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EFFECTS OF QUARRY PLANT ROCK DUSTS ON SOIL PHOSPHATE SOLUBILIZING BACTERIA AND ENZYME ACTIVITIES IN ISHIAGU, EBONYI STATE, NIGERIA

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Abstract

Effects of Quarry Plant rock dust on soil phosphate solubilizing bacteria and enzyme activities were investigated at Ishiagu, Ebonyi State, Nigeria. Soil samples were obtained from four points, 10m, 50m and 100m away from the plant with the fourth point over 350m away as the control. Results obtained showed decrease in pH, total phosphate, EC, CEC and temperature with increase in distance from the quarry plant while organic matter increased in the same direction. The trace elements, Ca, Mg and K were also highest nearest the plant but decreased with distance away from it. The variations in physicochemical parameters were statistically significant at $P < 0.05$. Total heterotrophic bacterial count ranged from 7.5×10^6 to 3.1×10^5 cfu/g, phosphate solubilizing bacterial count ranged from 1.4×10^6 to 3.4×10^4 cfu/g and nitrifying bacterial count ranged from 3.2×10^4 to 1.2×10^3 cfu/g. In each case, the 100m soil sample had the highest count, followed by the control while the 10m soil had the least counts. Bacteria belonging to seven genera were observed to solubilize phosophate and include *Bacillus, Erwinia,Flavobacterium, Alcaligenes Pseudomonas, Azotobacter* and *Arthrobacter*. All of these had higher prevalence rates in the 100m soil followed by the control soil. *Erwinia* and *Arthrobacter* were not observed in the 10m and 50m soil samples.*Flavobacterium* and *Azotobacter* were not also observed in the 10m soil. Determination of soil enzyme activities showed that dehydrogenase had the highest activities (34.84-16.2mg $g^{-1}24h^{-1}$) followed by urease ($3.67 - 2.04 mgg^{-1}24h^{-1}$) acid phosphase had activity range of 2.98 – 1.27 while alkaline phosphatase had 2.67 – 1.67 Umol-p-nitrol phenol. In all cases, 100m soil had the highest activities, followed by the control soil while 10m soil had the least. The enzyme activities correlated positively with the bacterial loads ($P < 0.05$). The results suggest positive effects of the rock dust on soil properties at low concentrations, so could be used for soil remineralization after depletion.

Key words: *Rock dust, soil enzyme, bacteria, quarry activities.*
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Introduction

Rapid industrialization and urbanization have resulted in the destruction of some habitats to improve others. Constructions of roads and buildings have engineered much demand for chips of granite (igneous rock) generally referred as chipping stones. To produce the chippings, quarries have emerged in various places producing rock dust which invariably alter the ecological balance of the receiving habitats, viz; air, water and soil (Saxena and Souza, 2005, Erkman, 2001)..

Microorganisms play vital roles in the biogeochemical transformations of various nutrients and minerals in the soil. These important intertwining transformations are affected by various natural and anthropogenic activities (Nwaugo *et al*, 2007, Das *et al*, 2007). It is now known that activities which modify the delicately balanced ecological equilibrium of any given habitat also affect its biogeochemical cycles. This is because the microorganisms mediate these reactions through their enzymes.

In soil fertility, phosphorus is a very limiting factor in the growth and yield of plants. It is perhaps, the most important element after nitrogen (Das *et al*, 2007, Chang and Yang, 2009). Most of the phosphate in soil occurs in the insoluble inorganic form, thus unavailable to plants. Only 10-25% of the total phosphate reaches the plants in soluble form (Saha and Biwas, 2009). The soil bacteria are therefore responsible for the continuous availability of the element as they solubilize the inorganic phosphate compounds. The phosphate solubilizing bacteria (PSB) which belong to diverse taxonomic groups differ in both structure and response to environmental changes (Ivanova *et al*, 2006, Souza *et al*, 2000, Behbahani and Behbahani, 2009).

Microbial transformation is mediated by enzymes and these enzymes are produced by both culturable and non-culturable organisms. Only 5-7% of these soil organisms are culturable (Pelczar *et al*, 2002). The use of enzymes in assessing soil biogeochemical transformation becomes important in determining soil biogeochemical activities. This

study was therefore designed to assess the effects of Quarry rock dust on soil phosphate solubilizing bacteria and enzyme activities in Ishiagu, a rural community of Ebonyi State, known for quarrying activities in Eastern Nigeria.

Materials and Methods

The study area is Ishiagu, a rural community of Ebonyi State, Nigeria while the western part of the community is known for heavy metal mining (Nwaugo *et al,* 2008), the northern parts is an established quarrying area. Ishiagu is located within latitudes 7°38' and 7°36'E and longitude 5°52' and 6°00'N. Ishiagu lies in the typical Guinea savanna area of Nigeria characterized by tall grasses and a few trees. The mean annual rainfall ranges from 1750 – 2000mm and the people are mainly farmers with a few working with the mining and quarrying companies.

Sample Collection

The top soil (0-15cm) was collected from 10m, 50m and 100m away from the Quarry plant site using sharpreks soil auger according to Dick *et al* (1990). A fourth sample, over 350m away served as control. At each sampling point, three samples were collected and pooled together to give one main sample.

The microbiological and enzyme activities of the soil samples were analyzed within 2-3 hours of collection. The soil samples for physiochemical parameters analysis were stored at 4°C and analyzed within 1-3 days of collection.

The soil temperature and pH were determined using the Jenway HANDA 1910 multipurpose tester (HANNA Instruments, Woonsuket, Rhodes Island, USA). The soil organic matter was determined using the loss of ignition method involving the use of the furnace (MAC 2000). The total phosphate, available phosphate and total $N0_3$ were determined according to Pansu and Gautheryron, (2006) in addition to EC and CEC of the soil samples. The trace elements (Ca, Mg and K) were determined following the description in UNEP manual (2004).

Microbiological Analysis

The soil physiological bacterial groups were determined using various culture media. Tryptone Soy Agar was used for total heterotrophic bacterial count, modified mineral salt agar for the nitrifying bacterial count and Pikovskay's media for the phosphate solubilizing bacterial count. The bacteriological loads of the soil samples were determined after ten-fold serial dilutions with 0.2ml of the desired dilution being inoculated on the various media using the spread plate technique according to Chessbrough (2002). The counting was done after 24 to 48 hours aerobic incubation.

The bacterial species observed in the Pikovskay's media (PSB) were characterized and identified to the genera level using morphological and microscopic features in addition to biochemical tests according to Holt *et al* (1994) and Chessbrough (2002).

Soil Enzyme Activity

The enzymes whose activities were assessed were dehydrogenase, urease, acid and alkaline phosphatases. The activity of dehydrogenase was determined by the reduction of Triphenyl tetrazolum chloride (TTC) to Triphenyl formazon (TPF) as described by Cassida *et al* (1964) and modified by Li *et al* (2005). 5.0g of 2mm sieved soil sample was mixed with 10ml of 0.25% aqueous TTC and incubated in sealed tubes at 25°C for 6 hours. The absorbance at 485nm of the methanol extract of the TPF formed was measured using methanol as blank. The results were expressed as TPF-1 dry soil 6h. The urease activity was determined using the spectrophotometric method as described by Kand der and Gerber (1998). This was based on the formation of NH_3-N from Ureas-amended soil at 37°C for 2 hours. The result was expressed as mg NH_3-Ng^{-1} dry soil 24h.

The activities of both acid and alkaline phosphates were determined by spectrophotometric estimation of the p-nitrophenol released by the enzymes after incubating the soil with buffered p-nitrophenol phosphate for 4 hours. The acid phosphatase was incubated at pH of 5.5 and alkaline

phosphatase at 11.0 according to Tabatabai and Bremear (1969). The reactions were stopped with $CaCl_2$ and NaOH before reading the resultant solution at 410nm.

Data Analysis

The results obtained in the study were subjected to statistical analysis to test their significance. Such tools used included ANOVA, correlation and standard deviation

Results

The results obtained in the soil physicochemical parameters are shown in Table1. All the parameters measured showed significant variations ($P < 0.05$) in either increasing or decreasing order in the various soil samples according to distance.

The soil pH which was 6.0 in control soil became 8.2 in 10m soil sample. The soil temperature changed from 28.2°C (control) to 30.2°C (10m soil). Total phosphate which was only 4.6mg/g in control increased significantly ($P<0.05$) to 6.1mg/g in 10m soil, while available phosphate changed from 0.37mg/g (10ml soil) 0.45 (50m soil) to the highest 0.64mg/g (10m soil (Table 1). Value of Total organic matter content was highest at the 100m soil (7.6mg/g) followed by control (7.2mg/g) while 10m soil had the least (4.7mg/g). A similar trend was observed in total nitrate values ranging from 1.8 – 3.2mg/g.

Results of the trace elements (Ca, Mg and K) showed highest values nearest the Quarry plant (10m soil) and lowest at the control. Of the elements, K had the lowest range of values, 1.07 – 0.31mg/g followed by Mg, 2.10 – 3.94mg/g while Ca had a range of 4.1 – 7.2mg/g. The EC and CEC also followed the same pattern with highest values nearest the Quarry site and lowest in the control (Table 1).

The total heterotrophic bacteria had the highest counts ranging from 7.5×10^6 cfu/g (100m soil), followed by 6.1×10^6 cfu/g (control) to the least 3.1×10^5 cfu/g in the 10m soil The same trends were observed in PSBC and NBC which had values from $2.2 \times 10^6 - 3.4 \times 10^4$ cfu/g and $3.8 \times 10^9 - 7 \times 10^3$ cfu/g respectively.

Phosphate solubilizing bacterial species isolated belonged to seven genera – *Bacillus, Pseudomonas, Erwinia,* and *Azotobacter.* Others were *Arthrobacter, Alcaligenes and Flavobacterium.* Two genera, *Erwinia and Flavobacterium* were absent from the 50m soil. Only three *(Bacillus, Pseudomonas* and *Alicaligenes)* were observed in the 10m soil (Table 3). Statistical analysis showed significant variations in prevalence of the organisms ($P > 0.05$). All the bacterial genera were observed in the control and 100m soil samples, though with higher prevalence in the 100m soils sample (Table 3).

Table 4 shows the values of the soil enzyme activities estimated. Dehydrogenase had the highest activities in all soil samples with a range ($16.2 – 34.84\ mg^{-1}6h^{-1}$) with the highest values in the 100m, followed by the control while the 10m soil sample had the least. Activities of acid phosphatase ranged from 2.98 to 1.27 µmol-p-nitrophenol and that of alkaline phosophatase ranged from 2.62 to 1.67 µmol-p-nitrophenol. The lowest values were in the 10m soil while the highest were in the 100m followed by control soil. The differences between alkaline and acid phasphatases were not statistically significant. Like other enzymes, urease had its highest activity in the 100m soil ($3.67mg^{-}g^{-1}\text{-}24^{h\text{-}1}$) followed by control ($3.44mg\text{-}g^{-1}24^{-h\text{-}1}$) and the least activity in 10m soil. Summation of the soil enzyme activities showed positive correlation with distance away from the quarry plant except the control i.e. the further the distance from Plant the more the enzyme activities.

Discussion

The results obtained in this study showed that stone quarrying activities affected soil physicochemical properties adversely at close ranges (high concentrations). The slight increase in temperature could be attributed to oxidation of the settled rock dust on soil and the heat released from the working machines. The increased pH observed in this study was similar to the observations of Szmidt and Ferguson (2004) and Zargari and Shaor (2008) who observed a similar effect from rock dust and cement factory waste impact on soil. Nwaugo *et al,* (2006) reported that the water from

abandoned quarry pit was alkaline as caused by the rock content.

The high total phosphorus contents observed near the Quarry site could be attributed to the rock dust content and its adsorption potential. Das *et al*, (2007) and Saha and Biwas, (2004) stated that rock dust contains some phosphate while Mortula *et al*, (2007) added that rock materials have high phosphorus adsorption potentials. Results obtained in this study tend to agree with these findings. Observations in the phosphate availability show that the high total phosphate near the Quarry site soil did not reflect high available phosphate. The ratio of available phosphate to total phosphate was highest in the 100m site sample (0.64:5.1 mg/g) and lowest in the 10m soil (0.31:6.1 mg/g). This portrays higher mineralization in the 100m soil sample compared to the other soil samples. It also shows that high total phosphate does not mean high available phosphate.

Recent studies by Chuaybamroong *et al*, (2000), Sharma and Dervez 2004) and Zerrouqi *et al*, (2008) opined that in rocks and lime, Ca and Mg contents are usually high. In this study, Ca had the highest values followed by Mg and then K. The high metallic content of the soil nearest the Quarry plant might have caused the increased EC and CEC observed in this study. This agrees with the views of Zerrouqui *et al*, (2008) and Zargari and Shoar (2008).

The microbiological analysis of the soil samples indicated that 100m soil had the highest bacterial load in all the three physiological groups examined, followed by the control while the least was in the 10m soil. This indicates that the conditions in the 100m soil were more favourable for microbial survival and proliferation than other soil samples. The proportions of the PSB and NB agree with Nwaugo *et al*, (2008). This is similar to the observation of Saha and Biwas (2009) that PSB are only 10 to 25% of the THB. This was the case in the soil samples examined except the 10m soil. Szmidt and Ferguson (2004) likened rock dust application on soil to remineralization, which caused increased microbial loads. In this study, the effect of the settled rock dust on soil caused the increase in the various bacterial groups examined only in the 100m distance. This suggests that the concentration of the rock dust on soil affects microbial loads.

Prevalence rates of the isolated PSB showed that all the bacterial genera were observed in control and 100m soil samples. The absence of *Erwinia*, and *Flavobacterium* from 50m soil shows that they were sensitive to the environmental changes that occurred in the soil at such distances from the Plant. The situation was more harsh in the 10m soil with the absence of two more *Azotobacter* and *Arthrotbactor*. This observation agrees with Ivanova *et al* (2006), Souza *et al*., (2000) and Behbahani and Behbahani (2009) that PSB differ in response to changes in environmental factors. The various prevalence rates were therefore due to the level of tolerance to the prevailing environmental conditions. The differences could be the cause of the observed low bacterial loads in the 10m and 50m soil samples earlier reported in this study.

Observations in the soil enzyme analysis buttressed the observation in bacterial loads. The enzyme activities therefore correlated positively with the bacterial loads. A similar observation has been reported by Nwaugo *et al*, (2007, 2008) and Li *et al*, (2005). All the enzymes examined dehydrogenase, urease, acid and alkaline phosphatases had their highest values in the 100m soil followed by the control while 10m soil has the least. This suggests that the highest biogeochemical transformations occur in the 100m soil and the least transformations took place nearest the Quarry plant. This is so because all biogeochemical activities are catalyzed by enzymes and these enzymes were mainly found in the 100m soil sample.

The activity of the dehydrogenase was quite reflective of the soil situation and agrees well with the bacterial load. Nwaugo *et al*, (2007 and 2008) and Li *et al*, (2005) had made similar observations. The activities of the urease tally with the organic matter content. The urease is known to metabolize organic matter releasing ammonia and Nitrate in the process. Its activities correlate positively with

organic matter content. Observations in this study indicate that both acid and alkaline phosphatases were affected adversely by the impaction of rock dusts from the Quarry plant as their activities decreased with nearness to the Quarry plant. Though acid phosphatase was more affected than alkaline phosphatase, the difference was not significant. Nwaugo *et al*, (2008) had stated that the activities of alkaline phosphatase increased with increased in pH in cattle market waste impacted soil. In this study, the number of the bacterial species producing it decreased, hence its activity also decreased unlike the case of compost application which caused increased in bacterial load generally.

In conclusion, this study suggests that application of rock dust only in small concentration could improve soil quality as high concentration could affect all biological and physicochemical parameters adversely. This could be exploited in remineralizing nutrient depleted agricultural soils in the tropics.

Table 1: The physicochemical properties of the various soil samples examined.

Parameters	10m	50m	100m	Control
pH	8.2	7.6	6.2	6.0
Temp. °C	30.2	29.4	28.3	28.2
Organic matter(%)	4.7	5.9	7.6	7.2
Total phosphate(mg/g)	6.1	5.6	5.1	4.6
Available phosphate(mg/g)	0.37	0.41	0.64	0.52
Total nitrate (mg/g)	1.8	2.3	3.2	3.0
K(mg/g)	1.07	0.83	0.46	0.31
Ca(mg/g)	7.2	6.4	4.4	4.2
Mg(mg/g)	3.94	3.07	2.30	2.10
EC	0.8	0.6	0.4	0.3
CEC	9.6	6.7	5.1	4.3

*Values are means of three replicates

Table 2: Bacteriological loads of the various physiological groups examined (cfu/g)

Microbial Group	Distance from Quarry Plant			Control
	10m	50m	100m	
THBC	$3.1x10^5$	$5.3x10^6$	$7.5x10^6$	$6.1x10^6$
PSBC	$3.4x10^4$	$4.1x10^5$	$2.2x10^6$	$1.4x10^6$
NBC	$1.2x10^3$	$2.1x10^4$	$3.8x10^4$	$3.2x10^4$

THBC = Total Heterotrophic Bacterial Count
PSB = Phosphate Solubilizing Bacterial Count
NBC = Nitrifying Bacterial Count
Values are means of three replicates.

Table 3: Prevalence of the various genera of the Phosphate solubilizing bacteria from the soil

	Bacterial Genera	Distance from Quarry Plant			Control
		10m	50m	100m	
1	*Bacillus*	50	80	100	100
2	*Pseudomonas*	50	80	100	100
3	*Erwinia*	NO	NO	50	40
4	*Arthrobacter*	NO	30	60	40
5	*Azotobacter*	NO	20	60	30
6	*Flavobacterium*	NO	NO	40	20
7	*Alcaligenes*	20	40	60	40

Figures are percentages of the prevalence rates from ten samples of each distance.
Values are of three replicates.
NO: = Not observed

Table 4: Soil enzyme activities of various soil samples.

Enzyme	Distance from Quarry Plant			Control
	10m	50m	100m	
Dehydrogenase mg-$g^{-1}6h^{-1}$	16.2	22.5	34.84	30.52
Acid phosphatase µmol-p-nitrophenol	1.27	1.43	2.98	2.84
Alkaline phosphatase µmol-p-nitrophenol	1.67	1.88	2.86	2.62
Urease mg-$g^{-1}24h^{-1}$	2.04	2.88	3.67	3.44

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