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Phytochemicals, Heavy Metals, and Antioxidant Vitamins Assessment in Tomatoes (*Solanum lycopersicum*) Cultivated Near Cement Company Firm of Sokoto, Nigeria

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ABSTRACT

Even at low concentrations, it is well known that vegetables can readily absorb metals from contaminated soil or deposits on air-exposed plant parts. These metals then build up at high levels in the edible parts of the vegetables. Vegetables are a significant part of the human diet; hence heavy metal poisoning of these food items cannot be understated. As a result, this study sought to quantify the amounts of phytochemicals, heavy metals, and antioxidant vitamins in tomatoes grown close to the Cement Company of Northern Nigeria (Sokoto Cement). Phytochemicals, heavy metals, and antioxidant vitamins were determined using standard analytical procedures. The study confirmed the presence of flavonoids, saponins, alkaloids tannins, steroids, and volatile oils. The heavy metals detected (Cu, Zn, Cd and Cr) were found below the WHO safe limits guidelines apart from Cr which exceed WHO safe limit guidelines. Substantial amounts of vitamin A, C and E were also observed. It was concluded that tomatoes cultivated in Kalambaina area are safe for consumption in terms of heavy metals.
Keywords: Antioxidant, Heavy metals, Phytochemicals, Pollution, Tomato, Vitamin

INTRODUCTION

Plants such as *Psidium guajava* and *Carica papaya* have long been our forefathers' first line of defense against diseases such as malaria, cholera, and diarrhea (Kar & Borthakur, 2008). They can easily be divided into groups based on their uses, such as those that are edible, those that are used as a source of spices or medicines, those that have ornamental value, etc. (Dalhat *et al.*, 2017). Tomato (*Solanum lycopersicum*) is a savory, red, edible fruit that originated in South America and spread around the world after the Spanish colonized America. Nowadays, it is widely farmed, often in greenhouses in kinder climates. The tomato fruit is frequently consumed in many forms, including as fresh, as a component of salads and sauces, or after being processed into purees, ketchups, and tomato soups (Aremu *et al.*, 2017). Tomatoes are high in minerals, vitamins, proteins, essential amino acids (leucine, threonine, valine, histidine, lysine, and arginine), monounsaturated fatty

acids (linoleic and linolenic acids), carotenoids (lycopene and -carotenoids), and phytosterols (β-sitosterol, campesterol, and β-stigmasterol) (Ali *et al.,* 2021). In Nigeria, tomatoes are mainly cultivated in the Northern region, precisely in the area called Sudan Savannah. This zone is affected by some level of environmental degradation from industrial activities, mostly Cement industries.

Environmental contamination from anthropogenic activity that is mostly caused by industrialization is a major problem. Different environmental elements like water, air, soil, and plants are harmed because of industrial and human activity (Singh *et al.,* 1980).

Heavy metals and other chemicals are released into the environment in significant quantities, particularly by industries amongst which the Cement Industry is a major player (Yaşar *et al*., 2010). Cement factories are known to emit a variety of pollutants, including dust, particulate matter, gases, and various heavy metals, all of which are harmful to the biotic environment, manifesting themselves in soil, plants, and terrestrial and aquatic flora and fauna (Akinci & Caliskan, 2010, Dalhat *et al*., 2017).

Food safety concerns caused by pollution have been recently reported worldwide (Li *et al.,* 2021). Food safety issues are mostly caused by biological agents such as viruses, bacteria, molds, protozoa, helminths (worms), and/or chemicals, which can cause both acute ailments (especially diarrheal diseases) and increase the risk of long-term illnesses for example, aflatoxins and heavy metals. Heavy metals are thought to pose the greatest threat to food safety of all the pollutants that have been reported. For instance, it was discovered that the levels of cadmium (Cd), lead (Pb), and arsenic (As) in vegetables and rice grown in polluted areas were significantly higher than the established safety limits (Odai *et al.,* 2008, Khan *et al.,* 2010).

Heavy metals are naturally occurring earth elements with densities more than 5 g/cm^3 and long biological half-lives. They cannot decompose biologically. In trace levels, several heavy metals are required to maintain the body's metabolism. On the other hand, toxic levels could happen. Heavy metals can enter our bodies in trace levels via food, water, and air. It is well-known that metals, even at low concentrations, can be readily absorbed by vegetables from contaminated soil or deposits on plant parts exposed to the air. These metals then build up at high levels in the edible parts of the vegetables. It is possible for heavy metals to contaminate crops when they are irrigated with tainted water, fertilized, or treated with pesticides that include metals. Contamination with heavy metals is also possible during crops transportation, harvesting, or storage (Obadahun *et al.,* 2021). Vegetables are a significant part of the human diet, hence heavy metal poisoning of these food items cannot be understated (Abdulmojeed and Abdulrahman, 2011).

A range of illnesses, particularly those affecting the heart, kidneys, neurological system, and bones, may result from eating heavy metal-contaminated fruits and vegetables. As a result, this study sought to quantify the amounts of phytochemicals, heavy metals, and antioxidant vitamins in tomatoes grown close to the Cement Company of Northern Nigeria (Sokoto Cement).

MATERIALS AND METHODS

Chemicals and Reagents

The reagents used for the study included sulphuric acid, hydrochloric acid, n-hexane, trioxonitrate acid, trichloroacetic acid (TCA), sodium hydroxide, acetic acid, ferric chloride, Wagners reagent, α^1, α^1-dipyridyl, potassium hydroxide, sodium chloride,

metaphosphoric acid, acetone, perchloric acid, chloroform, methanol, ethanol, indophenol, and distilled water. All chemicals used were of analytical grade. The tools used include Buck Scientific 210 Atomic Absorption Spectrophotometer and UV-Vis Spectrophotometer (752N).

Sample collection and preparation

Fresh tomato sample were collected in a clean plastic bag from Kalambaina area which is just less than one kilometer away from the main deport of Cement Company of Northern Nigeria in October 2021. The sample was identified and authenticated by the Department of Biological Science Sokoto State University, Sokoto. The collected sample was washed under a running tap, cut into smaller pieces using knife and then dried at 37^0C in an oven for approximately 2 weeks. After drying, the sample was crushed using clean mortar and pestle into a powder which was then stored at room temperature for the duration of the research.

Phytochemicals Screening

Alkaloids

The presence of alkaloids was investigated using the methods described by Wagner's (Wagners, 1991). 1 ml of the extract was 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.

Flavanoids

The determination for the presence of Flavanoids in the sample was conducted using alkaline reagent test by Okerulu *et al* (2017). 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.

Tannins

The determination of the presence of tannins in the test sample was carried out using Ferric chloride test described by Harbone (1973). 2 drops of 5% $FeCl_3$ was added to 1 ml of the extract. A greenish precipitate indicated the presence of tannins in the extract.

Test for cardiac glycosides (Keller-Killiani Test)

One milliliters (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 (Okerulu *et al*., 2017).

Saponins

The presence of saponins in the sample was done using Harbone (1973). 0.5 g of the extract was treated with 5 ml of distilled water and mixture was shaken vigorously, the production of foam which persisted in few minutes indicated the presence of saponins in the extracts.

Test for steroids (Lieber Mann Burchard Reaction)

Two milliliters (2 ml) of chloroform was added to 2 ml of plant extract and the mixture was shaken vigorously. The mixture was allowed to settle until two layers are formed. 1 ml of concentrated H_2SO_4 was carefully added alongside of the test tube. A reddish-brown ring at the interface indicates the presence of Steroids (Bashiru *et al*., 2017).

Test for Anthraquinones (Borntragers test)

Two milliliters (2 ml) of chloroform was added to 2 ml of plant extract in a test tube and shaken vigorously. 5 ml of 10% NH_3 was then added to the chloroform layer. The mixture was allowed to settle and observation was made. A bright pink color at the upper part of 2 layers formed indicates the presence

of free Anthraquinones (Okerulu *et al*., 2017).

Test for volatile oils

Two milliliters (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 (Harbone, 1973).

Determination of Heavy Metals

A sample of 0.5 g of the sample was digested for two hours at 95°C with 0.5 ml H_2SO_4, 0.6 ml concentrated HNO_3, and 1.8 ml concentrated HCl. The sample was chilled and made into a volume of 10 ml using deionized water, after which it was tested for the presence of Copper (Cu), Lead (Pd), Zinc (Zn), Cadmium (Cd) and Chromium (Cr) using a Buck Scientific 210 Atomic Absorption Spectrophotometer (Adekiya *et al.,* 2018). The mean and standard error of the mean were computed, each measurement was repeated three times (n=3).

Determination of antioxidant vitamins

Determination of Vitamin A

Five grams (5 g) of the sample was ground to fine paste and 1ml of saponification mixture (2 N KOH in 90% alcohol) was added. The tube was gently refluxed for 20 minutes at 60^0C and then cooled at room temperature followed addition of 20 ml of distilled water. Vitamin A was extracted with10 ml of petroleum ether in a separating funnel, twice. The organic extract was pooled and sodium sulphate (anhydrous) was added to remove the moisture for 30 minutes. Aliquot of ether was evaporated to dryness at 60^0C and the residue was dissolved in 1 ml chloroform.

Aliquot of the standard (Vitamin A acetate) was pipetted into a series of clean test tubes in the concentration range 1.5-7.5µg and the volume in each test tube was made up to 1 ml with chloroform. A 2 ml of Trichloroacetic acid (TCA) solution (prepared by dissolving 15 g TCA in 25 ml chloroform and stored in the dark) was added from fast delivery pipette, rapidly mixing the contents of the tube and the absorbance was immediately measured at 620nm in UV-Vis spectrophotometer (752N). Absorbance of the sample was also determined in triplicate in a similar manner. Standard graph was constructed by plotting the A_{620} in Y-axis and vitamin concentration in the X-axis and the amount of vitamin A/g in the sample was determined from the standard graph (Bayfield and Cole, 1980).

Determination Vitamin C

Vitamin C (Ascorbic acid) concentration of the sample was determined according to the procedure describe by Sadasivam and Balasubramanian (1987). A dye solution was prepared by dissolving 42 mg of sodium bicarbonate into small volume of distilled water and 52 mg of 2, 6-dichlorophenol indophenol was dissolved in it and the solution was made up to 200 ml with distilled water.

A 5 ml of working standard solution (100 µg/ml ascorbic acid prepared using 4% oxalic acid) was pipetted in to 100 ml conical flask. Ten milliliters (10 ml) of 4% oxalic acid was added and titrated against the dye (V_1). The end point was noted by the appearance of pink color which persists for few minutes.

A 5 g of ground sample was extracted in 4% oxalic acid and made up to 100 ml and centrifuged. To the supernatant (5 ml), 10 ml of oxalic acid was added and titrated against the dye (V_2 ml). Amount of Ascorbic acid mg/100g sample =

$$\frac{0.5\text{mg}}{\text{V1ml}} \times \frac{\text{V2}}{5\text{ml}} \times \frac{100\text{ml}}{\text{Wt. of sample}} \times 100$$

Determination of Vitamin E

A 0.5 g sample was weighed into stopper tube and 10 ml of 0.1N sulphuric acid was slowly added without shaking. The tube was stopped and allowed to stand overnight. Afterward, the contents of the tube was

vigorously shaken and filtered through Whatman No. 1 filter paper.

A 1.5 ml of the filtrate (in triplicate) was pipetted into centrifuge tubes labeled as test and 1.5 ml each of standard and distilled water was pipetted into centrifuge tubes labeled as standard and blank respectively. To the test and blank, 1.5 ml of ethanol was added and to the standard 1.5 ml of distilled water and then centrifuged. Xylene (1.5 ml) was added into all the test tubes, capped, mixed and centrifuged again. Carefully, 1 ml of xylene layer from each test tubes was transferred into another stopper tubes followed by 1 ml of 2, 2-dipyridyl reagent, stoppered and mixed. The extinction of the resulting mixture for the test and standard was read at 460nm against the blank. Then, 0.33 ml of ferric chloride solution was added, mixed and the absorbance of the test and standard was read against blank after 15 minutes at 520nm (Rosenberg, 1992).

The amount of vitamin E (µg/g) in the sample was calculated using the following formula:

$$\frac{\text{Ax520} - \text{Ax460}}{\text{As520}} \times 0.29 \times 15 \frac{\text{Vt}}{\text{Vu} \times \text{g}}$$

Where:
Ax520 = Absorbance of test at 520nm
Ax460 = Absorbance of test at 460nm
As520 = Absorbance of standard at 520nm
Vt = Total volume of homogenate
Vu = volume used and g = Weight of the sample.

Statistical Analysis

Results are expressed as mean ± standard error of mean, and the calculation was done using SPSS (Version 20). The results were compared with World Health Organization (WHO).

RESULTS AND DISCUSSION

Phytochemicals are important in the food, pharmaceutical, and dye industries. Some of them have pharmacological effects; flavones and tannins, for instance, are crucial components of many laxatives, medications, and colors (Dalhat *et al.,* 2017). The study sample's phytochemical analysis revealed a high content of flavonoids and saponins (Table 1). There is evidence that flavonoids and saponins have anti-inflammatory, cytostatic, analgesic, antibacterial, and analgesic properties (González-Madariaga *et al.,* 2020, Ullah *et al.,* 2020). Tannins, volatile oils, and alkaloids were found in moderate amounts (Table 1). Alkaloids are significant secondary metabolites that have considerable anti-cancer, analgesic, and cytotoxic potential (Nobori *et al.,* 1994, Pietta, 2000). While anthaquinones and cardiac glycoside were not found, a little amount of steroids were (Table 1).

The level of heavy metal concentration was shown in Table 2. Only chromium (Cr) was found to be slightly above the World Health Organization's (WHO) recommended limit, according to the current investigation. According to Dalhat *et al.,* (2017) the high level of Cr in the study sample may be related to its high level in the soil. However, the results are consistent with those of Bashiru *et al*. (2017), who found significant levels of Cr in *Sapinacea olaracea, Allium cepa*, and *Amarantus species* grown in the Kalambaina region. Cr is a necessary trace element that regulates the metabolism of carbohydrates, lipids, and proteins in its trivalent state (Cr^{3+}), but when it is in its hexavalent state (Cr^{6+}), it is poisonous, mutagenic, and carcinogenic (NIH, 2019, Wakeman *et al.,* 2017). The present investigation did not reveal the presence of lead (Pb). However, *Lactuca sativa* grown close to the cement mill was found to contain high levels of Pb, according to Dalhat *et al.,* (2017). This can be due to the plant's different capacity to absorb heavy metals from the soil or its close proximity to the facility. Although copper (Cu) is necessary for human existence, excessive concentrations of Cu have been linked to an

increased risk of cardiovascular illness, metal fume fever, dermatitis, hair and skin discolorations, respiratory tract infections, and certain other fatal conditions in people (Badaloni *et al.,* 2017, Lavigne *et al.,* 2019, Wang *et al.,* 2020). It has been demonstrated that, zinc (Zn) is involved in the production and breakdown of lipids, proteins, nucleic acids, and carbohydrates, is crucial for polynucleotide transcription and translation and, consequently, for the process of genetic expression (Dalhat *et al.,* 2017). Due to the high rate of soil-to-plant transfer of cadmium (Cd), these foods are the main sources of cadmium (Cd) contamination (Vergine *et al.,* 2017). It is a very toxic, non-essential heavy metal that is widely known for having a negative impact on cellular enzymatic systems, oxidative stress, and for making plants nutritionally deficient (Hassan *et al.,* 2020). It is important to note that the study plant's levels of Cu, Zn, and Cd are within the WHO-acceptable ingestion limits.

The antioxidant vitamins results in the research sample are shown in Table 3. Vitamin A is essential for normal gene expression, growth, and immune function as well as for maintaining epithelial cell processes (Lukaski, 2004). Vitamin C contains anti-inflammatory and immune-boosting characteristics, functions as a cofactor for mono- and di-oxygenases, and is needed for the synthesis of biological components such as collagen and catecholamines (Fujii, 2021, Spoelstra-de Man *et al.,* 2018).

Vitamin E is a powerful antioxidant that helps to protect cells from free radical damage. Red blood cells and muscles both require it for growth and optimal function (Lukaski, 2004). Vitamin A, C, and E concentrations were found to be 85.043, 234.062, and 19.086 µg/g in the sample, respectively (Table 3).

CONCLUSION

This study confirmed the presence of flavonoids, saponins, alkaloids, tannins, steroids and volatile oils. The presence of the heavy metals was also revealed (Cu, Zn, Cd and Cr). The amounts of heavy metals were currently below the WHO safe limits criteria, apart from Cr. Vitamins A, C, and E, which are antioxidant vitamins, were also found in significant amounts. In conclusion, tomatoes grown in the Kalambaina region are safe to eat in terms of heavy metals.

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Table 1. Result of phytochemical screening of *Solanum lycopersicum*

Parameter	Result
Flavonoids	+++
Saponins	+++
Alkaloids	++
Anthraquinones	ND
Tannins	++
Steroids	+
Volatile oils	++
Cardiac glycosides	ND

Keys: ND not detected, + slightly present, ++ moderately present, +++ abundantly present

Table 2. Heavy metal concentration in the *Solanum lycopersicum* sample

Heavy metal	Concentration (mg/kg)	FAO/WHO (mg/kg)
Cu	0.125±0.025	73.3
Pd	BDL	0.3
Zn	0.228±0.022	99.4
Cd	0.010±0.005	0.2
Cr	0.058±0.240	0.05

The results represent mean ± standard error of mean of triplicate experiment (n=3)
WHO STD = World Health Organization Standard (2011)

Table 3. Antioxidant vitamins concentration in *Solanum lycopersicum* sample

Parameter	Concentration (µg/g)
Vitamin A	85.043±5.77
Vitamin C	234.062±0.21
Vitamin E	19.086±1.73

The results represent mean ± standard error of mean of triplicate experiment (n=3)