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Phytochemical and Heavy Metal Analysis of Gongronema Latifolium, Talinum Triangulare and Amaranthus Hybridus

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Abstract

Heavy metals contamination of soil has continued to increase globally as a result of increase in anthropogenic actions. The phytochemical and heavy metals (Zn, Cu, As, Pb, Cd, Hg) content of three edible plant species grown in southern Nigeria and the health implications were evaluated. The heavy metal concentrations of *Gongronema latifolium, Talinum triangulare and Amaranthus hybridus* as well as the top soil were determined using Atomic Absorption Spectrophotometer. The consumption of vegetables is a very vital path to food chain by which toxic metals are transferred from the soil to human as well as other animals. Geo-accumulation index and Transfer factor were the parameters used to evaluate the extent of contamination of top soil and exposure by human via the food chain respectively. The results of phytochemical analysis of plants revealed the existence of some bioactive constituents and their corresponding concentrations are presented in increasing order of magnitude: anthocyanin< carotenoid < flavonoid < tannin < steroid < alkaloid. The soil analysis for heavy metals were performed and results demonstrated that zinc had the maximum concentration (103.1 – 174.0 mg kg-1) while mercury had the lowest levels (0.01 – 0.20 mg kg-1). The heavy metal concentrations in the soil samples are in the order; Zn > Pb > Cu > Cd > Ni > As > Hg. Geo-accumulation index analysis revealed that Pb and Cd were implicated in overall contamination of the soil samples but the control soil remained uncontaminated. The concentrations of heavy metal in the plant samples varied greatly with Zn having the maximum values (10.80 – 21.10 mg kg -1) whereas arsenic had the minimum concentration (0.01 – 0.03 mg kg -1). The heavy metal concentration in the plant samples are in the order; Zn > Cu > Pb > Cd > Ni > Hg > As. The concentrations of heavy metals in the selected plant samples evaluated were within the recommended standard limits apart for lead which was higher than the recommended value. Results revealed moderately high transfer factors and capacity of the vegetables investigated to accumulate copper, mercury and arsenic.

Keywords: Edible plants; *Gongronema latifolium; Talinum triangulare; Amaranthus hybridus*; Geo accumulation index.

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1. Introduction

The earth crust is a major reservoir of vast and valuable natural resources that is capable of sustaining agricultural activities. Soil is a product weathering of rock and lies above the solid geology but a medium for plant growth and other agricultural activities. The soil is contaminated due to civilization and activities of mankind. Combustion of coal, petroleum oil spillage, gas flaring, vehicular exhaust emission, solid and industrial effluents, municipal waste, practice of fertilizer applications and mining have been identified as sources of heavy metal contamination of the soil as well as the total environment [1-3]. For instance according to CDC and World health organization investigation on lead contamination in Zafara State, northern Nigeria, over 400 children died of lead contamination in May 2010. This was as a result of indiscriminate mining of gold which contained extremely high levels of lead by the natives. Leaf vegetables, otherwise called leafy greens, are plant leaves, tender petioles and shoots eaten as part of dietary requirement or food to mankind. More than a thousand species of plant with edible leaves have been cultivated on the soil.

Vegetables are very good source of B- vitamins, vitamins K, C, E, and A. Vegetables contain very low fat and carbohydrates but rich in fibers, and proteins. Edible vegetables are also rich in plant derived compounds, known as phytochemicals, which serves to protect the leafy vegetables from environmental stresses and changes in weather. Phytochemicals generally provides protection to many chronic ailments like diabetes, alzheimer's, cancer, and cardiac diseases in human. Flavonoids are a group of plant metabolite believed to have anti-oxidant properties, inhibit important viral enzymes and destroy pathogenic protozoans in human. Alkaloids are naturally occurring chemical compounds that contain nitrogen atom. Alkaloids exhibit some biological activities such as anti-malaria, antiasthma, anticancer, vasodilatory, analgestic, antibacterial and anti-hyperglycemic activities. Tannins exert physiological effects like to enhanced blood clotting, decrease blood pressure, reduces the serum lipid level [4]. Anthrocynides are generally associated with colours such as red or blue in plants and exhibit a range of activities including acting as antioxidants and fighting free radicals, anti-inflammatory, antiviral, anti-carcinogenic, neuroprotective and fostering eye health. Plant steroids include phytosterols and brassinosteroids amongst others. Steroids are signaling molecules which activate steroid hormone receptors in human and other animals. The biological benefits of carotenoids include anti-inflammatory, anticancer, maintaining healthy eye and good vision, enhancing immunity and protecting the skin from damage [5].

Vegetables cultivated on polluted soil are more likely to be contaminated with heavy metals [6]. Heavy metals at high levels constitutes very strong toxic effect are regarded as environmental pollutants [7-10]. Lead is a very toxic metal and contacts by human at high levels are linked to neurological disorders, digestive tract disorders, kidney disorder and sometimes death. Anaemia, liver dysfunction and kidney disorder have been traced to prolong exposure of high levels of copper. Exposure to mercury could be associated with pulmonary disorders, renal dysfunction and neurological disorder. Cadmium is a strong carcinogens, teragens, and mutagens, is associated with liver and kidney dysfunction. Health hazards associated with long term exposure to arsenic includes skin, lungs and bladder cancer. Others are neurotoxicity, diabetes and cardiovascular disease or circulatory system disorder [11].

Copper is an indispensable micronutrient needed for healthy growth of both plants and animals. In plants, copper is useful in water regulation, disease resistance and healthy seed production.

Zinc is an important trace and least toxic metal with numerous agricultural, biological as well as industrial applications and is mainly use for the corrosion protection of steel. Extreme concentration of Zinc in soil is associated with phyto-toxicity there by affecting the activity of not only weeds, but also earthworms and microorganisms [11].

Plants play very vital role in metal removal from the soil, a phenomenon which is appropriately described as phytoremediation. Metals are selectively filtered out from the soil and adsorbed through the plant roots then followed by sequential cation exchange [12]. Bioaccumulation of metals by plants depends on a series of factors such as variations in species and the growth stage of plants [13]. Other factors include translocation of metals, metal species and physiological adaptations of plants [12-17].

This research work was designed to assess the concentration of heavy metals in both soil and edible plants as well as uptake of metals by selected edible vegetables (*Gongronema latifolium, Talinium triangulare and Amaranthus hybridus*) in Aba metropolis.

2. Materials and Methods

2.1. Sample Collection and Preparation

This study was performed in Aba, the commercial nerve centre of Abia State, Nigeria. Soil samples were collected at depth of 0 - 30cm using soil augar, from farms in the neighborhood of Enyimba, Osisioma, World Bank housing estate OgborHill and Ohanku in Abia State. *Gongronema latifolium, Talinum triangulare* and *Amaranthus hybridus* samples were collected at the early hours of morning (7.00am Nigerian time) from the same farms, and analysis of heavy metal and some phytochemicals were conducted. Soil samples were labeled EY, OS, WB, OG and Control to represent Enyimba, Osisioma, World Bank housing estate, OgborHill and Ohanku correspondingly. The soil samples were dried in the air, ground to powder by the use of a pestle and mortar and then sieved to a particle size of 2 mm with the aid of a sieve. Pre-washed plant sample was rinsed with distilled water so as to remove any of possible contaminant on the surfaces of the leaves and dried in an oven at 75°C [18]. A known weighed of the dried leaves were thoroughly ground into finely divided particles prior to analysis for heavy metals.

2.2. Heavy Metal Analysis of Edible Plant Samples

Sample of the leafy vegetables was digested and analysed according to standard methods [19, 20]. Tri-acid digestion of dried plant sample was performed using HNO_3: H_2SO_4: $HClO_4$ (10:1:4 v/v). The resultant filtrate obtained was made up to 50 mL with distilled water followed by the analysis of heavy metals using atomic absorption spectrophotometer (FS240) with detection limit of 0.001 ppm [21].

2.3. Heavy Metal Analysis of the Soil Samples

2g of soil samples was taken in a 250 mL beaker and then digested with about 8.5 mL of aqua -regia on a sand bath for approximately 2 hours [21]. The supernatant solution obtained remained dried by evaporation followed by dissolution in 10 mL of 2% nitric acid [22]. Resultant solution was filtered and then made up to 50 mL with distilled water, and the total concentrations of the heavy metals were measured using atomic absorption spectrophotometer [19, 23].

2.4. Phytonutrients Analysis of Edible Plants

2.4.1. Analysis of Flavonoid

10 g of the sample were taken in to a round bottom flask, boiled and refluxed in 50 mL of 2 M HCl solution for 30 minutes. The mixture was left to cool and filtered, and then ethyl acetate solution was gradually added to the filtrate until precipitation was concluded. The precipitate was filtered and measured accordingly [21-24].

2.4.2. Analysis of Alkaloids

5g of plant sample and 200 mL of 20% ethanoic acid in ethanol solution (1:10) were taken into a 250 mL and left to stand for 240 minutes and then filtered. The filtrate was evaporated to $1/4^{th}$ of its original volume with the aid of a thermostatic water bath. Complete precipitation was then achieved by gradual addition of concentrated NH_4OH solution to the extract. The precipitate was filtered, dried at 78°C and measured using weighing balance [24-26].

2.4.3. Analysis of Tannin

500 mg of the plant sample was taken into a 100 mL wide open bottle which contains 50 ml of distilled water. The bottle and its content were shaken for 65 minutes with aid of a mechanical shaker followed by filtration. Distilled water was added to the filtrate in a standard volumetric flask up to the 50 mL mark. 5 mL of the filtrate was transferred into a cuvette using a pipette and 3 mL of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide were added. The absorbance at 120 nm wavelength was taken using a spectrophotometer. The absorbance of blank sample was measured under the same experimental condition [21, 27].

2.4.4. Analysis of Carotenoid

Laboratory blender was used to mix 10g of each plant sample in methanol. The resultant mixture was filtered and 25 mL of ether were added to the filtrate and the supernatant solution thoroughly shaken prior to addition of 25 mL of distilled water in a separating funnel. The ether layer was extracted and evaporated to dryness at temperature (42 to 48 ^{o}C) in a vacuum dessicator. The dried extract was saponified with 20 mL of ethanoic potassium hydroxide and left over night in a dark cupboard. The carotenoid content was dissolved in 25 mL ether and then washed twice each with 20 mL distilled water, the next day. The ether layer (carotenoid extract) was dried in a dessicator and then treated with light petroleum ether and left overnight in a freezer (-10 ^{0}C). Through centrifugation, precipitate of steroid was separated and the carotenoid extract was eventually dried in a previously washed and weighed evaporation dish, cooled in a dessicator and weighed [27, 28].

2.4.5. Analysis of Anthocyanin

5 g each of sample was boiled in 100 mL of 2M HCl solution for 30 minutes and then the hydrolysate was filtered using filter paper. Ethyl acetate solution and the filtrate were thoroughly mixed in a separation funnel and on standing, two distinct layers were separated. The extract was transferred into a crucible and heated to dryness in a water bath. Concentrated solution amyl- alcohol was added to the dried extract to release the anthocyanins and then filtered. The filtrate as well as alcohol extract were together dried in an evaporating dish. The residue was further dried in the oven for 35 min at 30°C [24-27]. The % anthocyanin content was calculated.

2.4.6. Analysis of Steroid Content

15 g each of the plant samples was dissolve in 150 mL deionized water, vigorously stirred and homogenized using a laboratory blender. The mixture was filtered using a filter paper. Ammonium hydroxide solution was used to elute the filtrate at pH 9.3 mL trichloromethane was used to dissolve 3 mL of eluate in a clean test tube and then transferred into a round bottom flask. 5 mL of acetic anhydride as well as 3 drops of concentrated hydrogen-tetraoxosulphate (VI) acid were poured in to a mixture of trichloromethane and eluate in a round bottom flask. Standard solution of sterol was prepared under the same experimental method and conditions as the sample solution. The absorbance of both the standard solution and prepared sample was measured at 420 nm using a spectrophotometer [21, 28].

2.5. Quality Control

Triplicate analyses were done on each sample and the mean was taken. The spectrophotometer was operated under optimal conditions: measurement mode (integrated), flame type (air/acetylene), air flow (13.0 $Lmin^{-1}$), acetylene flow (2.0 $Lmin^{-1}$) [21]. Slit width (0.5 nm), lamp current (4.0 mA), gain (79%). Certified Reference Materials were used to test the quality of analytical method, the same amounts of samples were used and under the same experimental conditions [19]. The mean recovery percentages of the reference material were presented in milligrams of metal per kilogram dry weight of sample thus; nickel (103%), zinc (97 %), lead (112%), copper (102%), cadmium (117%) and arsenic (99%) [20, 21]. A control soil was chosen at the outskirt of the town (Ohanku) and the same species of plants were cultivated, harvested and analysed. Analysis of variance (ANOVA) and person correlation coefficient were performed on experimental data so as to ascertain any significant variation among the concentrations of heavy metals in the samples and to establish the relationship among parameters evaluated using SPSS 17.0 respectively.

Geo-accumulation Index ($\mathbf{I_{geo}}$) was calculated using experimental data in order to evaluate the level of metal buildup in sediments and has remained very valuable in assessing the amount of metal contamination in soil samples [21, 29].

$$\mathbf{I_{geo}} = \text{In}\ [(C_m)\ /1.5\ x\ (B_m)]$$

The correction factor as a result of variation in the background value due to lithological differences is 1.5, C_m is metal concentration and B_m represents the Background value of the metal [30]. Values greater than 1 and as high as 6 indicates slightly to significantly very high contamination. There exists another index which is very valuable in assessing the movement of metal passing through soil to plant. This index is known as Transfer Factor (TF). Transfer factor therefore, can be defined as the concentration of metal in plant dry weight / concentration of metal in soil dry weight [21, 31-34].

3. Results and Discussion

The results of phytochemical determination of edible plant leaves evaluated are accessible in Table 1. The result shows that the phytochemical contents varied greatly. The highest (1.20g/100g) and minimum (0.80g/100g) levels of alkaloid were detected in *Talinium triangulare* and *Gongronema latifolium* respectively. *Gongronema latifolium* had

higher steroid content (0.30g/100g) and tannin concentration (0.21g/100g) whereas least values of (0.18g/100g and 0.11g/100g) were recorded in *Amaranthus hybridus* and *Talinium triangulare* respectively. Maximum (0.15g/100g) and minimum (0.10 g/100g) flavonoid contents were observed in *Amaranthus hybridus* and *Talinium triangulare* respectively. High levels of flavonoid in edible plants are exceedingly advantageous for the reason that it provides a protective therapy against diseases which includes ageing, cancer, atherosclerosis, inflammation, ischaemic injury and neuro-degenerative disorders [21, 28]. The concentration of anthocyanin ranged from 0.06 g/100g to 0.09 g/100g in the vegetables studied. These values were lower than those obtained for flavonoid, alkaloid and steroid. Low content of anthocyanin in the leaves of plants investigated in this study may be attributed to the absence of colour other than green in leafy vegetables [35].

Table-1. Phytochemical Composition of Edible Plant Leaves grown at control and contaminated soil

Phytonutrients	GL_1	$G L_2$	TT_1	TT_2	AH_1	AH_2
Anthocyanin	0.09	0.08	0.06	0.05	0.08	0.06
Steroid	0.30	0.15	0.24	0.20	0.18	0.15
Flavonoid	0.13	0.11	0.10	0.10	0.15	0.12
Tannin	0.21	0.17	0.11	0.09	0.16	0.15
Alkaloid	0.80	0.60	1.20	1.10	1.18	1.00
Carotenoid	0.06	0.05	0.08	0.06	0.12	010

GL Gongronema latifolium, TT Talinum triangulare,AH Amaranthus hybridus
Subscripts 1 and 2 depict contaminated and control sites respectively.

Analysis of soil samples for heavy metals at the time of planting of the leafy vegetables revealed a marked variation in the heavy metal content as the results are shown in Table 2. The range of concentration of these toxic metals were 8.30 ± 0.04 to 13.70 ± 0.01 mg kg^{-1} Cd; 70.20 ± 0.21 to 89.10 ± 0.31 mg kg^{-1} Pb; 1.80 ± 0.03 to 4.30 ± 0.02 mg kg^{-1} Ni; 31.20 ± 0.22 to 76.50 ± 0.06 mg kg^{-1} Cu; 103.10 ± 0.14 to 174.00 ± 0.10 mg kg^{-1} Zn; 0.40 ± 0.17 to 1.34 ± 0.20 mg kg^{-1} As and 0.09 ± 0.01 to 0.20 ± 0.01 mg kg^{-1} Hg. Comparatively, zinc metal concentrations exceeded the concentrations of other metals determined in this study and tend to exhibit highest value for soil Eyimba (EY). Conversely, mercury had the lowest values, with the highest value in soil WB and minimum concentration for soil OG. The levels of these toxic metals in contaminated soils were higher than those of control samples. The normal recommended range for lead concentration in soil is 18 to 36 mg kg^{-1} [24]. Copper content which exceeds 20 mg kg^{-1} constitutes toxic effect on plants [26].

Table-2. Concentration of heavy metals (mg kg^{-1}) in soil (Mean ± SD)

Metals	Control	EY	OS	WB	OG
Cd	0.03± 0.01	13.70 ± 0.01	10.30 ± 0.20	11.90 ± 0.17	8.30 ± 0.04
Pb	4.52 ± 0.20	70.20 ± 0.21	76.50 ± 0.01	85.80 ± 0.30	89.10 ± 0.31
Ni	1.02 ± 0.10	4.30 ± 0.02	3.50 ± 0.05	2.90 ± 0.12	1.80 ± 0.03
Cu	23.80± 0.04	49.10 ± 0.15	76.50 ± 0.06	63.20 ± 0.30	31.20 ± 0.22
Zn	89.60 ± 0.51	174.00 ± 0.10	156.10 ± 0.21	123.50 ± 0.20	103.10 ± 0.14
As	0.10± 0.00	1.34 ± 0.20	1.21 ± 0.10	0.70 ± 0.05	0.40 ± 0.17
Hg	0.01± 0.00	0.13 ± 0.01	0.17 ± 0.02	0.20 ± 0.01	0.09 ± 0.01

Geo-accumulation index and the contamination factors of toxic metals in soil are shown in Table 3. Geo-accumulation index calculated using experimental data exposed the fact that there was pollution of soil samples by Cd and Pb, having values which ranged from 5.50 to 6.01 and 1.07 to 1.31 correspondingly. Higher contamination factors were also observed for Cd and Pb across all the soil samples assessed except the control.

Table-3. Geo-accumulation index and Contamination Factor of Toxic metal in soil

Metals		Control	EY	OS	WB	OG
Cd	CF	0.20	91.33	68.67	79.33	53.33
	Igeo	-0.12	6.01	5.72	5.87	5.50
Pb	CF	0.28	4.39	4.78	5.36	5.57
	Igeo	-1.69	1.07	1.16	1.27	1.31
Cu	CF	0.34	0.70	1.09	0.90	0.44
	Igeo	-1.49	-0.76	-0.32	-0.51	-1.21
Zn	CF	0.68	1.32	1.18	0.94	0.78
	Igeo	-5.68	-5.01	-5.12	-5.40	-5.5
As	CF	0.20	0.27	0.24	0.14	0.08
	Igeo	-5.92	-3.33	-3.43	-3.98	-4.54
Hg	CF	1.65	1.63	2.13	2.50	1.13
	Igeo	0.08	0.08	0.35	0.51	-0.29

The metal concentrations in the edible plant leaves in the contaminated soil and control soil samples were determined and results are as shown in Tables 4 and 5 correspondingly. The concentrations of Cd ranged from 0.38 mg kg^{-1} to 0.60 mg kg^{-1}, 0.68 mg kg^{-1} to 0.80 mg kg^{-1} and 0.41 mg kg^{-1} to 0.90 mg kg^{-1} in *Amaranthus hybridus, Talinum triangulare and Gongronema latifolium* respectively. The mean concentrations of Cd in the plants studied ranged from 0.48 ± 0.11 to 0.72 ± 0.06 mg kg^{-1}. The concentrations of lead in various plant samples evaluated varied greatly with values which ranged from 3.90 to 5.00 mg kg^{-1}, 4.80 to 6.10 mg kg^{-1} and 3.80 to 6.20 mg kg^{-1} in

Amaranthus hybridus, *Talinum triangulare and Gongronema latifolium* respectively. Moreover, the minimum and maximum mean concentration of lead was recorded for *Amaranthus hybridus* (4.43 ± 0.48 mg kg^{-1}) and *Talinum triangulare (5.45* ± 0.06 mg kg^{-1}) correspondingly. The levels of Nickel were within the range 0.09 to 0.20 mg kg^{-1}, 0.10 to 0.72 mg kg^{-1}, and 0.20 to 0.40 mg kg^{-1} in *Gongronema latifolium, Talinum triangulare and Amaranthus hybridus*, respectively. The mean levels of Ni were lower in *Gongronema latifolium (*0.14 ± 0.05 mg kg^{-1}) and nevertheless, greater in *Talinum triangulare* (0.33 ± 0.28 mg kg^{-1}). These concentrations were not only within the recommended limits of Ni in plants (0.02 to 50 mg kg^{-1}) [32], but also comparable with values (0.20 – 0.27 mg kg^{-1} obtained in the control soil samples. The levels of Cu in plant samples ranged from 10.10 to 16.70 mg kg^{-1} (*Gongronema latifolium*), 9.80 to 14.00 mg kg^{-1} (*Talinum Triangulare*) and 10.20 to 13.80 mg kg^{-1} (*Amaranthus hybridus*). Lowest and highest mean levels of copper were recorded for *Amaranthus hybridus (*12.00 ± 1.49 mg kg^{-1}) and *Gongronema latifolium* (13.48± 2.75 mg kg^{-1}) respectively. These values were found to be higher than copper concentrations (9.10 - 10.90 mg kg^{-1}) obtained in the samples from the control station. Nevertheless, the levels of Cu found in the various plants determined were lower than the permissible range of concentrations (20 to 30 mg kg^{-1}) recommended by National Agency for Food and Drug Administration and Control (NAFDAC) and World Health Organisation (WHO). Zinc concentration in plant samples ranged from 10.80 to 21.10 mg kg^{-1}, 13.10 to 15.30 mg kg^{-1}, and 11.70 to 13.80 mg kg^{-1} for *Gongronema latifolium, Talinum triangulare* and *Amaranthus hybridus*, respectively. Furthermore, the Lowest and highest mean levels of zinc obtained in this study were 12.80 ± 0.94 mg kg^{-1} and 15.70 ± 4.94 mg kg^{-1} for *Amaranthus hybridus* and *Gongronema latifolium* respectively. These values were somewhat higher than the concentrations of the control samples (11.20 – 14.50 mg kg^{-1}) but within the normal range (50 mg kg^{-1} and 60 mg kg^{-1}) of zinc in plants as per NAFDAC/CODEX and WHO/FAO respectively [29, 36]. Results obtained for analysis of mercury in the leafy vegetables revealed very low concentrations compared to Zn, Cu, Ni, Pb and Cd. However, the mean mercury levels ranged between 0.02 to 0.03 mg kg^{-1}. These values were higher than the levels of mercury (0.01 mg kg^{-1}) obtained across the plants cultivated in the control station. This is an indication of mercury contamination of these plants which poses health risk to the unsuspecting consumers. The concentration of As (0.01 to 0.30 mg kg^{-1}) was higher in *Gongronema latifolium* and lower (0.01 to 0.15 mg kg^{-1}) in *Amaranthus hybridus,* while the mean levels ranged from 0.08 ± 0.06 to 0.15 ± 0.13 mg kg^{-1}. The concentrations of Cd were within the permissible value of 1.0 mg kg^{-1} (WHO/FAO) whereas the levels of arsenic were slightly above the recommended value 0.05 and 0.1 mg kg^{-1} (NAFDAC and CODEX) respectively [36]. The concentration of Cd in the plant leaves were below the 2.8 mg kg^{-1} recorded in the soil of Ado Ekiti, Southwestern Nigeria [37].

Table-4. Concentration of heavy metals (mg kg^{-1}) in Edible Plants Leaves

Metal	STATION	*Gongronema latifolium*	*Talinum triangulare*	*Amaranthus hybridus*
Cd	EY	0.90	0.80	0.60
	OS	0.55	0.68	0.40
	WB	0.41	0.71	0.55
	OG	0.82	0.69	0.38
	Mean± SD	0.67 ± 0.23	0.72 ± 0.06	0.48 ± 0.11
Pb	EY	6.20	5.70	4.20
	OS	5.91	6.10	5.00
	WB	5.50	4.80	3.90
	OG	3.80	5.20	4.60
	Mean± SD	5.35 ± 1.07	5.45 ± 0.57	4.43 ± 0.48
Ni	EY	0.20	0.30	0.40
	OS	0.15	0.10	0.20
	WB	0.10	0.18	0.30
	OG	0.09	0.72	0.24
	Mean± SD	0.14 ± 0.05	0.33 ± 0.28	0.29 ± 0.09
Cu	EY	16.70	11.90	10.20
	OS	12.90	13.50	12.30
	WB	14.20	9.80	13.80
	OG	10.10	14.00	11.70
	Mean± SD	13.48 ± 2.75	12.30 ± 1.89	12.00 ± 1.49
Zn	EY	21.10	15.30	13.30
	OS	18.60	13.10	12.40
	WB	12.30	14.40	13.80
	OG	10.80	13.60	11.70
	Mean± SD	15.70 ± 4.94	14.10 ± 0.96	12.80 ± 0.94
As	EY	0.10	0.10	0.09
	OS	0.30	0.20	0.15
	WB	0.20	0.10	0.07
	OG	0.01	0.10	0.01
	Mean± SD	0.15 ± 0.13	0.13 ± 0.05	0.08 ± 0.06
Hg	EY	0.01	0.02	0.04
	OS	0.05	0.01	0.02
	WB	0.02	0.03	0.03
	OG	0.01	0.02	0.01
	Mean± SD	0.02 ± 0.02	0.02 ± 0.01	0.03 ± 0.01

Similarly, the mean concentrations of lead, obtained from plant samples cultivated from soils other than the control, were above the value (2 mg kg^{-1}) recommended by WHO/FAO and NAFDAC for edible vegetables [38].

The variations in heavy metal profile in the result may be attributed to a number of factors such as anthropogenic activity, plant species and variation in soil properties including organic matter content, pH, temperature, electrolyte concentration and soil type. For instance, metals are more available to plants under acidic conditions than under alkaline conditions.

Table-5. Concentration of heavy metals (mg kg^{-1}) in Edible Plants Leaves (Control)

Metal	*Gongronema latifolium*	*Talinum triangulare*	*Amaranthus hybridus*
Cd	0.12	0.20	0.18
Pb	1.34	1.01	1.20
Ni	0.20	0.27	0.24
Cu	9.10	10.9	10.2
Zn	11.2	13.9	14.5
As	0.01	0.02	0.01
Hg	0.01	0.01	0.01

Table -6. Guidelines values for metals in food and vegetables (mg kg^{-1}) [21, 27]

Metals (mg kg^{-1})	WHO/FAO	Nafdac	EC/Codex	Normal Range in Plant
Cd	1	__	0.2	< 2.4
Cu	30	20	0.3	2.5
Pb	2	2	0.3	0.5 - 30
Zn	60	50	< 50	20 -100
As	-	0.05	0.1	-
Ni	_	_	_	0.02 -50
Cu	1	_	0.2	< 2.4

Mean comparison tests (ANOVA) were performed to check existence or not of difference between the results. Results of statistical analysis of the control plant samples revealed that there is no significant difference between the phytonutrients of *Gongronema latifolium* (GL_1), *Talinum triangulare* (TT_1) and *Talinum triangulare* (AH_1) at the Significant. level 0.978 > 0.05 with low F-Statistics ratio (0.023). Analysis of experimental results also revealed that there is no significant difference between the phytonutrients contents of plant samples from the contaminated soils (GL_1, TT_1, AH_1) and plant samples obtained from the control soils (GL_2, TT_2, AH_2) at the Significant. Level 0.370 > 0.05 with low F-Statistics ratio (1.062). Significant difference does not also exist between the metals concentrations of the control, EY, OS, WB and OG at the Significant level 0.817 >0.05 with low F-Statistics ratio (0.385).

Pearson correlation coefficient was used to establish the association between the phytonutrients and heavy metal concentrations (Table 3). Very strong positive correlation was observed between anthocyanins and arsenic; flavonoids and zinc as well as copper; tannins and arsenic, at 0.05 significant level (1-tailed). It was also noticed that steroids (Table 1) was significantly positively correlated with lead (Table 4) at r (1.000^{**} or 100.0% with significant <0.05). Results of Pearson correlation analysis also revealed association between metals. Al and Ca demonstrated significant negative correlation with Cd respectively whereas Pb was both significantly positive and negative correlated to Cd and Hg at 0.05 levels respectively.

The Transfer Factors of Cd, Pb, Ni, Cu, Zn, As and Hg for the plants investigated are presented in Table 7.

The results revealed a widespread variation in the transfer factors between plant species. Value of transfer factor for Cd varied from 0.035 to 0.091(*Gongronema latifolium),* 0.059 to 0.084 *(Talinum triangulare)* and 0.039 to 0.046 *(Amaranthus hybridus*). The transfer factor value for lead ranged from 0.043 to 0.086 *(Gongronema latifolium),* 0.056 to 0.081 *(Talinum triangulare)* and 0.046 to 0.065*(Amaranthus hybridus).*

Table-7. Transfer factor of Heavy metals from Soil to Edible Plants

Heavy Metals	Gongronema Latifolium				Talinum Triangulare				Amaranthus Hybridus			
	EY	OS	WB	OG	EY	OS	WB	OG	EY	OS	WB	OG
Cd	0.066	0.053	0.035	0.091	0.059	0.066	0.060	0.084	0.044	0.039	0.046	0.044
Pb	0.086	0.077	0.064	0.043	0.081	0.080	0.056	0.058	0.060	0.065	0.046	0.052
Ni	0.047	0.043	0.035	0.050	0.070	0.029	0.062	0.400	0.093	0.057	0.104	0.133
Cu	0.340	0.169	0.225	0.324	0.242	0.177	0.155	0.449	0.208	0.161	0.176	0.410
Zn	0.121	0.119	0.099	0.105	0.088	0.084	0.117	0.132	0.077	0.079	0.112	0.114
As	0.075	0.248	0.286	0.325	0.075	0.165	0.143	0.025	0.067	0.124	0.100	0.251
Hg	0.077	0.294	0.100	0.111	0.154	0.059	0.152	0.222	0.308	0.118	0.150	0.111

Nickel had a transfer factor values which ranged from 0.035 to 0.050 (*Gongronema latifolium),* 0.029 to 0.070 *(Talinum triangulare*) as well as 0.057 to 0.133 *(Amaranthus hybridus)* while the value of transfer factors for copper varied from 0.169 to 0.340 (*Gongronema latifolium),* 0.177 to 0.449 *(Talinum triangulare*) and 0.161 to 0.410 *(Amaranthus hybridus).* Zinc had moderate transfer factor values which ranged from 0.099 to 0.121 (*Gongronema latifolium),* 0.084 to 0.132 *(Talinum triangulare)* and 0.077 to 0.114 *(Amaranthus hybridus).* Values of transfer factor for arsenic varies from 0.075 to 0.294 *(Gongronema latifolium),* 0.025 to 0.165 *(Talinum triangulare),* and

0.067 to 0.251 *(Amaranthus hybridus)* whereas Hg had values of transfer factors from 0.077 to 0.294 (*Gongronema Latifolium),* 0.059 to 0.222 *(Talinum triangulare)* and 0.111 to 0.308 *(Amaranthus hybridus).* Among the metals studied, Cd had the least mean transfer value (0.057 ± 0.02) but copper recorded the highest mean transfer value (0.251 ± 0.10). Pb, Ni, Zn, As and Hg had lower to intermediate mean transfer value of 0.064 ± 0.01, 0.094 ± 0.10, 0.104 ± 0.02, 0.157 ± 0.10 and 0.155 ± 0.08 respectively. Low transfer factor indicates the robust sorption of metals to the colloids while higher transfer factor implies relatively poor retention in the soil or greater efficiency of vegetables to absorb metals [21, 33]. Higher transfer factor also demonstrates high bioavailability of metals to plants tissue. Low transfer factor is good especially for toxic metals. The lower the transfer factors of Hg, As, Pb and Ni in the edible plants tissues studied, the safer they are for consumption.

4. Conclusion

Geo-accumulation index and contamination factor revealed soil contamination by cadmium and lead. The leafy vegetables investigated demonstrated appreciable levels of phytochemicals: alkaloid, tannin, flavonoid, steroid, carotenoid and anthocyanin. The levels of zinc, copper, mercury and cadmium in the edible plants were within the permissible limits but concentrations of lead were significantly higher than the standard values (2 mg kg^{-1}) by WHO/FAO and NAFDAC. The levels of arsenic were higher than the recommended limit. It is interesting to note that the edible plants studied demonstrated very low capacities to accumulate cadmium and lead in their leaves despite very high concentrations in the soil. Metals with very high concentrations in the soil exhibit low TF values which signify more retention of metals in the soil and better proficiency of vegetables to absorb metals.

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