

Assessment of Heavy Metal Contamination in Green Leafy Vegetables Grown in Bangalore Urban District of Karnataka.

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Abstract

Randomly collected waste water, soil and green leafy vegetable samples were from five stations of Bangalore urban district were analysed for the heavy metals namely Cu, Zn, Pb, Cr, Cd and Mn using Atomic absorption spectrophotometer. Present study explains the extent of heavy metal contamination in two leafy vegetables viz., palak (*Beta vulgaris*) and coriander (*Coriandrum sativum*). Results showed that, palak leaves contain Cu, Zn, Pb, Cr and Mn in all the sampling points. Cr at S₁, S₂ and S₅ stations crossed safe value limit recommended by FAO/WHO. In coriander leaves Cu, Zn and Mn found in all stations. Pb concentration was exceedingly high in palak (28.43ppm to 149.50ppm) and coriander (54.69ppm to 75.50ppm) in all sampling stations. Cd was detected only at two stations in S₂ (0.81ppm) and S₄ (1.50ppm). Cr was detected at S₁, S₂ and S₃ stations and at S₂ station, Cr content in palak (70.79ppm) and coriander (127.27ppm) was alarmingly exceeding the allowable limit. Cytological studies indicated the formation of anaphase bridge, irregular metaphase, chromosome stickiness, precocious chromosome at anaphase and vagrant chromosomes. Soil samples do not revealed any appreciable increase in the concentration of heavy metals and found within the permissible limits but values were higher than control that shows contamination was mainly due to automobile exhaust, pesticides and industrial exhaust. Pb was detected in all the waste water samples and in S₁ (<0.05PPM) and S₂ (<0.05ppm) traces of Cd was detected. Physico-chemical parameters of waste water were also determined and factors such as pH, EC, TDS and DO found to exceed the drinking and irrigation water standards.

Key Words: Heavy metals, leafy vegetables, palak, coriander, waste water, soil, chromosome.

1. Introduction

Present day scenario suggests that majority of population suffer from malnutrition and therefore, noticeable nutrient deficient syndromes are visible in human beings. Variety of leafy vegetables used in balanced diet (116g/day) as they are rich in minerals and vitamins. Implication associated with heavy metal contamination is of great concern, particularly in agricultural production. These metals can pose a significant health risk to humans, particularly in elevated concentrations (Gupta & Gupta, 1998). Dietary exposure to heavy metals like, cadmium, lead, zinc and copper has been identified as a risk to human health through the vegetables consumption (Kachenko & Singh, 2006). Furthermore, consumption of heavy metals-contaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defenses, intrauterine growth retardation, impaired psycho-social behavior, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Arora et al., 2008). Utilization of sewage water in urban areas deserves special attention as it is making environment quite unsuitable for human health as well as animals and plants are also affected by heavy metals toxicity. Toxicological significance of heavy metals has been recognized several decades ago in developed countries (Tsuda et al., 1995). There is an opinion among the consumer that, green and big leaves as characteristics of good quality leafy vegetables. Recent reports indicated that heavy metals take driver's seat among the chief contaminants of leafy vegetables. Heavy metals are non-biodegradable and thermo stable and thus readily accumulate to toxic levels. Waste water irrigation may lead to the accumulation of heavy metals in agriculture soils and plants (Sharma et al., 2006; 2007). Untreated sewage water irrigation plays a pivotal role in significantly increasing heavy metals in soil and crops (Mapanda et al., 2005) and increases individual metal in soil by 2% to 80% and in crops by 14% to 90% (Sarabjeet Singh Ahluwalia & Dinesh Goyal, 2007). Main sources of heavy metals to vegetable crops are their growth media (soil, air, nutrient solutions) from which these are taken up by roots or foliage. Soil gets polluted due to waste water irrigation and

absorbed minerals settle in edible tissue of the vegetables (Lokeshwari & Chandrappa, 2006). Food safety issues and potential health risks make this as one of the serious environmental concerns (Cui et al., 2005). Though, metals are indispensable part of our environment and play positive role in various biological processes such as signaling, homeostasis and enzyme catalysis, higher concentration of metals tend to toxic effects since they are prone to bio-accumulation and bio-magnification along the food chain. Industrialization and urbanization as well as anthropogenic activities are main source for heavy metal contamination that undoubtedly affected lakes and tanks in Bangalore city. Satellite images from Survey of India indicated nearly 2,789 lakes have dried up and only 330 medium to large range live tanks are in Bangalore metropolitan region (Abida Begum & Harikrishna, 2010). Tank and lake water is degraded due to encroachment, eutrophication loads and silt discharge of untreated or partially treated waste water, discharge of organic, inorganic and toxic pollutants of industrial effluents. Considering the significance of heavy metals and consumption of vegetables, this investigation was carried out.

2. Materials and Methods.

2.1. Description of the Study Area



Fig.1. Study areas in Bangalore urban district of Karnataka.

Five experimental stations selected were Byramangala (S_1), Bellandur (S_2), Ramagondanahalli (S_3), Jigani (S_4) and Parappana Agrahara (S_5) areas (Fig.1). Tanks of these areas receive effluent discharges from industries manufacturing batteries, textiles, steel, paints, food processing and chemical products.

2.2. Collection and Preparation of Leafy Vegetable Samples



Fig.2. Palak leaf vegetation.



Fig.3. Coriander leaf

vegetation.

Two green leafy vegetables viz., palak (*Beta vulgaris*) (Fig.2) and coriander (*Coriandrum sativum*) (Fig.3) samples were collected randomly and washed thoroughly with tap water followed by distilled water to remove adsorbed elements. Samples were cut into small pieces, air dried for 2 days and kept in hot air oven at $100^{\circ}\text{C}\pm 1^{\circ}\text{C}$ for 4 hrs. Dried samples were grounded to powder and then pass through a 1mm sift. 0.5g of sample is taken in reference vessels, add 4ml of HNO_3 and 0.2ml of H_2O_2 and carousel was positioned into microwave unit. The system was pre-programmed for 1min. of microwave digestion at 250W power and another 5min. at 500W power and left to automatic ventilation for 10min. Digested solution was cooled, filtered using Whatman filter paper No. 40 and made up to 100ml with distilled water and stored in plastic bottles for analysis. Control leafy vegetables were obtained from areas where normal irrigation practices were followed.

2.3. Collection and Preparation of Water Samples



Fig.4. Storm water.

Random water samples (Fig.4) were collected separately from different tanks in pre-cleaned 100ml polythene bottles and 2ml of nitric acid was added as preservative. Water samples should be kept in insulated field kit containing ice and will be brought to laboratory. 10ml of water sample was taken in Teflon tubes and 1ml con. HNO_3 was added. Vessel is closed with valve and tightened. Then inserted into a single safety shield carousel and into microwave chamber. System was pre-programmed using Ethos D control terminal (equipped with software) for 5min. of digestion at 250W power and automatic ventilation for 2min. Then digested solution cooled and made up to 100ml with Milli-Q distilled water. Samples stored in pre-cleaned plastic bottles and used for analysis. Control samples were collected from station where normal water was used for irrigation.

2.4. Collection and Preparation of Soil Samples

Random soil sample of about ½ kg were collected at a depth of 0 - 25cm from 4 different places and stored in polythene covers. Samples were air dried at room temperature, ground to a fine powder using mortar and pestle and finally sift under 1mm nylon mesh. 0.25g of soil sample was poured into reference vessels. Then 2.5ml con.HNO₃ and 2.5ml of HF acid was added and inserted into a carousel and into microwave unit for digestion. System was pre-programmed for 6min. of microwave digestion at 300W powers and another 5min. at 500W power and automatic ventilation for 10min. Then solution was cooled, filtered using Whatman filter paper 40 and made up to 100ml with Milli-Q distilled water, samples were stored in pre-cleaned plastic bottles and used for analysis. Control samples were obtained from station where normal practices were followed.

2.5. Cytological Investigations

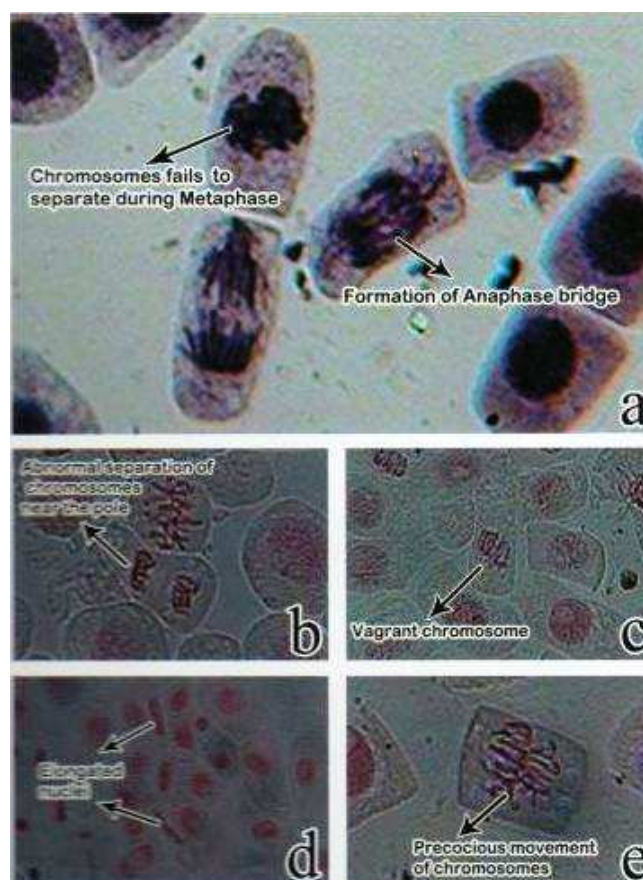


Fig.5. Chromosomal aberrations

Cytological investigation was carried out to test the effect of heavy metals on the plant material. Waste water collected from different locations were prepared with different concentrations of dilutions viz., 10%, 20%, 30%, 40% and 50% and taken in different jars and *Allium cepa* L. bulbs were allowed to germinate. Root tips raised in different concentrations were excised and fixed in 1:3 acetic alcohol at 10.30AM and preserved in 70% ethyl alcohol. Root tip squashes were made using 9:1 aceto-carmin and 1N HCL and chromosomal abnormalities were observed (Fig.5).

2.6. Experimental Methodology

Leafy vegetables namely palak and coriander, soil and water samples were collected randomly from five different stations (S₁-S₅) and control (S₆) to analyse heavy metals viz., Pb, Cu, Zn, Cd, Cr and Mn concentrations. Heavy metal analysis was carried out using atomic absorption spectrophotometer (Varian AA 525) at Azyme Biosciences Private Limited, Jayanagar, Bangalore – 560 041.

3. Results and discussion

3.1. Copper(Cu)

All six heavy metals present in *Beta vulgaris* L. in all the stations (S₁-S₅) including control. In palak, Copper concentration varied considerably ranged between 7.29ppm (S₁) to 34.70ppm (S₂). S₂ (34.70ppm), S₄ (34.66ppm) and S₅ (34.56ppm) stations have crossed the permissible limits (30ppm). In S₁, S₃ and control stations Cu concentration was far below the permissible limits of Indian Standard. In coriander, highest accumulation of Cu was recorded at S₂ (60.77ppm) and in other stations far below critical level and ranged from S₅ (3.58ppm) to S₁ (19.29ppm) and control showed 2.19ppm. In S₂ station there is twofold increases in the concentration of Cu (Table 1 & 2). Demirezen & Ahmet (2006) reported that, Cu concentration (22.19-76.50mg kg⁻¹) was found higher in leafy vegetables when compared to non-leafy vegetables in Turkey and it may be due to richness of chlorophyll. Fytianos et al., (2001) analysed various leafy vegetables including spinach (palak) and coriander from industrial areas and found no significant differences in metal concentration. Sharma et al., (2006) reported that, Cu concentration (2.25-5.42mg kg⁻¹) in vegetables grown in waste water areas of Varanasi, India are within safe limit. Present study revealed that, leafy vegetables accumulated below critical level and when compared to coriander, palak showed highest Cu concentration, control values were found to be much lesser than soil irrigated with waste water and present results are in conformity with the findings of Debopam Banerjee et al., (2010).

3.2. Zinc(Zn)

Zinc concentration in palak in all the stations varied considerably and except at S₄ (61.42ppm) in other stations it does not exceed the permissible limits of Indian Standard (50ppm). Availability of Zn ranged from 24.32ppm to 48.31ppm compared to control (21.11ppm). In coriander, Zn concentration exceeded the permissible limits at S₂ (73.72ppm) and S₅ (99.56ppm) stations, whereas S₁ (30.82ppm), S₃ (35.23ppm), S₄ (9.98ppm) and control (10.21ppm) showed values far below the permissible limits (Table 1 & 2). Zinc is an essential mineral that is naturally present in some foods, added to others and available as a dietary supplement. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of enzymes and plays a role in immune function, protein synthesis and wound healing. Zinc also supports normal growth and development. Sridhara Chary et al., (2008) have also found varied Zn concentration in leafy vegetables of waste water irrigated areas of Hyderabad, Andhra Pradesh. Abida Begum & Harikrishna (2010) observed similar trends in Zn concentration of leafy vegetables in periurban areas of Bangalore. Zn is present in appreciable amounts in leafy vegetables and appears to have higher uptake from continued sewage irrigated land in Bellandur, Bangalore urban district (Lokeshwari & Chandrappa, 2006).

3.3. Lead(Pb)

Lead level ranged from 28.43ppm (S₅) to 149.50ppm (S₄) in palak leaves. Lead concentration was exceedingly high in different stations S₁ (128.09ppm), S₂ (108.03ppm), S₃ (36.33ppm) including control (7.53ppm) and crossed safe limits. In coriander, maximum lead concentration was observed at S₄ (75.50ppm) and minimum at S₂ (54.69ppm) compared to control (10.23ppm). Accumulation of lead in all stations showed exceedingly high values compared to Indian standard. Lead concentration in palak is more compared to coriander and both crossed permissible limits and the situation is very much alarming (Table 1 & 2). Lead contents in coriander leaves were significantly (P<0.05) high compared to other vegetables and possible explanation is that, lead uptake can be promoted by soil pH and organic matter levels (Muhammad Farooq et al., 2008). Demirezen & Ahmet (2006) analysed leafy vegetables and reported high lead concentration (3.0-10.7mg/kg⁻¹) which poses health risk to human beings. Sharma et al., (2006) reported high lead concentration (17.54-25.00mg/kg⁻¹) in vegetables grown in waste water and revealed industrial areas are above safe limit (2.5mg/kg⁻¹) and present results were also in same line. Al Jassir et al., (2005) reported highest concentration of lead in coriander (0.171mg/kg⁻¹). Lead concentration in leafy vegetables was much higher than other vegetables which are coherent with the literature (Rahlenbeck et al., 1999). Accumulation of lead is mainly due to large number of small scale industries, vehicular emissions, re-suspended road dust and diesel generator sets. Uptake of lead in plants is regulated by pH, particle size and Cat-ion exchange capacity of soil as well as by root exudation and other physio-chemical parameters (Lokeshwari & Chandrappa, 2006). Present results are in conformity with Anita Singh et al., (2010) and Abida Begum & Harikrishna (2010) who found the presence of lead in leafy vegetables examined. The introduction of lead into the food chain may affect human health and thus, studies concerning lead accumulation in vegetables have increasing importance (Coutate, 1992) so also cadmium.

3.4. Chromium(Cr)

Chromium content in palak samples ranged from non-detectable (S_5 and control) to 28.59ppm (S_3) and S_2 (70.79ppm) it was exceeded permissible limit (20ppm) and in S_1 (0.39ppm) and S_4 (13.31ppm) stations Cr level was far below the permissible limit. In coriander, Cr concentration was high at S_2 (127.27ppm) and low at S_5 and S_1 stations. In other stations below detection level (BDL). Cr concentration in palak and coriander varied considerably, highest in palak at S_3 (28.59ppm) and in coriander at S_2 (127.2ppm) stations. (Table 1 & 2). Present findings revealed Cr in palak and coriander at S_2 are really a panic situation and urgent measures are required to thwart the condition. Chromium was not detected in S_5 and control and values observed were well within the permissible limits of Indian standard (Awashthi, 2000). Exposure to Chromium may occur through breathing air, drinking water, or eating food containing Cr or even through skin contact. In human beings and animals, it is considered to be an essential metal for carbohydrates and lipid metabolism within a certain range of concentrations (up to 200 μ g/day). However exceeding normal concentrations leads to accumulation and toxicity that can result in hepatitis, gastritis, ulcers and lung cancer (Garcia, E. *et al.*, 2001).

3.5. Cadmium(Cd)

Cadmium concentration in palak samples at S_2 (0.81ppm) and S_4 (1.50ppm) stations and in other stations below detection level. Cd was below detection level in coriander samples in all stations (S_1 - S_5) including control and in palak at S_4 station showed signs of exceeding the permissible limit (Table 1 & 2). Cd presence is a dangerous proposition and is a wakeup call for our ecosystem and human beings in general. Except at S_1 , S_3 , S_5 and control stations, presence of Cd in below detection level (BDL). Cadmium is the most toxic heavy metal because it bio-accumulates and may cause health disorders even at low doses (Nagajyoti *et al.*, 2010). Randwan & Salama (2006) found lowest Cd concentration in coriander and palak leafy vegetables. Use of contaminated water for irrigation, fertilizers, sewage and compost can remarkably increase the Cd uptake into plant tissues (Jackson & Alloway, 1991). Muhammad Farooq *et al.*, (2008) revealed that, based on plant species, their physical and chemical properties, plants can readily absorb Cd from soil where upon ingestion will enter into human food chain.

3.6. Manganese(Mn)

Manganese concentration in palak was recorded highest at S_1 (134.57ppm) and least at S_3 (71.57ppm) and control showed 87.21ppm and other stations viz., S_2 (117.80ppm) S_4 (91.38ppm) and S_5 (84.52ppm) respectively. Coriander exhibited higher Mn concentration at S_2 (358.67ppm) and least at S_3 (54.73ppm) and remaining stations S_1 (148.76ppm), S_4 (82.80ppm), S_5 (68.19ppm) and control (113.28ppm) showed mean concentration (Table 1 & 2). Manganese occurs naturally in many food sources, such as leafy vegetables, nuts, grains and animal products (IOM, 2002). Food is the most important source of manganese exposure in the general population (ATSDR, 2000; USEPA, 2002). Arora *et al.*, (2008) carried out a study to assess levels of heavy metals like iron, manganese, copper and zinc in vegetables irrigated with waste water and reported that, substantial buildup of manganese, copper and zinc in vegetables.

3.7. Soil

Cu was found in all stations (S_1 - S_5) concentrations ranging from 7.39ppm (S_2) to 35.32ppm (S_3). Control soils recorded 22.16ppm and values in all stations are well within Indian standard limits. Zn concentration was low in all stations and maximum at S_3 (32.58ppm) and minimum at S_2 (10.13ppm) compared to control (17.79ppm) and values hardly crossed permissible limit. Pb showed varied concentrations in all locations ranging from 23.03ppm (S_2) - 45.33ppm (S_3) compared to control (18.04ppm) and in all stations found below the permissible limit. Cr concentration is considerably high at S_3 (116.94ppm) and least at S_4 (53.03ppm) compared to control S_6 (16.78ppm) and values were below permissible limits. At S_2 , Cr was not detected. Cd is not detected in both land irrigated with waste water and bore well water. Mn concentration was high at S_3 (304.168ppm) followed by S_1 (248.30ppm), S_5 (121.08ppm) and S_4 (100.23ppm) stations compared to control (87.23ppm) and S_2 (25.16ppm) (Table 3). Heavy metal transportation from soil to roots largely depends on type and genetic features of soil forming rocks, granulometric soil composition, amount of organic matter, pH, absorption capacity, amount of $CaCO_3$ and other physical and chemical properties of soil (Habib Mohammad Naser *et al.*, 2009). Total metal concentration in soil does not necessarily correspond with metal bioavailability. Bioavailability depends on number of physical and chemical factors in the soil.

3.8. Water

Lead concentration in water samples was highest in all stations compared to control and values were higher than permissible limits. Zinc at S_1 to S_5 and cadmium at S_3 to S_5 was not detected except at S_1 (<0.05) and S_2 (<0.05). Magnesium and copper exceeded the WHO standard in all stations (Table 4). Water pH at S_1 (5.24), S_3 (5.16), S_4 (5.70) and S_5 (5.39) stations was acidic and at S_2 (7.10) station alkaline in nature. Primary water quality criteria laid down by

Central Pollution Control Board is 6.5-8.5 and water samples at all stations found below desirable limit compared. Electrical conductivity varied considerably, highest at S₃ (1690mv) and least at S₂ (1214Mv). Total hardness was maximum in S₂ (550mg/l) followed by S₅ (370 mg/l), S₃ (340 mg/l), S₁ (330 mg/l) and least at S₄ (210mg/l) and desirable limit of total hardness is (300mg/l). Except at S₄, other samples crossed permissible limits. Calcium was recorded varying results with highest concentration at S₂ (144mg/l) and least at S₄ (56mg/l). As per the characteristics of drinking water prescribed by BIS, it is found to be 75mg/l. Concentration of calcium at S₁ (60mg/l), S₄ (56mg/l) and S₅ (59mg/l) were found to be within prescribed limit. Magnesium was least at S₄ (16.8mg/l) and maximum at S₂ (45.6mg/l) stations. At S₄ (16.8mg/l) and S₅ (28.4mg/l) stations found below desirable limit and S₂ (45.6mg/l), S₁ (43.2mg/l), S₃ (36mg/l) stations exceeded prescribed drinking water limits. TDS found highest at S₂ (904.1mg/l) and lowest at S₄ (513.40mg/l). Total dissolved solids exceeded desirable limits in all the sampling stations S₁ (545.1mg/l), S₃ (691.4mg/l) and S₅ (616.30mg/l). Dissolved oxygen (DO) was least at S₂ (5.5ppm) and S₃ (5.5ppm) followed by S₁ (5.6ppm), S₅ (5.9ppm) and highest at S₄ (6.2ppm) stations. (Table 5). Lead concentration in water samples was highest in all stations compared to control and values were higher than permissible limits. Zinc at all the stations and Cadmium at S₃, S₄, S₅ was not detected except at S₁ (<0.05) and S₂ (<0.05). Magnesium and copper exceeded the WHO standard in all stations. Tanks receive waste from different industries and concentrations vary according to the types of activities upstream from time to time (Mwegoha & Khimpa, 2010). Highest concentration of TDS at S₂ may be due to high solids loading influent stream into lake and lowest TDS obtained at S₄ may be due to dilution effect. TDS are negatively charged and therefore attract more heavy metal ions, reducing their concentration in water. Higher electrical conductivity indicates higher concentrations of free metal ions in water. Rattan et al., (2005) have found higher concentrations of heavy metals in sewage effluents as compared to ground water. Many small scale industries such as dyeing, electroplating, metal surface treatment, fabric printing, battery, paints discharge their effluents into sewage water which may be the cause of elevated heavy metal. Bio-availability depends on the concentration of anions and chelating ligands present in water, pH, redox status and presence of adsorbent sediments (Prabhu, 2009).

3.9. Cytotoxic effect

Chromosomal aberrations observed in this work include C-mitotic effect, degenerating chromatin materials, anaphase bridge, precocious chromosome at anaphase, stickiness and vagrant chromosome. Fiskesjo & Levan (1993) reported that, *Allium* test has high correlation with other test system and could be used as an alternative to laboratory animal in toxicological research. Inhibition of germination and root growth was noticed at higher concentrations (40% and 50% dilution). Reduction in root growth is possibly due to accumulation of heavy metals in plant tissues and its interaction with minerals. Root growth retardation and root wilting is a result of suppression in cell division and chromosomal aberrations. Induction of lagging could be attributed to failure of normal organization and function of spindle apparatus. Dash et al., (1988) reported that, lead has diverse effect on the cell, some of which are enzyme inhibition, chromosome aberration and mutation leading to spindle impairment and malfunction. Present results are in conformity with Abu Ngozi & Mba (2011) who noticed various mitotic abnormalities such as anaphase bridge, multinucleated conditions and chromosome stickiness when *Allium cepa* L. was treated with different concentrations of pharmaceutical effluents containing Pb, Cu and Zn. Bruning & Chronz (1999) have reported that, high correlation between heavy metal amounts in natural and industrial environment to increased frequency of chromosome mutations.

4. Conclusions

The main source of pollution of water bodies is due to sewage and irrigation of contaminated water found to contain variable amounts of heavy metals leads to increase in concentration of metals in the soil and vegetation. Monitoring of water quality, soil and plant is inevitable to prevent potential health hazards of irrigation with sewage fed water. Results showed that, presence of heavy metals in palak and coriander leaves is in the order of Mn>Pb>Zn>Cu>Cr>Cd and present study would go a long way in providing a baseline data for the assessment of distribution of heavy metals in palak (*Beta vulgaris*) and coriander (*Coriandrum sativum*) grown in Bangalore urban district. Experiment revealed that, lethal effects of irrigated water may be attributed to the presence of heavy metals. There is considerable differences existed in the various leafy vegetables analysed and this may be due to topography and geographic position of area under irrigation and ability of the plants and their specific parts to accumulate metals as well. Therefore it is suggested that, regular survey of heavy metals should be done on all food commodities to protect the end user that might injure their health.

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References

- ATSDR. (2000), "Toxicological profile for manganese", Atlanta, GA, United States Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry.
- Abida Begum, & Harikrishna, S. (2010), "Pathogens and heavy metal concentration in green leafy vegetables". E-Journal of Chemistry, 7(S₁). [www. E-Journal.net](http://www.E-Journal.net)
- Abu Ngozi, E. & Mba, K. C. (2011), "Mutagenic city testing of pharmaceutical effluents on *Allium cepa* root tip meristems", *J. Toxicology and Environmental Health Sciences*, 3(2):44-51.
- Al Jassir, M. S., Sarkar, A. & Khaliq, M. A. (2005), "Deposition of heavy metal on green leafy vegetables sold on roadsides of Riyadh city, Saudi Arabia", *J. Environ. Contam. Tox.*, 75:1020-1027.
- Anita Singh, Rajesh Kumar Sharma, Madhoolika Agrawal & Fiona M. Marshall. (2010), "Risk assessment of heavy metal toxicity through contaminated vegetables from waste water irrigated area of Varanasi", *Indian Tropical Ecology*, 51(2):375-387.
- Arora, M., Kiran, B., Rani, S., Rani, A., Kaur, B. & Mittal, N. (2008), "Heavy metal accumulation in vegetables irrigated with water from different sources", *Food Chemistry*. 111: 811-815.
- Awashthi, S. K. (2000), "Prevention of Food Adulteration Act No. 37 of 1954", Central and State rules as amended for 1999, Ashok Law House, New Delhi.
- Bruning, T. & Chronz, C. (1999), "Occurrence of urinary tract tumors in miners highly exposed to Dimtrotoluene", *YOEM*, 3:144-149.
- Coutate, T. P. (1992), "Food. The Chemistry of its components". 2nd Edn. Cambridge. Royal Society of Chemistry, pp. 265
- Cui, Y. J., Zhu, Y. G., Zhai, R., Huang, Y., Qiu, Y. & Liang, J. (2005), "Exposure to metal mixtures and human health impacts in a contaminated area in Nanning, China", *Environ. Int.*, 31:784-790.
- Dash, S., Panda, K. K. & Pada, B. B. (1998), "Bio monitoring of low levels of mercurial derivatives in water and soil by Allium micronucleus assay", *Mutation Research*, 203:11-21.
- Debopam Banerjee, Himadri Bairagi, Sibabrata Mukhopadhyay, Anju Pal, Debabrata Bera & Lalitagauri Ray. (2010), "Heavy metal contamination in fruits and vegetables in two districts of West Bengal, India", *EJEAF Che.*, 9(9):1423-1432.
- Demirezen, D. & Ahmet, A. (2006), "Heavy metal levels in vegetables in Turkey are within safe limits for Cu, Zn, Ni and exceeded for Cd and Pb", *J. Food Qual.*, 29:252-265.
- Fiskesjo, G. & Levan, A. (1993), "Evaluation of the first ten MEIC chemicals in the Allium test", *ATLA*, 21:139-149.
- Fytianos, K., Ktasianis, K., Tziantafyllox, P. & Zacchariadis, G. (2001), "Accumulation of heavy metals in vegetables grown in an industrial area in relation to soil", *Bull. Environ. Contam. Toxicol.*, 67:423-443.
- Garcia, E. C., Cabrera, M. L., Lorenzo, Sanchez, J. and Lopez, C. (2001), "Daily dietary intake of chromium in Southern Spain measured with duplicate diet sampling", *Br. J. Nutr.*, 86:391-396.
- Gupta, U. C. & Gupta, S. C. (1998), "Trace element toxicity relationships to crop production and livestock and human health: Implication for management", *Commun. Soil Sci. Plant Anal.*, 29:1491-1522.
- Habib Mohammad Naser, N., Shil, C. N., Mahmud, U. M., Rashid, H. & Hossain, K. M. (2009), "Lead, cadmium and nickel contents of vegetables grown in industrially polluted and non-polluted areas of Bangladesh", *Bangladesh J. Agri. Res.*, 34(4):545-554.
- IOM. (2002), "Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc", Institute of Medicine, Food and Nutrition Board. Washington, DC, National Academy Press, pp. 10-1 to 10-22.
- Jackson, A. P. & Alloway, B. J. (1991), "The transfer of cadmium from sewage-sludge amended soils into the edible components of food crops", *Water, Air and Soil Pollution*, 57-58(1):873-881
- Kachenko A. G, Singh B. (2006), "Heavy metals contamination in vegetables grown in urban and metal smelter contaminated sites in Australia" *Water, Air and Soil Pollution*, 169:101-123.

- Lokeshwari, H. & Chandrappa, G. T. (2006), "Impact of heavy metal contamination of Bellandur lake on soil and cultivated vegetation", *Current Science*, 91(5):622-627.
- Mapanda, F., Mangwayana, E. N., Nyamangara, J. & Giller, K. E. (2005), "The effect of long term irrigation using waste water on heavy metal contents of soil under vegetables in Harare, Zimbabwe", *Agric, Ecosys, Environ.*, 107:151-165.
- Muhammad Farooq, Farooq Anwar & Umer Rashid. (2008), "Appraisal of heavy metal contents in different vegetables grown in the vicinity of an industrial area", *Pak. J. Bot.*, 40(5):2009-2016.
- Mwegoha, W. J. S. & Kihampa, C. (2010), "Heavy metal contamination in agricultural soils and water in Dar es Salaam city, Tanzania", *Afr. J. Environ. Sci. Technol.*, 4(11):763-769.
- Nagajyoti, P. C., Lee, K. D. & Sreekanth, T. V. M. (2010), "Heavy metals, occurrence and toxicity for plants: A review", *Environmental Chemistry Letters*, 8(3):199-216.
- Prabu, P. C. (2009), "Impact of heavy metal contamination of Akaki river of Ethiopia on soil and metal toxicity on cultivated vegetable crops", *EJEAF che.* 8(9):818-827.
- Rahlenbeck, S. I., Burberg, A. & Zimmermann, R. D. (1999), "Lead and cadmium in Ethiopian vegetables". *Bull. Environ Contam. Toxicol.*, 62(1):30-33.
- Randwan, M. A. & Salama, A. K. (2006), "Market based survey for heavy metals in Egyptian fruits and vegetables", *Food and Chemical Toxicology*, 44:1273-1278.
- Rattan, R. K., Datta, S. P., Chhonkar, P. K., Suribabu, K. & Singh, A. K. (2005), "Long-term impact of irrigation with sewage effluents on heavy metal content in soils, crops and groundwater-a case study", *Agriculture, Ecosystems & Environment*, 109(3 & 4):310-322.
- Sarabjeet Singh Ahluwalia & Dinesh Goyal. (2007), "Microbial and plant derived biomass for removal of heavy metals from wastewater", *Bioresource Technology*, (12):2243-2257.
- Sharma, R. K., Agarawal. M. & Marshall, F. M. (2006), "Heavy metal contamination in vegetables grown in waste water irrigated areas of Varanasi, India", *Bulletin of Environmental Contamination and Toxicology*, 77:311-318.
- Sharma, R. K., Agrawal, M. & Marshall, F. M. (2007), "Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India", *Ecotoxicology and Environmental Safety*, 66:258-266.
- Sridhara Chary, N., Kamala, C. T. & Samuel Suman, R. D. (2008), "Assessing risk of heavy metals from consuming food grown on sewage irrigated soils and food chain transfer", *Ecotoxicology and Environmental Safety*, 69:513-524.
- Tsuda, T., Inoue, T., Kojima, M. & Aoki, S. (1995), "Market Basket and duplicate portion estimation of dietary intakes of Cadmium, Mercury, Arsenic, Copper, Manganese and Zinc by Japanese adult", *J. AOAC Int.*, 78:1363-1368.
- USEPA. (2002), "Health effects support document for manganese", Washington, DC, United States Environmental Protection Agency, Office of Water.

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| Station | Copper | Zinc | Lead | Chromium | Cadmium | Manganese |
|-----------------|--------|-------|--------|----------|---------|-----------|
| S ₁ | 7.29 | 37.72 | 128.09 | 0.39 | BDL | 134.57 |
| S ₂ | 34.70 | 48.31 | 108.03 | 70.79 | 0.81 | 117.80 |
| S ₃ | 9.62 | 24.32 | 36.33 | 28.59 | BDL | 71.57 |
| S ₄ | 34.66 | 61.42 | 149.50 | 13.31 | 1.50 | 91.38 |
| S ₅ | 34.56 | 43.59 | 28.43 | BDL | BDL | 84.52 |
| Control | 16.28 | 21.11 | 07.53 | BDL | BDL | 87.21 |
| Indian Standard | 30.0 | 50.0 | 2.5 | 20.0 | 1.5 | --- |
| WHO | 40.0 | 60.0 | 5.0 | --- | 0.2 | --- |

Table: 1. Heavy metal concentration (ppm) in palak leaves

| Station | Copper | Zinc | Lead | Chromium | Cadmium | Manganese |
|-----------------|--------|-------|-------|----------|---------|-----------|
| S ₁ | 19.29 | 30.82 | 62.45 | 12.13 | BDL | 148.76 |
| S ₂ | 60.77 | 73.72 | 54.69 | 127.27 | BDL | 358.67 |
| S ₃ | 9.05 | 35.23 | 59.76 | BDL | BDL | 54.73 |
| S ₄ | 14.48 | 9.98 | 75.50 | BDL | BDL | 82.80 |
| S ₅ | 3.58 | 99.56 | 59.73 | 8.46 | BDL | 68.19 |
| Control | 2.19 | 10.21 | 10.23 | BDL | BDL | 113.28 |
| Indian Standard | 30.0 | 50.0 | 2.5 | 20.0 | 1.5 | --- |
| WHO | 40.0 | 60.0 | 5.0 | --- | 0.2 | --- |

Table: 2. Heavy metal concentration (ppm) in coriander leaves

| Station | Copper | Zinc | Lead | Chromium | Cadmium | Manganese |
|----------------|---------|-------|-------|----------|---------|-----------|
| S ₁ | 17.05 | 29.26 | 33.77 | 76.88 | BDL | 248.30 |
| S ₂ | 7.39 | 10.13 | 23.03 | BDL | BDL | 25.16 |
| S ₃ | 35.32 | 32.58 | 45.33 | 116.94 | BDL | 304.68 |
| S ₄ | 12.67 | 15.01 | 29.93 | 53.03 | BDL | 100.23 |
| S ₅ | 23.70 | 18.98 | 34.21 | 86.98 | BDL | 121.08 |
| Control | 22.16 | 17.79 | 18.04 | 16.78 | BDL | 87.23 |
| BIS-1991 | 135-270 | 300 | 250 | --- | 3 – 6 | --- |
| WHO | --- | 600 | 500 | --- | --- | --- |

Table: 3. Heavy metal concentration (ppm) in soil

| Station | Copper | Zinc | Lead | Chromium | Cadmium | Manganese |
|----------------|--------|------|-------|----------|---------|-----------|
| S ₁ | 0.036 | BDL | 0.444 | BDL | <0.05 | 0.144 |
| S ₂ | 0.170 | BDL | 0.380 | BDL | <0.05 | 0.161 |
| S ₃ | 0.026 | BDL | 0.652 | BDL | BDL | 0.243 |
| S ₄ | 0.012 | BDL | 0.366 | BDL | BDL | 0.153 |
| S ₅ | 0.036 | BDL | 0.418 | BDL | BDL | 0.234 |
| Control | 0.016 | BDL | 0.11 | BDL | BDL | 0.13 |
| BIS-1991 | 0.05 | 5.0 | 0.10 | 0.05 | 0.01 | 0.10 |
| WHO | 0.20 | 2.0 | 5.0 | 0.10 | 0.01 | 0.20 |

Table: 4. Heavy metal concentration (ppm) in waste water

| Parameters | S ₁ | S ₂ | S ₃ | S ₄ | S ₅ |
|----------------------|----------------|----------------|----------------|----------------|----------------|
| pH | 5.25 | 7.11 | 5.16 | 5.70 | 5.39 |
| EC | 1340Mv | 1214Mv | 1690Mv | 1279Mv | 1362Mv |
| Total Hardness(mg/l) | 330 | 550 | 340 | 210 | 370 |
| Ca (mg/l) | 60 | 144 | 76 | 56 | 59 |
| Mg (mg/l) | 43.2 | 45.6 | 36 | 16.8 | 28.4 |
| TDS (mg/l) | 545.1 | 904.1 | 691.4 | 513.4 | 616.30 |
| DO ppm | 5.6 | 5.5 | 5.5 | 6.2 | 5.9 |

Table: 5. Physico – chemical parameters of waste water