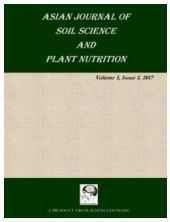

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Determination of Heavy Metals and Phytochemical Analysis of Some Selected Vegetables Grown at Kalambaina Area Sokoto

I. Bashiru[1*], M. H. Dalhat[2], A. M. Aminu[1], A. A. Sumayya[1], H. B. Zainab[1] and S. Zulkallaini[1]

[1]Department of Biochemistry, Sokoto State University, Sokoto, Nigeria.
[2]Department of Biochemistry, Usmanu Danfodiyo University, Sokoto, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author IB designed the study. Authors SZ, MHD and HBZ performed the statistical analysis. Authors IB and AMA wrote the protocol and wrote the first draft of the manuscript. Authors AAS and AMA managed the analyses of the study. Authors MHD, IB and AAS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Heavy metals and phytochemicals were investigated in some selected vegetables grown at Kalambaina area of Sokoto. The levels of heavy metals and phytochemicals were analyzed using Atomic Absorption Spectrometric (AAS) and standard analytical procedures respectively. The concentration of heavy metals were found to be higher in *Amarantus specie.* Pb; (1.330 ± 0.0014 mg/kg), Ni; (0.280 ± 0.0011 mg/kg), Cr; (0.110 ± 0.0012 mg/kg), Cd; (0.003 ± 0.0001 mg/kg) and *spinacea oleracea* Pb; (0.220 ± 0.0011 mg/kg), Ni; (0.020 ± 0.0002 mg/kg), Cr; (0.100 ± 0.0011 mg/kg) Cd; (0.010 ± 0.0001 mg/kg), however, low concentrations were observed in *Allium cepa* Pb; (0.080 ± 0.0011 mg/kg), Ni; (0.003 ± 0.0001 mg/kg), Cr; (0.130 ± 0.0020 mg/kg), Cd; (0.005 ± 0.0002 mg/kg). The results of the study for the three (3) vegetables revealed the concentrations of the heavy metals are within the World Health Organization (WHO) and Standard Organization of Nigeria (SON) safe limits guidelines with the exception of Cr and Pb. Phytochemical screening of

**Corresponding author: Email: ibrahim.bashiru@ssu.edu.ng;*

methanolic extracts of the vegetables showed the presence of alkaloids, saponins, tannins, flavonoids, phenolics and terpenoids. Therefore, this study revealed that the vegetables can be use as efficient source of secondary metabolites.

Keywords: Heavy metals; phytochemicals; Kalambaina; atomic absorption spectrometric.

1. INTRODUCTION

Plants are nature's gift to mankind in terms of providing us with food, oxygen, as well as shelter. Since time immemorial, 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. The Horticulturist divides the edible garden plant into vegetables and fruits [2].

Vegetables are important edible crops and are an essential part of the human diet. They are rich in nutrients required for human health, and are an important source of carbohydrates, vitamins, minerals, and fibers [3]. Heavy metals can be readily taken up by vegetable roots, and can be accumulated at high levels in the edible parts of vegetables, even heavy metal in soil at low levels. In many countries and regions, vegetables are exposed to heavy metals by various means, thus vegetable consumption can cause adverse health effects [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]. Despite of the bright prospects and enormous potential need of vegetables and fleshy fruits production in Nigeria, there are challenges such as Poor growth and harvesting techniques, lack of storage facilities, inadequate processing facilities, and improper packaging and pests' protection management procedures. These factors mentioned may lead to the contamination of the vegetables and fruits with the heavy metals. Anthropogenic activities can cause elevated levels of these metals in various parts of the ecosystem and thus environmental pollution by heavy metals may occur via various diffused and point sources [2]. Assessment of heavy metal compositions of vegetable is one of the most important method used for monitoring environmental pollution, 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 [6]. The present study is aim at investigating the levels of some heavy metals (Cd, Cr, Ni and Pb) and phytochemicals in edible portion of *spinacea oleracea*, *Allium cepa* and *Amaranthus specie* grown at kalambaina area 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, sodium chloride, perchloric acid, 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 bags from Kalambaina area Sokoto down Cement Company of Northern Nigeria (Fig. 1). The samples were identified and authenticated at the Department of Biological science Sokoto State University Sokoto. The collected samples were washed separately under running tap water before cut down into smaller pieces using knife and dried at 25-27℃ for about 2 weeks to a constant weight. 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 that was used for the extraction of the active ingredients from the plant parts in this research was extraction by evaporation using 70% methanol. This was done by suspending 50 g of the samples in 500ml of 70% methanol. The extraction was allowed for 24 hours at room temperature 25- 27℃ on shaker at rotation of 1500 rpm. Each sample was separated using Whatman filter paper No. 1 (150 mm), the filtered liquid was then dried in evaporator at 35℃ for about 4 hours in other to get methanolic extract [7].

Fig. 1. Map of the study area

2.4 Digestion Procedure

A 2.0 g of the samples were weighed into Kjeldahls flask mixed with 20 ml of concentrated sulphuric acid, concentrated perchloric acid and concentrated nitric acid in the ratio 1:4:40 by volume respectively and left to stand overnight. Thereafter, the flask was heated at 70℃ for about 40 min and then, the heat was increase to 120℃. The mixture turned to black after some time [8]. 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 [9]. 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 [10]. 1 ml of each extracts were treated with 2 drops of Wagner's reagent (2 g of iodine and 3 g of potassiuim 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. [11]. 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 [12] as reported by Mercy [13]. 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 Phenolics

The presence of phenolic in the samples was carried out using Ferric chloride test as reported by Mercy [13]. 2.0 ml of each extract were treated with 3-4 drops of 10% ferric chloride, formation of bluish blue color indicates the presence of phenolics compound in the extract.

2.6.5 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 [14]. 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.

2.6.6 Terpenoids

The presence of terpenoid in the samples was carried out according to method described by Salkowski [15]. 1 ml of each extract was treated with 2 ml of chloroform and few drops of sulphuric acid, it was shaken and allowed to stand for few minutes and appearance of reddish brown color at the lower layer indicates the presence of terpenoids in the extracts.

2.7 Statistical Analysis

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

3. RESULTS AND DISCUSSION

The concentration of heavy metals (Cd, Cr, Ni and Pb) were investigated in *Amarantus specie, Allium cepa and Spinacea oleracea* grown at Kalambaina Area, Sokoto. The results obtained (Table 1) showed that there is high concentration of Lead (Pb) in *Amarantus specie,* (1.330 ± 0.0014 mg/kg), *Spinacea oleracea* (0.220 ± 0.0011 mg/kg) and *Allium cepa* (0.080 ± 0.0011 mg/kg), the concentration of Pb is above the maximum allowable concentration (MAC) of World Health Organization (WHO) and Standard Organization of Nigeria (SON). Thus, *Amarantus specie* accumulate Pb more than other vegetables under study. High Pb concentration has shown to be commensurate with pollution or vehicles density [16]. Pb is an extremely toxic heavy metal that disturb various plant physiological processes and unlike other metals such as Zinc (Zn), Manganese (Mn) and Copper (Cu), it does not play any biological functions. Moreover, it fastens the production of reactive oxygen species (ROS), causing lipid membrane damage that ultimately leads to damage of chlorophyll, photosynthetic processes and suppresses the overall growth of the plant [17].

Our study also revealed that there is high concentration of Chromium (Cr) in *Allium cepa* (0.130 ± 0.0020 mg/kg), *Amarantus specei* (0.110 ± 0.0012 mg/kg) and *Spinacea oleracea* (0.100 ± 0.0011 mg/kg) (Table 1) when compared to the MAC of both WHO and SON (0.05 mg/kg and 0.05 mg/kg) respectively. Elevated Cr levels may be due to discharged of industrial wastes and ground water contamination which drastically increased the concentration of Cr in soil [18].

The concentrations of Nickel (Ni) in *Allium cepa* (0.003 ± 0.0001 mg/kg) and *Spinacea oleracea* (0.020 ± 0.0002 mg/kg) were low. However, high concentration was observed in *Amarantus specei* (0.280 ± 0.0011 mg/kg). The value obtained in *Allium capa* and *Spinacea oleracea* were below MAC of WHO and SON standard (0.05 mg/kg and 0.02 mg/kg). Ni concentration in this area may appear to be very low and this could be

Table 1. Concentration of some heavy metals in spinach, onion and amarantus

Metals (mg/kg)	*Spinacea olaracea*	*Allium cepa*	*Amarantus specie*	WHO	SON
Cd	0.010 ± 0.0001	0.005± 0.0002	0.003 ± 0.0001	0.005	0.003
Cr	0.100 ± 0.0011	0.130 ± 0.0020	0.110 ± 0.0012	0.05	0.05
Ni	0.020 ± 0.0002	0.003 ± 0.0001	0.280 ± 0.0011	0.05	0.02
Pb	0.220 ± 0.0011	0.080 ± 0.0011	1.330 ± 0.0014	0.03	0.01

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

Table 2. Qualitative phytochemical analysis of spinach, onion and *amarantus specie*

Phytochemical	*Spinacea oleracea*	*Allium cepa*	*Amarantus specie*
Alkaloids	-	-	+
Flavanoids	+	++	-
Saponins	-	-	++
Tannins	++	++	++
Phenolics	+	++	-
Terpenoids	++	+++	-

Keys: - Absent, + Slightly present
++ Moderately present, +++ Abundantly present

attributed to the high mobility of the metal, and that huge amount of it is required for its accumulation. Ni is one of many carcinogenic metals known to be environmental and occupational pollutant. Chronic exposure has been connected with increased risk of lung cancer, cardiovascular diseases, neurological deficits and developmental deficits in childhood and higher blood pressure [19].

Cadmium (Cd) concentrations obtained were (0.010 ± 0.0001 mg/kg) in *Spinacea oleracea,* (0.005 ± 0.0002 mg/kg) in *Allium cepa* and (0.003 ± 0.0001mg/kg) in *Amarantus specei* (Table 1) which revealed low MAC for WHO (0.05 mg/kg), but high SON (0.003 mg/kg). Cd is predominantly found in fruits and vegetables due to its high rate of soil-to-plant transfer [20]. It is highly toxic nonessential heavy metal that is well recognized for its adverse influence on the enzymatic systems of cells, oxidative stress and for inducing nutritional deficiency in plants [21].

Phytochemicals are biologically active occurring chemicals compound found in plants which provide health benefits for human further than those attributed to macronutrients and micronutrients [11]. The results of qualitative phytochemical screening of this research revealed the presence of biologically active compounds such as Tannins, Alkaloids, Saponins, Flavoniods and Phenolic at different levels.

The phytochemical screening showed the presence of alkaloids in *Amarantus specei* but absent in *Spinacea oleracea and Allium cepa* (Table 2). The presence of alkaloids in *Amarantus specei* indicated that the leaf can be used in the treatment of malaria, cold, cough, diabetes, and hypertension [22]. Alkaloids have been of great interest because of their pronounced physiological and medicinal properties (for examples, caffeine, nicotine, morphine, atropine and quinine). Several studies have reported the analgesic [23], antispasmodic and antibacterial properties of alkaloids [24].

Flavonoids were found to be present in *Allium cepa*, and Spinacea oleracea but absent in *Amarantus specie*. Flavonoids plays an important role in protecting biological system on macromolecules such as lipids, carbohydrates and proteins [25]. Extracts of Flavonoids are known to have anti-inflammatory, anti-allergic, anti-viral anti-spasmodic and diuretic effect [13].

Saponins were found to be present in *Amarantus specie* but absent in *Spinacea oleracea* and *Allium cepa* respectively. Saponins are known to be immune boosters. Extracts of plants rich in saponins are said to demonstrate anti-inflammatory, hemolytic, allelopathic, cholesterol lowering and anti-cancer properties [13]. Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [26].

Tannins were found to be present in all the vegetables under study. Tannins are chelating agents for metals and can form complexes with macromolecules through the process, essential substrates and enzymes of microorganisms are depleted leading to cell death. Tannins are good anti-microbial agent with precipitate protein thereby providing water proof layer on the skin when used externally or protect the underlying layers of the skin and limit the loss of fluid. They are also known to be good anti-viral agents [11].

Phenolic compounds are famous group of secondary metabolites with wide pharmacological activities. The phytochemical screening revealed the present of phenolic compound in *Allium cepa* and *Spinacea oleracea* but absent in *Amarantus specie*. Extract of plants rich with phenolic compounds plays an important role in biological activities such as

antiulcer, antidepressant, antioxidant, cytotoxic and antitumor [27].

Phytochemical screening also revealed the present of terpenoids in *Allium cepa* and *Spinacea oleracea* but absent in *Amarantus specie*. Plant extracts with terpenoids may have useful anticancer properties. They are also effective antioxidant and show strong anti-cancer activities [28].

4. CONCLUSION

The present study confirmed the presence of heavy metals (Cd, Cr, Ni and Pb) in vegetables (*Spinacea oleacea, Allium cepa* and *Amarantus specie*) grown at Kalambaina area Sokoto. However, the level of heavy metals were currently within the WHO and SON safe limits guidelines with the exception of Cr and Pb which exceeded the safe limit guidelines in all the vegetables. The study has also confirmed the presence alkaloids, flavonoids, tannins, phenolic and saponins in all the vegetables. The presence of many phytochemicals in the vegetables helps to enrich the immune system and other haematological parameters in human body.

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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