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Heavy metal health risk assessment and microbial menaces via dietary intake of vegetables collected from Delhi and national capital regions peri urban area, India

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Abstract
The present study was conducted to assess the risk to human health by microbial load and heavy metals (Cr, Cu, Ni, Zn, Cd and Pb) through the intake of locally grown vegetables irrigated by metal contaminated water and waste water in Delhi and NCR region. Twenty-two varieties of vegetables grown in the agricultural land were collected for analysis. The results revealed that concentrations of copper, chromium and zinc were uneven with different crops and vegetables. Nickel and lead concentrations were higher than the safe limits suggested by the different agencies. Hazard index for most of the vegetables was out of safe limit. Health risk index of all metals except lead was below the value of hazard. Metal pollution index value for brinjal, pigweed, bottlegourd, Armenian cucumber, cucumber, ridgegourd, red spinach, bittergourd, ladyfinger and radish were very high, indicating heavy metal contamination and negative impact on human health.

Key words: Metal pollution index, health risk index, hazard index, microbial load, heavy metal pollution.

Introduction
Due to social and health awareness, the demand of better quality vegetables is increasing among consumers. The perceptions of what is regarded as 'better quality' are however subjective. Mostly consumers consider dark green, big leaves, polished and undamaged vegetables as characteristics of good quality leafy vegetables [25]. However, the outer morphology of vegetables cannot guarantee safety from contamination [3]. In India, land disposal of sewage and industrial effluents has been implicated as the chief source of heavy metal enrichment of pasturelands and agricultural fields, particularly near sewage treatment works in urban areas [28]. The production of vegetables using wastewater for irrigation is practiced in peri urban but little is known on the contamination of these vegetables with heavy metals and microorganism [6]. Singh *et al.*[32] revealed that the metal pollution in water and soil of Delhi and peri urban area is continuously increasing day by day at alarming rate.The water having metal contamination got deposited in soil and from soil is uptake by the plants and vegetables. Due to non biodegradable nature, long half-life and their potential to accumulation different body parts heavy metals are very harmful. Most of the heavy metals are extremely toxic because of their solubility in water [4]. Even low concentrations of heavy metals have hazardous effects to human and fauna because there is no effective mechanism for their elimination from the body [10]. Heavy metals can cause epigastria pain, dermatitis, nausea, skin allergy, allergic sensitization, vomiting, lung and nervous system damage, mutagenic and carcinogenic properties, severe diarrhoea and haemorrhage in human beings [24, 27]. On the part of microbial load, vegetables are widely exposed to microbial contamination through wastewater irrigation and soil. In developing countries, continued use of untreated wastewater is a major contributing factor for pathogenic microbial contamination [29]. Heavy metals are ubiquitous due to excessive use in industrial applications. Effluents from industries contain significant amounts of toxic heavy metals, which are harmful to food web [5]. Accumulation of heavy metals in agricultural soils through wastewater irrigation may not only result in detrimental soil quality, but also affect vegetable quality and safety [19]. Vegetables have greater potential of accumulating heavy metals in their edible parts than grain or fruit crops [23]. Studies on the uptake of heavy metals by plants have shown that heavy metals can be transported passively from roots to shoots through the xylem vessels [16]. In addition, plant organs such as fruit and seed that have low transpiration rates did not accumulate heavy metals because the storage organs are largely phloem-loaded and heavy metals are poorly mobile in the phloem [8], the concentrations of heavy metals in vegetables per unit dry matter follows the order: leaves > fresh fruits > seeds. Contamination of the human food chains by heavy metals is not directly affected by the plants total uptake, but rather by the concentration in those parts that are directly consumed [18].Thus, in assessing exposure risks, heavy metal contents in roots and stems are of less importance than those in the edible parts [34]. Sensitivity of organisms to heavy metal toxicity depends on heavy metal accumulation rate in plants, intake rate in animals and age of the consuming organism amongst other factors [1]. The main objectives of this study were to determine the microbial quality and concentrations of Cu, Zn, Cd, Ni, Cr and Pb in vegetables grown around the Yamuna pushta region in Delhi-NCR, irrigated by heavy metal polluted water to estimate their intake rates by people consuming the vegetables.

Materials and Methods

Study area: The fifteen different sites (Palla, Christian ashram, Jagatpur, Sonia Vihar, Wazirabad, Shastri park, Indraprastha, Okhla, Noida, Basantpur, Nehru vihar, Daryia nalla, Punjabi bagh, Keshopura and Nilothi of Yamuna pushta regions in Delhi-NCR were selected for the vegetable sampling (Fig. 1). Twenty-two varieties of crops and vegetables were collected from the above mentioned sites.

Sample collection and treatments: Vegetables grown in the selected sites were collected during the period of February, 2013 – May, 2014. The details of different vegetables and crops analysed are mentioned in Table 1. After collection the samples were identified and packed into sterile polythene bags. In the laboratory samples were first cut into pieces and thoroughly washed with tap water following double distilled water. The samples were dried in an oven at 60ºC until constant weight obtained and grinded for further analysis.

Digestion and analysis of samples: Plant samples (1g) were digested after adding 15 ml of tri acid mixture (HNO_3, H_2SO_4 and $HClO_4$ in ratio 5:1:1) at 80ºC until a transparent solution was obtained [2]. After cooling, the digested sample was filtered using Whatman no. 42 filter paper and the filterate was finally maintained to 25 ml with double distilled water. Triplicate digestion of each sample was carried out together. The analysis was conducted using AAS4141 ECIL atomic absorption spectrophotometer.

Isolation of microorganisms: Twenty-five grams of each sample was weighed and washed in 100 ml of sterile distilled water. M-Endo agar was inoculated with 1 ml of the rinse water using the pour plate technique. The plates were allowed to solidify, inverted and incubated at 35ºC for 24 h for colony formation.

Data analysis

Daily dietary index: As food crops are contaminated by heavy metals so their daily intake needs to be evaluated for comparison as given by US-EPA. Daily dietary index was determined by the following formula [41]: DDI =X × Y × Z/B, where X = metal in vegetable; Y = dry wt. of the vegetable; Z = approximate daily intake; B = average body mass of the consumers.

Table 1. Vegetable samples collected from the 15 different sites of Delhi-NCR.

Common name	Scientific name	Edible part
Cucumber	*Cucumis sativus*	Fruit
Rice	*Oryza sativa*	Grain
Bittergourd	*Momordica charantia*	Fruit
Lady finger	*Abelmoschus esculentus*	Fruit
Radish	*Raphanus sativus*	Root
Ridgegourd	*Luffa luffa*	Fruit
Red spinach	*Basella alba*	Shoot
Mustard	*Brassica juncea*	Shoot and seed
Wheat	*Triticum aestivum*	Grain
Spinach	*Spinacia oleracea*	Shoot
Carrot	*Daucus carota*	Root
Armenian cucumber	*Cucumis melo*	Fruit
Pumpkin	*Cucurbita maxima*	Fruit
Bottlegourd	*Lagenaria siceraria*	Fruit
Sorghum	*Sorghum bicolor*	Grain
Beans	*Phaseolus vulgaris*	Fruit
Eddoe	*Colocasia esculenta*	Root
Cabbage	*Brassica oleracea*	Shoot
Pigweed	*Amaranthus palmeri*	Shoot
Brinjal	*Solanum melongena*	Fruit
Cauliflower	*Brassica oleracea*	Inflorescence
Tomato	*Solanum lycopersicum*	Fruit

Daily intake of metals: The value was determined by the formula: DIM = $C_{metal} \times C_{factor} \times D_{food\ intake}/B_{average\ weight}$, where, C_{metal} = heavy metal conc. in plants (mg/kg); C_{factor} = conversion factor; $D_{food\ intake}$ = daily intake of vegetables. The conversion factor of 0.085 is to convert fresh vegetable weight to dry weight [5].

Metal pollution index: Metal pollution index (MPI) was computed to determine overall heavy metal concentration in all vegetables analysed. This index was obtained by calculating the geometrical mean of concentrations of all the metals in the vegetables [14]: MPI (µg g^{-1}) = (Cf1×Cf2 × ……….× Cfn)1/n; where Cfn= concentration of metal "n" in the sample.

Health risk index: The health risk index was calculated as the ratio of estimated exposure of test vegetables and oral reference dose [44]. Oral reference doses were 4 ×10^{-2}, 0.3 and 1×10^{-3} mg/day for Cu, Zn, and Cd, respectively [40]; 0.004, 0.02, 1.5 mg/day for Pb, Ni and Cr, respectively [40]. Estimated exposure is obtained by dividing daily intake of heavy metals by their safe limit. An index

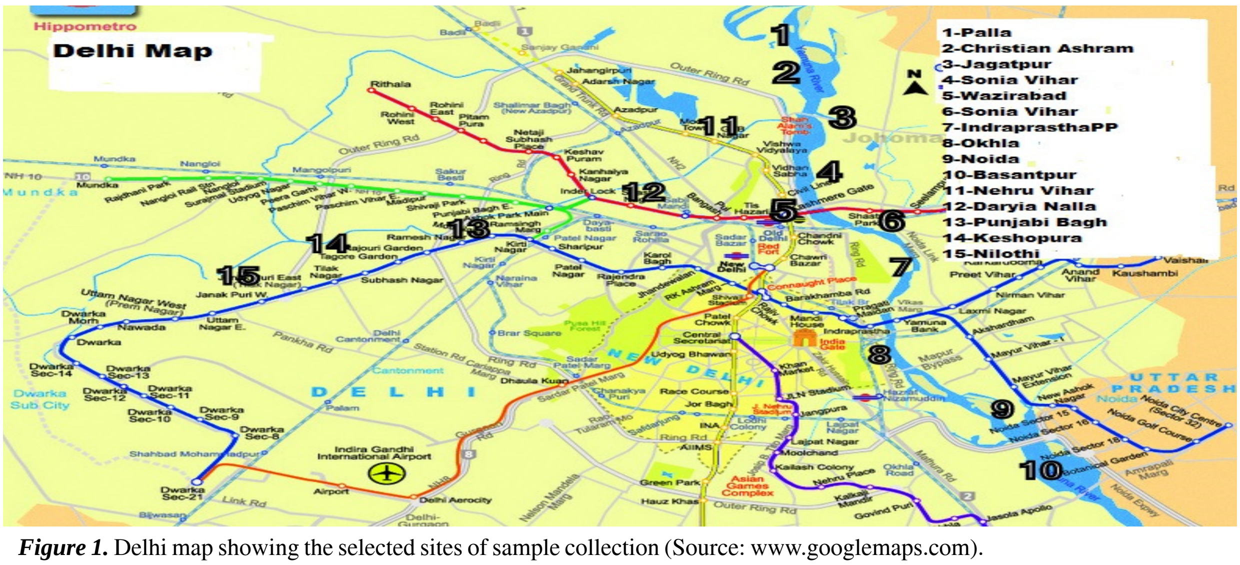

Figure 1. Delhi map showing the selected sites of sample collection (Source: www.googlemaps.com).

more than 1 is considered as not safe for human health [40]. The required amount of vegetables in our daily diet must be 300 to 350 g per person as suggested by WHO guideline [42]. A survey of 100 people was done for the average daily vegetable intake rate having an average weight of 70 kg. The average body weight was taken as 70 kg for adults according to WHO [43].

Hazard index (HI): To evaluate the potential risk to human health through more than one heavy metal, the hazard index (HI) has been developed [39]. The hazard index is the sum of the hazard quotients as described in the following equation: $HI = \Sigma HQ = HQCr + HQNi + HQCd + HQPb$.

Microbial load determination: For enumeration of microorganisms present in each sample, 10-fold serial dilutions of each rinse water were made and 1 ml of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} and 10^{-9} dilutions were pipette into sterile Petri dishes. The colonies were counted using a colony counter after 24 h incubation at 35ºC [35, 36].

Results and Discussion

Levels of heavy metals in vegetable samples: Due to use of metal contaminated water, i.e. Yamuna river water and wastewater for the irrigation purpose, the metals accumulated in soil and then by vegetables grown in that contaminated soil, and ultimately were taken up by humans [33]. The concentration of heavy metals showed variation among the different vegetables and crops collected from the fifteen different sites. Concentrations of heavy metals (Cu, Cr, Cd, Ni, Pb, Zn) in vegetables and crops (dry weight) are shown in Table 2.

The difference in heavy metal concentrations in different vegetables may be described to the difference in the morphology

Table 2. Concentration of heavy metals in vegetable samples (n = 4).

Samples	Metal concentrations (Mean ± standard mean error) (mg/kg)					
	Copper	Chromium	Nickel	Lead	Zinc	Cadmium
Cucumber	38.9±0.404	26.5±0.351	25.8±0.351	13.9±0.472	72.2±0.208	<0.01
Rice	34.2±0.208	26.2±0.208	24.1±0.152	7.05±0.704	56.1±0.152	<0.01
Bittergourd	42.7±0.099	29.6±0.057	6.07±0.574	33.3±0.458	56.9±0.529	<0.01
Lady finger	33.5±0.351	10.9±0.057	17.9±0.208	65.5±0.404	33.3±0.458	<0.01
Radish	26.3±0.321	35.3±1.050	20.9±0.416	15.15±0.165	44.4±0.360	<0.01
Ridgegourd	36.5±0.404	48.4±0.251	12.2±0.152	30.7±0.493	36.08±1.11	<0.01
Red Spinach	3.9±0.288	90.7±0.709	29.4±0.251	20.05±0.201	78.2±0.404	<0.01
Mustard	19.1±0.099	18.4±0.099	19.2±0.416	35.7±0.550	31.2±0.230	<0.01
Wheat	19.15±0.017	27.1±0.152	13.48±0.306	22.08±0.189	47.4±0.818	<0.01
Spinach	15.9±0.435	12.2±0.10	27.7±0.642	22±1	68.9±0.611	<0.01
Carrot	22.9±0.404	32±1.154	6.6±0.493	44.6±0.611	59.4±0.461	<0.01
Armenian cucumber	32.0±0.004	74±1.527	15.8±0.305	26.8±0.351	32.5±0.378	<0.01
Pumpkin	7.5±0.513	5.2±0.152	16.2±0.346	22.4±0.608	77.5±0.608	<0.01
Bottlegourd	37.7±0.472	49.3±0.435	22.9±0.608	25.5±0.351	39.6±0.529	<0.01
Sorgham	20.3±0.115	<0.06	4.4±0.305	20.6±0.556	46±0.577	<0.01
Beans	4.1±0.199	0.23±0.015	15.4±0.251	21.5±0.208	74.3±0.321	<0.01
Eddoe	27±1.527	5.5±0.30	1.9±0.404	10.3±0.305	12.9±1.011	<0.01
Cabbage	25.6±0.450	4.1±0.351	21.1±0.351	26.2±0.503	27.1±0.945	<0.01
Pigweed	35.6±0.351	<0.06	16.6±0.435	33.1±0.550	81.3±0.321	<0.01
Brinjal	33.7±0.709	<0.06	23.7±0.416	38.9±0.568	75.9±0.513	<0.01
Cauliflower	3.8±0.208	9.9±0.305	29.45±0.32	24.7±0.450	<0.01	<0.01
Tomato	40.3±0.472	8.2±0.115	17.5±0.503	12.05±0.437	86.2±0.208	<0.01
Indian standard (mg/kg)[5]	30	20	1.5	2.5	50	1.5
WHO-FAO (mg/kg)[18]	40	NA	NA	5	60	0.2

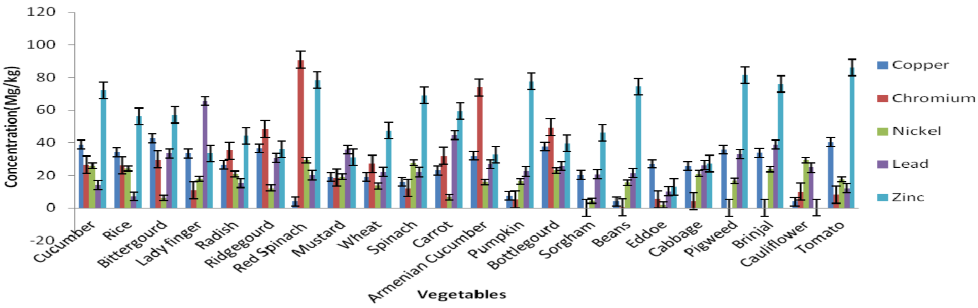

Figure 2. Variation of heavy metals (Cu, Cr, Ni, Pb, Zn) in different vegetables.

and physiology for heavy metals uptake, exclusion, accumulation and retention [20]. For all six metals, the values of copper, chromium and zinc were found uneven with different crops and vegetables. The values of nickel and lead were higher than the permissible limits in each and every vegetable grown in the Yamuna puhsta region. Cadmium was not detectable in all the sampled vegetables and crops (Fig. 2).

The concentration of copper had range of 3.8 - 42.7 mg/kg. The amount of copper was maximum in bitter gourd and minimum in cauliflower. Copper is an essential element for normal biological activities and also helpful in the enzymatic activities of biological system. The tyrosinase and aminooxidase enzymes are regulated through the copper in adequate quantity, but excessive intake of copper leads to hemolysis, hepatoxicity and nephrotoxicity [30]. According to an estimate, only 1.5-3 mg/day copper has been determined safe for human consumption [13].

In case of chromium the highest concentration was detected in red spinach (90.7 mg/kg) and lowest in beans (0.23 mg/kg). The daily intake of chromium of 50 to 200 µg/day has been suggested by US national Academy of Science.

For zinc, the maximum concentration was found in tomato (86.2 mg/kg) and minimum concentration in eddoe (12.9 mg/kg). Zinc is important for the enzymatic function. Zinc plays an important role in synthesis of proteins, DNA and insulin [7]. It is the second most abundant element in human next to iron. The recommended dietary intake of zinc is 15 mg/day for adult and for pregnant woman 30 mg/day [12].

Nickel and lead were above the permissible limits in all vegetables and crops. The maximum concentration of nickel was in red spinach and cauliflower (29.4 mg/kg) and minimum in eddoe (19 mg/kg). Nickel is known to be responsible for heart attacks, depression, haemorrhages, cancer and low blood pressure [22]. On the other hand, the maximum concentration of lead was in ladyfinger (65.5 mg/kg) and minimum in rice (7.05 mg/kg). Long-term exposure of lead leads to damage of brain and kidneys and ultimately death [21]. The metal accumulation is higher in vegetables in respect to cereal crops [31]. All the 22 samples accumulated metals, but 4 samples, i.e. brinjal, bittergourd, rice and cucumber, accumulated all the studied metals more than the permissible limits. As discussed earlier, the metal accumulation depends on plant physiology but the nature of metal binding efficiency of different metals is also one reason of difference in the uptake of metal by plants [12].

Daily dietary index (DDI): The values of DDI vary with the vegetables. The results reveal that the DDI was at very high level compared to the recommendation allowances for the metals in vegetables. The DDI varies with the type and varieties of vegetables. The amount of DDI above the allowances was found in case of Cr, Ni, Pb and Zn (Table 3). Most of vegetables had very high DDI, which proves that the vegetables grown in the Yamuna pushta regions are not good for human consumption or either peoples have to lower the intake of these contaminated vegetables.

Health risk index: The health risk index of metals (Cu, Cr, Cd, Ni, Pb, Zn) of 22 vegetables shows that copper, chromium, cadmium, nickel and zinc for all types of vegetables were lower than 1 (Fig. 3). The health risk index of lead was higher than 1, which poses greater risk of health for consumers. Except rice all other 21 studied vegetables had health risk index more than 1 in case of lead. This indicates that high HRI value of vegetables had great potential to health hazards. Earlier, it is reported that HRI values of lead, cadmium and zinc higher than 1 were found in some vegetables around Dinapur sewage plant, India. Also, Cui *et al.* [9] studied the vegetables of area Nanning, China, and the risk of cadmium and lead through consumption of vegetables. Jolly *et al.* [17] studied the area of Rooppur, Bangladesh, and found in vegetables HRI values for lead, zinc and cadmium higher than 1.

Metal pollution index: The study shows the following descending pattern of MPI in vegetables brinjal > pigweed> bottlegourd >Armenian cucumber >cucumber> ridgegourd> red spinach> bittergourd> ladyfinger> radish> carrot> rice> spinach> mustard> wheat>tomato> cabbage> sorghum> pumpkin> cauliflower> eddoe> bean (Fig. 4). Metal pollution index is suggested to be a reliable and precise method for metal pollution monitoring of wastewater irrigated areas [15]. From this we can conclude that the vegetables like brinjal, pigweed, bottlegourd, Armenian cucumber, cucumber, ridgegourd, red spinach, bittergourd, ladyfinger and radish accumulate more metals in the edible regions and hence are health hazardous to the peoples who rely on the vegetables grown on these areas.

Hazard index (HI): The index of hazard was calculated for the toxic elements like cobalt, chromium, nickel, cadmium and lead in different vegetables. Hazard index of vegetables shows the following ascending order eddoe> tomato> radish> cucumber> sorghum> red spinach> cabbage> spinach>pumpkin> cauliflower> pigweed>beans>mustard>Armenian cucumber > bottlegourd> ridgegourd> bittergourd> brinjal> carrot> ladyfinger> rice> wheat. The value of HI was more than 1 in all the vegetables which proves the great risk of health hazards in terms of toxic metals accumulation by vegetables. All the vegetables are not good for the consumption as per the hazard index (Fig.5).

Table 3. Daily dietary index in respect to recommended allowances.

Vegetables	Cu(DDI)[a]	Cr(DDI)[a]	Ni(DDI)[a]	Pb(DDI)[a]	Zn(DDI)[a]
Cucumber	194.5	132.5	129	69.5	361
Rice	171	131	120.5	35.25	280.5
Bittergourd	213.5	148	30.35	166.5	284.5
Lady finger	167.5	54.5	89.5	327.5	166.5
Radish	131.5	176.5	104.5	75.75	222
Ridgegourd	182.5	242	61	153.5	180.4
Red spinach	19.5	453.5	147	100.25	391
Mustard	95.5	92	96	178.5	156
Wheat	95.75	135.5	67.4	110.4	237
Spinach	79.5	61	138.5	110	344.5
Carrot	114.5	160	33	223	297
Armenian cucumber	160.03	370	79	134	162.5
Pumpkin	37.5	26	81	112	387.5
Bottlegourd	188.5	246.5	114.5	127.5	198
Sorghum	101.5	0	22	103	230
Beans	20.5	1.15	77	107.5	371.5
Eddoe	135	27.5	9.5	51.5	64.5
Cabbage	128	20.5	105.5	131	135.5
Pigweed	178	0	83	165.5	406.5
Brinjal	168.5	0	118.5	194.5	379.5
Cauliflower	19	49.5	147.25	123.5	0
Tomato	201.5	41	87.5	60.25	431
Average daily intake[b]	1.12	0.33	0.81	0.61	8.47
Recommended dietary allowances RDA (male)[c]	750-900	25-30	0.7-1	10 - 20	09- 11
RDA (female)[c]	750-900	15-20	0.6-1	10- 15	6- 8

[a]from the survey and questionnaire; [b]Tripathi *et al.* [37], Singh and Garg [33] ;
[c]Food and Nutrition Board [11]

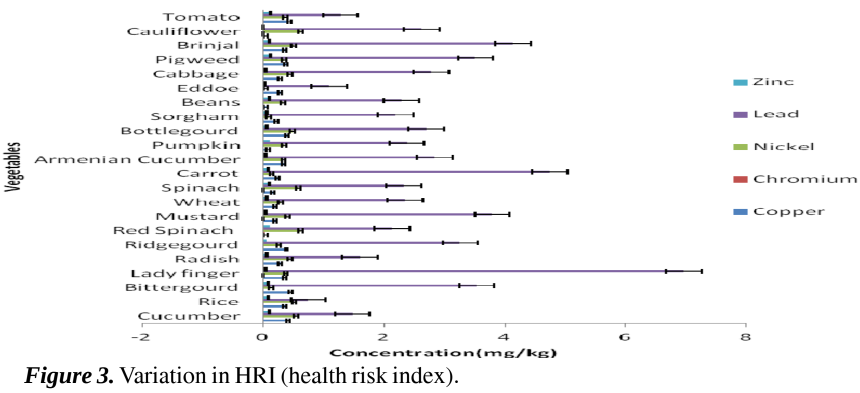

Figure 3. Variation in HRI (health risk index).

Microbial load of vegetables: All the 22 vegetables sampled in this study were contaminated. However, the microbial load of the vegetables varied with type (Fig. 6). Microbial load ranged from 1.1×10^5 to 5.3×10^8 cfu/ml (Fig. 6). Wheat and tomato had the lowest microbial load (1.1×10^5 and 4.6×10^6cfu/ml) of all the vegetables sampled, while cauliflower had the highest microbial load (5.3×10^8cfu/ml).

Conclusions

The metal pollution index value for brinjal, pigweed, bottlegourd, Armenian cucumber, cucumber, ridgegourd, red spinach, bittergourd, ladyfinger and radish is very high as compared to other vegetables analysed. This shows that either these vegetables should stop growing or we have to stop the contamination of water or refine it before irrigation. Health risk index for copper, chromium, nickel and zinc was less than 1 but for lead except rice all other 21 vegetables shows HRI value more than 1. In case of HRI, lead was found above the limit which proves that the vehicular discharge and lead industries are not under check, peoples should use more CNG and also contamination free irrigation water. Hazard index for all the vegetables was more than 1, which concluded the high potential risk of human health by consuming these contaminated vegetables. Among all the vegetables, wheat, rice, ladyfinger, carrot, brinjal, bittergourd, ridgegourd, bottlegourd and beans showed high value of HI. On account of microbial study it shows that the 22 vegetables and crops sampled and studied showed contamination of vegetables with microorganisms and were not good for the human consumption due to high microbial load. The overall study concludes that the metal accumulation in vegetables is quite high. The main cause of metal contamination is use of industrial grey and wastewater which is highly contaminated with metal and microbial load. The more contaminated water of irrigation the more concentration of metal in that soil and then to vegetables and to humans. The MPI, HRI and HI values in some vegetables for some elements were at alarming rate. The adverse effect can be reduced by consuming lesser amount of those vegetables. Moreover it is suggested that the irrigation water used should be treated well before using it in the field. For the treatment of water some biological agents were used to make the system ecofriendly. Accumulation of these metals in the vegetables can be toxic to the consumers when they are present in excess or cause metal related diseases when present in high quantities which are not suitable for the human health.

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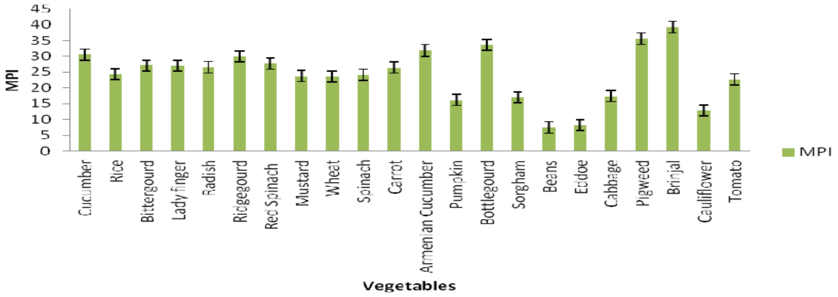

Figure 4. Metal pollution index of studied vegetables.

Figure 5. Hazard index of studied vegetables.

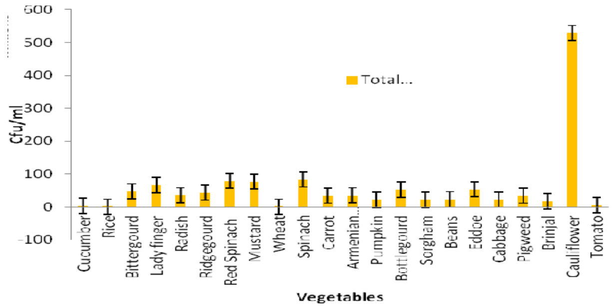

Figure 6. Total coliform count (cfu/ml) in vegetables.

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