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Effects of heavy metals contaminated irrigation water on antioxidant potentials of cabbage and lettuce grown along some rivers in Kano

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Abstract

The growing problem of water scarcity and absolute constraint in availability of fresh water for irrigation has made the use of wastewater necessary for irrigation in agricultural fields which represents an important route for transmission of heavy metals. The effects of heavy metals contaminated irrigation water from urban and industrial waste from Rivers Jakara and Getsi in Kano, Nigeria on the antioxidant potentials of cabbage and lettuce were studied. Soils, irrigation water, cabbage and lettuce were collected from three locations each along rivers Jakara (Zungeru Road, Airport Road and P.R.P) and Getsi (Gama, Gayawa and Getsi) sites. The levels of heavy metals (Zn. Cu, Cr, Fe, Pb, Cd, As, Ni, Co and Mn) were determined in the water, soil, cabbage and lettuce samples using Atomic absorption spectrophotometer. Total phenolics content was determined using UV-Visible spectrophotometer and antioxidant potential of cabbage and lettuce were assessed via 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS) and Ferric Reducing Antioxidant Power Assay (FRAP) analysis. All the heavy metals were detected in soil, water and the vegetable samples and were mostly found to be above the WHO/FAO approved limit except Mn and Hg in the soil, Zn, Cu, Cr and Ni in the irrigation water and Cu, Co, Ni and Mn in the vegetables. Total phenolic content was found to be significantly lower than the control in all the lettuce and cabbage except the cabbage collected from Zungeru road. The result of DPPH radical scavenging activity shows an increase with increasing concentration of the extracts and were found to be significantly lower than the control and the standard ascorbic acid solution. Similarly, ABTS and FRAP radical scavenging activity of the vegetables were observed to be lower than the control in all the samples analysed. From the results, it could be observed that the use of heavy metals contaminated water for irrigation purposes has led to the accumulation of heavy metals in the soil and their transfer to the edible parts of the vegetables and interfere with the capacity of the two vegetables studied to produce the right quality and quantity of antioxidant molecules to efficiently scavenge DPPH, ABTS and FRAP radicals.

Keywords: Antioxidant potential, Heavy Metals, Phenolic content, Radicals

EFFETS DE L'EAU D'IRRIGATION CONTAMINÉE PAR DES MÉTAUX LOURDS SUR LE POTENTIEL ANTIOXYDANT DU CHOU ET DE LA LAITUE CULTIVÉS LE LONG DE CERTAINES RIVIÈRES À KANO

Résumé

Le problème croissant de la rareté de l'eau et la contrainte absolue de la disponibilité de l'eau douce pour l'irrigation a rendu l'utilisation des eaux usées nécessaire pour *l'irrigation des champs agricoles qui représente une voie importante de transmission des métaux lourds. Les effets de l'eau d'irrigation contaminée par les métaux lourds provenant des déchets urbains et industriels des rivières Jakara et Getsi à Kano, au Nigéria, sur le potentiel antioxydant du chou et de la laitue ont été étudiés. Les sols, l'eau d'irrigation, le chou et la laitue ont été collectés à trois endroits chacun le long des rivières Jakara (Zungeru Road, Airport Road et P.R.P) et Getsi (Gama, Gayawa et Getsi). Les niveaux de métaux lourds (Zn. Cu, Cr, Fe, Pb, Cd, As, Ni, Co et Mn) ont été déterminés dans les échantillons d'eau, de sol, de chou et de laitue à l'aide d'un spectrophotomètre d'absorption atomique. La teneur totale en composés phénoliques a été déterminée à l'aide d'un spectrophotomètre UV-Visible et le potentiel antioxydant du chou et de la laitue a été évalué via le 2,2-diphényl-1-picrylhydrazyl (DPPH), le 2,2'-azino-bis (acide 3-éthylbenzothiazoline-6-sulfonique) (ABTS) et analyse du pouvoir antioxydant réducteur ferrique (FRAP). Tous les métaux lourds ont été détectés dans le sol, l'eau et les échantillons de légumes et se sont avérés pour la plupart supérieurs à la limite approuvée par l'OMS/FAO, à l'exception de Mn et Hg dans le sol, Zn, Cu, Cr et Ni dans l'eau d'irrigation et Cu, Co , Ni et Mn dans les légumes. La teneur totale en composés phénoliques s'est avérée significativement inférieure à celle du témoin dans toutes les laitues et les choux, à l'exception du chou collecté sur la route de Zungeru. Le résultat de l'activité de piégeage des radicaux DPPH montre une augmentation avec l'augmentation de la concentration des extraits et s'est avéré significativement inférieur au contrôle et à la solution standard d'acide ascorbique. De même, l'activité de piégeage des radicaux ABTS et FRAP des légumes s'est avérée inférieure à celle du contrôle dans tous les échantillons analysés. D'après les résultats, il a pu être observé que l'utilisation d'eau contaminée par des métaux lourds à des fins d'irrigation a entraîné l'accumulation de métaux lourds dans le sol et leur transfert vers les parties comestibles des légumes et interfère avec la capacité des deux légumes étudiés produire la bonne qualité et la bonne quantité de molécules antioxydantes pour piéger efficacement les radicaux DPPH, ABTS et FRAP.*

Mots-Clés : Potentiel antioxydant, Métaux lourds, Teneur en phénols, Radicaux

تأثير المعادن الثقيلة على تلوث مياه الري مضادات الاكسدة المحتملة

للملفوف والخس المزروع على طول بعض الأنـهار في كانو

نبذة مختصرة

مشكلة ندرة المياه المتزايدة والقيود المطلقة على توافر المياه العذبة لها جعلت الريمن استخدام المياه العادمة اللازمة للريفي الحقول الزراعية والتي تمثل طريقًا مهمًا لنقل المعادن الثقيلة .تمت دراسة آثار المعادن الثقيلة الملوثة لمياه الريمن النفايات الحضرية والصناعية من ريفرز جاكارا وجيتسي في كانو، نيجيريا على إمكان اتمضادات الأكسدة للملفوف والخس . مجمع التربة ومياه الريو الملفوف والخس من ثلاثة مواقع على طول كل من أنهار جاكارا) طريق Zungeruوطريق المطارو PRP) ومواقع Getsi (Gama و Gayawa و Getsi). تمت حديد مستويات المعادن الثقيلة (Zn. Cu ، Cr ، Fe ، Pb ، Cd ، As ، Ni ، Co ، Mn) في عينات الماء والتربة والكرنب والخسب استخدام مقياس الامتصاص الذري، وتمت حديد محتوى الفينولات الكليب استخدام الأشعة فوق البنفسجية -تمت قييم مقياس الطيف الضوئي المرئي وإمكان اتمضادات الأكسدة للملفوف والخس من خلال 2، 2-Diphenyl-1-picrylhydrazyl (DPPH)، 2 ، 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS) ومضادات الأكسدة

الحديدي كتحليل Power Assay (FRAP). تم الكشف عن جميع المعادن الثقيلة في عينات التربة والمياه والخضروات ووجدت في الغالب أنها أعلى من الحد المعتمد من منظمة الصحة العالمية / منظمة الأغذية والزراعة باستثناء المنغنيز والزئبق في التربة والزنك والنحاس والكروم والنيكل في مياه الريو النحاس والكوبالت، نيو من غنيز في الخضار .تم العثور على محتوى الفين ولا لكل يليكون أقلب كثير من السيطرة في كل الخس والملفوف باستثناء الملفوف الذي تم جمعه من طريق .Zungeru تظهر نتيجة نشاط الكسح الجذري DPPH زيادة مع زيادة تركيز المستخلصات ووجد أنها أقلب كثير من مجموعة التحكم ومحلول حمض الأسكوربيك القياسي. وبالمثل، لوحظ أن نشاط الكسح الجذري ABTS و FRAP للخضر واتكان أقل من التحكم في جميع العينات التي تمت حليلها . من النتائج يمكن ملاحظة أن استخدام المياه الملوثة بالمعادن الثقيلة لأغراض الري أدى إلى تراكم المعادن الثقيلة في التربة ونقلها إلى الأجزاء الصالحة للأكل من الخضار وتداخل معقدرة الخضار المدروسة. لإنتاج الجودة والكمية المناسبة من جزيئات مضادات الأكسدة لكبح جذور DPPH و ABTS و FRAP بكفاءة.

الكلمات الرئيسية: مضادات الأكسدة المحتملة، المعادن الثقيلة، محتوى الفينول، الجذور

Introduction

The growing problem of water scarcity and absolute constraint on the availability of fresh water for irrigation and the increasing consumption of vegetables as a result of awareness of their health benefits has made the use of wastewater necessary for irrigation in agricultural fields and the attendant transmission of heavy metals, organic pollutants and pathogens that are of public health and environmental concern (Jamila and Sule, 2020). The need for a year-round production of vegetables in or near urban areas led farmers in search of water for irrigation to often rely on urban wastewater (Drechsel *et al.,* 2002).

Point sources of contamination in the Rivers includes industrial effluents, municipal/domestic wastewater and abattoir waste, while non-point source includes animal defecation, storm water drainage and urban runoff (Odjadjare *et al.*, 2010). In Kano State Nigeria, wastewater discharge from Bompai Industrial Estate, which drains into River Getsi, contributes to a major source of the metropolitan irrigation water contamination (Bichi, 2000). Cabbage and lettuce were reported to contain significant amount of non-enzymatic antioxidant molecules such as Vitamin A, E, K and C and appreciable levels of enzymatic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APx) etc. (Sergio *et al*., 2020) that can act as free radical scavengers, decompose peroxide, quench singlet and triplet oxygen as well as inhibit some enzymes that may enhance oxidation (Lien et al. 2008).

The terms heavy metal, trace metals and trace elements are referred to the group of metals and metalloids of relatively high atomic number and mass (> 20 and 5 g cm-3, respectively) (Alloway, 2011). Some heavy metals such as zinc (Zn), copper (Cu), nickel (Ni), molybdenum (Mo), manganese (Mn), chromium (Cr) and iron (Fe) are essential trace elements as required in many structural and biochemical function in plants including plant growth, oxidation- reduction reactions, electron transport and many other metabolic processes (Kabata-Pendias, 2000). Non-essential metals such as lead (Pb), cadmium (Cd), mercury (Hg) and arsenic (As) with unknown biological function are also toxic to plants even at low concentrations (Shahid *et al*., 2017). Heavy metal stress causes several chemical, physiological and morphological changes in growth, photosynthesis, protein synthesis, lipid metabolism, respiration and energy

production of plants (Sharma and Dietz, 2009). The most frequently evidenced and earliest result of heavy metal stress in plant cells is the excessive generation of reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), singlet oxygen (½O_2), superoxide anion (O_2•-), hydroxyl (HO•), alkoxyl (RO•) and peroxyl (RO_2•) radicals (Schutzendübel and Polle, 2002).
Thus, this study aimed to assess the antioxidant content of cabbage and lettuce irrigated with heavy metals contaminated water along Rivers Jakara and Getsi.

Materials and Methods

Sample Collection

One (1) kg of the edible part of cabbage (*Brassica oleracea*) and lettuce (*Lactuca sativa*) samples were collected from six sampling sites, while control samples were collected from a farm in Kazaure Local Government Area of Jigawa State and were placed into cleaned polyethylene bags and transported to Biochemistry Laboratory of Bayero University, Kano for further preparations and analysis. The samples were collected from farmlands with the consent of the farmers. This was followed by a five-day air dying and was further dried in hot air oven at 40–50˚C for 24hours to achieve constant weight. The samples were ground into powder using pestle and mortar and sieved with a 2 mm mesh sieve. These were then stored in polyethylene bags in desiccators until digestion and analysis.

Geographical location of the study area

The Vegetable samples were collected from sites along Rivers Jakara and Getsi between Latitude 12^0 027'N to Latitude 12^0 638'N and Longitude 8^0 527' E and Longitude 8^0 563' E. River Jakara is the largest drainage network in the metropolitan Kano (Ibrahim and Saidu, 2010). Samples were collected from three (3) sites along River Jakara (Zungeru 8^0 527’ E, 12^0 027’N, Airport Road 8^0 535’ E, 12^0 638’N and P.R.P 8^0 552' E 12^0 056’N) and three (3) sites along River Getsi (Gama 8^0 560' E 12^0 036'N, Gayawa 8^0 568' E 12^0 053'N and Getsi 8^0 545' E 12^0 045'N)

Digestion of soil, irrigation water and vegetables for heavy metals determination

A 5g of the samples (soil, cabbage and lettuce) and 50Ml of the irrigation water were mixed with 9 mL of 10M HNO_3 and 3 mL of 10 M HCl and separately placed in a tightly capped vessels and placed in the microwave digestion system which was conducted at 180˚C for 45 minutes until a colorless solution was obtained (Bhavtosh and Shweta, 2013). The clear and colorless solution was passed through Whatman No. 42 filter paper whose volume was adjusted to 50mL using 2% HNO_3. The resulting solutions were analysed for heavy meals using Atomic Absorption Spectrophotometer.

Preparation of Aqueous Extracts

Five hundred (500g) of the powdered vegetables were soaked in 1000mL of distilled water. The mixture was allowed to stand for 72 hours with occasional shaking and concentrated using Vacuum Rotary Evaporator (IKA, RV10 Digital) under reduced pressure at 40ºC. The extracts obtained were used for the determination of antioxidant activity (Naima *et al.*, 2012).

Determination of Heavy metals content of irrigation water, soil and vegetables

The Heavy Metals Zn, Cu Cr, Fe, Pb, Cd, As, Co, Ni, Mn were determined using Atomic Absorption Spectrophotometer (AAS) manufactured by Perkin Elma LTD (model PinnAcle 900H) according to Manufacturer’s instructions. All samples were prepared and analysed in triplicate and the data obtained reported as mean ± SD. The blank was also prepared by following same procedures as per the optimum conditions established by Wilson *et al* (2005).

Antioxidant assays

Determination of Total Phenolic Compounds Content

The total phenolic compound content (TPC) of vegetable samples was measured using the Folin-Ciocalteu assay (Singleton *et al.*, 1999). A volume of 0.2 mL of the extract was introduced into test tubes followed by 0.2 mL Folin-Ciocalteu's reagent. The solution was then kept at dark for 5 min and then 1 mL sodium carbonate (15% w/v) was added. The tubes were covered with parafilm and kept again in the dark for 1 h. Absorption at 760 nm was measured with a UV-Visible spectrophotometer (PerkinElma, Model NIR 270) and compared to a gallic acid calibration curve. The results were expressed as mg gallic acid/g dried sample. Each assay was carried out in triplicate.

Determination of Antioxidant Activity

Antioxidant activity was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Method (Shen *et al.*, 2010). The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. The reduction of the radical is followed by a decrease in the absorbance at 517nm. A volume of 2 mL of aqueous solution of the extracts was put into test tubes and 2 mL of 1 mM DPPH solution was added. The tubes were covered with parafilm and kept in the dark for 1 h. Absorbance at 517 nm was measured with a UV-Visible spectrophotometer (PerkinElma, NIR 270) and compared to an ascorbic acid calibration curve. The results were expressed as mg ascorbic acid/g dried sample. Each assay was carried out in triplicate. The percentage inhibition of the DPPH radical was calculated using the following formula:

$$I\ \% = \frac{A_0 - A_t}{A_0} \times 100$$

where I = DPPH inhibition (%), A_0 = absorbance of control sample (t = 0 h) and A_t = absorbance of the tested sample at the end of the reaction (t = 1 h).

Determination of reduction of ABTS by the extract's antioxidants

The reduction of 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging was determined by the method of Manish *et al*., 2011**.** The ABTS radical cation decolourization method is based on the reduction of $ABTS^{\bullet+}$ radicals by antioxidants of the plant extracts tested. The reaction's mechanism involves the electron-donating ability and results in the decolorization of the radical. A 7mmol ABTS solution and a 2.45mmol solution of $K_2S_2O_8$ were mixed in a ratio of 1:1 and kept in the dark for 24 h. The mixture was then diluted in aqueous methanol in a ratio of 1:25. A volume of 20 µL aqueous extract was added to 2 mL of the mixture, and the mixture was kept at a standard temperature of 30 °C. The absorbance was measured at 734 nm in 0, 5, and 10 min after initial mixing. All solutions were used on the day of preparation, and all determinations were carried out in triplicate.

The percentage of inhibition of $ABTS^{\bullet+}$ was calculated using the following formula:

$$I\ \% = \frac{A_{t=0} - A_t}{A_{t=0}} \times 100$$

where I = ABTS inhibition (%), A_t=0 = absorbance of control sample (ABTS radical solution) and A_t = absorbance of a tested sample in 5 or 10 min. Antioxidant capacity was expressed in mg ascorbic acid/g dried sample

Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric reducing antioxidant power (FRAP) assay of the extract was measured using the method of Benzie and Strain (1998). 2mL of sample were mixed with 2mL of phosphate buffer (0.2M, pH 6.6) and 2mL of Potassium Ferrocynide (10mg/mL). The mixture was incubated at 50ºC for 20 minutes followed by addition of 2mL of trichloroacetic acid. The mixture was centrifuged at 3000rpm for 10 minutes to collect the upper layer of the solution. A 2mL from the upper layer collected was mixed with 2mL of distilled water and 0.4mL of 0.1% freshly prepared ferric chloride. After 10 minutes, the absorbance was measured at 700nm. A standard curve was plotted using an aqueous solution of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) (100– 1000 μM), with FRAP values expressed as micromoles of ferrous equivalent (μM Fe [II] per kg of sample).

Statistical Analysis

The data were analysed using the statistical package for social sciences (SPSS) version 16.0 and presented as mean ± SD. The acceptance level of significance was $P \leq 0.05$

Results and Discussions

Level of Heavy Metals Concentration in irrigation water Samples

The levels of heavy metals in water samples from six locations along River Jakara and River Getsi in Kano state are presented in Table 1.

The mean heavy metals concentrations of water samples collected from the six locations along Rivers Jakara and Getsi were found to fall within the limit approved by FAO/WHO except Pb, Cd, As, Fe and Co. Fe had the highest concentration of in Getsi followed by Pb in P.R.P. The third is As in Getsi while Cd had 0.61ppm in Gama. These values were significantly above ($p<0.05$) the recommended 5, 0.1, 0.1 and 0.03ppm for Fe, Pb, As and Cd respectively approved by FAO/WHO (FAO/WHO, 2011). The result also showed Zn, Cu, Cr and Ni having values below the approved limits.

The result above indicates a high level of iron, lead and arsenic which can be attributed to its release from activities of motor mechanics along the River Bank in Kofar Ruwa, Zungeru Road and Bomphai. The practice of recycling battery causes the discharge of lead into the environment. Arsenic has for long being regarded as an environmental contaminant and its use still persist. This can be attributed to the release of the heavy metals from the industries sited in Bomphai and the small to medium scale industries that dot the river banks of the two rivers. These industries produce paints, fabrication of metal products, lubricant oils and grease etc. Another factor is the point pollution practiced by most household along the rivers. In most houses, the domestic biological waste is channelled directly into the River via a pipe. This waste contains detergents, soap, cosmetics, pesticides, insecticides and drugs (Ahmed and Sadau, 2015).

Level of Heavy Metals Concentration in Soil Samples

The levels of heavy metals in soil samples from selected sites along Rivers Jakara and Getsi and FAO/WHO standard are presented in Table 2. All the six locations were found to be positive for all the heavy metals determined. The concentrations varied from one location to another, and the average is in this order of increasing concentration Fe >Mn >Cu > Zn > Cr > Pb > Ni > As > Cd > Co > Hg. The mean concentrations of heavy metals in various soil samples collected from the six locations were compared with the standards set for Soils by FAO & WHO, 2011 and other Standards (Vodyanitskii, 2016). Fe, As, Cd, Zn, Cr, Co, Cu and Pb in most of the soil samples exceeded the FAO & WHO standards in the soil indicating a varied properties of the soils and their capacities to retain heavy metals. Zn, Fe, Pb, Mn, Cr and Cu were found in most soil samples to have the highest concentrations.

The concentration of Chromium and Arsenic in the six sites where samples were collected show that there was a high level of these metals in farms along the two rivers that is above the recommended levels of 100mg/Kg and 14mg/Kg (FAO/WHO, 2011) while the levels of Nickel, Cobalt, Manganese and Mercury were found to be within the approved limit. The results indicate that the farmland soils from the study areas analysed in this study accumulated elevated concentrations of Fe, However, this finding is far below the value of 80000 mg/kg reported in soil by McGrath *et al*., (2003) and 46426.67mg/Kg by Gebeyehu and Bayissa (2020).

The activities of automobile mechanics, metal products fabrication and unregistered companies along the river bank contributes to the introduction of heavy metals and other environmental pollutants into the Environment.

The concentrations of copper were higher than those reported by Bello *et al.,* (2019). The high level of cadmium might be due to the use of cadmium-containing phosphate fertilizers and contamination from cadmium-containing dusts (Ewers, 1991).

The concentrations of cadmium obtained in this study were higher than recommended maximum limit for soil that is 3 mg/kg. From this study also it was found that various agricultural activities carried out in the areas were able to increase the Cd content in the soils. The relatively high levels of lead might have resulted from accumulation of lead through air pollution such as automobile exhaust fumes, Auto mechanic Garages, Illegal Battery recycling plants and from some pesticides, such as lead arsenates applied during cultivation. The values of Pb obtained in this study were lower than Bello *et al.*, (2019) and higher than Wodaje & Alemayehu (2017) and Abubakari *et al.*, (2017). Also the high levels of Cr and As could be attributed to presence of Tanneries, Tie and dye pits, paints, soap and dumping of industrial waste along the river canals.

The findings of this study agree with Dawaki and Shu'aibu, 2013 who reported that the soil along the river is severely polluted with Pb, Cd and Cr while Abdullahi and Mohammed (2020) reported that samples of soil and spinach recently collected around Kwakwachi along River Jakara were found to contain metals in the following order Ni>Cd>Pb>Zn>Cu>Cr.

Table 1: Levels of Heavy Metals of Irrigation water samples from Rivers Jakara and Getsi in parts per Million (ppm)

METAL/SITE	Zn	Cu	Cr	Fe	Pb	Cd	As	Ni	Co	Mn
ZUNGERU ROAD	0.22±0.015^{a}	0.03±0.01^{b}	0.03±0.002^{c}	13.7±0.002^{d}	0.29±0.09^{e}	0.25±0.08^{f}	0.83±0.12^{g}	0.07±0.01	0.27±0.09^{i}	0.10±0.03^{j}
AIRPORT ROAD	0.21±0.013^{a}	0.05±0.012^{b}	0.03±0.001^{c}	3.9±0.003^{d}	0.32±0.004^{e}	0.37±0.05^{f}	0.89±0.008^{g}	0.03±0.005	0.29±0.05^{i}	0.21±0.02
P.R.P	0.22±0.011^{a}	0.05±0.011^{b}	0.03±0.002^{c}	12.5±0.8^{d}	2.47±0.18^{e}	0.52±0.08^{f}	0.93±0.17^{g}	0.04±0.007	0.25±0.08^{i}	0.23±0.07
GAMA	0.20±0.018^{a}	0.05±0.01^{b}	0.04±0.008^{c}	11.1±0.02^{d}	0.55±0.02^{e}	0.61±0.11^{f}	0.62±0.14^{g}	0.12±0.04	0.15±0.09^{i}	0.19±0.06
GAYAWA	0.16±0.021^{a}	0.06±0.02^{b}	0.06±0.003^{c}	13.3±0.04^{d}	0.57±0.001^{e}	0.63±0.09^{f}	0.59±0.17^{g}	0.01±0.005	0.35±0.04^{i}	0.30±0.09^{j}
GETSI	0.19±0.011^{a}	0.06±0.06^{b}	0.09±0.01	26.5±0.21^{d}	0.87±0.005^{e}	0.52±0.13^{f}	1.03±0.32^{g}	0.02±0.006	0.33±0.08^{i}	0..29±0.08^{j}
WHO/FAO	2	0.2	0.1	5	0.1	0.03	0.1	0.2	0.05	0.2

Results are presented as Mean± standard deviation. Values bearing same superscript along the same column are significantly different from the WHO/FAO values. FAO Irrigation and Drainage (1985)

Table 2: Levels of Heavy Metals in Soil samples from selected sites along River Jakara and Getsi in mg/Kg.

METAL/SITE	Zn	Cu	Cr	Fe	Pb	Cd	As	Ni	Co	Mn
ZUNGERU ROAD	221.3±15.80[a]	311.5±7.62[b]	128.0±9.75	1302.9±86.59[d]	127.1±11.95[e]	28.4±6.80[f]	29.3±2.44	33.4±1.94	15.9±3.40[j]	333.2±0.82
AIRPORT ROAD	278.4±14.34	274.1±13.45[b]	176.5±4.54[c]	1416.8±77.37	110.6±9.26[e]	26.1±7.2[f]	27.2±2.52	39.0±2.51	19.2±2.07[j]	378.7±0.33
P.R.P	113.6±5.65[a]	259.2±7.28[b]	184.8±3.72[c]	1807.9±53.86[d]	97.0±2.72[e]	38.9±9.03[f]	30.8±5.62	29.2±3.73	17.1±4.18[j]	445.3±0.65
GAMA	87.5±5.88[a]	94.0±5.17[b]	199.0±7.93[c]	1793.7±210.39[d]	68.4±5.97	37.0±16.30[f]	42.2±3.41[g]	48.4±4.04	23.3±3.29[j]	330.1±1.24
GAYAWA	98.2±5.25[a]	88.2±2.59[b]	164.3±2.09[c]	2474.2±211.55	88.5±6.28[e]	39.3±8.03[f]	51.7±3.53[g]	63.4±2.59[h]	29.2±5.13[j]	443.5±0.61
GETSI	185.2±10.60[a]	374.9±10.88[b]	116.9±2.61	3483.2±475.51[d]	131.4±6.23[e]	44.9±0.69[f]	63.1±3.54[g]	57.1±2.57	18.6±4.02[j]	641.2±0.45
FAO/WHO (2011)	50	20	100	425	10	3	14	50	8	2000

Values are presented as Mean ± Standard Deviation. Values bearing same superscript along the same column are significantly Higher than the WHO/FAO values

Heavy metals content of vegetables

The heavy metal concentrations in the edible part of cabbage and lettuce collected from six farms along River Jakara and Getsi are listed in Table 2. The concentration of metals varied greatly among plant species and sample locations. The average concentrations of trace metals in all vegetable samples are in the following decreasing order: Fe >Zn > Cu > Cr > Co> Mn> Ni > Pb > As > Cd >Hg. The result shows that lettuce contain highest concentration of Fe, Zn, Cr, Cd and Arsenic in Getsi followed by Gayawa while the highest concentration of Fe in cabbage was in Gama and is significantly above ($p< 0.05$) the approved maximum level of 48mg/Kg, 20mg/kg, 1mg/kg, 0.2mg/kg and 0.1mg/kg respectively, while the level of Zinc in cabbage were found to be within the approved limit of FAO/ WHO.

The highest levels of Cr, Pb, Cd and As were found in cabbage collected from the three sites along River Getsi. However, the highest levels of Cu, Ni, Co, Mn and Hg were found in Airport Road, Gayawa, Getsi respectively. These levels were found to be not significantly different ($p<0.05$) from the FAO/WHO approved limit of 40, 10, 50, 500 and 0.3mg/kg for Cu, Ni, Co and Mn respectively.

The high level of Fe presented in Table 3 might be due to its abundance naturally in the soil because it is the fourth most abundant element in the earth's crust. This suggest that lettuce accumulate more Fe and Zn than cabbage while cabbage accumulates more Cr, Pb, Cd and As than lettuce. Doka *et al.*, 2020 reported heavy metals contamination in cabbage, lettuce, okra, spinach and pepper while Onions irrigated with river water contains number of organic contaminants (Ekevwe *et al*., 2017). In a study reported by Dawaki and Shu'aibu, 2013, the lettuce produced along the river contains Pb, Cd and Cr above FAO/WHO permissible limits while the Spinach produced along the river is severely contaminated with Pb and Ni (Abdullahi and Mohammed, 2020). Also, Lawal and Audu (2011) reported that spinach, okra, onions and tomatoes grown along the Jakara river contains higher concentrations of Co, Cu, Zn and Cr. However, in the current studies, Ni and Co contaminations were not observed. The result of this research is also higher than the levels of heavy metals in cabbage and lettuce in Ghananian markets reported by Bempah *et al* (2011).

Table 3: Level of Heavy Metals levels of Vegetables samples (Cabbage and Lettuce) collected from selected sites along River Jakara and Getsi in (mg/Kg)

	Heavy Metal	Zn	Cu	Cr	Fe	Pb	Cd	As	Co	Ni	Mn
	Zungeru	8.4±0.8	2.4±0.2	1.8±0.3	80±7.3	3.54±0.2 [e]	1.29±0.35 [e]	0.83±0.3 [e]	1.18±0.9	2.04±0.6	2.03±0.3
	Airport Rd	16.2±1.0	3±0.6	1.4±0.6	106±21	3.06±0.02	1.07±0.20 [e]	0.89±0.2 [e]	1.29±0.3	1.41±0.4	1.48±
CABBAGE	P.R. P	8.6±0.8	2.4±0.8	1±0.3	120.8±5.3	3.96±0.4 [e]	1.44±0.50 [e]	0.93±0.4 [e]	1.37±0.7	1.29±0.5	1.35±
	Gama	8.2±1.3	2±0.3	1.2±0.4	416±53 [d]	2.72±0.2 [e]	1.99±0.52 [e]	0.59±0.2 [e]	1.95±0.8	3.04±1.2	3.05±
	Gayawa	7.8±1.2	1.8±0.8	1±0.3	140.6±6.2	3.8±0.4 [e]	1.87±0.61 [e]	1.03±0.3 [e]	1.37±0.4	2.83±	2.38±
	Getsi	9.2±2.8	2.2±0.7	1±0.3	80±16	4.78±0.05 [e]	2.25±0.73 [e]	0.49±0.16 [e]	2.13±0.7	1.34±	3.17±
	Zungeru	16.2±3.1	5.2±1.2	3±1.2 [c]	356±59 [d]	4.62±0.27 [e]	2.61±0.83 [e]	1.92±0.5 [e]	3.05±1.02	1.18±0.5	1.65±0.8
	Airport Rd	18.8±2.9	6.2±0.5	2.4±0.6 [c]	68±21	3.72±0.15 [e]	2.4±1.13 [e]	2.05±1.1 [e]	2.12±	1.05±	1.40±
	P.R. P	20.4±4.7	4±1.1	2.2±0.7 [c]	366±42 [d]	3.74±0.6 [e]	2.67±0.88 [e]	2.51±1.1 [e]	1.94±	2.81±	2.49±
LETTUCE	Gama	13.8±2.5	3±0.4	2.6±0.3 [c]	624±71 [d]	4.76±0.6 [e]	3.21±1.18 [e]	1.03±0.6 [e]	1.34±	2.11±	3.31±
	Gayawa	25.4±5.1 [a]	5.6±0.8	3.6±1.3 [c]	748±82 [d]	6.12±1.3 [e]	3.60±1.49 [e]	3.21±0.18 [e]	1.48±	3.06±	3.11±
	Getsi	40.8±9.4 [a]	4.2±0.4	5±2.3 [c]	1950±116 [d]	6.26±0.8 [e]	4.12±1.32 [e]	3.84±0.17 [e]	1.38±	2.84±	4.27±
FAO/WHO		20	40	1	48	0.3	0.2	0.1	10	50	500

Results presented as Mean± standard deviation. Values bearing same superscript along the same column are significantly different from FAO/WHO-approved limits.

Total Phenolic Content

The results of total phenolic content of cabbage and lettuce grown along some Rivers in Kano are presented in Table 4. The result showed that the level of phenolic compounds in cabbage is significantly lower than the control except those collected from Zungeru Road while that of lettuce is significantly lower than the control in all samples collected from the six sites. The result further showed that the level of phenolic compounds is lowest in Getsi and P.R.P sites which also contains significant levels of some heavy metals.

Table 4: Total Phenolic Content (as Gallic acid equivalent) mg/dl of cabbage and lettuce grown along some Rivers in Kano

Location/ Vegetable	Zungeru Road	Airport Rd	P.R. P	Gama	Gayawa	Getsi	Control
Cabbage	0.41±0.18	0.34±0.12 [a]	023±0.02 [a]	0.16±0.04 [a]	0.19±0.07 [a]	0.11±0.06 [a]	0.51±0.23
Lettuce	0.38±0.02 [b]	0.46±0.02 [b]	0.24±0.01 [b]	0.32±0.05 [b]	0.37±0.03 [b]	0.27±0.03 [b]	0.73±0.29

The result is presented as mean ± standard deviation. Values having same superscript along the row are significantly different from the control

The role of phenolic compounds in the scavenging of free radicals associated with oxidative stress is well established (Fergusion *et al*., 2006). Georgiadou *et al* (2018) showed that levels of heavy metals in soils are inversely proportional to some physiological parameters of the plants leaves such as pigment and phytochemical content. Plants exposed to heavy metals produce increased amounts of reactive oxygen species (ROS) (Yadav, 2010). The overproduction of ROS can affect the redox status of cells which causes dramatic physiological challenges, leading to oxidative stress (Nadgórska-Socha, *et al*., 2013). Heavy metal-induced ROS can cause damage to plants, such as enzyme inhibition, protein oxidation and lipid peroxidation and possibly hampering their capacity to produce the needed phytochemicals to counter the effect of phenolic compounds inclusive (Cuypers *et al*.., 2011). In general, toxicity is affected by the bioavailability of the metals and interactions with other metals in the soil while soil uptake of heavy metals by plants depends on the type and level of soil contamination. Plants absorb large quantities of metals until they become toxic. The effects of contamination of the soil by Ni on plants could lead to decreased seed germination, growth reduction and limitation of transpiration and photosynthesis (Sreekanth *et al*., 2013). These effects inhibit the production of some vital secondary metabolite like diosgenin (De and De, 2011) known for its neuroprotective effects against aging-related deficits like memory improvement as well as metabolic disorder (Mafalda *et al*., 2016). To scavenge the overproduced ROS, plants employ specific mechanisms including activation of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione peroxidase, glutathione S-transferase (GSTs), dehydroascorbate reductase (DHAR), Glutathione reductase (GR), guaiacol peroxidase (GPX), shikimate dehydrogenase (SKDH), polyphenol oxidase (PPO), cinnamyl alcohol dehydrogenase (CAD), phenyl alanine ammonia- lyase (PAL) (Kovacik *et al.*, 2009) and non-enzymatic antioxidants such as carotenoids, glutathione, ascorbic acid, alpha-tocopherol, proline and many phenolic compound including flavonoids, anthocyanins, tannins, lignins, phenolic acid and related compounds, coumarin, flavenol, cinnamy acid, cinnamy alcohol, cinnamy aldehyde, etc. (Nicholson and Vermeris, 2011)

DPPH Radical Scavenging Activity

The results of DPPH of lettuce and cabbage collected from six locations each along Rivers Jakara and Getsi are presented in Figure 1 and 2 respectively. The result showed that with increasing concentration of the vegetable extracts, the values of DPPH radical scavenging activity were observed to be significantly lower than the control and the standard Ascorbic acid in all the samples. At the highest concentration of 10mg/dl, the extracts of lettuce collected from Airport Road and cabbage collected from Gama showed the highest radical scavenging activity. These were followed by samples collected from Zungeru and Gama for lettuce and cabbage respectively. Furthermore, the result showed that cabbage demonstrated a more efficient DPPH radical scavenging activity than lettuce

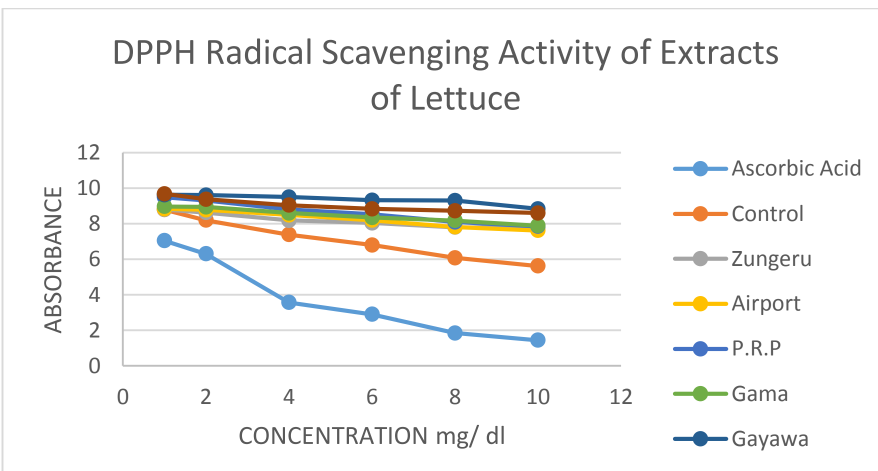

Figure 1: DPPH Radical Scavenging Activity of aqueous extract of lettuce grown along some Rivers in Kano. Lower absorbance indicates high radical scavenging activity

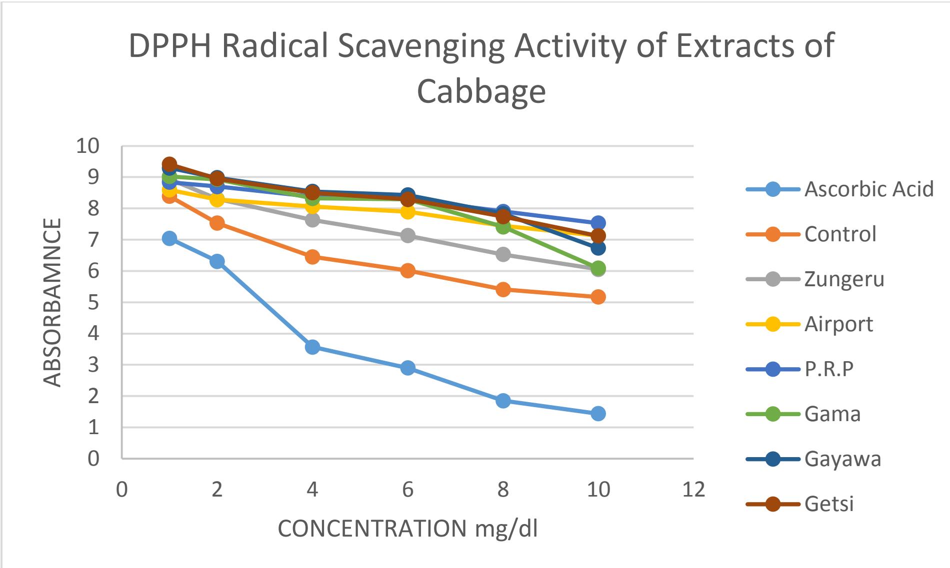

Figure 2: DPPH Radical Scavenging Activity of aqueous extract of Cabbage grown along some Rivers in Kano

From the result, it could be observed that the capacity of the vegetables known for high content of antioxidant molecules and enzymes has been compromised by the level of heavy metals in the soil and the vegetables. The levels of Zn, Pb, Cd, As, Co, Fe and Cu above the recommended FAO/WHO levels in the soil could lead to interference with the metabolic processes notably the inactivation of the enzymes involved in the biosynthesis of the antioxidants. However, Samrina *et al.* (2022) reported increased antioxidant enzymes activities such as superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in *Avicennia marina* and *Rhizophora mucronata* following exposure to multiple heavy metals reflecting a damage response to stress factors in those plants. The activation of these enzymes is an essential protective mechanism to reduce oxidative injury following exposure to heavy metals. In any case, oxidative stress was confirmed to be imposed in all heavy metal-treated samples demonstrated through higher levels of H_2O_2, with biochemical evidence for this induction being provided through the upregulated activity of major ROS metabolizing enzymes such as SOD, CAT and APX. SOD dismutases superoxide radicals to H_2O_2, while CAT and APX act as H_2O_2 scavengers (Gill and Tuteja, 2010). Similar results were found in the leaves of thyme (*Thymus vulgaris*) plants treated with the same heavy metals as the present report (Kulbat and Leszczynska, 2016).

ABTS Radicals Reduction Activity

The result of the scavenging activity of the extracts of lettuce and cabbage collected from six locations each along Rivers Jakara and Getsi against ABTS radical presented in Table 5.

Table 5: ABTS radical scavenging activity of extracts of lettuce and cabbage

LOCATION/ SAMPLE	ZUNGERU	AIRPORT	P.R.P	GAMA	GAYAWA	GETSI	CONTROL
LETTUCE	18.8±5.71*	17.4±7.24*	13.07±6.13*	15.8±4.38*	11.71±5.90*	13.62±5.08*	28.95±8.46
CABBAGE	26.7±8.36*	31.33±7,47*	22.88±5.83*	34.4±11.31*	20.16±6.04*	16.07±7,54*	44.39±10.78

Results presented as Mean ± Standard Deviation of % scavenging activity of ABTS radical. Values having * along the same row shows significant difference with the control.

The result showed that the highest activity was recorded in cabbage collected from Gama and lettuce collected from Zungeru which is followed by both cabbage and lettuce collected from Airport Road. The result showed that all the samples have values that are significantly lower than the control in both lettuce and cabbage.

From the result, it could be observed that the use of contaminated water for irrigation purposes along these two Rivers has significantly compromised the capacity of these vegetables to synthesize the right quantity and quality of phytochemicals to counter the effects of ABTS radicals when compared with the control. The growing of vegetables in heavy metals polluted soil may affect the biosynthesis of secondary metabolite. Chowardhara *et al* (2019) reported a severe incidence of phytotoxicity in plants treated with Zinc and Cadmium in Indian mustard, interfering with metabolic processes that inhibit seed germination, plant growth and development which could ultimately lead to plant death as concentration increases.

Metal toxicity is ascribed to three main reasons: (i) direct interaction with proteins due to their affinities for thioyl-, histidyl- and carboxyl-groups, causing the metals to target structural, catalytic and transport sites of the cell; (ii) stimulated generation of ROS that modify the antioxidant defense and elicit oxidative stress; and (iii) displacement of essential cations from specific binding sites, causing functions to collapse (Sharma and Dietz, 2009).

FRAP Radicals Scavenging Activity

The result of the scavenging activity of the extracts of lettuce and cabbage collected from six locations each along Rivers Jakara and Getsi against Ferric radical is presented in Table 6.

Table 6: Ferric radical scavenging activity of extracts of lettuce and cabbage (μM Fe [II] per kg of sample)

LOCATION/ SAMPLE	ZUNGERU	AIRPORT	P.R.P	GAMA	GAYAWA	GETSI	CONTROL
LETTUCE	103±22.3 ^{a}	105±28.7 ^{a}	85±19.7 ^{a}	65±20.5 ^{a}	92±25.6 ^{a}	85±18.6 ^{a}	260±42.3^{a}
CABBAGE	225±31.8^{b}	230±42.4 ^{b}	197±37.9 ^{b}	180±35.1 ^{b}	155±39.8 ^{b}	140±33.4 ^{b}	338±47.8 ^{b}

Results presented as Mean ± Standard Deviation of % scavenging activity of Ferric radical. Values bearing same superscript along the same row shows significant difference with the control.

The result showed that all the obtained values of FRAP radical scavenging activity are significantly ($p<0.05$) lower than the control. Furthermore, the result with the highest activity was observed in the Airport Road followed by Zungeru in lettuce and airport followed by Zungeru in cabbage samples.

The ability of plants to increase antioxidative protection to combat negative consequences of heavy metal stress appears to be limited since many studies showed that exposure to elevated concentrations of redox reactive metals resulted in decreased activities of antioxidative molecules (Velioglu,*et al*., 1998). This fact is also valid for U. *dioica* as shown in studies by many authors (Gulcin *et al*., 2010). As FRAP assay measures only non-enzymatic (reductants) antioxidants in the sample, there is an interesting relationship between metal content and obtained FRAP values which are valid for all investigated metals which are redox metals. Results obtained from FRAP assay in this study show that heavy metals induce oxidative stress as evident by the scavenging activity of cabbage and lettuce (in μmol $FeSO_4$ L-1) as in all cases levels for total antioxidant activity in samples exposed to metals are lower than the total antioxidant level in the control sample. Antioxidant systems and their significance for the acclimation of plants pollution and climatic stresses have been reviewed frequently with an emphasis on the responses of leaves (Darinka *et al*., 2013). Exposure to heavy metals also provoked responses of antioxidative systems, but the direction of response is dependent on the plant species, tissue analysed, the metal used for treatment, and also the intensity of the metal stress. However, some common reaction patterns can be found, for example, decreasing activities of antioxidative enzymes and molecules after the metal exposition (Shainberg, *et al.,* 2000). In most cases, exposure to heavy metals, Cd and some others as Cu, Ni, and Zn, initially resulted in a severe depletion of glutathione (GSH), which is only one example. This is a common response to Cd caused by an increased consumption of GSH for phytochelatin production and their role in sequestering heavy metals which is a mechanism that contributes to the protection from metal toxicity in different plants and in some fungi as well (Ishikawa *et al.,* 1997). Also, Gichner, 2003 reported a study that shows 61.9% lower antioxidant activity from the sample (leaves) and 33.5% lower antioxidants level for the stems of a studied plant.

Conclusion

The use of heavy metals contaminated water from urban and industrial waste contaminates the soil with toxic heavy metals beyond the recommended WHO/FAO limits and subsequently transfers some to plants grown on the soil to a level above the WHO/FAO limit. These phenomena lower the potentials of the plants studied to produce the right quantity and quality of antioxidant molecules to mitigate the effects of heavy metals stress thereby limiting their capacity to efficiently scavenge DPPH, FRAP and ABTS radicals.

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