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Research Paper

Comparative Study of Bioremediation of Organophosphate Pesticide Contaminated Soil using Landfarming Technique in Bauchi Metropolis.

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Abstract
Excessive and continuous dispersion of pesticide which are toxic heterogeneous compound in the environment results in environmental pollution with ecological effects (like increase in death rate of humans and animals) that requires remediation either by stimulating the indigenous microorganism with introduction of nutrients (biostimulation) or through the inoculation of an enriched mixed microbial culture into the soil (bioaugumentation). To demonstrate the potential use of bioremediation in soil contaminated with organophosphate pesticide (OPP), a laboratory study with the objective of evaluating and comparing the effects of bioattenuation, biostimulation, bioaugumentation and hybrid of bioaugumentation and biostimulation was performed. Each wooden Microcosm (A1-A4) contained 5% (w/w) of OPP artificially contaminated in the soil as a sole source of carbon and energy. After 10weeks of remediation, the results revealed that hybrid of bioaugumentation and biostimulation (A4) has the highest organophosphate pesticide removal rate (87.5%) followed by A3 (67.2%), A2 (59.4%), and the lowest organophosphate pesticide A1 (42.0%). Total Heterotrophic Bacteria Count revealed that bioremediation actually took place; A4 had the highest maximum bacteria growth (3.41E+05CFU/g) at the 6th week, followed by A3 (2.96 E+05CFU/g), A2 (2.85 E+05CFU/g), and A1 (1.71E+05CFU/g).

Keywords: *Bioremediation, Biostimulation, Bioaugumentation, Organophosphate Pesticide, wooden microcosms.*

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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). 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. They include herbicides, insecticides, rodenticides, fungicides, molluscides, nematicides, avicides, repellents and attractants used in agriculture, public health, horticulture, food storage or a chemical substance used for a similar purpose (REF). 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). In addition, it has been reported to be the world's largest selling herbicide in 2013, and Chinese manufacturers are the world's largest producers of glyphosate and its precursors (Steinrucken and Amrhein, 1980; Schonbrunn *et al.*, 2001; Stephen *et al.*, 2008; Mink *et al.*, 2012; Changpeng *et al.*, 2015). The United States Environmental Protection Agency and the European Union consider that there is no potential for the herbicide glyphosate to pose a health risk to humans. Furthermore, early epidemiological studies did not find associations between long term low level exposure to glyphosate and any disease (Aparico *et al*.,2013; Changpeng *et al*.,2015), but with the heavy use of fungicides, insecticides and herbicides such as the glyphosate formulation Roundup in agriculture, residues in soils are a growing problem (Zhang *et al*.,2012;

Piel *et al.*, 2012; He et al.,2014; Li et al.,2014; Changpeng *et al*.,2015). Indeed, a field test showed that lettuce, carrots, and barley contained glyphosate residues up to one year after the soil was treated with 4.15 kg of glyphosate per hectare (Albers *et al.*, 2009; Yamada *et al*., 2009; Changpeng *et al*., 2015). Glyphosate is readily degraded by soil microbes to carbon dioxide and amino methyl phosphonic acid (AMPA) that adsorbs strongly to soil and is not expected to move vertically below the top six inch soil layer. 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 examine, evaluate, and compare the methods of natural attenuation, biostimulation, bioaugmentation, and Hybrid biostimulation and bioaugmentation in the bioremediation of soil contaminated with organophosphate pesticide.

II. Materials and Methods

Soil 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 29^0C. The sieved soil was contaminated artificially with Organophosphate Pesticide to a pollutant level of 50,000ppm and its moisture content level was increased from 1.37 to 15 using distilled water. The soil matrix was properly mixed at ambient temperature (25-30^OC). Each of the contaminated soil (5kg) was stacked into four wooden boxes lined with polyethylene bags internally to prevent the leaching. Each box had dimension of 10cm height X 30cm length X 30cm width with soil layer 1.27cm deep. Various treatment options were prepared according to Table 1.

Table 1: Composition of Various Treatment Options.

Microcosm	Sample	Sample Mass(g)	Treatment
1	A_1	5000	Sample + No Heat + H_2O
2	A_2	5000	Sample + No Heat + Fortified Cow Dung+ H_2O
3	A_3	5000	Sample + Heat + S_1&S_2+ H_2O
4	A_4	5000	Sample + Heat + S_1&S_2+ Fortified Cow Dung + H_2O

Key Note:
S_1= *Bacillus subtilis*
S_2= *Pseudomonas aerouginosa*
Wooden microcosm under Organic manure treatment had a C: N: P ratio of 100:10:1

Experimental Design for the Bioremediation of Glyphosate Spiked Soil

Four wooden boxes used as bioreactors were prepared for each treatment as shown in Figure1, designated as bioattenuation (treatment A_1), biostimulation (treatment A_2), bioaugumentation (treatment A_3) and combined biostimulation and bioaugumentation (treatment A_4). Each bioreactor contained 5 kg of soil contaminated and well mixed with 100 ml of 50 g of Organophosphate pesticide (5% w/v) dissolved in water. The bioreactor under treatment A_2 and A_4 was amended with 8.0 kg of organic manure (cow dung)(biostimulation) and combined biostimulation and bioaugumentation. The bioreactor under treatment (A3 and A_4) was amended with 50 ml each of inoculum ($2.6\times10^5 CFU/g$ combine *Bacillus subtilis & Pseudomonas aeruogonisa)*) (bioaugumentation), respectively. The bioreactor under treatment A_1 was not amended with either Organic manure fertilizer and/or inoculum (natural bioattenuation). Soil in the bioreactor used for some experimental optional design was sterilized three times by autoclaving at 121^0C for 15 min. All the bioreactors with its contents were incubated at room temperature (28^0C $\pm2^0$C) for eight weeks under laboratory condition. The water (moisture) content of soil in each bioreactor was adjusted every week by addition of distill water in order to make up for water losses used by microbes to enhance their activities. The total bacterial counts for the treatments were carried out in representative soil composite samples using the standard serial dilution and nutrient agar-plate counting techniques (Lorch et al., 1995). In order to avoid anaerobic conditions, the samples in the microcosms were tilled and aerated daily to enhance oxygenation and kept moist during the experimental period. Samples will be withdrawn at intervals of 1 week for residual total pesticide content, total heterotrophic bacteria count and other physico-chemical analysis. The experiments were carried out in triplicates.

Plate 1: wooden microcosm stacked with different treatment options as presented in Table 1

Inoculum Preparation and Enrichment of Mixed Bacterial Culture

The pure isolate (*Bacillus subtlis and Pseudomonas aerugonisa*) in form of slants, was obtain from veterinary research institute in Vom, Jos, Plateau State. The slant was sub-culture using the streaking method on the nutrient agar in disposable petri dishes for 24 h in an incubator at 37^0C to monitor its cell growth. A loopful of each stock culture of *Bacillus subtlis* and *Pseudomonas aerugonisa* were respectively inoculated into 3 different 250 ml Erlenmeyer flasks containing 100 ml of freshly prepared sterile nutrient broth medium (0.8%) made up of yeast extract 2.0 g/L, peptone 5.0 g/L, NaCl 5.0 g/L, and agar 15.0 g/L, incubated at 37^0C for 48hrs to monitor a turbid suspension. 1mls of the stock solution was serially diluted into 9mls of sterile nutrient broth(same constituents above) in a serial dilution in triplicate for each stock solution to get a viable counts of 30-300cfu/ml. 200milliters ($2.3\times10^5 CFU/g$ *Bacillus subtilis*, $2.5\times10^5 CFU/g$ *Pseudomonas aeruogonisa* and $2.6\times10^5 CFU/g$ Combine *Bacillus subtilis & Pseudomonas aeruogonisa)* of the inoculums was inoculated on the surface of the microcosm and was also used for the bioaugumentation experimental design option to study the biodegradation of glyphosate in soil. All the microcosms were mixed three times every week for aeration for the Eight (8) weeks. After mixing, these microcosms were kept away from sunlight at room temperature in order to prevent rate of dehydration.

Extraction and Determination of Glyphosate Residual Content

5g each of soil contaminated with organophosphate pesticide was weighed on an electronic weighing balance and transferred into a centrifuge tubes with screw caps; 5 ml of n-hexane was added. The samples mixtures were vortexed briefly for 5 min, the mixtures were further shaken using a mechanical shaker for 10 min at room temperature. After settling, the Supernatants were transferred into a clean pre-weighed 50ml white reagent bottle. This procedure was repeated three times to bring the total solvent volume to 20 ml, The Supernatants was allowed to settle and then decanted into the 50ml white reagent bottle. The extract in the bottle was then allowed to settle for a period of 24 hours after which part of the extract was taken for the UV-VIS spectrophotometric analysis to measure the absorbance and concentration for the pesticide residual content at an optimum wavelength of 390nm.

Statistical Analysis

Mean concentrations of pollutant with different bioremediation strategies were statistically treated with Microsoft Excel 2010. One way between subjects ANOVA (Analysis of variance) was used to determine if there was a statistical significant difference in mean concentration of the treatment options within and between groups in the degradation of the Organophosphate pesticide in the contaminated soil sample.

III. RESULTS AND DISCUSSION.

Microbial Analysis

Addition of consortium of *Pseudomonas aerugonisa* and *Bacillus subtilis* on pesticide polluted soil offers an interesting possibility of enhancing decontamination of the soil after few weeks. Counts at the end of the investigation revealed that sample A4 in the Organophosphate Pesticide contaminated soil treated with

combined biostimulation and bioaugumentation strategies supported more bacteria growth. Figure 1 shows the variation of Total Heterotrophic Bacteria Counts (THBC) against bioremediation time, the counts are in order of A4> A3>A2> A1 with maximum count (A4) obtained at week 6. This indicated that in the soil, the addition of preselected bacteria (bioaugumentation) and nutrients (biostimulation) resulted in the highest number of bacteria at the beginning of remediation period as compared to natural attenuation (or bioattenuation). The lower bacteria population in natural attenuation treatment may be due to the presence of low nutrients (limiting nutrient) and the disturbance of nutrient ratio especially of C:N:P that is needed in the correct ratio for bacteria growth.

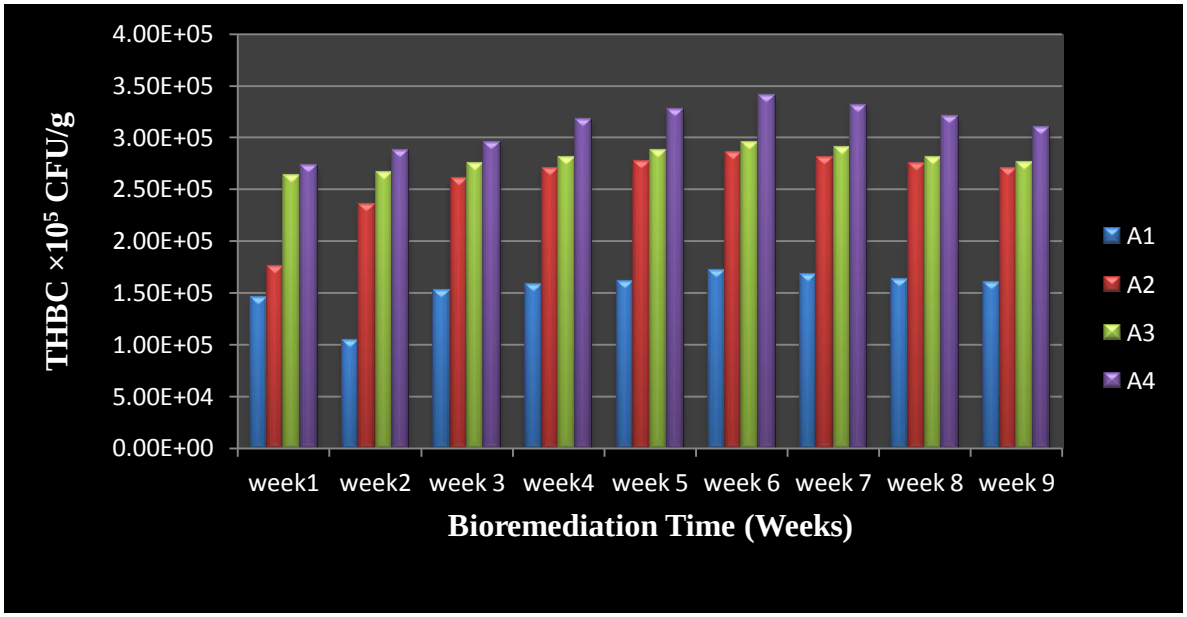

Figure 1: Variation of THBC with Bioremediation Time

The variation in concentrations of pesticide residual content (mm/kg and %) against bioremediation time is shown in Figure 2, biodegradation of the organophosphate pesticide started very fast during the first week of remediation in all the treatments and slowly continued up to the sixth week (day 42). The concentration of glyphosate was reduced from the initial concentration of 50000mg/kg to 29000 mg/kg, 20300 mg/kg, 16400 mg/kg, and 6500 mg/kg in six (6) weeks of remediation and corresponding to 42%, 59.4%, 67.2%, and 87% glyphosate reduction was achieved under A1, A2, A3 and A4 respectively. This observation revealed that during the glyphosate biodegradation in soil, addition of organic manure (Fortified Cow Dung) and bacterial inoculums individually resulted in a more effective bioremediation response than the natural attenuation. Similar observations have been reported for, naphthalene (Agarry and Oghenejoboh, 2015) and spent engine oil (Abdulsalam and Omale; 2009 and Abdulsalam et al., 2011). Nevertheless, bioaugmentation strategy elicited a higher biodegradation of glyphosate than the biostimulation treatment. This is in agreement with the observation of (Bento et al., (2003) and Agarry and Oghenejoboh, 2015) who reported that among the individual methods of natural attenuation, biostimulation, and bioaugmentation that were used for the remediation of a soil contaminated by diesel oil, bioaugmentation method elicited higher diesel oil degradation than others. In contrast, other workers have shown that biostimulation strategy enhanced the bioremediation of kerosene contaminated soil (Shabir et al., 2008; Agarry and Oghenejoboh, 2015), crude oil contaminated soil (Xu and Lu, 2010), spent engine oil contaminated soil (Abdulsalam et al.,2011) and lubricating oil contaminated soil (Agarry et al., 2013) than bioaugmentation. Generally, in this work, the combination of biostimulation (Organic Manure) and bioaugmentation treatment strategy (which has not been reported for glyphosate removal) showed relatively greater glyphosate reduction than other treatments during the whole period of remediation.

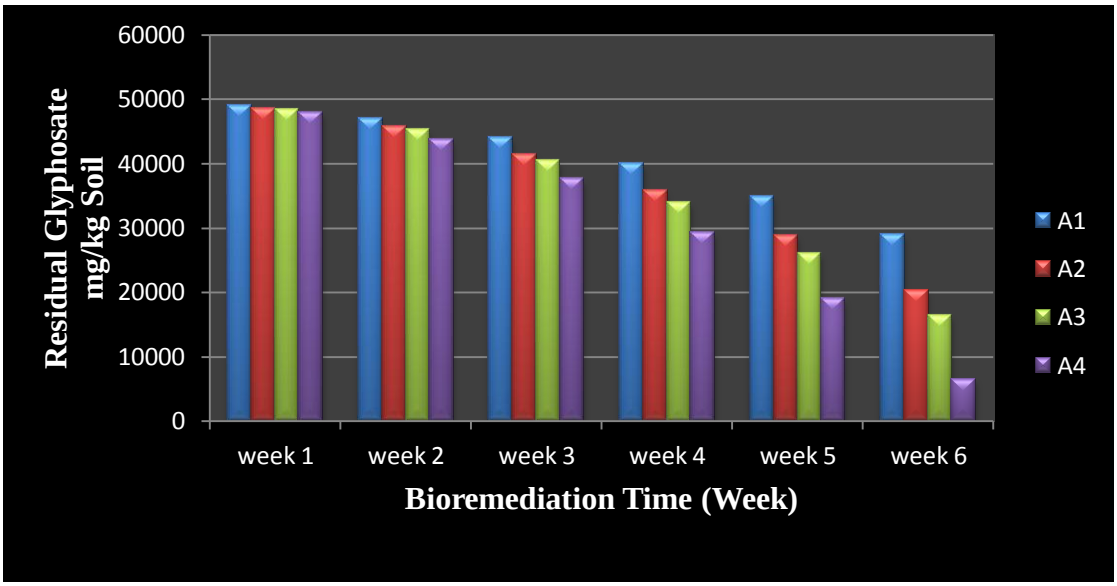

Figure 2: Variation of Residual Glyphosate content with Bioremediation Time.

IV. Conclusion

The work on enhanced aerobic biodegradation of organophosphate pesticide in soil was carried out successfully within the limits of experimental errors. The following conclusions can be drawn based on the findings from the work. The hybrid of bioaugumentation and biostimulation exhibited the highest degree of degradation at their various mean rate of the extent of percentages in pesticide concentrations removal, followed by Bioaugumentation treatment Options, then the soil treatment with Organic manure(cattle Dung) Biostimulation options and natural attenuation been the least removal rate of Organophosphate pesticide percentage concentration removal rate (combined Biostimulation and Bioaugumentation> Bioaugumentation> Biostimulation> Natural Attenuation). This same trend was also observed with the THBC analysis with the hybrid of bioaugumentation and biostimulation (A7) having the highest number of the bacterial count in the highest Exponential phase of the analysis at week 6. The low THBC counts in other treatment options could be attributed to availability of nutrients between the exogenous and native microbes. Therefore A7 was the best in cleaning OPP contaminated soil artificially.

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