

## Determination of Bio-concentration Factor of Heavy metal from Soil to Onion around Mojo, Meki and Ziway area of Oromia Ethiopian

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**Abstract:** This study was conducted on the transfer of heavy elements from soil to plant edible part is best described by considering the bio-concentration factor. The soil and onion sample were collected purposely and determined the concentrations of heavy metals (Fe, Cu, Zn, Mn, Cr, Cd, and Pb) mg/kg. The samples were extracted using wet digestion method and measured by with AAS. The result shows that all the results were below 1, that indicating the bio-concentration factors was high in soil relative to onion bulb. On the other word; BCF below 1.0 indicates high heavy metal concentration in soil in relation to levels in onion bulb.

**Key Words:** - AAS, Bio-concentration, Soil, Onion and Wet digestion

### INTRODUCTION

Heavy metal contamination is a major problem of the environment especially of growing medium sized cities in developing countries primarily due to uncontrolled pollution levels driven by causative factors like industrial growth and heavy increase in traffic using petroleum fuels. Heavy metal contamination may occur due to factors including irrigation with contaminated water, the addition of fertilizers and metal based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale (Duran et al., 2007).

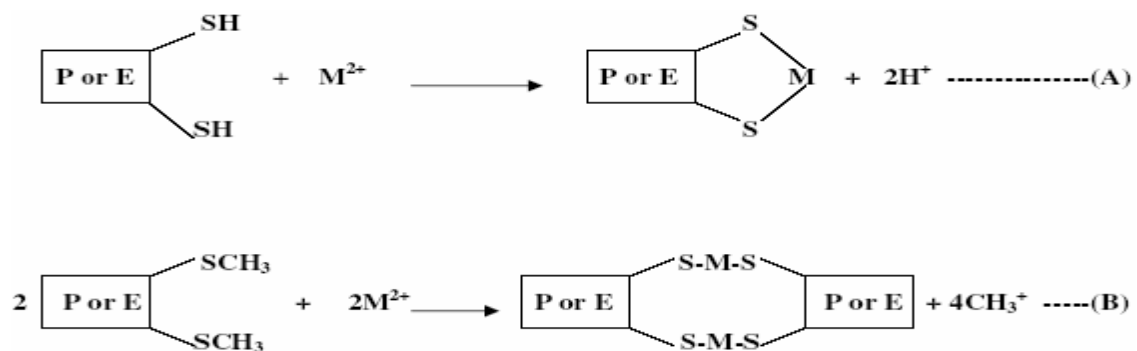
Crops can uptake toxic elements through their roots from contaminated soils, and even leaves can absorb toxic elements deposited on the leaf surface. The concentration of heavy metal in different parts of plants is heavily dependent on plant species. The ability of different plant species to accumulate heavy metals has been attributed to their genetic differences. Besides plant species, the availability of metals to plants will depend on their chemical speciation and is determined by the physical and chemical properties of the soil (Queirolo et al., 2000).

The transfer of trace elements from soil to plant edible parts is best described by considering the bio-concentration

factor. BCF is calculated as the ratio of the concentration of heavy metals in the vegetables to that in the corresponding soil where vegetables were obtained, all based on (dry weight) for each vegetable separately. The BCF of above 1.0 indicates higher uptake of heavy metals in vegetables than in the soil and BCF below 1.0 indicates high heavy metal concentration in soil in relation to levels in vegetables and therefore low uptake of heavy metals to vegetables. Several reports have dealt with BCF determinations for vegetables (Liu, et al., 2006). The objective of this study is to know the transfer of heavy metals from soil to onion and causes on human being.

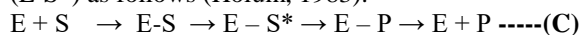
### Biochemistry of toxicity:

The poisoning effects of heavy metals are due to their interference with the normal body biochemistry in the normal metabolic processes. When ingested, in the acid medium of the stomach, they are converted to their stable oxidation states ( $Zn^{2+}$ ,  $Pb^{2+}$ ,  $Cd^{2+}$ ,  $As^{2+}$ ,  $As^{3+}$ ,  $Hg^{2+}$  and  $Ag^+$ ) and combine with the body's biomolecules such as proteins and enzymes to form strong and stable chemical bonds. The equations below show their reactions during bond formation with the sulphhydryl groups (-SH) of cysteine and sulphur atoms of methionine (-SCH<sub>3</sub>) (Ogwuegbu and Ijioma, 2003)



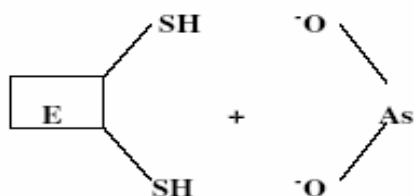
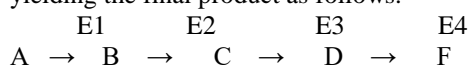
Where: (A) = Intramolecular bonding; (B) = Intermolecular bonding; P = Protein; E = Enzyme; M = Metal

The hydrogen atoms or the metal groups in the above case are replaced by the poisoning metal and the enzyme is thus inhibited from functioning, whereas the protein-metal compound acts as a substrate and reacts with a metabolic enzyme. In a scheme shown below (equation C), enzymes (E) react with substrates (S) in either the lock-and-key pattern or the induced-fit pattern. In both cases, a substrate fits into an enzyme in a highly specific fashion, due to enzyme chirality's, to form an enzyme-substrate complex (E-S\*) as follows (Holum, 1983).



(E = Enzyme; S = Substrate; P = Product; \* = Activated Complex)

While at the E-S, E-S\* and E-P states, an enzyme cannot accommodate any other substrate until it is freed. Sometimes, the enzymes for an entire sequence coexist together in one multi-enzyme complex consisting of three or four enzymes. The product from one enzyme reacts with a second enzyme in a chain process, with the last enzyme yielding the final product as follows.



The most toxic forms of these metals in their ionic species are the most stable oxidation states. For example,  $Cd^{2+}$ ,  $Pb^{2+}$ ,  $Hg^{2+}$ ,  $Ag^+$  and  $As^{3+}$ . In their most stable oxidation states, they form very stable biotoxic compounds with the body's bio-molecules, which become difficult to be dissociated, due to their bio-stabilities, during extraction from the body by medical detoxification therapy.

## MATERIALS AND METHODS

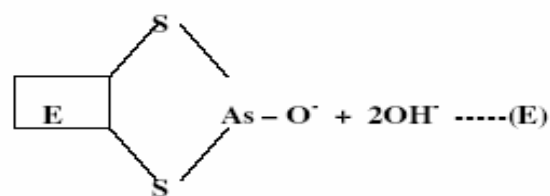
### Description of the study area:

The study was carried at Mojo (Lome woreda, Dunguge village), Ziway (Adami Tulu Jida combolcha wereda, Halaku-golba-boqe and Golbala-aluto villages) and Meki (Dugda Woreda, Shumi Gamo village).

There are five main reasons for selecting the study areas: (i) the areas are under continuous cultivation throughout the year and have been supplying significant portion of a wide variety of vegetables like tomato, onion, cabbage, green pepper, etc to the capital city(Addis Ababa), Hawassa, Adama and for local consumption since long time; (ii) modern farming practices such as mechanized farming, application of agrochemicals (fertilizers, pesticide, insecticides, fungicides, preservative, etc), and selected seeds are significant agricultural inputs used for getting better yield in the areas; (iii) The study areas are located in the region of the Great Rift Valley known to encompass various lakes and water bodies with poor drainage enhancing different minerals and salt deposits. (iv) The study areas are surrounded by many factories such as Mojo

The final product (F) goes back to react with the first enzyme thereby inhibiting further reaction since it is not the starting material for the process. Hence, the enzyme E1 becomes incapable of accommodating any other substrate until F leaves and F can only leave if the body utilizes it. If the body cannot utilize the product formed from the heavy metal - protein substrate, there will be a permanent blockage of the enzyme E1, which then cannot initiate any other bio-reaction of its function. Therefore, the metal remains embedded in the tissue, and will result in bio-dysfunctions of various gravities.

Furthermore, a metal ion in the body's metallo-enzyme can be conveniently replaced by another metal ion of similar size. Thus  $Cd^{2+}$  can replace  $Zn^{2+}$  in some dehydrogenating enzymes, leading to cadmium toxicity. In the process of inhibition, the structure of a protein molecule can be mutilated to a bio-inactive form, and in the case of an enzyme can be completely destroyed. For example, toxic  $As^{3+}$  occurs in herbicide, fungicides and insecticides, and can attack -SH groups in enzymes to inhibit their bioactivities as shown below (Ogwuegbu and Ijioma, 2003).



leather factory, floriculture farms around Meki and Ziway. These factories release effluent to the water used for irrigation. Lake Ziway is also drainage for two rivers from North Meki river which crosses Meki town and Katar river from South East direction. Besides, Awash river, which is mostly used for irrigation in the country, and Mojo River which is close to industrial zone are vicinity to the study areas. (v) There is no updated information on the effect of these factories on vegetables, water bodies and soil.

### Apparatus and instrument:

Chopping board and Teflon knife were used to cut onion bulb samples in to pieces while air-circulating ovens (WTC binder) were used for drying. Porcelain mortar, pestle and crucibles were used during pounding of the samples. Muffle furnace (Nabertherm) was used for dry-ashing process of vegetables samples. A metal concentration determination was done by flame atomic absorption spectrophotometer (FAAS) (Agilent Technologies, 200 Series AA) that used air-acetyleneas fuel-oxidant mixture and that was equipped with deuterium background corrector and hollow cathode lamps.

### Chemicals and reagents:

All the reagents and chemicals used in this study were analytical grade. 70%  $HNO_3$ , 70%  $H_2SO_4$  and 70%  $HClO_4$  was used. Stock standard solution of concentration 1000 mg/L in 2%  $HNO_3$  of the metals Cu, Zn, Cr, Pb, Fe, Mn and Cd standard solutions were used to prepare 100 mg/L intermediate standard solutions.

**Sample collection and preparations:**

All eighteen samples of soil and onion samples each of them three composite were collected using purposive sampling technique method from Mojo, Ziway, and Meki farmlands. Before sampling, there was survey assessment for identification of exact pollution source, distance cover, discharge sites and affected areas.

**Soil sample collection and preparation**

Soil samples were collected from all the corresponding sites of the plant materials at the depth of 0-25 cm using soil auger from agricultural fields. From each three main site, three sub-sites were taken for the purpose sampling. Five soil samples were collected from one sub-site and brought to one for composite. Therefore, three composite soil samples of 1kg were purposely collected from the three sub-sites in each three agricultural areas. Soil samples taken from each sub-sites were separately labeled and transferred into air tight polythene bags and brought to Holeta research center for laboratory analysis.

**Onion sample collection and preparation:**

Onion sample which corresponding sites of five soil samples were collected from one sub-site and brought to one for composite. Therefore, three composite onion samples of 1kg were purposely collected from the three sub-sites in each main sites of three agricultural areas (Mojo, Ziway, and Meki).

**Digestion of soil samples:**

1.0g of each of the ground soil samples were placed in block digester. 15 ml of 70% HNO<sub>3</sub>, 70% H<sub>2</sub>SO<sub>4</sub> and 70% HClO<sub>4</sub> mixture (5:1:1) of tri-acid were added and the content heated gently at low heat on block digester for 2 hrs at 80<sup>0</sup>C and modification was taken on the temperature to 150<sup>0</sup>C for 2hrs until a transparent solution was obtained. After cooling, the digested samples were filtered using what man NO. 42 filter paper.

**Digestion of Onion samples::**

Onion samples were digested using dry ashing method (Alam *et al.*, 2003). 0.5 g of grounded powder samples were weighted and transferred to a clean crucible, which was labeled according to the sample number and dry-ashing process was carried out in a muffle furnace by stepwise increase of the temperature up to 550<sup>0</sup>C and then left to ash at this temperature for 6 hr (Kalagbor and Opusunju, 2015). The samples were removed from the furnace and allowed to cool in the hood carefully. The ash was wetted with 1ml distilled water and 2.5ml conc. HCl was added. After cooling filtered using Whatman filter No.41.

**Analysis of samples:**

Concentrations of chromium (Cr), cadmium (Cd), Copper(Cu), Zink(Zn), Iron (Fe), Manganese (Mn) and lead (Pb) in the filtrate of digested soil and vegetables samples were estimated by using an Atomic Absorption Spectrophotometer. The instrument was fitted with specific lamp of particular metal. Working standard solutions of copper (Cu), zinc (Zn), Iron (Fe), Manganese (Mn), chromium (Cr), lead (Pb) and cadmium (Cd) were prepared from the stock standard solutions containing 1000 mg/L of element in 2N HNO<sub>3</sub>. The instrument was calibrated with calibration blank and five series of calibration standard solutions, these solutions were diluted for desired concentrations to calibrate the instrument.

**Data analysis:**

All the results of analysis were reported as mean  $\pm$  standard deviation of triplicate measurements. The data was computed using Statistical Package for Social Science (SPSS) statistic 10.0 (Microsoft window) for heavy metal analysis. The recorded data was subjected to analysis of variance (ANOVA), to assess the effect of vegetable type and site of production on the concentrations of heavy metal contaminant in the soil and Onion tested. ANOVA was used to test the existence of significant difference between means. In all statistical analyses, confidence level was held at 95%.

**RESULTS AND DISCUSSIONS**Table 1. Concentration of metal in onion bulb samples in mg/kg (mean  $\pm$  SD)

Meta ls	Sites			FAO/WHO (2001mg/kg)
	Mojo Onion bulb	Meki Onion bulb	Ziway Onion bulb	
Cr	4.87 $\pm$ 0.50 <sup>a</sup>	4.13 $\pm$ 0.12 <sup>b</sup>	3.33 $\pm$ 0.12 <sup>c</sup>	2.3
Cu	3.93 $\pm$ 0.61 <sup>a</sup>	1.33 $\pm$ 0.50 <sup>c</sup>	0.87 $\pm$ 0.12 <sup>c</sup>	73.3
Zn	12.42 $\pm$ 0.29 <sup>a</sup>	7.71 $\pm$ 0.61 <sup>c</sup>	13.47 $\pm$ 0.24 <sup>e</sup>	99.4
Pb	0.33 $\pm$ 0.12	ND	ND	0.3
Cd	0.05 $\pm$ 0.02	0.03 $\pm$ 0.01	0.06 $\pm$ 0.02	0.2
Mn	8.20 $\pm$ 0.20	13.40 $\pm$ 0.20	7.53 $\pm$ 0.42	500
Fe	20.87 $\pm$ 0.64 <sup>a</sup>	24.33 $\pm$ 0.61 <sup>c</sup>	0.80 $\pm$ 0.20 <sup>e</sup>	425.5

The concentration of Cr in onion bulb was above the permissible level of (2.3mg/kg) FAO/WHO 2001at Mojo(4.87mg/kg), Meki(4.13mg/kg) and Ziway (3.33 mg/kg). The other metals were within the ranges of FAO/WHO 2001.

**Concentrations of metals in soil samples:**

Concentrations of metals in the soil collected from Mojo, Meki and Ziway farmlands in which vegetable was planted is given in (Table 2).

Table 2. Concentration of metals in soil samples with (mean ± SD), n=3

Metals	Sites			U.A.EPA 1993 (mg/kg) max. con.
	Mojo	Meki	Ziway	
Cr	39.13 ± 0.32 <sup>a*</sup>	18.37 ± 0.15 <sup>b</sup>	7.03 ± 0.10 <sup>c</sup>	3000
Cu	35.88 ± 1.81 <sup>a</sup>	32.80 ± 0.33 <sup>a</sup>	19.25 ± 3.29 <sup>b</sup>	4300
Zn	145.66 ± 4.22 <sup>a</sup>	153.30 ± 5.75 <sup>a</sup>	92.40 ± 7.86 <sup>b</sup>	7500
Pb	0.833±0.289	ND	ND	420
Cd	0.095±0.005	0.083 ±0.029	0.133 ±0.029	85
Mn	1264.82±0.635 <sup>a</sup>	1546.42±0.317 <sup>b</sup>	789.43±0.840 <sup>c</sup>	2000mg/kg ( Itana 2002.
Fe	27,427.33±3.21 <sup>a</sup>	25,922.67±6.25 <sup>b</sup>	9,947.00±3.50 <sup>c</sup>	1500 mg/kg (FAO 1998)

ND- not detected, \* Means followed by different letters within the same row are significantly different at 5% probability level

Fe (27,427.33±3.21mg/kg) is the highest in Mojo soil compared to from Meki (25,922.67±6.25mg/kg) and Ziway(9,947.00±3.5(mg/kg) Farms. This result indicated that concentration of Fe was above the allowable value (1500 mg/kg) (FAO 1998).

In this study the concentration of Fe, Mn and Cr in between Mojo, Meki and Ziway showed significantly different at p<0.05. Zn and Cu have no significant difference at p<0.05between Mojo, Meki; but it showed significance difference with Ziway. The detection of Pb(Meki and Ziway) in soil samples were below detection limit. Except

Fe, all the concentration of metals was below permissible level of (U.A.EPA 1993).

**Bioconcentration factor of metals between soil and onion bulb:**

Heavy metals have the capability to translocate from the soil to the edible parts of the food crop and can be determined by the bioaccumulation factor (BF) (Liu, *et al.*, 2006).

BF= Heavy metal concentration in the food crops edible parts.....eqn. 1

Heavy metal concentration in the soil

Where; BF bioconcentration factor

Table 3. Bio-concentration factor (BCF) of metals between soil and onion bulb (mean ±SD),n=3

Metal	Sites		
	Mojo	Meki	Ziway
Cr	0.1243 ± 0.01185	0.2250 ±0.00458	0.4739 ± 0.01272
Cu	0.1101 ± 0.02093	0.0407 ± 0.01554	0.0455 ± 0.00579
Zn	0.0853 ± 0.00311	0.0504 ± 0.00576	0.1467 ± 0.01577
Pb	0.3961 ± 0.00000	ND	ND
Cd	0.5263 ± 0.14826	0.3000 ± 0.10000	0.4667 ± 0.17638
Mn	0.0065 ± 0.00016	0.0087 ± 0.00013	0.0095 ± 0.00052
Fe	0.0008 ± 0.00002	0.0008 ± 0.00002	0.0001 ±0.0000

ND not detected

All the results were below 1, that indicating the bioconcentration factors was high in soil relative to onion bulb. On the other word; BCF below 1.0 indicates high heavy metal concentration in soil in relation to levels in onion bulb and therefore low uptake of heavy metals to vegetables. Several reports have dealt with BCF determinations for vegetables (Liu, *et al.*, 2006). Among the different metals, Cd showed the maximum transfer factor value (Table 3), which ranged from 0.5263 (Mojo) to 0.3000 (Meki). (Lokeshwari and Chandrappa, 2006) reported that Cd was retained less strongly by the soil and hence it is more mobile than other metals. Hence, among all elements, transfer factor of Cd was highest for assayed from the sampling sites, which showed that Cd is more mobile than the other metals.

**Analysis of variance (ANOVA):**

Variations in the mean levels of metals between the samples were tested whether it was from just a random error or treatment (i.e. difference in mineral contents of soil, onion and water) using one way analysis of variance (ANOVA). Significant differences were obtained (p< 0.05) at 95% confidence levels for Zn, Mn, Fe and Cr in onion bulbs and soil at Mojo, Meki and Ziway. However, the variations for

Pb (soil and onion), Cu, and Cd were not significant different (p< 0.05) in the samples at the three area.

**CONCLUSIONS**

In this study, soil and onion bulb samples were analyzed for the concentration of heavy metals(Cu, Zn, Cr, Fe, Mn, Pb and Cd) for determination of bio-concentration. The bio-concentration factorwas high in soil relative to onion bulb. On the other word; BCF below 1.0 indicates high heavy metal concentration in soil in relation to levels in onion bulb and therefore low uptake of heavy metals to vegetables.

The result of Bio-concentration factors indicates high heavy metal concentration in soil in relation to levels in onion bulb and therefore low uptake of heavy metals to onion. The results generally indicate that the consumption of these onion bulb cause the health hazards respective to Cr and Pb.

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