**BIOACCUMULATION OF SINGLE AND BINARY APPLICATIONS OF CADMIUM AND ZINC CONTAMINANTS IN OKRA (***Abelmoschus esculentus***)**

**ABSTRACT**

The pathways of toxic exposure are mostly from air, water, soil and food. Of all these pathways, soil remains the principal pathway toxic wastes like dyes and heavy metals are discharged into the food chain. Soil contaminated with heavy metals is now a universal challenge leading to retardation in plant growth and yield. A study of single and binary application of zinc and cadmium metals concentration was compared at different levels in okra parts (fruit, leaf, stem and root). The samples under different levels of concentration of these metals were digested using a 1:4 mixture of concentrated HClO4 and HNO3. The acid digests were analyzed using The Flame Atomic Absorption Spectrophotometer.

Contaminating the okra plant with increasing concentration of cadmium as contaminants 1, 2 and 3 shows a decrease in the growth area, percentage moisture content, fresh weight and root length which varies between 8.10 – 7.77cm2; 76.28 – 72.15%; 36.60 – 33.50g; 17.43 – 13.43cm respectively. Contaminants 4, 5 and 6 also show an inhibition of the growth area from 7.97cm2 to 5.34cm2; percentage moisture content from 73.64 – 69.97%; fresh weight from 47.00 – 35.60g; root length from 18.20 – 16.70cm respectively. On the contrary, the mixture of both metals as applied to contaminants 7 – 9 rather favor the growth area, percentage moisture content, fresh weight and root length of the okra plant from 7.97 – 8.55cm2; 70.38 – 74.41%; 30.45 – 38.25g; 14.34 – 17.90cm respectively. The root and fruit parts accumulated cadmium more than the stem and leafy parts. It was also observed that the leaf part has highest retention for zinc. The presence of Zn leads to decrease in the bioaccumulation of Cd in the case of binary application of metals. It is evident that estimated daily intakes of metals in okra fruit under all the contaminants examined were significantly lower than the recommended values.

**Key words:** Bioaccumulation, Heavy metals, Vegetable, Okra, Wastewater

**INTRODUCTION**

Okra is a vegetable plant majorly cultivated and consumed in Asia and Africa. It belongs to the family called the Malvaceae. Water is essential for the growth and productivity of okra, which is consumed all year round. The accessibility of this crop in dry season is hindered by poor preservative techniques and absence of rainfall during dry season hence the need for irrigation practices.

Okra is called bhindi in India; In Thailand it is referred to as krajiab kheaw and lady’s finger in England (Ndunguru & Rajabu, 2004). Alternatively, it is called quiabo in Portuguese and Angola; quimbombo in Cuba; gombo in France. It is named qiu kui in Taiwan (Siemonsma & Kouame, 2000). The binomial nomenclature for okra is *Abelmoschus esculentus* (Kumar *et al.,* 2010). Okra is rich in proteins, carbohydrates, fibres, vitamins and minerals. Its fruits can be cooked, fried, boiled or eaten raw. In medicine, okra serves as a substitute for plasma and expander of the blood volume (Kumar *et al.,* 2010).

Concern has recently been raised about the rise in environmental damage and universal public health issues linked to heavy metal pollution. Effluents and wastes from domestic and industrial operations constitute the principal bases of heavy metals which supports its constant increase in both the hydrosphere and the lithosphere. Heavy metals possess high density and these are toxic for living organisms irrespective of their concentrations due to their buildup in the food ecosystem (Ahmed *et al.,* 2019; Oves, *et al.,* 2012; LWTAP, 2004). Due to the heavy metals buildup and their exposure into the food chain, growing vegetables alongside brooks across large cities has become a major global concern in recent decades.

These heavy metals have been a serious threat for the plant production as they lead to the alteration in various physiological, morphological, and biochemical changes and diverse toxicity symptoms such as chlorosis and necrosis in plants after accumulation (Forti et al., 2007). When present in higher concentrations, heavy metals interfere with the essential biomolecules of cells as nuclear proteins and DNA that ultimately produce excessive numbers of reactive oxygen species (ROS). These ROS impose severe morphological, metabolic, and physiological abnormalities in plants. The elevated content of such heavy metal in soils is an alarming issue in agricultural production due to the undesirable effects on food safety and marketability.

In Nigeria, soil polluted with heavy metals is gradually increasing due to the scarcity of rains and the use of recycled wastewater for irrigation as is often the case in semiarid regions (Forti et al., 2007).

**MATERIALS AND METHODS**

Analytical grade reagents and distilled water were used throughout the study.

**Preparation of soil for planting**

Sandy-loam was used as the soil for planting the vegetable which was collected on the same location and at the same time. It was then thoroughly mixed (to ensure an even distribution of the soil nutrient) and sieved to remove large particles for proper aeration and water percolation.

**Planting Processes**

Seeds of Okra (*Abelmoschus esculentus*) were acquired from National Horticultural Research Institute (NIHORT), Ibadan, Oyo State, Nigeria. The seeds were washed thoroughly in distilled water; a clean and new suitable plastic pots, dimension (30cm × 40cm) were also provided. After the planting of the okra seeds into the pots at 2cm depth wetting with ordinary water follows and this was done daily and consequently until it begins to germinate.

The plant pots were arrayed into four groups based on the heavy metal contaminants to be applied. The first group was the control, with no introduction of heavy metal contaminant. The second group was cadmium contaminant introduced at concentrations of 10, 20 and 40ppb respectively. Then the same process was repeated for zinc contaminant in the third group but at concentrations of 20, 40 and 80 ppm respectively. The last group has the mixture of both heavy metals but in this case, the concentrations of the zinc was kept constant at 40ppm while cadmium’s was varied at 10, 20 and 40ppb respectively, making the total number of each group to be nine (9) i.e. there are three pots for each concentration of heavy metals contaminants. All the pots were ensured to be labelled according to the concentration of the heavy metals applied. The application of solution of the contaminants begun two weeks after germination and this was done on weekly basis for four consecutive times.

The pots are then classified thus: For singly application: cadmium with 10ppb is regarded as contaminant 1; cadmium with 20ppb is regarded as contaminant 2; cadmium at 40ppb is regarded as contaminant 3; zinc at 20 ppm is regarded as contaminant 4; zinc at 40 ppm is regarded as contaminant 5; zinc at 80 ppm is regarded as contaminant 6. For binary application: zinc at 40 ppm and cadmium at 10 ppb is regarded as contaminant 7; zinc at 40 ppm and cadmium at 20 ppb is regarded as contaminant 8 and zinc at 40 ppm and cadmium at 40 ppb is regarded as contaminant 9.

**Measurement of Parameters**

The parameters determined for this work include; the growth, percentage moisture content, metal concentration in okra parts using Atomic Absorption Spectrophotometer (AAS) and Health risk assessment of heavy metals.

In determining the growth parameter, the shoot length and the stem widths were measured and recorded weekly until the harvest period with the aid of measuring tape and vennier caliper respectively.

Percentage moisture content was determined on the whole okra crop system. After it was carefully uprooted, it was rinsed with distilled water, air dried, then weighed and recorded. This stands for the fresh weight. It was then oven dried at 80°C to a constant weight and recorded. The heavy metals parameter was determined to assess the accumulated concentration using the Atomic Absorption Spectrophotometer (AAS) by firstly digesting the dried samples with HNO3 and HClO4 mixture in a ratio of 1:4.

**Sample Digestion**

A 0.5g of ground dried sample from each plant part of the okra vegetable was weighed and transferred into 150ml conical flask followed by adding 10ml diacid mixture of 1:4 (Perchloric acid and Nitric acid) and thereafter kept overnight for partial digestion. The partially digested content in the conical flask was heated over a thermostated hot plate maintained at 110–1500C for about 1 hour in a fume cupboard for complete digestion. This procedure was done until the content became colourless. After this, the flask was brought down and cooled for 5-10 minutes and the digested aliquot was diluted with distilled water, filtered using (Whatman No. 42) and made up to 50ml in a volumetric flask. Subsequently, this was poured into 50 mL plastic vial in preparation for Atomic Absorption Spectroscopy analysis to evaluate for the zinc and cadmium metals concentrations (Singh *et al.,* 1999).

**Concentration of Heavy Metal in okra using A.A.S. Analysis**

The analyses were done at wavelength of 213.90nm and 228.80 nm for zinc and cadmium respectively, and results were covered to definite concentration of metals in samples using;

Concentration (mg/Kg) = $\frac{RV X VE}{WS}$ (i)

Where RV = Read Value (mg/L); VE = Volume of extract (mL);

WS = Weigh of the sample digested (g).

**Percentage Moisture Content**

Percentage Moisture Content is the moisture content of the samples before analysis;

The % moisture content is calculated thus:

% MC = $\frac{W₁ -W₂}{W₂}$ x 100% (ii)

Where W1 = weight of fresh samples (g),

 W2 = weight of dry samples (g)

**Health risk assessment of heavy metals**

Health risk assessment entails relevant evaluation in research that is associated with human safety (Aigberua et al., 2018). The estimated daily intake of metals (EDIM) is used to assessment the health risk of the heavy metals. The assessment was calculated for two groups involving adult (with 60kg average body weight) (Iwegbue et al., 2015) and children (having 35kg average body weight) (Aigberua et al., 2018; Kigigha et al., 2017). The consumption rate of 40g was used which was established on the per capital consumption of 14.6 kg per annum for the estimation of dietary intake of metals. The provisional tolerable weekly intake (PTWI) was used as a standard for each metal exposure (Iwegbue et al., 2013).

**Estimated daily intake of Metal (EDIM)**

The daily intake of heavy metals was evaluated to know the average daily concentration of metal into the body system of the consumer. The weight of humans can affect the tolerance of contaminants. The ingestion of metals on a daily basis is dependent on both the metal concentration in food and the daily food consumption (Iwegbue et al., 2015; Udowelle et al., 2017; Aigberua et al., 2018)**.** The average daily intake of vegetable for adults and children were 0.345 and 0.232 kg/person/day respectively (Wang *et al*., 2005).

EDIM = $\frac{CM ×DFI}{BW}$ (iii)

Where EDIM is the estimated daily intake of Metals (µg/kg bw/day), CM is the Concentration of heavy metal in the samples (okra fruit) (mg/kg), DFI = Daily food intake (kg person-1 day-1), and BW is the Body weight (kg person-1).

**RESULTS AND DISCUSSION**

**Table 1: Effect of Zinc and Cadmium on okra growth**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **METAL/CONC** | **GROWTH****AREA (cm2)**  | **% MC** | **FRESH WEIGHT (g)** | **ROOT LENGTH (cm)** |
| CONTROL | 7.99±0.02 | 76.26 | 35.30±0.01 | 18.20±0.30 |
| Contaminant 1 | 8.10±0.11 | 76.28 | 36.60±1.30 | 17.43±0.24 |
| Contaminant 2 | 8.05±0.06 | 72.36 | 34.20±1.10 | 15.73±1.65 |
| Contaminant 3 | 7.77±0.22 | 72.15 | 33.50±1.80 | 13.43±3.24 |
| Contaminant 4 | 7.97±0.02 | 73.64 | 47.00±5.22 | 18.20±0.00 |
| Contaminant 5 | 7.68±0.31 | 72.36 | 43.00±4.20 | 18.13±0.07 |
| Contaminant 6 | 5.34±1.75 | 69.97 | 35.60±4.50 | 16.70±1.65 |
| Contaminant 7 | 7.97±0.02 | 70.38 | 30.45±4.64 | 14.34±3.20 |
| Contaminant 8 | 8.00±0.01 | 71.29 | 37.85±3.70 | 17.72±0.73 |
| Contaminant 9 | 8.55±0.56 | 74.41 | 38.25±3.33 | 17.90±0.40 |

**N.B:** Contaminants 7, 8 and 9, zinc is kept at constant concentration of 40ppm while cadmium is varied at concentrations 10ppb, 20ppb and 40ppb respectively. |  |  |  |  |  |

Table 1 shows the growth area of okra before contamination and after contamination. The result shows that when contaminated with cadmium, there is increase in the growth area for contaminants 1 and 2 when compared with the control but decreased with increase concentration of cadmium contaminant. The result for zinc shows that at contaminants 4 and 5, the growth area is lower than that of the control which may be due to bioaccumulation but at contaminant 6, there was a significant reduction in the area when compared with the control. It was deduced that excessive concentration of the metals will lead to the inhibition of the growth area of the okra plant. On the contrary, the mixture of both metals (Zn + Cd) rather favor the growth area of the okra plant and this effect could be explained from the fact that zinc will displace cadmium when they combine (as in from electrochemical series) thereby favoring the growth area since the concentration of the zinc was kept constant for the binary application. It should be noted that zinc is essential to vegetable growth at moderate concentration.

It is noteworthy that the increased singly applications of cadmium and zinc contaminant concentrations lead to decrease in the percentage moisture content of the okra fruit. It was also observed that the introduction of contaminated water decreases the root length of the plant while the binary application shows an increasing trend.

**Table 2: Concentrations of cadmium contaminants (mg.Kg-1) in the okra parts**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Control** | **Contaminant 1** | **Contaminant 2** | **Contaminant 3** |
| **FRUIT** | 0.40 | 3.15 | 6.54 | 0.83 |
| **LEAF** | 1.14 | 2.48 | 1.27 | 0.14 |
| **STEM** | N.D. | 0.02 | 5.34 | 0.27 |
| **ROOT** | 12.07 | 11.34 | 4.42 | 61.78 |

**N.D. – Not detected**

The result in table 2 shows the highest accumulation of cadmium at the root part of the okra plant for control, contaminant 1 and contaminant 3 singly applications with concentrations of 12.07, 11.34 and 61.78 mg.Kg–1 respectively. The fruit part accumulates cadmium more than other parts at contaminant 2 application with concentration of 6.54 mg.kg–1.

The stem and leafy parts seem not to retain cadmium because the concentration was reducing even at the application of higher concentrations of contaminants. The root and fruit parts accumulated cadmium more than the stem and leafy parts.FAO/WHO maximum permissible value of cadmium is 0.2 mg/kg or 200 ppb.

**Table 3: Concentrations of zinc (mg.Kg-1) in the okra parts**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Control** | **Contaminant 4** | **Contaminant 5** | **Contaminant 6** |
| **FRUIT** | 16.31 | 16.90 | 18.60 | 21.03 |
| **LEAF** | 38.65 | 39.92 | 60.80 | 74.94 |
| **STEM** | 6.00 | 27.43 | 32.67 | 36.80 |
| **ROOT** | 15.27 | 32.04 | 61.98 | 73.99 |

The result in table 3 shows that all parts accumulated zinc but the root part accumulated the highest metal (61.98 mg.Kg–1) at singly application of contaminant 5. At a persistent pattern, all parts showed increasing concentration of metal at increasing contaminant application. This is evident where the concentration of zinc at the fruit part rose from 16.90 to 21.03 mg.Kg–1; at the leaf part, 39.92 to 74.94 mg.Kg–1; the stem part, 27.43 to 36.80 mg.Kg–1 and the root part, from 32.04 to 73.99 mg.Kg–1. It was also observed that the leaf part has highest retention for zinc at control and contaminants 4, 5 and 6 applications with concentrations of 38.65, 39.92, 60.80 and 74.94 mg.Kg–1 respectively. The fruit part showed least accumulation of the metal. FAO/WHO maximum permissible value of zinc is 99.4 mg/kg or ppm

**Table 4: Mixture of zinc and cadmium concentrations (mg.Kg-1) in the okra parts**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Control** | **Contaminant 7** | **Contaminant 8** | **Contaminant 9** |
| **FRUIT (Zn)** | 16.31 | 26.71 | 86.14 | 14.92 |
|  **(Cd)** | 0.40 | N.D. | N.D. | N.D. |
| **LEAF (Zn)** | 38.65 | 76.32 | 71.27 | 53.10 |
|  **(Cd)** | 1.14 | 0.75 | N.D. | 0.88 |
| **STEM (Zn)** | 6.00 | 44.91 | 27.56 | 34.47 |
|  **(Cd)** | N.D. | 4.95 | 0.09 | 0.24 |
| **ROOT (Zn)** | 15.27 | 59.14 | 84.21 | 52.34 |
|  **(Cd)** | 12.07 | 1.51 | 8.49 | 4.43 |

**N.D. – Not Detected**

The table 4 shows that all parts of the plant accumulated both cadmium and zinc in various amount. Cadmium is accumulated mostly at the root part of the okra plant considering its concentrations of 12.07, 1.51, 8.49 and 4.43 mg.Kg–1 for control, contaminant 7, 8 and contaminant 9 respectively. The stem part also demonstrated cadmium accumulation with concentrations of 4.95, 0.09 and 0.24 mg.Kg–1 for contaminants 7, 8 and 9 respectively. The fruit part showed no significant retention of cadmium concentration which makes it safe for consumption. The leaf part showed accumulation for control, contaminants 7 and 9 as 1.14, 0.75 and 0.88 mg.Kg–1 respectively.

Zinc accumulated mostly at the leaf part of the plant with concentrations of 38.65, 76.32, 71.27 and 53.10 mg.Kg–1 for control, contaminants 7, 8 and 9 binary applications. The only instance of concerned accumulation at the fruit part is seen at concentration of 86.14 mg.Kg–1 in contaminant 8 and the root part has 84.21 mg.Kg–1 for the same contaminant. All the parts of the plant accumulated zinc in varying concentrations.

It was observed from this study, that the presence of a particular metal can lead to decrease in the bioaccumulation of the other metal in any case of binary application of metals. The similarities of zinc and cadmium made them the metals of interest since they belong to the same group IIB in the periodic table.

**Table 5: Estimated Daily Intake of Metals in Okra fruit**

|  |  |
| --- | --- |
| **Contaminants** | **EDIM** |
| **Cd** | **Zn** |
| **Adult** | **Children** | **Adult**  | **Children** |
| **Control** | 0.0023 | 0.0027 | 0.0938 | 0.1081 |
| **Contaminant 1**  | 0.0181 | 0.0209 | -- | -- |
| **Contaminant 2** | 0.0376 | 0.0434 | -- | -- |
| **Contaminant 3** | 0.0048 | 0.0055 | -- | -- |
| **Contaminant 4** | -- | -- | 0.0972 | 0.1120 |
| **Contaminant 5** | -- | -- | 0.1070 | 0.1233 |
| **Contaminant 6** | -- | -- | 0.1209 | 0.1394 |
| **Contaminant 7** | 0.0000 |  0.0000 | 0.1536 | 0.1770 |
| **Contaminant 8** | 0.0000 | 0.0000 | 0.4953 | 0.5710 |
| **Contaminant 9** |  0.0000 | 0.0000 | 0.0858 | 0.0989 |
| **MTDI****CDPM****FAO/WHO** | 0.20.050.2 | 0.20.050.2 | 602050 | 602050 |

The estimated daily intake of the metals were evaluated according to the average concentration of each metal in the okra fruit and the consumption rate. The estimated daily intake and recommended daily intake values of the studied metals from consumption of okra both in adult and children were shown in Table 5.

The EDI of Cd (singly application) for adult and children in contaminants 1 – 3 were 0.0181 & 0.0209; 0.0376 & 0.0434 and 0.0048 & 0.0055 mg/day respectively.

Cadmium is a metal with high toxicity and it is a non-essential element in foods and water. It accumulates mostly in the liver and kidneys resulting to dysfunction of the kidney with increased secretion of proteinuri etc (Adesuyi et al., 2015). According to FAO/WHO (2001), the safe limit for Cd consumption in vegetables is 0.2 mg/kg. The concentration of Cadmium in this study is equal to the permissible levels by FAO/WHO in vegetable. The most common sources of cadmium in plants and vegetables are sewage sludge application, deposition from fossil fuel combustion, phosphate fertilizers etc (Adesuyi et al., 2015).

The EDI of Zn (singly application) for adult and children in contaminants 4 – 6 were 0.0972 & 0.1120; 0.1070 & 0.1233 and 0.1209 & 0.1394 mg/day respectively.

The EDI of Cd and Zn in binary application of contaminants 7 – 9 showed that Cd was not detected for both adult and children therefore confirming the displacement of cadmium by zinc in a combined study since the concentration of the zinc was kept constant for the binary application. Zn indicated an EDI for both the adult and children for binary application as 0.1536 & 0.1770; 0.4953 & 0.5710 and 0.0858 & 0.0989 mg/day. Zinc is essential to vegetable growth at moderate concentration. The trends in the data further suggest the influence of the metal combination.

Zinc is an essential element in human diet and it is necessary to sustain the functioning of the immune system. It is taken up actively by roots (Adesuyi et al., 2015). Zn was quite abundant in all the sampled parts but it does not exceed the Chinese Department of Protective Medicines safe limit in vegetable of 20 mg/kg (Asdeo and Loonker, 2011).

It is very clear from the table that the estimated daily intakes of metals in okra fruit under all the contaminants examined, both for singly and binary applications were significantly lower than the MTDI, Chinese department of Protective Medicine (CDPM) and FAO/WHO values recommended indicating that the okra fruit pose no health risks to consumers and it is safe for consumption.

**CONCLUSION**

The majority of the physical characteristics and heavy metal concentrations found in Okra vegetable samples grown using single and binary applications of heavy metal contamination were observed to be in the range of the permissible limits as suggested by FAO/WHO for vegetable cultivation. This suggests because of the high concentration of heavy metals, eating okra grown by irrigation along a riverbank during the dry season poses a risk to the public's health. Even for heavy metals whose concentrations are within advised limits, there is always a chance that they could pose a health risk because heavy metals can accumulation in the body over time and cause damage to delicate organs.

Metabolic processes like enzymatic activity, redox catalysis, electron transfer make zinc very essential when associated with okra growth (Gupta et al., 2013; Ahamad et al., 2020). On the other hand, Cd is harmful to plant metabolism and are amongst the highest 20 toxic substances as reported by the Agency for Toxic Substances and Disease Registry (ATSDR) (Yan et al., 2020; Alsafran et al., 2021).

Most vegetable species are heavy metal-sensitive at all stages of their lifecycle, cadmium have significant effects on photosynthetic pigments (Saadaoui et al., 2022).

**RECOMMENDATION**

Finding any risk connected to wastewater irrigation, regular risk assessments should be carried out. It is important to design and apply efficient risk management measures in order to reduce the potential negative effects on crop quality and human health.

Therefore, the study recommended routinely assessing and inspecting the heavy metals levels in okra obtained from the study-site, likewise for the heavy metals found to be within the safe limit, as the accumulation of these metals in soil and water used for cultivation and irrigation may increase undetected and pose a threat to public health, the values of which would only be known through experimental investigations.

Nowadays, cleaning up contaminated soils with the aid of heavy metal-absorbing plants (phytoremediation) is a green approach that encompasses phytodegradation, phytoextraction, rhizo-filtration, phytovolatilization and phytostabilization for heavy metals in soil–food crop subsystems.

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