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Determination and risk assessement of heavy metals via intake of spinach *(Amaranthus Caudatus)* from wastewater used for irrigation in Bauchi suburb

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Abstract
This research determined the concentration of some selected heavy metals (Zn, Mn, Cd, Cu, Cr and Pb) in irrigation and non-irrigation soil, wastewater used for irrigation, and irrigated and non-irrigated Spinach (*Amaranthus caudatus*) using Atomic Absorption Spectrophotometer (AAS). The research reveals that, the heavy metal concentrations in wastewater (0.01-0.39 for Zn, 1.36-1.27 for Mn, Cd ND, 0.05-0.07 for Cu, 0.05-0.42 for Cr and 0.01 for Pb in mg/dm^3 at Gwallaga, and 0.0-0.04 for Zn, 0.17-0.23 for Mn, Cd ND, 0.05-0.09 for Cu, 0.0-0.11 for Cr and 0.0 for Pb in mg/dm^3 at Gombe road) were within the permissible limits of World Health Organization [WHO] (2001), except Mn and Cr of Gwallaga wastewater. The concentrations in soil (50.0-83.0 for Zn, 508.0-554.0 for Mn, Cd ND, 13.5-17.0 for Cu, 17.5-23.0 for Cr and 0.0-1.0 for Pb in mg/kg at Gwallaga irrigation soil, 56.5-64.0 for Zn, 513.0-556.55 for Mn, Cd ND, 10.0-11.5 for Cu, 8.5-12.5 for Cr and 0.0-0.5 for Pb in mg/kg at Gombe road irrigation soil) were within the permissible limits of WHO (2001). The concentrations in *Amaranthus caudatus* (23.5-35.7 for Zn, 35.5-63.5 for Mn, Cd ND, 11.0-17.7 for Cu, 0.0 for Cr and 0.0-0.5 in mg/kg at Gwallaga irrigation site, and 24.0-75.5 for Zn, 52.5-133.0 for Mn, Cd ND, 8.5-13.0 for Cu, 0.0 for Cr and 0.0-0.5 for Pb in mg/kg at Gombe road irrigation site, and 25.0-25.5 for Zn, 74.5-75.5 for Mn, Cd ND, 5.0-5.5 for Cu, 0.0 for Cr and 0.0 for Pb in mg/kg at non-irrigation site) were within the permissible limits of WHO (2007). The enrichment factor (EF) shows that, Cu has the highest EF value (1.6727 and 1.4450) while Cr and Pb have least EF value (0.0000). Therefore there is high translocation of Cu from soil to the *Amaranthus caudatus* plant. The Metal Pollution Index (MPI) reveals that, the *Amaranthus caudatus* cultivated during rainy season (non-irrigation) have higher MPI, therefore may pose hazards to human health. The Health Risk Index (HRI) for metals of this research were found to be less than 1. This research recommended to be monitoring plants cultivated during rainy season (non-irrigation).

Keywords: Determination, concentration, *Amaranthus Caudatus*, wastewater

Introduction

Production of vegetables through irrigation system and consumption of these vegetables have increased. It is taken both cooked and raw form but vegetables containing toxic metals can cause detrimental effect on human health (Jolly *et al.*, 2013.) [7]. Increase in intake of heavy metals by human beings is due to the increasing environmental pollution. Air, soil, and water pollution are contributing to the presence of harmful elements such as cadmium, lead and mercury in foodstuff (Oriskwe *et al.*, 2012) [10]. Heavy metals have important positive and negative roles in human life (Sharma *et al.*, 2008) [12]. Elements are essential micro-nutrients and have a variety of biochemical functions in all living organisms. Metals like iron, copper, zinc and manganese are essential metals for human, since they play an important role in biological system, but the essential heavy metals can produce toxic effect when their intake is excessively elevated (Arora *et al.*, 2008) [4]. Heavy metals are hazardous elements to human health. Although there is no clear definition of what a heavy metal is, density is in most cases taken to be the defining factor (Jarup, 2003) [6]. Heavy metals are elements that have density higher than the density of water.

Vegetables are consumed enormously by human being all over the world (Jolly *et al.*, 2013) [7]. Production of vegetables through irrigation and consumption of it has increased because it is one of the major components of human diets. Vegetables are one of the major components of human diets, as it is the source of essential nutrients, antioxidants and metabolites in food items (Jolly *et al*., 2013) [7]. Continuous irrigation lead to heavy metals accumulation in the edible and non-edible parts of these vegetables (Lente *et al.*, 2014) [8]. Food safety issues and potential to health risks make this as one of the most serious environmental concerns (Vousta *et al.*, 1996) [13]. Waste water use for irrigation leads to the accumulation of heavy metals in soil and consequently into the vegetables (Anita *et al*., 2010) [3]. Heavy metals, in general are non-bio-degradable, have long biological half-lives and have the potential for accumulation in different body organs leading to chronic toxic effects. Due to non-biodegradable and persistent nature, heavy metals are accumulated in vital organs in the human body such as the kidneys, bones and livers and are associated with numerous serious health disorder (Druibe *et al.*, 2007). Metals such as lead, mercury, cadmium and copper are cumulative poisons, which cause environmental hazards and are reported to be exceptionally toxic (Sharma *et al.,* 2009) [11]. Metals like iron, zinc, and manganese are essential metals for humans, since they play an important role in biological systems, but the essential heavy metals can produce toxic effect when their intake is excessively elevated (Harmanjit *et al.,* 2011).This accumulation of heavy metals in the vital organs in the human body is through dietary intake. Contamination of foods by heavy metals has become an inevitable challenge these days.

Since production of vegetables through irrigation system and consumption of these vegetables have increased. And consumption of foodstuff polluted with heavy metals is a major food chain route for human exposure (Anita *et al.*, 2010) [3]. The contamination of food by heavy metals have become a serious problem these days. This is because heavy metals are accumulating in the vital organs in human body which lead to a serious health problem. Ingestion of contaminated vegetables is one of the ways in which heavy metals enter human body, hence there is need to investigate the heavy metal concentration. Thus the concentration of some selected heavy metals in Spinach (*Amaranthus caudatus*) from wastewater used for irrigation will be determined and also the risk of those heavy metals will be assessed.

Materials and Methods
Materials
All reagents/chemicals and materials used were of analytical grade

Methodology
Study sites
The irrigated samples were collected from two sites Gwallaga and Gombe road which are among the Bauchi suburb area. The samples collected include; Wastewater used for irrigation, irrigated and non-irrigated soils and irrigated and non-irrigated vegetable (spinach).

Sampling
Water Sampling
Water used for irrigation were collected monthly (January, February and March, 2015) using a pre-acid washed polypropylene bottles. The samples were collected in place where the water is stagnant (Anita *et al.*, 2010) [3]. 500ml of water were collected and 5ml of concentrated HNO_3 was added in the water sample to prevent microbial activity. The samples were then kept in refrigerator before digestion.

Soil Sampling
Soil samples of the irrigated water site were collected monthly (January, February, and March, 2015) using a polyethylene bags. The soil were collected by digging out 5x5x10 cm size, from 5 areas randomly. The samples were air dried, crushed and passed through 2mm mesh size sieved and stored at ambient temperature before digestion.

Vegetable Sampling
The vegetable crop (*Amaranthus caudatus*) at the irrigated water site were collected monthly (January, February and March, 2015). Five areas were randomly marked at the experimental site and the edible portion of test vegetable were collected. The sample were taken to the laboratory and washed with distilled water to remove the soil particles adhered to the sample of the vegetable. The samples were cut into piece, and placed in an oven at a temperature of 40-60°C until a constant weight was achieved. The samples were then ground and sieved and stored before digestion.

Digestion of Samples
Water
The irrigation water sample (50ml) were digested with 50ml of concentrated HNO_3 at 80°C until the solution becomes transparent (APHA, 2005) [2]. The solution were filtered through what man No 1 filter paper and the total volume were maintained to 50ml with distilled water.

Soil and Vegetable
Soil and vegetable (5g) were digested with 50ml of tri acid mixture (HNO_3, H_2SO_4 and $HClO_4$ in 5:1:1ratio) at 80°C until transparent solution was obtained (Allen *et al.*, 1986) [1]. The solutions were then cooled and filtered using what man No. 1 filter paper and the filtrate were maintained to 50ml with distilled water.

Heavy Metal Analyses
Concentration of manganese copper cadmium, lead zinc and chromium in the filtrate of digested water, soil, vegetable samples were estimated using atomic absorption spectrophotometer. The instrument was calibrated using manually prepared standard solution of respective heavy metals and the blank.

Data Analysis
Enrichment Factor
To examine the translocation of heavy metal from the soil to the edible portion of test plants, and to show the difference in metal concentration in the plants, the enrichment factor (EF) were calculated by using the formula given by Buat-minerd and Chasselet (1979) (Anita *et al.*, 2010) [3].

$$EF = \frac{\text{Conc of metal in edible part at irrigated water site /conc of metal in soil at irrigated water site}}{\text{Conc of metal in edible part at the control site/conc of metal in soil at the control site.}}$$

Metal Pollution Index (MPI)
To examine overall heavy metal concentrations of all the metals in the crops analyzed in the irrigated site, metal pollution index (MPI) were calculated. This were done by calculating the geometrical mean of concentrations of all the metals in the vegetable (Anita *et al.*, 2010) [3].

$$\text{MPI of } \textit{Amaranthus caudatus} \text{ (mg/kg)} = (CF_1 \times CF2 \times \text{------------} \times CFn)^{1/n}$$

Health Risk Index (HRI)
The daily intake metal were determined by conducting a survey where the people having average body weight at Gwallaga site Gombe road site were asked for their daily intake of vegetables from the experimental area in each month of sampling (Anita *et al.*, 2010) [3]. The average daily intake of vegetables were calculated. The health risk index were calculated at ratio of estimated exposure of test crops and oral reference dose (Cui *et al.*, 2004). The oral reference dose are 0.30 for Zinc 0.001 for Cadmium, 0.04 for Copper 0.004 for Pb and 1.5 for Chromium, 0.033(mk/kg/day) for Mn (USEPA, 2006) [14]. The estimated exposure were obtained by dividing daily intake of heavy metals by their safe limits. An index more than 1 is considered as not safe for human health (USEPA, 2002) [14].

$$HRI = \frac{\text{Daily intake metal}}{\text{Reference Dose}}$$

Where

$$\text{Daily intake metal (DIM)} = \frac{C_{metal} \times D_{food\ intake} \times C_{factor}}{B_{average\ weight}}$$

C_{metal} = represent the heavy metal concentration in plant (mg/kg)
$D_{food\ intake}$ = represent daily intake of vegetables
$B_{average\ weight}$ = represent average body weight
C_{factor} is used to convert fresh vegetable weight into dry weight and is given as 0.085

Statistical Analyses

The concentrations of heavy metals or the data obtained from irrigation soil and vegetables were subjected to one-way analysis of variance (ANOVA) test for assessing the significant difference. The significant difference at 95% level were determined.

Result and Discussion

Key
G = Gwallaga irrigation site
R = Gombe road irrigation site
N= Non irrigation site
ND= Not detected
Wtr = Wastewater
Amr= *Amaranthus caudate*

Table 1: Concentration of Heavy Metals (mg/dm^3) in wastewater used for irrigation

	concentrations					
Samples	**Zn**	**Mn**	**Cd**	**Cu**	**Cr**	**Pb**
G_1Wtr	0.01± 0.00	1.36± 0.0	ND	0.07± 0.00	0.12± 0.0	0.01± 0.0
G_2Wtr	0.03± 0.00	1.17± 0.0	ND	0.07± 0.00	0.42± 0.0	0.01± 0.0
G_3Wtr	0.39± 0.00	1.60± 0.0	ND	0.05± 0.00	0.05± 0.0	0.01± 0.0
R_1Wtr	0.00	0.23± 0.0	ND	0.05± 0.00	0.11± 0.0	0.00± 0.0
R_2Wtr	0.04± 0.00	0.22± 0.0	ND	0.09± 0.00	0.10± 0.0	0.00± 0.0
R_3Wtr	0.02± 0.00	0.17± 0.0	ND	0.06± 0.00	0.00± 0.0	0.00± 0.0

Values are mean ± standard deviation (n=3)

Table 2: Concentration of heavy metals (mg/kg) in Irrigation and non-irrigationSoil

	Concentrations					
Samples	**Zn**	**Mn**	**Cd**	**Cu**	**Cr**	**Pb**
G_1 Soil	83.0 ± 0.00	511.5 ± 0.00	ND	15.5 ± 0.00	23.0 ± 0.00	1.0 ± 0.00
G_2 Soil	50.0 ± 0.00	554.0 ± 0.00	ND	13.5 ± 0.00	17.5 ± 0.00	0.0
G_3 Soil	56.5 ± 0.00	508.0 ± 0.00	ND	17.0 ± 0.00	17.5 ± 0.00	0.0
R_1 Soil	57.0 ± 0.00	566.5 ± 0.00	ND	11.5 ± 0.00	12.5 ± 0.00	0.0
R_2 Soil	64.0 ±0.00	513.0 ± 0.00	ND	8.5 ± 0.000	8.5 ± 0.00	0.5 ± 0.00
R_3 Soil	56.5 ± 0.00	545.5 ± 0.00	ND	10.5 ± 0.00	8.5 ± 0.00	0.0
N Soil	19.5 ±0.00	561.0 ± 0.00	ND	8.0 ± 0.00	16.0 ± 0.00	1.0 ± 0.00

Values are mean ± standard deviation (n=3)

Table 3: Concentration of heavy metals (mg/kg) in Irrigated and non-irrigated *Amaranthus caudatus*

	Concentrations					
Samples	**Zn**	**Mn**	**Cd**	**Cu**	**Cr**	**Pb**
G_1 Amr	31.5 ± 0.00	35.5 ± 0.00	ND	11.0 ± 0.00	0.0	0.0
G_2 Amr	37.5 ± 0.00	63.50 ± 0.00	ND	13.5 ± 0.00	0.0	0.0
G_3 Amr	23.5 ± 0.00	40.0 ± 0.00	ND	17.5 ± 0.00	0.0	0.5 ± 0.00
R_1 Amr	24.0 ± 0.00	72.0 ± 0.00	ND	8.5 ± 0.00	0.0	0.0
R_2 Amr	75.5 ±0.00	133.0 ± 0.00	ND	13.0 ± 0.000	0.0	0.0
R_3 Amr	50.0 ± 0.00	52.5 ± 0.00	ND	13.0 ± 0.00	0.0	0.0
N Amr	25.5 ±0.00	74.0 ± 0.00	ND	5.5 ± 0.00	0.0	0.5 ± 0.00

Values are mean ± standard deviation (n=3)

Table 4: Enrichment Factor of Heavy Metals in *Amaranthus caudatus*

Gwallaga Vegetables						
Vegetables	**Zn**	**Mn**	**Cd**	**Cu**	**Cr**	**Pb**
G Amr	0.3801	0.6727	-	1.4450	0.0	0.0
R Amr	0.6560	0.1209	-	1.6727	0.0	0.0

Table 5: Metal Pollution Index

Samples	MPI(mg/kg)
G Amr	8.213
R Amr	9.957
N Amr	21.602

Table 6: Health Risk Index

	Zn	Mn	Cd	Cu	Cr	Pb
G Amr	0.0316	0.4363	-	0.1175	0.0000	0.0154
R Amr	0.0516	0.8090	-	0.0875	0.0000	0.0155
N Amr	0.0258	0.6945	-	0.042	0.0000	0.0000

Heavy metal concentrations are very high in soil compare to the concentrations in wastewater as shown on Table 2 and 1 above. This because heavy metals tends to accumulate in the soil through continuous irrigation (Marshall *et al.,* 2007). This research shows the presence of all the heavy metals determined except Cd which was not detected and the absence of cadmium might be due to the detection limit of the machine. All the samples shows Mn have higher concentrations as shown on Table 1, 2 and 3. This because manganese makes up about 1000ppm (0.1%) of the earth's crust making it the most abundant element (Emsley, 2001). The heavy metal cocentrations obtained in *Amaranthus caudatus* were below the safe limit of WHO (2007). Table 4 present the enrichment factor (EF) calculated, higher EF suggest poor retention of metal in the soil and more translocation to the plant. In this research Cu has highest EF which implies that there is more translocation of Cu from soil to the *Amaranthus caudatus*. The metal pollution index (MPI) as presented on Table 5 shows that non-irrigated Amaranthus caudatus have higher MPI than the irrigated one, there Amaranthus caudatus cultivated during raining season might pose risk to human health. The health risk index calculated as presented on Table 6 shows that the Amaranthus caudatus is safe for human consumption because all the values obtained are less than 1. The analysis of variance reveals that the heavy metal concentration of the soil and vegetable of the irrigated and non-irrigated site are significant at (P≤0.05).

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