PHYSICOCHEMICAL, NUTRITIONAL AND BIOCHEMICAL IMPACT OF COW DUNG ON HEAVY METAL POLLUTED QUARRY MINING SITE: A CASE STUDY OF ISHIAGU MINING SITE

Abstract

The present study evaluated physicochemical, nutritional and biochemical impact of cow dung on heavy metal polluted quarry mining site using standard analytical methods. Physicochemical results shows an increase in soil pH, temperature and other physicochemical parameters in the mining site relative to control ($P \leq 0.05$). Nutritional studies shows increase in vitamin, mineral and phytochemical composition of vegetables treated with cow dung while biochemical assay indicates a reduction in high density lipoprotein as well as liver enzyme concentrations compared to control ($P \leq 0.05$). Findings from this study reveals the nontoxic potentials of these vegetables planted in cow dung amended soils. This also indicates these vegetables when grown in cow dung amended soils have reduced phyto accumulation potentials to heavy metals. This however may be due to nutrient availability and hence we recommend that cow dung may be a remediating, non-phytotoxic agent in heavy metal polluted sites.

Keywords: biochemical impact, phytotoxic agent, heavy metals, nutrition

Introduction

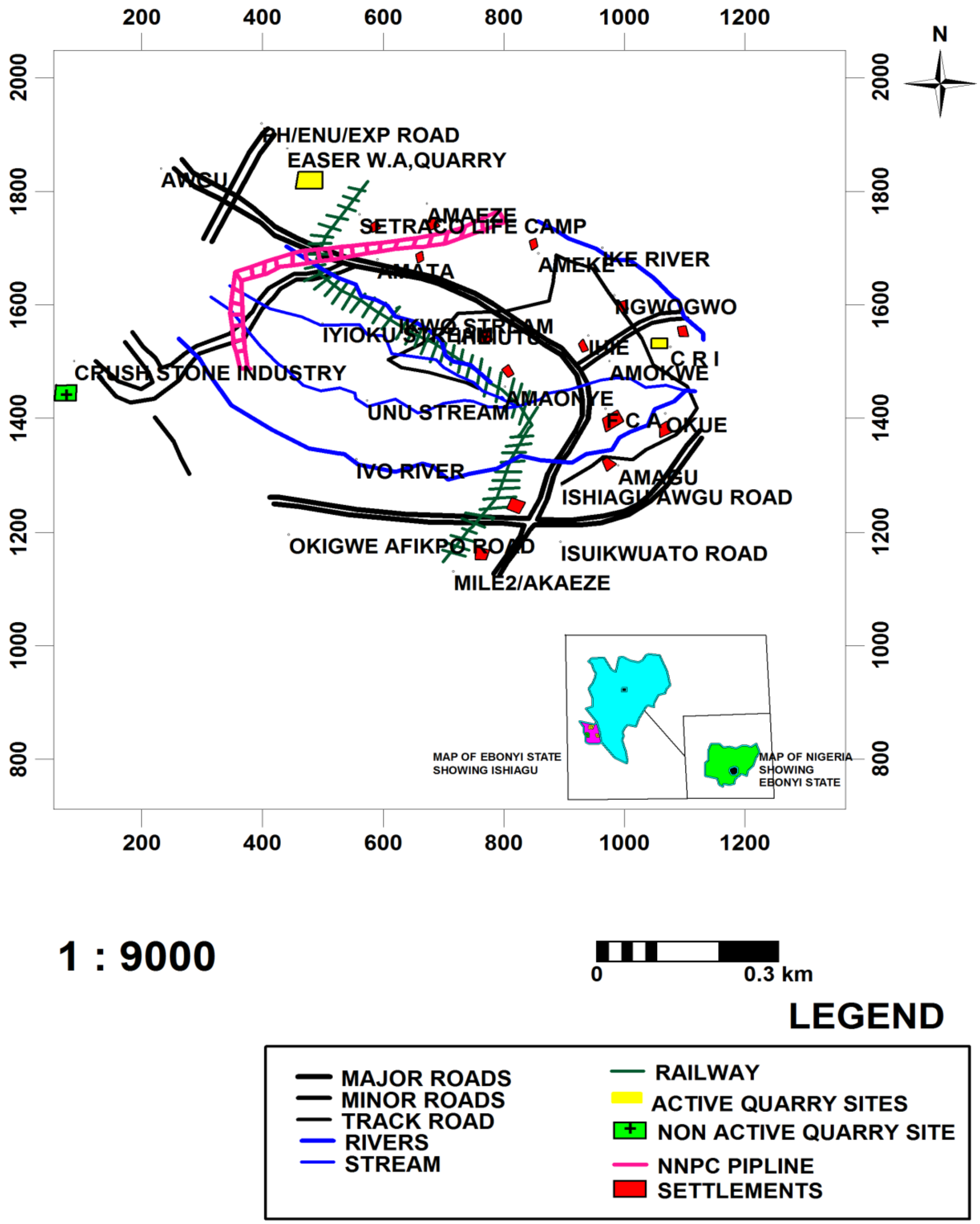
Mining activities generate a variety of wastes whose presence in soils have adverse effects on plant growth, such as low water infiltration rates, rough surfaces, poor aeration, high level of heavy metals, low fertility, salinity and extremes of pH. Ezeh (2007) and Nwaugo *et al*.,(2007a) reported that mining activities are sources of environmental pollution and degradation through the introduction of chemicals above their threshold limit into the environment as wastes generated in the mining process are often discharged into the surrounding environment especially in developing countries. Environmental impacts of such mining operations are potentially long lasting due to destruction of vegetation, surface runoff of organic matter and the overall degradation of soil structure affecting plant and microbial growth (Monty and Amiya, 2012). The release of such contaminants has been reported to pose significant potential environmental and human treat to people living around such environment (Laidlaw and Filippelli, 2008). Plants grown on land polluted with mining wastes can absorb heavy metals in form of mobile ions present in the soil solution through their root or through foliar absorption (Oluyemi *et al*.,2008). These absorbed metals get bioaccumulated in the roots, stems, fruits and leaves of plants which eventually get into the human system. Vegetables are one pathway for toxic metal mobilization into the human system which can result to DNA, kidney and liver damage, as alteration of body metabolism, skin and lung cancer caused by the mutagenic ability, neurotic effect as well as anemia from lead poisoning (Duruibe *et al.*, 2007). The determination of elemental status of cultivated land is necessary to identify yield limiting deficiencies of essential nutrients and level of polluted soils. 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 (Ezeh and Chukwu, 2011). This study therefore aimed at remediating the impact of heavy metals on soil and human health using cow dung.

Materials and Methods

Study Area

This study was carried out with quarry mining contaminated soil samples from Ishiagu in Ivo local Government Area of Ebonyi State while the cow dung was collected from Lokpa cattle market in Umuneochi L.G.A Abia State. The rural settlers in Ishiagu are mainly peasant farmers with most farm products as cassava, vegetables, yam, cocoyam and rice. Quarry sites of leads and zinc are the major industrial activities in Ishiagu. These ever increasing quarry, lead and Zinc industries dispose their wastes into nearby farm lands and these farms are cultivated by the rural settlers.

Fig I. Chrush rock Quarry mining site Ishiagu (Site of soil sample collection)



MAP OF ISHIAGU QUARRY SITES SHOWING SAMPLING LOCATIONS

Quarry mining contaminated soil collection

Soil samples were collected from three (3) different locations from the quarry mining site. The soil samples were thoroughly mixed before collection at a depth of 0-90cm. Points of collection were 100m (Sample B) 200m (Sample C) away from discharge point (Sample A) along the flow of the mining effluent using metal auger. Soil samples were transferred into labeled polythene bags for analysis while the control soil was collected far away (about 500m) from an unimpacted area devoid of mining activities.

Collection of cow dung

Cow dung was collected from Lokpa cattle market in Umuneochi Local Government Area of Abia State.

Plant Seedling Collection:

The plant seeds *Cucurbita pepo* and *Taliferia occidentalis* used for this analysis were bought from Agricultural Development Program office Umuahia, Abia State.

Planting of Plant Seedlings

The method of planting was potted planting.

Cultural Conditions.

Seeds of the vegetables were placed spaciously to avoid undue competitions among plants. In order to ensure optimum plant growth and yield, weeds were removed on weekly intervals from the pots.

Preparation of Soil Samples for Analysis.

Soil samples were sieved and stored at 4^{o}C until analyses, while the soil sample for determination of physicochemical analysis were air dried, crushed with a wooden roller, sieved with 2mm sieve and stored in a soil sac for immediate use

Preparation of vegetable Samples for Analysis.

Vegetable samples collected were washed with distilled water to remove sand and dust particles. The samples were then cut to separate the roots and shoots. The plant samples were oven dried at 60^0C before using them for analysis while the samples for phytochemical analysis were air dried and used for analysis.

Preparation of animal feed with cow dung treated vegetables

The animal feed was composed of 60% plant samples and 40% normal feed and 40% plant sample and 60% normal feed. Samples A = 60% *Cucurbita pepo* and 40% normal rat chow. Sample B = 60% *Taliferia occidentalis* and 40% normal rat chow, Sample C = 40%*Cucurbita pepo* and 60% normal rat chow while sample D =40% *Taliferia occidentalis* and 60% normal rat chow.

Determination of physicochemical properties

Soil temperature and moisture was determined by the method of (APHA, 1998) While soil pH was determined by the method of Bates (1954) using air dried soil sample. The cation exchange capacity, exchangeable acidity, soil sulphate, phosphate and nitrate were determined by ammonium saturation method described by Dewis and Freitas (1970). Soil organic carbon/organic matter of the samples were obtained according to the method outlined by Walkely and Black (1934). Soil electrical conductivity was determined using digital electrical conductivity meter according to the method described by Whitney (1998).

Determination of heavy metals in soil and vegetable samples

Heavy metals were determined by the method described in APHA (1998) using Atomic Absorption Spectrophotometer (AAS).

Determination of vitamin composition of vegetables: Thiamine (B1), Riboflavin (B2) , Nicotinamide (B3), Ascorbic Acid(c) (mg) and tocopherol were determined by the method of Association of Official Analytical Chemist (AOAC, 2005).

Determination of mineral composition of vegetables: Sodium (Na), Zinc(zn), Iron **(Fe),** Copper (CU), Calcium (Ca), Potassium (k), Magnesium (Mg) and Phosphorus (p) were determined by the method of Association of Official Analytical Chemist (AOAC, 2005).

Determination of proximate composition of vegetables

Moisture, protein , lipids, fibre, ash and carbohydrate were determined by the method of Association of Official Analytical Chemist (AOAC, 2005).

Phytochemical Screening of vegetables

The methods described by Odebiyi and Sofowora (1978) were used to test for the presence of saponins, phenolics and alkaloids; Lieberman Burchad reaction as described by Herburne (1973) and Sofowara, (1993) was used for glycosides, flavonoid and oxalate determination. The method of Adetugi and Popoola (2001) was used for the determination of tannins while quantitative phytochemical constituents of vegetables were assayed using gas chromatography Flame ionization detector (GC-FID)

Biochemical analysis

High density lipoprotein and low density lipoprotein cholesterol were determined by the method of Fossati and Prencipe, (1982) using Randox Reagent kit while serum triglyceride and total cholesterol was determined by the method described by Fossati and Prencipe, (1982). Serum total protein was determined by the method described by Henry *et al.* (1974). Albumin concentration was determined by the method of Bromocresol Green (BCG) as described by Doumas *et al.* (1971). Alkaline phosphatase (ALP) activity was determined by the method described by Bassey *et al*., (1946) using commercial diagnostic kit (Randox, United Kingdom), Serum alanin aminotransferase (ALT) and serum aspartate aminotransferase (AST) activity was determined according to the method of Reitman and Frankel (1957)

Statistical analysis

The statistical analysis of result was done using students package for social sciences (SPSS) version 20 computer software and data collected was analyzed using Analysis of Variance (ANOVA). Means were separated using One Way Analysis of Variance

Results.

Location	Site	Temperature (°C)	pH	CEC (cmol/kg)	Organic Mater (%)	Organic Carbon (%)	EC (µS/cm)	Moisture (%)
Control		26.53±0.00^{a}	7.37±0.00^{i}	39.74±0.00^{j}	80.43±0.001^{i}	46.65±0.00^{d}	26.12±0.00^{a}	20.88±0.00^{a}
	A	27.07±0.02^{e}	6.03±0.02^{b}	18.13±0.02^{b}	46.18±0.15^{a}	76..66±0.02^{a}	18.93±0.13^{j}	55.54±1.01^{i}
	B	27.04±0.01^{d}	5.04±0.00^{a}	24.29±0.10^{f}	22.12±0.40^{c}	19.93±0.33^{g}	87.68±0.13^{i}	49.65±2.11^{g}
	C	27.01±0.01^{c}	5.04±0.06^{c}	25.79±0.11^{g}	34.35±0.03^{f}	13.37±0.25^{c}	73.13±1.74^{h}	45.88±3.29^{d}
	D	28.00±0.01^{h}	5.09±0.02^{d}	15.38±0.01^{a}	20.07±0.03^{b}	11.66±0.17^{b}	66.80±0.11^{g}	59.42±1.05^{j}
LSD		0.023	0.0079	0.155	0.216	0.0512	0.9366	0.0376

Table 1: Physicochemical properties of quarry mining soil and cow dung

Values are mean of triplicate determination ±standard deviation. Means down the column having different superscript are significantly different (P<0.05). Site A= cow dung, , B= soil sample from discharged point, C= soil sample 100m away from discharged point while sample D = soil sample 200m away from discharged point. CEC= Cation Exchange Capacity of soil samples, EC=Electrical Conductivity of soil samples.

Table 2: Level of trace metals in vegetables grown in cow dung treated soils (mg/kg)

	Cucurbita pepo						*Taliferia occidentalis*					
Sample	Lead	Cadmium	Chromium	Nickel	Manganese	Zinc	Lead	Cadmium	Chromium	Nickel	Manganese	Zinc
Control	0.04 ±0.00^{a}	0.03 ±0.00^{a}	0.11 ±0.00^{a}	0.04 ±0.00^{a}	0.16 ±0.00^{a}	0.01 ±0.00^{a}	0.08 ±0.00^{a}	0.09 ±0.00^{a}	0.15 ±0.00^{a}	0.09 ±0.00^{a}	0.06 ±0.00^{a}	0.85 ±0.00^{a}
A	0.16 ±0.12^{b}	0.07 ±0.06^{b}	0.20 ±0.10^{b}	0.42 ±0.15^{d}	0.19 ±0.10^{b}	0.29 ±0.01^{b}	0.97 ±0.06^{c}	0.76 ±0.00^{a}	0.85 ±0.14^{c}	0.99 ±0.12^{d}	0.65 ±0.17^{c}	1.10 ±0.31^{c}
B	0.82 ±0.25^{d}	0.19 ±0.12^{c}	0.50 ±0.07^{d}	0.32 ±0.06^{c}	0.53 ±0.05^{c}	0.23 ±0.02^{c}	1.07 ±0.47^{d}	0.64 ±0.07^{c}	0.90 ±0.06^{b}	0.79 ±0.06^{c}	0.82 ±0.07^{b}	1.50 ±0.21^{b}
C	0.44 ±0.26^{c}	0.25 ±0.15^{d}	0.39 ±0.15^{c}	0.20 ±0.06^{b}	0.75 ±0.03^{d}	0.53 ±0.06^{d}	0.88 ±0.11^{b}	0.49 ±0.05^{b}	0.85 ±0.19^{d}	0.75 ±0.03^{b}	1.05 ±0.17^{d}	1.28 ±0.34^{d}
LSD	0.361	0.190	0.185	0.166	0.195	0.054	0.462	0.097	0.233	0.124	0.363	0.478

Values are mean of triplicate determination ±standard deviation. Means down the column having different superscript are significantly different (P<0.05). Site A= soil sample from discharged point, B= soil sample 100m away from discharged point, C= soil sample 200m away from discharged point

Table 3. Shows preliminary phytochemical constituents of *Taliferia occidentalis* and *Cucurbita pepo*

Phytochemicals	*Cucurbita pepo*	*Taliferia occidentalis*
Phenols	++	++
Glycoside	+	+
Tannins	++	++
Flavonoids	+	+
Steriod	+	+
Oxalate	_	+

Table 4 GC-Fid Carotenoid content of vegetables grown in cow dung treated soils (mg/100g)

Name of compound	*Cucurbita pepo*	*Taliferia occidentalis*
Malvidin	0.0857	0.1125
Carotene	0.0325	0.0657
Lycopene	0.09492	0.12172
Beta-crytoxanthin	0.3328	0.3596
Lutein	0.2544	0.2812
Anther-xanthin	0.0871	0.1139
Asta-xanthin	0.0125	0.0457
Viola-xanthin	0.4874	0.5142
Neo-xanthin	0.6624	0.6892
Xanthophylls	0.1584	0.1852

Table 5: GC-Fid Phenolic content of vegetables grown in cow dung treated soils (mg/100g)

Name of compound	*Cucurbita pepo*	*Taliferia occidentalis*
4-hydroxy benzaldehyde	0.0167	0.1920
4-hydroxybenzoic acid	0.0693	0.3340
4-hydroxybenzoic acid methyl ester	0.0920	0.4510
Vanillic acid	0.0237	0.5960
Gallic acid	0.1420	0.2170
Ferulic acid	0.0431	0.7900
Capsaicin	0.0400	0.3990
Rosmarinic acid	0.0927	0.5680
Tannic acid	0.044	0.0900

Table 6: GC-Fid Flavonoid content of vegetables grown in cow dung treated soils (mg/100g)

Name of compound	*Cucurbita pepo*	*Taliferia occidentalis*
Catechi	0.4611	0.6005
Resveratrol	0.2119	0.3513
Genistein	0.0072	0.1322
Daidzein	0.0348	0.1742
Apigein	0.027	0.1124
Butein	0.3698	0.5092
Naringenin	0.0002	0.1392
Biochanin	0.1182	0.0212
Luteolin	0.0178	0.1572
Kaemferol	0.2828	0.4111
(-) Epicatechin	0.0808	0.2200
(-)Epigalocatechin	0.0038	0.1490
Quercetin	0.6508	0.7902
Gallocatechin	0.1484	0.009
(-) Epicatechin -3-gallate	0.167	0.0215
(-)Epigallocatechin-3-gallate	0.1212	0.0182
Isorhamnetin	0.1258	0.2652
Robinetin	0.2868	0.4262
Ellagic acid	0.0932	0.0462
Myricetin	0.2848	0.4242
Baicalein	0.0522	0.0872
Nobicalein	0.3618	0.5012
Kaempferol -3,7,4,-trimethyl ether	0.0008	0.1402
Quercetin-3,7,4′–trimethyl ether	0.2128	0.3522
Baicalin	0.1221	0.0173
Tangeretin	0.1269	0.0125
Quercetin-3,7,3′,4′-tetramethyl ether	0.1385	0.0009
Artemetin	0.1952	0.0498
Hyperoside	0.0908	0.2302
Silymarin	0.1968	0.3362
Kaempferol-3-Arabinoside	0.1468	0.2862
Quercitrin	0.0672	0.2066
Naringin	0.1299	0.0095
Isoquercetin	0.128	0.0114
Oriebtin	0.7613	0.9007
Rutin	0.2895	0.4289
Isoorientin	0.90931	1.04871
Hesperidin	0.4611	0.6005

Table 7.GC-Fid Lignin content of *Cucurbita pepo* vegetable grown in cow dung treated soils (mg/100g)

Name of compound	*Cucurbita pepo*	*Taliferia occidentalis*
2-ally1-5ethoxy-4-methoxyphenol	0.003	0.262
(9E, 12E, 15E)-9, 12, 15-Octadecatrien-1-01	0.205	0.054
Apigenin-4,7-methyl ether	0.226	0.315
Dehydroabietic acid	0.034	0.207
Retusin	0.013	0.254
Galgravin	0.112	0.329
Epieudesmin	0.13	0.389
Sakuranin	0.221	0.48

Table 8 Vitamin composition of *Cucurbita pepo* grown in cow dung treated soils (mg/100g)

Location	Retinol	Tocopherol	Niacin	Ascorbic acid
Control	0.04 ± 0.00^{a}	0.91 ± 0.00^{b}	1.25 ± 0.00^{d}	1.31 ± 0.00^{b}
A	0.14 ± 0.10^{c}	1.04 ± 0.01^{a}	2.13 ± 0.20^{b}	2.03 ± 0.35^{c}
B	0.07 ± 0.03^{b}	1.20 ± 0.06^{c}	5.15 ± 0.15^{d}	2.73 ± 0.55^{d}
C	0.08 ± 0.01^{b}	1.24 ± 0.06^{d}	4.82 ± 0.35^{c}	1.41 ± 0.26^{a}
LSD	0.00099	0.00083	0.0407	0.0662

Values are mean of triplicate determination ± standard deviation. Means down the column having different superscript are significantly different (P<0.05). Site A= soil sample from discharged point, B= soil sample 100m away from discharged point, C= soil sample 200m away from discharged point

Table 9 Vitamin content of *Taliferia occidentalis* grown in cow dung treated soils (mg/100g)

Location	Retinol	Tocopherol	Niacin	Ascorbic acid
Control	0.006 ± 0.00^{a}	0.11 ± 0.00^{a}	1.14 ± 0.00^{a}	1.27 ± 0.00^{a}
A	0.007 ± 0.02^{b}	0.15 ± 0.02^{b}	1.77 ± 0.38^{b}	2.22 ± 0.25^{d}
B	0.029 ± 0.17^{c}	0.19 ± 0.05^{c}	1.83 ± 0.27^{c}	1.92 ± 0.17^{c}
C	0.044 ± 0.03^{d}	0.15 ± 0.00^{b}	1.84 ± 0.46^{c}	1.40 ± 0.52^{b}
LSD	0.0022	0.0050	0.0614	0.0322

Values are mean of triplicate determination ± standard deviation. Means down the column having different superscript are significantly different (P<0.05). Site A= soil sample from discharged point, B= soil sample 100m away from discharged point, C= soil sample 200m away from discharged point

Table 10. **Proximate composition of vegetables grown in cow dung treated soils (%)**

	Cucurbita pepo	*Taliferia occidentalis*
Ash	4.78	6.97
Moisture	80.72	76.38
Lipid	0.55	1.22
Fiber	2.03	3.34
Carbohydrate	11.07	11.17
Protein	0.85	0.92

Table 11. **Mineral composition of vegetables grown in cow dung treated soils (mg/100g)**

	Cucurbita pepo	*Taliferia occidentalis*
Calcium (Ca)	2.90	3.18
Sodium (Na)	2.82	1.97
Iron (Fe)	1.83	2.03
Potassium (K)	0.74	1.06

Table 12. Lipid profile of male albino rats fed with vegetables from heavy metal polluted sites treated with cow dung

Groups	**HDL**(mg/dl)	**Cholesterol**(mg/dl)	**TAG**(mg/dl)	**LDL**(mg/dl)	**VLDL**(mg/dl)
Group A	82.80±1.80^{a}	140.74±1.58^{a}	166.76±4.22^{a}	24.59±3.07^{a}	33.23±0.82^{a}
Group B	84.60±3.65^{b}	142.18±5.74^{b}	167.27±3.44^{b}	24.32±3.90^{b}	34.26±0.69^{b}
Group C	87.28±3.19^{a}	143.31±10.17^{a}	168.67±6.09^{a}	23.50±8.59^{a}	35.54±1.22^{a}
Group D	84.54±2.19^{b}	142.46±4.54^{a}	165.17±4.12^{a}	22.29±4.74^{a}	35.63±0.83^{a}

Results represent mean ± standard deviation. Different superscripts represent significant difference at 90% confidence level. Values in the same column having the same superscript are not significantly ($P<0.05$) different. Samples A = 60% *Cucurbita pepo* and 40% normal rat chow. Sample B = 60% *Taliferia occidentalis* and 40% normal rat chow, Sample C = 40%*Cucurbita pepo* and 60% normal rat chow while sample D =40% *Taliferia occidentalis* and 60% normal rat chow.

Table 13 . Renal function assessment of male albino rats fed with vegetables from heavy metal polluted sites treated with cow dung

	Creatinine (mg/dl)	**Urea (mg/dl)**

Group A	0.90 ± 0.08^{c}	34.00 ± 3.16^{f}
Group B	0.69 ± 0.08^{a}	38.00 ± 1.58^{f}
Group C	0.78 ± 0.02^{b}	36.00 ± 2.24^{f}
Group D	1.12 ± 0.10^{d}	30.00 ± 1.58^{e}

Results represent mean ± standard deviation. Different superscripts represent significant difference at 90% confidence level. Values in the same column having the same superscript are not significantly ($P<0.05$) different. Samples A = 60% *Cucurbita pepo* and 40% normal rat chow. Sample B = 60% *Taliferia occidentalis* and 40% normal rat chow, Sample C = 40%*Cucurbita pepo* and 60% normal rat chow while sample D =40% *Taliferia occidentalis* and 60% normal rat chow

Table 14. Liver enzymes of male albino rats fed with vegetables from heavy metal polluted sites treated with cow dung

Groups	**Parameters**		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Group A	33.43 ± 2.82^{a}	19.29 ± 3.04^{a}	52.90 ± 0.72^{a}
Group B	34.14 ± 6.15^{b}	18.00 ± 2.65^{b}	50.64 ± 3.92^{b}
Group C	31.57 ± 2.64^{c}	20.43 ± 1.99^{c}	51.84 ± 3.16^{c}
Group D	33.14 ± 3.02^{d}	21.00 ± 3.32^{c}	53.97 ± 1.72^{c}

Results represent mean ± standard deviation. Different superscripts represent significant difference at 90% confidence level. Values in the same column having the same superscript are not significantly ($P<0.05$) different. Samples A = 60% *Cucurbita pepo* and 40% normal rat chow. Sample B = 60% *Taliferia occidentalis* and 40% normal rat chow, Sample C = 40%*Cucurbita pepo* and 60% normal rat chow while sample D =40% *Taliferia occidentalis* and 60% normal rat chow.

Discussion

Soil pH is a master variable that affects soil physical, chemical and biological properties. Soil pH which determines the basic and acidic properties of soil influences the availability and uptake of nutrients needed by plant for growth (Lad and Samant, 2015). The pH values obtained for the various soils contaminated with quarry mining effluent discharge as shown are generally acidic. The acidity marginally declines from the epicenter and distances away along the waste plume path. This could be attributed to high level of pollutants caused by mining effluent at the discharge points. Low acidic soil pH has been shown to increase metal phyto-availability (Tshibangu *et al.*, 2014). The results suggest that indiscriminate discharge of mining effluents into these farms have contributed immensely to acidic pH as observed. Low acidic pH in mine water has also been reported by Aroh *et al.*,(2007) who evaluated physicochemical properties of pit-water from Ishiagu lead/ zinc (Pb/Zn) mine. Soil becomes acidic with considerable loss of soil nutrient when contaminated with mine water.

Soil pH recorded from this study showed high acidity compared to control ($P<0.05$). This low acidic pH obtained from the study could be traced to mine water discharge and loss of soil nutrients. Low soil acidity observed from this study may hinder the release of available essential plant nutrient and reduce crop yield.

Soil temperature plays an important role in many processes which takes place in soil such as chemical reactions and biological interactions. High soil temperature recorded from this study may affect carbon turn over and organic matter. High soil temperature has been reported to predispose soils to slower microbial metabolism and carbon mineralization which is usually favoured by lower soil temperature (Osuji and Adesiyan, 2005). Temperature raise has been reported to increase metal activity in soil solution as well as absorption rates in plants through high evaporation and transpiration from the plant. Change in soil temperature can strongly affect plant growth, biomass, nutrient uptake by plants and microbial activities.

Electrical conductivity is the measure of the number of ions (dissolved salts) present in soil solution. Results showed high electrical conductivity in quarry mining effluent discharge soils compared to control ($P<0.05$). Findings also revealed that electrical conductivity decreased distance away from discharge points. This result implicates the discharge points as having more soluble salts than distances away from source of contamination. This could be due to increased level of trace metals at the discharge points. This agrees with the findings of Akubugwo *et al.*, (2010) who reported high electrical conductivity in mine water contaminated soils. Significant high values of electrical conductivity recorded from this study may be due to excessive level of trace metals observed from the results while cation exchange capacity is defined as a measure of all the exchangeable cations the soil can absorb which plays vital role in soil fertility. Cation exchange capacity has been reported to give the soil a buffering capacity which may slowdown the leaching of nutrients. Results obtained in this study for cation exchange capacity were relatively low at the discharge points but increased distances away from discharge points. This can be attributed to low organic matter recorded from the discharge point and high level of trace metals at the discharge points as observed from this study. Soils with low cation exchange capacity have been shown to develop deficiencies of essential nutrients while high soil cation exchange capacity hinders the leaching of soil nutrients (Akubugwo *et al.*, 2010). Cation exchange capacity of the mining effluent discharge soils shows a positive relationship with increasing pH.. Soils with high pH usually have high cation exchange capacity.

The moisture content of soil is defined as directly proportional to the water holding capacity of soil. Results showed that moisture content decreased distance away from discharge points. High moisture content of the soil samples was expected considering the overall climatic predisposition of the area under study. Soil moisture content has been reported to stimulate plant productivity and microbial driven processes (Vargas *et al.*, 2012). High soil moisture content may have effect on the organic matter decomposition through indirect reduction of soil ventilation.

Soil organic matter is often considered a key quality factor in soil and is highly correlated to numerous factors influencing soil productivity and environmental sustainability. Organic carbon/organic matter content of soil are the reservoir of plants essential nutrients for growth and development. Okalebo *et al.,* (1993) noted that the level of soil organic matter influences a number of soil processes and is indicative of the soil as a rooting environment. Anikwe and Nwobodo,(2002) opined that high organic carbon/organic matter is directly related to high soil productivity. Results obtained from this study showed that soil organic carbon/ organic matter were adversely affected by high metal concentration. The results indicates high organic carbon/organic matter content in the control soils compared to quarry mining effluent discharge soils ($P<0.05$). The presence of high organic matter in soil has been reported to be beneficial for plant productivity and for structural stability of the soil (Surendra and Rana, 2010). Soil organic carbon/ organic matter content obtained from the study increased distance away from the discharge points. This could be due to lesser effect of trace metals distance away from discharge points. Monty *et al.,* (2012) also observed a gradual increase in organic matter/carbon content in a nutrient deficient soil with increasing distance. Nwaugo *et al*., (2007b) noted that soils with low pH have reduced metal solubility which directly rubs off soil organic matter. Generally, wet and cool climates exhibit reduced decomposition and promote organic matter accumulation, while warmer climates favour accelerated decomposition of organic matter (Frimpong *et al.*, 2014). Organic carbon levels in mined soils are often low due to the disruption of ecosystem functioning, depletion of soil organic pool and loss of litter layer during mining activities (Dutta and Agrawal, 2002).

Phytochemicals form part of the natural plant defense system against infection, microbial invasions and free radical generation and is one of the initial responses of plants to stress (Michalak, 2006). Dey *et al*. (2015) noted that phytochemical constituents of plants vary heavily depending on variation in season, soil constituents as well as cultivar. This explains the changes observed on many researches on phytochemical components of different plants. Findings from the present study also showed an increase in the amount of phytochemicals. This increase especially in phenolic compounds could be due to protective function of these compounds against trace metal stress by metal chelation and ROS scavenging as well as amendment of cow dung. This is in agreement with the findings of Pritesh *et al*., (2013); Abdussalam *et al.,(*2015) who reported significant increase in phenolic compounds in vegetables treated with cow dung. Many authors have reported induction of phenolic metabolism as a response of plants to stress (Hegazy *et al.*, 2013). Phenolic compounds are known to have strong antioxidant activity in plants growing under conducive environment. It has been suggested that their antioxidant act resides chiefly in their chemical structure. Theunissen *et al*.,(2010) reported that phenolic compounds are known to be synthesized in response to various environmental stress such as nutritional stress, pest and diseases and temperature. Similarly, the induction of phenolic compound biosynthesis has been reported in wheat in response to nickel toxicity (Diaz *et al.*, 2001) and in maize in response to aluminum (Winkel-Shirley, 2002). Flavoniod are important secondary plant metabolites that possess strong antioxidant activity due to their ability to scavenge reactive oxygen species and inhibit oxidative stress (Shiva and Jung-Ho, 2014). The results showed an increase in flavonoid concentration of vegetables. This could be due to increased heavy metal stress that triggers the biosynthesis of the flavonoids as flavonoids have been reported as efficient metal chelators (Brunetti *et al.,* 2013). Carotenoid content of the vegetables grown in quarry mining effluent discharge soils increased. This increase could be due to stress as carotenoid has been reported to play important role in detoxification of ROS (Hegazy *et al.*,2013). Carotenoid increase could be due to its antioxidant role in protection of chlorophyll reduction under trace metal stress. Similar findings in carotenoid increase in plants under metal stress has also been reported by Hegazy *et al.,*(2013). Accumulation of carotenoid is often regarded as a mechanism to counter stress in plants. Lignin content of the vegetables grown in the mining effluent discharge soils showed an increase in relation to control. This could also be due to increased metal stress in the sites which elicit lignin biosynthesis. Buchanan *et al.,(*2000) reported that phenolics are the precursor of lignin hence increased phenolic content due to heavy metal stress is directly related to lignin biosynthesis.

Vitamins are antioxidants that play important role in cellular defense strategy against stress (Fikriye and Omer, 2005). Findings from this study showed significant increase in vitamin composition of vegetables grown in the mining effluent treated soils compared to control ($P<0.05$). The results also indicates significant increase in the concentration of tocopherol, ascorbic acid, retinol and niacin composition of the vegetables grown in quarry mining effluent discharge soils ($P<0.05$). This may be due to increased oxidative stress leading to increased biosynthesis of the vitamins as the serve as free radical scavenger. This agrees with the findings of Fikriye and Omer (2005) who reported an increase in retinol, tocopherol and ascorbic acid content in vegetables due to exposure to heavy metals. Several authors have also reported that the content of ascorbic acid is also activated following exposure of vegetables to heavy metal stress (Ivanov *et al.,* 2012). Hegazy *et al*., (2013) reported an increase in vitamin C content of bean plant with increased accumulation of uranium and thorium. This increase in the level of vitamins analyzed in dry season could be due to its antioxidant based function in plant metabolism.

Results of proximate analysis shows significant increase in percentage composition of carbohydrate, fats and protein, ash, fiber and moisture content of vegetables grown in cow dung treated soils. This increase could be due to increased level of nutrient availability. .Results of this study revealed a decrease in protein content of vegetables compared to control ($P<0.05$). This may be due to inhibition of protein synthesis or due to unavailability of essential components of amino acids or inhibition of amino acid mobilization to the site of protein synthesis or may be due to interaction of trace metals with thiol residues of proteins and its replacement with trace metals in metalloproteins. Impaired protein synthesis has been reported in plants under heavy metal stress (Abdussalam *et al.,* 2015). Analysis of percentage carbohydrate composition of the vegetables shows decrease in carbohydrate content of vegetables ($P<0.05$). This decrease in carbohydrate content may be a metabolic signal in response to trace metal stress

Calcium is needed in the development of bone and teeth and it regulate heart rhythm, helps in normal blood clotting, maintain proper nerve and muscle functions and lower blood pressure (Fagbohun *et al.*, 2012). Iron plays a major role in haemoglobin synthesis. Sodium is associated with potassium in the body in maintaining acid-base balance and nerve transmissions (Yussuf *et al.,* 2007). The presence of sodium recorded from this study may be an indication that cow dung improved the soil fertility indices and the vegetables may be effective in treatment of hypertension. Potassium has been reported as an important mineral nutrient in the control of hypertension and in the reduction of risks of stroke (Emelike and Unegbu, 2015). This implicated the fertility improvement of cow dung on heavy metal polluted soils and t the vegetables maybe helpful in controlling hypertension and stroke.

The kidneys are involved in vital functions associated with homeostatic processes of the human body, such as filtration of blood, regulation of acid, water and electrolyte balance, regulation of hormone levels in the blood, and removal of waste products and unwanted substances from plasma. Kidney function tests are used for the assessment of kidney diseases, acid-base disorders, and water balance. Use of physical examination alone in the evaluation of kidney function is limited and imprecise; therefore, laboratory measurement of various substances in plasma and urine is required for the accurate assessment of kidney function. Determinations of importance include glomerular filtration rate (GFR), urine protein levels, disturbances in electrolytes, and serum concentrations of creatinine, urea, and uric acid (Iweala *et al*., 2013). Assay for liver enzymes namely ALT, AST and ALP is important in assessing optimal liver function . Increase in the level of liver enzymes in the serum is an indication of liver dysfunction (Iweala *et al*., 2013). Findings from this study reveals the nontoxic potentials of these vegetables planted in cow dung amended soils. This also indicates these vegetables when grown in cow dung amended soils have reduced phytoaccumulation potentials to heavy metals. This however may be due to nutrient availability and hence we recommend that cow dung may be a remediating, non phytotoxic agent in heavy metal polluted sites.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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