aureus*	*B. subtilis*	*P. aeruginosa*
Grams reaction	+	+	-
Shape	Cocci	Rods	Rods
Catalase	+	+	+
Coagulase	+	-	-
Starch hydrolysis	-	+	-
Oxidase	-	-	+
MSA	+	-	-
VP	-	+	+
MR	+	-	-
Indole	-	-	-
Citrate	-	+	+

Table 4. Degradation of Herbicides by *Staphylococcus aureus* at 600 nm optical density

Herbicides	Control (nm)	Degradation	% Degradation
Weed off	0.233	0.232±0.225	3.43
Glyphosphate	0.150	0.138±0.072	47.83
Atrazine	0.130	0.128±0.120	6.25

Table 5. Degradation of Herbicides by *Pseudomonas aeroginosa* at 600 nm optical density

Herbicides	Control (nm)	Degradation	% Degradation
Weed off	0.233	0.232±0.220	5.17
Glyphosphate	0.150	0.122±0.037	69.67
Atrazine	0.130	0.125±0.111	11.20

Table 6. Degradation of herbicides by *Bacillus subtilis* at 600 nm optical density

Herbicides	Control (nm)	Degradation	% Degradation
Weed off	0.233	0.230±0.218	5.22
Glyphosphate	0.150	0.115±0.020	82.61
Atrazine	0.130	0.121±0.113	6.61

degradation experiments for each organism. *Bacillus* had the highest percentage of degradation (82.61%) of herbicides especially in the glyphosate herbicide and this is due to the fact that *Bacillus* have competent degradative enzyme, produce spores and they are able the utilize and break the major ingredient in glyphosate which is the (*N*-(phosphonomethyl)-glycine) and use it as a sole source of phosphorus [7,8,9]. *Pseudomonas* also had a

higher degradation percentage of (69.67%) in the herbicide glyphosate, this may be due to their biofilm formation which serves as a protective mechanism against hostile environmental conditions [15,9]. Lastly *Staphylococcus aureus* had a percentage degradation of 47.83% on glyphosphate. There was low degradation of the other herbicides by the bacterial isolates, according to Wackett, et al. [16] atrazine biodegradation can occur by Hydrolysis of the C-Cl bond followed by the ethyl and isopropyl groups, catalyzed by the hydrolase enzymes and Dealkylation of the amino groups. Weed-off which had the major ingredient as paraquat dichloride did not unergo degradation by any of the bacterial isolates. The results of this study indicates that *Bacillus subtilis* is a promising bio-inoculant for plant growth promotion, biological control of plant disease and glyphosate degradation, and it is useful for the bioremediation of soils polluted with difficult-to-hydrolyze organophosphorus chemicals [17]. To a certain extent, GP can be destroyed by aboriginal microbial communities, but the degradation efficiency remains low and depends both on the conditions of this process and its duration and on the micro organisms' adaptation to GP [9,14].

5. CONCLUSION

In the assessment of the biodegradation of herbicides by bacteria isolated from the soil. Bacterial were isolated, identified and characterized to determine their degradation potentials of herbicides. It was discovered that microorganisms isolated from soil were able to degrade herbicides in the environment. *Pseudomonas and Bacillus* had higher degrading abilities especially in the herbicide glyphosate. The use of herbicides in the environment is important but control measures have to be applied to prevent damaging of the soil ecosystem.

6. RECOMMENDATIONS

It's recommended that:

i. Farmers should choose herbicides like glyphosate that are least harmful and to apply at minimum effective quantity.
ii. Further research on biodegradation of other herbicides by microorganisms from the soil should be studied.
iii. The use of bio- herbicides should be encouraged.
iv. Farmers should be more enlightened on the use of herbicides.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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