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Profiling Of the Selected Residual Soil Enzymes Associated With Glyphosate Impacted Top Soils (Short Communication)

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ABSTRACT: The edaphic enzyme profiles associated with compounded top herbicide; Glyphosate impacted and control soils sourced from a commercial farm setting and a fallow land were determined using routine methodologies. The commercial farm establishment and the fallow farm were sited in Amukpe and Adavware communities both located in Sapele Local Government Area, Delta State, Nigeria. The amylase and invertase content of the soils ranged from 11.43 µg glucose/g soil ± 0.24 to 52.15 µg glucose/g soil ± 0.68 and 55.46 µg sucrose/g soil ± 1.25 to 548.13 µg sucrose/g soil ± 15.56 respectively. The mean protease, urease, and dehydrogenase activities varied from 59.24 µg tyrosine/g soil ± 1.59 to 122.65 µg tyrosine/g soil ± 0.59, 6.34 µg NH_4^+/g soil ± 0.22 to 25.63 µg NH_4^+/g soil ± 0.46 and 5.61 µg TPF/g soil ± 0.18 to 13.86 µg TPF/g soil ± 0.26. The differences in soil enzymatic activity amongst the glyphosate exposed soils and the control soil were statistically significant ($p<0.001$). The usage of glyphosates impacted negatively on the analyzed soil invertase, protease, amylase, and urease activities but had no observable effect on dehydrogenase activity.

Keywords: Commercial farm establishment, Compounded, Amukpe, Glyphosate, Soil enzyme activity

Introduction

Soil is known to contain free enzymes, immobilized extracellular enzymes, and enzymes within microbial cells (Mayanglambam *et al*., 2005). These enzymes can serve as indices of biological equilibrium, fertility, quality and variations in the biological attributes of soil as a consequence of pollution (Bucket and Dick, 1998; Kucharski *et al*., 2000; Antonious, 2003). Burns (1982) stated that the function of soil enzyme activities can be defined by their relationships with soil and other environmental factors i.e., acid rain, heavy metals, pesticides, and other industrial chemicals that can impact directly or indirectly on their activities. It is well documented that microorganisms play a critical role in decomposition of several inorganic and organic compounds frequently used in agriculture (Carney and Matson, 2005). Microbial enzymes have been known to play an important role in the decomposition process. Essential microbial enzymes known to take part in the mineralization/decomposition of soil organic matter include cellulases, proteases, ureases, phosphatases (Albiach *et al*., 2000; Kunito *et al*., 2001), dehydrogenases, invertases, glucosidases, and lipases. Dilly and Nannipieri (2001), attributed cellulases with the decomposition of cellulose moieties present in plant residues

which are deposited above soil (the litter layer), while Masciandaro and Ceccanti (1999) posited that nitrogen fertilization was the most important management strategy for agricultural soil improvement, but urea was the most frequently utilized source of organic N amendment, which can be easily hydrolysed to ammonium and carbon (IV) oxide by urease. Organic nitrogen is also known to directly impact the distribution and activity of soil-borne proteolytic enzymes (Cenciani *et al*., 2008).

Herbicides are extraneous to soil component pools, and expectedly affect soil enzyme behavior and catalytic efficiency (Sannino and Gianfreda, 2001). Several researches have indicated that herbicides can cause qualitative and quantitative changes in enzyme activities (Saeki and Toyota, 2004; Sebiomo *et al.,* 2011; Xia *et al.,* 2011). Enzyme activity can be influenced by soil conditions such as organic matter content (Kandeler *et al*., 1999), moisture (Bergstrom *et al*., 1998), and temperature (Tscherko *et al*., 2001). Hussain *et al*. (2009) reported that the impact of pesticides on soil enzymes especially extracellular enzymes is unclear primarily due to their multidimensional behavior in complex soil medium and the greater complexity of soil microbial and biochemical interactions. For these reasons, researchers have encountered challenges in the evaluation of the impacts of pesticide usage on extracellular enzyme activities in soils (Nannipieri, 1994). The negative impact of pesticides on soil enzymes like hydrolases, oxidoreductases, and dehydrogenase activities has been documented (Monkiedje and Spiteller, 2002; Menon *et al*., 2005). Glyphosate (N-phosphonomethylglycine) ($C_3H_8NO_5P$) is a systemic herbicide routinely employed globally in the control of broad spectrum of weeds in farmlands and pastures respectively (Šantric *et al*., 2018). It can also be described as a non -selective, broad-spectrum herbicide and crop desiccant. Globally, the herbicide has been described as the most commonly utilized herbicide and is known to be rapidly inactivated in soil, both by degradation and adsorption processes (Benslama and Boulahrouf, 2013). In Nigeria, glyphosate is commercially available under several brand names which include; Roundup®, Rodeo® and Pondmaster® respectively.

In several parts of Nigeria, extensive commercial farm establishments utilize appreciable amounts and types of herbicides in their day to day routine specifically in weed elimination at these respective farm holdings. As the different tiers of the Nigerian Government has over the years engage in several aggressive sensitization programmes aimed at nudging individuals and corporate firms to make substantial investments in the commercial farming sector, there is a need to ascertain the effect of regular herbicide application on the activities of the soil biome specifically, the free and bound enzyme profiles. The necessity of these researches is not far-fetched based on the important contributions these soil enzymes play in the recycling of soil organic matter. This research was aimed at the determination of the edaphic enzyme profiles associated with compounded herbicide impacted and control soils.

Materials and methods

***Description of study area*:** Several farming settlements such as Adagbrasa, Amukpe, Adavware, Elume, Ogiedi, Ughorhen, and Ikeresan are known to abound in Sapele Local Government Area of Delta State, Nigeria. Amukpe is located within Longitude E 5º 42’ 55.76” and Latitude N 5º 51’ 38.75” with elevation above sea level being 11meters. The vegetation pattern of this area is consistent with the tropical rainforest zone except for drainage streams where swampy areas are known to exist (Fig. 1). The commercial farm establishment sited in Amukpe, is an integrated farm registered as a company limited by guarantee in September, 2005, by the Delta State Government.

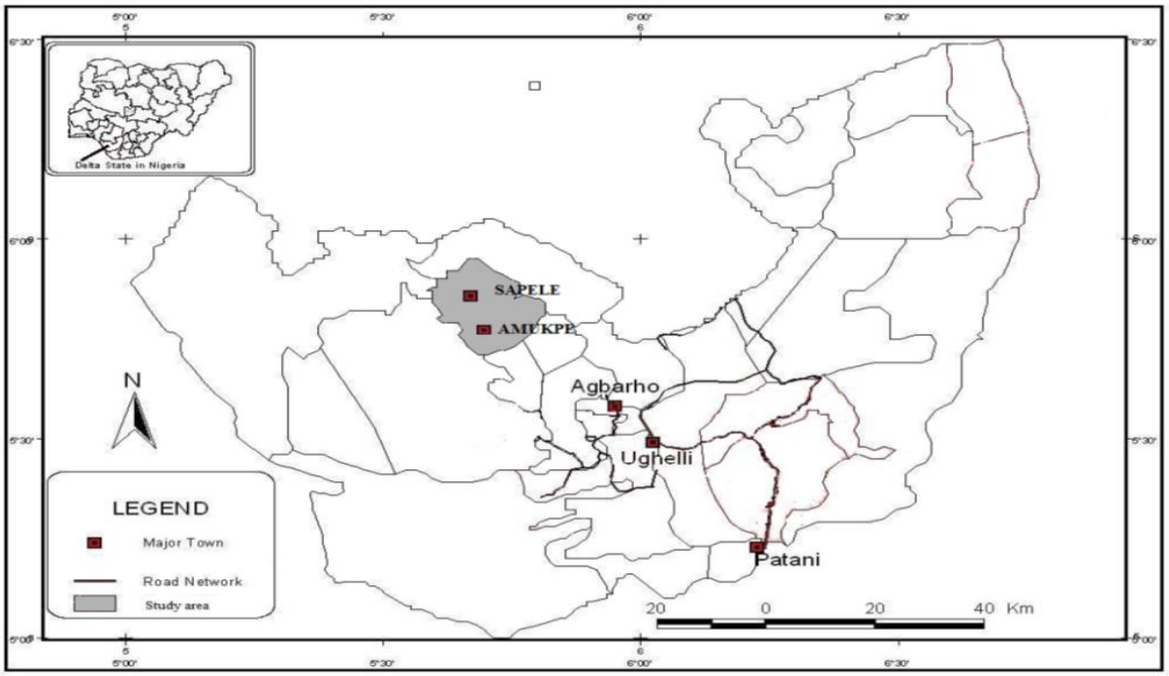

Fig. 1: Map of Delta State, Nigeria, showing the study area

***Collection of top soils*:** Top soils were sourced from several sampling points in an actively farmed land within the commercial farm premises sited in Amukpe, Sapele LGA, Delta State. Prior to soil sampling, verbal consent was sought and obtained from the overall farm manager of the agricultural establishment. With the aid of a soil auger, 100kg of the top soil were obtained from the respective sampling stations at a uniform depth of 0-15 cm; Station one; watermelon and tomatoes farm Station two; Maize farm Station three; Cassava farm. Another 100kg of fallow soil which has been left for an unknown time period, which served as the control were collected from Adavware community, near Amukpe. These soil samples were placed in labeled sterile polyethylene bags and transported to the laboratory for analysis.

Determination of the enzyme profile of the soils

Dehydrogenase and invertase activity: The dehydrogenase and invertase activity of the soils were evaluated using the procedure described by Margesin (2005) and Guo *et al.* (2009).

Urease, amylase and protease activity: The edaphic urease, protease, and amylase activities were ascertained in accordance with procedures described by Somogyi (1952), Ladd and Butler, (1972) and Guo *et al.* (2009).

Statistical analysis of the mean enzyme values: Analysis of variance (ANOVA) of the respective mean enzyme value was conducted ($\alpha = 0.001$). Duncan Multiple Range (DMR) tests were conducted to locate the cause of any significant differences in the mean values.

Results and discussion

The amylase content of the soils ranged from 11.43 µg glucose/g soil ± 0.24 for the bulked experimental soil collected from Station 3 to 52.15 µg glucose/g soil ± 0.68 for the control soil obtained from Station 1 (Table 1).

Amylase activity displayed a decreasing trend when compared to that of the control. Similar decreasing trends were also exhibited in the invertase, protease and urease activities respectively. The variation in soil amylase activity with respect to herbicide exposure was found to be statistically significant ($p<0.001$). The difference in amylase activity in various herbicide treated soil could be attributed to herbicide induced change in amylase activity and the possible unavailability of nutrients thus, inducing stress (Perucci and Scarponi, 1994; Anigboro and Tonukari, 2008). Besides, certain groups of microorganisms are known to initiate the decomposition of applied herbicide(s) after a few days (Milosevic and Govedarica, 2002).

Table 1: Mean enzyme activity from the control (Adavware) and experimental (herbicide impacted) soils

Enzyme (unit)	Bulked Control soil from Station 1	Bulked experimental soil from Station 1	Bulked experimental soil from Station 2	Bulked experimental soil from Station 3
Amylase (µg glucose/g soil)	52.15 ± 0.68^{a}	12.16 ± 0.21^{b}	$12.40 \pm 0.20^{b,c}$	$11.43 \pm 0.24^{c,d}$
Invertase (µg sucrose/g soil)	548.13 ± 15.56^{a}	55.46 ± 1.25^{b}	$94.552 \pm 1.03^{c,d}$	$79.00 \pm 4.62^{b,c,d}$
Protease (µg tyrosine/g soil)	122.65 ± 0.59^{a}	85.11 ± 5.73^{b}	59.24 ± 1.59^{c}	70.46 ± 3.61^{d}
Urease (µg NH_4^+/g soil)	25.63 ± 0.46^{a}	9.08 ± 0.31^{b}	8.12 ± 0.17^{c}	6.34 ± 0.22^{d}
Dehydrogenase (µg TPF/g soil)	5.61 ± 0.18^{a}	11.02 ± 0.06^{b}	12.02 ± 0.14^{c}	13.86 ± 0.26^{d}

*TPF = Triphenyl formazon), Mean values succeeded by alphabets; b, c and d are statistically significant ($p<0.001$)

The invertase activity of the soils ranged from 55.46 µg sucrose/g soil ± 1.25 for the experimental soil collected from Station 1 to 548.13 µg sucrose/g soil ± 15.56 for the control soil obtained from Station 1 (Table 1). Amylase and invertase activities are usually selected for their importance in soil carbon cycling. The invertase activity in control soil was found to be highest amongst all enzymes analyzed. The invertase activity in the soils exposed to herbicide showed a significant decrease as compared to that of the control soil. A possible reason for the decrease in soil invertase activity may be attributed directly to the cellular destruction effected by the applied herbicide (Perucci and Scarponi, 1994) accompanied by the reduction in nutrient mobilization and glucose level (Anigboro and Tonukari, 2008). Further, invertase has an extracellular origin and the death of some microorganisms may cause a decline in the production and excretion of enzymes which could result in the reduction of soil invertase activity (Perucci *et al.,* 1999).

The protease profile of the soils ranged from 59.24 µg tyrosine/g soil ± 1.59 for the experimental soil collected from Station 2 to 122.65 µg tyrosine/g soil ± 0.59 for the control soil obtained from Station 1 (Table 1). Mean protease activity detected in the herbicide treated soil showed a decrease in comparison to the control site. The protease activity is known to be associated with proteolytic bacteria distribution (Sardans *et al.,* 2008; Anjaneyulu *et al.,* 2011; Subrahmanyan *et al.,* 2011), and the amount of proteinaceous substrate availability, NH_4-N accumulation (Sardans and Penuelas, 2005; Tischer, 2005) in soil organic matter.

The urease content of the soils varied from 6.34 µg NH_4^+/g soil ± 0.22 for the experimental soil collected from Station 3 to 25.63 µg NH_4^+/g soil ± 0.46 for the Control soil obtained from Station 1 (Table 1). The urease activity in herbicide treated soils showed a significant decrease as well. The urease activity was found to be lower as compared to other enzyme activities among the treated sites. It is known that appreciable urease activity occurs in soils. This trend has led to the conclusion that native soil urease are mainly extracellular and are particularly persistent because of their association with inorganic and organic soil colloids (Gianfreda *et al.,* 1995). A considerable amount of total activity of an enzyme (including urease) in soil may be ascribed to an enzymatic fraction located either within proliferating and non-proliferating cells or attached to or contained within cellular matrices (Nannipieri, 1994). This enzymatic fraction does not contribute to the measured activity of soil enzyme, because it is not easily detectable in specific enzyme assays. Thus, it could be assumed that such urease fractions predominate in soils.

The dehydrogenase activity of the soils varied from 5.61 µg TPF/g soil ± 0.18 for the control soil obtained from Station 1 to 13.86 µg TPF/g soil ± 0.26 for the experimental soil collected from Station 3 (Table 1).The soil dehydrogenase activity showed an increase in the herbicide treated soil as compared to the control soil. The increase in soil dehydrogenase activity for the herbicide treated soil might be due to the increase in microbial community composition with the capability of utilizing the herbicide as a carbon source. Dehydrogenase is an intracellular enzyme involved in microbial oxygen metabolism. This activity is dependent on the metabolic state of soil biota and could serve as a good indicator of soil microbial activity (Garcia *et al.,* 1994).

Besides, if the herbicide utilized for weed control was applied at recommended rates or dosages, it could have resulted in the observable non-inhibitory effects on dehydrogenase activity (Rao and Raman, 1998). Dehydrogenase activity is the most sensitive to the combined toxic effect of heavy metals and polycyclic aromatic hydrocarbons (PAHs) in soil (Madejon *et al.,* 2001; Maliszewska-Kordybach *et al.,* 2003; Shen *et al.,* 2005). More so, it can also be due to the microbial multiplication on increased supply of nutrients available in the forms of microorganisms killed by the herbicides (Vandana *et al.,* 2012).

Conclusion

The compounded top soils exposed to varying amounts of glyphosate had significantly lower invertase, protease, amylase and urease activities in comparison with the bulked control (fallow) soil. However, dehydrogenase activity increased in the bulked experimental soils in comparison with the control soil. The usage of glyphosates impacted negatively on the analyzed soil invertase, protease, amylase, and urease activities but had no observable effect on dehydrogenase activity.

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