ASSESSMENT OF THE IMPACT OF HERBICIDE CONTAMINATED TOP SOILS ON ITS PHYSIOCHEMICAL, MICROBIOLOGICAL AND ENZYMATIC PROPERTIES

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K. E. Enerijiofi* and E. Obade
Department of Biological Sciences, Samuel Adegboyega University, PMB 001, Ogwa, Edo State, Nigeria
Corresponding author: kingsmore1luv@gmail.com; kenerijiofi@sau.edu.ng

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Abstract: Herbicides not only control weeds but also affect soil microorganisms which are responsible for numerous biological processes essential for crop production. The study aimed at determining the effect of herbicide contaminated soils on its physicochemical, microbiological and some enzymatic properties. Standard methods were used for soil sampling and analyses. The physicochemical parameters recorded were pH (5.17 – 5.41), electrical conductivity (98.00 – 237.00 uS/cm), particle size (89.21 – 91.11%) and heavy metals with iron being the highest at all contaminated locations (44.10 – 106.65 mg/kg). There was high significant difference in physicochemical parameters at locations A, C and E ($P<0.001$). The three impact assessment indices revealed the same pattern of heavy metals contamination; $Pb^{2+} > Cr^{2+} > Fe^{2+} > Cu^{2+} > Zn^{2+} > Mn^{2+} > Ni^{2+}$. Location E had the highest heterotrophic bacterial and fungal counts of 4.19×10^6 and 1.85×10^6 cfu/g, respectively while location A had the least of 2.78×10^6 cfu/g and 1.13×10^6cfu/g for bacterial and fungal respectively. The microorganisms identified were *Pseudomonas fluoriscens*, *Penicillium* sp. and *Aspergillus niger*, 13.64%; *Bacillus* sp. 11.36%; *Staphylococcus aureus, Corynebacterium*, *Rhizopus* and *Candida* species, 9.09%; *Serratia* sp. 6.82% and *Escherichia coli*, 4.55%. *Pseudomonas flouriscens* produced the highest enzyme, maltase with concentrations of 0.313, 0.407, 0.421 and 0.429 mg/ml at days 2, 4, 6, and 8, respectively closely followed by invertase with 0.363, 0.421 and 0.429 mg/ml at day 4, 6 and 8, respectively. Herbicide application had negative impact on the soil properties studied. This study recommends that maltase, invertase and amylase enzymes produced by *Pseudomonas fluoriscens* could be employed as biocatalysts for treating herbicide contaminated soils so as to guarantee environmental safety and public health for all.

Keywords: Crop production, environmental sustainability, heavy metals, herbicide

Introduction

Soil is the main regulator of herbicide movement in the ecosystem and several herbicides have not only been introduced as pre- or post-emergence unwanted plants eradicators but have also created unwanted residues in soil ecosystem which are biologically harmful (Ayansina and Oso, 2006). This has led to significant changes in the microbial population and their activities by inducing microbial ecological inbalance and affecting soil fertility (Derksen *et al.*, 2002; Enerijiofi *et al.*, 2017a; Riaz *et al.*, 2007). The reliance of herbicides to support agriculture has led the concern of their ecotoxicological impact particularly on the microbial populations. As the soil microbes are sensitive to low concentrations of chemicals and speedy response to soil agitation, they are described as indicators of soil pollution (Shen *et al.*, 2005).

The effects of herbicides on microbial population growth, is either motivative or depressive depending on the type and concentration, microbial species and environmental conditions applied on the soil (Zain *et al*., 2013). Soil microbial communities varies tremendously and the relation between their diversity and function influences soil stability, productivity and bounciness (Sebiomo *et al*., 2011). Many chemical and biological activities such as adsorption phenomena, chemical and biological degradation determine the retaining or transporting of herbicides on the soil surface. While all these processes are consistent, it is of great importance to first know adsorption from the time when it controls the bioavailability of herbicides in the soil.

The microbial soil community composition is important, because they play an important role in carbon flow, nutrient cycling and litter decomposition, which in turn disturb soil fertility, plant growth and also occupy a unique position in biological cycles in terrestrial habitat (Tripathi *et al.*, 2006; Pandey *et al.*, 2007). Any alteration in their population and activities may affect nutrient cycling and availability of soil microbial biomass (Wang *et al.*, 2008). This work was aimed at assessing the impact of over four years herbicide application on the receiving soils physiochemical, microbiological and some enzymatic properties.

Materials and Methods

Study area

The study area is Ujiogba, situated in Esan West local Government Area of Edo State. It lies approximately on latitude 6.52911, longitude 6.1477 and altitude 157 m. The soil type is precisely reddish-brown in colour and it's fertile for farming which is the main occupation of the people.

Sample collection

Composite soil samples were collected from depths of 0 - 15cm using sterilized auger from five different locations in over four years herbicide contaminated farm land in Ujiogba Community. Soil samples free of herbicides contamination were also collected and served as control. In each of the sampling sites, garbage were removed before collection. They were transported to the Laboratory for analyses (Enerijiofi *et al*., 2017b).

Physicochemical and heavy metals analyses

The physicochemical properties of the soil samples: pH, electrical conductivity, chloride, sulphate, nitrate, phosphate, ammonium nitrogen, moisture, organic carbon, and total nitrogen were determined according to the method of APHA, (2011), particle size by (Bouyoucos, 1962). The cations and heavy metals concentrations were determined using Jenway flame photometer model PFP-7 and Shimadzu atomic absorption spectrophotometer, model PG 550 respectively by aspiration (Enerijiofi *et al*., 2017b).

Assessment of the impact of herbicide on heavy metals in the receiving soil

Three indices were employed to assess the impact of herbicide on the receiving soils (Enerijiofi *et al.*, 2017b).

(i) Contamination factor

$C_f = C^i_{0\text{-}1} / C^i_n$. Where $C^i_{0\text{-}1}$ is the mean content of metals from at least 5 contaminated samples and C^i_n is the pre- industrial (control) concentration of individual metals.

(ii) geo-accumulation index

I-geo = log (Cn / 1.5Bn). Where Cn is the concentration of the heavy metal in the contaminated soil sample and Bn is the concentration of the metal in the unpolluted (control) sample. The factor 1.5 is introduced to minimize the effect of the possible variations in the control

(iii) Quantification of concentration

$$\frac{x - x_c 1}{x} x 100\%$$

Where x = average concentration of the metal in the contaminated soil sample and x_c = average concentration of the metal in the control samples.

Microbiological analysis

Aliquot 1 ml of appropriate ten - fold serial dilution (10^{-3}, 10^{-6} and 10^{-9}) of the herbicide contaminated soil samples and control were inoculated into plates containing nutrient agar (oxoid) with fuscin for bacterial growth and potato dextrose agar (oxoid) containing streptomycin for fungal growth. The inoculated plates were incubated at 37^0C for 24 h in an incubator and at room temperature of 28^0C for 72 h for bacterial and fungal enumerations respectively using colony counter (Model- Labtech) and expressed in colony forming units per gram (cfu/g) of the soil samples (Enerijiofi *et al.*, 2017a). The purified bacterial isolates were characterized culturally, morphologically and biochemically (Cheesbrough, 2006; Holt *et al.*, 1994) while the fungal isolates were also characterized macroscopically and microscopically using lactophenol cotton blue stain (Fawole and Oso, 2001).

Assay for microbial enzymes

Soil invertase and protease concentrations from herbicides treated soils was determined according to the method of Guan *et al.* (1987). Soil maltase and amylase concentrations were determined as described by Baboo *et al.* (2013) while soil dehydrogenase activity was also determined (Burns, 1978).

Statistical analysis of data

Conventional statistical methods were used to calculate means and standard errors. Data were statistically tested for one-way analysis of variance (ANOVA) and Duncan's multiple range test was applied for comparing means according to the method of Ogbeibu (2005).

Results and Discussion

Table 1 shows the physicochemical parameters of the soil samples contaminated with herbicides. The pH ranged from (5.17 – 5.41), electrical conductivity (98.00 – 237.00 uS/cm), Sulphate (28.25 – 59.25 mg/kg), Potassium (41.81 – 87.69 mg/kg), Iron (44.10 – 106.65 mg/kg), moisture (26.46 – 63.99%), Organic carbon (1.08 – 2.61%) and particle size (89.21 – 91.11%). There was high significant difference in physicochemical parameters at locations A, C and E ($P<0.001$). The impact assessment indices are as shown in Table 2 with lead having the highest for contamination factor, geo – accumulation index and quantification of concentration with 29.60, 19.73 and 96.62%, respectively. This was followed by chromium with 12.71, 8.47 and 92.13%. The result of the microbial analysis is recorded in Table 3. Soil samples from location E had the highest of 5.52 x 10^6 and 2.43 x 10^6 cfu/g for bacterial and fungal counts respectively followed by Location D with 4.19 x 10^6 and 1.85 x 10^6 cfu/g bacterial and fungal counts, respectively. The predominant microbial isolates were *Bacillus* sp., *Pseudomonas fluorescens*, *Aspergillus niger*, *Penicillium* sp. and *Rhizopus* sp. Figure 1 recorded the percentage frequency of occurrence of bacterial and fungal isolates. *Pseudomonas fluoriscens*, *Penicillium* sp. and *Aspergillus niger* were the highest (13.64%) followed by *Bacillus* sp. (11.21%). The enzyme assay result revealed that *Pseudomonas flouriscens* produced the highest enzyme (maltase) at day 2, 4, 6, and 8 with concentration of 0.313, 0.407, 0.421 and 0.429 mg/ml followed by invertase with 0.36, 0.421 and 0.429 mg/ml at day 4, 6 and 8, respectively (Fig. 2).

Table 1: Physicochemical parameters of the soil samples

Physicochemical parameters	Units	Location A	Location B	Location C	Location D	Location E	Control	FEPA effluent 1991
pH		5.17	5.33	5.27	5.41	5.29	5.77	6-9
EC	uS/cm	113.00	122.00	237.00	98.00	162.00	55.00	1000
Cl	mg/kg	24.86	26.84	52.14	21.56	35.64	12.10	600
Sulphate	mg/kg	28.25	30.50	59.25	24.50	40.50	13.75	50
Nitrate	mg/kg	22.60	24.40	47.40	19.60	32.40	11.00	1.5
Phosphate	mg/kg	15.82	17.08	33.18	13.72	22.68	7.70	5.0
Ammonium nitrogen	mg/kg	2.26	2.44	4.74	1.96	3.24	1.10	
Calcium	mg/kg	7.91	8.54	16.59	6.86	11.34	3.85	100
Magnesium	mg/kg	18.08	19.52	37.92	15.68	25.92	8.80	100
Sodium	mg/kg	28.36	30.62	59.49	24.60	40.66	13.81	50
Potassium	mg/kg	41.81	45.14	87.69	36.26	59.94	20.35	0.2
Zinc	mg/kg	1.81	1.95	3.79	1.57	2.59	0.88	1.0
Copper	mg/kg	4.52	4.88	9.48	3.92	6.48	2.20	1.5
Chromium	mg/kg	10.40	11.22	21.80	9.02	14.90	1.06	0.5
Lead	mg/kg	0.39	0.66	0.11	0.94	0.86	0.02	
Manganese	mg/kg	4.07	4.39	8.53	3.53	5.83	1.98	0.5
Iron	mg/kg	50.85	54.90	106.65	44.10	72.90	24.75	20
Nickel	mg/kg	6.78	7.32	14.22	5.88	9.72	3.30	1.0
Moisture	%	30.51	32.94	63.99	26.46	43.74	34.85	
Organic carbon	%	1.24	1.34	2.61	1.08	1.78	0.61	
Total Nitrogen	%	0.14	0.15	0.29	0.12	0.20	0.07	
Clay	%	7.98	7.04	7.45	6.85	6.97	5.12	
Silt	%	2.58	2.96	3.34	2.04	2.59	1.26	
Sand	%	89.44	90.00	89.21	91.11	90.44	93.62	
Mean ± SE		21.6±5.78	23±6.1	40.5±10.7	19.4±5.3	29.1±7.7		
ϰ		476.49	45.45	161.31	33.82	72.000		
P Value		0.000	0.005	0.000	0.088	0.000		
Significant level		P<0.001*	P>0.001	P<0.001*	P>0.001	P<0.001*		

P>0.001 No significant difference, P<0.001* high significant difference

Table 2: Average contamination factors (CF), geo-accumulation index (L-geo), uantification of concentration (QoC) and Background values (BC) of heavy metals in soils

Soil parameters	CF	L-geo	QoC (%)	BC
Zn^{2+}	2.66	1.77	62.43	0.88
Cu^{2+}	2.66	1.78	62.43	2.20
Cr^{6+}	12.71	8.47	92.13	1.06
Pb^{2+}	29.60	19.73	96.62	0.02
Mn^{2+}	2.66	1.77	62.43	1.98
Fe^{2+}	2.66	2.12	62.43	24.75
Ni^{2+}	2.66	1.77	62.43	3.30

Table 3: Microbiological Analyses of Soil Samples

Sample ID	THBC ($x10^6$ cfu/g)	THFC ($x10^6$ cfu/g)	Microbial distribution
Location A	2.78	1.13	*Escherichia coli*, *Pseudomonas fluoriscens*, *Aspergillus niger*, *Corynebacterium* sp., *Bacillus* sp., *Penicillium* sp. and *Rhizopus* sp.
Location B	3.37	1.40	*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluoriscens*, *Aspergillus niger Corynebacterium* sp., *Bacillus* sp., *Serratia* sp., *Penicillium* sp., *Candida* sp.
Location C	3.65	1.39	*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluoriscens*, *Aspergillus niger*, *Bacillus* sp., *Serratia* sp., *Penicillium* sp., *Candida* sp. and *Rhizopus* sp.
Location D	4.19	1.85	*Escherichia coli*, *Pseudomonas fluoriscens*, *Aspergillus niger*, *Corynebacterium* sp., *Bacillus* sp., *Serratia* sp., *Penicillium* sp., and *Rhizopus* sp.
Location E	5.52	2.43	*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas fluoriscens*, *Aspergillus niger*, *Corynebacterium* sp., *Bacillus* sp., *Serratia* sp., *Penicillium* sp., *Rhizopus* sp. and *Candida* sp.
Control	1.54	1.04	*Staphylococcus aureus*, *Pseudomonas fluoriscens*, *Aspergillus niger*, *Bacillus* sp., *Penicillium* sp., and *Rhizopus* sp.

Legend: THBC: Total heterotrophic bacterial count; **THFC:** Total heterotrophic fungal count

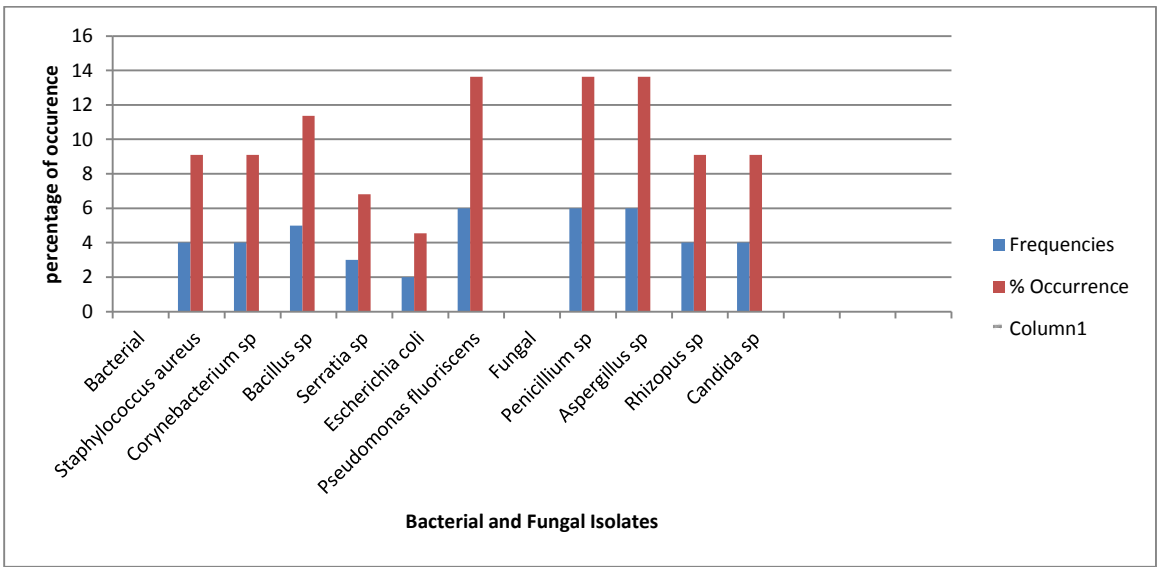

Fig. 1: Percentage Frequency of Occurrence of Bacterial and Fungal Isolates

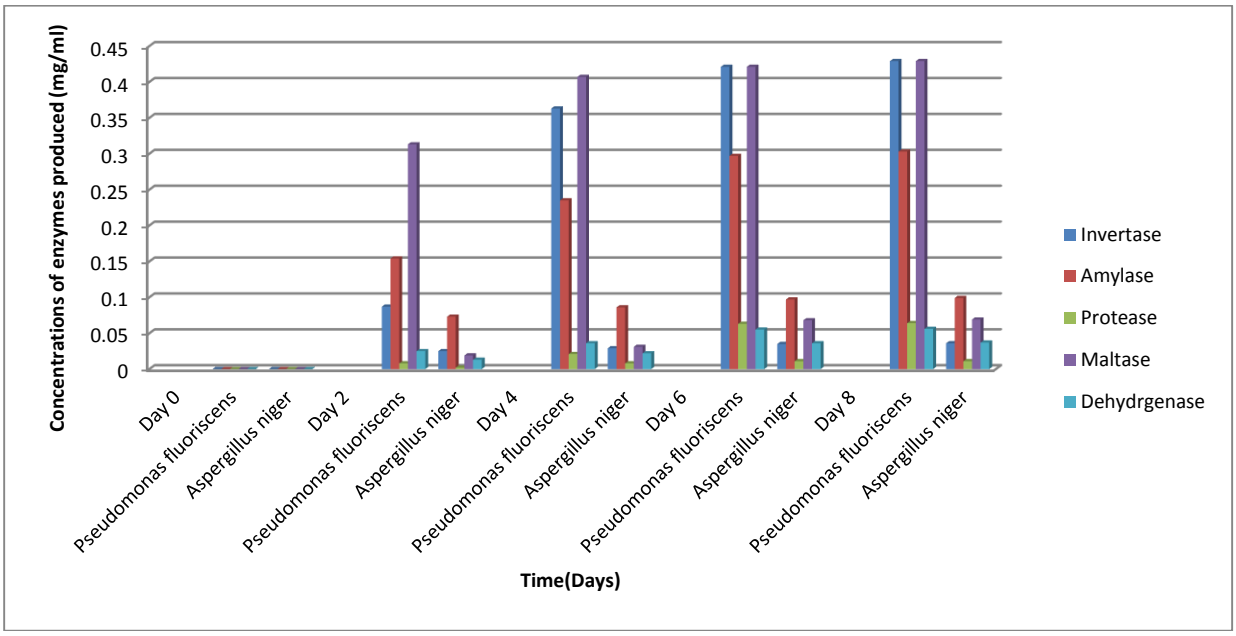

Fig. 2: Concentrations of various microbial enzymes production (mg/ml)

The result of physiochemical parameters of herbicides contaminated soils at the different locations revealed that all assayed parameters were higher than the control except pH which was lower. The lower pH values reported for the contaminated soils could be due to the effect of herbicides in the soil. Also, heavy metals such as copper, chromium, manganese, iron and nickel were higher than the control values and FEPA (1991) set limit. This finding was not surprising as heavy metals are components of these herbicides which are being added to the soil when the herbicides are sprayed. The high acidity reported in the herbicide impacted soils could interfere with nutrient cycling in the environment to the extent that deposition and dissolution of nutrient may take place. (Sebiomo *et al.*, 2011). Also, the acidic pH recorded determines the availability of nutrients and promotes vertical movement of heavy metals as reported earlier (Akpoveta *et. al*., 2010). The particle size distribution revealed that the soils were sandy. This sandy nature of the soil encourages easy movement of heavy metals down the soil depth. Locations A, C and E had significant difference in the physicochemical parameters studied.

The contamination factor showed highest for lead and chromium which revealed high contamination, while iron, copper, zinc, manganese and nickel showed considerable contamination. The values for lead and chromium clearly indicates that contamination in the soil arose from anthropogenic inputs of herbicide application. The pollution status in the herbicide contaminated soils expressed in terms geo-accumulation index showed very high pollution with lead followed by chromium and to a lesser degree with iron, copper, zinc, manganese and nickel. The Quantification of concentration pattern were consistent with the two earlier assessment indices which followed the same pattern of heavy metals contamination. This points to similar source of anthropogenic inputs of these heavy metals into the soil as earlier reported (Enerijiofi *et al*., 2017b). However the heavy metals abundance were Pb^{2+}> Cr^{2+} > Fe^{2+} > Cu^{2+}> Zn^{2+} > Mn^{2+} > Ni^{2+}.

The total heterotrophic bacterial and fungal counts from the herbicide impacted soils were higher than the control soil. This high counts could be due to the presence of extra nutrients. This study documented a diverse range of bacterial and fungal isolates from the contaminated soils which varied from one location to another. These variation may have arose from differences in the concentration of nutrient in the different location of the farm as occasioned by the sloppy nature of the farm. These opportunistic microbial species of human and public health importance isolated in this study are similar to the ones earlier documented by Ayansina and Oso, (2006) from herbicide contaminated soils. Some of these isolates are associated with degradation of organic matter, while others like *Candida* species and *Aspergillus niger* are pathogenic especially in immunocompromised individuals. According to Uraih, (2004), *Aspergillus niger* is known for aflatoxin production which destroys the liver by inducing fatty acid metamorphosis of its cells.

Pseudomonas fluoriscens produced the highest concentrations of maltase, amylase and invertase, enzymes compared to *Aspergillus niger*. This trend followed through particularly from day 2 to day 8. The high production ability of *Pseudomonas fluoriscens* to produce maltase, amylase and invertase enzymes was an indication that soils were highly rich in substrate that could be metabolised by these high yielding enzymes, hence their high biosynthesis. Enzymes production by most microorganisms are growth associated and usually induced in the presence of its substrate in the culture medium. Generally enzymes production takes place during the log phase of microbial growth and its concentration decreases towards the end of the log phase or during the stationary phase. Metal ions particularly Ca^{2+} can either stimulate or inhibit microbial enzyme production and function (El Khattabi *et al.*, 2003).

Conclusion and Recommendation

This study revealed that herbicides applications increased the concentrations of the physicochemical and microbiological parameters above the control. This study recommend harnessing the potentials of maltase, amylase and invertase enzymes produced by *Pseudomonas fluoriscens* as biocatalyst for decontaminating herbicide contaminated soils as this will reduce public health risks to man and its environment.

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