

Assessment of Some Major and Heavy Metal Contents in Green Pepper (*Capsicum Annum*) and Beetroot (*Beta Vulgaris*) Grown in the Vicinity of the Industrial Area of Ejersa, Ethiopia

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Abstract

Treated waste water were used for irrigation purpose in the vicinity of industrial area of Ethiopia Tannery. The vegetables, beetroot (***beta vulgaris***) and green pepper (***capsicum annum***) growing in the area of the Ethiopia Tannery were tested for its heavy metal contents. For this purpose three samples of waste water from different sites along the channel of the tannery were collected and analyzed by flame atomic absorption spectrophotometry (FAAS). Similarly soil and vegetables were brought from the same area and analyzed to assess their heavy metal contamination. The concentration of heavy metal in vegetables were found that Cu 8.5 mg/Kg in beetroot and 9.5 mg/Kg in green pepper, Cd 0.96 mg/Kg in beetroot and 1.2 mg/Kg in green pepper, Zn 132.4 mg/Kg in beetroot and 100.9 mg/Kg in green pepper, Ca 3739.7 mg/Kg in beetroot and 99 mg/Kg in green pepper and Mg 6262.7 mg/Kg in beetroot and 399 mg/Kg in green pepper. It was found that Cu 0.019-0.027, Cd 0.018-0.023, Cr 3.33-7.95, Zn 0.17-0.29 mg/L and Pb below detection limit of the instrumentation in various treated waste water samples. The results are also compared with the levels of metals in the data from literature. The cadmium content obtained in vegetables showed that the level is higher than the FAO/WHO limits.

Keywords: vegetables, *Waste water*, *Heavy metals*, *Flame atomic absorption Spectrophotometry*

1. Introduction

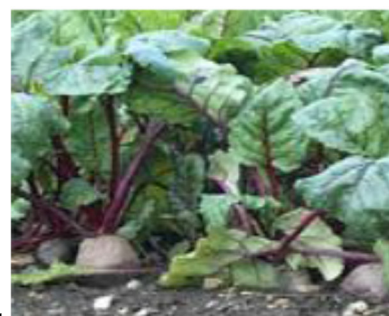
Vegetables and fruits have many similarities with respect to their composition, methods of harvesting and cultivation, storage properties and processing. In true botanical sense, many vegetables are considered as fruits.

Bell pepper or green pepper is a cultivar group of the species *Capsicum annum* (chili pepper). Cultivar of the plant produces fruits in different colors, including red, yellow and orange. The fruit is also frequently consumed in its unripe form, when the fruit is still green. Bell peppers are sometimes grouped with less pungent pepper varieties as "sweet peppers" (Demirezen and Ahmet, 2006). Peppers are native to Mexico, Central America and northern South America. Pepper seed was later carried to Spain in 1493 and from there spread to, African and Asian countries. Today, Mexico remains one of the major pepper producers in the world (Sawidis *et al.* 2001).

Beetroot a plant is native to Southern Europe. It has dense, elongated goosefoot shaped, glossy, mid-green leaves with bright red veins and dark red stalks originating from the root. The leaves are olive green on the upper surface sometimes red on both sides if kept in bright light. Its root is a tap-root, which is edible and pushes deeply in the ground. Roots can be round shaped, cylindrical or tapered. Their color can be white, yellow or red according to the color of the flesh. The leafy tops can also be used as a tasty spinach substitute (Demirezen and Ahmet, 2006).



a



b

Fig.1 (a) green, yellow and red pepper from top to down, (b) beetroot

Waste water irrigation is known to contribute significantly to the heavy metal contents of soils. Waste water expands pollutant from their original or polluted area to non-polluted area (Sawidis *et al.* 2001). If the plants die and decay, heavy metals taken into the plants are redistributed, so the soil is enriched with the pollutants. Uptake and accumulation of elements by plants may follow two different paths i.e., through the roots and foliar

surface (Szpyrkowicz *et al.* 2005).

The uptake of metals from the soil depends on different factors such as their soluble content in it, soil pH; plant growth stages, types of species, fertilizers and soil types (Rajamani 1999). Most of the laboratory research on bio-sorption of heavy metals indicates that no single mechanism is responsible for metal uptake. In general, two mechanisms are known to occur, via “adsorption”, which refers to binding of materials onto the surface and “absorption”, which implies penetration of metals into the inner matrix (Rajamani *et al.* 1999).

Disposal of sewage water and industrial wastes is a great problem. Often it is drained to the agricultural lands where it is used for growing crops including vegetables. These sewage effluents are considered not only a rich source of organic matter and other nutrients but also they elevate the level of heavy metals like Fe, Mn, Cu, Zn, Pb, Cr, Ni, Cd and Co in receiving soils (Rajamani *et al.* 1999). As a result, it leads to the contamination of the food chain, because vegetables absorb heavy metals from the soil, polluted air and water. One important dietary uptake pathway could be through crops irrigated with contaminated wastewater. Heavy metals are not easily biodegradable and consequently can be accumulated in human vital organs. This situation causes varying degrees of illness based on acute and chronic exposures (Sawidis *et al.* 2001).

The characteristics of tannery wastewater vary widely depending on the nature of the adopted tanning process, the amount of water used, the process of hide preservation, the hide processing capacity and the in-plant measures followed to reduce pollution. Treatment of this waste water by conventional biological methods is often inadequate to remove pollutants completely, especially ammonia and tannins, the latter being characterized by low biodegradability, which is common in poly-phenolic compounds. Meanwhile, the biological treatment of wastewaters containing resistant and toxic compounds requires a long duration of time (USEPA 1996).

2. Material and Methods

2.1. Description of sample site

The Ethiopia tannery is located to south of Addis Ababa about 77 Km near Modjo town in the vicinity of lake Koka. The Ethiopia Tannery is a public enterprise originally established under the technical co-operation agreement signed between the governments of Ethiopia and Czechoslovakia on December 11, 1959. The tannery is located in Koka by the lake of the same name near Modjo about 90 Km South of Addis Ababa on a site of some 200,000 square meters.

2.2. Apparatus and Instruments

Ceramic pestle and mortar were used to grind and homogenize the dried vegetables sample and soil sample. A drying oven (Ov 150 SS England) was used to dry vegetables sample. A digital analytical balance (ADAM®) with $\pm (0.01)$ g precision was used to weigh vegetables and soil samples. 1000 mL of round bottomed flasks were used for reflux digestion. Micropipette (superior marinfeld) was used for measuring different amounts of acid mixtures and standard solutions. A muffle furnace (CARBOLITE CWF 1300) was used to ash vegetables sample. (Buck scientific 210VGP AAS, Este Norwaik, U.S.A) atomic absorption Spectrophotometry equipped with deuterium background correctors was used for the analysis of the analyte metals (Cu, Pb, Cr, Cd, Zn, Ca, Mg) using air-acetylene flame.

Stock standard solution of 1000 mg/L in 2 % nitric acid of the metals Cu, Pb, Cr, Cd, Zn, Ca and Mg (scientific venture) were used for preparation of calibration standards and in the spiking experiments. Nitric acid (Uni-chem 69-72 %) hydrochloric acid (Uni-chem 35.4 %) and hydrogen peroxide (Uni-chem 30%) were used for digestion of samples. Deionized ($<1\mu\text{s/cm}$) water was used throughout this work.

2.3. Sample collection and preparation

Samples of green pepper and beetroot, treated waste water and soil for polluted area were randomly collected from effluent irrigated fields in the vicinity of the Ethiopia Tannery Share Company. Different sizes of both vegetables sample of green pepper (*Capsicum annum*) and beetroot (*Beta vulgaris*) were randomly collected from irrigated field of along the shores of Ethiopia tannery. Vegetable samples were thoroughly washed to remove all adhered soil particles. Samples were cut into small pieces by using plastic knife and air dried for 1 day. For the preparation of bulk samples, the vegetable samples were collected from different site in same land area were mixed in ceramic crucibles and dried in oven at 80 °C for 72 hours. The reason why two vegetables were selected is only these vegetables are cultivated by using treated waste water for irrigation purpose (Tiller, G., 1989).

Soil samples at the surface level (0-20 cm in depth) were collected at the spot during vegetables sampling by using wood shovels and were brought to the laboratory in polyethylene plastic bags for analysis. Treated waste water samples were collected at discharge point, 200 m from discharge point and at irrigated land. Since the treated waste water discharged from the tannery is continuous flow in order to find out the representative sample, the treated waste water were sampled by difference of 6 hr for each sampling points. Finally treated waste water was collected in prepared highland bottles and were brought laboratory for analysis (Wang *et al.* 2008)

2.4. Digestion of vegetable samples

The vegetable samples were dried in an oven at 80 °C for 72 hrs and their dry weight was determined. The dry samples were crushed in a mortar and the resulting powder was digested by weighing 0.5 g of the oven-dried ground sample and sieved (<1 mm) into an acid-washed porcelain crucible and placed in a muffle furnace for 4 hrs at 500 °C. 10 mL of 6 M HCl was added, covered and then heated on a steam bath for 15 min. 1 mL of HNO₃ was added and evaporated to dryness by continuous heating for 1 hr to dehydrate silica and completely digest organic compounds. Finally, 5 mL of 6 M HCl and 10 mL of water were added and the mixture was heated on a steam bath to complete dissolution. The mixture was cooled and filtered through a Whatman No.41 filter paper into a 50 mL volumetric flask and made up to the mark with distilled water (Akan *et al.* 2009).

2.5. Digestion of waste water samples

A 100 mL of treated waste water was transferred into the beaker and 2 mL of concentrated nitric acid and 5 mL of concentrated hydrochloric acid were added to the beaker. The sample was covered with watch glass and heated on a hotplate at 95 °C until the volume has been reduced to 20 mL. The beaker was removed and allowed to cool. Finally the mixture was filtered through a Whatman No.41 filter paper into 50 mL volumetric flask and made up to the mark with deionized water (Rohrbough 1986).

2.6. Digestion of soil samples

Soil samples were mixed thoroughly to achieve homogeneity and sieved (< 2 mm). 1 g (dry weight) was weighed by using digital balance and transferred to refluxing flask. 10 mL of 1:1 HNO₃ was added to the sample and refluxed for 15 min. 5 mL of conc. HNO₃ was added to the sample and refluxed for 30 min and evaporated to 5 mL then allowed to cool. 2 mL of distilled H₂O and 3 mL of H₂O₂ were added to the cooled sample and refluxed for 2 hrs until general sample appearance was unchanged by continuous addition of 1 mL of H₂O₂ (2 times). 10 mL of HCl was added to the sample digest and refluxed for 15 min. The digest were filtered through a Whatman No. 41 filter paper and the filtrate were collected in 100 mL volumetric flask and made up to the mark with deionized water (USEPA 1996).

2.7. Determination of metals in vegetable, effluent and soil samples

For the determination of metals in both vegetables (green pepper and beetroot), effluent and soil a series of four working standard solutions were prepared from the 10 mg/L of intermediate solution of their respective metals, which were prepared by diluting the stock standard solutions of the metals with deionized water. Optimum acetylene and air flow rates were chosen to obtain suitable flame conditions. Other conditions such as slit width, wave length, and lamp current were selected for each hollow cathode lamp according to the manufacture's recommendation.

Four point calibrations were established by introducing the prepared working standard solutions in the flame atomic absorption Spectrophotometry. Immediately after calibration of the instrument, the reagent blank and the sample solutions were aspirated into the atomic absorption spectrophotometer consecutively and a minimum of three readings were taken for each sample solution and reagent blank solution.

Table 1: Instrumental operating conditions for the determination of metals in vegetable, soil and waste water sample solutions by FAAS

Elements	Parameters				
	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Sample energy (KJ)	Instrumental detection limit
Cu	324.7	0.7	1.5	3.854	0.005
Pb	217	0.7	3	2.504	0.04
Cd	228.9	0.7	2	3.267	0.01
Cr	357.9	0.7	2	3.556	0.04
Zn	213.9	0.7	2	3.083	0.005
Ca	422.7	0.7	2	3.904	0.01
Mg	285.2	0.7	1	3.837	0.05

Method detection limit

Limit of detection is the smallest mass of analyte that can be distinguished from statistical fluctuation in a blank, which is usually corresponding to the standard deviation of the blank solution times a constant. Five reagent blank (HNO₃, H₂O₂, HCl), six reagent blank (HNO₃, HCl) and three reagent blank (deionized water, HNO₃, HCl) samples were digested for soil, vegetables and influents respectively following the same procedure as the respective samples and each of samples were determined for the elements of interest (Cu, Pb, Cr, Cd, Zn) for soil and effluents and (Cu, Pb, Cr, Cd, Zn Ca and Mg) for vegetable samples by atomic absorption Spectrophotometry. The pooled

standard deviation for each element was calculated from the respective reagent blank measurements to determine method detection limit.

3. Results and Discussion

3.1 Calibration of the instrument

Calibration curves were prepared to determine the concentration of metals in the samples solution. A series of standard working solution were prepared from the 10 mg/L intermediate standard solutions of their respective metals and the solutions were aspirated into the atomizer and absorbance were recorded. Concentration of the working standards and values of correlation coefficient of the Concentration of the working standards and values of correlation coefficient of the calibration graph for each metal are listed in table (1).

Table 2: Concentration of working standards and value of correlation coefficient

Element	Concentration of standard (mg/L)	Correlation coefficient
Cu	0.01, 0.1, 0.2, 0.4	0.99994
Pb	0.05, 0.1, 0.2, 0.3	0.99965
Cd	0.02, 0.04, 0.06, 0.1	0.99938
Cr	0.05, 0.5, 1.5, 3	0.9999
Zn	0.01, 0.1, 0.5, 1	0.99969
Ca	0.2, 1, 2, 4	0.99949
Mg	0.1, 0.5, 1, 2	0.99914

3.2. Validation of the digestion procedure

Method validation is the process of providing that the analytical method is acceptable for its intended purpose. Therefore analysts are increasingly encouraged to validate analytical procedures and to estimate the uncertainty associated to the results. Since there is no certified reference material (for vegetable, effluent, and soil) samples in our laboratory, the validity of the digestion procedure for vegetable, effluent and soil samples were checked by carrying out with lower traceability, such as spiked samples. As shown in tables (3-5) the percentage recovery ranges 90-104 %, 90-96 % and 94.4-110 % for vegetable, effluent and soil samples respectively.

Table 3: Recovery test for vegetable samples

Metal	Conc. in sample (mg/kg)	Amount added (mg/kg)	Conc. in spiked (mg/ kg)	% Recovery
Cu	8.90 ± 0.30	1	9.95 ± 0.70	105
Cd	0.96 ± 0.10	0.5	1.44 ± 0.33	96
Zn	100.27 ± 8.00	10	109.87 ± 1.40	96
Ca	99.01 ± 4.00	10	109.56 ± 0.80	105
Mg	392.00 ± 0.700	10	401.05 ± 3.00	90

Table 4: Recovery test for waste water samples

Metal	Conc. in sample (mg/kg)	Amount added (mg/kg)	Conc. in spiked (mg/ kg)	% Recovery
Cu	8.90 ± 0.30	1	9.95 ± 0.70	105
Cd	0.96 ± 0.10	0.5	1.44 ± 0.33	96
Zn	100.27 ± 8.00	10	109.87 ± 1.40	96
Ca	99.01 ± 4.00	10	109.56 ± 0.80	105
Mg	392.00 ± 0.700	10	401.05 ± 3.00	90

Table 5: Recovery test for soil sample

Metal	Conc. in sample (mg/kg)	Amount added (mg/kg)	Conc. in spiked (mg/ kg)	% Recovery
Cu	22.7 ± 0.60	0.10	22.79 ± 0.68	90.00
Pb	11.1 ± 0.50	0.10	11.19 ± 0.40	96.00
Cd	2.7 ± 0.04	0.10	2.80 ± 0.06	95.00
Cr	225 ± 3.00	5.00	229.65 ± 0.80	92.60
Zn	220.8 ± 1.00	5.00	224.68 ± 0.50	95.40

3.3. Determination of metals

The concentration of metals in aliquot of digested of soil and vegetable samples were calculated using the following equation.

$$C = \frac{A}{W} \times V$$

Where C= total metal concentration (mg/L) or (mg/Kg)

A = mg/L of metal in digested sample

W = weight of digested sample (g)

V = final volume of digested sample solution (mL)

The level of heavy (Cu, Pb, Cd, Cr and Zn) and major (Ca & Mg) metals are determined in vegetables (*Capsicum annuum* & *Beta vulgaris*), treated waste water and soil are reported as mean of three measurements with the corresponding standard deviation for each metal in a given sample as given in the following tables (6-8)

3.3.1 Concentration of heavy and major metals in vegetables

The level of heavy (Cu, Pb, Cd, Cr and Zn) and major (Mg and Ca) metals (Mean \pm SD) (mg/kg) in vegetables (*Beta vulgaris* and *Capsicum annuum*)

Table 6: concentration of heavy and major metals in vegetables

Metal	Beetroot (<i>Beta vulgaris</i>)	Green pepper (<i>Capsicum annuum</i>)	FAO/WHO 2005 Recommended values
Cu	8.9 \pm 0.3	9.5 \pm 0.6	73.3
Pb	ND	ND	0.3
Cd	0.96 \pm 0.1	1.2 \pm 0.4	0.2
Cr	ND	ND	2.3
Zn	132.4 \pm 10.0	100.27 \pm 8.0	99.4
Ca	3739.7 \pm 7.0	99 \pm 4.0	1000 ^b
Mg	6262.9 \pm 31.0	392 \pm 4.0	400 ^b

^bsource. RDI (reference daily intake) in gram

ND is not detected.

The mean concentration of copper in the vegetables are **8.9** mg/kg in beetroot to **9.5** mg/kg in green pepper. The recent study conducted by (Prabu *et al.* 2009) reported that the mean concentrations of copper of Akaki area were 32.25 mg/kg in beetroot and 17.21 mg/kg in green pepper. These values are higher than the values in the present study i.e. **8.9** mg/kg and **9.5** mg/kg in beetroot and green pepper respectively.

The cadmium concentration determined in beetroot (*Beta vulgaris*) and green pepper (*Capsicum annuum*) are **0.96** mg/kg and **1.2** mg/kg respectively which is above the maximum value cited by FAO/WHO. The study conducted by (Prabu *et al.* 2009) had concentration of cadmium 0.254 mg/kg in beetroot and 0.18 mg/kg in green pepper at Akaki River irrigated farm. These values are lower than the values obtained in both vegetables (0.96 mg/kg in beetroot and 1.2 mg/kg in green pepper) in the present study respectively. The mean concentration of cadmium reported by (Fisseha 2002) had Cd concentration of 0.08 mg/kg in lettuce at peacock farm and also 0.04 mg/kg in beetroot again at peacock farm, whereas the content of metal in this study higher (**0.96** mg/kg) in green pepper and (**1.2** mg/kg) in beetroot.

Zinc was determined both in beetroot and green pepper, **132.40** and **100.27** mg/kg respectively. Prabu Pc.etal (2009) shows concentration of zinc 90.3 mg/kg in beetroot and 65.9 mg/kg in green pepper which is lower than the present study, **132.4** mg/Kg and **100.27** mg/kg in beetroot and green pepper respectively.

Concentration of calcium determined in beet root (*Beta vulgaris*) and green pepper (*Capsicum annuum*) were found to be **3739** and **99** mg/kg respectively.

The concentration of magnesium were determined in beetroot and green pepper (**6262.9** mg/kg and **392** mg/kg) respectively which indicates that the highest level in both vegetables. As shown from the figure (2), beetroot is more accumulators of Zn, Ca and Mg while Cd and Cu more accumulated in green pepper. From the graph it can be seen that the trend of occurrence of the metal concentration in green pepper sample analyzed is in order of Mg > Zn > Ca > Cu > Cd. This trend suggests that green pepper have higher concentration of magnesium, calcium and zinc than copper and cadmium. The trend of occurrence of metal concentration in beetroot sample analyzed is in order of Mg > Ca > Zn > Cu > Cd. Beetroot is more accumulator of major elements (Mg and Ca) than green pepper.

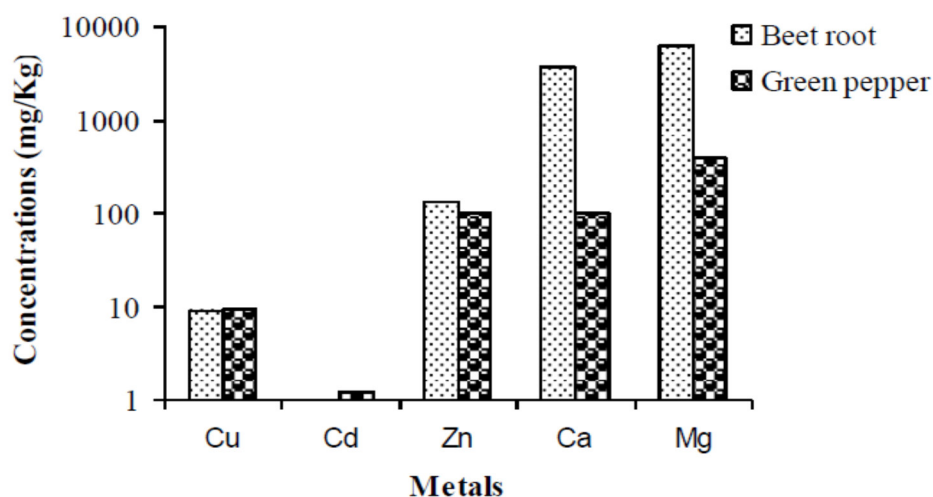


Figure 2: Level of heavy metals in vegetables (*Beta vulgaris* and *Capsicum annum*)

The contents of heavy metal in the treated waste water which used for irrigation purpose where analyzed

Table 7: The level of heavy metals (mean \pm SD) (mg/L) in treated waste water samples.

Elements	Sampling site			Guide lines		
	S1	S2	S3	FEPA (mg/L)	WHO (mg/L)	NEQS (mg/L)
Cu	0.023 \pm 0.003	0.027 \pm 0.005	0.019 \pm 0.008	1.00	1.50	1.00
Pb	ND	ND	ND	1.00	1.50	0.10
Cd	0.018 \pm 0.005	0.023 \pm 0.008	0.020 \pm 0.005	1.00	1.50	0.10
Cr	7.948 \pm 0.300	3.330 \pm 0.200	6.400 \pm 0.2	1.00	1.50	1.00
Zn	0.270 \pm 0.001	0.170 \pm 0.030	0.290 \pm 0.040	1.00	1.50	5.00

FEPA= federal environmental protection agency (Nigeria) WHO = world health organization NEQS = national environment quality standards (Pakistan) Where ND is not detected

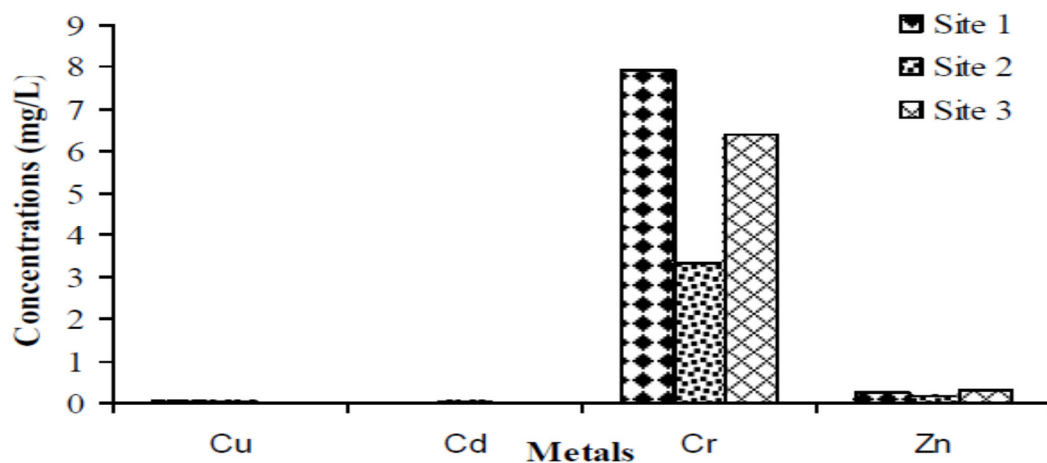


Figure 3: Level of heavy metals in treated waste water (effluent) in mg/L Where S1, S2 and S3 are sampling sites one, two and three respectively.

Table 8: The level of heavy metals (mg/kg) in soil

Metal	Concentration (mean \pm SD) (mg/kg)	guidelines mass in soil (mg/kg)
Cu	22.7 \pm 0.6	100 ^a
Pb	11.1 \pm 0.5	100 ^a
Cd	2.7 \pm 0.04	3 ^a
Cr	225.0 \pm 3.0	100 ^a
Zn	220.8 \pm 1.0	300 ^a

^asource = USEPA 2001

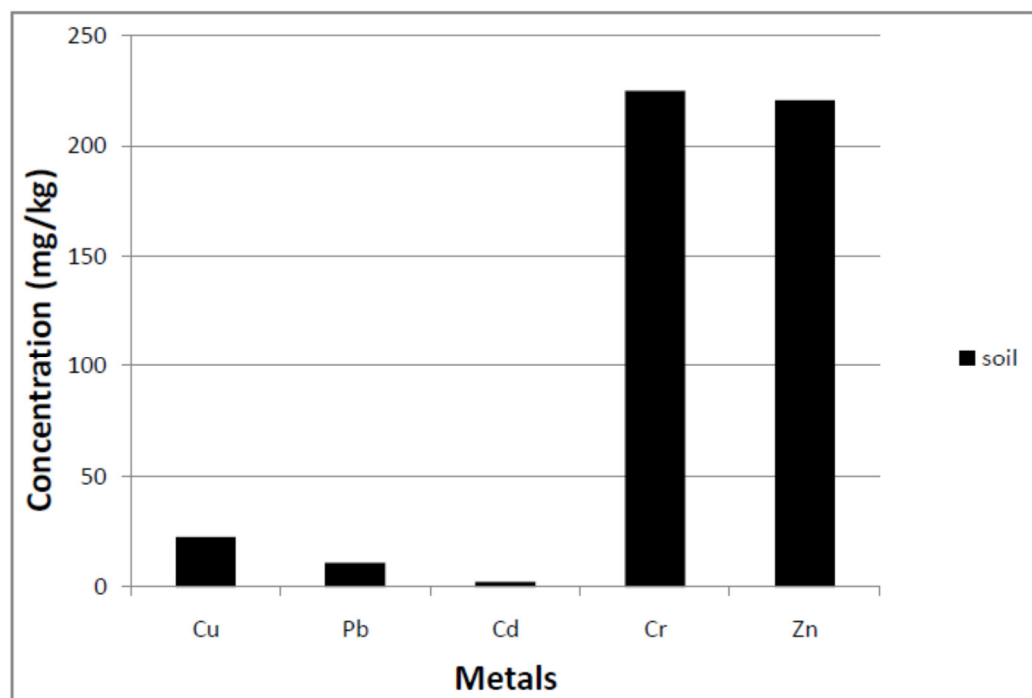


Figure 4: Distribution heavy metal in soil in the vicinity of the Ethiopia Tannery

From the graph, the result show that the metal concentration in soil samples analyzed is in order of Cr > Zn > Cu > Pb > Cd.

Conclusion In this study two kind vegetable samples were analyzed for their contents of Cu, Pb, Cd, Cr, Zn, Ca and Mg. Soil and waste water samples were also analyzed for contents of Cu, Pb, Cd, Cr and Zn. The reference digestion method for all sample analyzed was found effective for all of the elements and as it was evaluated through recovery experiments. Good recovery percentage was obtained (90-110) for the elements identified. The average concentration of zinc and cadmium in both vegetables (green pepper and beetroot) were found higher than the literature values. The concentration of chromium was found higher in the effluents than the concentration of other metals analyzed, which suggests that the waste water discharged from the tannery is contaminated with chromium. This is due to chrome tanning or any other chrome related activities in the tannery. Except cadmium and zinc which are above FAO/WHO value all other metals were below the maximum values recommended by FAO/WHO.

In order to identify the cause for the excessive cadmium contents in green pepper and beetroot found, more compressive studies are required. Generally more compressive studies are required to investigate the cause of metal content in vegetables.

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