Original Research Article

COMPARATIVE STUDY OF MINERAL AND PHYTOCHEMICAL ANALYSIS OF SOIL AND *LACTUCA SATIVA* GROWN IN THE VICINITY OF CEMENT COMPANY OF NORTHERN NIGERIA (SOKOTO CEMENT) AND USMANU DANFODIYO UNIVERSITY SOKOTO (KWALKWALAWA)

ABSTRACT

Environmental pollution is a major issue which confronts industry and business in today's world on daily basis. Industrial activities are leading cause of metals emission, often associated with soil and plant metal concentration in adjacent regions. Cement industry is one of the 17 most polluting industries listed by the central pollution control board (CPCB). Impact of dust deposition from Cement Company of Northern Nigeria on the proximate and phytochemical concentrations of lettuce (Lactuca Sativa) was studied. A comparative study of heavy metal concentration and phytochemicals of *Lactuca sativa* and soil samples from Kalambaina (Industrial area) and Kwalkwalawa (non-Industrial area) were estimated using atomic absorption spectroscopy (AAS) and standard analytical procedures respectively. Result of quantitative phytochemical analysis revealed significant difference ($P<0.05$) in all parameters. Heavy metal values of Pb(0.012±0.002 mg/g), Zn(0.043±0.003 mg/g), and Ca(706.860±14.980 mg/g) in *Lactuca sativa* collected from Kalambaina revealed significant difference ($P<0.05$) when compare to samples collected from Kwalkwalawa and WHO standard. In addition, the heavy metal concentration in soil collected from Kalambaina showed significant difference ($P<0.05$) when compare to samples collected from Kwalkwalawa; with the highest value recorded in Ca (974.25±48 mg/g) which might be as a result of activities in the cement industry. Conclusively, plants grown at cement industries might be safe for consumption.

Keywords: Environmental pollution, Micronutrients, Phytochemicals, Atomic absorption spectrometric, Ash, Moisture

1.0 Introduction

Environmental pollution is a major issue associated with industry and business in today's world on daily basis. Different industrial activities are degrading various environmental components like water, air, soil and vegetation [1]. The environmental pollution as a result of cement industry could be defined as the adverse effect induced on water, air and land through various activities, starting from mining activity of the raw material (lime stone, dolomite etc.) up to its crushing, grinding, and other processes developing in a cement plant [2].

Cement is a fine, gray or white powder which is largely made up of Cement Kiln Dust (CKD), a by-product of the final cement product, usually stored as wastes in open-pits and landfills. Exposure to cement dust

for a short period may not cause serious problem, however prolonged exposure can cause serious irreversible damage to plants and animals. Dust which is emitted during cement processes are eventually deposited on soil, sediment, water and plants. The dust emissions from cement and other related industries therefore have to be given attention for control [3]. This is necessary in view of the pollution load and its impact on the environment. Pollutants have devastating effects on plants when taken by direct absorption through leaves or by water through the roots [4]. Heavy metals are natural constituents in nature, usually occurring in low concentration under normal conditions. Heavy metals contamination of vegetables cannot be underestimated as these food stuffs are important components of human diet [5]. Excessive concentration of both essential and heavy metals from pollutants, therefore, may result in phytotoxicity in plants [5]. The metals which when present in excessive amounts are mostly toxic to plants are Hg, Cu, Pb, Cd and Ni.

Plants are nature's gift to humans in terms of providing us with food, oxygen, as well as shelter. Since time immemorial [6], they have served as the first line of defense used by our forefathers to fight diseases such as Diarrhea, Cholera and Malaria [1]. Plants are conveniently separated into those which are edible, those which serve as a source of drugs or spices, and those that are of ornamental value etc. Although almost intensively cultured plant rightly comes under the domain of horticulture, primary effort is centered on the various traditional "garden" plants [6]. *Lactuca sativa* is an annual plant of the daisy family *Asteraceae*. It is most often grown as a leaf vegetable, but sometimes for its stem and seeds. Generally grown as hardy annual, lettuce is easily cultivated, although it requires relatively low temperatures to prevent it from flowering quickly. It can be plagued with numerous nutrient deficiencies, as well as insect and mammal pests and fungal and bacterial diseases [4-6]. L. *sativa* crosses easily within the species and with some other species within the Lactuca genus; although this trait can be a problem to home gardeners who attempt to save seeds, biologists have used it to broaden the gene pool of cultivated lettuce varieties. L. *sativa* is most often used for salads, although it is also seen in other kinds of food, such as soups, sandwiches and wraps; it can also be grilled. Assessment of heavy metal compositions of vegetable is one of the most important method used for monitoring environmental pollution [6], as the deficiency or excess of the elements is known to cause a number of serious metabolic growth, physiological and as well as toxic effect [7]. The present study was aimed at investigating the levels of some heavy metals (Pb, Zn, Ca, Cr and Cu), phytochemicals, Ash and moisture contents in edible portion of *L. sativa* grown in the vicinity of Cement Company of Northern Nigeria (Sokoto Cement) Sokoto.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

The reagents used for the study included hydrochloric acid, trioxonitrate acid, sodium hydroxide, ferric chloride, Wagners reagent, sulphuric acid, mayer's reagent, fehling's solution, sodium chloride, perchloric acid, ammonium sulphate, chloroform, methanol, and distilled water. All other chemicals used were of analytical grade and purchased from standard manufactures.

2.2 Samples Collection

Fresh samples were collected in a pre-cleaned plastic bag from Kalambaina area Sokoto down Cement Company of Northern Nigeria (Fig. 1) and Kwalwalawa area around Usmanu Danfodiyo University Sokoto. The samples were identified and authenticated at the Department of Biological science, Usmanu Danfodiyo University. The collected samples were washed separately under running tap water before cut down into smaller pieces using knife and dried at 25-27^{o}C for approximately 2 weeks. The samples were grounded into a fine powder, sieved through 2 mm sieve and stored in plastic jar labeled for analysis.

2.3 Extraction of Plant Samples

The procedure used was extraction by evaporation involving 5 g of the dried samples were put in a 250 ml cornical flask and 100 ml of distilled water was added and was covered with Aluminium foil. It was allowed to stand for 72 hours and filtered using Whatman No.1 filter paper in a separate 250 ml cornical flask. The filtrate was used for qualitative Pytochemical analysis [8].

2.4 Digestion Procedure

A 2.0 g of the samples were weighed into Kjeldahls flask mixed with 20 ml of concentrated sulphuric acid and helder tablet. The flask was heated at 70°C for about 40 min and then, the heat was increase to 120°C. The mixture turned to black after some time [9]. The digestion was completed after the solution became clear and white fumes appeared. The digest was diluted with 20 ml of distilled water and boiled for 15 min. Solution was then allowed to cool, it was transferred into 100 ml volumetric flasks and diluted to the mark with distilled water. The sample solution was then filtered through a Whatman filter paper No.1 (150 mm) into a screw capped polyethylene bottle, the procedure was repeated for all the samples.

2.5 Determination of Heavy Metals

The method applied for the assessments of heavy metal concentration of each sample of plant materials, after the digestion of the samples was by using the Atomic Absorption Spectrometric (AAS) techniques as described previously [10]. Each measurement was repeated 3 times, the mean and standard deviation were calculated (n=3).

2.6 Phytochemicals Screening

2.6.1 Alkaloids

The presence of alkaloids in each sample was investigated using the methods described by Wagner's [11]. 1 ml of each extracts were treated with 2 drops of Wagner's reagent (2 g of iodine and 3 g of potassium iodine were dissolved in 20 ml of distilled water and made up to 100 ml with distilled water). Formation of brown precipitate indicates the presence of alkaloids in the extracts.

2.6.2 Flavanoids

The determination of the presence of flavanoids in the samples was done using alkaline reagent test by Okerulu et al. [12]. 3 ml of each extract were treated with 1 ml of 10% NaOH solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids in the extracts.

2.6.3 Saponins

The presence of saponins in the test samples was done using Harbone method [13] as reported by Mercy [14]. 0.5 g of each extract were treated with 5 ml of distilled water and mixture was shaken vigorously, the production of foam which persisted in few minutes indicated the present of saponins in the extracts.

2.6.4 Tannins

The determination of the presence of tannins in the test sample was carried out using Ferric chloride test described by Harbone [12] as reported by Osagie [8]. 2 drops of 5% $FeCl_3$ was added to 1 ml of each extract. A greenish precipitate indicated the presence of tannins in the extracts.

Figure 1: Map of Cement Company of Northern Nigeria

2.6.5 Test for Cardiac Glycosides (Keller-Killiani Test)

1 ml of the filtrate was added to a test tube and then 2 ml of 3.5% $FeCl_3$ was added. The mixture was shaken for 1 minute and then 1 ml of concentrated H_2SO_4 was poured down the wall of the test tube so as to form a lower layer. A reddish brown ring at the interface indicates the presence of Cardiac Glycosides [12].

2.6.6 Test for Saponin Glycosides

2 ml of the filtrate was added to a test tube, and then 2 ml of fehling's solution was added. A bluish-green precipitate shows the presence of Saponin Glycosides [9].

Preparation of Chloroform layer for Steroids and Anthraquinones

5 ml of the filtrate was added to a test tube, then 5 ml of chloroform was added, and the mixture was shaken vigorously for 1 minute. The mixture was allowed to settle until two layers are formed. The upper layer was separated from the lower layer (Chloroform layer) and discarded. The lower layer was used to test for Steroids and Anthraquinones [14].

2.6.7 Test for Steroids (Lieber Mann Burchard Reaction)

1 ml of concentrated H_2SO_4 was added to 1 ml of the chloroform layer in a test tube and the mixture was allowed to settle. A reddish-brown ring at the interface indicates the presence of Steroids [6].

2.6.8 Test for Anthraquinones (Borntragers Test)

5 ml of 10% NH_3 was added to the remaining chloroform layer in and shaken. The mixture was allowed to settle and observation was made. A bright pink colour at the upper part of 2 layers formed indicates the presence of free Anthraquinones [12].

2.6.9 Test for Volatile Oils

2 ml of the filtrate was added to a test tube and 2 ml of 10% HCl was added. A white precipitation indicates the presence of Volatile oils [13].

2.6.10 Test for Glycosides

1 ml of the filtrate was added to a test tube and 0.5mls of 50% H_2SO_4 for 15 minutes. After boiling, the mixture was allowed to cool and then it was neutralized with 1 ml of 10% NaOH. 2 ml of fehlings' solution was added to the mixture and observation was made. A brick-red precipitate indicates the presence of glycosides [9].

2.7 Quantitative Pytochemical Analysis

2.7.1 Test for Tannins

Powdered sample (0.1g) was put in a 100cm^3 cornical flask and 52cm^3 volumetric flask. The residue was washed several times and the combined solution made up with distilled water to 0, 1, 2, 3, and 4 cm^3 of the standard tannic acid and 10cm^3 of the sample solution in a 50cm^3 volumetric flask, 2.5 cm^3 Folin-Denis reagent and 10 cm^3 of Na_2CO_3 solution were added and made to volume with distilled water. The flask was allowed to stand for 20 minutes after which optical density was measured at 760 nm. The calibration curve was plotted from which the concentration of tannic acid in the sample was extrapolated [8].

2.7.2 Determination of Alkaloid

5 g of powdered plant sample was extracted with 100ml of methanoic: water (1:1; V: V) mixture and solvent evaporated. The resultant residue was mixed with 20ml of 0.0025M H_2SO_4 and partitioned with ether to remove unwanted materials. The aqueous fraction was basified with strong NH_3 solution and then extracted with excess chloroform to obtain the alkaloids fraction or separated by filtration. The chloroform extraction was repeated several times and the extract was concentrated to dryness. The alkaloid was weighed and the percentage was calculated with reference to initial weight of powder [11].

$$\%\text{Alkaloid} = \frac{Weight\ of\ alkaloid\ residue}{weight\ of\ sample}\ X\ 100$$

2.7.3 Determination of Flavonoids

5 g of powdered sample was hydrolysed by boiling in 100ml of hydrochloric acid solution for about 35 minutes. The hydrolysate was filtered to recover the extract (filtrate). The filtrate was treated with ethylacetate drop wise until in excess. The precipitated flavonoids were recovered by filtration using a weighed filter paper after drying in oven at 100^{0}C for 30 minutes; it was cooled in a dessicator and reweighed. The difference in weighed gave the weighed of flavonoids which was expressed as the percentage of the weight of sample analysed.

$$\%\text{Flavonoid} = \frac{W2-W1}{5g}\ X\ 100$$

Where 5g = weight of sample

W_1 = weight of empty filter paper

W_2 = weight of filter paper + sample precipitate

2.7.4 Determination of Saponins

5 g of powdered sample was placed in a 250ml cornical flask containing 20ml of 50% alcohol. The mixture was boiled under reflux for 30minutes. The filtrate was allowed to cool at room temperature thereby resulting in the precipitation of saponins. The separated saponins were collected by decantation

and suspended in about 2ml of alcohol and filtered. The filter paper was immediately transferred to a dessicator containing anhydrous calcium chloride and the saponins were left to dry. They were weighed with reference of extract used.

%Saponins = $\frac{W2-W1}{5g} X$ 100

Where

5g = weight of sample

W_1 = weight of filter paper

W_2 = weight of filter paper + sample precipitate.

2.8 Moisture Content Determination

Clean and dried petri-dishes were placed in an oven for about 30 minutes, cooled in a dessicator and each was weighed empty. 2kg each of the dried samples were placed in a separate petri-dish and weighed. The petri-dishes and the content were then placed in an oven at 105^{0}C for 24hours. The percentage moisture of a given sample was calculated using the formula in equation

$$\%Moisture = \frac{W2 - W3}{W2 - W1} X\ 100$$

Where W_1 = weight of empty petri-dish

W_2 = weight of petri-dish plus dried sample

W_3 = weight of petri-dish plus sample after drying [18].

2.9 Ash Content Determination

The term ash refers to the residue left after combustion of the oven dried sample and it is a measure of the total mineral content. The ash content of the samples was determined by heating porcelain crucibles in a muffle furnace to about 500^{0}C, cooled in a dessicator and weighed. The 2g each of the air-dried samples were transferred into separate crucibles and weighed. The crucibles containing the samples were placed in a muffle furnace and the temperature raise to 600^{0}C for 3 hours. The crucibles containing the ashes were allowed to cooled and weighed [10].

The percentage ash of a given sample was calculated using the formula in equation

$$\%Ash = \frac{W3 - W1}{W2 - W1} X\ 100$$

Where W_1 = weight of empty crucible

W_2 = weight of crucible plus dried sample

W_3 = weight of crucible plus sample after drying

2.10 Determination of the pH of the Soil Samples

The process was carried out by weighing 20g of each air-dried soil sample into separate 50cm^3 beakers. This was followed by addition of 20cm^3 of distilled water, which was allowed to stand for 30 minutes, stirring occasionally with a glass rod. The electrode of a pH meter already calibrated with pH 7.0 and pH 4.0 buffer solutions was dipped into each beaker containing the partly settled suspensions and readings were taken. The electrode was rinsed with distilled water and wiped dry with a clean filter paper each after reading [15].

2.11 Statistical Analysis

The collected data were subjected to statistical tests of significance using one way Anova followed by dunnett comparison test ($P<0.05$) to assess results in the lettuce samples. Probabilities less than 0.05 ($P<0.05$) were considered significant. Statistical analyses were done using IBM SPSS (v20) statistical software. Results are Presented in Mean ±Standard Deviation (n=3).

3.0 RESULTS AND DISCUSSION

Table 1: Moisture and Ash contents (%) in dry weight of *Lactuca sativa* samples.

Sample	Moisture (%)	Ash (%)
A	45.33±1.33^a	35.5±2.18^a
B	13.30±1.31^b	19.9±1.02^b

Sample A = *Lactuca sativa* obtained from Kwalkwalwa

Sample B = *Lactuca sativa* obtained from Kalambaina industrial estate (Sokoto cement)

Superscipt a&b in sample A and B respectively show significant difference ($P<0.05$)

Table 2: Qualitative phytochemical analysis of *Lactuca sativa* samples.

Phytochemical	A	B
Flavonoids	+	+
Saponins	+	+
Alkaloids	+	+
Anthraquinones	ND	ND
Tannins	+	+
Steroids	+	+
Volatile oils	ND	ND
Cardiac Glycosides	+	+
Saponin Glycosides	+	+

+ = Present

ND = not detected

A = *Lactuca sativa* obtained from Kwalkwalwa

B = *Lactuca sativa* obtained from Kalambaina industrial estate (Sokoto cement)

Table 3: Quantitative phytochemicals estimation of the *Lactuca sativa* samples.

Sample	Flavonoids	Saponins	Alkaloids	Tannins	Steroids	Saponin glycosides	Cardiac glycoside
A (%)	6.91±0.15[a]	5.04±0.71[a]	3.47±0.96[a]	2.29±0.15[a]	1.92±0.45[a]	4.10±0.32[a]	2.09±0.62[a]
B (%)	4.76±0.32[b]	2.65±0.51[b]	5.00±0.27[b]	0.30±0.01[b]	0.45±0.36[b]	1.71±0.16[b]	2.09±0.58[b]

Sample A = *Lactuca sativa* obtained from Kwalkwalwa

Sample B = *Lactuca sativa* obtained from Kalambaina industrial estate (Sokoto cement)

Superscipt a&b in sample A and B respectively show significant difference ($P<0.05$)

Table 4: Micronutrient Metals of *Lactuca sativa* collected from Kwalkwalawa and Kalambaina.

Metals	Sample A (mg/g)	Sample B (mg/g)	WHO/FAO STD (mg/day)
Pb	ND	0.012±0.002[bc]	0.002
Zn	0.008±0.002[a]	0.043±0.003[bc]	2.2
Ca	278.58±8.230[a]	706.860±14.980[bc]	400 – 500
Cr	0.001±0.001	0.002±0.001	0.002 - 0.005
Cu	0.005±0.001[a]	0.008±0.002[b]	0.03

Sample A = *Lactuca sativa* obtained from Kwalkwalwa

Sample B = *Lactuca sativa* obtained from Kalambaina industrial estate (Sokoto cement)

WHO/FAO values in mg/day are based on a 60kg body weight of adult

a = Comparison between A and WHO/FAO

b = Comparison between B and WHO/FAO

c = Comparison between Sample A and B

ND = Not Detected

Superscipts a, b&c in sample A, B and WHO/FAO STD respectively show significant difference ($P<0.05$)

Table 5: Micronutrient Metal of Soils collected from Kwalkwalawa and Kalambaina.

Sample	Pb (mg/g)	Zn (mg/g)	Ca (mg/g)	Cr (mg/g)	Cu (mg/g)
A	0.002±0.001[a]	0.029±0.045[a]	353.86±27[a]	0.008±0.003[a]	0.010±0.001[a]
B	0.031±0.002[b]	0.069±0.083[b]	974.25±48[b]	0.038±0.002[b]	0.027±0.003[b]

A = Soil obtained from Kwalkwalwa

B = Soil obtained from Kalambaina industrial estate (Sokoto cement)

Superscript a&b in A and B show statistically significant (P<0.05)

Absorption of metals by plants in industrial areas lead to phytotoxicity, the present study revealed significant difference (P<0.05) in the metal concentration in plant found in industrial and non-industrial areas. The result revealed a significance difference (P<0.05) in moisture and ash contents of sample A (*Lactuca sativa* obtained from Kwalkwalawa) and sample B (*Lactuca sativa* obtained from Sokoto cement) (Table 1). The moisture content is very low when compared with the control sample. There is an increase in ash content in sample B when compared with sample A. The values are within the range of some common Nigerian vegetables [16]. Also, the ash content is very high (19.9%) when compared with that of sample A. The values are within the range of some Nigeria vegetables [17-19].

Pytochemicals are relevant in medicine, food, and dye industry. Some of them have pharmacological effects for example, flavones, and tannins form important ingredients of several laxatives and medicine and in dyes. Qualitative estimation of phytochemicals of A and B revealed the presence of flavonoids, saponins, alkaloids, tannins, steroids, cardiac glycosides, and saponins glycosides (Table 2). This agrees with report by Miroslav and Vladimir [20]. Similarly, quantitative phytochemical analysis revealed presence of flavonoids, saponins, alkaloids, tannins, steroids, cardiac glycosides and saponin glycosides, in addition significant difference (P<0.05) was observed between the two groups (Table 3).

Comparative studies of *Lactuca sativa* collected from sample A (*Lactuca sativa* obtained from Kwalkwalawa) and B (*Lactuca sativa* obtained from Sokoto cement) showed significant difference (P<0.05) was seen between the two samples in the comparison of Heavy metal concentrations except Cr (Table 4). Pb has no biological role, it is a poisonous affecting almost every organ and system in the human body [21] and interferes with some metals such as Cd, Zn, and Fe. Prolonged exposure of Pb can lead to kidney damage. The maximum acceptable concentration (MAC) of Pb in food by WHO is 0.002mg/day when compared to Pb concentration in sample B (0.012±0.002 mg/g) whereas no detection in sample A. Similar reports were observed by Amusan *et al.* [22], Miller-Ihli and Baker [23] and contrary

result was obtained by Anthony and Balwant [24]. Cu is an essential element in trace amount because it plays a major role in the enzyme function as a co-enzyme, but in large amount is toxic. Cu concentrations in sample A (0.005±0.001 mg/g) and sample B (0.008±0.002 mg/g) are negligible when compared to 0.03 mg/day MAC of Cu. Cr in trivalent state (Cr^{3+}) is an essential trace element that potentiates insulin action and influences carbohydrate, lipid and protein metabolism. However, in hexavalent state (Cr^{6+}), it has toxic, mutagenic, carcinogenic effects [25]. The concentration of Cr in sample A (0.001±0.001 mg/g) and B (0.002±0.001 mg/g) are with range of MAC intake for Cr (0.002-0.005 mg/day).

Zn participates in the synthesis and degradation of carbohydrates, lipids, protein, and nucleic acids and has shown to play an essential role in polynucleotide transcription and translation and thus in the process of genetic expression. The concentrations of Zn of both samples are within recommended MAC. Ca in sample A was found to be 278.58±8.230 mg/g and 706.860±14.980 mg/g in sample B. The latter is above MAC 400-500 mg/g for Ca. Some of the studied results showed values below than other reports [26] [2].

The mineral analysis of soil collected from kwalkwalawa (sample A) and Pb concentration is 0.002±0.001 mg/g in sample A (soil from kulkwalawa) and 0.031±0.002 mg/g in sample B (soil from kalambaina) (Table 5). Similar result was reported [22] [29]. The Cr concentration in sample A cultivated soil is 0.009 and 0.038 mg/g in sample B cultivated soil respectively. Low values were reported by Uwah *et al.* [29]. The comparison of both soils showed significant difference in Zn and Cu. reported by Uwah *et al.* [30] but the value is low when compared with the values obtained during autumn in Torun Poland by Buszewski *et al.* [29] and also, the value of 0.102mg/g by Amusan *et al.* [22] [30][32].

4. CONCLUSION

Our results revealed that the alkaline dust emitted from the cement plant causes uptake of metals by the plant and decreased phytochemical concentrations. The level of Ca was very high in the plant and exceed limit required by WHO/FAO. Heavy metal levels especially Pb which is toxic exceeds limits required by WHO in plants and in soil. This showed that industrial activities increased the concentration of metals in the environment which can increase the possibilities of environmental pollution. However, the pollution is not always attributed to industrial depositions, the excessive use of fertilizers, manures, pesticides, herbicides, and other agro-chemicals as well as the use of waste water by irrigating the soil can cause increase in the metal contents. Therefore consumption of this plant as food may pose health hazards to human at the time of study.

5. RECOMMENDATIONS

Further studies should be carried out to determine the concentration of heavy metals in soil and water of the study area. The study should also be extended to different areas of Kalambaina. Further studies should also be carried out to assess heavy metals in bloods of people living around Kalambaina area.

There is need for regular monitoring of heavy metals in plant tissues (is essential) in order to prevent excessive build-up of these metals in the human food chain. Control SPM and other emissions should be given top priority to maintain the ecosystem around the unit in its natural or near to natural form. Proper maintenance on the various process equipment and machine efficiency ensure reduction in the generation of dust and gases during various operations. This would reduce adverse impact on vegetation and human life. Cement industries faces a lot of problems due to mining activity. To overcome this problem, they should start back-filling of abandoned mine as soon as they complete the mining of a particular area.

CONFLICT OF INTEREST

No conflict of interest declared.

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