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Absorption of some macro-element and heavy metals by leaves of spinach *Amarantus hybridus* L

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ABSTRACT

Vegetables are the fresh and edible portions of herbaceous plants. They are important food and highly beneficial for the maintenance of health and prevention of diseases. These vegetables however contain heavy metals that can affect the availability of the nutrients and results to toxicity. This research is aimed to determine the rate of absorption of some heavy metals by spinach (*Amaranthus hybridus* L) irrigated with different water sources. Thermometer, Secchi disc and pH meter immersing methods were adopted for estimating temperature, transparency and pH respectively. After nursery proper, a total of 150 seedlings were randomly selected and divided into 3 groups of ten polypots each, each group 1, 2 and three are treated with different water source (distilled, tap and sewage water respectively) Standard chemistry protocol AAS (atomic absorption spectroscopy) were used to determine heavy metals concentration. The results for the determination of water quality indicators revealed sewage water had higher values of Temperature than distilled and tap water respectively. The results showed that sewage water was highly acidic while distilled and tap water was slightly alkaline. The results further showed that distilled and tap water had higher values of Transparency than sewage water. The analysis of accumulation of chemical elements in leaf showed that *Amaranthus hybridus* L revealed significant ($P<0.05$) increase in concentration of zinc and lead in groups irrigated with sewage water compared to distilled and tap water irrigated groups. Also, concentration of Sodium significantly ($P<0.05$) increase in groups irrigated with sewage water compared to distilled and tap water irrigated groups While, concentration of potassium and magnesium significantly ($P<0.05$) increase in groups irrigated with sewage compared to distilled water irrigated groups. In conclusion, the present study entailed that all sample water has richer plant chemical nutrient contents, hence favours better growth and development of plants. However, following the high concentration of some toxic heavy metals in the leaves of *Amaranthus hybridus* L irrigated with sewage, it is not advisable to consume such leafy vegetable, due to the possibility of chronic health hazards to the consumers.

Keywords: Heavy metals, Absorption, *Amarantus hybridus*, macro-elements

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1. INTRODUCTION

Globally, water has been a major requirement for the growth and development of biotic life (Vörösmarty et al., 2010) Water has for long exerted strong influence on the choice of place for human settlement. Of all human activities, agriculture and industrial activities have been identified as the highest consumer of water (Aulakhet al., 2009). With time, increase in population and sophisticated advancements in man's industrial sectors have resulted in drastic scarcity of available natural/clean water (Rosen, 2000). Hence, the use of waste water either in the raw state or purified form in agricultural activities, especially in the off season vegetable crop production and ornamentals became imperative for decades in Europe, North America and Australia (Raschid-Sally and Jayakody, 2009). Following the success of propagating vegetable crops, ornamentals and lawn maintenance, using waste water, the practice became recognized in many other countries like India, China, Middle East etc. (Raschid-Sally and Jayakody, 2009).

Nigeria by virtue of its population size generates several tonnes of agricultural, industrial, municipal and domestic wastes that overwhelms the nation's waste disposal machinery and pose an environmental pollution problem (Okhuoya et al., 2010). These so called wastes constitute a negative factor both in the economic evaluation of existing industrial and agricultural operations and because of the adverse environmental effects resulting from their disposal (Dixon et al., 2013). Sadly, much of this waste is either burned, shredded or used as landfill or for improvement of soil quality, even though these wastes constitute a potentially valuable resource and can be recycled for the production of edible food for man (Porteous, 2008). Although physical and chemical technologies may, in some cases, play important associated roles for handling these wastes (Gadd, 2009). Biotechnological approaches are essential for the emergence of practical conversion processes which can be applied to situations in developing countries throughout the world where large scale capital intensive operations are inappropriate (Ruane et al., 2010).

Heavy metals are naturally occurring compounds, present in different food substances in varying amounts depending on the kind of food, mode of its irrigation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances (Jiménez, 2006). The use of pesticide, herbicide and fertilizer and irrigation water stimulate the concentration of this heavy metals, although there is limitation in the use of pesticide, herbicide and fertilizer in northern Nigeria especially in rural areas mainly because of accessibly and cost of the chemicals and also with regard to fertilizer, available alternatives are abundant i.e. (the use of local manure) (Onakpa et al., 2018). As such irrigation might be the major source of heavy metals. Therefore the purpose of this article is to access the concentration of heavy metals absorption in leaf (*Amaranthus hybridus* L.) irrigated with distilled water, tap water and sewage water. *Amaranthus hybridus* L quickly comes to mind and thus is selected for this study, because of its popularity in most diets, particularly in the northern states of Nigeria.

2. MATERIALS AND METHODS

Study Area

All experiments were conducted at the laboratory and Botanical garden Department of Plant Sciences and Biotechnology of Kebbi State University of Science and Technology, Aliero, Kebbi State, Nigeria. It is located at latitude 12.3061°N and longitude 4.4920°E, Aliero Local Government shares common boarders with Gwandu Local Government area on the Northern, Jega Local Government area by the Southwest and by East is Tambuwal Local Government of Sokoto State. The North West is by Birnin Kebbi Local Government (Uzondo, 2007).

Collection and Identification of Plant Sample

The study is carried out in the Botanic Garden of the Department of Plant Science and Biotechnology Kebbi State University of Science and Technology Aleiro, Nigeria. Seeds of *Amaranthus hybridus* L were purchased from Aleiro Central Market. The seeds were identified and confirmed in the Herbarium of the Department of Plant Science and Biotechnology following the procedure after which the seeds were subjected to viability test, as described by Bewely and Black, (1985). The seeds were soaked in water for fifteen minutes after which, seeds that floated are decanted while seeds that sank are obtained, air-dried and packed in brown envelope before storing in a dessicator for four days, to enable other processes of raising the seedlings to be accomplished.

Pre-nursery and Nursery Proper

Broadcasting method was employed at pre-nursery stage. Four beds (1m x 10 m) were made in the Botanic Garden, Kebbi State University of Science and Technology Aleiro. In preparing the beds, one bag (15 kg each) of composted poultry manure are ploughed into each bed, after which the seeds were sowed and then watered with the aid of watering can while nursery proper was conducted according to the method described by Nzekweet al., (2016).

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Transplanting of Seedlings from the Nursery

A total of one hundred and fifty (150) seedlings of near uniform size and vigorous growth are extracted from the nursery bed with the aid of transplanting fork. The seedlings are planted in thirty poly pots at the rate of five seedlings per poly pot filled with 1:1 mixture of Top Soil and Poultry Droppings. The potted seedlings are displayed in a screened house in the Botanic Garden to prevent other water sources (Rainwater) from intervening with the treatments. The potted seedlings were watered with distilled water daily and were allowed to grow for four (4) days to allow the seedlings recover from transplanting shock. And then the seedlings were divided into different treatment groups and the experiment was conducted as follows:

Groups	Treatment
Groups 1	Serves as control and receive distilled water in ten (10) poly pots containing five (5) seedlings each.
Group 2	Received tap water in ten (10) poly pots containing five (5) seedlings each.
Group 3	Received sewage water in ten (10) poly pots containing five (5) seedlings each.

Determination of Water Quality

The temperature is taken by immersing the thermometer into the water samples (distilled water, Sewage water and tap water) collected in a beaker. Values were recorded in ^{o}C transparency was measured with Secchi disc and recorded in centimetre (cm) as described by Chinedu et al., (2011).

Preparation of Samples for Heavy Metals Determination

Sample (leaves) was dried, milled and two grams of sample is weighed into a clean crucible. This is ashed in a furnace at a temperature of 450^{o}C for about 3 hrs. The ashed sample is allowed to cool before they were dissolved in 5ml of 30% HCl in a 50 ml flask. Then made up to mark i.e. (50 ml) with de-ionized water and then filtered (Hossner, 1996).

Determination of Heavy Metals and Macro Element

The determination of heavy metals (copper, zinc, mercury, arsenic, iron, cadmium and lead) was conducted according to the method described by Hortwiz and Latimer, (2005). Also, macro elements (magnesium, calciumnitrogen, chloride and phosphate) were determined according to the method described by Hortwiz and Latimer, (2005) while potassium was determined following the methods of Jan et al., (2017).

Data Analysis

The data generated from the study are presented as Mean ± Standard error of mean and subjected to one-way analysis of variance (ANOVA) and statistical difference between means were seperated using Duncan multiple comparison test using statistical package for social science (SPSS) version 20. Values are considered statistically significant at $P<0.05$.

3. RESULTS

Water Quality Indicators

The results for the determination of water quality indicators are presented in (Table 1). Sewage water had higher values of Temperature than distilled and tap water respectively. The results showed that sewage water was highly acidic while distilled and tap water was slightly alkaline. The results further showed that distilled and tap water had higher values of Transparency than sewage water.

Table 1 Water Quality Indicators of the Water Samples

Indicators	Distilled Water	Tap Water	Sewage Water
Temperature (^{O}C)	25.00	26.00	31.00

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Transparency(cm)	70.00	65.50	33.8
Ph	6.8	6.90	3.8

Accumulation of Heavy Metals in Leaves of *Amaranthus hybridus* L Treated with Deferent Water Samples

The results of the analysis of the accumulation of chemical elements in the leaves of *Amaranthus hybridus* L is presented in (Table 2). The results showed that *Amaranthus hybridus* Lirrigation with distilled, tap and sewage water accumulates toxic heavy metals (Zinc, Cadmium, Copper, Arsenic and Lead,) and other essential elements (Potassium, Magnesium, Calcium, Sodium, Iron chloride and Phosphate). However the concentration of zinc and lead significantly (P<0.05) increase in groups irrigated with sewage water compared to distilled and tap water irrigated groups. Also, concentration of Sodium significantly (P<0.05) increase in groups irrigated with sewage water compared to distilled and tap water irrigated groups While, concentration of potassium and magnesium significantly (P<0.05) increase in groups irrigated with sewage compared to distilled water irrigated groups.

Table 2 Accumulation of Heavy Metals in Leaves of *Amaranthus hybridus* L

Chemical elements	Sewage Water (PPM)	Tap Water (PPM)	Distilled Water (PPM)
Zinc	3.57 ± 1.17^{b}	1.03 ± 0.44^{a}	-0.14 ± 0.09^{a}
Cadmium	0.49 ± 0.17^{a}	0.10 ± 0.06^{a}	0.11 ± 0.05^{a}
Cupper	-2.16 ± 0.34^{a}	-2.55 ± 0.12^{a}	-2.45 ± 0.09^{a}
Arsenic	-109.90 ± 65.87^{a}	-102.99 ± 14.34^{a}	-67.44 ± 10.16^{a}
Lead	3.55 ± 0.98^{b}	-1.39 ± 0.15^{a}	-0.47 ± 0.24^{a}
Calcium	29.69 ± 7.61^{a}	86.94 ± 27.52^{ab}	167.97 ± 29.66^{b}
Iron	21.05 ± 16.98^{a}	3.92 ± 2.33^{a}	1.44 ± 0.07^{a}
Potassium	442.34 ± 59.18^{b}	272.71 ± 56.19^{ab}	245.79 ± 35.57^{a}
Magnesium	143.22 ± 11.56^{b}	100.02 ± 20.32^{ab}	74.23 ± 8.42^{a}
Sodium	23.00 ± 5.12^{b}	10.95 ± 2.82^{a}	8.40 ± 0.71^{a}
Chloride	13.83 ± 1.20^{a}	13.57 ± 1.23^{a}	15.10 ± 1.55^{a}
Phosphate	9.91 ± 0.73^{a}	9.83 ± 0.83^{a}	9.22 ± 0.08^{a}

Values are presented as mean ± SEM (n = 3) values in column having same superscript are not significantly different at (P>0.05) using One-Way ANOVA, followed by Duncan multiple comparison test with SPSS version 20.0.

Comparism of Heavy Metals Concentration with FEPA Standard in Leaves of *Amaranthus hybridus* L

The results of the analysis of the comparism of chemical elements with FEPA in the leaves of *Amaranthus hybridus* L is presented in (Table 3). The results revealed sewage water has high concentration of zinc, lead, iron and phosphate (3.56, 3.55, 29.69, 21.05 and 9.91mg/l) respectively which are above Federal Environmental Protection Agency Standard (FEPA), while tap water has a slightly high zinc concentration of 1.03 and high calcium and phosphate concentration of 86.94 and 983mg/l respectively.

Table 3 Comparism of Heavy Metals Concentration with FEPA Standard in Leaves of *Amaranthus hybridus* L

Chemical Elements	Sewage Water (mg/l)	Tap Water (mg/l)	Distilled Water (mg/l)	FEPA Standard mg/l
Zinc	3.56 *	1.03 *	-0.14	<1.0

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Cadmium	0.49	0.10	0.11	<1.0
Cupper	-2.16	-2.55±	-2.45	<1.0
Arsenic	-109.90	-102.99	-67.44	<1.0
Lead	3.55 *	-1.39	-0.47	<1.0
Calcium	29.69 *	86.94 *	167.97 *	3.0
Iron	21.05 *	3.92	1.44	20.0
Potassium	442.34	272.71	245.79	3500-4700
Magnesium	143.22	100.02	74.23	200
Sodium	23.00	10.95	8.40	2300
Chloride	13.83	13.57	15.10	200-600
Phosphate	9.91 *	9.83 *	9.22 *	5.0

Key: value denoted with * are above acceptable standard, NA= Not available

4. DISCUSSION

According to (Passow, 2002) transparency is how easily light can pass through liquid substance e. g water which depends on the amount of particles in the water. The results of the present study showed that Sewage water had lower values of the water quality indicators (Transparency), than both tap and distilled water. The results agreed with the work of Nweze and Eji, (2005) who reported that sewage water collected from the University of Nigeria, Nsukka sewage oxidation pond had heavy load of dissolved and decomposing matter. An article working on the same sewage water source reported the presence of a host of pathogens (Mbagwu, 2003). Thus, the presence of these materials and organisms in sewage water resulted in the depletion of the quality of sewage water. The presence of the decomposing materials also reduced the sewage water transparency as observed in the present study. Temperature affects the solubility and reaction rates of chemicals. In general, the rate of chemical reactions increases with increasing water temperature (Akiya and Savage, 2002).

In the present study, toxic heavy metal accumulation in *Amaranthus hybridus* L irrigated with distilled, sewage and tap water respectively showed that the leaves which is mostly consumed by man as leafy vegetable, when irrigated with sewage water accumulated higher values of some toxic heavy chemical elements (Cu, Zn, Pb, Cd and Ar) than *Amaranthus hybridus* L grown using distilled and tap water. Although the results revealed that the values accumulated by the leaves of the species irrigated with sewage water are above the acceptable level recommended by the Federal Environmental Protection Agency (FEPA) however, this values may even persist on prolong usage of sewage water for irrigating this plants (Khanna, 2011). There are several reports on the absorption and accumulation of toxic heavy metals in plant tissues irrigated with sewage water or any other waste and water pollutants (Example, petroleum products). Ali and Khan, (2009) reported that toxic heavy metals present in water used in growing food crop plant species may enter food chain, thus posing a serious health hazard to the consumers of such food items. Chandra and Kumar, (2017) reported the accumulation of toxic heavy chemical elements in the leaf of *Amaranthus* spp irrigated with sewage water. Kora, (2020) reported high concentration of toxic heavy chemical elements and high load of pathogens in sewage water used in raising food crops in self-help farming practices by rural farmers. The results of the present study which showed that *Amaranthus hybridus* L accumulated heavy metals in its leaves irrigated with sewage water to levels beyond those irrigated with distilled and Tap water respectively although not above FEPA recommended standards implied that the heavy metals may have entered the food chain. Thus, continual utilization of such food materials using sewage water may in future pose serious health hazards.

Numerous reports have shown that consumption of food materials (leafy vegetables, fruits, water, mineral drinks, etc) that accumulated heavy metals at a concentration that is above the recommended acceptable standards of Federal Environmental Agency (FEPA) or the United State Environmental Agency (USEPA) are associated with different types of health hazards (Maryam, 2011).

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5. CONCLUSION

In conclusion, following high concentration of some toxic heavy metals in the leaves of *Amaranthus hybridus* L irrigated with sewage, it is not advisable to consume such leafy vegetable, due to the possibility of chronic health hazards to the consumers. Additionally, based on the information on the health hazards associated with producing vegetables using sewage water and the findings of the present study, it can be concluded that the use of sewage water in irrigating vegetables as well as the consumption of such vegetables should be discouraged.

Ethical approval

Not applicable.

Informed consent

Not applicable.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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