

# **Heavy metals and pathogens in green leafy vegetables**

**Submitted as a major project in partial fulfillment of the requirements for the award of the degree of**

**MASTER OF SCIENCE  
IN  
BIOTECHNOLOGY**

**By**

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## Candidate's Declaration

I, hereby declare that the work presented in the dissertation entitled “Heavy metals and pathogens in green leafy vegetables” in partial fulfillment of the requirement for the award of the degree of Masters in Biotechnology, Department of Biotechnology and Environmental Sciences, Thapar Institute of Engineering and Technology, Patiala, is an authentic record of my own work during the period of five months from January 2006 to May 2006, under the supervision of Dr. Dinesh Goyal, Associate Professor, Department of Biotechnology & Environmental Sciences, Thapar Institute of Engineering and Technology. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Place: Patiala

Date: 19-06-06

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This is to certify that the above statement made by the candidate is correct and true to the best of our knowledge.

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## **Certificate**

This is to certify that the thesis entitled “Heavy metals and pathogens in green leafy vegetables” submitted by Harmanjit Kaur in partial fulfillment of the requirements for the award of Degree of Masters of Science in Biotechnology to Thapar Institute of Engineering and Technology (Deemed University), Patiala, is a record of student’s own work carried out by her under my supervision and guidance. The report has not been submitted for the award of any other degree or certificate in this or any other University or Institute.

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## ABSTRACT

Presence of heavy metal and bacterial pathogen in randomly collected samples of green leafy vegetables from agricultural field in and around Rajpura and Patiala city was detected. In palak (*Beta vulgaris* L), Coriander (*Coriandrum sativum*) and yellow sarsoo (*Brassica campestris*) heavy metals Fe, Zn, Cd, Cr and Pb were analyzed by tri-acid digestion method and total bacteria and coliforms were enumerated on TSA (Tryptone Soya Agar) and VRBA (Violet Red Bile Agar) media respectively. The presence of heavy metals was in the order of Fe>Zn>Pb>Cu>Cd.

Nearly 16% of samples of palak were showing higher levels of Cd than the permissible limit of 1.5 mg/kg as per Indian Standard of Food Adulteration Act (PFA), 1954. All the samples including palak, coriander and bathua (grown in different plots) and samples of palak, cabbage and yellow sarsoo purchased from the market had Cd within the safe limits. Among all these green leafy vegetables nearly all these were high in Zn, Pb and Fe having crossed the permissible limits of PFA. The safe limits recommended for Pb is 2.5 mg/kg whereas for Zn is 2.5 mg/kg and none of the vegetable samples were in this range. The metal content in vegetables from agricultural areas indicates high levels of soil contamination and there is a potential danger of heavy metal accumulation particularly Pb and Cd in vegetables grown in vicinity of Village Bhappal, Kami-kalan and Bahadurgarh. The result indicates that the consumers are purchasing vegetables with high level of heavy metals (HMs).

The total bacterial count in randomly collected samples of palak ranged from  $45 \pm 17.3$  cfu/g to  $2.68 \pm 0.91$  Log cfu/g, in cabbage from  $12.7 \pm 1.2$  cfu/g to  $1.05 \pm 0.3$  Log cfu/g whereas in case of brassica was  $26.8 \pm 6.4$  cfu/g to  $1.2 \pm 0.08$  Log cfu/g which is an indication of improper pre-harvest and post harvest handling.

## 1. INTRODUCTION

Metal-contaminated soils are potentially harmful to plants, animal and humans. Harmful effects are often related to the free-metal concentration in the soil solution. Immobilization is a useful method to improve the quality of metal-contaminated soils by transforming free-metal ions into species that are less mobile and less toxic. The effect of many immobilizing products can be attributed to sorption on the surface of the material (Oste *et al.*, 2000).

The use of sewage sludge as farm fertilizers, encouraged in recent years by changes in U.S. Environmental Protection Agency (U.S. EPA) policy, has raised concerns among some scientists regarding food safety and long-term soil productivity. The U.S. EPA risk assessment for entry into human diet of three of the most toxic metals, cadmium (Cd), mercury (Hg) and lead (Pb) utilized uptake coefficients (UCs), to calculate the amount of each metal that could enter food crops from the soil. UC was calculated as the increment of metal concentration in the edible part of the crop per unit increase of metal loading to the soil. UC values used by the U.S. EPA underestimated the actual risk posed by uptake into crops.

Both general and specific investigations of soil and crop heavy metal contamination have been carried out. The results indicate that levels of Cd, Hg and Pb in soil and some in crops were greater than the Standards laid down by Chinese government. Among different species tuberous vegetables seemed to accumulate a larger portion of heavy metals than leafy and fruit vegetables, except celery. Furthermore the endosperm of both wheat and rice crops had the highest portion of Cd and Cr. Rice endosperm and wheat chaff accumulated the highest Pb, although the concentrations of all three metals were variable in different parts of the grains. For example, 8.3, 6.9, 1.4 and 0.6 mg kg<sup>-1</sup> of Pb were found in chaff, cortex, embryo and endosperm of wheat compared with 0.11, 0.65, 0.71 and 0.19 mg kg<sup>-1</sup> in the same parts of rice, respectively.

Untreated sewage water used for irrigation was the major cause of increasing soil and crop metals. Short periods of the sewage water irrigation increased individual metals in soils by 2 to 80 % and increased metals in crops by 14 to 209 % (Murray *et al.* 1998). Atmospheric deposition, industrial or municipal wastes, sewage sludge improperly used as fertilizers and metal-containing phosphate fertilizers played an important role including some specific areas in high metal content in crop plants.

An assessment of metal exposure to population subgroups living on and growing food on urban sites was analyzed and uptake of cadmium, copper, nickel, lead and zinc for a selection of commonly grown allotment and garden vegetables was observed. Predicted concentration of metals in the vegetables were used to assess the risk of exposure to human populations from homegrown food sources. In the present investigation it was observed that all the metals except Cd had exceeded the permissible limit designed by PFA. The heavy metals and pathogen content in some random samples of green leafy vegetables were carried out to assess level of contamination and associated potential risks.

## 2. REVIEW OF LITERATURE

Several studies have indicated that vegetables, particularly leafy crops grown in heavy metals contaminated soils have higher concentrations of heavy metals than those grown in uncontaminated soils (Guttormsen *et al.*, 1995; Dowdy and Larson, 1995). A major part of soil contamination is through atmospheric deposition of heavy metals from sources such as: metaliferous mining, smelting and industrial activities. Other non-point sources include inputs such as fertilizers, pesticides, sewage sludge, organic manures and composts (Singh, 2001).

Prolonged exposure to heavy metals such as cadmium, copper, lead, nickel and zinc can cause deleterious health effects in humans (Reilly, 1991). Metal contamination of garden soils may be widespread in urban areas due to past industrial activity and that of fossil fuels (Chronopoulos *et al.*, 1997; Sanchez-Camazano *et al.*, 1994; Sterrettet *et al.*, 1996). Heavy metals may enter the body through inhalation of dust, direct ingestion of soil and consumption of food plants grown in metal-contaminated soil (Cambra *et al.*, 1999; Dudka and Miller, 1999; Hawley, 1985). Potentially toxic metals are also present in commercially produced foodstuffs [Department of Environment, Food and Rural Affairs (DEFRA), 1999]. Exposure to potentially toxic metals from dust inhalation or soil ingestion is usually modeled simply as the concentration of a contaminant measured in the soil multiplied by the quantity of dust inhaled or soil digested (Konz *et al.*, 1989).

Under Part II of Environment Protection Act 1990, the UK government favors a “suitable for use” approach to redevelopment (DETR). Land is contaminated only if the current or intended use of a site has the potential to cause an unacceptable health risk to human occupants or the environment. Under the U.K. Town and Country Planning Act 1990 (DETR, 2000), this approach requires that land be assessed for redevelopment on a site-specific basis. At present, concentration of metals in the soil is compared to metal-

specific “trigger values”. In the past these trigger values were based on total contaminant concentration in the soil. More recently, the introduction of Contaminated Land Exposure Assessment in April 2002 has replaced these trigger values with generic soil guidance values. These are considered as an improvement on the previous values and for Cd at least categories are employed where food plants are to be grown. Where a soil exceeds these values, it is recommended that a risk assessment or remediation be performed.

Potential risk to population subgroups living on and consuming vegetables grown on large urban sites were assessed and was considered that young children are highly exposed (Ryan and Chaney, 1995). The potential cancer risk from Cd and Ni are addressed as a result of occupational inhalation exposure (Goyer and Clarkson, 2000). There have been a number of studies, which have investigated atmospheric deposition of heavy metals in soil and or in vegetables growing in the vicinity of industrial areas (Gzyl 1995, Voutsas *et al.*, 1996). These studies indicate high concentrations of heavy metals in vegetables grown in vicinity of industries and identify leafy vegetables at greatest risk of accumulating elevated concentrations.

The content of toxic substances such as heavy metals has been increased in natural waters and agricultural soils, in particular in industrialized countries. When agricultural soils become polluted with heavy metals, food crops may accumulate too high amounts of the metals and thus may not be safe as households and agriculture itself, which causes pollution. Municipal sludge is a valuable organic manure and soil conditioner and has been used as a fertilizer over decades (Towers and Paterson, 1997), but at the same time sludge may contain heavy metals and organic pollutants, which are harmful to crops and microorganisms in soil. Contaminated crops also reduce quality of the feed and food. (McLaughlin *et al.*, 1999; Giller *et al.*, 1998; Das *et al.*, 1997; McBride 1995).

### **Sources of Heavy metal contamination**

Heavy metal depositions are associated with a wide range of sources such as small-scale industries (including battery production, metal products, metal smelting and cable-coating industries), brick kilns, vehicular emissions, re-suspended road dust and diesel

generator sets. Coal combustion is also an important source of contamination, because Indian coal is of relatively poor quality and has high heavy metal contents. Additional potential sources of heavy metals in field location in urban and peri-urban areas include irrigation water contaminated by sewage and industrial effluents leading to contaminated soils and vegetables. Other sources include unsafe or excess application of pesticides, fungicides and fertilizers such as sewage sludge (Krishna Murti, 1989).

## **2.1 Some major disasters caused by heavy metals in the past**

### **2000, The Baia Borsa Accident**

The pollution of the second huge mine accident occurred in Hungary at the surviving part of Tisza river. In the border zone the dissolved zinc content of the river grow to 230  $\mu\text{g/l}$  and the lead was 130  $\mu\text{g/l}$ . The length of the pollution was approximately 10 kilometers. The amount of pollutants attached to suspended particles was much higher than the amount of dissolved heavy metals and were well above the threshold value for very polluted water (e.g. 2900  $\mu\text{g/l}$  lead). The pollution contains aluminum and copper along with zinc and lead. The severe flood worsens the situation. The high level of pollution exists despite the very high water volume in the river system (1200m<sup>3</sup>/sec.) which indicates its high magnitude (UNEP experts).

### **1932, Minamata**

Chisso's chemicals works into Minamata Bay in Japan released sewage containing mercury. The mercury accumulated in sea creatures, eventually leading to mercury poisoning (Lenntech, 1998).

### **1952, Minamata Syndrome**

The first incidents of mercury poisoning appear in the population of Minamata Bay in Japan, caused by consumption of fish polluted with mercury, causing over 500 fatalities. Since then, Japan has had the strictest environmental laws in the industrialized world.

**1986, Sandoz**

Water used to extinguish a major fire carried 30 t fungicides containing mercury into the Upper Rhine and killed over a stretch of 100 km (Pollution of the Rhine at Basel, 1986).

**1998, Spanish nature reserve**

Spanish nature reserve contaminated after environmental disaster, toxic chemicals were released in water from a burst dam belonging to a mine contaminate the Coto de Donana nature reserve in southern Spain. Sulphur, lead, copper; zinc and cadmium flow down the Rio Guadimar. Experts estimate that Europe's largest bird sanctuary, as well as Spain's agriculture and fisheries, will suffer permanent damage from the pollution (Lenntech, 1998).

**2006, Heavy metals in fish species**

More than 40 per cent of fish species in East China's province were found to be contaminated with heavy metals, according to a survey released by Jiangsu Environment Monitoring Center Cadmium, lead, mercury, chromium and zinc were the five kinds of heavy metals found in 41 per cent of species in the sample survey (Lenntech, 1998).

**2003, Coca Cola contaminated with heavy metals**

Kandhari Beverage: has hazardous quantities of heavy metals in the samples taken from the plant and tested in the Board's Central Testing Laboratory at Parwanoo. Kandhari Beverages, the authorized bottlers of soft drink Coca Cola at Baddi in this district does not have sludge storage licence required under the HP Environment Protection Act 1986 (The Tribune, 2003).

**2.2 Environmental and health risks****(A) Cadmium**

Cadmium is produced as an inevitable by-product of zinc (or occasionally lead) refining, since these metals occur naturally within the raw ore. However, once collected the cadmium is relatively easy to recycle. The most significant use of cadmium is in nickel/cadmium batteries, as rechargeable or secondary power sources exhibiting high

output, long life, low maintenance and high tolerance to physical and electrical stress. Cadmium coatings provide good corrosion resistance, particularly in high stress environments such as marine and aerospace applications where high safety or reliability is required; the coating is preferentially corroded if damaged. Other uses of cadmium are pigments, stabilizers for PVC, in alloys and electronic compounds. Cadmium is also present as an impurity in several products, including phosphate fertilizers, detergents and refined petroleum products.

In the general non-smoking population the major exposure pathway is through food, via the addition of cadmium to agricultural soil from various sources (atmospheric deposition and fertilizer application) and uptake by food and fodder crops. Additional exposure to humans arises through cadmium in ambient air and drinking water.

In humans, long-term exposure is associated with renal dysfunction. High exposure can lead to obstructive lung disease and has been linked to lung cancer, although data concerning the latter are difficult to interpret due to compounding factors. Cadmium may also produce bone defects (osteomalacia, osteoporosis) in humans and animals. In addition, the metal can be linked to increased blood pressure and effects on the myocardium in animals, although most human data do not support these findings.

### **(B) Chromium**

Chromium is used in metal alloys and pigments for paints, cement, paper, rubber and other materials. Low-level exposure can irritate the skin and cause ulceration. Long-term exposure can cause kidney and liver damage and damage to circulatory and nerve tissue. Chromium often accumulates in aquatic life, adding to the danger of eating fish that may have been exposed to high levels of chromium.

### **(C) Copper**

Copper is an essential substance to human life, but in high doses it can cause anemia, liver, kidney damage, stomach and intestinal irritation. People with Wilson's disease are at greater risk for health effects from overexposure to copper. Copper normally occurs in drinking water from copper pipes, as well as from additives designed to control algal growth.

#### **(D) Lead**

Lead in the environment arises from both natural and anthropogenic sources. Exposure can occur through drinking water, food, air, soil and dust from old paint containing lead. In the general non-smoking, adult population the major exposure pathway is from food and water. Food, air, water and dust/soil are the major potential exposure pathways for infants and young children. For infants up to 4 or 5 months of age, air, milk formulae and water are the significant sources.

Lead is among the most recycled non-ferrous metals and its secondary production has therefore grown steadily in spite of declining lead prices. Its physical and chemical properties are applied in the manufacturing, construction and chemical industries. It is easily shaped and is malleable and ductile.

In humans exposure to lead can result in a wide range of biological effects depending on the level and duration of exposure (Lenntech, 1998).

Various effects occur over a broad range of doses, with the developing fetus and infant being more sensitive than the adult. High levels of exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of hemoglobin, effects on the kidneys, gastrointestinal tract, joints and reproductive system and acute or chronic damage to the nervous system. Lead can have small, subtle, sub clinical effects, particularly on neuropsychological developments in children.

#### **(E)Mercury**

Mercury is a global pollutant with complex and unusual chemical and physical properties. The major natural source of mercury is the degassing of the Earth's crust, emissions from volcanoes and evaporation from natural bodies of water.

Worldwide mining of the metal leads to indirect discharges into the atmosphere. The usage of mercury is widespread in industrial processes and in various products (e.g. batteries, lamps and thermometers). It is also widely used in dentistry as an amalgam for fillings and by the pharmaceutical industry. Concern over mercury in the environment arises from the extremely toxic forms in which mercury can occur.

The main sources of mercury emissions in the UK are from the manufacture of chlorine in mercury cells, non-ferrous metal production, coal combustion and crematoria. UK emissions of mercury are uncertain and it is estimated that the range is from 13 to 36 tonnes per year. Emissions are estimated to have declined by around ¾'s between 1970-1998, mainly due to improved controls on mercury cells and their replacement, and the fall in coal use.

Whilst there has been a decline in the level of European emissions of mercury, emissions from outside of Europe have started to increase – increasing the level of ambient concentrations in the continent.

Mercury is a toxic substance, which has no known function in human biochemistry or physiology and does not occur naturally in living organisms. Inorganic mercury poisoning is associated with tremors, gingivitis and/or minor psychological changes, together with spontaneous abortion and congenital malformation.

Monomethylmercury causes damage to the brain and the central nervous system, while foetal and postnatal exposure have given rise to abortion, congenital malformation and development changes in young children.

#### **(F) Nickel**

Small amounts of Nickel are needed by the human body to produce red blood cells, however, in excessive amounts can become mildly toxic. Short-term overexposure to nickel is not known to cause any health problems, but long-term exposure can cause decrease in the body weight, heart and liver damage and skin irritation.

The EPA does not currently regulate nickel levels in drinking water. Nickel can accumulate in aquatic life, but its presence is not magnified along food chains.

#### **(G) Antimony**

Antimony is a metal used in the compound antimony trioxide, a flame retardant. It can also be found in batteries, pigments and ceramics and glass. Exposure to high levels of antimony for short periods of time causes nausea, vomiting and diarrhea. There is little

information on the effects of long-term antimony exposure, but it is a suspected human carcinogen. Most antimony compounds do not bio-accumulated in aquatic life (Lenntech, 1998)

**Table 2.3 Characteristics of some microbial pathogens linked to outbreaks of produce-associated illness.**

<b>Microorganism</b>	<b>Incubation Period</b>	<b>Symptoms</b>	<b>Infectious Dose</b>	<b>Sources</b>
<i>Clostridium botulinum</i>	12-36 hr	Nausea, vomiting, fatigue, dizziness, dryness of mouth and throat, muscle paralysis, difficulty swallowing, double or blurred vision and breathing difficulty	Intoxication growth and toxin production in food.	Soil, lakes, Streams, decaying vegetation.
<i>Listeria monocytogenes</i>	1 day to 5 or more week	Febrile gastroenteritis in healthy adults; may lead to spontaneous abortion or stillbirth in pregnant women.	Unknown dependent upon health of individual	Soil, food-processing environment.
<i>Salmonella spp.</i>	18-72 hr	Abdominal pain, Diarrhea, chills, Fever, nausea, vomiting.	10-100,000	Animal and human feces, cross contamination from raw meat, poultry or eggs

Source: U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001

## **2.4 Survival and multiplication of pathogens on raw produce**

The survival and growth of pathogens on fresh produce is governed by the type of the organism and the produce and environmental conditions in the field and during post-harvest. In general pathogens only survive and do not grow on the uninjured outer surface of fresh fruits or vegetables, mainly afforded by the plant's natural barriers like, cell wall and wax layer. In some cases the pathogen incidence even declines on the outer surface. In the field the physical environment of leaf surface is inhospitable for the growth and survival of bacteria because of lack of nutrients and moisture, temperature and humidity fluctuations and ultraviolet light exposure (Dickinson, 1986). Change in environmental conditions, however can greatly influence bacterial population. Presence of free moisture on the leaves from precipitation, dew and irrigation may promote their survival and multiplication (Blakeman, 1981; Andrews, 1992; Beattie and Lindow, 1999). Certain conditions such as light particularly the shorter wavelengths, can damage the bacterial cells (Webb, 1976; Jagger, 1981; Sundin *et al.*, 1996; Sundin and Jacobs, 1999). Frequent exposure to fast fluctuating environmental conditions, sometimes induces stress compatibility and results in the development of hardy pathogens. The results suggest that human pathogens are less likely to develop stress resistance (O'Brien and Lindow, 1988).

### **Sources of pathogenic microorganisms and conditions that influence their survival and growth on fresh produce.**

#### **Pre-harvest**

Soil, irrigation water, green or incompletely composted manure, air, wild and domestic animals, human handling, plant protective measures involving pesticides.

#### **Post-harvest**

Cross contamination occurs during storage, preparation and display, dirty harvesting equipment, mixing of wash and rinse water, unkept conditions and unhygienic handling during sorting, packing, cutting for further-processing, transport conditions, improper storage and display (U.S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001).

### **3. Materials and Method**

#### **3.1 Collection of leafy vegetable samples**

**Market:** Vegetable samples were collected from places in and around Rajpura and Patiala. The samples collected include Spinach (*Beta vulgaris* L), Brassica (*B. campestris*), Cabbage (*B. oleracea*). 45 samples in total were collected to assess the heavy metal contamination of vegetable being purchased during the period from Feb to April 2006 from the markets. Different sampling sites selected were the vegetable markets of Rajpura and Patiala and villages like Kami-Kalan, Banur and Bathonia.

**Field sampling:** Samples were taken from a wide range of agricultural field production sites in Patiala District. The samples collected include Spinach, Coriander and Chenopodium. The most important vegetable production areas were identified on the basis of field surveys. The areas were divided as: Patiala, Rajpura, Villages-Bathonia, Kami-Kalan, Bhappal, Banur, Main village, distillery unit near Main village, Bhadak and Bahadurgarh.

#### **3.2 Determination of heavy metals in leafy vegetables**

Tappis Test method was used for the analysis of heavy metal content.

Vegetable samples were oven dried at 80°C and grounded to fine powder. Samples were digested using tri-acid digestion process to extract the heavy metals and the resulting solutions analyzed for concentration of Lead, Copper, Zinc and Cadmium with the highly sensitive atomic absorption spectrophotometer (*GBC 932 AA Australia*).

### **3.3 Field Experiment**

Palak, Coriander and Chenopodium were grown in micro-size plots to study the uptake potential for different metals Fe, Zn, Pb, Cu, Ni, Cd, and Cr. These plants were grown in alkaline soils and the plots were divided into two halves. One was inoculated with nitrogen fixing and phosphate solubilizing biofertilizer, whereas other was kept as control. The period of plantation varied from February to April.

### **3.4 Analysis of Nursery soil**

Soil from micro-plots was analyzed for physico-chemical parameters: WHC, soil texture, pH, electrical conductivity, available sulphur, phosphorus and organic carbon and heavy metal.

### **Water Holding Capacity**

#### **Procedure:**

1. Soil was air-dried and crushed in a porcelain mortar.
2. It was sieved through mesh-size of 1.5 mm.
3. A filter paper was placed at the bottom of a keen box and weighed.
4. Keen box was filled with soil by continuous tapping.
5. It was placed in petridish containing water up to  $\frac{1}{4}$  depth and was left overnight.
6. Weight of the keen box containing wet soil was taken.
7. Then it was dried in oven at 105°C and weighed again.

#### **Calculations and Result**

Weight of box + filter paper =  $W_1$

Weight of box + dry soil =  $W_2$

Weight of box + soil after moisting it =  $W_3$

Weight of dry soil =  $W_2 - W_1$

Weight of moisture absorbed =  $W_3 - W_2$

Moisture absorbed by filter paper =  $W_4$

Moisture held by soil =  $W_3 - W_2 - W_4$

Water holding capacity of soil =  $(W_3 - W_2 - W_4) \times 100 / (W_2 - W_1)$

### 3.5.2 Soil Texture

#### Procedure

1. 50g dried sieved soil was mixed with 50g sodium oxalate.
2. The mixture was put in a measuring cylinder and water was added to make the final volume 1 lt.
3. Immediately hydrometer was dipped into it.
4. Initial Reading was taken after 40sec and then it was left undisturbed and final reading was taken after 2hrs.

#### Observation

Time	Initial Reading	Correction Factor ( $^{\circ}\text{F}-68$ ) 0.2	Final Reading
40sec	X	CF	X + CF
2hrs	Y	CF	Y + CF

#### Result

**Silt (%)** = IR - FR

**Clay (%)** = FR

**Sand (%) = 100 - (Clay + Silt)**

### **3.5.3 Soil Reaction (pH)**

#### **Procedure**

1. 20g of air-dried sieved soil was taken in a 100 ml beaker and 40ml distilled water was added to it.
2. It was thoroughly stirred for 10 seconds using a glass rod.
3. Further stirred for four to five times during the next 30 seconds.
4. Suspension was allowed to settle for 30 seconds.
5. Electrodes were immersed and final readings were taken.

### **3.5.4 Total Soluble Salts (EC)**

#### **Procedure**

1. 20g of air-dried sieved soil was taken in a 100ml beaker and 40ml distilled water was added to it.
2. It was thoroughly stirred for 10 seconds using a glass rod.
3. Further stirred for four to five times during the next 30 seconds.
4. Suspension was allowed to settle for 30 seconds.

5. EC Meter was calibrated using 0.01M KCl solutions.
6. Electrode was dipped and value was taken.

### **3.5.5 Total Organic Carbon (Walkey and Black, 1934)**

#### **Procedure**

1. 1.0 g of 0.2 mm-sieved soil was taken in a 500 ml conical flask.
2. 10 ml of 1N  $K_2Cr_2O_7$  was added and was swirled for a while.
3. 20 ml of  $H_2SO_4$  was added and the flask was kept for 30 min.
4. Finally into this 200 ml of distilled water was added.
5. 10 ml of phosphoric acid, 0.5 g of NaF and 1 ml of diphenylamine indicator were added and the contents were titrated with Ferrous ammonium sulphate solution till the end point appeared (blue to grassy green). Blank without soil was also run.

#### **Calculation**

Organic carbon % =  $10(B-T) \times 0.003 \times 100 / \text{wt of soil} \times B$

B = Volume in ml of Ferrous ammonium sulphate solution required for blank titration.

T = Volume in ml of Ferrous Ammonium sulphate solution required for soil sample.

### **3.5.6 Available Phosphorus**

Suitable for saline alkaline soils (Olsens *et al.*, 1954)

## Reagents

### 1. Extracting solution

2.22 g of  $\text{NH}_4\text{F}$  and 4.16 ml  $\text{HCl}$  were dissolved in water and final volume was made 2 L.

### 2. Reagent A

12 g ammonium molybdate was dissolved in 250 ml of distilled water.

0.2908 g antimony potassium tartrate was dissolved in 100 ml of distilled water.

To these two solutions 1000ml of 2.5 M  $\text{H}_2\text{SO}_4$  was added and finally mixed thoroughly and made total volume 2000ml with distilled water

### 3. Reagent B

1.056 g ascorbic acid was mixed in 200 ml of reagent A.

### 4. Standard Stock (50mg/L)

0.2195 g  $\text{KH}_2\text{PO}_4$  was dissolved.

### 5. Working standard (1 mg/L)

20 ml of (50 mg/L P) solution was diluted to 1lt

## Procedure

1. 2.5 g of 2 mm air-dried soil was taken in a 100 ml flask and 25 ml of extracting solution was added.
2. Flask was stoppered and immediately shaken for 5 min on shaker.
3. The suspension was filtered through whatman no.42 filter paper.
4. 2 ml of aliquot of filtrate was transferred to 100 ml flask.
5. 20 ml of distilled water was added.
6. 8 ml of reagent B was mixed with 20 ml of distilled water.
7. Blank was prepared using 2 ml extracting solution in a place of soil extract.
8. Standard curve measure 0.2, 5, 10, 20, 25 and 30 ml of standard P solution in a 6 different 50 ml volumetric flask. 2 ml extracting solution was added.
9. Added 8 ml of reagent B and water to bring volume to 50 ml. The P concentration of these solutions will be 0, 0.04, 0.10, 0.20, 0.30 and 0.40 m/L respectively.

11. Absorbance was taken at 882 nm.

### **3.5.7 Available Sulphur (Jackson, 1967)**

#### **Reagents**

##### **Extracting Solution**

1.986 g of Calcium chloride dihydrate ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ) was mixed with distilled water and finally diluted to 1lt.

#### **1. Barium Chloride**

Barium chloride crystals were grinded until they pass through 30-mesh sieve.

#### **2. Gum acacia solution (0.25%)**

0.25g gum acacia was dissolved in distilled water and was diluted to 100ml.

#### **3. Standard Sulphur solution**

0.5434g of the reagent grade Potassium sulphate ( $\text{K}_2\text{SO}_4$ ) was mixed in distilled water and was diluted to 1lt.

#### **Procedure**

1. 10g of air-dried sieved soil were transferred to 150 ml of conical flask.
2. 50 ml of 0.15%  $\text{CaCl}_2$  solution was added and shaken for 30 min on electric shaker.
3. The suspension was filtered through whatman no.42 filter paper.
4. 20 ml of the filtrate was pipetted in 25 ml volumetric flask.
5. 10 ml of 0.15%  $\text{CaCl}_2$  and 1 ml 0.25% of gum acacia were added.
6. Total volume was made within 5-20 min of development of turbidity and absorbance was read at 420 nm.
7. Blank without soil was also run.

#### **Calculations**

Weight of soil taken =10 g

Volume of extract ant added =50 ml

First dilution =5 times  
Volume of aliquot taken =20 ml  
Final volume =25 ml  
Second dilution =1.25 times  
Total dilution =5 X 1.25 =6.25 times  
Absorbance reading =A  
ppm of S from curve =Y  
ppm of S in soil = Y x 6.25 ppm  
Kg of S/ha =ppm x 2.24

### **3.6 Enumeration of bacterial pathogens in green leafy vegetables**

Vegetables weighing 25 g were washed with 50 ml of sterile Butterfield's phosphate buffer. Washing was done by slowly rubbing the vegetable in the buffer solution. 1 ml of the washed solution was taken and finally appropriate dilutions were made and were then enumerated by plating on suitable medium.

- **For Total Bacterial count**

Total bacterial count was done on Tryptone Soya agar medium. 25 g of vegetable samples were added to 50 ml Butter fields Phosphate Buffer and washed in it for about 5 min further serially diluted the same sample in butter field's phosphate Buffer, appropriate dilutions were spread on tryptone soya agar plates in triplicates. All plates were incubated at 37°C for 24-48 hrs. Colonies in each plate were counted, averaged and expressed as cfu/g and converted to log.

- **For Faecal coliforms**

Total faecal coliforms were enumerated on Violet red Bile Agar medium. For enumerating the total coliforms, appropriate dilutions were prepared from samples as above and were spread plated on to Violet Red Bile Agar plates in triplicates. All plates

were incubated at 37°C for 24-48 hrs. Violet colored colonies were counted and results were expressed as cfu/g and converted to log.

### **Butterfield's phosphate buffer**

Stock solution

$\text{KH}_2\text{PO}_4 = 34\text{g/L}$

Distilled water = 500 ml

Adjusted pH = 7.2 with 1n NaOH and made volume to 1L with distilled water and autoclaved for 15 min at 121°C.

### **Tryptone Soya Agar**

Composition (g/L)

Casein enzymatic hydrolysate = 17.0

Papaic digest of soybeans meal = 3.0

NaCl = 5

Dipotassium phosphate = 2.50

Dextrose = 2.50

Agar = 15.0

Distilled water = 1L

Final pH = 7.2

### **Violet Bile Salt Agar**

Composition (g/L)

Peptone = 7

Yeast Extract = 3

NaCl = 5

Glucose = 10

Bile salt = 1.5

Neutral Red = 0.03

Crystal violet = 0.002

Agar = 13

pH 7

The ingredients were suspended in distilled water and the mixture was mixed properly and was left for few minutes. The pH was adjusted to 7.4. The contents were heated in a microwave oven with continuous agitation until agar was melted properly before autoclaving.

## 4. RESULTS AND DISCUSSION

### 4.1 Metals in random samples of green leafy vegetables

In randomly collected samples of palak (*Beta vulgaris* L), cabbage (*B. oleracea*) and yellow sarsoo (*B. campestris*) from 45 different vendors within the city of Rajpura and Patiala different heavy metals Fe, Zn, Pb, Cr and Cd were analysed. Leafy vegetables were preferred for sampling since past research indicates that they accumulate heavy metals at greater extent than other vegetables (Jindasa *et al.*, 1997). The study showed that the concentration of metals greatly varied in palak collected from vendors (Table 1). Zn varied from 24 to 69.55 mg/kg, Cd varied from 0.2 to 3.35 mg/kg, Fe varied from 5 to 12.5 mg/kg. Whereas Cr and Ni were below detection limit in all the samples. Stastical analysis showed that the trend for the heavy metals in palak samples was in the order Fe>Zn>Pb>Cu>Cd.

In cabbage Fe ranged from 0 to 3925 mg/kg, Zn varied from 2.2 to 59.6 mg/kg , Pb was from 0.04 to 5 mg/kg, Cd was from 1.05 to 6.95 mg/kg whereas Cr and Ni were below detection limit in all and the trend was Fe>Zn>Pb>Cu>Cd.

In yellow sarsoo samples Cd ranged from 0.85 to 37.6 mg/kg, Zn from 26 to 69.8 mg/kg. Fe was from 1.06 to 44.8 mg/kg, Pb was from 9.1 to 50 mg/kg whereas Cr and Ni were below detection limit in all the samples.

In all the three green leafy vegetables a similar trend in metal content was observed i.e. Fe>Zn>Pb>Cu>Cd however their values in all vegetables were different. Fe was found highest in palak with a mean of  $24\pm 3.5$  mg/kg followed by cabbage with a mean of  $17.45\pm 3.3$  mg/kg and yellow sarsoo was  $17.35\pm 3.3$  mg/kg. Zn was highest in yellow sarsoo with a mean of  $35.6\pm 4.03$  mg/kg followed by palak with a mean of  $24\pm 3.5$  mg/kg and cabbage having  $16.3\pm 5.04$  mg/kg. Cd was highest in cabbage with a mean of  $23.5\pm 6.6$  mg/kg followed by palak with a mean of  $18.75\pm 5.3$  mg/kg and minimum in brassica i.e.  $17.35\pm 3.3$  mg/kg. Cu was maximum in case of palak with a mean of  $56.25\pm 5.5$  mg/kg, followed by cabbage with a mean of  $5.3\pm 0.52$  mg/kg and  $3.95\pm 0.37$  mg/kg was for yellow sarsoo which was least.

The metal content in all the vegetables was above as defined by nutritiondata.com and elook.org. The Zn concentration permissible for human intake as according to prevention of food adulteration act, 1954 is 2.5 mg/kg whereas in yellow sarsoo Zn was from 26 to 69.8 mg/kg. Similar was the case with other metals in leafy vegetables.

The order of vegetables according to their metal content along with their mean was Fe was highest in palak (24 mg/kg), followed by cabbage (17.45 mg/kg) and value in yellow sarsoo was (17.45 mg/kg). Zn was highest in yellow sarsoo (35.6 mg/kg), followed by palak (24 mg/kg) and value in cabbage was (16.3 mg/kg). Cd was highest in cabbage (23.5 mg/kg), followed by palak (18.7 mg/kg) and followed by yellow sarsoo (17.35 mg/kg). Pb followed a similar trend as that followed by Cd i.e. highest in cabbage (24.75 mg/kg), followed by palak (14.04 mg/kg) and yellow sarsoo (5.6 mg/kg). Cr and Ni were below detection limit in all the samples.

In cabbage Cd concentration was 17.35 mg/kg whereas the standards fixed by Indian Prevention of Food Adulteration Act, 1954 and European Union standards is 1.5 mg/kg. Zn concentration recommended by Indian Prevention of Food Adulteration Act, 1954 and European Union standards is 50 mg/kg so the concentration of Zn was well

within the range in all the samples. The daily intake of Fe by human body ranges between 0.08 to 0.18  $\mu\text{g}$  per day. If it exceeds the limit then various diseases like loss of taste and smell, sterility, poor wound healing and hair loss occurs (Genova diagnostics, 2004).

The maximum level allowed by Prevention of Food Adulteration Act (PFA), India for Pb, Zn and Cd is 2.5, 5.0 and 1.5 mg/kg respectively. Nearly 16% of the samples these i.e. only some of the samples of palak were showing higher levels of Cd than the permissible limit of 1.5 mg/kg as per Indian Standard of Food Adulteration Act (PFA), 1954. Rest of the samples including palak, coriander and bathua (grown in different plots) and samples of palak, cabbage and yellow sarsoo (taken from market) were safe since they had Cd within the safe limits. Among all these green leafy vegetables nearly all were higher in Pb and Fe having crossed the permissible limits of PFA. The safe limits recommended for Pb is 2.5 mg/kg whereas for Zn is 2.5 mg/kg and none of the vegetable samples were in this range. The result indicates that the consumers are purchasing vegetables with high level of heavy metals (HMs).

#### **4.2 Metals in random samples of green leafy vegetables collected from agricultural fields around Rajpura city**

Vegetables were collected from different agricultural fields around Rajpura city to study the metal concentration in the fresh farm produce. The concentration of Fe in yellow sarsoo was found to be as high as 62.45 mg/kg (Table 2). The maximum concentration of Fe was found in samples of villages Banur (S2), followed by Main village (S5) which was 44.85 mg/kg. Lowest concentration of Fe was reported in samples from village Bathonia where it was 2.4 mg/kg. In samples from village Kami-Kalan (S1) the concentration of Fe in yellow sarsoo was 12.8 mg/kg whereas it was 7.3 mg/kg in samples from village Bhadak (S4). Cd concentration in brassica samples from Kami-Kalan (S1) was 37.6 mg/kg and 1.1 mg/kg in samples from Bathonia (S3). It was 8.98 mg/kg in samples from village Banur (S2) and 1.45 mg/kg in samples from village of Bhadak (S4). Zn was maximum in samples from village Banur (S2) that was 421 mg/kg and was minimum in samples from Kami-Kalan (S1). Zn was 44.9 mg/kg, 53.3 mg/kg

and 41.8 mg/kg in samples from Bathonia, Bhadak and main village respectively. Cu was maximum in samples from village Banur i.e. 81.8 mg/kg and minimum in samples from village Bathonia i.e. 2.4 mg/kg. Similar trend was seen for Pb. Banur (S2) was having 170 mg/kg of Pb whereas Kami-Kalan was 3.25 mg/kg. Statistical analyses showed that the order of metal content in brassica was  $Zn > Pb > Cu > Fe > Cd$ .

Palak samples showed Fe concentration in the range of 23.35 mg/kg in samples from Banur (S2) to 3.8 mg/kg in samples from Main village (S5) and 7.75 mg/kg from village Bathonia (S3) and village Bhadak (S4) (Table 3). Cd varied from 6.45 mg/kg in samples from village Banur (S2) to 0.0 in case of samples from village Bathonia (S3). Cd in Main village (S5) samples was 0.6 mg/kg and in samples from Bhadak village (S4) was 0.55 mg/kg. Zn varied from 246 mg/kg in samples from village Banur and was below detection limit in samples from village Bathonia. Pb varied from 81 mg/kg in samples of village Banur and 6.2 mg/kg in samples from village Bhadak. Pb was 27.75 mg/kg in samples from village Kami-Kalan and 10.15 mg/kg in samples from village Bathonia. Cu was maximum i.e. 16.85 mg/kg in samples from village Kami-Kalan (S1) and minimum i.e. 5.9 mg/kg in samples from village Main. Samples from village Banur has 39.55 mg/kg of Cu whereas samples from village Bathonia has 6.85 mg/kg of Cu. Trend in metal content was  $Zn > Pb > Cu > Fe > Cd$ .

Cabbage had showed Fe concentration of 7.45 mg/kg in samples from village Bathonia (S3) that was 2.25 mg/kg in samples from village Main (S5) and 6.75 mg/kg in samples from Banur (S2) and 4.55 mg/kg in samples from village Bhadak (S4) (Table 4). Zn samples varied from 47.35 mg/kg in samples from village Bathonia (S4) to 26.05 mg/kg in samples from village Main and were 27.15 mg/kg in samples from village Bhadak (S4). Cd was 0.55 mg/kg in samples from village Kami-Kalan (S1) and 0.3 mg/kg in samples from village Banur (S2) whereas in other places like Bathonia, Main and Bhadak Cd was below detection limit. Cu varied from 16.3 mg/kg in samples from Bathonia (S3) to 8.2 mg/kg in samples from Bhadak (S4) and village Banur (S2) 9.7 mg/kg. Pb was 60.3 mg/kg in samples from village Bathonia (S3) and 34.95 mg/kg in samples from village Banur (S2) whereas it was 29 mg/kg in samples from village Main (S5). Trend in metal content was  $Pb > Zn > Cu > Fe > Cd$ .

The study showed that the concentration of metals in different samples from different villages were different, indicating varied metal uptake. In all vegetables from different places it was found that Banur (S2) had high concentration of Fe, Zn, Cu and Pb. Samples from Village Kami-Kalan (S1) had higher concentration of Cd whereas Cr and Ni was below detection limit. High concentration of these metals in vegetables was due to high content of metals in the soil as these places were located near industries and high content may be due to irrigation by metal contaminated water released from industries in the vicinity. A major pathway of soil contamination is through atmospheric deposition of heavy metals from point sources such as metalliferous mining, smelting and industrial activities. Other non point sources of contamination affecting predominately agricultural soils are due to various inputs such as fertilizers, pesticides, sewage sludge, organic manure and compost (Singh, 2001). Additionally foliar uptake of atmospheric heavy metals from emission gas also been identified as an important pathway of heavy metal contamination in vegetable crops (Bassuke, 1986; Salim *et al.*, 1992)

### 4.3 Field experiment

In field plot experiment leafy vegetables i.e. palak, coriander and bathua were grown to study the uptake potential for different metals Fe, Zn, Pb, Cu, Ni, Cd, and Cr in the presence of beneficial soil microbes such as *Azotobacter* and *Pseudomonas striata*.

In palak (*Beta vulgaris* L) uptake of Fe was found to be highest (Table 5). The control, without biofertilizer had 225 mg/kg of Fe whereas the one, which was provided with biofertilizer, had 473 mg/kg of Fe. Zn was found to be 67.1 mg/kg in treated whereas in control it was 54.25 mg/kg. Pb was observed to be 25.95 mg/kg in treated and 31.65 mg/kg in control. Cd was 1.95 mg/kg in treated and 0.2 mg/kg in control. Whereas in Cu was 10.25 mg/kg in treated plant and 16 mg/kg in case of control plant. The presence of Cr was not detected in any vegetable. Heavy metal pattern in the treated plants as well as untreated was in the order Fe>Zn>Pb>Cu>Cd.

In coriander (*Coriandrum sativum*) Fe was 170 mg/kg while in control it was 162 mg/kg. Cu in treated ones was 14.1 mg/kg and in control was 6.1 mg/kg. Zn in treated was 51.7 mg/kg and in control was 24.3mg/kg. Lead was 17.8 mg/kg in treated and 37.2 mg/kg in case of control. Cd was 0.35 mg/kg in treated whereas in control was 0.5 mg/kg. Cr and Ni were below detection limit. Heavy metals were in the order of Fe>Zn>Pb>Cu>Cd which was same in treated and untreated.

In case of Bathua (*Chenopodium sp.*) Fe was 560 mg/kg in treated and 116 mg/kg in case of control. Zn was 26.45 mg/kg in treated whereas in case of control it was 29.2 mg/kg. Cu was found to be 8mg/kg and 13.5 mg/kg in treated and control respectively, whereas Cd was 0.65 mg/kg and 6.4 mg/kg in treated and control respectively. Lead was 34.15 mg/kg in case of treated and 25.1 mg/kg in control. Cr and Ni were below detection limit in both treated and untreated. The trend in heavy metal was Fe>Pb>Zn>Cu>Cd in case of treated whereas in case of control was Fe>Zn>Pb>Cu>Cd.

Concentration of all the metals Fe, Cd, Zn, Cu and Pb was higher in plants grown on treated soil (which was provided with biofertilizers). Several studies have shown that vegetables, particularly leafy crops, grown in heavy metal contaminated soils have higher concentrations of heavy metals than those grown in uncontaminated soil (Guttormsen *et al.*, 1995; Dowdy and Larson, 1995).

#### **4.4 Analysis of Nursery soil**

Nursery soil was analyzed for their physico-chemical parameters. These parameters include: pH of the soil was 8.6 and electrical conductivity was 256 ( $\mu\text{S cm}^{-1}$ ). Available sulphur was 0.2 mg/kg, Available nitrogen was 0.002%, phosphorus was 2.12 mg/kg and organic carbon was 0.090%. Percentage of clay, silt and sand was 38.4, 6 and 55.6 respectively (Table 6).

Heavy metal analysis was done for soil with treated (with biofertilizer) and control (without biofertilizer) (Table 8). In palak Fe concentration was 473 mg/kg in case of treated and 225 mg/kg in control. Zn was also higher in case of treated i.e. 67.1 mg/kg

whereas in control was 54.25 mg/kg. Similar trend was for Cd, which were 1.95 mg/kg in treated and 0.2 mg/kg in control. The trend was bit different with Pb and Cu where concentration of metal was more in case of control. Pb was 25.95 mg/kg and 31.65 mg/kg in case of control and treated respectively. Similarly Cu was 10.25 mg/kg in treated and 16 mg/kg in control. In coriander Fe was 170 mg/kg in treated and 162 mg/kg in control. Zn was 51.75 mg/kg and Cu was 14.1 mg/kg in treated whereas in control Zn was 24.3 mg/kg and Cu was 6.8 mg/kg. Pb and Cd followed a different trend in concentration. Pb was 17.8 mg/kg and Cd was 0.25 mg/kg in case of treated. In case of control Pb was 37.2 mg/kg and Cd was 0.5 mg/kg. In case of chenopodium trend was same as in other cases where Pb, Zn and Cu was higher in control as compared to treated. Fe & Cd was 560 mg/kg & 0.65 mg/kg in treated and 116 mg/kg & 0.4 mg/kg in control.

The metal concentration in the nursery soil followed almost a similar trend in case of all the metals. In some cases the concentration was more for control as compared to treated. The reason for this could be that the uptake of metals by plants depended on the availability of metals in the soil to the plants (Khairiah *et al.*, 2004). However, only soluble, exchangeable and chelated metal species in the soil are mobile and hence, more available forms for plants (Arambarri, 1999). Elevated levels of heavy metal in soils may lead to their uptake by plants, which depends not only on heavy metal content in soils but is also determined by soil pH, metal content in soil, organic matter and clay contents and fertilizers. These parameters cannot change the total amount of heavy metals but can significantly affect the bioavailable part.

#### **4.5 Bacterial pathogens in randomly collected samples of green leafy vegetables**

Randomly collected samples of palak, yellow sarsoo and cabbage from 45 different vendors located in the city were enumerated for bacterial pathogens mainly to assess level of post harvest contamination primarily of coliforms and total bacteria. Great variation in the bacterial count was observed in all the samples (Table 7).

In palak the log cfu/g of total bacterial count varied from 0.9 to too numerous to count (TNTC) while coliforms were observed to be present in negligible number to 2.28. The cfu/g of palak samples was in the range of 8 to TNTC for total bacterial count and between 0.9 to TNTC for coliforms. Brassica also showed total bacterial count in terms of log cfu/g from negligible to 2 for total bacteria present and between negligible to 2.05 for coliforms. In terms of cfu/g the count was between negligible to 48 for total bacterial count and from negligible to 120 for coliforms. In terms of log cfu/g cabbage showed a variation between negligible to 2.0 in both the cases i.e. for total bacterial count and coliforms. The cfu/g values ranged between negligible to 104 for total count and coliforms.

The mean values showed the following order for coliforms and total bacterial count for the three vegetables. Total bacterial count was highest in palak followed by brassica and cabbage and the coliforms were highest in yellow sarsoo followed by palak and cabbage.

The number of pathogens found on the surface of palak, yellow sarsoo and cabbage showed a great variation. The count does not depend upon the vegetable type rather than it depends on the survival and growth of pathogens on the fresh produce and environmental conditions in the field and thereafter, including storage conditions. Environmental conditions, however, could greatly influence bacterial population; the presence of free moisture on leaves from precipitation, dew or irrigation might promote survival and growth of bacterial population (Bvlackeman, 1981; Andrews, 1992; Beattie and Lindow, 1995.) The other sources of contamination could be pre-harvest and post-harvest conditions. Pre-harvest conditions can come from irrigation water, improperly composted manure used as fertilizer, faecal contamination from human and domestic animals.

#### **4.6 Bacterial pathogens in green leafy vegetables grown in micro plots**

In palak the log cfu/g of total bacterial count was 1.6 while coliforms were observed to be present in negligible number (Table 8). The cfu/g of palak samples for nitrogen fixers and phosphate solubilisers varies from 0.9 to 0.20 for treated. Log cfu/g for total bacterial count and coliforms for coriander varied from 1.5 to negligible. Bathua also showed total bacterial count in terms of log cfu/g from negligible to 2.18 for total bacteria present and between 0.50 to 0.80 for coliforms. In terms of cfu/g the count for nitrogen fixer and phosphate solubilisers was negligible.

**(A) Table 1: Heavy metals in random samples of green leafy vegetables (mg/kg of dry weight)**

<b>HEAVY METAL</b>	<b>Sarsoo</b>	<b>Palak</b>	<b>Cabbage</b>
	<b>Mean±SE</b>	<b>Mean±SE</b>	<b>Mean±SE</b>
<b>Iron (Fe)</b>	17.35±3.3	24±3.5	17.45±3.8
<b>Cadmium (Cd)</b>	17.35±3.3	18.7±5.3	23.35±6.6
<b>Lead (Pb)</b>	14.04±2.3	5.6±0.9	24.75±7.2
<b>Zinc (Zn)</b>	35.6±4.03	24±3.5	16.3±5.04
<b>Copper (Cu)</b>	3.95±0.37	56.25±5.5	5.3±0.52
<b>Chromium (Cr)</b>	<0.05	<0.05	<0.05
<b>Nickel (Ni)</b>	<0.04	<0.04	<0.04

**(B) Table 2: Heavy metals (mg/kg of dry wt) in random samples of green leafy vegetable (Brassica) collected from villages around Rajpura (S1: Kami -Kalan, S2: Banur, S3: Bathonia, S4: Bhadak, S5: Main)**

<b>Samples</b>	<b>Cd</b>	<b>Zn</b>	<b>Fe</b>	<b>Cu</b>	<b>Pb</b>	<b>Cr</b>	<b>Ni</b>
<b>S1</b>	<b>37.6</b>	<b>26</b>	<b>12.8</b>	<b>3.25</b>	<b>17.5</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S2</b>	<b>8.98</b>	<b>421</b>	<b>62.45</b>	<b>81.8</b>	<b>170</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S3</b>	<b>1.1</b>	<b>44.9</b>	<b>2.4</b>	<b>8.9</b>	<b>0</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S4</b>	<b>1.45</b>	<b>53.3</b>	<b>7.3</b>	<b>8.85</b>	<b>24.85</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S5</b>	<b>2.1</b>	<b>41.8</b>	<b>44.85</b>	<b>11.4</b>	<b>20.65</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>Average ± SE</b>	<b>10.246±7</b>	<b>117.4±7 7.2</b>	<b>25.96±11</b>	<b>22.84±15</b>	<b>46.6±31</b>	<b>-----</b>	<b>-----</b>

**(C) Table 3: Heavy metals (mg/kg of dry wt) in random samples of green leafy vegetable (Palak) collected from villages around Rajpura (S1: Kami -Kalan, S2: Banur, S3: Bathonia, S4: Bhadak, S5: Main)**

<b>Samples</b>	<b>Cd</b>	<b>Zn</b>	<b>Fe</b>	<b>Cu</b>	<b>Pb</b>	<b>Cr</b>	<b>Ni</b>
<b>S1</b>	<b>1.0</b>	<b>94.5</b>	<b>11.65</b>	<b>11.65</b>	<b>27.75</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S2</b>	<b>6.45</b>	<b>246</b>	<b>23.35</b>	<b>39.55</b>	<b>81</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S3</b>	<b>0.0</b>	<b>0.0</b>	<b>7.75</b>	<b>6.85</b>	<b>10.15</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S4</b>	<b>0.55</b>	<b>47.75</b>	<b>7.75</b>	<b>10.3</b>	<b>6.2</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S5</b>	<b>0.6</b>	<b>33.95</b>	<b>3.8</b>	<b>5.9</b>	<b>17.65</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>Average ± SE</b>	<b>1.72±1.21</b>	<b>84.44±43.8</b>	<b>10.86±3.4</b>	<b>14.85±6.3</b>	<b>28.55±13.8</b>	<b>-----</b>	<b>-----</b>

**(D) Table 4 Heavy metals (mg/kg of dry wt) in random samples of green leafy vegetable (Cabbage) collected from villages around Rajpura (S1: Kami -Kalan, S2: Banur, S3: Bathonia, S4: Bhadak, S5: Main)**

<b>Samples</b>	<b>Cd</b>	<b>Zn</b>	<b>Fe</b>	<b>Cu</b>	<b>Pb</b>	<b>Cr</b>	<b>Ni</b>
<b>S1</b>	<b>0.55</b>	<b>28.75</b>	<b>2.45</b>	<b>9.7</b>	<b>29.95</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S2</b>	<b>0.3</b>	<b>26.3</b>	<b>6.75</b>	<b>9.45</b>	<b>34.95</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S3</b>	<b>0.0</b>	<b>47.35</b>	<b>7.45</b>	<b>16.3</b>	<b>60.3</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S4</b>	<b>0.0</b>	<b>27.15</b>	<b>4.55</b>	<b>8.2</b>	<b>32.15</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>S5</b>	<b>0.0</b>	<b>26.05</b>	<b>2.25</b>	<b>7.55</b>	<b>29</b>	<b>&lt;0.05</b>	<b>&lt;0.04</b>
<b>Average ± SE</b>	<b>0.17±0.11</b>	<b>0.17±0.11</b>	<b>31.11±1.08</b>	<b>10.24±0.45</b>	<b>37.27±5.9</b>	<b>-----</b>	<b>-----</b>

**(E) Table 5 Heavy metals in green leafy vegetables grown in different plots (mg/kg of dry wt) with and without inoculation of biofertilizers.**

<b>Samples</b>	<b>Zn</b>	<b>Pb</b>	<b>Cd</b>	<b>Fe</b>	<b>Cu</b>
Palak (Inoculated)	<b>67.1</b>	<b>25.95</b>	<b>1.95</b>	<b>473</b>	<b>10.25</b>
Palak (Control)	<b>54.25</b>	<b>31.65</b>	<b>0.2</b>	<b>225</b>	<b>16</b>
Coriander (Inoculated)	<b>51.75</b>	<b>17.8</b>	<b>0.35</b>	<b>170</b>	<b>14.1</b>
Coriander (Control)	<b>24.3</b>	<b>37.2</b>	<b>0.5</b>	<b>162</b>	<b>6.8</b>
Bathua (Inoculated)	<b>26.45</b>	<b>34.15</b>	<b>0.65</b>	<b>560</b>	<b>8</b>
Bathua (Control)	<b>29.2</b>	<b>25.1</b>	<b>0.4</b>	<b>116</b>	<b>13.35</b>

**(F) Table 6: Bacterial pathogens in random samples of green leafy vegetables**

SNo.	Palak				Cabbage				Yellow sarsoo			
	TSA		VRBA		TSA		VRBA		TSA		VRBA	
	cfu X10 <sup>3</sup>	Log cfu/gm	cfu X10 <sup>5</sup>	Logcfu/gm	cfu X10 <sup>5</sup>	Log cfu/gm	cfu X10 <sup>5</sup>	Log cfu/gm	cfu X10 <sup>5</sup>	Log cfu/gm	cfu X10 <sup>5</sup>	Log cfu/gm
1	72	1.8	176	2.2	8	0.9	--	--	16	1.2	8	0.9
2	296	2.4	192	2.28	--	--	8	0.9	8	0.9	8	0.9
3	TNTC	TNTC	256	2.4	16	1.2	16	1.2	--	--	8	0.9
4	112	2.0	--	--	--	--	8	0.9	8	0.9	16	1.2
5	--	--	8	0.9	TNTC	TNTC	8	0.9	8	0.9	--	--
6	16	1.2	40	1.6	8	0.9	8	0.9	24	1.38	16	1.2
7	8	0.9	136	2.1	16	1.2	TNTC	TNTC	8	0.9	8	0.9
8	8	0.9	--	---	8	0.9	TNTC	TNTC	16	1.2	TNTC	TNTC
9	--	--	--	--	8	0.9	8	0.9	--	--	8	0.9
10	16	1.2	--	--	16	1.2	--	--	104	2.01	48	1.68
11	16	1.2	--	--	24	1.3	TNTC	TNTC	8	0.9	16	1.2
12	TNTC	TNTC	80	1.9	16	1.24	104	2.01	48	1.68	56	1.7
13	--	--	8	0.9	--	--	8	0.9	80	1.9	8	0.9
14	8	0.9	56	1.7	8	0.9	16	1.2	16	1.2	16	1.2
15	--	--	56	1.7	--	--	16	1.2	56	1.7	--	--
16	TNTC	TNTC	84	1.9	8	0.9	104	2.01	8	0.9	84	1.92
17	TNTC	TNTC	24	1.3	--	--	8	0.9	--	--	8	0.9
18	56	1.7	112	2.0	16	1.24	16	1.2	8	0.9	112	2.04
19	8	16	0.9	1.2	8	0.9	8	0.9	16	1.2	--	--
20	104	2.0	184	2.2	--	--	--	--	24	1.38	112	2.04
<b>Average ± SE</b>	45±17.3	2.68±0.9	94.19±17.9	1.752±0.1	12.3±1.2	1.05±0.03	24±7.7	1.14±0.08	26.8±6.4	1.244±0.085	33.25±8.5	1.28±0.1

-- --: No Growth  
TNTC: Too numerous too count

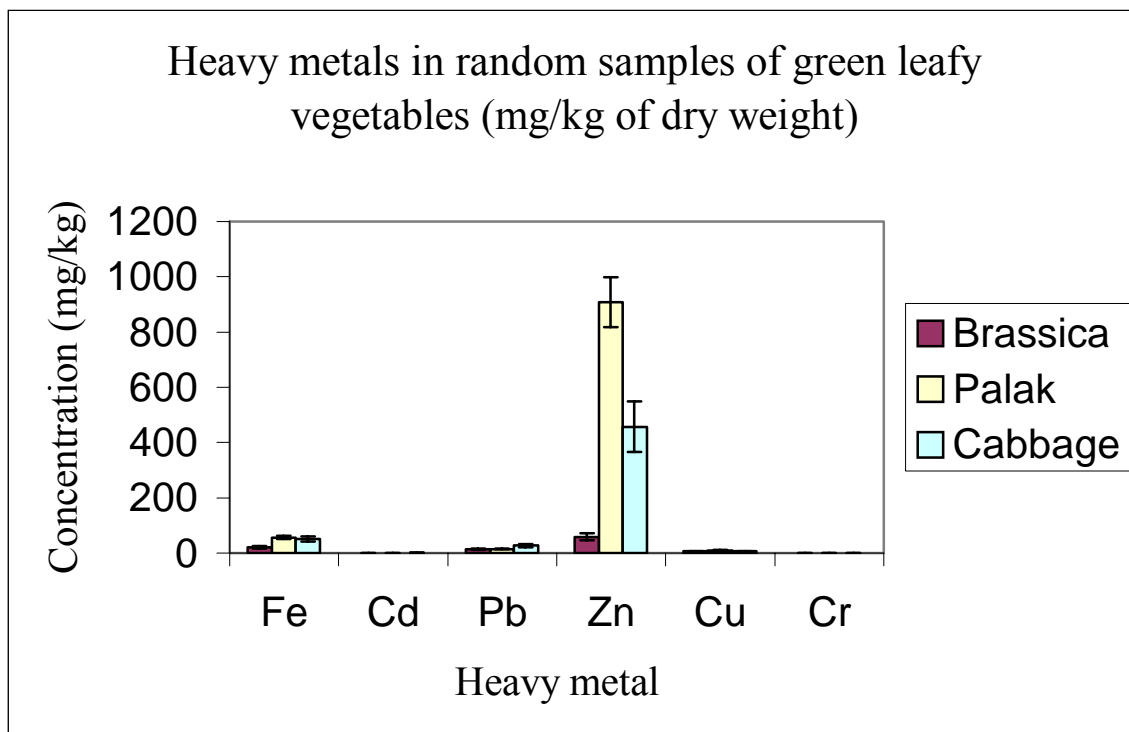
**(G) Table 7: Bacterial pathogens in green leafy vegetables grown in micro-plots with and without inoculation of bacterial biofertilizers**

Samples	TSA		VRBA		Pikovskaya		Jensen	
	Cfu X10 <sup>5</sup>	Log cfu/gm	cfu X10 <sup>5</sup>	Log cfu/gm	Cfu X10 <sup>5</sup>	Log cfu/gm	cfu X10 <sup>5</sup>	Log cfu/gm
Palak (Inoculated)	40	1.6	3.2	0.50	8	0.9	1.6	0.20
Palak (Control)	64	1.8	Nil	Nil	Nil	Nil	0.8	>0.09
Coriander (Inoculated)	32	1.5	Nil	Nil	Nil	Nil	0.8	>0.09
Coriander (Control)	128	2.1	3.2	0.50	Nil	Nil	Nil	Nil
Bathua (Inoculated)	Nil	Nil	3.2	0.50	Nil	Nil	TNTC	TNTC
Bathua (Control)	152	2.18	6.4	0.80	Nil	Nil	24	0.38

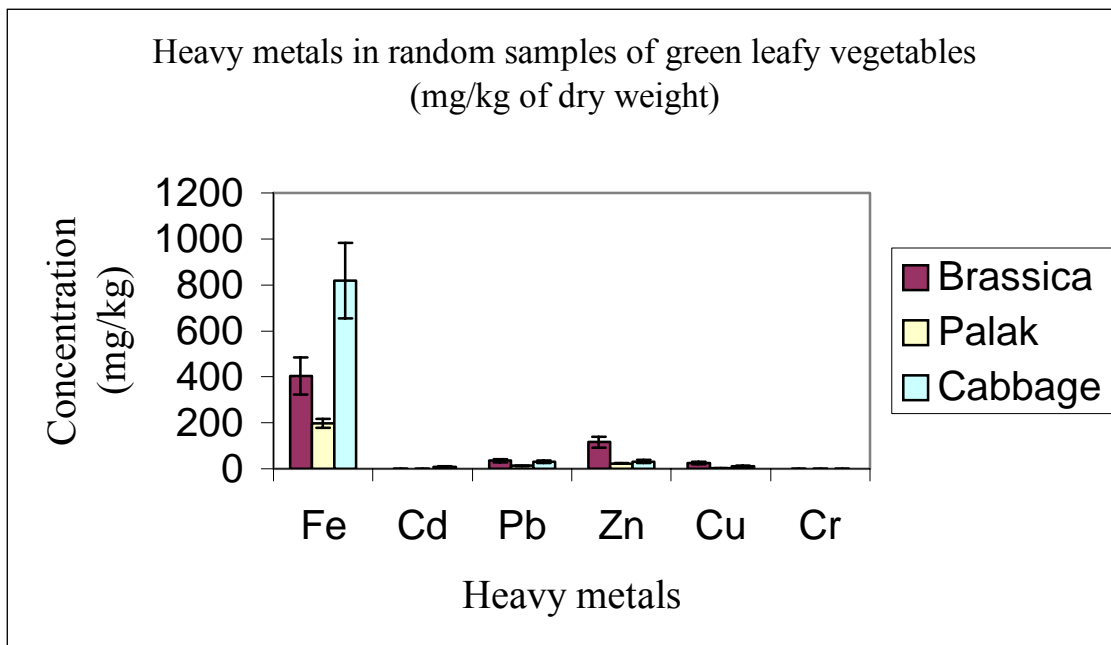
**(H) Table 8 Physico-chemical characterization of soil**

EC ( $\mu\text{S cm}^{-1}$ )	256
pH	8.6
Available S (mg/kg)	0.23
Available P (mg/kg)	2.12
Organic Carbon (%)	0.09

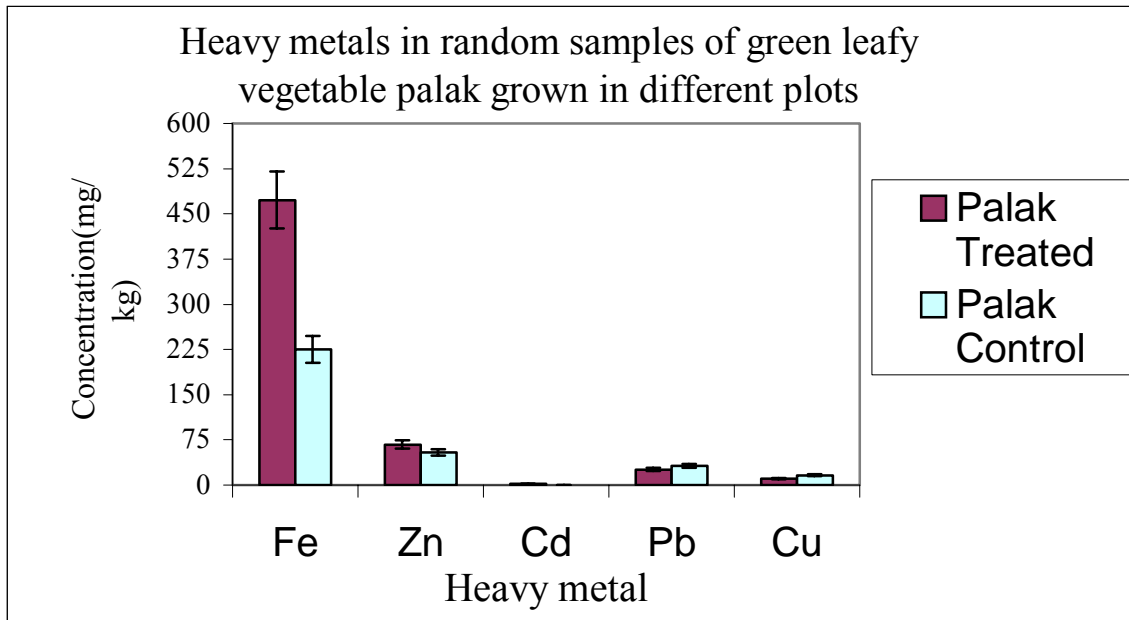
**Fig 1 Heavy metals in random samples of green leafy vegetables**



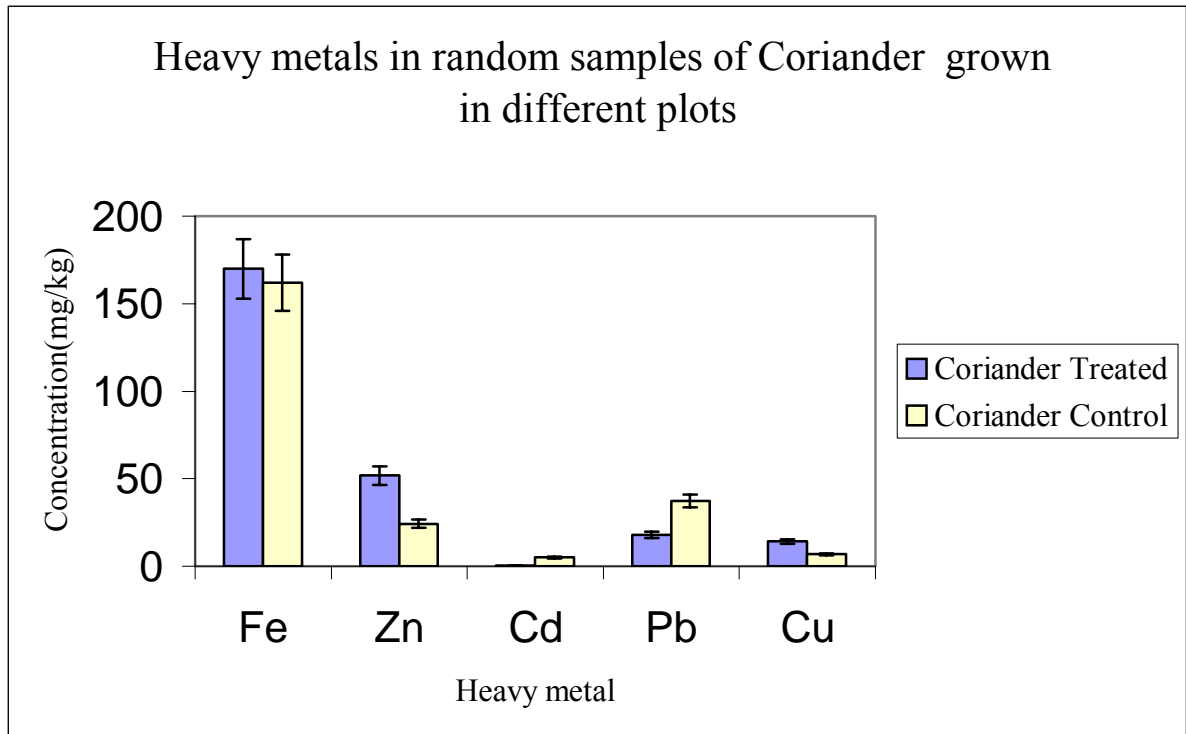
**Fig 2 Heavy metals in vegetables collected from villages around Rajpura**



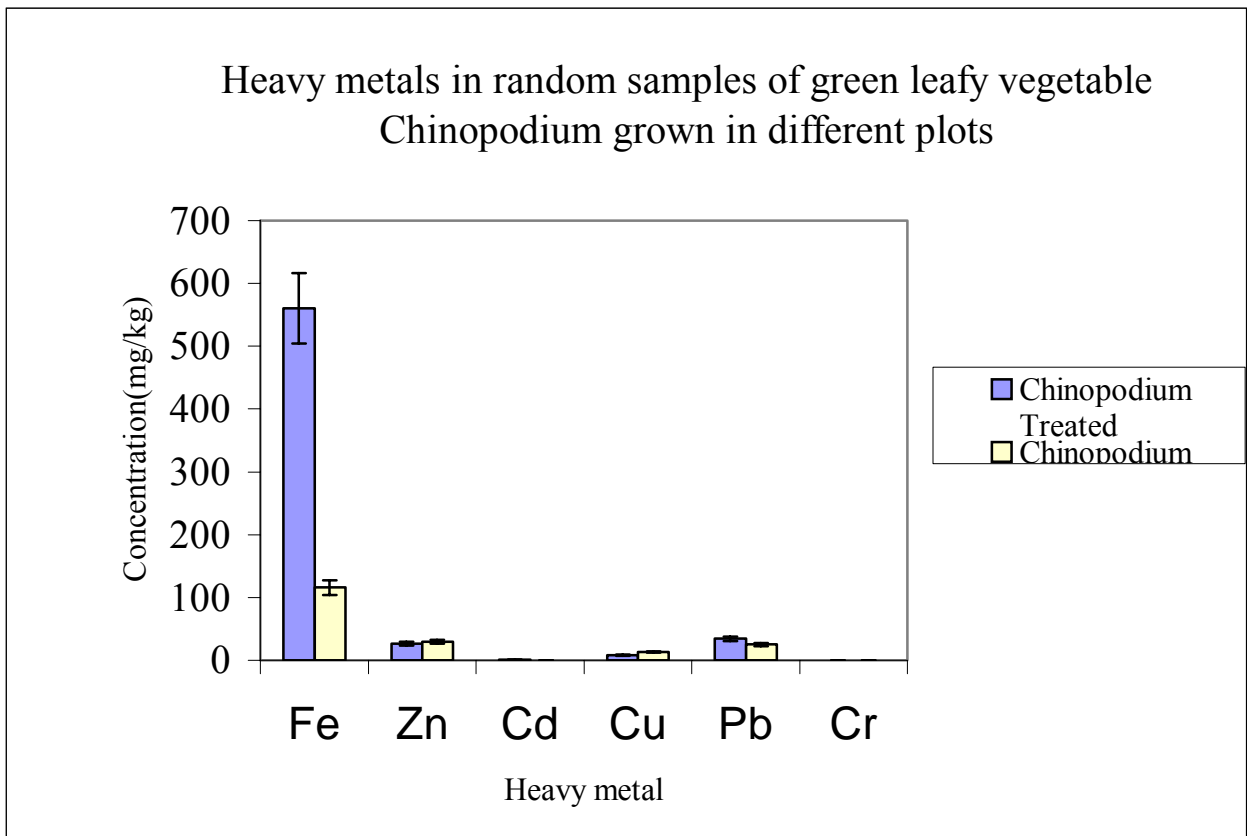
**Fig 3 Heavy metals in random samples of Palak grown in different plots**



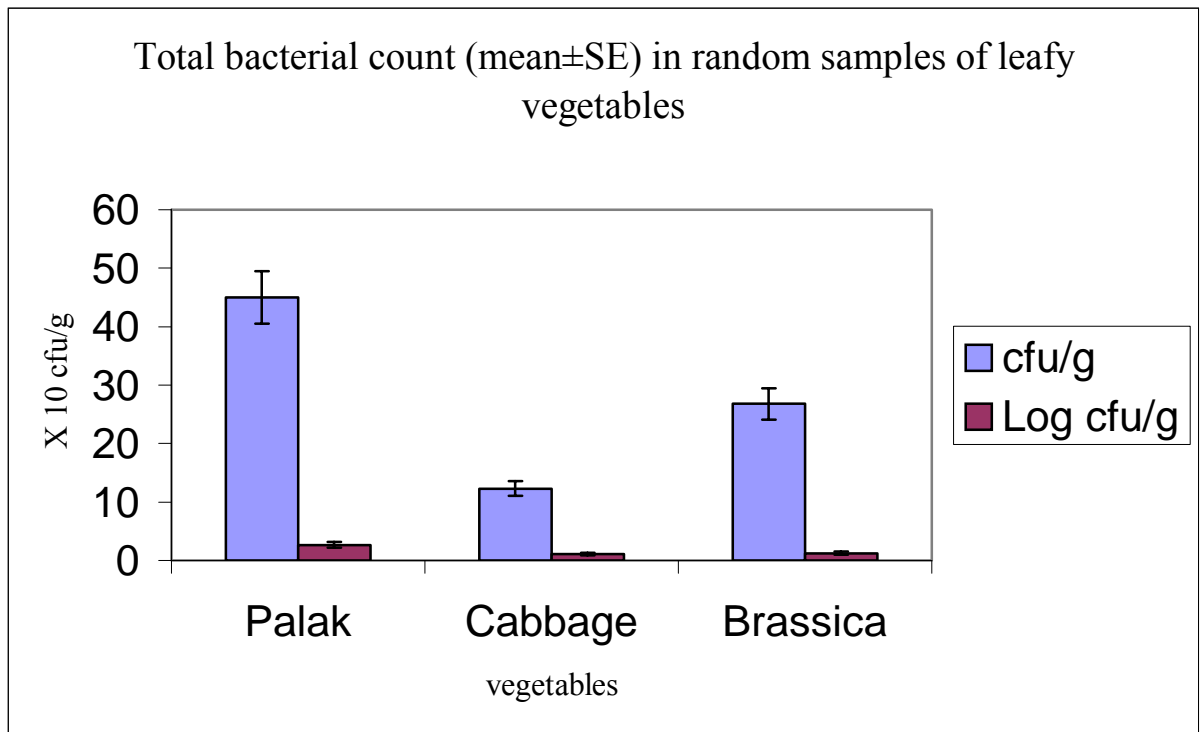
**Fig 4 Heavy metals in random samples of Coriander grown in different plot**



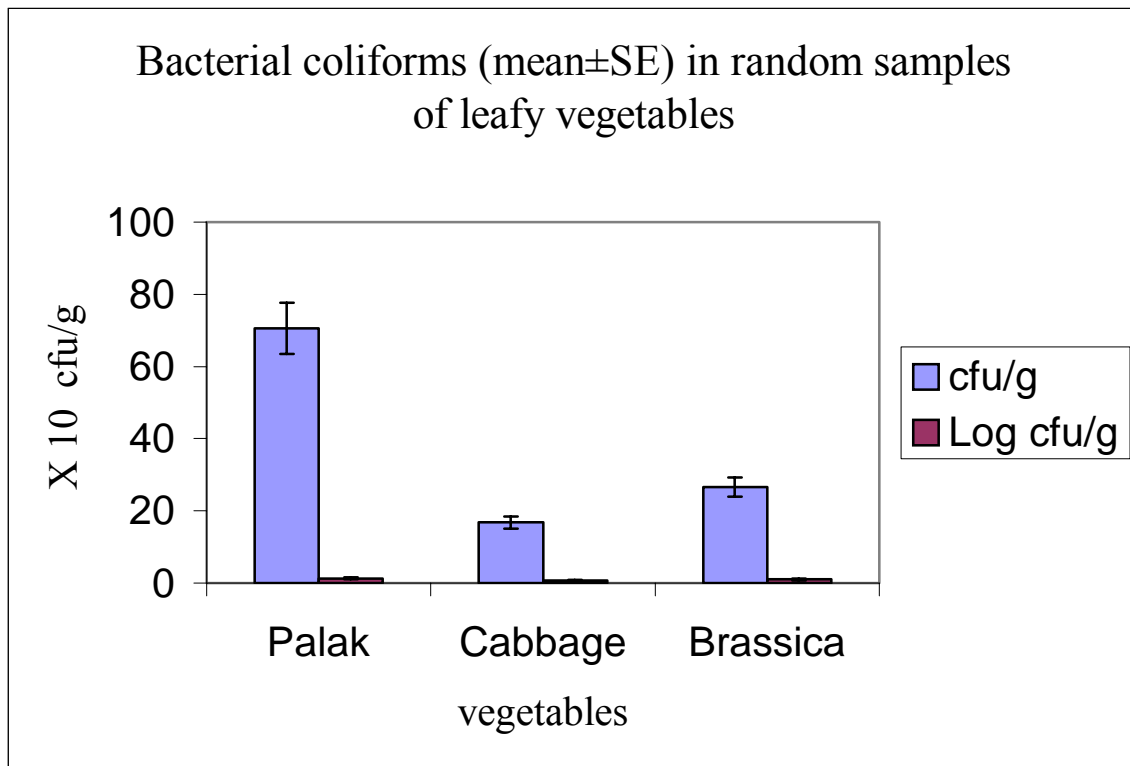
**Fig 5 Heavy metals in random samples of Chinopodium grown in different plots.**



**Fig 6 Bacterial pathogens in random samples of green leafy vegetables**



**Fig 7 Bacterial coliforms in random samples of green leafy vegetables**



## CONCLUSION

1. The metal concentration in the green leafy vegetables from the market was higher than the permissible limits of Indian Prevention of Food Adulteration Act (PFA), 1954. Nearly 16% of samples of palak were showing higher levels of Cd than the permissible limit of 1.5 mg/kg as per Indian Standard of Food Adulteration Act (PFA), 1954. Rest of the samples including palak, coriander and chenopodium (Grown in different plots) and samples of palak, cabbage and brassica (taken from market) were since they had Cd within the safe limits. Among all these green leafy vegetables nearly all were higher in Zn, Pb and Fe having crossed the permissible limits of PFA. The safe limits designed for Pb is 2.5 mg/kg whereas for Zn is 2.5 mg/kg and none of the vegetable samples were in this range. The result indicates that the consumers are purchasing vegetables with high level of heavy metals (HMs).

2. Different agricultural field soils have different concentration of metals thus the crop (vegetables) grown in such soils have different concentration of metals in them. There is a danger of heavy metal accumulation of Cd and Pb in the vegetables grown in the vicinity of Village Bhappal, Kami-kalan, and Bahadurgarh.

3. The metal content in vegetables with the addition of biofertilizer was higher than the vegetables that were lacking biofertilizer. Uptake of Fe, Zn was more in case of palak, which was provided with biofertilizer. However the uptake of Pb and Cu was more in case of control as compared to treated. Similar trend was with coriander and chinopodium.

4. The total bacterial count in randomly collected samples of palak ranged from  $45 \pm 17.3$  cfu/g to  $2.68 \pm 0.95$  Log cfu/g, in cabbage from  $12.7 \pm 1.2$  cfu/g to  $1.05 \pm 0.3$  Log cfu/g

whereas in case of brassica was  $26.8 \pm 6.4$  cfu/g to  $1.2 \pm 0.08 \log$  cfu/g which is an indication of improper pre-harvest and post harvest handling

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