Physicochemical and Microbial Analysis of Glyphosate Degraders of Selected Agricultural Soil in Bauchi

Adukwu Amedu[1]*, Surajudeen Abdulsalam[1], Babagana Gutti[2] and Idris Muhammad Misau[1]

[1]*Department of Chemical Engineering, Faculty of Engineering, Abubakar Tafawa Balewa University; PMB 0248, Bauchi, Bauchi State, Nigeria.*

[2]*Department of Chemical Engineering, Faculty of Engineering, University of Maiduguri, P.M.B 1069, Maiduguri, Borno State, Nigeria*

**Corresponding Author*

Abstract: **- Excessive use of pesticides has been known to be hazardous to the environment, affect soil fertility as well may impart toxicity in living beings. This study is aimed at isolation of bacteria consortium that is capable of utilizing/degrading glyphosphate pesticide as a sole source of carbon using pour plate method and also to access the physiochemical characteristics of glyphosphate degrader of selected agriculture soil in Bauchi. Three pure bacteria isolate were identified and characterized based on their morphological and biochemical characteristics on the enhancement medium. The strains were presumptively identified as *Pseudomonas aeruginosa, Pseudomonas putida* and *Bacillus subtilis,* while six pure isolates were identified on the general purpose medium, the strains are *Pseudomonas aeruginosa, Bacillus subtilis, Micrococcous letus, Staphylococcus auerus, Proteus vulgaris* and *Pseudomonas putida*. The total heterotrophic bacteria count was 4.2×10^{9} $cfug^{-1}$ indicating that the density of the indigenous bacteria in the soil was adequate for effective bioremediation since it exceeded the minimal value of 1.00E+5 required. The physical and chemical properties/ parameters of the selected soil were analyzed. The result showed that the soil texture was loamy sand by employing the use of ATSM soil classification triangle. All other parameters (Soil pH, bulk and particle density, temperature, soil porosity and total organic matter) analyzed fell within the acceptable limit required for microbial degradation except the soil moisture content and nutrients (carbon, nitrogen and Phosphorus) that needs to be augmented, these factors are limiting for effective bioremediation requirements for optimum growth and proliferation of microbes.**

Keywords: **bacteria, biodegradation, glyphosphate, nutrients, organophosphate, pesticides**

I. INTRODUCTION

Pesticide is widely used in Nigeria; there have been an increase in the usage of pesticide since its introduction in the early fifties for cocoa production. It has been estimated that about 125,000 - 130,000 metric tons of pesticides are applied every year in Nigeria (Asogwa and Dongo, 2009; Agarry *et al.*, 2013). The excessive use of pesticides leads to accumulation of a huge amount of residues in the environment, thereby posing a substantial health hazard for the current and future generations due to uptake and accumulation of these toxic compounds in the food chain and drinking water (Mohammed, 2009;Olawale *et al.*,2011; Agarry *et al*.,2013). Pesticides are organic compounds manufactured and used for pest control (Ortiz – Hernandez and Sanchez-Salinas, 2010).

Pesticide therefore can be defined as any chemical substance or mixture of substances intended for preventing, destroying, repelling, or mitigating the effect of any pest of plants and animals. Pesticides can be classified into five chemical groups which are organochlorine, organophosphate, carbamate, synthetic pyrethroids, avermectin and formamidine (Agarry *et al.*,2013;Naqvi *et al*., 2011; Nsikak and Aruwajoye, 2011; Natala and Ochoje, 2009). Organophosphate pesticides are a group of highly toxic heterogeneous compounds that share a phosphoric acid derivative chemical structure widely used for plant protection and pest control. There are currently 140 organophosphate compounds being used as pesticides and as plant growth regulators around the world (Kang *et al*., 2006; Agarry *et al.*, 2013). These compounds are components of more than 100 types of commercially available pesticides (such as Paraoxon, Parathion, Malathion, Diazinon, Glyphosate and Dichlorvos). The toxicity of pesticides from exposure to contaminated food is mostly unknown but there is growing evidence of cancer, neurological damage, endocrine disruption and birth defects consequential from exposure (Miller and Sharpe, 1998; IARC, 2001; ATSDR, 2005, Agarry *et al*., 2013).

The use of microorganisms in the degradation and detoxification of many toxic xenobiotics, especially pesticides, is an efficient tool for the decontamination of polluted sites in the environment (Mohammed, 2009; Olawale *et al.*, 2011). Biological decontamination methods

are preferable to conventional approaches (chemical treatment, recycling, pyrolysis, incineration and landfills) because in general, microorganisms degrade numerous environmental pollutants without producing toxic intermediates (Pieper and Reineke, 2000; Farukawa, 2003; Olawale *et al.*,2011). In view of compelling evidence of health effects on humans based on studies especially in developed countries and weak implementation of government policy on regulation / ban or surveillance program for pesticides levels in the environment and foods in Nigeria, there is need for evaluation of organophosphate pesticides detoxification. The objective of this study is to analyze the physicochemical parameters, isolate, identify and characterize an organophosphate pesticide degrading bacterial consortium obtained from a contaminated agricultural soil and to test its potential use in bioremediation of soil contaminated with Glyphosate pesticide.

II. MATERIALS AND METHODS

Materials

The materials used for this study includes: digital weighing balance, pH meter, atomic absorption spectrophotometer (AAS), hand held digital alarm thermometer, hydrometer, express autoclave, schwa batch Incubator, analytical grade glyphosate 41% (Glycel brand name), nutrient agar and other chemicals used as media components (products of Sigma-Aldrich, USA) as well as Chemical (Magnesium Sulphate, Calcium chloride, mono-potassium phosphate, diammonium hydrogen phosphate, potassium nitrate, ferric chloride and Distilled Water) needed for Biochemical test.

Sample Collection

An un-impacted soil samples with little or no history of pre and post treatment of pesticide from Agricultural and Bio-resources Engineering Farms (Soil and Water Field), Abubakar Tafawa Balewa University (ATBU), Bauchi State were collected from the surface layer of the vadose zone 15 to 30 cm below the land surface. The soil samples were air-dried, homogenized, passed through a 2 mm (pore size) sieve to enhance proper mixing and extract consisting mainly of stones and dead plant debris discarded, the sample was then stored in a polyethylene bag at room temperature of 29^{o}C.

Methods

Soil samples were characterized for their physicochemical and microbial parameters according to standard methods. The soil texture was determined by methods as described by Abdulsalam (2011). Total organic carbon and total nitrogen of soil were determined using Walkley-Black and Macro-Kjeldahl methods, respectively (Black (1965), APHA (1985)). Soil pH was determined using pH meter fitted with a combined glass pH and reference electrode (APHA (1985)). Soil moisture content was determined by evaporation on Whatman filter paper No. 1 at 103°C to 105°C in an electrical oven. Available phosphorus was determined using Bray No. 1 method (Black (1965), APHA (1985)). The Total bacterial counts for the treatment was carried out in representative soil composite samples (Triplicate) using the standard serial dilution method (John, 1982). The average number of colonies was calculated by

$$Number\ of\ cfug^{-1} of\ soil = \frac{Average\ \ number\ \ of\ colonies\ \times dilution\ \ factor}{initial\ \ weig\,ht\ of\ soil} \quad \text{....Equ 1}$$

The total hydrocarbon-degrading bacteria (THDB) population was finally determined by the pour plate method (Colores *et al*., 2000). In this method, 10 g of soil was transferred into 250-ml Erlenmeyer flasks containing 100 ml of mineral salt medium (MSM). The MSM (Kaster *et al*., 1994) consists of 2.13 g Na_2HPO_4,0.5 gNH_4Cl, 0.2 g $MgSO_4{\cdot}7\ H_2O$, 1 .3 g KH_2PO_4, 0 .0 1 g $FeSO_4{\cdot}7H_2O$, 0.1 g NaCl, 20 g agar, and 1% glyphosphate in 1-L de-ionized water, pH 7.4. The flasks were shaken for 3 days at 180 rpm and 28 ± 2°C. After the third day, 10 ml of the supernatant was transferred to 125 ml of fresh medium, Thereafter, serially diluted samples (0.1 ml) were plated on nutrient agar medium (Oxoid) supplemented with 50 µg/ml nystatin to suppress the growth of fungi. The oil agar plates were incubated at 30°C for 5 days, and the colonies were counted and randomly picked. Pure isolates were obtained by repeated sub-culturing on nutrient agar (Oxoid). The bacterial isolates were characterized using microscopic techniques and biochemical tests. The identities of the isolates were determined by comparing their characteristics with those of known taxonomy as described in Bergey's manual of determinative bacteriology (Kreig *et al.*, 1994).

III. RESULTS AND DISCUSSION

Microbial Analysis

The total heterotropic bacteria count was 4.2× 10^9 cfug^{-}1 indicating that the density of the indigenous bacteria in the tested soil was adequate for effective bioremediation since it exceeded the minimal value of 1.00E+5 required (Abdulsalam *et al*., 2011). Below this value, landfarming technique of bioremediation may still be effective as long as the existing bacteria are stimulated using nutrients or the soil is amended to increase the bacteria population (USEPA, 1994; EPA, 2014). The bacteria isolated were identified and characterized based on colonial morphology, microscopic morphology and biochemical characteristics by comparing their characteristics with those known taxonomy as described by bergy's manual of determination bacteriology(Racke *et al*., 1998; Hassan and Ahmed 2014) as shown in Table 1 below. Three bacteria strain (*Pseudomonas putida*, *Pseudomonas aeruginosa* and *Bacillus subtilis*) which were able to grow in minimal media capable of utilizing organophosphate pesticide as a sole carbon source for growth isolated from agricultural soil

sample in the presence of glyphosate pesticide were generated through enrichment procedure. While six bacteria strain (*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Micrococcous letus*, *Staphylococcus auerus*, *Proteus vulgaris* and *Pseudomonas putida.*) was also identified from the general purpose medium as shown in Table 2. Biochemical and growth characterization of the three isolates were further investigated. The result showed that the three isolates were short rod Gram Negative, motile *Pseudomonas putida*, *Pseudomonas aeruginosa* and Gram negative for *Bacillus subtilis*. The three isolates showed positive reactions for catalase, citrate and oxidase tests. Feng and his co-workers in 1998 reported for the first time isolation of a pure culture of bacteria capable of using 3, 5, 6-trichloro-2pyridinol (TCP) as the sole source of carbon and energy under aerobic conditions (Bhagobaty et al., 2007; Olawale *et al* ., 2013). It has been reported also that Pseudomonas strain ADP inoculated to soil contaminated with 1500µg of atrazine g-1 resulted in mineralization of over 60% of 10µg of the pesticide in 49 days (Yanze-Korotchou and Gschwind, 1995; Olawale et al ., 2013).

Table 1: Summary of Biochemical Characteristics Test by Bergey's Manual

BACTERIA							TRIPPLE SUGAR IRON				
	GRAM REACTION	**CATALASE**	**CITRATE**	**UREASE**	**INDOLE**	**OXIDASE**	**GLU/GLY**	**LACTOSE**	**SUCROSE**	**H2S**	**GAS PROD FROM GLU/GLY**
P.aeruginosa	-	+	+	-	-	+	-	-	-	-	-
B.subtilis	+	+	+	-	-	+	+	+	+	-	-
M.letus	+	+	+	+	-	-	-	+	+	-	-
S.aureus	+	+	+	+	-	-	+	+	+	-	-
P. vulgaris	-	+	+	-	+	-	-	-	+	+	-
P.putida.	-	+	+	+	-	+	+	-	-	-	-

KEY:

P. aeruginosa: Pseudomonas aeruginosa

B. subtilis: Bacillus subtilis

M. letus: Micrococcous letus

S. auerus: Staphylococcus auerus

P. vulgaris: Proteus vulgaris

P. putida: Pseudomonas putida

Table 2: Summary of Isolation, Identification and Characterization of Glyphosphate Degrading Bacteria.

Bacteria	General Purpose Medium	Enhancement Medium
Pseudomonas aeruginosa	**Present**	**Present**
Bacillus subtilis	**Present**	**Present**
Micrococcous letus	**Present**	**Absent**
Staphylococcus auerus	**Present**	**Absent**
Proteus vulgaris	**Present**	**Absent**
Pseudomonas putida	**Present**	**Present**

Analysis for Physico-chemical Parameters

The results of the physicochemical characteristics of the selected soil are presented in the Table 3, the soil texture is Loamy Sand by employing the use of ATSM soil classification triangle. This soil type is consistent for effective bioremediation because of its low clay and silt contents. The soil pH was also within the acceptable limit of 5.5-8.5. The high value of soil porosity is advantageous because of the ease of oxygen, water flow and nutrient supply to the soil matrix (Less and Senior 1994; Abdusalam *et al*., 2011) on the other hand, the soil moisture and soil nutrient such as (C and N) needs to be augmented, these factors are limiting for effective bioremediation. Low moisture content can give high water absorption capacity. The value of the moisture content fell out of the range (25-30%) requirement for optimum growth and proliferation of microbes (Pandey *et al.*, 2012)

Table 3: Physico-Chemical Characteristics of Soil Sample

Parameters	Value/Type
Soil Texture	Loamy Sandy
Soil pH	6.35
Particle Density(g/cm^3)	2.6
Bulk Density(g/cm^3)	1.2
Soil Porosity	54.9
Total Organic Carbon (TOC) (%)	0.72
Total Organic Matter (%)	1.26
Available Phosphorous (mg/kg)	3.6
Nitrogen (mg/kg)	2600
Soil Temperature (^{O}C)	27
Soil Moisture Content (%)	14.5

IV. CONCLUSION

The present study reports isolation of a bacteria consortium that is capable of utilizing glyphosphate pesticide as a sole source of carbon. Utilization of xenobiotics compounds by soil microbes is a crucial phenomenon by which these compounds are removed from the environment, thus preventing environmental pollution. The result of the present study suggest that bacteria which were isolated are capable of growing in an environment where there is addition of pesticide and thus useful for soil and water bioremediation, in addition, the result obtained from the physiochemical analysis suggests that most parameters met with the requirement for microbial growth except for moisture content and soil nutrient(carbon, nitrogen and phosphorus) that needs augmentation in order to meet the requirement for the optimum growth and proliferation of microbes.

REFERENCES

[1]. Abdulsalam,S., Bugaje,I.M., Adefila,S.S. and Ibrahim,S., (2011). Comparison of Biostimulation And Bioaugumentation For Remediation of Soil Contaminated With Spent Motor Oil. Int. J.Environ.sci.tech., 8(1). 187-194.

[2]. Agarry, S.E., Olu-arotiowa, O.A., Aremu, M.O. and Jimoda, L.A. (2013). Biodegradation Of Dichlorovos (Organophosphate Pesticide) In Soil By Bacteria Isolates. Journal of Natural Science Research Issn 2224-3186 (paper) ISSN 2225-0921(online) Vol 3, No 8. (Pp12-17)

[3]. Agency for Toxic Substances and Disease Registry (ATSDR) (2005). Toxicological profile for Hexachlorocyclohexane. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. Alexander, M. (1999). Biodegradation and bioremediation. Academic Press, San Diego, USA. Pg 453

[4]. APHA (1985): Standard Methods for Examination of Water and Wastewater .American Public Health Association, Washington D.C

[5]. Asogwa, E.U and Dongo, L.N (2009). Problem associated with pesticide usage and application in Nigerian Cocoa production: a review. A fri. j. agri. Res. 4(8): 675-683

[6]. Pandey, B. and Fulekar, M.H. (2012). Bioremediation technology: A new horizon for environmental

[7]. Bhagobaty, R. K., S. R. Joshi and Malik, A. (2007). Microbial Degradation of Organophosphorus Pesticide:Chlorpyrifos (Mini-Review). The Internet Journal of Microbiology4:29.

[8]. Black, CA (1965): Method of Soil Analysis II, pp. 573-590. American Society of Agronomy, Madison

[9]. Colores, G.M.; Macur, R.E.; Ward, D.M. and Inskeep, W.P. (2000). Molecular analysis of surfactant-driven microbial population shifts in hydrocarbon-contaminated soil. Appl. Environ. Microbiol. 66, 2059 –2964

[10]. EPA, (2014). Report Pollution and Environmental Incidents. Available: www.epa.nsw.gov.av ISBN 9781743596074 12P

[11]. Furukawa, K.(2003). "Super bugs" for Bioremediation Trends in Biotechnology..21:187-190.

[12]. Hassan, S. and Ahmad, G.A (2014). Factors Controlling Degradation of pesticide in the soil environment; A Review Agric Sci dev., Vol(3), No(8), Pp 273-278.

[13]. International Agency for Research on Cancer (IARC) (2001). In: IARC Monographs on the Evaluation of Carcinogenic Risk to Humans.

[14]. Kang, D.G., Choi., S.S. and Cha, H.J. (2006). Enhanced biodegradation of toxic organophosphate compounds using recombinant Escherichia coli with sec pathway driven peri plasmic secretion of organophosphorous hydrolase. Biotechnol. Prog 22: 406-410

[15]. Less, Z M. and Senior, E. (1995), Bioremediation. A Practical Solution to Land Pollution: In Clean Technology and the Environment, Chapman and Hall, New York pp 121-146

[16]. Miller, W.R. and Sharpe, R.M. (1998). Environmental estrogens and human reproductive cancers. Endocr. Relat. Cancer, 5: 61-69.

[17]. Mohammed, M.S. (2009). Degradation of Methomyl by the Novel bacteria strain stenotrophomonas maitophilia MI. Electronic J. Biotechnol 12(4): 1-6

[18]. Naqvi, T.A., Kanhar, N.A., Hussain, M., Shar, A. and Ahmed, S. (2011). Microcosm studies for the biodegradation of carbaryl in soil. Pak. J. bot.,43(2): 1079-1084

[19]. Natala, A.J and OCHOJE, O.S. (2009). Survey of pesticide used in the control of ectoparasites on farm animals in kaduna state, northern Nigeria. J. animal and plant sci 4(1): 276-280

[20]. Nsikak, A.J. and Aruwajoye (2011). Assessment of contamination by organochlorine pesticides in solanum lycoperiscum L. and capsicum annum L: A market survey in Nigeria. A fri. J. Environ. Sci. Technol. 5(6): 437-442.

[21]. Olawale, A.k. and Akintobi, O.A. (2011). Biodegradation of Glyphosate Pesticide by Bacteria Isolated from Agricultural Soil. Pp 124-128. Available: http://www.science pub.net/report. (Pp 124-128)

[22]. Ortiz – Hernandez, M.L. and Sanchez- Salinas, E. (2010). Biodegradation of the Organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soil in Mexico, Rev. Int. Contam. Ambient, 26(1), 27-38(2010).

[23]. Pieper, D. H., and Reineke, W. (2000). Engineering Bacteria for bioremediation. Current Opinion in Biotechnology..11: 262-270

[24]. Racke, K.D., Coats, J.R. and Titus, K.R., (1988). Degradation of chlorpyrifos and its hydrolysis products, 3,5,6-trichloro-2-pyridinol, in soil . J. Environ.Sci. Heal. B. 23, 527- 539.

[25]. US EPA (1994). How to Evaluate Alternative Cleanup Technologies for Underground Storage Tank Site: A guide for corrective action plam reviewers, EPA 510-B-95-007, Chapter V: Lnadfarming, United States Environmental Protection Agency, Office of Underground StorageTanks (OUST), Washington DC; available at www.epa.gov/swerust1/pubs/tums.htm

[26]. Yanze-Kontchou, C., and Gschwind N. (1995). Mineralization of the herbicide atrazine in soil inoculated with a Pseudomonas strain. J. Agric.Food Chem. 43:2291-2294.