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Effects of calcium carbide waste on soil bacteria and enzymes

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Abstract: ***The effects of calcium carbide waste on some groups of soil microorganisms and soil enzymes were studied using standard microbiological and biochemical techniques. Soil samples were collected from a vehicle maintenance area as well as from an adjacent farm land (control) during the dry and rainy seasons. The population of all the bacterial groups in the different seasons was affected adversely by calcium carbide waste. Total heterotrophic bacteria (THB), being a representative of the bacterial groups decreased from 3.6 x 10^6 ±0.35 cfu/g to 4.1 x 10^4 ±0.09 cfu/g in the dry season, and from 4.1 x 10^6±0.02cfu/g to 1.1 x 10^5±0.01cfu/g during the rainy season. There was also significant decrease in the activity of the enzymes in different seasons. The enzymes most adversely affected in the different seasons include acid phosphate, urease and lipase. The effect of calcium carbide waste on the parameters studied was more severe during the dry season.***

Keywords: Calcium carbide, enzymes, total heterotrophic bacteria.

1. Introduction

Environmental sustainability depends largely on a sustainable soil ecosystem, because soil is the key component of natural ecosystem (Adedokun and Ataga, 2007; Adenipekun, 2008; Onuh *et al.,* 2008). Soil is a dynamic natural body developed as a result of pedogenic processes during and after weathering of rocks, consisting of mineral and organic constituents, possessing definite physical, chemical mineralogical and biological properties, having a variable depth over the surface of the earth and providing a medium for land plants (Biswas and Mukherjee, 1991; Kolwzan *et al*., 2006), capable of supporting life. The biological activities in the soil are largely in the topsoil, and the topsoil receives the greatest impact from pollutants. Soil pollution is caused by materials, mostly chemicals that are out of place or are present at concentrations higher than normal which may have adverse effects on humans or other organisms. Most environments in Nigeria and to a large extent in the whole of West Africa are subject to an increasing pollution load from the discharge of different kinds of effluents resulting from anthropogenic activities which have become a major threatening factor to the quality of soil. Vehicle maintenance area wastes like spent engine oil, spent calcium carbide, and automotive battery wastes are common environmental pollutants/toxicants.

Calcium carbide is a grayish-white powder that is used by welders to generate acetylene gas which is burned to produce high energy light (Lavoie, 1980). Calcium carbide waste is a by-product of an acetylene gas production process (Makaratal *et al*., 2010). The waste may contain Cu, Pb, Fe, Mn, Ni and Zn ions (Ibrahim, 2002). Calcium Carbide wastes have toxic effect on microorganisms, which could ultimately affect the higher organisms which depend on microbes and their by-products for growth and development (Lavoie, 1980). Microorganisms are involved in all nutrient cycling (Willey, 2008). Microorganisms can achieve this feat because they possess enzymes, which may be exo-enzymes or endo-enzymes. Soil enzymatic activity is the driving force behind all biochemical transformations in soil (Tabatabai, 1986; Serrano *et al.,* 2006.). Any factor that affects microorganisms and soil enzymes adversely will also affect soil fertility.

The aim of this work was to determine the effects of spent calcium carbide on some groups of soil bacteria, and soil enzymes during different seasons.

2. Materials and Methods

Soil samples were collected using disinfected trowel from 0-15 cm depth in soils polluted with spent calcium carbide, and adjacent agricultural land for the control samples. A set of soil samples was collected during the dry season (December – March) and another set of soil samples was collected during the rainy season (April – October), from Orji Mechanic Village Owerri North. The co-ordinates of Owerri North Local Government Area are longitude $7^0$07′ E and latitude 5^o33′ N. Bulked or composite soil samples from impacted and control sites were air-dried, ground, sieved (2mm) and stored at room temperature (28±2^{O}C) for 24h.

Microbiological Analysis

The bacterial density and diversity were determined by estimation of the population of different groups of bacteria and identification of the heterotrophic bacteria.

Estimation of Total Heterotrophic Bacteria (THB)

Population of heterotrophic bacteria in the impacted and control soil samples were enumerated as described by Okpokwasili and Okorie (1988).

Estimation of Nitrifying Bacteria (NB).

One gram (1g) of soil sample was put into 9 ml of sterile physiological saline and shaken properly. Ten-fold serial dilution of each sample was prepared according to

Cheesbrough (1987). Bacterial growth was obtained after 48-72h incubation at room temperature (28±2^{o}C) (Nwaugo *et al*., 2004).

Estimation of Lipolytic Bacteria (LB)

This was carried out according to Mohan *et al*., 2008. Aliquots of diluted soil samples were plated on tributyrin agar for 24h, and the formation of halo zones around the colonies on tributyrin agar was considered as positive result for the test.

Enumeration of Phosphate Solubilizing Bacteria (PSB)

The method described by US Patent (2003) was used. The soil samples were inoculated on NBRI-BPB medium. Production of yellow halo around the colonies was taken as positive result.

Enumeration of Cellulolytic Bacteria (CEB)

The organisms were enumerated by plating serially diluted soil samples on cellulose agar which contained carboxy methyl cellulose (CMC), according to the method of Hatami *et al*., (2008).

3. Enzyme assay

Acid and Alkaline Phosphatases

The method described by Alef and Nannipieri (1995) was employed. This is based on the colorimetric estimation of the p-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5 for acid phosphatase activity and pH 11 for alkaline phosphatase activity) sodium p-nitrophenyl phosphate solution and toluene.

Lipase Assay

The method described by Onilude *et al*., (2010) was employed in this assay.

Urease Assay

This was assayed using the method described by Alef and Nannipieri (1995).

Cellulase Assay

The assay method described by Alef and Nannipieri (1995) was used. The method was based on the determination of released reducing sugars after the incubation of soil samples with carboxy methyl cellulose salt solution (CMC) for 24h at 50^{o}C.

Assay for Dehydrogenases

The assay method as described by Cassida *et al*., (1964) was used.

Statistical Analysis

SPSS was used to carry out a paired sample T-test to analyze the data and make inferences.

4. Results and Discussion

Calcium carbide waste caused a decrease in the population of all the bacterial groups in the different seasons as shown in Table 1. In the dry season, THB, with the highest population of 3.6 x 10^{6} ±0.35 cfu/g decreased to 4.1 x 10^{4} ±0.09 cfu/g. NB had the least population in the control, 3.0 x 10^{3} ±0.02cfu/g, while LB had the least count in the impacted soil, 1.2 x 10^{2} ±0.02 cfu/g. In the rainy season, NB had the lowest population in the control (3.9 x 10^{3}±0.19 cfu/g) while LB had the least (1.4 x 10^{2} ±0.19 cfu/g) in the impacted soil. THB had the highest density in both the control and the impacted soil (4.1 x 10^{6}±0.02cfu/g and 1.1 x 10^{5}±0.01cfu/g). The microbial loads were higher during the rainy season than in dry season. This could be because of changes in environmental factors (Liu *et al*., 2010). Hoyle and Murphy (2006) reported that absence of water and readily available carbon sources during the dry season lead to limited microbial activity in the soil. The population of NB decreased, as was also reported by Nwaugo *et al*., (2004), who observed that CaC_2 was adverse to nitrite-oxidizing bacteria, because they are destroyed in high alkaline environment. This invariably affects nitrification which will eventually affect soil fertility.

Calcium carbide waste brought significant decrease in the activities of enzymes as depicted in Table 2. During the dry season, dehydrogenase had the highest value (30.10±0.04 mg g^{-1} $6h^{-1}$) which decreased to 26.10±002 mg g^{-1} $6h^{-1}$. Acid phosphatase had the least value in the impacted soil (1.21±0.02 µmol –p- nitrophenol). Alkaline phosphatase decreased from 3.10±0.15 – 2.0±0.02 µmol-p-nitrophenol, urease: 3.40±0.02 – 2.40±0.17 mg g^{-1} $2h^{-1}$, cellulase: 3.70±0.04 – 2.40±0.19 mg g^{-1} $6h^{-1}$, lipase: 2.70±0.04 – 1.90±0.04 g^{-1} 30 min. In the rainy season, dehydrogenase activity was highest in the control and in the impacted soil (33.47±0.11 mg g^{-1} $6h^{-1}$ and 20.72±0.32 mg g^{-1} $6h^{-1}$ respectively). Lipase showed the least activity in the control (3.00±0.17 g^{-1} 30 min) and in the impacted soil (1.40±0.19 g^{-1} 30 min). Acid phosphatase decreased from 3.80±0.23 µmol-p-nitrophenol in the control to 2.17±0.28 µmol-p-nitrophenol in the impacted soil, alkaline phosphatase: 3.60±0.02 – 2.40±0.09 µmol-p-nitrophenol, cellulase: 4.10±0.19 – 2.30±0.06 mg g^{-1} $6h^{-1}$, and urease from 3.50±0.17 – 1.60±0.09 mg g^{-1} $2h^{-1}$. The increase in metallic ions (Ibrahim, 2002) may have poisoned or denatured the enzymes. DHA had the highest activity, while acid phosphate had the least.

Table 1: Effect of Calcium Carbide on Microbial Load of Soil (cfu/g) in Different Seasons

	Dry Season		Rainy Season	
Microbial Group	**Control**	**Impacted Soil**	**Control**	**Impacted Soil**
Nitrifying Bacteria	$3.3x10^{3}$±0.27	$2.1x10^{2}$±0.02	$3.9x10^{3}$±0.13	$2.7x10^{2}$±0.21
Phosphate Solubilizing Bacteria	$1.7x10^{4}$±0.18	$2.1x10^{2}$±0.15	$2.1x10^{4}$±0.02	$2.4x10^{2}$±0.19
Total Heterotrophic Bacteria	$3.6x10^{6}$±0.35	$4.1x10^{4}$±0.09	$4.1x10^{6}$±0.02	$1.1x10^{5}$±0.01
Cellulolytic Bacteria	$3.4x10^{4}$±0.18	$2.1x10^{2}$±0.18	$3.9x10^{4}$±0.31	$1.1x10^{3}$±0.01
Lipolytic Bacteria	$3.2x10^{4}$±0.02	$1.2x10^{2}$±0.02	$3.5x10^{4}$±0.33	$1.4x10^{2}$±0.19

Table 2: Effects of Calcium Carbide Waste on the activity of soil Enzymes in different seasons

	Dry Season		Rainy Season	
Enzymes	**Control**	**Impacted Soil**	**Control**	**Impacted Soil**
Dehydrogenase (mg g^{-1} $6h^{-1}$)	30.10±0.04	26.10±0.02	33.47±0.11	20.72±0.32
Acid Phosphatase (µmol-p-nitrophenol)	3.40±0.19	1.21±0.02	3.80±0.23	2.17±0.18
Alkaline Phosphatase (µmol-p-nitrophenol)	3.10±0.15	2.40±0.02	3.60±0.02	2.40±0.09
Urease (mg g^{-1} $2h^{-1}$)	3.40±0.02	2.00±0.17	3.50±0.17	1.60±0.09
Cellulase (mg g^{-1} $6h^{-1}$)	3.70±0.04	2.40±0.19	4.10±0.19	2.30±0.06
Lipase (g^{-1} 30 min)	2.70±0.04	1.90±0.04	3.00±0.17	1.40±0.19

5. Conclusion

Calcium carbide waste had adverse effect on the soil microorganisms since the populations of all the groups were higher in the control than in the polluted soil. Also, the activity of the enzymes decreased in the polluted soil.

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