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# Effects of mining effluent contaminated soil treated with fertilizers on growth parameters, chlorophyll and proximate composition of *Cucurbita pepo* vegetable

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**Key words:** Mining effluent, *Cucurbita pepo,* contaminated.

**Abstract**

The present study evaluated the effect of mining effluent contaminated soil treated with fertilizers on growth parameters, chlorophyll content and proximate composition of *Cucurbita pepo*. Effluents of Crush rock, Crush stone and Ezza quarry sites in Ishiagu, Ebonyi State Nigeria were used for the study. Soil samples including the control were treated with urea fertilizer (sample E), NPK 20.10.10 (sample F), while sample D was without fertilizer. Results showed that plants treated with urea fertilizer showed significant increase ($p<0.05$) in all the parameters evaluated when compared with other treatments and are in the order E>F>D. Results suggest that mining effluent impacted negatively on growth, chlorophyll and proximate composition of *Cucurbita pepo* vegetable.

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## Introduction

The introduction of harmful substances into the environment has been shown to have many adverse effects on human health, agricultural productivity and natural ecosystem (Gabriella and Anton, 2005). Soil is one of the repositories for anthropogenic wastes. When agricultural soils are polluted, these metals are taken up by plants and consequently accumulate in their tissues (Trueby, 2003).The implication associated with heavy metal contamination is of great concern particularly in agricultural production system.

Plants as essential components of the ecosystem and agrosystems represent the first component of the terrestrial food chain due to their capacity of toxic metal accumulation when they grow on soils polluted with such metals (Mohammad *et al.,* 2011). Heavy metal toxicity is one of the major abiotic stresses leading to hazardous effects in plants. The sensitivity of plants to heavy metals depends on the interrelated network of physiological and molecular mechanisms such as: (i) uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents. (ii) Efflux of heavy metals from cytoplasm to extranuclear compartments including vacuoles (Mohammad *et al.,* 2011).

The common consequences of heavy metal toxicity is the excessive accumulation of Reactive Oxygen Species (ROS) and Methylglyoxal (MG), both of which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, DNA damage or interaction with other vital constituents of plant cell. Heavy metals through their actions disturb plant metabolism, affecting respiration, photosynthesis, stomata opening and plant growth (Ana-Irina *et al.,* 2008). Vegetables constitute an important part of human diet and are rich sources of vitamins, minerals, trace element, and fibre and also have antioxidant activities.

*Cucurbita pepo* belongs to the family of *Cucurbitaceae* and is an important vegetable in the South-East due to its nutritional composition. Ezeh and Chukwu (2011) reported that over the years there has been a noticeable increase in the activities of quarry mining companies in ishiagu and wastes generated are carelessly discharged into the surrounding environment which may contaminate these farms.

This is important especially in Ishiagu because the inhabitants are essentially farmers and large quantities of crops and vegetables are produced not only for local consumption but also for food supplies to other parts of Nigeria. Obiekezie *et al.*,(2006) reported that Ishiagu has remained a metal mining community for over 30 years with its attendant problems unaddressed. These mining activities generate soils and effluents with extremely high concentration of heavy metals that might have adverse effect on ecosystem and human health. The aim of the present study therefore, is to evaluate the effects of mining effluent contaminated soil treated with fertilizers on growth, total chlorophyll and proximate composition of *Cucurbita pepo* grown in the soil.

## Material and methods

*Study area*

The study was carried out with mining effluent contaminated soil samples from Ishiagu in Ivo Local Government area of Ebonyi State, South-East Nigeria. Effluents of Crush stone, Crush rock and Ezza quarry sites were used for the potted planting of *Cucurbita pepo* vegetable.

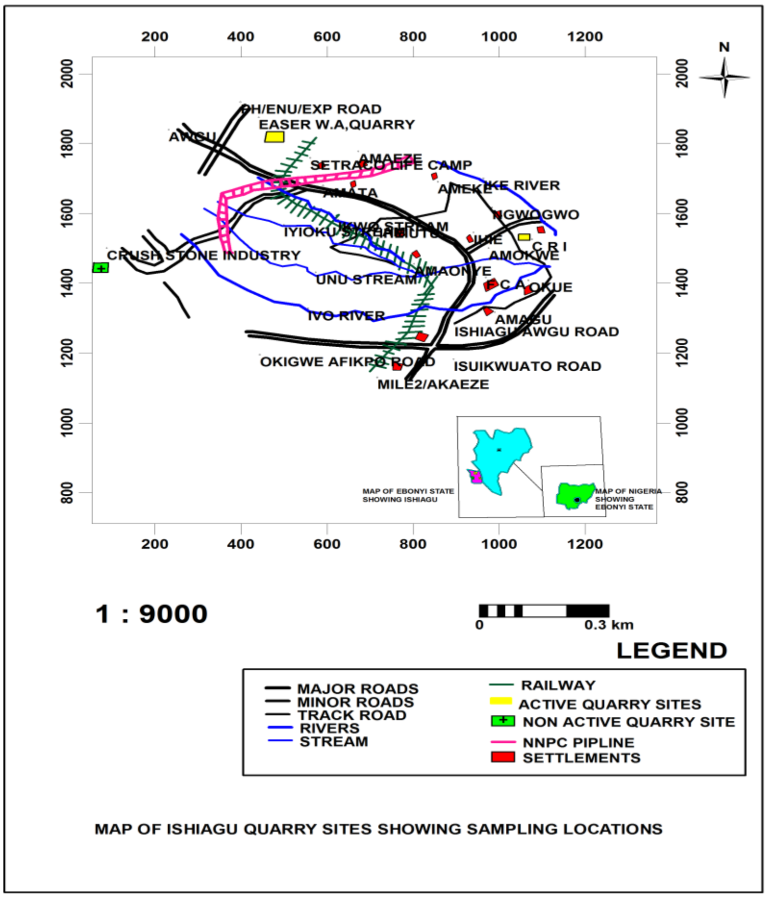


**Fig. 1.** Map of Ishiagu showing sample locations. (Source: Aroh *et al.*, 2007).

*Soil and plant sample collection*

Soil samples were collected at a depth of 0-30cm and were homogenized before collection at three different spots using auger. The soil samples from the three (3) spots in each mining sites were homogenized and divided into three (3) giving a total of twelve (12) soil samples for potted planting of Cucurbita pepo vegetable, While the control soil was collected from an unimpacted area devoid of mining activities. The plant seedlings were bought from Agricultural Development Program (ADP) Umuahia, Abia State.

*Planting of seedling and application of fertilizers*

The plant seedlings were planted in the pots containing mining effluent contaminated soil and the control soil respectively.

Urea fertilizer and N.P.K 20-10-10 used for this study were bought from Agricultural Development Program (ADP) Umuahia, Abia State. Two (2) out of the three (3) pots from each spot including the control were applied N.P.K 20-10-10 fertilizer(Sample F) and urea fertilizer(Sample E) while the other pot served as the control without any fertilizer application(Sample D). This was done, four weeks from the date of planting.

*Measurement of plant growth parameters*

Plant growth parameters measured were, Plant Height, Plant Collar Diameter, Plant Leaf Area and Plant Internodes.

*Measurement of Plant Height (cm)*

This was measured with the aid of a meter rule from the base of the stem at the root-collar to the terminal bud of the main stem.

*Measurement of plant Leaf Area ($cm^2$)*

This was measured with the aid of a meter rule by multiplying leaf length by leaf width. (Width x Length)

*Measurement of plant Collar diameter (mm)*

This was obtained with a venier caliper 15-100-100 monostat to the nearest 0.01mm

*Measurement of plant Internodes (cm)*

This was measured with the aid of a meter rule by measuring the length between plant leaves.

*Determination of plant total chlorophyll content*

This was determined by the method described by Hendrik and Yvonne (2011). Exactly 0.15g of fresh leaf was weighed into the mortar that was placed on ice. A bit of quartz sand was added to the mortar and ground until a homogenous mixture. 20ml of 80% acetone was added to the mixture. The suspension was quantitatively transferred to a centrifugation tube and centrifuged for about 5 min at maximum of 4500 rpm. The supernatant was transferred into a 25ml volumetric flask. The absorbance was read off using spectrophotometer at different wave lengths.

Determination of proximate composition of Cucurbita pepo

Moisture content, total ash, fiber content was determined by the method described by James (1995), while protein and lipid contents were determined by the method described by Chang (2003). Carbohydrate content was done by the method of Change, (2003).

Statistical analysis

Data collected were subjected to statistical analysis using one way analysis of variance (ANOVA) procedure and difference in mean were separated using Least Significant Difference (LSD) as described by Onuh and Igwemma (2000).

**Results and Discussion of plant height, internodes, leaf area and collar diameter**

**Table 1.** Growth performance of Cucurbita pepo grown in mining effluent contaminated soil treated with fertilizers.

| LOCATION | SITE | PLANT HEIGHT (cm) | PLANT INTERNODES (cm) | LEAF AREA ($cm^2$) | COLLAR DIAMETER (mm) |
|---|---|---|---|---|---|
| CONTROL | D | $4.26^{b}$ | $0.79^{cdef}$ | $24.21^{cd}$ | $5.37^{c}$ |
| | E | $4.78^{a}$ | $1.18^{a}$ | $37.17^{a}$ | $7.05^{a}$ |
| | F | $4.53^{a}$ | $1.15^{a}$ | $33.74^{ab}$ | $6.60^{ac}$ |
| EZA QUARRY | D | $2.89^{g}$ | $0.52^{f}$ | $22.48^{de}$ | $4.26^{d}$ |
| | E | $3.82^{c}$ | $0.83^{cde}$ | $30.17^{b}$ | $6.46^{abc}$ |
| | F | $3.53^{d}$ | $0.79^{cdef}$ | $29.02^{bc}$ | $5.72^{bc}$ |
| CRUSH ROCK QUARRY | D | $2.32^{h}$ | $0.62^{def}$ | $11.88^{f}$ | $4.01^{d}$ |
| | E | $3.61^{cd}$ | $1.37^{a}$ | $18.58^{e}$ | $6.19^{abc}$ |
| | F | $3.03^{fg}$ | $1.08^{abc}$ | $18.72^{e}$ | $6.15^{abc}$ |
| CRUSH STONE QUARRY | D | $2.22^{h}$ | $0.56^{ef}$ | $13.43^{f}$ | $4.17^{d}$ |
| | E | $3.39^{de}$ | $0.86^{bcd}$ | $20.11^{de}$ | $5.75^{bc}$ |
| | F | $3.22^{ef}$ | $0.71^{def}$ | $19.28^{de}$ | $5.68^{bc}$ |
| LSD | | 0.27 | 0.29 | 5.06 | 1.11 |

Value of are mean Means in the same column, having the same letter(s) are not significantly different ($P<0.05$) using Least Significant Difference (LSD)

Legend:

D = *Cucurbita pepo* grown in soil without fertilizer

E = *Cucurbita pepo* grown in soil treated with Urea fertilizer

F = *Cucurbita pepo* grown in soil treated with NPK 20.10.10 fertilizer

Table 1. Shows growth performance of *Cucurbita pepo* grown in mining effluent contaminated soil treated with fertilizers. The test samples showed significant decrease in the growth parameters when compared to control ($p<0.05$). The results are in the

order E>F>D. Results showed that mining effluent has impacted negatively on the plants. This could be attributed to hampered nutrient uptake and oxidative stress. This is in consonance with Onwuchekwa *et al.*, (2009) who reports that soil becomes acidic with considerable loss of nutrients when contaminated with mine water. Results also showed that plants treated with urea fertilizer showed significant increase when compared to those treated with NPK 20.10.10 fertilizer and plants without fertilizer. This could be attributed to high nitrogen content (46%) in urea fertilizer and its ability to release its nutrient faster than NPK 20.10.10. This agreed with Sahar *et al.*,(2012) who evaluated the improvement of corn yield by seed bio fertilization and urea application.

**Table 2.** Total chlorophyll content of *Cucurbita pepo* grown in mining effluent contaminated soil treated with fertilizers

| LOCATION | SITE | TOTAL CHLOROPHYLL (mg/ml) |
|---|---|---|
| | D | 28.746[i] |
| | E | 40.737[a] |
| CONTROL | F | 39.367[b] |
| | D | 18.358[l] |
| | E | 30.939[g] |
| EZA QUARRY | F | 28.755[h] |
| | D | 27.175[j] |
| CRUSH ROCK QUARRY | E | 35.729[c] |
| | F | 33.143[e] |
| | D | 25.878[k] |
| CRUSH STONE QUARRY | E | 34.671[d] |
| | F | 31.608[f] |
| LSD | | 0.002 |

Values are mean of triplicate determination
Means in the same column, having the same letter(s) are not significantly different (P< 0.05) using Least Significant Difference (LSD)
Legend
D = Cucurbita pepo grown in soil without fertilizer
E = Cucurbita pepo grown in soil treated with Urea fertilizer
F = Cucurbita pepo grown in soil treated with NPK 20.10.10 fertilizer

Table 2. Shows concentration of total chlorophyll content of Cucurbita pepo grown in mining effluent contaminated soil treated with fertilizers. The highest total chlorophyll content was recorded from site E (urea fertilizer), while the lowest total chlorophyll content was recorded at site D (without fertilizers). Results suggest that both the test samples and the control samples showed significant increase in site E when compared to site F and D. This could be attributed to high nitrogen content (46%) in urea fertilizer and its ability to release its nutrients faster. This agreed with Sahar *et al.*,(2012); Nayana and Malode, (2012) that the greatest and lowest leaf chlorophyll were obtained under highest and non-urea application. Nitrogen content in plants can be assessed by the measurement of chlorophyll. Similar decrease in chlorophyll under heavy metal stress was reported in Brassica Juncea L. (John *et al.*, 2009), in sun flower (Zengin and Munzuroglu, 2006) and in almond (Elloumi ., 2007). This decline in chlorophyll content in plants exposed to heavy metal stress is believed to be due to inhibition of important enzymes. α–aminolevulinic acid and protochlorophyllide reductase associated with chlorophyll biosynthesis (Elloumi ., 2007).

**Table 3**. Proximate composition of *Cucurbita pepo* grown in mining effluent contaminated soil treated with fertilizers.

| LOCATION | SITE | %MOISTURE | % CRUDE FIBER | %PROTEIN | %LIPID | (%) TOTAL CARBOHYDRATE | TOTAL ASH (%) |
|---|---|---|---|---|---|---|---|
| | D | $6.02^{c}$ | $0.40^{b}$ | $1.09^{ab}$ | $0.17^{d}$ | $91.93^{j}$ | $6.41^{c}$ |
| CONTROL | E | $6.27^{a}$ | $0.45^{a}$ | $1.19^{a}$ | $0.26^{a}$ | $91.45^{l}$ | $6.64^{a}$ |
| | F | $6.23^{b}$ | $0.44^{a}$ | $1.19^{a}$ | $0.23^{b}$ | $91.52^{k}$ | $6.61^{b}$ |
| | D | $5.67^{i}$ | $0.33^{d}$ | $0.56^{e}$ | $0.12^{f}$ | $92.74^{c}$ | $6.02^{g}$ |
| EZA QUARRY | E | $5.76^{g}$ | $0.36^{c}$ | $0.99^{cb}$ | $0.13^{ef}$ | $92.37^{h}$ | $6.13^{e}$ |
| | F | $5.78^{f}$ | $0.35^{c}$ | $0.99^{cb}$ | $0.17^{d}$ | $92.41^{g}$ | $6.12^{ef}$ |
| | D | $5.63^{j}$ | $0.25^{g}$ | $0.77^{d}$ | $0.08^{g}$ | $92.79^{b}$ | $6.11^{f}$ |
| CRUSH ROCK QUARRY | E | $5.82^{de}$ | $0.32^{d}$ | $0.89^{cd}$ | $0.21^{c}$ | $92.34^{i}$ | $6.23^{d}$ |
| | F | $5.73^{h}$ | $0.30^{e}$ | $0.89^{cd}$ | $0.17^{d}$ | $92.56^{e}$ | $5.98^{h}$ |
| | D | $5.77^{fg}$ | $0.23^{h}$ | $0.88^{ch}$ | 0.14e | $92.96^{a}$ | $5.77^{j}$ |
| CRUSH STONE QUARRY | E | $5.83^{d}$ | $0.28^{f}$ | $0.99^{cb}$ | $0.26^{a}$ | $92.59^{d}$ | 5.88i |
| | F | $5.81^{c}$ | $0.32^{d}$ | $0.99^{cb}$ | $0.18^{d}$ | $92.51^{f}$ | $5.98^{h}$ |
| LSD | | 0.015 | 0.017 | 0.182 | 0.016 | 0.005 | 0.018 |

*Values are mean of triplicate determination.*
*Mean in the same column, having the same letter (s) are not significantly different (P<0.05) using Least Significant Difference (LSD).*

*Legend*
D = Cucurbita pepo grown in soil without fertilizer
E = Cucurbita pepo grown in soil treated with Urea fertilizer
F = Cucurbita pepo grown in soil treated with NPK. 20.10.10 fertilizer

5.61 g and the flush percentage from 71.42 % to 87.16%. These informations are important because the higher the weight of the fruit and the lower the weight of the endocarp, the olive yield is higher (Mehri and Mehri Kamoun, 2007).

Proximate result showed increased nutritional value of sites treated with urea fertilizer (Site E) when compared to sites (D and F). This could be attributed to high Nitrogen content (46%) in urea fertilizer, increased uptake of nutrients from the soil and the ability of urea fertilizer to increase soil structure and nutrient availability to plants. This agrees with Yildirim ., (2007) who evaluated the effect of foliar urea application on quality, growth, mineral uptake and yield of broccoli. Results suggest that urea fertilizer would be an advisable treatment to vegetables.

*Graphical representation of Cucurbita pepo growth parameters after 8 weeks from the date of planting.*

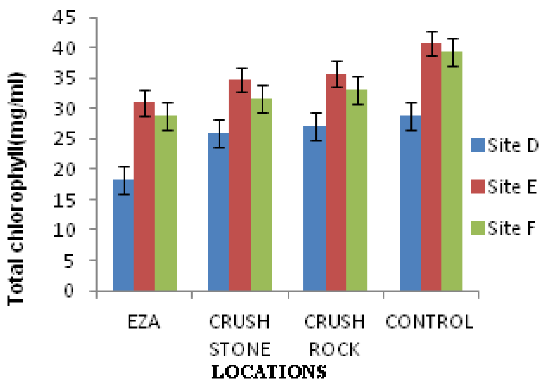


**Fig. 1.** Total chlorophyll content (mg/ml) of *Cucurbita pepo* after 8weeks.

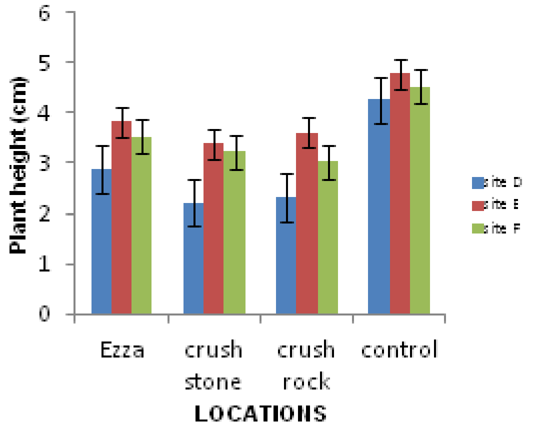


**Fig. 2.** Plant height (cm) of *Cucurbita pepo* after 8 weeks.

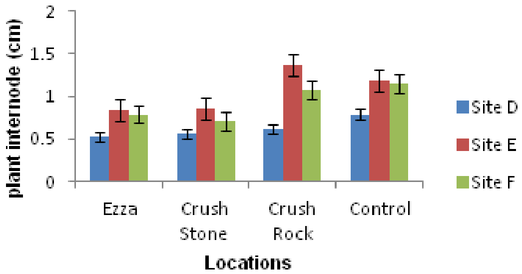


**Fig. 3.** Plant internode (cm) of *Cucurbita pepo* after 8 weeks.

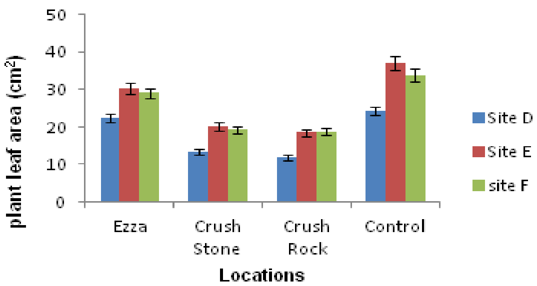


**Fig. 4.** Plant leaf area (cm²) of *Cucurbita pepo* after 8 weeks.

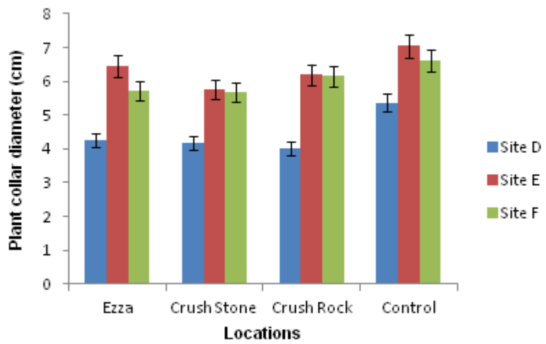


**Fig. 5.** Plant collar diameter (cm) of *Cucurbita pepo* after 8 weeks.

## Conclusion

The contamination of agricultural soil by mining effluent eventually contaminates the soil and these pollutants readily accumulate in soil which eventually is taken up by plants. This adversely affects plants growth and yield. However, the application of urea fertilizer is preferable to NPK 20:10:10 on vegetables and plants planted in and around the quarry mining areas. This however does not suggest and encourage planting of vegetable around mining areas as these vegetables are likely to accumulate the heavy metals which may pose a significant health risk.

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