Health Risk Assessment of Heavy Metals via Consumption of Vegetables grown in soil of agricultural field

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Abstract: In India, a large number (more than 50) of vegetables are frequently consumed because of their high nutritional value. However, disruptions of biological and metabolic processes in the human body are what cause heavy metal poisoning of vegetables in industrial areas. The health risk assessment for heavy metals is a particularly efficient technique since it may reveal details regarding possible risks from produce contaminated with heavy metals. Different studies employ various methodologies for assessing health risk. The risk of health issues due to eating common vegetables poses to human healthiness because of the existence of heavy metals (Cr, Cu, Pb, Cd, and Ni). Lagenaria siceraria and Solanum melongena that are harvested from the field is described in the present study. Along with the methodologies employed for the health risk assessment, one of these procedures is the chronic daily intake of metals (CDI). The hazard quotient (HQ) is used to evaluate health risk. All of the analysed components' predicted daily intakes were incredibly low.

Keywords: Heavy Metals, Vegetables, Contamination, Chronic Daily Intake, Hazard quotient.

1.Introduction

Vegetables must be checked for heavy metal contamination because they are crucial parts of the human diet [1]. In addition to being a great source of vitamins, minerals, and fibre, vegetables also contain healthy antioxidants and metabolites that function as buffers for the acidic waste products of digestion [2]. Vegetables ingested both necessary and harmful components from the soil, but [3]. Due to eating contaminated vegetables with heavy metals (HMs), dangers to human health like cancer and renal damage are frequently documented [4]. But consuming plants contaminated with heavy metals could be detrimental to one's health. Heavy metal contamination of food products is one of the most important aspects of food quality assurance [5]. As a result of a better understanding of the risk these metals provide to food chain contamination, worldwide and national legislation on food quality have reduced the maximum permissible levels of dangerous metals in food items [6].

The common vegetables Amaranthus tricolour L., Chenopodium album L., Spinacia oleracea, Coriandrum sativum, Solanum lycopersicum, and Solanum melongena significantly accumulate the heavy metals Cr, Ni, Co, Cd, and Pb [7]–[14]. Contamination, enrichment, health risk, and heavy metal sources Cr, Co, Pb, Ni, and Cd in two vegetables—Lagenaria siceraria and Solanum melongena—grown in soil from Hisar city, Haryana, India, are detailed in the current paper. The rapid and haphazard industrial and urban expansion that has occurred in developing countries like China [15] and India [16] has led to elevated levels of heavy metals in the urban environments of those countries. Heavy metals are environmental pollutants that are persistent and non-biodegradable. They can be deposited on surfaces and later consumed by vegetables. Plants absorb heavy metals by depositing them on the portions of the plant that are exposed to the air from polluted environments and contaminated soil [17]. Numerous investigations have revealed heavy metals to be one of the main contaminants in vegetables [18–19]. Vegetable heavy metal poisoning may also result from irrigation with tainted water [20–21].

Vegetable surfaces may become contaminated with heavy metal pollutants from numerous industries and vehicles during the processes of production, transportation, and marketing. Scientists have discovered greater levels of heavy metals in vegetables sold in markets in Riyadh, Saudi Arabia, as a result of air deposition [22]. Some reports claim that the levels of heavy metal contamination in the common vegetables sold in Varanasi, India, markets might be considerably increased by air deposition [23].

Chronic accumulation of heavy metals in the human kidney and liver may develop from long-term exposure to dangerous levels of heavy metals through diet, interfering with various biochemical processes and leading to diseases of the heart, nervous system, kidneys, and bones [24,25]. Some heavy metals, like Cu, Zn, Mn, Co, and Mo, function as micronutrients for human and animal growth when present in minute levels, whereas others, like Cd, As, and Cr, act as carcinogens, and Hg is connected to the emergence of anomalies in newborns [26,27,28]. Some assert that chronic cd use caused ovarian, prostate, and kidney cancers [29].

Chromium, cadmium, nickel, and lead are only a few of the metals to which the general public is now more exposed as a result of the expansion of different industrial sectors and the rise in demand for metals. Chromium compounds have teratogenic, mutagenic, embryotoxic, and carcinogenic long-term consequences. As they enhance the likelihood of trivalent chromium interacting with DNA, internal activities that convert hexavalent chromium to trivalent chromium might be viewed as activating chromium's carcinogenic qualities [30]. Cadmium can alter genetic material, particularly in the chromosomes of mammalian cells. Cadmium accumulates particularly well in the kidneys, which are a vital organ. Nickel compounds are classified by the International Agency for Research on Cancer as a Group 1 carcinogen. Hematopoietic diseases, hemic acid synthesis, suppression of haemoglobin formation, and shortened red blood cell life are examples of how harmful lead is to the human body. Reticulocytosis and anaemia are noted in later stages. Lead also impairs renal functions, the neurological system, and the digestive system [31]. Producers obtain the herbs they utilise from both industrial crops and specific sources, whose herbs may include heavy metal contamination.

The current study provides information on the levels of heavy metals (Cr, Co, Cd, Ni, and Pb) in some important Indian vegetables, including Lagenaria siceraria and Solanum melongena, which are grown locally in rural regions and sold in open markets in cities. Due to air depositions in urban areas, the amounts of heavy metals during transport and marketing may increase, which would greatly contaminate vegetables at market locations compared to where they were produced. The Prevention of Food Adulteration (PFA) act (30, 50, 1.5, and 2.5 lg/g, respectively; 32) and the European Union's guidelines for food contamination were compared to the levels of Cu, Zn, Cd, and Pb discovered in the vegetables. (EU) (0.1 and 0.3 lg/g, respectively, for Cd and Pb). Based on the amount of vegetables consumed on a daily average, the contribution of heavy metal contamination from dietary intake of the studied vegetables is also evaluated.

2. Materials and Methods

2.1 Study area

For present investigation soil and plant samples were collected from Haryana (India). The state of Haryana is in the north-west part of India. Though Haryana lies in the sub-tropical belt still climate is Arid to Semiarid.

For the process of soil and crop sampling, area of a field is selected from Hisar district, The normal annual rainfall of the district is 420 mm village. Hisar District is one among 22 Districts of Haryana State, India. Hisar District Administrative head quarter is Hisar. It has been designated as a counter-magnet city for the National Capital Region to emerge as an alternative focus of growth to Delhi and is situated 161.2 kilometres to the west of New Delhi, India. The overall population of the Hisar district is 17,43,931. It is 2nd Largest District in the State by population. Hisar district is divided into 04 Tehsils, 258 Panchayats, 268 Villages. Narnaund Tehsil is the smallest Tehsil with 1,40,880 population. Hisar Tehsil is the Biggest Tehsil with 10,69,309 population. There is total 268 villages in Hisar district. The town chosen for sampling is not a heavy metal-prone region, but for the study, the influence of soil characteristics and the uptake of heavy metals by crops from a normal soil will be an important element of the study.

2.2 Sample Collection

Vegetable agricultural samples were gathered throughout the month of April 2021. Food crops were collected during their separate harvesting seasons in order to assess the transfer and translocation factor of heavy metals. The details of the samples collected are given below:

1. **Lagenaria siceraria (Bootlegourd/ Lokki):** Samples were collected during its harvesting season (August-September) for the year (2021). A total of 10 samples were collected each weighing 500 - 1000g. The collected samples were cleaned, sealed and stored as discussed above.
2. **Solanum melongena (Brinjal):** Vegetable samples from designated sampling locations were gathered throughout the year's harvest season (Dec.–Jan. (2021). Total 10 samples were collected each weighing 500 - 1000g. After removal of extraneous matter like soil pebbles, stones and other debris the samples were sealed in plastic containers, taken to the laboratory and stored till further analysis.
3. **Soil sample**: The equivalent subsurface soil samples from the rooting zone were also obtained in order to assess the transfer and translocation factor of heavy metals from soil to the various environmental matrices above. Throughout various sampling seasons, a total of 40 soil samples were taken. Four sub-samples of soil from a rectangular grid of 0.5 m2 area were taken from a depth of 5–10 cm, and they were then combined to create a representative sample. In order to remove the metal contamination, non-metallic spades were used to gather the soil samples, each weighing 500g. After the removal of foreign bodies, samples were packed in fresh plastic bags. In preparation for further investigation, soil samples were air dried, powdered, and then sieved with a 2.0 mm mesh size.

3. SAMPLE PROCESSING

3.1 Soil Samples Processing

Soil samples were air dried, grounded and stored in plastic containers that were air tight for analysis. After removal of extraneous matter like pebbles and plant roots etc. At a temperature of 110°C, soil samples were dried in an oven until the sample weight remained constant. Then the samples were ground in grinder with care to avoid cross contamination. After milling, the whole samples were sieved using 2.0 mm sieve.

- For heavy metals analysis, processed samples were stored in air tight plastic containers to have moisture free conditions till further analysis.

3.2 Agricultural Sample Processing

3.2.1 Vegetable Samples Processing

- The samples' fresh weight was noted shortly after collection.
- Vegetables' various parts (roots, shoots, and fruits) were dried in a drying oven at a temperature of 110°C until the sample weight remained constant.
- Following drying, the sample's dry weight was noted, and 100 g of dry subsamples were heated to 350–400 °C in a muffle furnace until the ash turned white.
- The ashed samples were then stored in moisture free conditions till further analysis

3.3 Sample Analysis

3.3.1 Heavy Metals Analysis

3. Sample Digestion

Atomic Absorption Spectrophotometer (SenSAAGBC, Australia) was used to evaluate the total Cd, Ni, Cr, Co, and Pb contents of agricultural vegetables and soil samples. Concentrations were then represented in mg kg-1 on a dry weight basis. Each sample of agricultural crop ash weighed 0.2 g, and 5.0 ml of a 9:1 (v/v) diacid (HNO3 and HClO4) mixture was added to it. Similarly, 5.0 ml of the diacid mixture was added to 0.5g of each soil sample. After taking the sample mixture in clean Teflon containers, it was digested on a hot plate the following day while it was left open at room temperature. When digestion was finished, the containers were quantitatively transferred into glass beakers and chilled to room temperature. The digests were then heated to a temperature of 130 to 150 °C, evaporated to dryness on a hot plate, and the residue was dissolved in double-distilled water to the appropriate volume (50 ml). After being transferred to polypropylene bottles, extracted solutions were stored in the refrigerator until analysis.

To create all of the working standards for analysis, approved standard solutions containing 1000 mg/L were diluted. Atomic Absorption Spectrophotometer (AAS) employed air as support and acetylene gas as fuel. With the exception of chromium, which was measured using a reducing nitrous oxide flame, all examples used an oxidising flame. Using an Atomic Absorption Spectrophotometer (SenSAAGBC, Australia), the extracts were examined for the presence of eight heavy metals, including Cd, Ni, Cr, Co, and Pb. Quality assurance and control procedures were used during the processing and analysis of the samples to guarantee the validity of the findings. Double distilled water was used to prepare standards, prepare samples, and rinse glassware in order to prevent sample contamination. To eliminate analytical bias, reagent blanks were examined, and the data were then blank adjusted. Replicated measurements had relative standard deviations (RSDs) of 0.999. After every ten samples were tested, standards of the relevant metals were run to guarantee the accuracy of the results.

3.4 Health Risk Assessment from Consuming fruits in vegetables (Bottlegourd and Brinjal)

- **Chronic Daily Intake**

In terms of mass of a substance per unit body weight per unit time, averaged over a lengthy period of time (a lifetime), CDI represents the population's exposure to a substance.

The Chronic Daily Intake of Heavy Metals through the Consumption of Grains (Wheat and Rice) was determined utilising the (USEPA, 2010).

$$\text{CDI (Ingestion)} = \frac{Cm * Fir * Ef * Ed}{WAB * TA}$$

Where CM is the amount of a heavy metal present in fruits (such as bottlegourd and brinjal) per kilogramme of dry weight.

FIR is the ingestion rate for an adult residing in the study area (0.147 kilogramme per day for bottlegaurd and 0.178 kg per day for brinjal). (FAO, 2014)

EF is the exposure frequency (365 days year^{-1})

ED is the exposure duration (70 years for adults)

WAB is the average body weight (60 kg for Indian adults) (NNMB, 2002)

TA is the average exposure time for non-carcinogenic effects (ED × 365 days year^{-1}).

The oral reference dose for Hg, As, Cd, Pb, Cu, Mn, Zn, Cr and Fe reported was 0.0001, 0.0003, 0.001, 0.004, 0.04, 0.14, 0.3, 1.5 and 15 mg/kg/day, respectively [16].

- **Total Hazard Quotient**

RfDo is the oral reference dose (mg kg1 day1) and is an estimation of the daily exposure to which the human population is likely to be exposed without any appreciable risk of deleterious effects during a lifetime (USEPA, 2010). The RfDo values used were 1.010-3, 2.0102, 4.0103, and 1.5. THQ has been calculated as the ratio between the estimated dose of a contaminant and the dose level below which there will not be any appreciable dose (USEPA, 2010).

$$THQ = \frac{CDI}{RfDo}$$

Where CDI stands for chronic daily intake

RfDo stands for oral refrence doses

4. Results & Discussion

4.1. Heavy Metal Concentration in Soils

Each soil sample was examined in triplicate, and Table 1 shows the mean concentrations of the heavy metals Cr, Co, Cd, Ni, and Pb. The soils' Cr, Co, Cd, Pb, and Ni concentrations varied from 0.11 to 0.201, 5.351 to 6.102, 3.80 to 4.37, 8.48 to 9.60, and 8.22 to 8.53 mg/kg, with mean values (p = 0.05) of 0.139 to 0.0226, 5.652 to 0.250, 4.212 to 0.180, 9.203 to 0.438, and 8.367 to 0.093, respectively. In the soil of the study area, Co, Pb, and Ni were three metals that were much more enriched than other heavy metals.

Table 1. Range and mean of heavy metals in agricultural soil

Statistics	Metals				
	Ni	Pb	Cd	Cr	Co
Min.	8.221	8.488	3.806	0.111	5.351
Max.	8.534	9.604	4.376	0.201	6.102
Mean ± SD	8.367±0.093	9.203±0.438	4.212±0.178	0.139±0.026	5.652±0.250

Concentration of Heavy Metals in Vegetables

Each vegetable has two species that were examined. In the veggies, the permissible levels for Cr, Co, Cd, Pb, and Ni were 2.3, 0.2, 0.1, 0.2, and 67.9 mg/kg, respectively. The study area's brinjal was found to include elements such as Cr, Ni, Cd, Pb, and Co. Angiosperm Solanum melongena (Brinjal), a member of the Solanaceae family, is a widely produced vegetable crop throughout the tropics and subtropics, even in areas with high levels of pollution. The average concentration of Pb (0.193 - 0.071) was higher than that of Ni (0.184 - 0.0126), Co (0.044 - 0.023), Cd (0.022 - 0.006), and Cr (0.00) (n = 5). The findings make it evident that the samples of tested brinjal vegetables had no Cr and a significant Pb concentration respectively mg/kg. The study area's brinjal was found to have equivalent levels of Cr, Cd, Co, Pb, and Ni to those reported for other regions of the nation [33].

Originally from tropical Africa, the bottlegourd (Lagenaria siceraria), also known as white-flowered gourd or calabash gourd, is a climbing or running vine of the gourd family (Cucurbitaceae) that is grown in warm climes all over the world for its attractive and practical hard-shelled fruits. The young fruits can be consumed and are typically prepared like vegetables. Vegetable samples from ten sampling stations from the study area were collected during harvesting season. All the collected samples were analyzed for Ni, Pb, Cd, Cr and Co content. Heavy metals in the samples of brinjal collected during April - May, 2021 was: (Pb) 0.122 - 0.187 mg kg^{-1} , (Ni) 0.014 - 0.04 mg kg^{-1} , (Cd) 0.014 - 0.03 mg kg-1 , (Co) 0.016 - 0.054 mg kg-1 and Cr was not measurable. The following was the order of the mean value of the heavy metal concentration (mg kg-1) in the bottlegourd samples that were taken: According to Table 3, Pb (0.0146 0.023) is followed by Co (0.036 0.014), Ni (0.024 0.009), Cd (0.019 0.004), and Cr (0.00). The findings make it abundantly evident that the above-mentioned vegetable sample Pb and Cr level was highest and lowest, respectively.

Table 2. Concentration of heavy metals (mg kg^{-1}) in fruit of Solanum melongena

S. No.	Heavy Metals	Mean
1	Ni	0.184 ± 0.126
2	Pb	0.193 ± 0.071
3	Cd	0.022 ± 0.006
4	Cr	0.00±0.000
5	Co	0.044 ± 0.023

Table 3. Concentration of heavy metals (mg kg^{-1}) in fruit of Lagenaria siceraria

S. No.	Heavy Metals	Mean
1	Ni	0.024 ± 0.009
2	Pb	0.146 ± 0.023
3	Cd	0.019 ± 0.004
4	Cr	0.00±0.000
5	Co	0.036 ± 0.014

Heavy metal Chronic Daily Intake (CDI) with consumption of bottlegourd fruit

For identifying the underlying health hazards, estimation of the exposure amount and tracking the pathways of contaminants to the target species are crucial. By consuming bottlegourd fruits (2021) grown at studied sampling locations in the study area an adult will intake 0.00013635 – 0.0000707 mg kg^{-1} day^{-1} of Ni, 0.00094435 – 0.0006161 mg kg-^{1} day^{-1} of Pb, 0.00010605 – 0.000101 mg kg^{-1} day^{-1} of Cd, 0.00025755 – 0.0000808 mg kg-^{1} day^{-1} of Co and Cr value was nil and with mean CDI (mg kg^{-1} day^{-1}) of heavy metal via bottlegourd fruit (2021) ingestion was in order: Co (0.000183315) > Ni (0.000124735) > Cd (0.000100495) > Pb (0.00073831),(table 4). Maximum permissible limits (mg kg^{-1} day^{-1}) of Cd, Pb, Ni, Co and Cr through ingestion of vegetables are 0.2, 0.3, 67.9, 50 and 0.1 respectively. The current study's findings showed that the mean CDI of metals ingested was lower than the threshold CDI values.

Table 4. Chronic daily intake (mg kg-1 day-1) of heavy metals via ingestion of bottle gourd fruit

Statistics	Metals				
	Ni	Pb	Cd	Cr	Co
Min.	0.0000707	0.0006161	0.000101	0	0.0000808
Max.	0.00013635	0.00094435	0.00010605	0	0.00025755
Mean	0.000124735	0.00073831	0.000100495	0	0.000183315

For identifying the underlying health hazards, estimation of the exposure amount and tracking the pathways of contaminants to the target species are crucial. By consuming brinjal fruit (2021) grown at studied sampling locations in the study area an adult will intake 0.001843 – 0.000192 mg kg^{-1} day^{-1} of Ni, 0.001742 – 0.000631 mg kg^{-1} day^{-1} of Pb, 7.07E-05 – 0.000101 mg kg^{-1} day^{-1} of Cd, 5.56E-05 – 0.000106 mg kg^{-1} day^{-1} of Co and no value was observed in Cr with mean CDI (mg kg^{-1} day^{-1}) of heavy metal via brinjal fruit (2021) ingestion was in order: Pb (0.000975) > Ni (0.000933) > Co (0.000223) > Cd (0.000112) (Table.5). Vegetables may include up to the following maximum permitted levels of Cd, Pb, Ni, Co, and Cr: 0.2, 0.3, 67.9, 50, and 0.1, respectively. The current study's findings showed that the mean CDI of metals ingested was lower than the threshold CDI values.

Table 5. Chronic Daily Intake (CDI) of heavy metals via ingestion of brinjal fruit

Statistics	**Metals**				
	Ni	Pb	Cd	Cr	Co
Min.	0.00606	0.1818	0.0303	0	0.303
Max.	0.009595	0.269814	0.037033	0	0.488167
Mean ± SD	0.00623675	0.210945714	0.033498333	0	0.61105

Total Hazard Quotient (THQ) of heavy metals via ingestion of bottlegourd fruit

The THQ of individual heavy metal to the target population via ingestion of bottlegourd fruit (2021) was in the range of 0.009595 – 0.00606 (Ni), 0.269814 – 0.1818 (Pb), 0.037033 – 0.0303 (Cd) and 0.488167 – 0.303 (Co)). The mean value of overall THQ of discrete heavy metal to the aim population via ingestion of bottlegourd fruit (2021) was in the order: Co (0.61105) > Pb (0.210945714) > Cd (0.033498333) > Ni (0.00623675) and Cr value was nil (Table 6). The target group is unlikely to experience negative health consequences from consuming brinjal fruit on a daily basis because the THQ of the examined heavy metals was less than unity.

Table 6. Total hazard quotient of heavy metals via ingestion of bottlegourd fruit

Statistics	**Metals**				
	Ni	Pb	Cd	Cr	Co
Min.	0.0000707	0.0006161	0.000101	0	0.0000808
Max.	0.00013635	00094435	0.00010605	0	0.00025755
Mean	0.000124735	9.203±0.438	0.000100495	0	0.000183315

Total Hazard Quotient (THQ) of heavy metals via ingestion of brinjal fruit

The THQ of discrete heavy metal to the marked population via ingestion of brinjal fruit (2021) was in the range of 0.098233 – 0.05757 (Ni), 0.497786 – 0.180357 (Pb), 0.057233 – 0.0505 (Cd) and 1.329833 – 0.606 (Co) respectively and no value was observed for Cr. The mean value of overall THQ of every heavy metal to the aimed population via ingestion of brinjal fruit (2021) was in the order: Co (0.744033) > Pb (0.278615714) > Ni (0.04663675) > Cd (0.03737) (Table 7). The target group is unlikely to have negative health consequences from ingesting examined heavy metals on a daily basis because their THQ was less than unity.

Table 7. Total hazard quotient of heavy metals via ingestion of brinjal fruit

Statistics	Metals				
	Ni	Pb	Cd	Cr	Co
Min.	0.05757	0.180357	0.0505	0.111	0.606
Max.	0.098233	0.497786	0.057233	0.201	1.329833
Mean	0.04663675	0.278615714	0.03737	0	0.744033333

4. Conclusion

Co, Cr, Cd, Pb, and Ni were the most harmful metals found in the soil in the study area. They can accumulate in vegetables, and extended consumption of them may interfere with a person's biological and metabolic functions. It was discovered that all of the studied veggies had no harmful effects on the persons consuming these. As all studied veggies has low CDI and THQ values. To map the risks to local residents' health, a thorough analysis of the CDI values for the numerous green vegetable varieties growing in the study area can be done. Through this study it was concluded that there is no risk of consuming veggies grown on this field.

References:

1. Ogunkunle, A. T. J., Bello, O. S., & Ojofeitimi, O. S. (2014). Determination of heavy metal contamination of street-vended fruits and vegetables in Lagos state, Nigeria. International Food Research Journal, 21(6)
2. Devi, P. B., Vijayabharathi, R., Sathyabama, S., Malleshi, N. G., & Priyadarisini, V. B. (2014). Health benefits of finger millet (Eleusine coracana L.) polyphenols and dietary fiber: a review. Journal of food science and technology, 51, 1021-1040.
3. Islam, E. U., Yang, X. E., He, Z. L., & Mahmood, Q. (2007). Assessing potential dietary toxicity of heavy metals in selected vegetables and food crops. Journal of Zhejiang University Science B, 8(1), 1-13.
4. Zhuang, P., McBride, M.B., Xia, H., Li, N. and Li, Z. (2009) Health Risk from Heavy Metals via Consumption of Food Crops in the Vicinity of Dabaoshan Mine, South China. Science of the Total Environment, 407, 1551-1561.
5. Khanna, P. (2011). Assessment of heavy metal contamination in different vegetables grown in and around urban areas. Research journal of environmental toxicology, 5(3), 162.
6. Gall, J. E., Boyd, R. S., & Rajakaruna, N. (2015). Transfer of heavy metals through terrestrial food webs: a review. Environmental monitoring and assessment, 187, 1-21.
7. Gupta, A.K. and Sinha, S. (2007) Phytoextraction Capacity of the Chenopodium album L. Grown on Soil Amended with Tannery Sludge. Bioresource Technology, 98, 442-446.
8. Kundu, R., Bhattacharyya, K. and Pal, S. (2012) Arsenic Intake and Dietary Risk Assessment of Coriander (Coriandrum sativum L.) Leaves in the Gangetic Basin of West Bengal. Journal of Spices and Aromatic Crops, 21,125-129.
9. Naser, H.M., Sultana, S., Mahmud, N.U., Gomes, R. and Noor, S. (2011) Heavy Metal Levels in Vegetables with Growth Stage and Plant Species Variations. Bangladesh Journal of Agricultural Research, 36, 563-574.
10. Oluwatosin, G.A., Adeoyolanu, O.D., Ojo, A.O., Are, K.S., Dauda, T.O. and Aduramigba-Modupe, V.O. (2010) Heavy Metal Uptake and Accumulation by Edible Leafy Vegetable (Amaranthus hybridus L.) Grown on Urban Valley Bottom Soils in Southwestern Nigeria. Soil and Sediment Contamination, 19, 1-20

11. Pathak, C., Chopra, A.K. and Srivastava, S. (2013) Accumulation of Heavy Metals in Spinacia oleracea Irrigated with Paper Mill Effluent and Sewage. Environment Monitoring and Assessment, 185, 7343-7352
12. Swati, N., Srivastava, R.C. and Agarwal, K.M. (2012) Accumulation of Heavy Metals by Solanum melonuma Irrigated with Wastewater. International Journal of Agriculture Environment and Biotechnology, 5, 329-332.
13. Singh, S. and Kumar, M. (2006) Heavy Metal Load of Soil, Water and Vegetables in Peri-Urban Delhi. Environmental Monitoring and Assessment, 120, 79-91
14. Yusuf, K.A. and Oluwole, S.O. (2009) Heavy Metal (Cu, Zn, Pb) Contamination of Vegetables in Urban City: A Case Study in Lagos. Research Journal of Environmental Sciences, 3, 292-298.
15. Khanna, P. (2011). Assessment of heavy metal contamination in different vegetables grown in and around urban areas. Research journal of environmental toxicology, 5(3), 162.
16. Inoti, K. J., Fanuel, K., George, O., & Paul, O. (2012). Assessment of heavy metal concentrations in urban grown vegetables in Thika Town, Kenya. African Journal of Food Science, 6(3), 41-46.
17. Akan, J. C., Kolo, B. G., Yikala, B. S., & Ogugbuaja, V. O. (2013). Determination of some heavy metals in vegetable samples from Biu local government area, Borno State, North Eastern Nigeria. International Journal of Environmental Monitoring and Analysis, 1(2), 40-46.
18. Jafarian-Dehkordi, A., & Alehashem, M. (2013). Heavy metal contamination of vegetables in Isfahan, Iran. Research in pharmaceutical sciences, 8(1), 51.
19. Sharma, R. K., Agrawal, M., & Marshall, F. (2007). Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicology and environmental safety, 66(2), 258-266.
20. Lokeshwari, H., & Chandrappa, G. T. (2006). Impact of heavy metal contamination of Bellandur Lake on soil and cultivated vegetation. Current science, 622-627.
21. Lokeshwari, H., & Chandrappa, G. T. (2006). Impact of heavy metal contamination of Bellandur Lake on soil and cultivated vegetation. Current science, 622-627.
22. Al Jassir, M. S., Shaker, A., & Khaliq, M. A. (2005). Deposition of heavy metals on green leafy vegetables sold on roadsides of Riyadh City, Saudi Arabia. Bulletin of environmental contamination and toxicology, 75(5), 1020-1027.
23. Sharma, U., & Chow, E. W. (2008). The attitudes of Hong Kong primary school principals toward integrated education. Asia Pacific Education Review, 9, 380-391.
24. Kramer, I. R., Pindborg, J. J., & Shear, M. (1992). The WHO histological typing of odontogenic tumours. A commentary on the second edition. Cancer, 70(12), 2988-2994.
25. Toledano, M. B., Hansell, A. L., Jarup, L., Quinn, M., Jick, S., & Elliott, P. (2003). Temporal trends in orchidopexy, Great Britain, 1992-1998. Environmental Health Perspectives, 111(1), 129-132.
26. Feig, A. L., & Lippard, S. J. (1994). Reactions of non-heme iron (II) centers with dioxygen in biology and chemistry. Chemical Reviews, 94(3), 759-805.
27. Mantzoros, C. S., Tzonou, A., Signorello, L. B., Stampfer, M., Trichopoulos, D., & Adami, H. O. (1997). Insulin-like growth factor 1 in relation to prostate cancer and benign prostatic hyperplasia. British journal of cancer, 76(9), 1115-1118.
28. Pitot, H. C., Dragan, Y. P., Teeguarden, J., Hsia, S., & Campbell, H. (1996). Quantitation of multistage carcinogenesis in rat liver. Toxicologic pathology, 24(1), 119-128.
29. Hartwig, A. (1998). Carcinogenicity of metal compounds: possible role of DNA repair inhibition. Toxicology Letters, 102, 235-239.
30. Singh, A., Sharma, R.K., Agrawal, M. and Marshall, F.M. (2010) Risk Assessment of Heavy Metal Toxicity through Contaminated Vegetables from Waste Water Irrigated Area of Varanasi, India. Tropical Ecology, 51, 375-387.
31. Da Silva, A. A., Do Carmo, J., Dubinion, J., & Hall, J. E. (2009). The role of the sympathetic nervous system in obesity-related hypertension. Current hypertension reports, 11(3), 206-211.
32. Dickson, R., Awasthi, S., Williamson, P., Demell week, C., & Garner, P. (2000). Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. Bmj, 320(7251), 1697-1701.
33. Rahman, M. M., Asaduzzaman, M., & Naidu, R. (2013). Consumption of arsenic and other elements from vegetables and drinking water from an arsenic-contaminated area of Bangladesh. Journal of hazardous materials, 262, 1056-1063.