Assessment of Essential and Non-Essential Metals Concentration in Some Selected Edible Vegetables Irrigated with Municipal Waste Water in Mayham, Adigrat, Estern Tigray – Ethiopia

Gebregziabher Brhane¹, Kassa Belay², Kiflom Gebremedhin², Taame Abraha², Tassew Alemayehu², Teklay Mezegebe², and Mebrahtu Hishe²

¹Department of chemistry, Adigrat University, Adigrat, Tigray, P.Box. 50, Ethiopia

²Department of Biology, Adigrat University, Adigrat, Tigray, P.Box. 50, Ethiopia

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ABSTRACT: This paper presents the concentration of Cu, Zn, Fe, Pb and Cd in vegetables, soil and water samples. Samples were collected from Mayham (Adigrat, Tigray region). Total acid (7ml mixture of HNO_3 , H_2SO_4 , $HClO_4$) digestion method was employed and determination was made by flame atomic absorption spectroscopy. The percentage recoveries of the metals were in the range of 89% to 100% in vegetable, 84% to 100% in water and 82% to 103% in soil sample. The range of concentration($\mu g/g$) of the metals on dry weight basis are: Cd 1.18-1.45 in vegetables, 1 in soil and 9 in water; Cu 9-18 in vegetables, 15-17 in soil and 4.3 in water; Pb 1.67-5.01 in vegetable, 3-5 in soil and 2.6 in water; Zn 40-398.5 in vegetable, 59-66.8 in soil and 9.2 in water; Fe 218.25-4987.5 in vegetables, 23705.75 – 29248.5 in soil and 177.5 in water sample. The result obtained imples that the plant is rich in iron, zinc and copper and has small concentration of non-essential trace elements like lead and cadmium.

KEYWORDS: vegetable, acid digestion, metals, Mayham garden, FAAS.

1 INTRODUCTION

Food safety is a major public concern worldwide. During the last decades, the increasing demand for food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins. Food safety issues and potential health risks make this as one of the most serious environmental concerns. Heavy metal accumulation in plants depends upon plant species, and the efficiency of different plants in absorbing metals is evaluated by either plant uptake or soil-to plant transfer factors of the metals. Vegetables constitute essential components of the diet, by contributing protein, vitamins, iron, calcium and other nutrients which are usually in short supply. However intake of heavy metals contaminated vegetable may pose a risk to human health. Heavy metal, namely cadmium (Cd), lead (Pb), zinc (Zn), copper (Cu) and other has been identified as a risk to human health through the consumption of vegetable crops. Heavy metals are given special attention throughout the globe due to their toxic and mutagenic effects even at very low concentration [1, 2].

Heavy metals are among the major contaminants of food supply and may be considered the most important problem to our environment. Such problem is getting more serious all over the world especially in developing countries such as North and South Africa, Zimbabwe, Nigeria, Tanzania and Egypt. Heavy metals are non-biodegradable and persistent environmental contaminants, which may be deposited on the surfaces and then absorbed into the tissues of vegetables. Plants take up heavy metals by absorbing them from deposits on the parts of the plants exposed to the air from polluted environments as well as from contaminated soils. Contamination of vegetables with heavy metal may be due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, the harvesting process,

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storage and/or at the point of sale (market). Human beings are encouraged to consume more vegetables and fruits, which are beneficial for health. Publicity regarding the high level of heavy metals in the environment has created apprehension and fear in the public as to the presence of heavy metal residues in their daily food. Keeping in the potential toxicity and persistent nature and cumulative behavior as well as the consumption of vegetables and fruits, there is necessary to test and analyze these food items to ensure the levels of these contaminants meet agreed international requirements [3]. Heavy metals may enter the human body through inhalation of dust, consumption of contaminated drinking water, direct ingestion of soil and consumption of food plants grown in metal-contaminated soil. [4].

Lead and cadmium are among the most abundant heavy metals and are particularly toxic. The excessive content of these metals in food is associated with etiology of a number of diseases, especially with cardiovascular, kidney, nervous as well as bone diseases. Other metals such as copper and zinc are essential for important biochemical and physiological functions and necessary for maintaining health throughout life, but if theses metals have excessive concentration above the WHO value, which cause a diseases (5).

The health effect of these metals is less studied though a number of people are consuming vegetable day to day in its raw and sauce form that may result the accumulation of trace metals in human body. However, to the extent of assessment done, there is no literature report on the determination of the levels of heavy metals in Ethiopian vegetables (cabbage, lettuce and potato). Hence, this research is intended to determine the concentration of trace metals (cadmium, copper, zinc, iron and lead) in edible vegetables that are commonly grown.

1.1 OBJECTIVE OF THE STUDY

1.1.1 GENERAL OBJECTIVE

The general objective of this study is to assess the concentration of essential and non- essential metals in municipal waste water irrigated vegetables grown in Adigrat town, particularly in Mayham.

1.1.2 SPECIFIC OBJECTIVE OF THE STUDY

To determine the concentration of essential (Cu, Zn and Fe) and non essential (Pb and Cd) metals in soil, water and edible vegetable samples treated with sewage water for irrigation.

To compare the level of essential and non-essential metals among the soil, water and vegetables.

To assess the health of metals.

2 METHODOLOGY

2.1 DESCRIPTION OF STUDY AREA

The sample for this study was collected from Adigrat town which is located in the Northern part of Ethiopia, Tigray region 898km far from Addis Ababa and 115 km away from north of mekelle town, the capital city of Tigray regional state. It is found between 14°16.453' N latitude and 039°, 27.654'E longitude with altitude of 2457 masl.

2.2 CHEMICALS AND INSTRUMENTATION

2.2.1 INSTRUMENTATION

The instrument used was fully automated PC- controlled true double-beam atomic absorption spectrometer with fast sequential operation for fast multi element air acetylene flame AA determinations. Its feature have 4 lamp positions and automatic lamp selection, operated with specter AA base and pro-soft ware versions with each different hallow cathode. Therefore the general setting and optional parameters of atomic absorption spectroscopy used in this study are listed as follow.

Element	Wavelength(nm)	Slit Width (nm)	Lamp Current (mA)	Flame type
Cd	228.8nm	0.2	4.0	Reducing
Pd	230.1nm	0.4	10.0	oxidizing
Cu	324.8nm	0.5	4.0	Air/acetylene
Zn	213.9nm	1.0	5.0	Air/acetylene
Fe	248.3nm	0.2	5.0	Air/acetylene

Table2.1. Work	ing Conditions	of Atomic Abso	orption Spectroscopy
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2.2.2 CHEMICALS

Distillated water, H_2SO_4 , $HClO_4$, concentrated HNO_3 , stock solutions of $Cd(NO_3)_{2_2}$, $Cu(NO_3)_{2_2}$, $CaCO_3$, $Pb(NO_3)_{2_2}$, $Fe(NO_3)_2$ and $Zn(NO_3)_2$ was used in the experiment.

2.2.3 APPARATUS AND INSTRUMENTS

Poly ethylene bags, different size volumetric flasks, oven, mixer grinder, beakers, digital analytical balance, Crucibles, pipettes, thermometer, muffle furnace, filter Paper No.41, porcelain mortar, and flame atomic absorption spectrophotometer (FAAS).

2.2.4 FLAME ATOMIC ABSORPTION SPECTROMETRY (FAAS)

FAAS is a method of detecting and measuring metallic elements. It is the most widely used technique for analysis of trace metals in contaminated wastes. The introduction of FAAS has produced a rapid and relatively inexpensive method for the quantitative determination of metals at trace level (1-100 ppm) in a wide variety of samples. This technique is based on the vaporization of the analyte sample by aspirator of the solution into the flame. The samples to be investigated have to be broken in to their atoms. This is done by aspirating the sample solution into a hot flame. Before it enters the flame the solution is dispersed into a mist of very fine droplets, which evaporates in the flame. At least a part of the vaporized molecules must dissociate into atoms of the element to be measured. Light of certain wave length produced by a special type of light source or lamp is passed through the long axis of a flat flame and into a spectrometry. The atom dispersed in the flame, absorbs some of the radiation. They do not absorb all the line emitted by the lamp, since nearly all the atoms are in their ground state. Therefore only those emission lines that correspond to transitions from the ground state will be absorbed consequently, the beam of radiation coming out of the sample misses the radiation in the corresponding wave length, which is a measure of the characteristics of the sample. The instrument to be used is the buck model 210VGP atomic absorption spectrophotometer [6].

2.3 SAMPLE COLLECTION

2.3.1 COLLECTION OF WATER SAMPLE

Water sample collection was performed by the distance of 50cm in order to get representative sample and 50 cm depth in order to exclude the dust materials. Finally the freshly collected water sample was mixed together and was taken the composite sample for digestion process. Water samples means that waste water used for irrigation was collected along with the blank (distilled water) in a 100 ml pre acid - washed polypropylene bottle and 1 ml of *8concentrated HNO₃ was added to the sample to avoid microbial activity [2].

2.3.2 COLLECTION OF SOIL SAMPLE

Soil samples were also be collected in triplicate by digging out a monolith of 10 x 10 x 15 cm size from 4 different fields from waste water irrigation sites.

2.3.3 COLLECTION OF VEGETABLE SAMPLE

Vegetables samples were air dried, crushed, passed through a 2 mm mesh sieve and were stored at ambient temperature for analysis. Edible parts of different vegetables were collected from the experimental sites. Leafy vegetables such as

cabbage, potato and lettuce, only edible portion of each of the test vegetables were collected. After washing with clean tap water to remove the soil particles, vegetable sample will be oven dried at 80° c to constant weight. The dried sample was ground, passed through 2 mm sieve and stored at room temperature before analysis [3].

2.4 SAMPLE PREPARATION AND TREATMENT

For heavy metal extraction, 1 g dried sample of vegetables or soil was digested in 15 ml of HNO_3 , H_2SO_4 and $HClO_4$ mixture (5:1:1) at 80 °C until a clear and colorless solution will be obtained. A water sample (50 ml) was digested with 10 ml of concentrated HNO_3 at 80 °C until the solution became a clear and colorless solution. These a clear and colorless solution was filtered using Whatman number 42 filter papers and diluted to 50 ml with distilled water.

The concentrations of Cd, Cu, Pb, Zn, Fe, and Ca in the filtrate was determined by using flame atomic absorption spectrophotometer (Model 2380, Perkin Elmer, Inc. Norwalk, CT, USA), fitted with a specific lamp of particular metal using appropriate blanks solution [8].

2.5 PREPARATION OF STANDARD SOLUTIONS

Determination of the metal concentration in the experimental solution was based on the calibration curve. In plotting the calibration curves lead, cadmium and chromium stock solutions of 1000 ppm were prepared by dissolving 1.6 g of Pb(NO₃)₂, 2.74 g Cd(NO₃)₂.4H₂O and 2.83 g K₂C-/*9+/8r₂O₇ in de- ionized water respectively. Blank solutions were prepared for the methods and, for the standard working solutions, to prepare 100 ppm, 10 mL of the standard Pb(NO₃)₂, Cd(NO₃)₂.4H₂O and K₂Cr₂O₇ stock solution were pipetted and added into 100 mL calibrated flasks finally diluted with de-ionized water and the solution was mixed thoroughly. Next, to prepare 50 ppm standard solution of each metal, 50 mL of each of 100 ppm stock solution was pipetted into 100 mL volumetric flasks and diluted with de-ionized water. Finally to prepare 0.0, 0.5, 1.0, 2.0, 4.0, 6.0 ppm aliquots of this standard working solution 0.0, 0.5, 1.0, 2.0, 4.0, 6.0 mL was pipetted from 50 ppm standard solution into 50 mL calibrated flasks and made up to volume with De-ionized water [9].

2.6 ANALYTICAL PROCEDURE FOR HEAVY METAL ANALYSIS BY FAAS

Soil, water and vegetable samples were analyzed for heavy metals using FAAS. The heavy metals analysis adjustment of the operating condition was very essential target. Wavelength, slit width, limit of detection was adjusted for the analysis of the metals Pb, Cd, Fe, Ca, Zn and Cu. 1000 mg/l Standard solutions of metals was prepared in 0.1 N HNO₃ for calibration curve from the standard salt of each metal in 1000 ml volumetric flask. From this stock solution 100 mg/l of each metal was freshly prepared by diluting in 100 ml volumetric flask with distillated water and then the working solution (10 mg/l) of each metal was prepared. For the determination of these metals, four solutions was prepared for each sample from each source and four standard solutions was made for each metal which is shown below and rinse blank (distilled water) was used to flush the uptake system to reduce memory interferences [10].

Metals	Concentration of standards (mg/kg)		
Pb	0.2, 0.4, 0.8, 1.2		
Cd	0.1, 0.3, 0.7, 1.1		
Zn	0.5, 1.0, 3.0, 5.0		
Cu	0.10, 1.0, 2.0, 4.0		
Fe	0.5, 1, 2, 4		

Table2. 2. Standard concentration of the metals to be analyzed by FAAS

2.7 DATA ANALYSIS

The statistical analysis of all data was conducted using SPSS software (version 16.0). One way ANOVA was used to determine the significance difference of the metals among soil, water and vegetables at P < 0.05 of significance level.

3 RESULT AND DISCUSSION

3.1 VALIDATION OF EXPERIMENTAL RESULTS

3.1.1 DETERMINATION OF DETECTION LIMITS

Method detection limit defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero. Detection limit is the lowest concentration level that can be determined at 95% confidence level [6] or the minimum concentration that can be detected by the analytical method with a given certainty [9]. A general accepted definition of detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal. In this study the detection limit of each element was calculated as three times the standard deviation of the blank (3σ blank, n = 5), as summarized in **Table 3.1**.

element	MDL	LOQ
Cd	0.001	0.01
Cu	0.03	0.3
Pb	0.002	0.02
Zn	0.002	0.02
Fe	0.03	0.3

3.1.2 RECOVERY TESTS

The efficiency and accuracy of the optimized methods were evaluated by analyzing the digests of spiked samples. 0.02, 0.02 ppm of Pb, Cr and 0.2 ppm Cd, respectively, were taken from stock solution of each metal and spiked in a 250 mL Erlenmeyer flask containing 1g spice sample. The recoveries of metals in the spiked spice samples were 92 to 103 %. The results are given in Table 6. Generally, good recoveries were obtained for all metals, (particularly in Garlic for metals like Cd, Pb and Cr). In Cd the percentage recovery for all samples except Fenugreek were not calculated due to results obtained was not within the method detection limit. Each determination was carried out at least three times in order to ensure precision. The relative standard deviations were less than 10% for all measurements.

$\% Recovery = \frac{Amount after Spike - Amount before Spike}{Amount Added} x 100 [9]$

Table 3.2: the percentage recovery test

Metal	Vegetables			water	soil		
	Cabbage	Lettuce	Potato		Cabbage	Lettuce	Potato
Pb	95	96	97	96	99	98	103
Cu	94	93	95	98	95	97	99
Cd	92	94	94	84	99	82	85
Fe	100	99	93	95	93	98	91
Zn	96	92	89	100	92	100	96

3.2 DISCUSSION

Optimum method was selected for sample digestion from the tested procedure with preconditions producing clear and colorless solutions with minimum reagent volume, less digestion time and digestion temperature. The method fulfilling such conditions considered to be optimum [11]. The efficiency of methods used for sample preparation was evaluated with spiked recoveries and the detected heavy metals indicated a recovery above 90% with relative standard deviations below 10% showing the method used was efficient.

3.2.1 DISTRIBUTION OF HEAVY METALS IN VEGETABLES OF MAYHAM GARDEN

All the three trace elements evaluated in this study were above detection limits in the edible vegetable and the method detection limits of each element were calculated as MDL = 3α blank.

NՉ	element	site			
		cabbage	lettuce	Potato	Detection limit
1	Cd	1.20±0.003	1.45±0.002	1.18±0.01	0.001
2	Cu	12.50±0.23	18.75±0.22	9.75±0.1	0.03
3	Pb	2.56±0.01	5.01±0.11	1.67±0.02	0.002
4	Zn	40.25±0.6	398.5±0.88	40.00±0.66	0.002
5	Fe	466.50±0.9	4987.50±0.77	218.25±0.75	0.03

Table 3.3. Distribution of trace elements (mg elements/kg dry mass) in vegetables of the mayham garden (mean± s.d for n=3).

As can be observed from **figure 2** the distribution of trace metals varied as follows: Cd concentration varies in the order: lettuce > cabbage > potato, Pb, Zn and Fe was similarly varied as lettuce > cabbage > potato.



Figure 3.1. Distributions of essential and non essential metals on the vegetables

3.2.2 DISTRIBUTION OF TRACE METALS IN SOILS OF MAYHAM GARDEN

Heavy metals uptake and accumulation occurs mainly from water or soil due to the direct contact of with those vegetables I n the providing of available nutrients. The efficiency of assimilation in different plant organisms might be affected by many factors such as: ecological needs, habitat, and biology of plant organisms [12]. Due to this the concentration distribution in the soil of each vegetable of mayham garden is listed in the table below.

Table 3.4. Distribution of trace elements (mg elements/kg dry mass) in the soil of the vegetables found in mayham garden (mean± s.d
for n=3)

Nº	element	site			
		cabbage	lettuce	Potato	Detection limit
1	Cd	1.00±0.001	1.00±0.001	1.00±0.001	0.001
2	Cu	16.00±0.02	17.30±0.32	15.30±0.45	0.03
3	Pb	5.00±0.03	3.00±0.8	5.00±0.91	0.002
4	Zn	66.80±0.07	61.00±0.9	59.00±0.6	0.002
5	Fe	29248.50±0.4	26177.75±1.5	23705.75±1.5	0.03

As can be shown in the above table the concentration variation found in the soil of each vegetable was observed slightly difference in some of those metals as well as greater difference was observed in two of those metals while the same in Cd metal. As it was tried to mention in the above the same concentration of Cd metal was recorded in the soil of each of the three vegetables. Whereas, the slight concentration difference found in Zn was placed as cabbage > lettuce > potato, Cu place as lettuce > cabbage > potato, Fe concentration was similarly varied as Zn. Out of the trace metals that they were

determined in the mayham garden Pb concentration found in the soil of each vegetables like potato and cabbage is equal in concentration but in lettuce it was less than the two vegetables.



Figure 3. Distribution concentrations of metals in soil sample of the garden

3.2.3 DISTRIBUTION OF THE TRACE METALS IN WATER FOUND IN THE VEGETABLE GARDEN

Water may be one of the contributors in order to increase the trace metal accumulation of vegetables. Hence, the concentration of each trace metals in water is listed in the table below.

Table 3.5. Distribution of concentration of metals in water sample of mayham garden

Sample	Trace metals						
	Cd	Cu	Pb	Zn	Fe		
Water	9±0.3 μg/l	4.3±0.1 μg/l	2.6±0.04 μg/l	9.2±0.2 μg/l	177.51±1.5µg/l		

3.2.4 STATISTICAL ANALYSIS OF VARIANCE (ANOVA)

Analysis of variance (ANOVA) is powerful statistical technique which can be used for the separation and estimation of the different causes of variation of more than two means for different experiments. The possible sources of variation are due to the random error in measurement, which causes a different result to be obtained each time a measurement is repeated under the same conditions. As the means vary from one sample to another, ANOVA tests whether there is significance difference between the samples means and thus enabling to explain the cause of error. ANOVA is used to test hypothesis about difference between two or more means where there is one variable or factor being considered and replicate data from changing the level of the variables are available [13]. For this study, the significance of variation between samples was analyzed using one-way ANOVA which can be made using detail calculations following a statistical formula or by computer using excel and SPSS software. For this study SPSS (version 16) was used for statistical analysis to know the presence or absence of significant difference in mean concentration of each metal between each vegetable analyzed sample among the three sites. For P > 0.05 the levels of each particular metal in all vegetables as well as soil together with water were not significantly different except the concentration of Fe in all the sample ingredients. This is due to the presence of Fe metal in vegetables, soil and water naturally even though it might not beer any effect as it becomes essential.

3.2.5 COMPARISON BETWEEN THE AVERAGE HEAVY METAL CONTENT OF VEGETABLES OF THIS STUDY WITH THAT OF LITERATURE AND WHO VALUES

There are some reports from different countries on the analysis of the metal contents of the vegetables. It is important to compare the result obtained from the analysis of the three vegetables in this study with the values sited in other countries and WHO guideline values. Currently almost half of the concentration of trace and heavy metals that are determined in this study were in the permissible range of the international guidelines listed below. Hence, Cd, Pb and Fe were above the

maximum permissible -certified reference approved by FAO/WHO. Whereas the rest trace metals concentration were within the reference range that are found in the international guidelines. The concentration of Fe metal couldn't beer an adverse effect on the health of the society. Why is that as Fe is essential metal in nature and so has different metabolically activities of the body of human as well as in hemoglobin and so on. However, the concentration of Pb and Cd which heavy metals in nature might pose an adverse effect on the health of the society as they are toxic in small concentration in nature.

As per the finding the concentration of Cd and Pb exceeds above the international guidelines. So that this study was the pointer to be careful all over the society used in that garden.

NՉ	element	site						
		Maximum allowable limits of elements in fruits and vegetables mg/Kg dry weight	cabbage	lettuce	Potato	Detection limit		
1	Cd	0.2	1.20±0.003	1.45±0.002	1.18±0.01	0.001		
2	Cu	40	12.50±0.23	18.75±0.22	9.75±0.1	0.03		
3	Pb	0.3	2.56±0.01	5.01±0.11	1.67±0.02	0.002		
4	Zn	60	40.25±0.6	398.5±0.88	40.00±0.66	0.002		
5	Fe	425	466.50±0.9	4987.50±0.77	218.25±0.75	0.03		

Table 3.6. FAO/WHO guideline values for the maximum concentration of heavy metals in vegetables [11].

4 CONCLUSIONS AND RECOMMENDATION

4.1 CONCLUSIONS

Determination of heavy metals concentration in vegetables and food products is important for health risk assessment during food consumption. Heavy metals are not only affecting the nutritive values of vegetables but also have deleterious effect on human beings using these food items. The levels of trace and heavy metals such as Fe, Pb, Cd, Cu and Zn concentration were studied lettuce, potato and cabbage vegetables, and the permissible levels with international guidelines for safe food were compared. The optimum procedure selected for digestion process produced good recovery results ranged from 91.09±1.3 up to 104±1.37 with RSD below 10% which shows the efficiency of method used. The concentration of Fe was found in the higher concentrations compared to other metals analyzed or significantly different at 95% confidence level than the rest metals studied in this study. However, the levels of the concentration of Fe, Pb and Cd exceeded the permissible level set by FAO/WHO specifications in all vegetable samples collected from mayham gardens, but Cu and Zn were below the recommended levels. The higher concentration of Pb and Cd above the permissible level in vegetables used for human food may pose health risk to consumer. However, the concentration of Fe hasn't any health effect on the body of the people's they take from the garden. Concentration results of metals between samples were also compared using one-way ANOVA to be proved statistically. The results of ANOVA indicated that there were not significantly different in the levels of most metals between the three samples each of lettuce, potato and cabbage with their soil and water of the garden except the concentration of Fe in all the sample ingredients.

Generally, the levels of metals in similar vegetable samples differed between the three sampling site, that may be due to variation in sources and processes of contaminations that could attribute to metals contamination to take place during preharvest and post-harvest process. Possible sources during pre-harvest include from soil type, fertilizers, pesticides, municipal wastewater and water used for irrigation, while post harvest sources may include contamination through air pollution and during transport to the market or at the point of sale.

4.2 RECOMMENDATION

Lastly the researcher recommends the following

- It is recommended that these types of vegetables should not be cultivated in farms and fields nearby urban areas which have heavy vehicle movements and irrigated with questionable water quality which could be sources of heavy metals contamination.
- This study further suggests that to reduce the health risk, vegetables should be washed properly before consumption as washing can remove a significant amount of aerial contamination from the vegetable surface.
- Markets establishments for vegetable sale should be away from motor vehicle parking and movement areas, as motor vehicle emissions can be a source of heavy metal contamination in vegetables at road side market.
- Use of good agricultural practices under supervision for proper fertilizer application.
- Further works should be carried out in the soil samples where the vegetables are grown, irrigation water and compost or fertilizers on the availability of metals to different vegetable, as it could be useful to take remedial measures by regulatory agencies of the town to abate the metal pollution and also restrict the cultivation of vegetables on contaminated soils.

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