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Heavy metal content of some leafy vegetable crops grown with waste water in southern suburb of Tehran-Iran

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Abstract

Vegetables are the main source of many beneficial substances such as vitamins, minerals, fiber and various antioxidant compounds in human diet. Heavy metals contamination of vegetable crops is always of great concerns, which cause significant reduction in their quality. In this study, accumulation of heavy metals of Cd, Pb, Ni, Cr, Ar, Co, Cu and Zn in green leafy vegetable crops of coriander, garden cress, lettuce and spinach were evaluated under waste water irrigation in fields located in southern suburb of Tehran-Iran. In vegetable samples that were grown under waste water irrigation, the concentration of all heavy metals were significantly higher than WHO-FAO permissible limits for these heavy metals in edible plant tissues. Among the vegetable crops spinach and garden cress accumulated more of heavy metals compared to coriander and lettuce plants. Central leaves of lettuce, however, showed the lowest heavy metal content. This indicates that a national strategy is required toward more safe production of vegetable crops, or to change the cultivation program toward industrial crops in such polluted areas.

Key words: Contamination, heavy metal, leafy vegetable, waste water, environment

Introduction

Vegetable crops are the major source of minerals, vitamins, antioxidants and many other beneficial food factors for human nutrition. Leafy vegetable crops are more important in supplying healthy beneficial compounds in human diets rather than other crops; however, they are also more susceptible to contamination with higher dosage of heavy metals or other toxins (Naieji and Souri 2014). Contamination of vegetable crops is a great threat for human health. Over their consumption, if there is contamination, greater risk is posed to human health rather than beneficial effects. So, this must be taken into account in prescription of vegetable's diets by food specialists (Naieji and Souri 2014).

Heavy metals such as lead, cadmium and arsenic are natural component of earth's crust; however, various human activities toward modernism and industrialization have increased their soil concentrations, their environmental abundance and bioavailability to a hazardous threshold (Gyekye 2013, Sharma et al. 2008). Therefore, heavy metals are life toxic elements which widely distributed in soil, water and air due to anthropogenic activities and natural processes (Mandal and Suzuki 2002). Some heavy metals such as zinc, iron, copper, manganese are among the essential nutrient elements which are required for normal plant growth and functioning (Marschner 1995). These micronutrients in higher available dosage could be toxic for normal plant growth, and is considered as heavy metal (Marschner 1995, Clemens 2006). In addition, there is no known biological role for most of heavy metals such as Cd in plant and human physiology, so increasing to a critical level in tissues; they become extremely toxic for biological functions (Benavides *et al.* 2005). The toxic and hazardous wastes are of great concern. Industrial activities generally generates huge amounts of waste water containing heavy metals and many other toxics, which over their direct or indirect applications, significant pollution occurs on environment (Bose and Bhattacharyya 2008). Generally, about 30% of toxic and hazardous wastes are from the metal industries (Sapari *et al.* 1996).

Nowadays, irrigation of vegetable fields using wastewater is a major agricultural practice in areas near the inhabitant

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locations, particularly around large-populated cities (Naieji and Souri 2014, Sharma *et al.* 2007). This practice is the main contributing factors to heavy metals build up in tissues of vegetable crops (Khan *et al.* 2008, Sharma and Shukla 2013). Climate change and reduced precipitation toward desertification contribute to complicate the heavy metal pollutions of environment and foods around metropolitan cities. In many arid and semi-arid parts of the world, application of waste water for irrigation of crops particularly vegetable crops, is the main choice in fresh vegetable production for high demanding local markets (Sharma *et al.* 2008, Sapari *et al.* 1996).

Vegetables absorb heavy metals primarily from the soil by their root system, as well as by their foliage from the air (Voutsa *et al.* 1996). Continues and gradual uptake of heavy metals can lead to accumulation of toxic metals in edible plant parts. However, the uptake, translocation from the roots and accumulation of heavy metals in vegetative shoots are influenced by many factors including; climate, plant species, water and soil pollutions and atmospheric deposition (Voutsa *et al.* 1996). Several clinical disorders and physiological problems could arise over their build up in plant, animal and human tissues (Khan *et al*. 2008; *Wang et al.* 2005). Heavy metals are not degradable substances and continuously accumulate inside the soil over their entrance from effluent waste water and atmospheric deposition (Sharma *et al.* 2007).

In India, studies on heavy metal content of plant samples of sewage-irrigated area around populated cities in leafy vegetables were found with very high levels of heavy metal contamination including Cd, Zn, Cu, Mn and Pb (Sharma *et al*. 2007, Sharma *et al*. 2008, Singh *et al.* 2010, Tiwari *et al*. 2011). A similar research was carried out in Delhi and its surrounding areas on 'Vegetables eating up vegetarians' found the presence of deadly heavy metals in vegetable samples collected from across the capital (Delbari and Kulkarni 2014). Tehran with a population near to 14 million inhabitants is a very large city located at southern parts of the Alborz Mountains in Iran. The industrial parts generally located at the southern edge of the city. The nearby also are located the main vegetable production areas which has important role in fresh supply of vegetables for this metropolitan city. In this metropolitan city several thousands of tons of waste and by products are generated annually from domestic and industrial sources. The stream of waste water from domestic and industries, due to geographical features, end to the region and generally is used for production of various vegetable crops during 8-9 months of the year. The present study aimed to evaluate the heavy metal accumulation and concentrations in four leafy green vegetable crops grown in polluted fields in this region (south of Tehran-Iran).

Material and Methods

Sample collection: Samples of four leafy vegetable crops; namely garden cress (*Lipidium sativum* L.), coriander (*Coriandrum sativum*), lettuce (*Lactuca sativa*) and spinach (*Spinacea oleracea*) were randomly collected from a growing field in southern suburb of metropolitan city of Tehran-Iran. This region consisted of several towns and many villages which contribute as the main production area of fresh vegetable crops for markets in Tehran and other nearby cities. Vast vegetable cultivations exist in the region which those fields are mainly dependent (direct or indirectly) on streams of domestic and industrial wastewater for irrigation. In addition, intensive applications of chemical fertilizers of phosphorus, nitrogen and to less extent potassium fertilizers are general among the farmers.

The physicochemical properties of wastewater sample taken from the site are presented in Table 1. At the sampling time, four samples of surface soil (0-30 cm depth) were also collected from the field. Soil samples were air dried and passed through a 2 mm sieve and then was mixed, from which one kg sample was taken for soil analysis. The physicochemical characteristics of soil under cultivation are presented at Table 2. Plant samples were collected over a period of three months from September to November in 2012. For each vegetable crop, five samples (as replications) were randomly collected from the field. This includes leaves containing petiole and blade, as well as fresh vegetative shoots for all four vegetable crops. For lettuce also the inner leaves including leaves of 3-5 cm of apical shoot was also sampled separately.

Sample preparation: The edible parts of these leafy vegetable crops were used for heavy metal determination. After transferring of samples to the laboratory using paper bags, the old or discolored leaves were removed, and then collected vegetable samples were gently washed using distilled water to remove any possible dust on the leaves. The samples were then de-watered by filter paper and cut in to small pieces, and oven-dried at 60 °C for 48 h. A whole leaf including blade and petiole was used for analysis. After drying samples were powdered using a grinding machine and the samples then were transferred into small plastic bags and stored in dry conditions. At the time of measurement, one gram of each sample was transferred to a crucible and placed in a furnace at 550 °C for 6 hours for dry ashing. The full digestion of ash was carried out using

HCL and HNO_3 solutions. The mixture was heated at 70 °C for 20 min until a light-colored solution was achieved. The residue was washed and filtered into 50 ml flask using distilled water. Atomic absorption spectrophotometer was used to estimate and measure the levels of these heavy metals in the vegetables foliage. Each sample solution was run in duplicate to give higher creditability of the obtained results. The same procedure was followed for each sample and the appropriate dilution factors were used in the calculation. Standard solutions of lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), arsenic (As), cobalt (Co), zinc (Zn) and copper (Cu) were prepared from the stock solution of 1000 mg/l in 2N HNO_3 from MERK grade of chemicals. A blank sample was also included for better correction and calculation of concentration of various heavy metals. Accordingly, the calibration curves were prepared for each element. Data were analyzed using SPSS 16 and differences among treatments were determined at 5% level by Duncan's test. Graphs were prepared using EXCEL Microsoft.

Results

The results showed that cadmium concentration was significantly higher in spinach compared to other vegetable crops (Fig. 1). Garden cress and coriander plants showed almost similar concentrations of cadmium. Lettuce accumulated relatively less Cd compared to other vegetable crops. The lowest Cd concentration was in inner leaves of lettuce (Fig. 1). The trend of Cd concentration for these vegetable crops was in following order: Spinach > garden cress= coriander > lettuce > inner leaves of lettuce. The range of cadmium in plant foliages were also from 0.1 to 4.6 mg/kgDW.

Determination of lead concentrations revealed that two vegetable crops of spinach and garden cress had the significantly maximum Pb concentration, which showed significant difference to coriander and lettuce plants

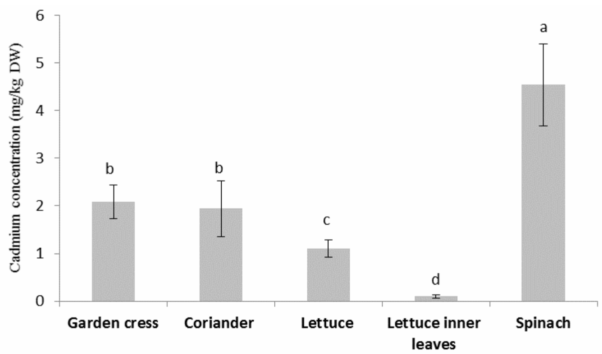

Fig. 1: Cadmium concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

(Fig. 2). The lowest Pb concentrations were observed in inner leaves of lettuce plants. However, the trend of Pb concentrations was seen in following orders: spinach e" gardencress > coriander > lettuce > inner leaves of lettuce. The range of lead in plant foliages were from 2 to 49 mg/kgDW. Measurement of arsenic concentration of plant foliages (Fig. 3) showed that the maximum arsenic concentration was in foliages of spinach plants which showed no significant difference to garden cress plants. There was significantly less arsenic in coriander and lettuce plants, and the arsenic concentration was reduced more in inner leaves of lettuce (Fig. 3). The range of measured arsenic in plant foliages were from 0.1 to 0.97 mg/ kg DW.

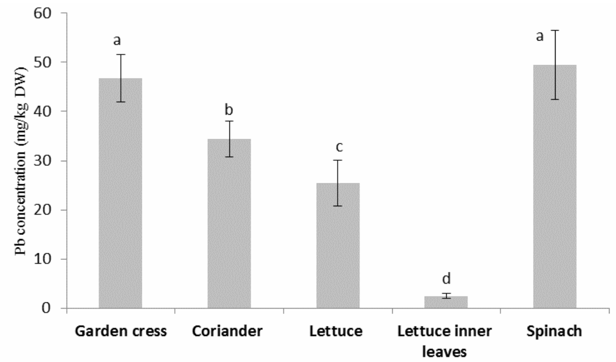

Fig. 2: Lead concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

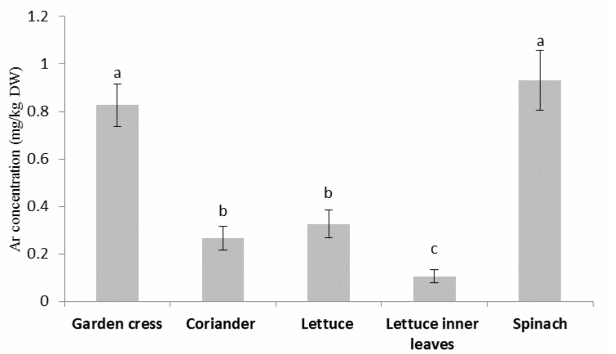

Fig. 3: Arsenic concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

Determination of chromium (Fig. 4) in plant shoot tissues of these vegetable crops showed that the significant highest Cr concentration was in garden cress which showed no significant difference compared to spinach plants. The coriander and lettuce plants had significantly less Cr concentrations compared to garden cress plants. The lowest chromium levels were recorded in inner leaves of lettuce plants (Fig. 4). The range of chromium

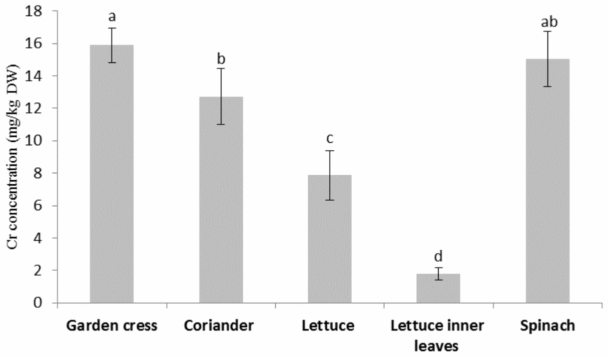

Fig. 4: Chromium concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

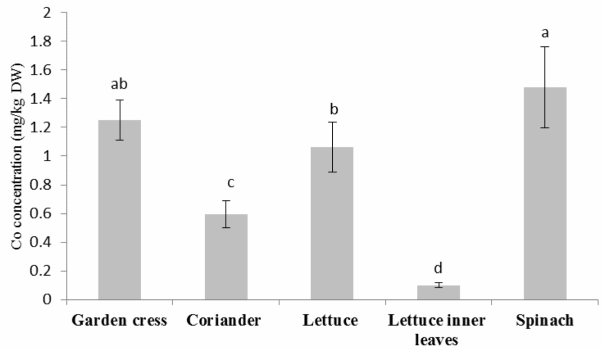

Fig. 6: Cobalt concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

concentrations in plant foliages were from 1.8 to 15.9 mg/ kg DW. Measurement of nickel concentration (Fig. 5) in plant foliages showed that spinach plants had the significantly maximum Ni concentration in their foliages, followed by garden cress, lettuce and coriander plants. Coriander plants showed significantly less amounts of nickel compared to spinach, garden cress and lettuce plants. However, the lowest levels of Ni were observed in inner leaves of lettuce plants. The range of nickel concentrations in plant foliages were from 0.26 to 7.1 mg/ kg DW (Fig. 5).

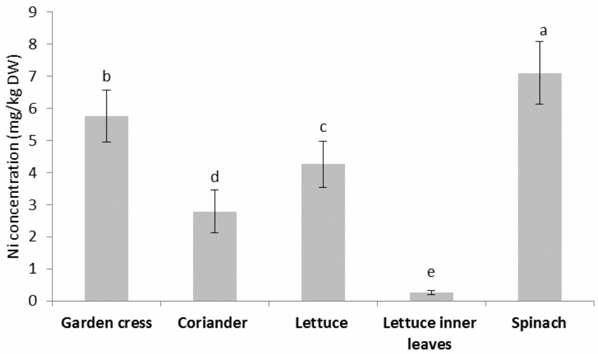

Fig. 5: Nickel concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

Cobalt concentration (Fig. 6) was highest in spinach among four vegetable crops, which showed significant difference with lettuce and coriander plants. Also coriander plants accumulated significantly less cobalt compared to lettuce plants. Nevertheless, inner leaves of lettuce had the least cobalt concentration among the vegetable samples (Fig. 6). Zinc concentration of vegetable crops showed that similar to other heavy metals, the significant maximum Zn was observed in spinach followed by lettuce, garden cress and coriander plants. The lowest Zn concentration was in central inner leaves of lettuce (Fig. 7). However, determination of copper concentration (Fig. 8) in edible foliages of these vegetable crops revealed that garden cress plants had the maximum Cu levels followed by spinach, coriander, and lettuce plants. The lowest Cu levels were recorded in inner leaves of lettuce plants (Fig. 8).

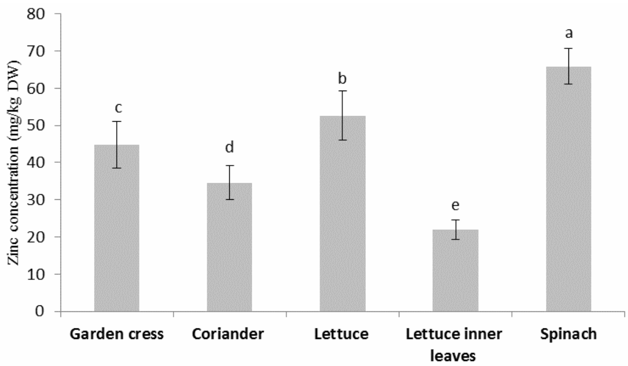

Fig. 7: Zinc concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

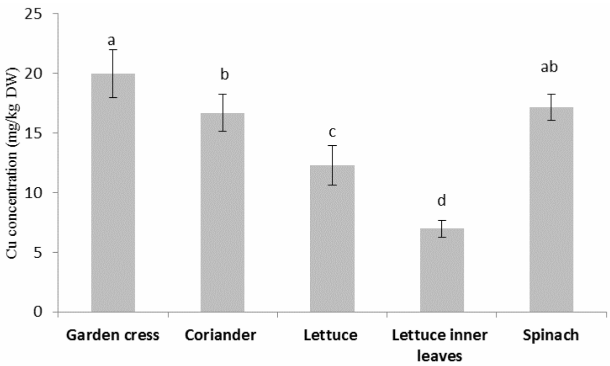

Fig. 8: Copper concentration of foliages in different vegetable crops irrigated using wastewater. Bars with at least one common letter are not significantly different at 5% level of Duncan test.

Discussion

In present study plants were sampled from a field in which vegetables were growing using (domestic and industrial) wastewater. The heavy metal levels of waste water presented in table 1, and it shows that high levels of heavy metals, beyond permissible limits, exist in the wastewater. Similarly, significant amount of those heavy metals were also measured in the soil sample (Table 2). Determination of foliage heavy metal content showed that the concentrations of Cd, Pb, As, Ni, Cr, Co, Zn and Cu were significantly higher compared to standard references (FAO 1989). Irrigation of these crops using wastewater resulted in significantly high accumulation of these metals in plant foliages in all four vegetable crops. This could be a real threat on consumer's health over their consumption. Plant growing in polluted environments can accumulate metals at high concentrations causing a serious risk to human health when these foods are consumed (Wenzel and Jackwer 1999). Vegetable crops similar to other plants are able to take up heavy metals and accumulate them in their tissues; however, this uptake and cumulative concentrations of heavy metals even could be significantly higher in vegetables rather than other agricultural crops (Marschner 1995).

Under field conditions, the uptake, translocation and accumulation of heavy metals may be controlled by various factors (Voutsa *et al.* 1996, Sapari *et al.* 1996). Increased levels of heavy metals in soil and water samples are quite expected when wastewater is used for irrigation (Sapari *et al.* 1996, Khan *et al.* 2008, Sharma Shukla 2013). Metals can be also accumulated in the soil at toxic levels due to long term application of wastewater (Sharma *et al.* 2007). Sewage sludge is a main source of heavy metals at the starting point of pollution and contamination. Based on pollution source, absorption from the air by plant foliages could be also significant (Khan *et al.* 2008). In fact, all types of municipal solid waste and compost contain more heavy metals than the background concentrations present in soil, and will increase their contents in amended soil (Smith 2009). Elevated levels of heavy metals in soils may lead to uptake by plant, however there is not generally found high relationship between concentration in soils and in plants, because it depends on many different factors such as soil metal bioavailability, plant growth and metal distribution to plant parts (Wenzel and Jackwer 1999). Nevertheless, treating and refining of wastewater is very vital if the wastewater aimed to be used for crop irrigation. Increase in heavy metal concentration of plants due to wastewater irrigation is a well-known phenomenon (Bose and Bhattacharyya 2008, Maleki *et al.* 2008 2014, Khan *et al.* 2008, Sharma *et al.* 2007, Smith 2009, Sharma and Shukla 2013). Moreover, elevated levels of heavy metal concentrations also in tissues of various vegetable crops due to irrigation using wastewater have been widely observed (Delbari and Kulkarni 2014, Gyekye 2013, Maleki *et al.* 2008, Kananke *et al.* 2014, Sharma *et al.* 2007 2008, Singh *et al.* 2010). Similarly, high levels of chromium, lead in various plant and vegetable crops near the Tehran have been reported (Pouring and Noori 2012). High levels of cadmium, lead, copper and chromium were reported in vegetable crops of leek, sweet basil, parsley, garden cress, and tarragon grown in western city of Sanandaj-Iran, (Maleki *et al.* 2008, 2014).

Vegetables absorb heavy metals primary from the soil by their root system. Plants can also absorb heavy metals from the air through their foliages (Khan *et al.* 2008, Voutsa *et al.* 1996), which could be in significant amounts for especial metals which are pollutants of certain industries such as cadmium and lead (Tallis *et al.* 2011). In many parts of Iran, especially in the region under study, frequently dusty air in critical levels of pollutions is very common since last decade. Traffics and vehicles were suggested as the main and industrial factories as the second source of arising dusty air in metropolitan Tehran.

Heavy metal content of plants normally increases with closed distance to high ways in many populated areas (Delbari and Kulkarni 2014, Sharma and Agarwal 2007, Singh *et al.* 1997). It was also shown that vegetable contamination of heavy metals alongside of a high way in Tehran was significant. As the maximum cadmium concentration was 5 mg/kg in parsley, maximum lead

Table 1. The physicochemical properties of wastewater sample from the site

pH	EC	TDS (mg/l)	Cd (mg/l)	Pb (mg/l)	As (mg/l)	Cr (mg/l)	Ni (mg/l)	Zn (mg/l)	Cu (mg/l)	SAR
7.56	4.37	3737	8.6	14.8	0.26	6.7	4.8	13.1	4.6	12.5

Table 2. The physicochemical properties of soil from the cultivated field

Texture	pH	EC	Cd (mg/kg)	Pb (mg/l)	As (mg/l)	Cr (mg/l)	Ni (mg/l)	Zn (mg/l)	Cu (mg/l)	Co	SAR
Loamy	7.23	13.4	14.3	281	6.4	26.3	22.1	75.2	28.4	11.3	12.5

(640 mg/kg) and chromium 2670 mg/kg DW were in spinach. Maximum zinc was in sweet basil with an amount of 1.193 mg/kg, and maximum Cu and Ni were in spinach 0.90 mg/kg and 0.23 mg/kg (Delbari and Kulkarni 2014). Significant contamination with heavy metals in different green leafy vegetables collected in markets in Colombo, Sri Lanka was reported (Kananke *et al.* 2014), which is mainly due to industrial effect.

Plants can accumulate higher amounts of metals if they are grown in polluted soils. Prolonged exposure to heavy metals such as cadmium, copper, lead, nickel and zinc can cause deleterious health effects in humans (Singh 2010). However, in present study spinach and garden cress plants have accumulated significantly more heavy metals compared to lettuce and coriander crops. These two are well known plants regarding phytoremediation. The use of garden cress as an indicator for biomonitoring of urban soil due to its simplicity, sensitivity, and cost-effectiveness, has been suggested (Gyekye 2013). In all sampling sites, inner leaves of lettuce plants recorded less amount of heavy metal concentration. Plants also have different sensitivity to various heavy metals. The responses of various plant tissues are also different for each heavy metal. Root growth and root length is adversely affected by heavy metals for Allium cepa and for garden cress plants (Arambaši *et al*. 1995, Lux *et al.* 2010). Nevertheless, vegetable crops are capable of mobilizing the heavy metals within the soil, more than any other crop.

Industrial activities and domestic wastes generate huge amounts of wastes containing a wide range of heavy metals and toxins which must be treated and removed safely instead of using them for agricultural food production. In present study, collected vegetable crops in fields under waste water irrigation showed considerable high levels of heavy metals in their shoot tissues. If there would be programs to use waste water for irrigation, first they must get primary, secondary and more refining treatments to bring down the toxic levels and use this refined and treated wastewater for production of landscape plants or for industrial crops, but not for fresh green vegetables which are consumed directly and raw, contributing significantly on health conditions of human.

सारांश

सब्जियों अनेकों लाभदायक पदार्थों जैसे–विटामिन्स, खनिज लवण खाद्य रेशा एवं मानव भोजन के कई एन्टी आक्सीडैन्ट के महत्वपूर्ण स्रोत हैं। सब्जियों में भारी धातुओं का प्रदूषण सबसे चिन्तनीय है जिससे सार्थक रूप से गुणवत्ता में गिरावट आती है। इस अध्ययन में भारी धातुओं जैसे–कैड्यियम, लेड, निकल, क्रोमोनियम, आर्सेनिक, कोबाल्ट, कापर एवं जस्ता मुख्यतया पत्तीदार सब्जियों जैसे धनिया, गार्डेन क्रेस, लेट्यूस तथा पालक का प्रक्षेत्र मूल्याँकन तेहरान–ईरान के दक्षिणी उपनगर में उपशिष्ट सिंचाई जल से करने पर किया गया। अपशिष्ट जल सिंचाई से उगायी गयी सब्जियों के खाने योग्य पौध उत्तक के प्रतिदर्श में विश्व स्वास्थ्य व संगठन विश्व कृषि संगठन के संस्तुत सीमा की सार्थक मूल्य से ज्यादा पाया गया। सब्जियों में पालक एवं गार्डेन क्रेस की तुलना में धनियां तथा लेट्यूस पत्ती में भारी धातुओं का संग्रहण ज्यादा था। लेट्यूस के पत्तियों के केन्द्रीय भाग में सबसे कम भारी धातु पाये गये। इससे स्पष्ट होता है कि अधिकतम सुरक्षित सब्जी उत्पादन हेतु राष्ट्रीय नीति की आवश्यकता है अथवा ऐसे प्रदूषित क्षेत्रों में औधोगिक फसलों के उगाने का कार्यक्रम निर्धारित करना चाहिए।

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