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SELECTED HEAVY METALS IN SOME VEGETABLES PRODUCED THROUGH WASTEWATER IRRIGATION AND THEIR TOXICOLOGICAL IMPLICATIONS IN EASTERN ETHIOPIA

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ABSTRACT

Vegetables widely consumed in some areas of eastern Ethiopia such as cabbage (*Brassica oleraceae* var. capitata L.), potato (*Solanum tuberosum* L.), and khat (*Catha edulis* Forsk.) are cultivated through irrigation with wastewater. The purpose of this study was to analyse the contents of selected toxic heavy metal (Cr, Co, Cd and Pb) of the vegetables, the effluents used to irrigate the crops, and the soils on which the crops were grown, using flame atomic absorption spectrometry (FAAS). The optimized wet digestion procedure was employed to solubilise the metals from the samples. The validation was performed by spiking the samples with a standard solution of each metal having a known concentration and the percentage recovery values in the range of 91.0–98.3% for soil, 92.0–102% for effluent, and 89.0–101% for vegetable samples. The following concentrations (mg kg^{-1}) of the metals were found in the edible parts of the cabbage, potato, and khat plants, respectively: Cr [less than method detection limit (<MDL)- 17.13], (11.96-14.21), and (9.04-15.54); Co (5.72-9.72), (5.15-8.72), and (<MDL-8.87); Cd (1.15-2.46), (1.22-1.46), and (0.38-3.22); Pb (5.48-11.95), (5.43-7.78), and (4.49-11). The concentrations in the effluent samples (mg kg^{-1}) ranged from 0.17-0.26, 0.57-1.02, 0.04-0.08, and 0.82-2.52 for Cr, Co, Cd, and Pb, respectively. Similarly, concentrations (mg kg^{-1}) of the metals in the soil samples were in the ranges of 25.71-41.45, 17.69-23.59, 0.79-2.56, and 26.04-47.29 for Cr, Co, and Cd, and Pb, respectively. The study revealed that the concentrations of all metals in the vegetables, except Co, were found to be above the safe limits set by different international organizations for consumption, posing a serious health hazard to humans. Therefore, regular monitoring of effluents, soils, and vegetables are essential to prevent excessive build-up of the toxic heavy metals in food. Thus, the health risk and the extent of heavy metal contamination can be reduced.

Keywords: Vegetables, wastewater, safe limits, irrigation

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INTRODUCTION

Vegetables are important ingredients in the human diet and contain essential nutrients and trace elements that have potential health benefits [1]. Environmental pollution has caused the contamination of soil; on the other hand, wastewater irrigation resulted in significant infusion of non-essential elements in agricultural lands [2]. The main cause of the infusion is the waterways through which non-essential elements are leached out of the soil and taken by the vegetation. If plants decay, these potential toxic elements are redistributed, and accumulate in agricultural soils. Long-term irrigation with wastewater leads to a build-up of heavy metals in soils and foods [3].

Exposure of vegetables or plant products to various metal containing components has varying health implications [4]. Furthermore, consumption of food and vegetation contaminated with heavy metals can seriously deplete some essential nutrients in the body causing a decrease in immunological defences, intrauterine growth retardation, impaired psycho-social behaviour, disabilities associated with malnutrition, and a high prevalence of upper gastrointestinal cancer [5]. Based on the effect of heavy metals on consumers, different organizations have proposed maximum permissible limits of the metals in edible vegetation, wastewater, and soils as shown in Table 1[6 – 10].

It has been reported that sewage effluents contain significant amounts of major plant nutrients and thus fertility levels of soil are increased under sewage irrigation of crops like cabbage, radish, cauliflower, spinach [11]. However, studies of crops (cabbage, radish, chandaliya) irrigated with untreated sewage water revealed the presence of toxic metals like Pb, Cr, Cd, Ni, Fe, Co, Zn, Co, thereby reducing soil fertility and agricultural outputs [12]. In several parts of rural and urban Ethiopia, where effluent water is available, people cultivate vegetables with this type of water. This is true for areas around the towns of Aweday, Harar, Haramaya, as well as the vicinity of the main campus of Haramaya University (HU) in eastern Ethiopia.

The present study was undertaken to assess the extent of toxic trace heavy metal contamination in selected vegetables grown under irrigation with sewage water in various fields of Harari region near Harar, Aweday, and Haramaya towns of the Oromia National Regional State as well as the vegetable producing areas in the vicinity of the main campus of Haramaya University (HU). The study was necessary as a large number of people consume the produce and no research has been conducted to elucidate the extent of the problem in the region.

MATERIALS AND METHODS

Experimental Sites

Vegetable, soil, and effluent water samples were collected from different areas of East Hararghe Zone of the Oromia Regional State *viz.*, Aweday town (9^{o}21'36.75''N, 42^{o}02'44.01''E), Haramaya town (9^{o}23'30.33''N, 42^{o}00'40.82''E), the vicinity of Haramaya University (HU) (9^{o}25'05.69''N; 42^{o}02'45.65''E), where toilet and laundry sewage from student dormitories flow into farmlands, and Harar town (9^{o}18'16.54''N,

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42^{o}07'57.55"E). Analysing the heavy metals was conducted in the Chemistry and Soil Laboratory of Haramaya University.

Chemicals, Reagents and Instruments

All chemicals and reagents used were of analytical grade. HNO_3 (69−72%, Fine-Chem Mumbai-391780, India) and 70% $HClO_4$ (A.C.S. reagent, Aldrich, UK) were used for the digestion of the vegetable samples (cabbage, potato and khat). HNO_3 (69−72%, Fine-Chem Mumbai-391780, India), 70% $HClO_4$ (A.C.S. reagent, Aldrich, UK) and H_2SO_4 (Fine-Chem Mumbai - 400 02 India) were employed during digestion of the soil samples. HNO_3 (69−72%, Fine-Chem Mumbai-391780, India) and 36-37% HCl (A.C.S. reagent, Aldrich, UK) were used in digestion of the effluent samples. Stock standard solutions 1000 mg/L, containing 2% HNO_3, of the metals Pb, Co, Cd and Cr (Buck Scientific Puro-GraphicTM, USA) were used for preparation of the working solutions (which were immediately prepared before analysis) for calibration and in spiking experiments. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked in 4 M HNO_3 solution and rinsed several times with deionized water. Deionized water was used throughout the experiment for preparation and dilution of the sample solutions.

Air circulating oven (Genlab Limited, UK), electronic blending device (K-M20, IKA-WERKE, Germany), ceramic mortar and pestle (Halden wanger, Germany), a digital analytical balance (Mettler Tolendo, Switzerland), stainless steel Auger, Kjeldahl block digester (Gallen kamp, England) were used. Flame atomic absorption spectrophotometer (Buck Scientific Model 210VGP AAS, East Norwalk, USA) with air-acetylene flame and deuterium background correction was used for the analysis of the target metals. The operational conditions of flame atomic absorption spectrometry (FAAS) are given in Table 2.

Sample collection and preparation

The effluent, soil, and vegetable samples were collected from January to April 2012. About 2 kg leaf samples of cabbage (*Brassica oleraceae* var. capitata), khat (*Catha edulis* Forsk), and potato (*Solanum tuberosum* L.) were collected, packed, labelled and transported to the laboratory. The samples were washed with tap water to eliminate soil and other dirt; the edible parts were separated from the other portions, rinsed with distilled deionized water, shredded (cabbage and khat leaves) and minced (potato tubers). The samples were air-dried in paper bags and then ground, sieved, homogenized, and heated in an oven at 105 ^{o}C to constant weights. The contents were cooled and placed in clean paper bags and stored in desiccators until digestion.

Soil samples (about 1 kg) were collected from 0-15 cm depth in triplicates from the sites where the plants originated with an auger and placed in clean polyethylene bags. The composite soil samples were air-dried in a dry and dust-free place at room temperature (25 ^{o}C) for 5 days, followed by oven drying to constant weights. The samples were then ground with a mortar and pestle to pass through a 2-mm sieve and homogenized. The dried, sieved, and homogenized soil samples were stored in clean and dry containers till digestion.

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Effluent samples were collected from the four areas *viz*. Harar, Aweday, Haramaya town and the vicinity of Haramaya University from surfaces where effluents were directed to vegetable farms. The effluents were filled in to plastic bottles (250 mL), which were rinsed with the effluent water several times. The collected effluent water samples were taken to the laboratory and acidified with conc. HNO_3 (5 mL L^{-1}) and stored in a clean area before analysis.

Calibration procedure

Calibration curves were prepared to determine the concentration of the heavy metals in the sample solutions. Intermediate standard solutions (10 mg/L) of each heavy metals were prepared from stock standard solutions containing 1,000 mg L^{-1} of Co, Cd, Cr and Pb. Appropriate working standards were prepared for each of the metal solution by serial dilution of the intermediate solutions using deionized water. Potassium chloride solution was added as an ionization suppressor for determining the concentration of Cr. Analytical wavelengths, colour of the flame and slit width were adjusted according to the instrument operation manual to attain its better sensitivity and working standards were then aspirated one after the other into the flame atomic absorption spectrometry and their absorbance was recorded. Calibration curves were plotted with four points for each of the trace heavy metals standard using absorbance against concentrations (mg/L). Immediately after calibration using the standard solutions, the sample solutions were aspirated into the FAAS instrument and direct reading of the metal concentrations was recorded.

Digestion of the plant samples

To obtain a clear sample solution that is suitable for the analysis using flame atomic absorption spectrometry, different digestion procedures for the plant samples (cabbage, potato, and khat) were assessed using concentrated HNO_3 and $HClO_4$ acid mixtures by varying the volume of the acid mixture, digestion time, and temperature of the method. Optimized procedure was selected based on usage of lesser reagent volume, shorter digestion time, and reasonably mild temperature for obtaining clear solutions of the resulting digests. Among the different digestion procedures tested, digesting a plant sample weighing 0.5 g with 5 mL of 3:2 (v/v) mixture of concentrated HNO_3 and concentrated $HClO_4$ heated at 250 °C for 55 minutes on a Kjeldahl digestion block was chosen. After the digestion was completed, the digest was cooled and filtered into 50 mL volumetric flasks using Whatman no. 41 filter paper to remove any suspended and turbid matter. After rinsing, the solutions were diluted to the mark with deionized water. The digestion was made in triplicates for each of the vegetable samples collected from the four sampling sites. Digestion of a reagent blank was performed along with the vegetable samples keeping all digestion parameters the same. All the digested and diluted samples were stored in a refrigerator at 4ºC until analysis.

Digestion of the soil samples

A digestion method reported by Allen *et al* .[13] was used for the digestion of the soil samples after making a slight modification on the procedure to obtain clear solutions of the digest. Accordingly, a soil sample weighing 1 g was digested on Kjeldahl digestion block by adding 7 mL of tri-acid mixture (HNO_3, H_2SO_4, and $HClO_4$ in 5:1:1 (*v/v/v*) ratio) at 100 ºC for 40 min and then at 160 ºC for 50 min to obtain a transparent solution

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of the digest. The digested sample was filtered using Whatman no. 41 filter paper after cooling, and the filtrate was finally made up to 100 mL with deionized water.

Digestion of the effluent samples

United State Environmental Protection Agency (US EPA) 3005A [14] method was used for digesting the effluent water samples used for irrigating the vegetables. A 50 mL aliquot of well mixed effluent samples was digested in a beaker covered with a watch glass by adding 1 mL of concentrated HNO_3 and 2.5 mL of concentrated HCl and heated on a hot plate at 90 ^oC until the volume was reduced to about 15 mL. Then the beaker was removed and cooled. The solution was filtered and finally diluted to 100 mL with distilled-deionized water. The level of heavy metals in the filtrate was determined by FAAS.

Method Detection Limit

Nine replicate blank samples were digested following the same procedures utilised for digesting the vegetable, soil, and effluent samples. Each blank was assayed for its metal contents (Cr, Co, Cd and Pb) by FAAS. The standard deviations (SD) of the nine replicate blanks were calculated to determine the method detection limit (MDL) and limit of quantification (LOQ). Method detection limit (MDL) was calculated as three times the standard deviations (MDL = 3SD) and LOQ was calculated as ten times the standard deviation (LOQ = 10SD)[15]. The MDL values obtained were compared with the instrument detection limit (IDL) and found to have greater values in all cases (Table 3).

Analytical method validation

Efficiency of the optimized procedure used for digesting the vegetable samples, soil, and effluent samples were checked by spiking the pre-treated vegetable, soil and effluent water samples with standard solutions of each metal having a known concentration. The spiked vegetables, soil, and effluent samples were digested following the same procedure employed in the digestion of the respective samples. Accordingly, a 0.5 g of cabbage sample was spiked with 5 mg kg^{-1} Cr and Pb and 1 mg kg^{-1} Cd and Co. The same amount (0.5 g) of potato and khat leaf samples were spiked with 5 mg kg^{-1} Cr, Co, and Pb, and 1 mg kg^{-1} Cd. For the soil sample, 1 g was spiked with 10 mg kg^{-1} Cr and Pb, 5 mg kg^{-1} Co and 1 mg kg^{-1} Cd. However, the effluent water sample (50 mL) was spiked with 0.5 mg kg^{-1} Cr and Cd and 1 mg kg^{-1} Co and Pb. For the vegetables, effluent, and soil samples, the recovery was performed in triplicates.

Transfer factor (TF)

Transfer factor (TF) was calculated to understand the risk associated with wastewater irrigation and consequent heavy metal accumulation in the edible portions of the test vegetables following a standard procedure [16].

$$\mathrm{TF} = \frac{\text{Concentration of metal in the edible part}}{\text{Concentration of chemical element in soil under irrigation with wastewater}}$$

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Statistical Analysis

Analysis of variance (ANOVA) was used to test the level of significance at $\alpha = 0.05$[17]. Means were separated using the least significant difference test at 5% level of significance. A correlation test was carried out between the investigated metals for the vegetable materials and among metals detected in the soil and vegetables to associate the distribution of metals in the soil samples and their availability and accumulation in the vegetables at $\alpha = 0.05$.

RESULTS

Evaluation of the analytical method

In this study, the method validation was made by a spiking experiment in which known quantities of the metal standard solution were added to the samples to be studied. Percentage recovery values for individual analysis for soil, effluent, and vegetable samples are presented in Table 4. The percentage recovery values of the metals for soil, effluent, and vegetable samples were found to be within the range of 91.0–98.3%, 92.0–102%, and 89.0–101%, respectively. These ranges are within the acceptable range [18] which confirmed the validity of the method utilized in the current study.

Metals in the effluent samples

The data presented in Figure 1 show that high concentrations (mg kg^{-1}) of the toxic heavy metals were found in the effluent samples from some of the sites. The concentration of Cr in the effluent was found to be the lowest (0.17 mg kg^{-1}) in the samples collected from Haramaya town and the highest (0.26 mg kg^{-1}) in the samples collected from both Harar and Haramaya University (HU). The concentration of the same metal was found to be 0.21 mg kg^{-1} in the effluent collected from Aweday site. The concentration of Cd in the effluents varied from 0.04 mg kg^{-1} for the sample collected from Haramaya site to 0.05 mg kg^{-1} for the sample collected from Aweday, 0.06 mg kg^{-1} for the sample collected from Harar town. The highest concentration of the metal (0.08 mg kg^{-1}) was observed in the effluent sampled from HU site. The concentration of Co in the effluent ranged from 0.57 mg kg^{-1} from Harar and Haramaya towns to 0.71 mg kg^{-1} from HU and 1.02 mg kg^{-1} from Aweday town. However, the highest concentration in the effluent sample was found to be that of Pb, which ranged from 0.82 mg kg^{-1} for the sample collected from Haramaya town to 1.12 mg kg^{-1} for the sample collected from Harar town, and 1.72 mg kg^{-1} for the sample obtained from Aweday town. The highest concentration (2.52 mg kg^{-1}) of this heavy metal was detected in the effluent collected from the vicinity of Haramaya University. The heavy metals with the next highest concentration in the effluents were Co and Cr. The concentration of Cd was found to be the lowest in the effluent waters collected from all sites.

Metals in the soil samples

The concentrations of heavy metals (Cr, Co, Cd, and Pb) are shown in Figure 2. The concentrations of the metals analysed varied in the soil samples. High Cr concentration (41.45 mg kg^{-1}) was observed for the HU site and the least concentration (25.71 mg kg^{-1}) was recorded for the Aweday site. However, Cr concentration in soils of Haramaya and Harar sites were 29.52 mg kg^{-1} and 37.62 mg kg^{-1}, respectively. The concentration of Cd in the soil samples was found to be 0.79, 1.38, 1.43 and 2.56 mg kg^{-1} for Haramaya, Harar, Aweday and HU sites, respectively. Similarly, the soil from which the vegetables originated revealed high Co concentration (23.59 mg kg^{-1}) from the HU site. This was closely followed by the Co concentration collected from Harar and Aweday sites, which amounted to 17.69 mg kg^{-1} and 20.65 mg kg^{-1}, respectively. The lowest Co concentration (17.69 mg kg^{-1}) was observed for the Haramaya site. The soil sample from HU site contained high Pb concentration (47.29 mg kg^{-1}) followed by soil from Aweday site (31.25 mg kg^{-1}). The lowest and comparable Pb concentration (26.04 mg kg^{-1}) was recorded in soil samples of Haramaya and Harar towns. Among the targeted heavy metal analytes, Cr was found to be the highest in concentration followed by Pb and Co. The concentration of Cd was found to be the lowest in the soil samples from all the sampling sites.

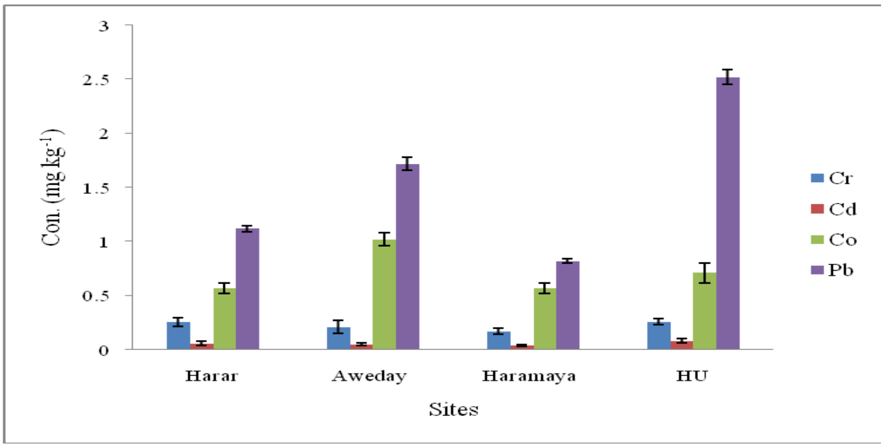

Figure1: Concentrations of heavy metals in the effluent samples
HU = the vicinity of Haramaya University

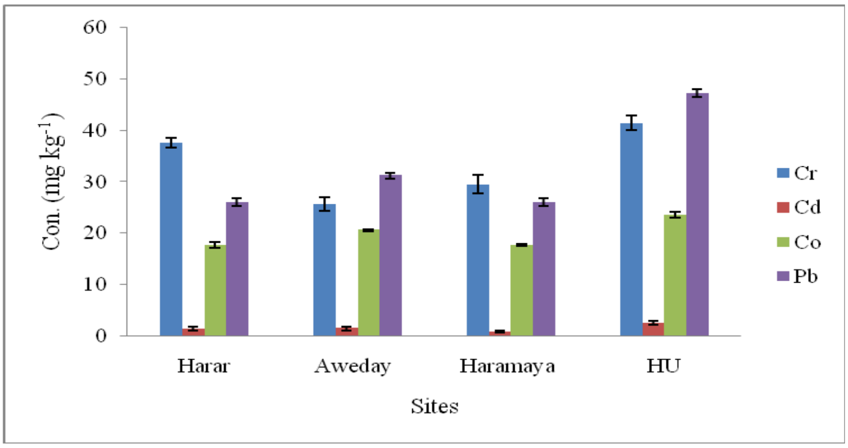

Figure 2: Concentrations of heavy metals in the soil samples of the plant origins
HU = the vicinity of Haramaya University

Metals in the vegetables

The concentrations of the metals (Cr, Co, Cd and Pb) in the investigated vegetables are given in Figures 3-5 for the cabbage, potato, and khat samples, respectively. The metal concentrations (mg kg^{-1}) in cabbage samples were found to be <MDL–17.13, 5.72–9.72,

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1.15-2.46 and 5.48-11.95 for Cr, Co, Cd and Pb, respectively (Figure 3). The concentrations (mg kg^{-1}) of heavy metals in potato samples from Aweday and the vicinity of HU sites ranged from 11.96-14.21, 5.15 -8.72, 1.22-1.46, and 5.43-7.78 for Cr,Co,Cd and Pb, respectively (Figure 4). The metal concentrations (mg kg^{-1}) in khat samples were found to be 9.04–14.54, <MDL–8.87, 0.38-3.22, and 4.49-10.95 for Cr, Co, Cd and Pb, respectively (Figure 5).

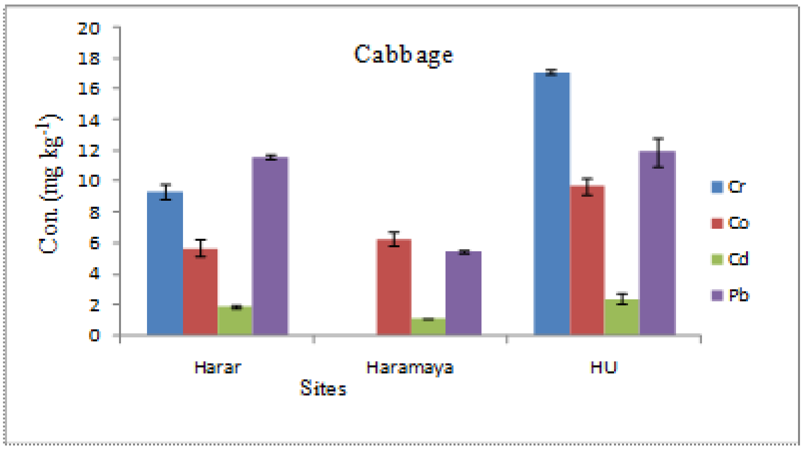

Figure 3: Concentrations of heavy metals in cabbage
HU = the vicinity of Haramaya University

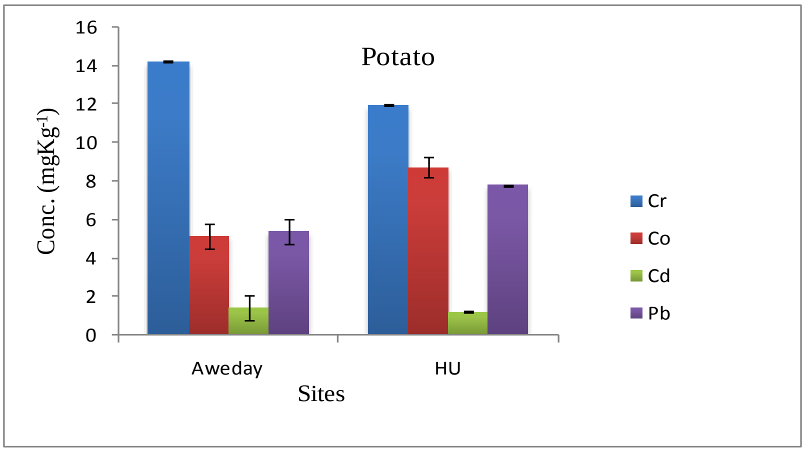

Figure 4: Concentrations of the heavy metals in Potato
HU = the vicinity of Haramaya University

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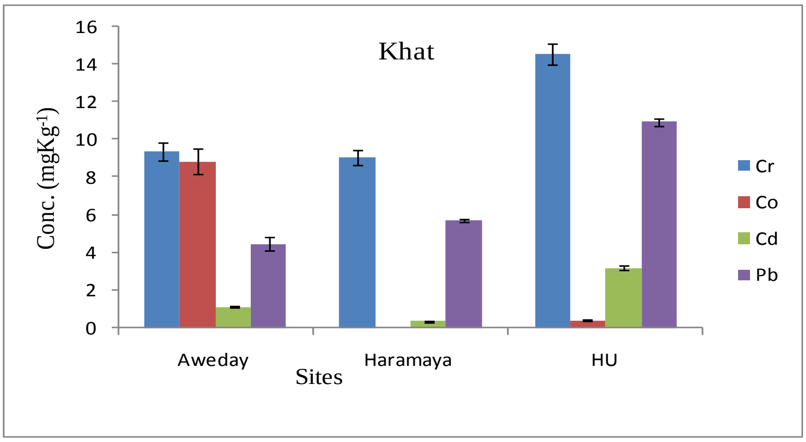

Figure 5: Concentrations of the heavy metals in Khat
HU = the vicinity of Haramaya University

Comparison of metal concentrations in the vegetables with other edible and medicinal plants

The levels of metal concentration in the vegetables (cabbage, potato and khat) were compared with other edible and medicinal plants reported in various parts of the world as summarized in Table 5. Based on the comparison, the results of the present study are in agreement with most of the reported values.

Transfer Factor (TF)

Among the different metals, Cd showed the maximum transfer factor value (Table 6), which ranged from 0.48 (khat from Haramaya) to 1.46 (cabbage from Haramaya) and it was minimum for Co, ranging from 0.02 (khat from HU) to 0.43 (khat from Aweday). The transfer factor for Cr and Pb ranged from 0.25 (cabbage from Harar) to 0.55 (potato from Aweday) and 0.14 (khat from Aweday) to 0.45 (cabbage from Harar). The transfer factors of heavy metals for the different vegetables in the current study were compared with similar and other edible plants (Table 7).

Statistical Evaluation

Pair-wise statistical analyses of the results were made to verify whether there were significant differences in concentrations of the heavy metals in the effluent water, soil, and vegetable samples assayed with the sampling sites. For the present study, the significance of variation within sample and between samples has been studied using a one-way ANOVA [19]. At 95% confidence level, for the effluent samples, no significant difference ($P > 0.05$) was observed for Cr (except for those from Harar/Haramaya and Haramaya/HU), Cd (except for the samples from Haramaya/HU) and Co (except for the samples from Harar/Aweday and Aweday/HU). However, significant differences ($P < 0.05$) were observed for Pb between the samples from the various sampling areas. For the case of soil samples, significant differences ($P < 0.05$) were observed for most of the investigated metals except Cd (for the samples from Harar/Aweday, Harar/Haramaya and Aweday/Haramaya), and Co and Pb (for those from Harar/Haramaya). For the cabbage samples, significant differences ($P < 0.05$) were observed for Cr, Cd (except for the samples from Harar/HU) and Co (except for the samples from Harar/Haramaya) and

Pb (except for those from Harar/HU). In the case of potato samples, significant differences ($P < 0.05$) were observed for Cr, Cd, Co and Pb for different sampling sites. Similarly, all metals had significant variations ($P < 0.05$) for khat with the sampling sites except for Cr which did not vary significantly ($P > 0.05$) for the samples from Aweday/Haramaya.

DISCUSSION

Levels of metals in the effluent samples

The effluent analysis revealed significant differences in the concentrations of heavy metals in the samples collected from the various sampling areas. The differences may be attributed to the fact that the contaminating analytes depended on sources of the wastewater discharged to the irrigation water. Heavy metals in the sewage water are associated with small scale industries such as colouring, electroplating, metal surface treatments, fabric printing, battery and paints, releasing Cd, Co, Cr, Pb, Zn, Ni and other heavy metals into water channels, which are accessed for irrigation [20].

The concentrations of Cd and Co detected in the effluent waters exceeded the safe limits set by international organizations *viz*., [6, 7, 9]. The concentration of Pb in all the effluent water samples was found to be under the safe limit [7], but above the limit when compared with other international organizations ([6, 9]). However, Cr exceeded the safe limit according to [10], but was below the limit according to [6, 9]. The results obtained show that the effluent samples are profoundly contaminated with these heavy metals. Therefore, attention should be focussed on regular monitoring and control of wastewater used for irrigation.

Levels of metals in the soil samples

For the soil samples, the concentration of heavy metals considered in the current study *viz*., Cd, Cr, Co and Pb were found to be below the safe limits set by different organizations ([6, 8]). The critical level of Cd in the soil for plant growth is 3 mg Cd/kg soil [21]. However, the concentration of Cd was found to be high and approaching the safe limits in the soil samples collected from HU site according to the standard sets ([6, 8]). The concentration of Cd in the soil may be increased due to the application of sludge [21]. Cadmium (Cd) is also added to the soil in small amounts in phosphate fertilizers [22]. The relatively higher concentration of Cd in soils sampled from the vicinity of Haramaya University may be attributed to the higher sewage sludge emanating from the residences of staff and students as well as the higher use of phosphate fertilizers in the research and nearby farms in the area. This indicates that the level of cadmium in the soil needs to be managed.

Levels of metals in the vegetables

Except for cabbage samples collected from Haramaya site (<MDL), the concentrations of Cr detected in all of the vegetables analysed in this study were higher than the limit levels in food by FAO/WHO guidelines [23]. The concentration of Cr was observed to be the highest for cabbage samples collected from HU site and it was below method detection limit in cabbage samples from Haramaya site. However, the amounts in all samples were below the permissible limit set by Indian standards [6]. Among the

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investigated vegetables, cabbage was found to be more Cr-loaded than potato and khat. Higher concentration of this heavy metal was obtained in vegetables from HU site which might be due to sewage sludge discharged to the environment from laboratories as well as from toilets and laundries of student dormitories and staff residences of the University.

The concentrations of Co detected in all of the vegetables analysed, in this study, were far lower than the stipulated permissible levels in food by FAO/WHO guidelines [10]. Based on these results, it can be summarized that the vegetables are safe to consumers in terms of Co. However, amongst the investigated vegetables, cabbage was found to be more cobalt-loaded than potato and khat, and high concentration of the heavy metal was obtained from vegetable samples collected from the vicinity of Haramaya University.

All samples of the current study contained Cd concentration above the maximum permissible limit set by different organization, which is 0.1 mg kg^{-1}[10] and 0.2 mg kg^{-1} [7]. Among the sampling sites, the samples from the HU site contained higher Cd concentration which might be attributed to the sewage sludge discharge from the University's laboratories, student dormitories, garage and cafeterias. Cadmium is a toxic metal and can cause serious health problems. Recently, attention has been focused on its availability in water, soil, milk, dietary products, medicinal plants, and herbal drugs [24]. The most common sources for Cd in soil and plants are phosphate fertilizers, non-ferrous smelters, lead and zinc mines, sewage sludge application and combustion of fossil fuels [25]. Leafy vegetables (khat and cabbage) contained the highest levels of Cd which shows higher accumulation of this metal in the aerial parts of the plants than the roots. It has been reported that Cd is a highly mobile metal, easily absorbed by the plants through root surface and moves to wood tissue and transfers to upper parts of plants [26].

The vegetable samples also contained Pb above the maximum permissible limit sets ([6, 7, 10]). Hence, the vegetables collected from the four sampling sites (Aweday, Harar, Haramaya, and HU) are not safe to consume in terms of Pb content. Cabbage accumulated the highest amount of Pb followed by khat and potato samples. The three samples collected from HU site accumulated higher Pb than samples from the other sites which might be due to the effluents from laboratories and toilets from the University. Lead is a toxic element that can be harmful to plants, although plants usually show the ability to accumulate large amounts of Pb without visible changes in their appearance or yield. In many plants, Pb accumulation can exceed several hundred times than the threshold of maximum level permissible for humans [27]. On the whole, all vegetables that were studied in this study were contaminated by Pb and are bound to be unsafe for human consumption.

Transfer Factor (TF)
Variations in transfer factor among the different vegetables may be attributed to differences in the concentration of metals in the soil and differences in element uptake by different vegetables [28]. Among all the vegetables, transfer factor of Cd was highest for assayed vegetables from the four sampling sites, which showed that Cd is more mobile than the other metals. Consistent with this suggestion, it was reported that Cd was retained less strongly by the soil and hence it is more mobile than other metals [29]. Transfer factor of 0.1 indicates that the plant is excluding the element from its tissues.

The greater the transfer coefficient value than 0.50, the greater will be the chances of vegetables for metal contamination by anthropogenic activities [30]. Hence, most of the metals might be affected by anthropogenic activities based on the transfer factor calculated in the current study. The transfer factor does not present the risk associated with the metals in any form. Therefore, the degree of toxicity of heavy metals to human beings depends upon their daily intake [28].When compared with a previous study Tyokumbur & Okorie [31], Jolly *et al*.[32] and Gebrekidan *et al*.[33], the current study showed a higher ease of transfer factor that can be attributed to differences in location and samples [31], and anthropogenic activities in the area.

CONCLUSIONS

The results of this study demonstrated that Cr was found in the highest concentration in the vegetables studied from among the investigated toxic heavy metals. The range of metals in the vegetables ranged from <MDL-17.13, 1.22-14.21, and <MDL-14.54 in cabbage, potato and khat, respectively. The respective soil samples contained higher concentrations of all assayed metals than the corresponding plant materials except for the case of Cd in some samples (cabbage from Harar and Haramaya, potato from Aweday and khat from Haramaya University) in which higher concentrations of the metal were detected in the plant samples than in the corresponding soil samples. In general, the results revealed that high concentrations of the toxic heavy metals were found in the vegetable samples, which are above the international safe limits. This reveals that consuming the vegetables produced in these areas poses health risks to humans. Therefore, people should be advised against consuming vegetables cultivated through irrigation with wastewater. It is also important to make farmers in the area aware of the danger of using wastewater for growing crops, and alternative sources of clean water should be sought for farmers to irrigate their crops during the dry seasons.

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Table 1: Guideline for safe limits of heavy metals in soil, water, and edible plants

Sample	Standards	Metals			
		Cr	Co	Cd	Pb
Soil (mg/kg)	Indian [6]	-	-	3-6	250-500
	WHO/FAO [7]	-	-	-	-
	European Union [8]	150	-	3.0	300
Water (mg/L)	Indian [6]	0.05	-	0.01	0.10
	European Union [8]	-	-	-	-
	FAO [9]	0.10	0.05	0.01	5.0
Plant (mg/kg)	Indian [6]	20.0	-	1.5	2.5
	WHO/FAO [7]	-	-	0.2	5.0
	FAO/WHO [10]	-	50.00	0.10	0.30

Table 2: Flame atomic absorption spectrometry instrument operational parameters

Metal	Wavelength (nm)	Slit width (nm)	Lamp current (mA)
Cr	357.9	0.7	2.0
Co	240.7	0.2	4.5
Pb	283.3	0.7	2.0
Cd	228.9	0.7	2.0

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Table 3: MDL and LOQ for the assayed samples

Sample	Metal	MDL	LOQ	IDL
Effluent	Chromium	0.175	0.584	0.050
	Cadmium	0.028	0.094	0.005
	Cobalt	0.146	0.487	0.050
	Lead	0.304	1.014	0.100
Soil	Chromium	0.277	0.922	0.050
	Cadmium	0.037	0.123	0.005
	Cobalt	0.137	0.457	0.050
	Lead	0.513	0.712	0.100
Vegetables	Chromium	0.175	0.584	0.050
	Cadmium	0.032	0.107	0.005
	Cobalt	0.139	0.463	0.050
	Lead	0.362	1.207	0.100

Table 4: Percentage recovery values ($\bar{x} \pm SD$, n =3) of vegetables, effluent water and soil samples

Samples		Metals			
		Cr	Cd	Co	Pb
cabbage	CIS[a]	9.4	1.9	5.7	12
	AA[b]	5	1	1	5
	AR[c]	4. 8±0.23	0.96±0.04	1.1±0.11	4.9±0.08
	R[d](%)	95.6	96.0	106	98.2
potato	CIS[a]	14	1.5	8.7	7.8
	AA[b]	5	1	5	5
	AR[c]	5.1±0.06	0.89±0.05	4.9±0.21	4.8±0.12
	R[d](%)	101	89.0	97.2	96.6
khat	CIS[a]	9	3.2	8.9	11
	AA[b]	5	1	5	5
	AR[c]	4.7±0.1	0.9±0.07	4.7±0.06	4.9±0.02
	R[d](%)	94.6	94.0	94.2	99.4
effluent	CIS[a]	0.3	0.06	1.0	2.5
	AA[b]	0.5	0.5	1	1
	AR[c]	0.5±0.03	0.5±0.01	0.97±0.02	0.9±0.05
	R[d](%)	92.0	102	97.0	93.0
soil	CIS[a]	37.6	1.4	18	26
	AA[b]	10	1	5	10
	AR[c]	9.8±0.13	0.9±0.09	4.9±0.03	9.2±0.07
	R[d](%)	98.3	91.0	97.6	92.1

$\bar{x}$ = mean, SD = standard deviation, n = number of replicates, [a]concentration in sample, [b]concentration added, [c]concentration recovered, [d]recovery

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Table 5: Comparison of metal concentration (mg/kg) in the vegetables with other edible and medicinal plants from the literature

Scientific name of the plant	Concentration (mg/kg) of metals in plants				Reference
	Cd	Pb	Cr	Co	
Spinach	<0.06	0.98	<0.05	0.35	
Carrot	<0.06	0.72	<0.05	<0.27	Jolly *et al*.[32]
Radish	0.65	0.51	1.68	<0.27	
Cabbage	0.18	3.82	0.43	0.14	Gebrekidan *et al*., [33]
Potato	0.18	2.58	0.39	0.10	
Lettuce	0.30	1.55	0.32	0.21	
Cabbage	0.97	5.31	-	-	Mohamed *et al*. [34]
Potato	0.99	6.19	-	-	
Cabbage	1.79	7.14	4.33	-	Jafarian *et al*.[35]
Potato	0.30	UDL[a]	6.00	-	
Lettuce	1.79	UDL[a]	4.33	-	
Coriander	0.01	0.40	4.20	0.20	
Radish leaf	0.01	0.40	4.70	0.50	Maleki *et al*.[36]
Dill	0.005	0.40	3.80	0.20	
Spinach	15.24	16.20	-	-	
Cabbage	2.97	13.01	-	-	Yadav *et al*., [37]
Radish	18.92	17.26	-	-	
Cabbage	1.2-2.5	5.5-12	<MDL[b]-17	5.7-9.7	
Potato	1.2-1.5	5.4-7.8	12-14	5.2-8.7	Present study
Khat	0.4-3.2	4.5-11	9.0-15	<MD[b]-8.9	

[a]under detection limit, [b]less than method detection limit

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Table 6: Transfer factor of the Chemical elements

Chemical elements	Vegetables	Sites			
		Harar	Aweday	Haramaya	HU
	Cabbage	0.25	-	-	0.41
Chromium	Potato	-	0.55	-	0.29
	Khat	-	0.36	0.31	0.35
	Cabbage	0.32	-	0.36	0.41
Cobalt	Potato	-	0.25	-	0.37
	Khat	-	0.43	-	0.02
	Cabbage	1.39	-	1.46	0.96
Cadmium	Potato	-	1.02	-	1.26
	Khat	-	0.8	0.48	1.26
	Cabbage	0.45	-	0.21	0.25
Lead	Potato	-	0.17	-	0.16
	Khat	-	0.14	0.22	0.23

Table 7: Comparison of transfer factors (TF) for heavy metals from soil to vegetable tissues with similar and other edible plants from the literature

Name of the plant	Concentration (mg/kg) of metals in plants				Reference
	Cd	Pb	Cr	Co	
Amaranthus caudatus	-	0.58	0.58	0.38	Tyokumbur and
Corchrus olithorus	-	1.20	0.46	0.37	Okorie [31]
Carrot	-	0.048	-	0.020	Jolly *et al.* [32]
Amaranthus	1.161	0.064	0.02	0.036	
Cabbage	0.25	0.56	0.01	0.01	Gebrekidan *et al.* [33]
Potato	0.26	0.78	0.01	0.01	
Cabbage	0.96-1.46	0.21-0.45	0.25-0.41	0.32-0.41	Present study
Potato	1.02-1.26	0.16-0.17	0.29-0.55	0.25-0.37	
Khat	0.48-1.26	0.14-0.22	0.31-0.36	0.02-0.43	

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