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# **INVESTIGATION OF HEAVY METAL ACCUMULATION AND NUTRITIOUS STATUS CHANGES IN LEAFY VEGETABLES GROWN ON CONTAMINATED AND NON-CONTAMINATED SOILS COLLECTED FROM INDUSTRIAL AREA OF BHARUCH DISTRICT, GUJARAT, INDIA**

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Present research is aimed towards investigation of heavy metal accumulation and nutritious activity changes in leafy vegetables from surroundings of industrial area which is polluted with sewage, domestic effluents and industrial wastes flows through the Bharuch district, Gujarat, India. Available heavy metals content of contaminated soils, results revealed that Pb, Cd and Ni ranged from 10.52 to 84.20 mg kg<sup>-1</sup>, 73.52 to 184.80 mg kg<sup>-1</sup> and 65.88 to 150.68 mg kg<sup>-1</sup>, respectively, while the corresponding values were 19 to 2.18 mg kg<sup>-1</sup>, 0.06 to 3.98 mg kg-1 and 0.26 to 3.14 mg kg-1, respectively, non-contaminated soils. However, surrounding villages of contaminated soils areas as all, fifty, twenty-one and twenty-four samples were noted to cross maximum permissible limit of Cd, Pb and Ni, respectively. While Pb, Ni and Cd were found within maximum permissible limit and are safe in soils of non-contaminated areas. The qualitative analysis showed higher amounts of total soluble protein, total phenol, total carbohydrates and ascorbic acid in the edible portion of leafy vegetables grown in non-contaminated soils conditions than contaminated soils while, due to stress conditions of different heavy metal content, Proline concentration was found in maximum on leafy vegetables grown in contaminated soils. Heavy metals accumulation of all leafy vegetables with contaminated soils caused a significant increase in all elements like Cd, Pb and Ni except significant increase was noticed for Fe and Zn. Accumulation trend of metals in all vegetables: Cadmium > Lead > Nickel. **ABSTRACT**

*Key words :* Heavy metal, Contaminated soil, Leafy vegetables, Nutrients status.

# **Introduction**

Green leafy vegetables are predominantly known for their high nutritional content, which have biochemical characteristics and are mostly consumed for health and nutritional benefits. Generally, an industrial belt contains different hazardous heavy metals, *viz*., Cu, Zn, Pb, Fe, Ni, Cd and Mn (Devkota *et al*., 2000; Sharma *et al*., 2006). Contamination of soils by metal-containing substance can result from either the decisions of local farm management to use agricultural sprays, fertilizers or waste products as soil amendments, that is, delebrated pollution, or from sources incidental to modern society, such as factory and power station emission, which are often beyond local control. Many researchers and scientists from India and abroad, after studying soil (Kumar and Srikantaswamy, 2012) and plant samples (Sharma *et al*., 2009) from industrial and adjacent areas, reported varying degrees of contamination of heavy metals in soilwater-plant systems, which ultimately were polluting not only aquatic resources and lives but also agricultural and other plants/crops which later on became a part of food chain and thereby becoming harmful to human and animal kingdom. Vegetables constitute an important part of the human diet since they contain proteins, vitamins, as well as carbohydrates, minerals and trace elements. Green leafy vegetables are predominantly known for their high nutritional content and are mostly consumed for health and nutritional benefits. It is known that serious systemic health problems can develop as a result of excessive accumulation of dietary heavy metals such as Cd, Cr and Pb in the human body. Consumption of heavy metalscontaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defenses, intrauterine growth retardation, impaired psycho-social behaviour, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Arora *et al*., 2008). These elements can be phytotoxic and harmful to vital microbial processes in nutrient cycling (Oliveira *et al*., 2005). Chemical analyses provide information only referring to the accumulation of these contaminants in soils, but they will not indicate the consequences they have on soil biochemical composition and metabolism. Keeping all the above points in view, a pot experiment was planned on the evaluation of heavy metal accumulation and bio-chemical changes in leafy vegetables grown on contaminated and non-contaminated soils collected from industrial area of Bharuch district, Gujarat.

#### **Materials and Methods**

#### **Overview of study area**

First year soil survey was conducted from leafy vegetables growing areas around Ankleshwar and Bharuch region. Identify various locations of both the areas for collected representative soil samples. Total ten locations were identified from Bharuch and Ankleshwar area and collected soil samples of contaminated area from Amla khadi, Pungam, Piraman, Sajod, Bhadkodra, Sakarpor, Kosamadi, Umervada, Ankleshwar and Sanjali. Total three location of non-contaminated areas were found *i.e.* college farm (CF), National Agricultural Research Project, Bharuch (NARP) and Regional Cotton Research Station (RCRS), in Bharuch area and collected 15 nos. of soil samples from each their location (5 samples were collected randomly).

## **Soil Sampling and their analytical procedure**

To fulfil the objectives of present research GPS based representative surface (0-15 cm) soil samples were collected from above 10 locations. The location of the study areas are shown in figure 1. From each location, 5 samples were collected randomly (Total 50 nos.). Utmost care was taken to keep the soil samples free from contamination from the soils of other samples. Collected samples were mixed well to make a composite representative soil sample for each area and packed in a polythene bag with tag details *viz*. name of place, date of collection, etc. and bags were brought to the laboratory for further laboratory studies. Soil samples were allowed to dry in shade for two-three days and then after passing through a 2-mm sieve were preserved. Then samples were grounded to pass through a 0.25-mm sieve for DTPA- Extractable soil Pb, Ni, Cd were determined by Atomic Absorption Spectrophotometer following the method as suggested by Lindsay and Norvell (1978). 0.5 g grounded (powdery) plant / crop samples were digested with 15 ml Di-acid mixture on a hot plate for 48 hours. After the digestion, temperature was brought down to room temperature and then filtered. From filtrate, toxic metal (Pb, Ni and Cd) concentrations were determined by using AAS following standard procedure. All the collected soil samples were air dried, processed for analysis. All the chemical analysis was done through standard methods. Soil chemical analysis was done and afterwards identified contaminated and non-contaminated soils samples on the basis of maximum permissible limits of heavy metal concentration as given by WHO (1996). The maximum allowable limits of heavy metals in plants have been established by standard regulatory bodies such as World Health Organization (WHO), FAO and Indian Standard as Pb- 2.5 ppm, Ni- 1.5 ppm and Cd- 1.5 ppm.

### **Plant sampling and their analytical procedure**

Plant samples were collected from various places of contaminated areas of Pungam, Sajod, Sakarpor, Kosamadi and Umervada of Bharuch district. The numbers of samples were collected as per availability of their individual location. For the plant samples collection, different leafy vegetables (Fenugreek, Spinach, Amaranthus, Coriander and Dill leaves) were collected from above areas. Plants collected at each site were carefully and repeatedly hand-washed and rinsed in deionized water to eliminate the dust that had accumulated on the aerial parts. Vegetable were weighed after washing with de-ionized water and blotting dry with tissue paper. Plant parts were then oven-dried at 60°C for 72 hours weighed and ground. The samples were dried and powdered in a pestle and mortar prior to analysis. The micronutrient (Fe and Zn) and toxic heavy metal content (Ni, Pb and Cd) was determined only from the fresh vegetables (un-stored and un-cooled) after wet digestion of the powdered samples. These samples were analyzed by an atomic absorption spectrophotometer. Vegetables observed in this experiment were root, stem and leaf. Analysis of different chemical parameters was followed by standard operating procedure. The mean



**Fig. 1 :** Sampling locations of the study area.

concentrations of heavy metals in various vegetable species collected from the study area were compared with the standard set for vegetables. In this experiment, different leafy vegetables collected and analyzed heavy metal accumulation in root, stem and leaf of Fenugreek (*Trigonella foenum-graecum* L.), Spinach (*Spinacia oleracea* L.), Amaranthus (*Amaranthus tricolor*), Coriander (*Coriandrum sativum* L.) and Dill leaves (*Anethum graveolens*). Crop differs in their ability to absorb and accumulate heavy metals in different plant parts. The susceptible crops exhibits phytotoxicity early where as tolerant crops can accumulate and pass on to the food chain. Hence, the heavy metal contents in plants require monitoring and application rate should be restricted accordingly. The maximum allowable limits of heavy metals in plants have been established by standard regulatory bodies such as World Health Organization (WHO), FAO and Indian Standard as Pb- 2.5 ppm, Ni-1.5 ppm and Cd- 1.5 ppm. The concentrations of heavy metals in all of the vegetable samples analyzed were higher than the Indian Standard values.

## **Results and Discussion**

**Season and experiment No. 1 (Survey) :** Initial status of soil samples collected from industrial belt of Bharuch district are presented in Table 1. The result revealed that status of available Cd in contaminated soils area *i.e.* A, B, C, D, E, F, G, H, I and J, showed 'high to very high' and varied widely from 18.91 to 39.60, 31.26 to 84.20, 16.90 to 24.02, 12.90 to 33.52, 10.52 to 29.68, 24.60 to 34.68, 20.73 to 29.20, 22.70 to 50.80, 20.60 to  $23.00$  and  $18.90$  to  $29.30$  mg  $kg<sup>-1</sup>$ , respectively, with corresponding mean values of 26.54, 50.55, 19.07, 17.24, 18.75, 27.75, 23.47, 30.26, 21.41 and 23.92 mg kg<sup>-1</sup>, respectively. However, with respect to mean Cd content, soil samples from contaminated soils of various locations can be arranged as:  $B < H < F < A < J < G < I < E < C$  $\langle$  D. The result revealed (Table 1) that Cd (mg kg<sup>-1</sup>) was found in all the samples (50 nos.) of was 10.52 to 84.20

mg kg<sup>-1</sup> (with mean value 28.24 mg kg<sup>-1</sup>). However, out of 50 soil samples collected from different contaminated areas, all the samples exhibited Cd content above the maximum permissible limit  $(3.0 \text{ mg kg}^{-1})$ . Now, coming to non-contaminated soils (Table 1) areas of College Farm, NARP and RCRS available Cd varied widely from 0.72 to 2.18, 0.25 to 0.64 and 0.19 to 0.63 mg  $kg^{-1}$ , respectively, with corresponding mean values of 1.31, 0.40 and 0.36 mg  $kg<sup>-1</sup>$ , respectively. However, out of 15 soil samples collected from different non- contaminated areas, all the samples exhibited Cd content below the maximum permissible limit  $(3.0 \text{ mg kg}^{-1})$ . The heavy metals concentrations were lower than the values reported for typical non-contaminated soils (Bowen, 1966). The maximum observed Cd concentration was higher than the Indian Standard. The result of this study was supported with Misra and Mani (2008) reported that the fertilizers contain certain amount of heavy metal as well as such as lead and cadmium, which may ultimately pollute the soil. The comparison of the data from the present study with earlier findings of Singh *et al*. (2004) at Dinapur, Varanasi suggested that the range of concentration of Cd in soil was higher. The maximum observed Cd concentration was higher than the Indian Standard.While, in case of Pb status (Table 1) ranged from 'low to high' showing 98.88 to 143.90, 91.02 to 184.80, 79.32 to 94.76, 75.12 to 152.26, 81.12 to 123.32, 73.52 to 157.12, 77.52 to 110.00, 81.12 to 95.86, 89.46 to 95.12 and 89.32 to103.64 mg kg-1, respectively in A, B, C, D, E, F, G, H, I and J, respectively from Amla khadi, Pungam, Piraman, Sajod, Bhadkodra, Sakarpor, Kosamadi, Umervada, Ankleshwar and Sanjali, with corresponding mean values of 117.98, 133.83, 85.89, 98.59, 99.70, 102.75, 84.23, 85.98, 92.23 and 95.01 mg kg<sup>-1</sup>, respectively. However, the soil samples arranged in ascending order of mean Pb  $(mg kg<sup>-1</sup>)$  content were as: B  $(133.83) < A (117.98) < F (102.75) < E (99.70) < D$  $(98.59) < J (95.01) < I (92.23) < H (85.98) < C$  $(85.89)$  < G  $(84.23)$ . However, out of 50 soils samples collected from different contaminated areas, 21 (twentyone) samples exhibited Pb content above the maximum permissible limit (100 mg  $kg^{-1}$ ). The reason for the high lead concentration may be due to traffic burden and industries as reported by Mishra and Tripathi (2008).Now, coming to non-contaminated soils (Table 1) areas, all the samples exhibited Pb content below the maximum permissible limit (100 mg kg-1). Available Ni content in Amla khadi, Pungam, Piraman, Sajod, Bhadkodra, Sakarpor, Kosamadi, Umervada, Ankleshwar and Sanjali, was 'high to very high', depicting wide range from 72.36 to 75.66, 68.02 to 78.29, 72.74 to 150.68, 68.03 to





**B:** Below and **A:** Above, **MPL**= maximum permissible limit \* denote as based on as per WHO (1996).

75.80,71.78 to 77.18, 65.88 to 81.98, 80.03 to 107.98, 76.32 to 149.38, 75.67 to 88.38, 82.02 to 96.58 mg kg-1 , respectively with corresponding mean values of 73.76, 72.86, 87.90, 71.79, 74.03, 71.48, 95.00, 100.79, 81.31 and 88.89 mg kg<sup>-1</sup>, respectively. However, the soil samples arranged in ascending order of mean Ni content (mg kg-

<sup>1</sup>) were as: I  $(100.79) < H (95.00) < J (88.89) < C$  $(87.90) < I$   $(81.31) < E$   $(74.03) < A$   $(73.76) < B$  $(72.86)$  < D $(71.79)$  < F $(71.48)$ . However, out of 50 soils samples collected from different contaminated areas, 24 (twenty-four) samples exhibited Ni content above the maximum permissible limit  $(80 \text{ mg kg}^{-1})$ . The lowest

 $(73.52 \text{ mg kg}^{-1})$  and the highest  $(184.80 \text{ mg kg}^{-1})$  value of Ni content were recorded in F (Sakarpor) and B (Pungam) village, respectively. The total Ni content in the lithosphere is around 80 mg  $kg<sup>-1</sup>$  (Baker, 1990) and average Ni content in the surface soil ranged from 5.00 to 700 ppm with an average value of 19 ppm as reported by Shacklette and Boerngen, (1984). Whereas, the average value of Ni in the world soils as reported by Vinogradov (1959) was 40 mg  $kg<sup>-1</sup>$  and the range is from 5.0 to 500 mg kg-1 (Adriano *et al*., 1980). The degree of metals was found in cultivated soils of agriculture land of the industrial belt in the present investigation exceed the permissible levels reported by different scientist like Kabata - Pendias and Pendias (1992) and Temmerman *et al*. (1984). This variation of result might probably be due to the variations of heavy metals concentration in irrigation water and other agronomic practices of the respective area. Kayastha (2014) reported the irregular pattern showed on concentration of heavy metals in soils. Ruqia Nazir *et al.* (2015) was reported that in all soil samples content of Cd, Pb and Ni heavy metals above the permissible limits set by WHO.

**Season and experiment No. 2 (Pot experiment) :** In the first year of soils survey, soil chemical analysis was done and afterwards identified contaminated soils samples on the basis of maximum permissible limits of heavy metal concentration as given by WHO (1996) and later on, those soils were found contaminated/polluted, so soil samples were taken and used for the pot experiment in the second year of experimentation under protected cultivation (green house).

In respect of Cd, Pb and Ni accumulation, all plant parts of leafy vegetables recorded significantly higher nutrient accumulation in contaminated soil as compared with non-contaminated soil (Table 2). In case of individual effects of different leafy vegetable crops grown, significantly highest Cd accumulation of leaves (4.48 ppm) and root (0.93 ppm) were recorded in Spinach (V2) and Coriander (V4) crops, respectively. The significantly higher Cd accumulation of stem was (2.46 ppm) in spinach (V2) vegetables and it was statistically at par with V2: Spinach (2.39 ppm) crop. The interaction effect (Table 2) of different leafy vegetable crops and soil type impart significant influence on Cd accumulation. Interaction effect between soil type and vegetables type were found significantly superior and highest Cd content of leaves  $(8.81$  ppm) and root  $(1.78$ ppm) found in S1V3 (contaminated soil + Amaranthus) and S1V4 (Contaminated soil + Coriander), respectively. However, in case of root, significantly higher Cd accumulation (4.55 ppm) was found under S1V2

(contaminated soils + Spinach) and it was statistically at par with leafy vegetables of Dill leaves grown under contaminated soils (4.51 ppm) (Table 2).

Significantly higher Pb accumulation was recorded in contaminated soil as compared with non-contaminated soil. In respect of individual effect of different leafy vegetable crops grown, significantly higher Pb accumulation of leaves (4.77 ppm) and root (1.10 ppm) were recorded in Spinach (V2) and Dill leaves (V5), respectively and it was statistically at par with Coriander (4.58 ppm) and Spinach (1.08 ppm) in above chronologically order. The significantly highest Pb accumulation (1.57 ppm) in the stem was found in Amaranthus (V3). The interaction effect of different leafy vegetable crops and soil type (Table 2) on Pb accumulation of leaves, stem and root was found significantly and value of Pb in leaves and stem recorded highest in combination of S1V3 (contaminated soils + Amaranthus) and S1V3 (contaminated soils + Amaranthus), respectively with corresponding value of 9.42 ppm and 3.08 ppm, respectively. However, in case of root (Table 2), interaction effect of different leafy vegetable crops and soil type impact significantly and higher Pb accumulation (2.11 ppm) was found under S1V5 (contaminated soils + Dill leaves) and it was statistically at par with Spinach crop grown under contaminated soils (2.04 ppm).

Significantly higher Ni accumulation was recorded in contaminated soil as compared with non-contaminated soil. In case of individual effect of different leafy vegetable crops, significantly highest Ni accumulation of leaves  $(3.24$  ppm), stem  $(0.46$  ppm) and root  $(0.50$  ppm) recorded in Fenugreek (V1), Amaranthus (V3) and Coriander (V4) crops, respectively. The interaction effect of different leafy vegetable crops and soil type (Table 2) on Ni accumulation of leaves, stem and root were found significantly superior and accumulation value of leaves, stem and root were highest in combination of S1V1 (6.23 ppm), S1V3 (0.89 ppm) and S1V4 (0.96 ppm), respectively. For comparing of heavy metals accumulation of different parts like leaf, stem and root, the higher concentration was found in leaf as compared to stem and root. Barman and Lal (1994) reported the higher accumulation of heavy metals of Pb and Cd in edible parts than in non-edible plant parts. Singh *et al*. (2012) suggested that the diverse vegetable crop species also showed marked differences in respect of metal uptake and their distribution to various plant parts especially to the edible part, which could be emphasized for selection of vegetable crops for cultivation on metals contaminated soils depending on their metal uptake

**Table 2 :** Effect of soil type and leafy vegetables crop on accumulation of Cd, Pb and Ni by leaf, stem and root of different leafy vegetable crop.

<b>Treatments</b>	$Cd$ (ppm)			Pb(ppm)			Ni(ppm)		
	Leaf	<b>Stem</b>	<b>Root</b>	Leaf	<b>Stem</b>	<b>Root</b>	Leaf	<b>Stem</b>	<b>Root</b>
Soil type (S)									
S1: Contaminated	7.25	3.47	0.74	8.00	2.24	1.64	3.78	0.62	0.61
S2: Non-Contaminated	0.16	0.33	0.17	0.19	0.12	0.10	0.22	0.05	0.08
$SEm \pm$	0.07	0.04	0.01	0.06	0.02	0.02	0.03	0.01	0.01
CD at $5%$	0.20	0.12	0.03	0.19	0.07	0.05	0.08	0.02	0.02
<b>Vegetables (V)</b>									
V1: Fenugreek	3.69	1.67	0.39	3.62	0.98	0.97	3.24	0.21	0.21
V2: Spinach	3.61	2.39	0.31	4.27	1.13	1.08	2.60	0.36	0.24
V3: Amaranthus	4.48	1.56	0.45	4.77	1.57	0.56	1.37	0.46	0.39
V4: Coriander	2.91	1.42	0.93	4.58	1.21	0.66	1.68	0.29	0.50
V5: Dill leaves	3.85	2.46	0.17	3.23	1.01	1.10	1.11	0.34	0.39
$SEm \pm$	0.11	0.06	0.01	0.10	0.04	0.03	0.04	0.01	0.01
$CD$ at $5\%$	0.31	0.18	0.04	0.30	0.10	0.08	0.12	0.03	0.03
S×V interaction effect									
$SEm \pm$	0.15	0.09	0.02	0.14	0.05	0.04	0.06	0.01	0.01
CD at $5\%$	0.44	0.26	0.05	0.42	0.15	0.11	0.17	0.04	0.04
CV <sub>0</sub>	6.96	7.99	6.51	6.06	7.31	7.41	5.11	6.60	7.08

**Table 3:** Effect of soil type and leafy vegetables crop on quality assessment of different leafy vegetable crop.



potential and their transportation/distribution to edible part.

An assessment of data (Table 3) of quality parameters of edible portions of different leafy vegetables revealed that significant influence on quality parameters *viz.* total soluble protein, total phenol, Proline, Ascorbic acid and total carbohydrates of leafy vegetables crops grown on non-contaminated soils were recorded higher as compared with contaminated soils.

An examination of data (Table 3) effect of individual effect of different leafy vegetables explained that

significantly highest total soluble protein (12.29 mg/ 100 g), total phenol (22.95 mg/100 g), total carbohydrates  $(273.21 \text{ mg}/100 \text{ g})$  and Ascorbic acid  $(167.75 \text{ mg}/ 100 \text{ g})$ recorded under Fenugreek (V1), Spinach (V2), Coriander (V4) and Fenugreek (V1) crops, respectively. The interaction effect (Table 3) of different leafy vegetable crops and soil type on quality assessment of leafy vegetables was found significantly superior and highest total soluble protein  $(13.50 \,\text{mg}/ 100 \,\text{g})$ , total phenol  $(41.38$ mg/ 100 g) total carbohydrates  $(321.47 \text{ mg}/ 100 \text{ g})$  and Ascorbic acid (224.60 mg/ 100 g) were recorded in combination of S2V1 (non-contaminated soils + Fenugreek), S2V2 (non-contaminated soils + Spinach), S2V4 (non-contaminated soils + Coriander) and S2V1 (non-contaminated soils + Fenugreek), respectively. Inferior quality of all the qualitative parameters was recorded in different leafy vegetables grown under contaminated soils. In case of individual effects of soil type, significantly highest Proline content (6.56 mg/ 100 g) was recorded in contaminated soil as compared with non-contaminated soil. However, the effect of different leafy vegetables was found significantly and recorded higher (4.98 mg/ 100 g) in Amaranthus (V3) crop and it was statistically at par with Fenugreek crop (4.74 ppm) Whereas, interaction effect between soil type and crop type was found significantly (Table 3); higher Proline (7.42 mg/ 100 g) recorded in Fenugreek crop grown under contaminated soil (S1V3) and it was statistically at par with Amaranthus crop grown under contaminated soil (7.28 ppm). However, significantly, lower Proline content (2.68 mg/ 100 g) was recorded in S2V3 and it was statistically at par with S2V4 and S2V2.

#### **Conclusion**

An examination of data expounded that varying leafy vegetable crops and soil type exerted their significant influence on available lead (Pb) and nickel (Ni) content. Significantly, highest available Pb  $(16.80 \text{ mg kg}^{-1})$  and  $Ni (19.17 \text{ mg} \text{ kg}^{-1})$  were recorded in contaminated soil as compared with non-contaminated soil. The qualitative analysis showed higher amounts of Total soluble protein, Total phenol, Total carbohydrates and Ascorbic acid in the edible portion of leafy vegetables grown in noncontaminated soils conditions than contaminated soils while, due to stress conditions of different heavy metal content, Proline concentration was found in maximum on leafy vegetables grown in contaminated soils. Heavy metals accumulation of all leafy vegetables with contaminated soils caused a significant increase in all elements like Cd, Pb and Ni except significant increase was noticed for Fe and Zn. Accumulation trend of metals in all vegetables: Cadmium > Lead > Nickel. Spinach

and Fenugreek indicated the highest metal absorption capacity compared to other vegetables studied.

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