



Evaluation of Heavy Metals Uptakes of Lettuce (*Lactuca sativa* L.) Under Irrigation Water of Akaki River, Central Ethiopia

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Abstract: The present study was implemented to remediate heavy metal levels in lettuce grown by Akaki river water in 45 pots. The soil for the pot experiment collected from the Akaki area and the water used for lettuce growing from the upper, middle, and lower part of Akaki River with five treatment for three locations: groundwater (control), 25, 50, 75, and 100% Akaki river water with groundwater combination set as treatment. The results obtained from this pot experiment in lettuce samples Cd, Pb, Fe, Zn, Cr, and Cu concentration in the range of (0.047-0.263), (0.42-6.55), (339.83-420.00), (2.96-13.44), (0.95-7.87) and (1.68-7.49) (mg /Kg) respectively, all heavy metal concentration except groundwater irrigated lettuce sample above-recommended level set by WHO (1999). Whereas, Concentration of these metals in the soil samples (mg/kg) was found to be Cd (0.47-3.47), Pb (8.00-118.00), Fe (13557.30-16800.00), Zn (40.00-224.67), Cr (4.91-39.36) and Cu (35.00-149.88), out of Cd and Fe heavy metal concentration in Akaki river water irrigated soil samples except 100% Akaki river water irrigated soil sample was below the recommended level set by /FAOWHO (2001). Similarly, concentrations (mg/L) of the metals in the Akaki river water samples were found to be in the ranges of 0.18-0.28, 1.40-2.67, 0.97-1.40, 0.037-0.087, 0.037-0.080, and 01-0.14 for Fe, Zn, Cu, Cd, Pb, and Cr, respectively. The concentrations of heavy metals in the Akaki river water and lettuce samples were above the recommended limit of both WHO and FAO. Also, Cd and Fe for the soil samples were greater than WHO and FAO recommended limit. Based on facts obtained from this study we suggest concerned official body (ies) take the necessary precaution measures for cleaning the Akaki river water.

Keywords: Heavy Metals, Lettuce, Akaki River Water

1. Introduction

Heavy metals (HM) are extremely persistent in the environs. HMs are non-biodegradable and non-thermo degradable and they are readily accumulate to toxic levels in environment [1].

Wastewater and likely to increase markedly during the next few decades as water stress intensifies [2-4]. The aim of wastewater irrigation are that it delivers a consistent source of irrigation water for farmers and it has the helpful aspects of adding valuable plant nutrients and organic matter to soil [5], which are creating both opportunities and problems for the farming sector [6, 7]. Plants have a natural tendency to take up metals. Some of them like Cu^{2+} , Co^{2+} , Fe^{2+} , Mo^{2+} ,

Mn^{2+} , and Zn^{2+} are essential plant micronutrients [8], while few others like Hg^{2+} , Cd^{2+} , Ni^{2+} and Pb^{2+} are toxic to plants. Conversely, those like toxic HMs harmfulness effect are vary from genotype to genotype of the same crop [5]. Leafy vegetables are highly metal accumulator with compare to root vegetables and legumes [9].

Vegetables found in Akaki area are lettuce; spinach, carrots, radish, cabbage, potato, tomato, etc. are frequently grown in Peri-urban areas of Akaki and irrigated by the city effluents (Akaki river water). The direct use of untreated wastewater is common in developing countries, which are due to the lack of alternative water sources, The use of city effluents for irrigation purpose may also result in accumulation of macro (Na^+ , K^+ , Mg^{2+} , Ca^{2+}) and micro-essential elements (Fe^{2+} ,

Mn^{2+} , Zn^{2+}) as well as trace and toxic elements (Pb^{2+} , Ni^{2+} , Cd^{2+} , Cr^{2+} , and Co^{2+}) in phytotoxic concentrations [10], which may adversely affect the human metabolism. Therefore, wastewater application on agricultural lands and their heavy metal accumulation by vegetables is a cause of serious concern due to the potential public health impacts. It is often argued that heavy metals such as Cd^{2+} , Ni^{2+} or Pb^{2+} in sludge, when applied to soils, may enter in the food chain through plants or animals, contaminate surface and groundwater, and thus cause health hazards [11, 12]. This study was therefore; mainly focused to formulation suitable delusion of contaminated Akaki river water with irrigation or groundwater for the production of lettuce.

2. Materials and Methods

2.1. Study Area

Akaki river catchment is found in central Ethiopia sideway of the western border of the main Ethiopian Rift Valley. The river catchment is found $8^{\circ} 46' - 9^{\circ} 14' N$ and $38^{\circ} 34' - 39^{\circ} 04' E$, and the of river catchment are 1500 km^2 [13]. Addis Ababa, which lies within the Akaki catchment, has a fast population growth, abandoned development and industrialization, poor sanitation, untreated waste dumping, which results in a serious deterioration of surface and groundwater quality. As it is the country's commercial, manufacturing and cultural center, large quantities of solid, liquid and gaseous wastes are generated and released into the environment of the city, most often untreated [14]. Aba Samuel reservoir was assembled in 1939 at the middle part of Akaki River. It was the first hydropower station in Ethiopia, but it was abandoned in 1970s, because of many years of lack of maintenance, siltation and pollution issues [15]. It was rebuilt and revived in 2016. The local people in the Akaki River catchment and Aba Samuel reservoir use the water for irrigation, drinking water for cattle, washing clothes, waste disposal site and other domestic needs without information on the level of water quality parameters [16]. Therefore, this study has been conducted in some parts of Upper (Akaki sub city), middle (Aba Samuel reservoir) and lower (Abo sera area before joining of Awash River).

2.2. Soil Collection

Soil sample were collected from 0-20 cm depth with an auger from Alkali district for laboratory analysis and pot experiment around the Akaki river that are 2 km away from irrigation farm in the area, the soil was possibly non contaminated with heavy metals [17]. Then the samples were transported to the DZARC soil lab for lab analysis and pot experiment. Larger soil particles and debris were eliminated from the soil samples and then samples were air dried at room temperature ($2^{\circ}C$) for 7 days. Then the sample separated in to pot experiment and lab analysis. The sample for lab analysis were milled with a mortar and pestle to pass through a 2 mm sieve and homogenized. The prepared samples were placed in polyethylene bags until the time of

analysis.

2.3. Akaki River water Sampling

The water was collected from three stations of Akaki River namely: upper, middle and lower part of the river. Samples were collected for three months from December, 2018 to February, 2019 at ten days interval for pot experiment irrigation water. The water samples were collected from 0.5 meter below the water surface and collected in clean and dry polyethylene bottles. For heavy metal analysis from upper, middle and lower part of the river water samples were collected and immediately acidified by 1 ml nitric acid, for the purpose of keeping metals in the samples and avoid the adsorption container walls [18].

2.4. Experimental Setup

For the determination levels of HMs in lettuce it was irrigated with five different combination of wastewater (treatments) collected from 3 different point of the Akaki River (upper, middle and lower part of the river). The treatments (T) were be made by dissolving calculated amount of wastewater (Akaki river water (WW)) in irrigation water (ground water IW)) in the Debre Zeit Agricultural Research Center i.e., control, 25, 50, 75, and 100%. Those treatments were then designated by this abbreviation IW, 75%IW+WW, 50%IW+WW, 25%IW+WW and WW respectively. Each treatment were replicated 3 times. Lettuce grown in 45 pots contained well-mixed possibly non-contaminated garden soil collected from Akaki area. They were then arranged in proper distance to avoid any possible external contamination along with the provision of possible uniform light condition. 4 healthy and uniform seeds of lettuce were sown at one-centimeter depth at an equal distance. Pots were arranged in a completely randomized design (CRD) in green house, and were then equally irrigated with respective treatments of wastewater and irrigation water. All standard agricultural practices were made during the course of germination, growth and development. Before initiation of flowers, plant samples were made by taking three leaves each from all treatments and replicates. Each treated plant sample was washed separately with 1% of HCl. They will be then followed by 3-4 washings with de-ionized water to remove the foreign materials. These samples were then spread on blotting paper and air-dried, then oven dried at $70^{\circ}C$. Then the samples for lab analysis were ground with a mortar and pestle and homogenized samples were placed in polyethylene bags until the time of analysis [17]. And the soil sample from each pot were collected and follow the proper preparation method for laboratory analysis.

2.5. Digestion of Soil, Water and Lettuce Samples

2.5.1. Water Samples Digestion

A 50 mL filtered aliquot of water sample was pipetted into a digestion flask. The metal percentage found in the water was determined by digestion in 3 mL concentrated HNO_3 and 3 mL H_2O_2 below $80^{\circ}C$ for 1 h until a clear solution was observed.

The clear solution was diluted to 100 mL volumetric flask with distilled water and blank digestion was also carried out in the same way [19]. All samples were digested in triplicates. For heavy metal analysis FAAS was used.

2.5.2. Lettuce Samples Digestion

A 0.5 g powdered lettuce sample, 10 mL HNO_3 -HCl- H_2O_2 (8:1:1, v/v/v) were added in a borosilicate digestion flask. The mixture were heated at 120°C over 3 h on block digester. After digestion completed, the clear and colorless solution were filtered into 50 mL volumetric flask. Each digestion tube were rinsed with distilled water to collect any possible residue, and added to the volumetric flask which made up to volume with distilled water. Then dilute samples were stored in 100 mL plastic bottles (high density polyethylene) until analysis. The blank solution was prepared by taking a mixture of 8 mL HNO_3 , 1 mL HCl and 1 mL H_2O_2 and treating similarly as that of the sample [17, 20].

2.5.3. Soil Samples Digestion

The 0.5 g dried and homogenized soil samples were transferred in to 100 mL digestion flask. In each of these flasks, 5 mL of deionized water and 30 mL of a mixture HNO_3 (69%) and 37% HCl with volume ratio of 5:1 were added. The sample dissolved in the acid mixture was digested in digestion hood (at 200°C) for 1 h and kept to cool. After adding 2 mL of H_2O_2 to the cold digestion mixture, the final, the mixture was filtered out through filter paper to a 100 mL volumetric flask and diluted to the mark with deionized water [21]. Heavy metal content determination was done by FAAS. The blank reagent was also digested following the same procedure as the soil sample.

2.6. Soil Physicochemical Property Analysis

Selected Soil physicochemical property were conducted on experimental soil before sowing and after harvesting, following standard soil analytical procedures according to their parameters.

The pH was measured potentiometrically in the supernatant suspension of 1:2.5 soils to liquid ratio using distilled water (pH- H_2O), [22]. Electrical conductivity (EC) was measured using digital conductometer from the supernatant used for determination of pH- H_2O [23]. Organic carbon (OM) was determined by Walkley-Black wet oxidation method of the organic matter with potassium dichromate in the presence of sulphuric acid. The residual dichromate was titrated against ferrous sulphate to determine the amount of dichromate consumed by organic carbon or organic matter [24]. Total nitrogen (TN) was determined by Kjeldahl method using sulfuric acid and mixed catalyst to convert organic nitrogenous to ammonium sulfate. The distillation process was conducted in the presence of 10% sodium hydroxide to evolve ammonium in the form of ammonia gas [25]. Soil P content was determined before sowing and after harvesting, using Olsen method [26]. The extracts were determined spectrophotometrically with blue ammonium molybdate method in the presence of ascorbic

acid which is used as reducing agent. Cation exchangeable capacity (CEC) was determined, from extracts which were obtained from residue of exchangeable cations, after removal of unbounded ammonium salt with 97% alcohol and then extracted with 10% NaCl. The distillation was conducted using Kjeldahl distillation apparatus and the quantification made by titration method, [27].

2.7. Statistical Analysis and Data Interpretation

All the data generated from the pot experiments were subjected to statistical analysis using (SAS 9.0). Significant difference among treatment means was assessed using the least significant difference (LSD) test at 0.05 probability level [28].

3. Result and Discussion

3.1. Physico-Chemical Analysis of Soil Samples

The mean concentrations of pH, electrical conductivity (EC), total nitrogen, organic matter, and available phosphorus Cation exchangeable capacity before sowing were 7.89, 0.17 dm/S, 0.12%, 2.37%, 8.50 mg/kg and 24.23 cmol/kg, respectively and after harvesting of lettuce from pot experiment were also the above listed parameter were determined and presented in Table 1. The pH values of the soil samples range from 7.77 -8.22 are moderately alkaline. Consequently, it shows that alkaline ranges of soils are known to limit the mobilization of HMs and thus reduce the uptake of heavy metals by plants [29]. In general, most of HMs are less available to plants under alkaline conditions than under acid conditions.

The electrical conductivity values of the soil samples range from 0.11 -0.17 dS/m. Conductivity is a degree of the ability of aqueous solution to carry an electric current that depends on the presence and total concentrations of ions, their mobility and valance and on the temperature [30].

The Available P values of the soil samples before sowing and after harvesting range from 8.17 -12.42 mg/kg in medium range before sowing and after harvesting the pot experiment [31]. The nitrogen content of the soil before sowing and after harvesting was found to be low as rated by London [32].

Organic matter accumulates at the soil surface, mainly as a result of decomposing plant material. Whilst the organic matter content of soils is often small compared to that of clay, the organic fraction has a significant influence on metal binding. The mechanisms involved in the retention of metals by organic matter appear to include both complexation and adsorption, i.e. inner sphere reactions may take place as well as ion exchange [33]. Increase in soil organic matter content leads to elevation of soil adsorption capacity hence enhancing the accumulation of trace metals. Organic matters can therefore, be considered as an important medium through which heavy metals are incorporated into the soil [34].

The CEC is an important parameter of soil because it gives an indication of the type of the dominant clay minerals

present in the soil and its capacity to retain nutrients against leaching. The CEC is strongly affected by the nature and amount of mineral and organic colloids present in soil. Soils

with large amount of clay and organic matter have higher CEC than sandy soil low in organic matter.

Table 1. Soil physicochemical properties after harvesting of lettuce.

	Parameter	%TN	%OM	P mg/kg	EC dS/m	PH	CEC cmol/kg
	IW	0.097d	1.69b	8.17c	0.12c	8.04ab	27.34bc
upper water	75%IW+WW	0.103cd	1.83ab	9.70b	0.13c	8.18a	27.25c
	50%IW+WW	0.117bc	1.93ab	10.07b	0.14b	8.05ab	32.03ab
	25%IW+WW	0.123ab	1.94ab	10.20b	0.15b	7.95b	33.41a
	WW	0.137a	2.13a	12.17a	0.17a	7.90b	32.67a
	CV	6.716	10.46	9.59	4.30	1.28	8.29
	LSD (0.05)	0.015	0.38	1.27	0.01	0.19	4.77
middle water	IW	0.090c	1.90b	8.97b	0.12b	8.17a	27.58c
	75%IW+WW	0.087bc	1.87b	8.95b	0.12b	8.09ab	33.17a
	50%IW+WW	0.103b	1.90b	9.05b	0.12b	8.09ab	29.17bc
	25%IW+WW	0.120a	2.09ab	9.21b	0.012b	7.98bc	28.43c
	WW	0.123a	2.34a	12.42a	0.13a	7.89c	31.20ab
	CV	8.456	10.56	10.43	2.83	0.93	4.56
Lower water	LSD (0.05)	0.017	0.38	1.06	0.01	0.14	2.5
	IW	0.097b	1.78b	8.34b	0.12	8.22a	27.65b
	75%IW+WW	0.093b	2.02ab	9.44ab	0.12	8.12c	27.73b
	50%IW+WW	0.093b	1.89ab	8.64b	0.11	8.18b	26.72b
	25%IW+WW	0.137a	1.99ab	10.02ab	0.12	7.95d	35.31a
	WW	0.130a	2.12a	11.37a	0.12	7.91e	33.73a
	CV	7.78	8.12	14.48	3.11	0.21	24.98
	LSD (0.05)	0.016	0.3	1.94	N	0.03	3.67

Where IW, 75%IW+WW, 50% IW+WW, 25%IW+WW and WW refer to soil sample taken from 100% ground water (irrigation water), 75% ground water + 25% Akaki River water, 50% ground water + 50% Akaki River water, 25% ground water + 75% Akaki River water and 100% Akaki River water

irrigated lettuce growing pot, respectively. Values are given as means of triplicates \pm SD. The mean values in the same column having different superscript letters are significantly different from each other at 5% confident interval.

Table 2. Mean concentration of Cd, Pb, Fe, Zn, Cr and Cu of soil samples after lettuce harvesting in acid digestion method ($n=3$ in mg/kg).

	S. code	Cd	Pb	Fe	Zn	Cr	Cu
Upper Akaki water	IW	0.47c	8.00d	13557.30d	40.00e	5.60e	45.00e
	75%IW+WW	3.08b	14.00d	14097.00cd	77.90d	21.60d	88.17d
	50%IW+WW	3.19b	36.00c	14466.70c	108.00c	25.52c	108.33c
	25%IW+WW	3.23b	101.60b	15060.00b	159.67b	30.48b	133.67b
	WW	3.43a	118.00a	16800.00a	224.67a	39.36a	149.88a
	CV	3.77	8.49	2.05	12.93	5.30	6.21
Middle Akaki water	LSD (0.05)	0.19	8.88	570.75	22.72	2.45	12.28
	IW	0.53e	7.00d	13593.30c	50.00e	4.91c	35.00e
	75%IW+WW	2.62d	33.40c	13898.00c	97.67d	22.56b	55.00d
	50%IW+WW	2.84c	42.50b	13921.30c	141.33c	24.00b	93.50c
	25%IW+WW	3.05b	40.90b	14986.70b	160.67b	29.79a	112.33b
	WW	3.22a	49.30a	15613.30a	206.00a	29.84a	130.00a
Lower Akaki water	CV	2.25	5.15	1.34	3.93	5.15	9.29
	LSD (0.05)	0.10	3.35	363.38	9.70	2.16	14.90
	IW	0.53d	8.00	13576.00e	46.67e	5.42c	45.00c
	75%IW+WW	2.26c	15.00d	14103.30d	86.67d	8.80c	53.50c
	50%IW+WW	2.81b	37.20c	14886.70c	122.67c	16.00b	62.83b
	25%IW+WW	2.87b	44.60b	15753.30b	144.33b	20.00b	66.00b
	WW	3.23a	49.00a	16240.00a	201.67a	29.52a	143.50a
	CV	6.28	4.65	1.48	4.42	15.11	6.27
	LSD (0.05)	0.28	2.69	414.30	10.02	4.54	8.76
	FAO/WHO [36]	3	100	5000	300	50	100
	USEPA, [44]	3	300	-	300	150	140
	EU, [43]	3	300	-	200	-	50

3.2. Levels of Heavy Metals in Soil Samples After Harvesting of Lettuce From Pot

The concentrations of Cd, Pb, Fe, Zn, Cr and Cu in the

digested samples of soil were determined by FAAS. The concentrations of these metals before planting were 0.61, 8.12, 13601.00, 52.01, 6.01 and 54.12 in mg/kg respectively and the concentration of the metal after harvesting are

presented in Table 2 in all treatments the concentration of iron were much higher than other. As shown in Table 2, the recorded results of accumulated heavy metals in soil showed that iron, zinc and copper showed relatively higher values in the all five treatment (IW, 75%IW+WW, 50%IW+WW, 25%IW+WW, WW) and three location upper, middle and lower Akaki river) compared to other heavy metals. Those directs wastewater might contain more sources of these metals. In generally, heavy metals like, Lead (Pb), and cadmium (Cd) have no beneficial effects in humans, and there is no known homeostasis mechanism for them [17]. They are generally considered the most toxic to humans and animals; the adverse human health effects associated with exposure to them, even at low concentrations, are diverse and include, but are not limited to, neurotoxic and carcinogenic. The metals considered in this study include the metals which are micro-nutrient such as iron, zinc, cobalt and copper and the non-essential/toxic heavy metal which are toxic to plant when present in the soil at concentrations above tolerance levels for the non-essential metals, Cr, Pb and Cd are recognized as health hazardous and all have caused major health problems as a result of environmental pollution [17].

The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

3.2.1. Chromium in Soil Samples

Chromium plays a vital role in the metabolism of cholesterol, fat, and glucose. Its deficiency causes hyperglycemia, elevated body fat, and decreased sperm count, while at high concentration it is toxic and carcinogenic [35]. As shown in Table 2 above, the Cr contents in the soil samples after pot trials were found to be within the range of 4.91 and 39.36 mg/kg. The highest and lowest contents of Cr occurred in the upper Akaki river irrigated soil and irrigated water irrigated soil, respectively. Cr permissible level in farm field set by WHO/FAO [36] is 50 mg/kg. But, the levels of Cr in detected soil samples from pot experiment might be safe for human health. But the increment of Cr concentration in Akaki river water irrigated pot soil samples were increase rapidly.

3.2.2. Cadmium in Soil Samples

Cadmium is also a non-essential heavy metal. It is extremely toxic even at low concentration. It causes learning disabilities and hyperactivity in children [37]. As shown in Table 2, the experimental results showed that Cd concentration in soil samples occurred in the range of 0.47 and 3.43 mg/kg. The highest and lowest contents of Cd occurred in the upper Akaki river irrigated soil and irrigated water irrigated soil, respectively. WHO/FAO [36] permissible limit of cadmium in soil is 3 mg/kg. So, the concentration of cadmium found in upper Akaki river (75% IW+WW, 50% IW+WW, 25% IW+WW and WW), middle Akaki river (25%IW+WW and WW) and lower Akaki river (WW) irrigated soil were above the permissible limit set by WHO/FAO [36] it might be harmful for human health.

3.2.3. Zinc in Soil Samples

The natural range of zinc in soils is 10 – 300 mg/kg [38]. It is the basic component of a large number of different enzymes and plays structural, regulatory, and catalytic functions. As shown in Table 2 above, the soil concentration of zinc in this study was within these natural ranges with values ranging between 40.00 mg/kg to 224.67 mg/kg. WHO/FAO permissible limit of zinc in soil is 300 mg/kg. So, the concentration of zinc we obtained now is found to be below the permissible limit set by WHO/FAO [36] in all treatment irrigated soil samples.

3.2.4. Iron in Soil Samples

Iron is the most abundant and most essential constituent for all plants and animals. On the other hand, at high concentration, it causes tissues damage and some other diseases in humans. It is also responsible for anemia and neurodegenerative conditions in human being [39]. As shown in Table 2 above, the results indicate that soil samples contained Fe in the concentration range of 13557.30 and 16240.00 mg/kg. WHO/FAO [36] permissible limit of iron in soil is 5000 mg/kg. So, the concentration of iron found in all soil samples from pot experiment might be harmful for human health, even though in irrigated water irrigated soil samples the concentration of Fe was above the permissible level.

3.2.5. Lead in Soil Samples

Lead is one of the more insistent metals and is predictable to have a soil retention time of 150 to 5000 years [40]. It is a non-essential HM. High level accumulation of Pb in body causes anemia, colic, headache, brain damage, and central nervous system disorder [41]. As shown in Table 2, the soil samples contained Pb concentrations in the range of 7.00–118.00 mg/kg. WHO/FAO [36] permissible limit of lead in soil is 100 mg/kg except 100% upper Akaki river water irrigated soil samples might be safe for human health. But the increment of Pb concentration in Akaki river water irrigated pot soil samples were increase rapidly.

3.2.6. Copper in Soil Samples

Being an essential trace element, it is necessary for many enzymes. High concentration of Cu causes metal fumes fever, hair and skin decolorations, dermatitis, respiratory tract diseases, and some other fatal diseases in human beings [42]. As shown in Table 2 above, highest level (149.88 mg/kg) of Cu was found in 100% upper Akaki river water irrigated soil and the soil irrigated with irrigation water had the smallest level (35.00 mg/Kg) of Cu. WHO/FAO [36] allowable limit of lead in soil is 100 mg/kg. Also the concentration of copper in upper Akaki river (50% IW+WW, 25% IW+WW and WW), middle Akaki river (25% IW+WW and WW) and lower Akaki river (WW) irrigated soil was above the concentration permissible limit set by EU [43]; USEPA [44] as shown in Table 2.

3.3. Concentration of Metals in the Lettuce Samples

The concentrations of Cd, Pb, Fe, Zn, Cr and Cu in lettuce plant collected from experimental pots are presented in Table

3. From the study, the concentration of all metals in the detected sample were accumulated to greater or lesser extents.

Table 3. Mean concentration of Cd, Pb, Fe, Zn, Cr and Cu of lettuce samples in wet digestion method (n=3 in mg/kg).

	S. code	Cd	Pb	Fe	Zn	Cr	Cu
Upper Akaki water	IW	0.047d	0.51d	345.60c	2.96e	0.98e	2.39d
	75%IW+WW	0.233c	0.78d	352.43cd	5.19d	4.32d	4.41c
	50%IW+WW	0.223b	2.00c	361.67c	7.20c	5.1c	5.42b
	25%IW+WW	0.237c	6.53b	384.75b	9.51b	5.66b	6.03b
	WW	0.263a	6.55a	420.00a	11.66a	7.87a	7.49a
	CV	1.550	5.32	2.18	5.52	5.67	7.82
	LSD (0.05)	0.010	0.31	15.32	0.76	0.51	0.76
Middle Akaki water	IW	0.050d	0.42d	339.83c	3.15d	0.97c	1.68e
	75%IW+WW	0.200c	1.94c	348.03b	6.51c	4.58b	3.09d
	50%IW+WW	0.230b	2.35b	350.12b	8.89b	4.80b	4.88c
	25%IW+WW	0.230b	2.36b	384.67a	8.97b	5.71a	5.89b
	WW	0.250a	2.75a	391.00a	11.30a	5.97a	6.63a
	CV	2.240	5.40	1.07	2.90	6.52	3.02
	LSD (0.05)	0.010	0.20	7.34	0.42	0.54	0.25
Lower Akaki water	IW	0.043d	0.46e	340.07e	3.18e	0.95d	2.32c
	75%IW+WW	0.190c	0.83d	353.41d	5.78d	1.76c	2.65c
	50%IW+WW	0.220b	2.10c	372.17c	8.14c	3.2b	3.13b
	25%IW+WW	0.230b	2.48b	394.50b	9.54b	4.00b	3.30b
	WW	0.250a	2.72a	406.00a	13.44a	5.77a	7.18a
	CV	2.87	4.11	1.42	4.83	13.55	5.67
	LSD (0.05)	0.01	2.31	9.99	0.73	0.80	0.40
	WHO [45]	0.20	0.5	150	1.5	1.20	2.0
	CMH [48]	0.05-0.2	0.1-0.3	-	-	0.5-1.0	-
	FAO [49]	0.01	5.00	-	2.00	-	0.2

The means in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

3.3.1. Cadmium in Lettuce

Cd concentration in lettuce samples collated for pot experiment were ranged of 0.043-0.263 mg/kg in all three part of the river water irrigated lettuce samples as shown in Table 3. The concentration of Cd was maximum (0.263 mg/kg) in lettuce sample irrigated with 100% upper Akaki river water, this, result was higher than permissibility level set by WHO [45]. Cadmium is more solvable as compared to other metals so, it can accumulate more into the vegetables tissues [46]. The permissible limits of Cd is (0.20 mg/Kg) in the plant tissue proposed by WHO [45].

3.3.2. Lead in Lettuce

Results show that the levels of lead in the lettuce studied had a range of 0.42 to 6.55 mg/kg as shown in Table 3. A similar study that is conducted by Girmaye [47] reported that lead concentrations of lettuce ranged from 2.3-5.30 mg/kg sample by which super passed the maximum permissible level of Pb set by WHO [45]; CMH [48]; FAO [49]. In this study, the high level of Pb in lettuce suggests that the Akaki river water used for irrigation was not good for irrigation of crops in general and leafy vegetables in particular.

3.3.3. Iron in Lettuce

In this study, Fe concentration from the lettuce samples for pot trials varied between 339.83-420.00 mg/kg. The concentration of Fe was maximum (420.00 mg/kg) in lettuce sample irrigated with 100% middle Akaki river water, this, result was higher than permissibility level set by WHO [45]

and WHO [45] permissible limit is 150 mg/kg (Table 3). Fe is vital element for all plants has many significant biological roles in the processes as diverse as photosynthesis, chloroplast development and chlorophyll biosynthesis [50]. In humans, increased body stores of iron have been shown to increase the risk of several estrogen-induced cancers [51].

3.3.4. Zinc in Lettuce

In this study, results show that the levels of zinc in lettuce had a range of 2.96-11.66 mg/kg, middle Akaki river water irrigated lettuce 3.15-11.30 mg/kg and lower Akaki River irrigated lettuce 3.18-13.44 mg/kg and WHO [45] permissible limit is 1.50 mg/kg (Table 3). All the ventures exhibited very high concentration compared to the permissible limit set by WHO [45]; CMH [48]; FAO [49]. The concentration may be as a result of the number of factories wastewater immersing in to Akaki River.

3.3.5. Chromium in Lettuce

In this study the chromium contents in lettuce samples collected from pots experiment irrigated with Akaki river water were obtained to have ranged in Upper Akaki water irrigated lettuce 0.97±0-7.87 mg/kg, middle Akaki river water irrigated lettuce 0.97-5.97 mg/kg and lower Akaki river irrigated lettuce 0.95-5.77 mg/kg these, result were higher than permissibility level set by WHO [45] except the irrigation water irrigated lettuce sample in all three part of the river Table 3.

3.3.6. Copper in Lettuce

In this study, Cu concentration from the lettuce samples from pot experiments varied between 1.68-7.49 mg/kg and WHO [45] permissible limit is 2.0 mg/kg (Table 3). High levels of Copper

present in the parts of the plants may be due to the absorption ability of the plants to get the trace heavy metals from the polluted soils. Cu is especially important in seed production, disease resistance, and regulation of water [17].

3.4. Level of Heavy Metals in Akaki River Water Samples

The distribution of heavy metals (Fe, Zn, Cu, Cd, Pb and Cr) in Akaki river water has been evaluated. Akaki river

samples were collected from the upper, middle and lower part of Akaki River and taken to the laboratory for chemical analysis. As shown in Table 4, the result heavy metals in the Akaki river water samples were found to be in the order of $Zn > Cu > Fe > Cr > Pb > Cd$ in the three part of the river. Moreover the concentration of each heavy metal was higher than the permissible limit set by WHO [52] which could pose a huge threat to human health and the natural environment.

Table 4. Mean concentration of Fe, Zn, Cu, Cd, Pb and Cr in mg/L of Akaki river sample (n=3).

S. code	Fe	Zn	Cu	Cd	Pb	Cr
ground water	0.080c	0.67d	0.63c	0.004d	0.017d	0.07c
Upper Akaki	0.28a	2.67a	1.40a	0.087a	0.080a	0.14a
Middle Akaki	0.24a	1.80b	1.10b	0.060b	0.053b	0.11b
Lower Akaki	0.18b	1.4c	0.97b	0.037c	0.037c	0.10b
CV	9.79	11.32	11.15	6.950	7.890	7.33
LSD 0.05	0.04	0.37	0.23	0.007	0.007	0.02
WHO [52]	0.30	0.03	0.01	0.003	0.050	0.10
USEPA [44]	-	2.00	1.00	0.005	0.015	0.10

The mean values in the same column having different superscript letters are significantly different from each other at 5% confidence interval.

3.4.1. Iron Akaki River Water

Concentrations of iron in the Akaki river samples at upper, middle and lower part of the river were 0.28, 0.24 and 0.18 mg/L, respectively. Iron concentrations in three samples were above the recommended maximum concentration for irrigation [52] Table 4. The high concentration of iron in irrigation water contributes to soil acidification and loss of availability of phosphorus and molybdenum when applied to the soil [53].

3.4.2. Zinc Akaki River Water

Zinc concentration in Akaki river samples at upper, middle and lower part of the river were 2.67, 1.80 and 1.4 mg/L, respectively. The samples exceeded the recommended maximum concentration for crop irrigation WHO, [52]. Zinc is the least toxic element in the human diet it is required to maintain the proper functioning of the immune system, normal brain activity and is essential in the growth and development of the foetus, but a very high concentration of zinc is very toxic, hence injurious to the human body [54].

3.4.3. Copper Akaki River Water

As shown in Table 4, Cu levels in Akaki River samples at the upper, middle and lower part of the river were 1.40, 1.10 and 0.97 mg/L, respectively. These values were found to be higher than optional limit of Cu for irrigation water set by WHO [52] (Table 4).

3.4.4. Cadmium Akaki River Water

The results show that the Cd concentrations in the Akaki river samples at upper, middle and lower part of the river were 0.087, 0.060, 0.037 mg/L, respectively. The concentrations of Cd obtained in the present study are above the permissible limit set by WHO [52] USEPA [44] for crop irrigation. Cadmium is considered to be hazardous metal because of its toxicity and accumulation capacity in the

living system [4].

3.4.5. Lead Akaki River Water

Lead concentration in Akaki River samples at upper, middle and lower part of the river were 0.080, 0.053 and 0.037 mg/L, respectively. The lead value were of to be higher than recommended limit of Pb for irrigation water set by WHO [52] and USEPA [44] (Table 4). This makes the water unsuitable for human consumption as Pb is known to be toxic even at low levels with resultant ill-health effects as chronic exposure has been linked to growth retardation in children [55]

3.4.6. Chromium Akaki River Water

The levels of Cr in the Akaki river samples at the upper, middle and lower part of the river were 0.14, 0.11 and 0.10 mg/L, respectively. These values are above the permissible limit set by WHO [52] and USEPA [52] (Table 4) for crop irrigation. Cr is a toxic element due to its harmful effect on human health, mainly in its hexavalent form [53].

4. Conclusion

As stated previously, the major aim of this study was to find out solution for heavy metal toxicity levels of Akaki River water for irrigation purpose. The Akaki River water, lettuce growing soil and lettuce samples that were growing with upper, middle and lower part of Akaki River water with ground water by the combination of control, 25, 50, 75, and 100% Akaki river water with ground water combination set as treatment) were subjected to wet-digestion and the concentration of detected metals were determined via FAAS. The level of heavy metals in upper, middle and lower from part of Akaki river water is above the recommended level set by WHO and USEPA for irrigation water. The concentration heavy metals in all diluted and undiluted upper, middle and lower Akaki River water irrigated lettuce samples were above the recommended levels set by WHO. But the concentration heavy metals in soil samples that lettuce were grown is within the recommended levels set by FAO/WHO, USEPA

and EU in the other hand the levels of Fe was above the recommended levels set by the above organization. Based on facts obtained from this study we suggests that this formulation used for drip irrigation. And concerned official body (ies) to take the necessary precaution measures for cleaning the polluted Akaki River water.

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