HEAVY METALS AND PATHOGENS IN LEAFY VEGETABLES

A

DISSERTATION

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Masters of Science in Biotechnology

Under the Guidance of
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CERTIFICATE

This is to certify that the thesis by Navneet Jaj (Roll no 3030112) in partial fulfillment of the requirements for the award of Degree of Master of Sciences 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

Heavy metal and presence of bacterial pathogen in randomly collected samples and from agricultural areas in and around Patiala City was conducted. In palak (*Beta vulgaris* L), fenugreek (*Trigonella foenum-graecum*) and coriander (*Coriandrum sativum*) heavy metals Fe, Zn, Cd, Cr, Ni and Pb were analysed by wet digestion method and total bacteria and coliforms were enumerated on TSA (Tryptone Soya Agar) and VRBA (Violet Red Bile Salt Agar) media. All vegetables had metal content in the order Fe> Zn> Pb> Cr> Ni> Cd. Only 32% palak, 26% of coriander and 23% of fenugreek samples contained Pb concentration below the permissible limit of 2.5 mg/Kg as per Indian Standard of Food Adulteration Act (PFA), 1954. Among all green leafy vegetables nearly 67-76% of samples were high in Pb, 18-39% samples were high in Zn, which crossed Indian permissible limit of 50 mg/Kg, and 6-9% samples were high in Cd, which was beyond 1.5 mg/Kg. The results indicate that the consumers are purchasing vegetables with high level of heavy metals (HMs). The total bacterial count in vegetables ranged between 250-109 cfu x10^4 and 5.7-6.0 log cfu/g and coliforms were in the range of 29-87 cfu x 10^4 and 4.8-5.6 Log cfu/g, which is an indication of improper pre harvest and post harvest handling. The metal content in vegetables from agricultural areas indicate 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 Main Village, Bahadurgarh, Sullar road, Khusropur, Bhanari and Thapar Technology Campus. Pot culture experiment showed that heavy metals were accumulated at high levels in these vegetables when irrigated with synthetic water containing high concentration of heavy metals.

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1. INTRODUCTION

World has seen significant changes in eating habits with the consumption of fresh produce increasingly becoming important in the daily dietary requirement of people. There has been a phenomenal increase in the demand for organically produced foods. Green leafy vegetables are the examples of minimally processed foods, which many times carry high risk of contamination and warrant maintenance of hygienic conditions during their production from farm to table.

In production and processing of fresh produce, quality and hygiene are the most important criteria for reducing consumer risk. Leafy vegetables are often eaten raw or with minimal processing and, if contaminated with pathogenic bacteria, may lead to health hazard. In recent years several surveys of food-born pathogens and indicator bacteria in these products have revealed their variable occurrence. Some reports are also available on the survival and transfer of the most emerging food borne pathogens. Contamination of vegetables may take place at all stages of production and processing and originate from sources like soil, faeces, water, ice, animals and harvesting and processing equipment. Soil fertilized with farmyard manure or sewage water poses a higher risk of contamination with pathogenic bacteria like *E.coli O157, Salmonella* and *Listeria monocystogens.* Scientific evidence suggests that *E.coli O157* can survive in avian manure up to 21 months, which means that it can also be a source of contamination. *Salmonella* has been detected in vegetables from farms, wholesale markets, supermarkets and small shops. For fresh cut vegetables that are eaten raw, there is no treatment that can be relied upon for decontamination.

Human exposure to soil borne pathogens is a matter of general health concern. Heavy metals and trace elements are found naturally in undisturbed soils and, in fact, are required for vital metabolic functions by plants. Rapid industrialization and extraction of metals from deposits has increased their levels in the environment including the soil. Many of these metals undergo chemical transformation during processing and find way to the effluent and waste dumps contaminating soil, air and ground water. The term heavy metal refers to any metallic elements that are mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb).

The metals originate in the soil from three major sources i) withering rocks from which soil is formed, ii) air carrying them as suspended particles and iii) disposal of contaminated water and solid waste. Metals are an indispensable part of our environment and play positive role in various bio*log*ical processes such as signaling, homeostasis and enzyme catalysis. However, at higher concentration, they tend to have toxic effects. Heavy metals impede functioning of liver, brain and lungs showing characteristic symptoms since they are prone to bioaccumulation and biomagnification along food chain. Vegetables growing around heavy metal threatened areas are at higher risk of accumulating toxic levels of heavy metals. Reports from Punjab and other places show high degree of heavy metal contamination in vegetables. The Buddha nullah is the most contaminated water body of Ludhiana City and the vegetables grown in its vicinity have alarming levels of the carcinogenic heavy metals, because they are irrigated and washed with the contaminated water from nullah. Scores of studies carried out at Punjab Agricultural University revealed that these vegetables carry a high lead of heavy metals. More so, in monsoon season these vegetables become a source of water-borne diseases like gastroenteritis, cholera and jaundice as the nullah receives the domestic sewage from surrounding inhabitation.

A survey on the nature and pollution level revealed the presence of alarming concentration of heavy metals, including lead, nickel and chromium, in the nullah water. After ingestion these metals get accumulated in soft organs of the body like liver and kidney and cause cancer over a period of time (The Tribune, July 27, 2004). Similarly 1,000 acres of farms in Mansa District are being irrigated with water mixed with sewage, containing high quantities of lead, zinc and other toxicants. The water contaminates the soil and the heavy metal gets accumulated in the vegetables posing a grave health hazard. In this case the wastewater comes from a sewerage plant at Sirsa road, near New Anaj Mandi and is used by the farmers at Khokhar, Ramditta, Chotti Khokhar, Vadi Khokhar and other villages (The Hindustan Times, January 20, 2005).

Therefore, in the current investigation metal content and pathogen analyses were carried out in randomly collected fresh farm produced samples of green leafy vegetables palak (*Beta vulgaris L*), fenugreek (*Trigonella foenum-graecum*) and coriander (*Coriandrum sativum*).

2. REVIEW OF LITERATURE

Urban food security in India is a matter of growing concern. Urban poverty is reflected in the nutritional status of the urban populace, whose intake of important nutrients frequently lies below the minimum recommended daily allowance. Over the past few decades there has been a change in the focus on nutritional health in India encompassing malnutrition to widespread chronic shortages of micronutrients, particularly iron and vitamin A. While these deficiencies are not directly life threatening, they cause serious functional disorders particularly in women and children. It is important to encourage the consumption of highly nutritious fruits and vegetables. Studies have shown that awareness is gradually increasing about the nutritional benefits of green leafy vegetables like palak. While we endeavor to increase the production and consumption of fresh vegetables, it is necessary that the consumer get safe food.

Soil acts as a sink for water pollutants especially the heavy metals. The heavy metals being immobile get accumulated in the soil endangering crops and vegetables (Athar and Vohra, 1995). Plants absorb and accumulate heavy metals from the soil and water, which up to certain level are essential for their growth and development. These metals include Mg, Fe, Mn, Zn Cu, Mo and Ni (Langille and Machean, 1976). These cannot be substituted by other elements, as they are specific to many biochemical processes. Co and Mo are needed for the activity of nitrate reductase and nitrogenase enzymes, Cu and Zn are integral to oxidases and anhydrases in proteins and carbohydrate metabolism. Fe mediates electron transport during photosynthesis and Mn is crucial for O_2 photoproduction. Ni is involved in hydrogenase and vanadium in lipid metabolism (Athar and Vohra, 1995). Certain plants also have the ability to accumulate heavy metals, having no known bio*log*ical consequence. These include Cd, Cr, Pb, Co, Erg, Se and Hg (Hanna and Grant, 1962; Baker and Brooks, 1989)

2.1 Uptake of heavy metals by plants

Heavy metals are mobile and can be taken up easily by the plants. They occur in the soil in soluble form as salts (Bloemen, *et al.*, 1995). However, only soluble, exchangeable and chelated metallic species in the soil are mobile and in available form (Arambarri, *et al.,* 1999). Mobility is an important factor in regulating the availability and solubility of heavy metals in the soil and soil solution. The mobility depends on their speciation in the soil, which in turn depends on parameters such as organic matter and mineral composition, pH and Eh of the soil (Boisson, *et al*., 1999). Mobility of heavy metals is also related to their immobilization in the solid soil. Metal accumulation in plant depends on plant species, growth stages and types of soil and metal, soil conditions, weather and environment (Zalidis *et. al.,* 1999)

2.2 Heavy metals and human health

Metal uptake by plants may pose risks to human health when vegetables are grown on or near contaminated areas. Several studies have indicated that vegetables, particularly the leafy crops, grown in heavy metals contaminated soils had high concentration of heavy metals than in uncontaminated soil (Guttormsen et al 1995; Dowdy and Larson, 1995). The health risk depends on the physical status and chemical composition of the waste material and type, quantity and part of the vegetable consumed. Although, some heavy metals are essential for human metabolism, at higher concentration they act as toxicants. Prolonged intake of unsafe concentration of heavy metals leads to disruption of numerous bio*log*ical and biochemical processes in the human body. Hg and Pb have been associated with the developmental abnormalities in children (Marshall *et. al.,* 2003).

The situation may become more alarming in vegetarians, for whom major part of the diet comprises fresh and green vegetables. Urbanized vegetarians are at higher risk due to very high level of pollution caused by industries, automobiles and reduced green cover (Cobb *et. al*., 2000). Toxic and nutrient element chart is given below.

Toxic and nutrient element chart

Metals	Sources	Daily intake (μg/d)	Symptoms of imbalance
Zinc (Zn)	Oysters, soybeans, wheat germ, seeds, nuts, lamb, beef, chicken, eggs, milk, yeast, root vegetables.	9-11	Deficiency may result in decreased growth, loss of taste smell, sterility, poor wound healing, hair loss, kidney disease, diabetes, nightblindness.
Iron (Fe)	Organ meats, poultry, shellfish, dark green leafy vegetables, molasses, and dried fruit. Legumes, nuts and seeds.	8-18	Microcystic anemia occurs late in deficiency. Plasma ferritin a good early indicator. Hemochromatosis and liver damage with toxic levels.
Magnesium (Mg)	Oatmeal, buckwheat, unpolished grains, nuts, dark green leafy vegetables, chocolate, milk	360-420	Deficiency results in muscle weakness. May be related to depression, hypertension and heart attack
Molybdenum (Mo)	Buckwheat, beans, grains, nuts, legumes, meats and vegetables.	43-45	High molybdenum along with low copper intake may cause copper deficits and resultant anemia. Low molybdenum may impair sulfation detoxification pathways.
Cadmium (Cd)	Shellfish, liver, tobacco, kidney meats. Nickel-Cadmium batteries, PVC plastics, fungicides.	0.0004	Hypotension, hypertension, fatigue, anemia, diarrhea, vomiting, nausea, emphysema. Target organs are the liver, placenta, kidneys, lungs, brain and bones.
Lead (Pb)	Dolomite soil, pesticides, batteries, cable coverings, pipes drains, hair coloring		Microcystic anaemia, glycosuria, cognitive dysfunction, anorexia, insomnia. Target organs include the brain, bone, blood, kidneys and thyroid gland.
Nickel (Ni)	Sludge, stainless steel manufacturing.	0.02	Dermatitis, allergies, increased inflammation.
Chromium (Cr)	Organ meats, fish, whole grains, cheese, mushrooms	24-35	Hypoglycemia and hyperglycemia improve with chromium supplementation. May be deficient in cardiovascular disease and prolonged stress.
Potassium (K)	Almonds, vegetables, milk, potatoes, lentil, beans, beef, palak	1600-5000	Deficiency: irregular/rapid heart beat, kidney disease, ovarian cysts, joint pains, hyperglycemia. Excess: Hypotension, reactive hypoglycemia.
Sodium (Na)	Ubiquitous in prepared foods, cheeses, canned food	1000- 3500	Excess: edema, hypertension, stroke dizziness, gout headache
Copper (Cu)	Organ meats, shellfish, whole grain products, seeds, nuts.	890-900	Toxic levels cause nausea, behaviour problems, vomiting and diarrhea.

Courtesy: Genova Diagnostics, 2004

2.3 Some major disasters caused by heavy metal contamination in the past

1932

Minamata

Sewage containing mercury was released by Chisso's chemical works into Minamata Bay in Japan. The mercury accumulated in sea creatures, leading eventually to mercury poisoning in the population.

1952

Minamata syndrome

In 1952, the first incidents of mercury poisoning appeared in the population of Minamata Bay in Japan, caused by consumption of fish polluted with mercury, bringing over 500 fatalities. Since then, Japan promulgated strict environmental laws for the industrialized world.

1986

Sandoz

Water used to extinguish a major fire carried c.30 t fungicide containing mercury into the upper Rhine. Fish were killed over a stretch of 100 km. The shock drove many FEA projects forward.

1998

Spanish nature reserve contaminated after environmental disaster

Toxic chemicals in water from a dam at a mine, contaminated the Coto de Donana nature reserve in southern Spain as mud containing large amount of sulphure, lead, copper, zinc and cadmium flew down to the river Rio Guadimer. Europe's largest bird sanctuary and Spain's agriculture and fisheries suffered permanent damage from the pollution.

2.4 Sources of contamination

Accumulation of heavy metals in the edible parts of the vegetables constitutes a direct pathway for their incorporation into the human food chain (AOAC, 1984). Heavy metals may be present as deposits on the plant surface, cell inclusions and components of cell organelles. For all these heavy metal loaded effluent forms the main source. Plants are able to take up metal in quantities more than their physio*log*ical needs. While this may not have adverse effect on plant itself, it may prove dangerous for the consumer (Athar and Vohra, 1995).

2.5 Pathogens in fresh produce

The normal practice of harvesting and collecting fresh vegetables does not have any effective microbes elimination step, resulting in minor to major contamination of potentially hazardous microorganisms. Storage conditions of the untreated harvested vegetables aggravate the problem by encouraging the growth of the contaminants. All these contaminating microorganisms are not linked with the produce-associated illness, but under right conditions, they may prove to be hazardous. Food borne illness depends upon the type, quantity and survival of the pathogen until the time of consumption. The infective dose (minimum numbers of organisms necessary to cause illness) is very low in many cases, which means that the microorganism needs to exist only and may not multiply in the produce. For example, many pathogenic bacteria, intestinal parasites and viruses multiply only in the human or animal body and the contaminated vegetables act merely as carriers of their propagules. Contamination of raw fruits and vegetables with pathogens is mainly caused by the domestic sewage, which carries a very heavy lead of coliform bacteria, intestinal parasites and viruses, contributed by the human faeces. It is estimated that on dry weight basis as much as 40 % of human excreta is constituted by microbes. Characteristics of some microbial pathogens linked to outbreaks of produce-associated illness are given below.

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

Microorganism	Incubation Period	Symptoms	Infectious Dose	Source
Clostridium botulinum	12-36 hr	Nausea, vomiting, fatigue, dizziness, dryness of mouth and throat, muscle paralysis, difficulty swallowing, double or blurred vision, drooping eyelids, and breathing difficulties	Intoxication growth and toxin production in food	Soil, lakes, streams, decaying vegetation, reptiles
Escherichia coli O157:H7	2-5 d	Bloody diarrhea, abdominal pain. Can lead to hemolytic uremic syndrome and kidney failure especially in children and the elderly	10-1000	Bloody diarrhea, abdominal pain. Can lead to hemolytic uremic syndrome and kidney failure especially in children and the elderly
Salmonella spp.	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
Listeria monocytogenes	1 d to 5 or more wk	Febrile gastroenteritis in healthy adults; may lead to spontaneous abortion or stillbirth in pregnant women; severe septicemia and meningitis in neonates and immunocompromised adults; mortality may be 20 to 40 %.	Unknown dependent upon health of individual	Soil, food processing environments

Courtesy: (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001)

2.6 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 protection 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).

2.7 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 walls 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 leaves from precipitation, dew and irrigation may promote their survival and multiplication (Blakeman, 1981; Andrews 1992; Beattie and Lindow 1995, 1999). Certain conditions, such as sunlight, particularly the shorter ultraviolet wavelengths, can damage bacterial cells (Webb 1976; Jagger 1981; Sundin and others 1996; Sundin and Jacobs 1999). Frequent exposure to fast fluctuating environmental conditions, sometimes induces stress compatibility and results in the development of hardy pathogens. High ultraviolet tolerance in *Pseudomonas syringae* and high osmotic potentials in *Escherichia herbicola* are some such examples. The results suggest that human pathogens are less likely to develop stress resistance (O'Brien and Lindow, 1988). Many of the human pathogens have an enteric source and therefore may be unsuccessful as plant surface colonizers as compared to the more suited plant microbial populations (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001).

2.8 Bacterial pathogens of concern

Escherichia coli

Enterotoxigenic *E. coli* is a common cause of travelers' diarrhoea, an illness invariably experienced while visiting the developing countries. Enterohemorrhagic *E. coli* O157:H7 is recognized as an important food borne pathogen. The infectious dose is very low and sequential to gastroenteritis can include bloody diarrhoea (hemmorrhagic colitis) and hemolytic uremic syndrome. It grows rapidly in several types of raw fruits and vegetables, particularly when stored at 12°C (53.6°F) and above. (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001).

Salmonella sp.

The genus *Salmonella* has over 2700 serotypes. Animals and birds are the natural reservoirs. Surveys of fresh produce have revealed the presence of several *Salmonella* serotypes capable of causing human infection. (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001).

Shigella sp.

Shigellosis is usually transmitted from person-to-person but can also occur through consumption of contaminated water and foods, including fruits or vegetables that have received little or no heat treatment. Several outbreaks of shigellosis have been attributed to the consumption of contaminated raw vegetables. (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001).

Staphylococcus aureus

Staphylococcus aureus has been detected on fresh produce and ready-to-eat vegetable salads and is transmitted through food handlers. However, enterotoxigenic *S. aureus* does not compete well with other microorganisms normally present on fresh produce, so incipient spoilage caused by nonpathogenic microbiota would likely precede the development of high populations of this pathogen (U. S. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 2001).

3. MATERIALS AND METHODS

3.1 Collection of leafy vegetable samples

Samples of green leafy vegetables were collected randomly from vendors in and around Patiala City and from agricultural areas of Main Village, Bahadurgarh, Sullar road, Khusropur, Bhanari and Thapar Institute of Engg. and Tech. Campus. Vegetables chosen for the study were: palak (*Beta vulgaris* L), Fenugreek or methi (*Trigonella foenum-graecum*) and Coriander (*Coriandrum sativum*).

3.2 Determination of trace elements and heavy metals in plant samples.

Vegetable samples were washed in running tap water then with distilled water and were carefully dried in oven at 70 ^{o}C for 24 hr. Preweighed samples were ground in a pestle and mortar followed by wet digestion with HNO_3 and $HClO_4$ (Tappi Test method, 1989) in the ratio 3:1. The samples were digested on a hot plate at a temperature corresponding to 100^{o}C for 3-4 hrs. Heating was done such that it did not boil. Heating was done till it dried up completely and whitish brown dry mass was obtained. It was then cooled and the precipitate/digest mixture was extracted in acid water mixture (HCl: distilled water in the ratio 3:1) and filtered through whatman filter paper No 42, volume was made up to 50 ml. The filtrate was analyzed for metal content using AAS (GBC 932 AA). The various metals along with their sensitivity limits are as follows: Cr -0.05 μg ml^{-1}, Fe- 0.05 μg ml^{-1}, Ni- 0.04μg ml^{-1}, Pb- 0.06 μg ml^{-1}, Zn-0.008 μg ml^{-1}.

3.3 Pot culture experiment

Uptake of heavy metals by plants in a metal contaminated and normal soil was studied in pot culture experiment. Vegetables palak, fenugreek and coriander were grown in earthen pots (12”diameter) in two replication and were irrigated with synthetic effluent containing heavy metals in following concentrations: Fe -150.00 ppm, Zn- 50.00 ppm, Cr- 5.00 ppm, Cd- 5.00 ppm, Cu-2.50ppm, Pb- 2.50ppm, Ni-2.50ppm. In controls normal water was used for irrigation. The plants were grown for a period of three months from January to March 2005 and were then harvested completely. The soil in the pots was tilled and through porous soil, plants were carefully removed with their roots. The plants from each pot (even in case of replicates) were kept in separate glass vessel. After this these plants were thoroughly washed under running tap water so that no soil particles remained. The roots and shoots were then separated carefully from each other. The excess water was shedded off and with the help of tissue paper they were dried. Root and shoot from all these plants were weighed and were then kept for overnight drying at a temperature of 80^{o}C overnight. They were then again weighed. After this root and shoot were ground with the help of pastel and mortar and 1 g of the grounded sample was used for digestion purpose. After digestion these were analysed for heavy metals.

3.4 Analysis of nursery soil

Soil characterization was done for physical and chemical parameters. pH and electrical conductivity (Jackson, 1967) was be determined by soil, suspension method with a soil water ratio of 1:2. Organic carbon content was determined as per the protocol (Walkely and Black, 1934), available N, P, S (Jackson, 1967). Analysis of heavy metals was done in HNO_3/$HClO_4$ digested samples (Tappi Test method, 1989) and analysed by Atomic Absorption Spectrophotometer (GBC 932).

3.5 Determination of available sulphur in soil (Jackson, 1967)

10 g of dried and sieved soil was taken in a 50 ml of conical flask. 0.15 % $CaCl_2$ solution was added and a flask was kept on shaker at 120 rpm for 30 minutes. The solution was then filtered through whatman filter paper 42. 20 ml of the filtrate was taken in a vol. flask and added 1 g $BaCl_2$. Solution was swirled and added 1 ml of 0.25 % solution of gum acacia and made the final volume to 25 ml. The solution was shaken well and then OD was taken at 420 nm.

Calculations

Weight of soil taken	= 10 g
Volume of extractant added	= 50 ml
First dilution	= 5 times
Volume of aliquot taken	= 20 ml
Final volume	= 25 ml
Second dilution	= 1.25 times
Total dilution = 5x 1.25 times	= 6.25 times
Absorbance reading = A	
ppm of S from standard curve against A value	

ppm of S in soil = Yx6.25 ppm
Kg of S/ha = ppmx2.24

3.6 Determination of organic carbon in soil (Walkely and Black (1934) rapid titration method)

1.0 g of 0.2 mm sieved soil was taken in dry 500 ml conical flask.10 ml of 1 N $K_2Cr_2O_7$ was pippetted and swirled for a while, then 20 ml of H_2SO_4 was run. The flask was allowed to stand for 30 minutes and then 200 ml of distilled water was added. After incorporation of 10 ml of ortho-phosphoric acid and 0.5 gm of NaF and 1 ml of diphenylamine indicator, the contents were titrated with 0.5 Ferrous ammonium sulphate solution till the colour flashes from blue violet to green. A blank was also run without soil.

Calculations

Organic C (%) = 10(B-T) x0.003x100/wt of soil x B
B = Vol. in ml of Ferrous ammonium sulphate solution required for blank titration.
T = Vol. in ml of Ferrous ammonium sulphate solution required for soil sample.

Organic (C) = 10(B-S)/B x (0.003x100/wt of sample)

3.7 Determination of available nitrogen in alkaline soil (Jackson, 1967)

In around bottom flask, 5 g of the soil was taken. To this 5 ml of water was added followed by 2.5 ml each of 0.32% $KmnO_4$ and 2.5% NaOH. Glass beads were added to prevent bumping during boiling. The contents were distilled in a Kjeldhal assembly at a steady rate and liberated ammonia was collected in a conical flask containing 5 ml of boric acid solution. With the absorption of ammonia the pinkish colour turned to green. The distillate was collected and was titrated with 0.02 N H_2SO_4 to the original shade (pinkish).

Calculation

Mineralizable N (kg/ha)= $Rx0.02x1/5x0.014x2.24x10^6$ = Rx1.36
Where R = volume of 0.02 N H_2SO_4 required for titration.

3.8 Determination of available phosphorous (Jackson, 1967) (Suitable for saline alkaline soil)

Reagents:

1. Extracting solution: (0.5M $NaHCO_3$)
   84 g $NaHCO_3$ was dissolved in distilled water and made up to 2L
   (Adjusted pH 8.5 with 1M NaOH)

2. Reagent A:
   12 g ammonium molybdate was dissolved in 250 ml of distilled water. 0.2908 g antimony potassium tartarate was dissolved in 100 ml of distilled water. Added these two solutions in 1000 ml of 2.5M H_2SO_4 and made the volume to 2000 ml with distilled water.

3. Reagent B:
   1.056 g ascorbic acid was dissolved in 200 ml of reagent A and mixed well.

4. Sulphuric acid (2.5 M)
   140 ml of concentrated H_2SO_4 was diluted to 1 L with distilled water.

5. Stock standard P solution (50 mg/L P)
   0.2195 g of KH_2PO_4 was dissolved in 1 L distilled water.

6. Working standard P solution (1 mg/L)
   20 ml of solution was diluted to 1 L.

Procedure for available phosphorous

2.5 g of dried and sieved soil was taken in 100 ml flask to which 50 ml of extracting solution was added and was kept on shaker at 120 rpm for 30 minutes. The solution was filtered through whatman 42 filter paper. 10 ml of the aliquot of the filtrate was transferred to a 100 ml beaker. To it, added 15.5 ml distilled water and 1 ml of 2.5 M H_2SO_4 and 8 ml of reagent B were added. Then 15.5 ml of distilled water was added. Similarly blanks were prepared. OD was taken at 882 nm within 10 minutes of sample preparation.

Calculations

P in soil (mg/kg) = P in extract (mg/L) x 20(the standard soil to solution ratio) X 5 (dilution factor).

3.9 Determination of heavy metals (Tappi test method, 1989)

Reagents:

Concentrated HNO_3

Perchloric acid ($HClO_4$)

Procedure:

1. Took 1 g of soil sample in 150 ml beaker.
2. Slowly added 15 ml of HNO_3.
3. Slowly added 5 ml of $HClO_4$ and a few glass beads and heated on a hot plate such that no boiling occurred. Since there is generation of fumes so the digestion was carried out in a digestion chamber. Heating was done till the contents dried up and turned into dry white dry mass.
4. The samples after digestion were treated with acid water mixture and filtered through whatman filter paper No 42.
5. Cooled it and made the final volume to 50 ml with 50% HCl i.e. HCl: D/W (1:1).
6. The filtrate was analyzed for metal content using AAS (GBC 932 AA).

3.10 Enumeration of bacterial pathogens in green leafy vegetables

Portion of vegetables weighing 25 gm were diluted as 1:10 with 50 ml of sterile Butterfield's phosphate buffer which were subsequently diluted fivefold with the same. Appropriate dilutions were then enumerated by plating on suitable medium.

1. Total aerobic bacterial count was done on Tryptone Soya Agar medium: 25 g of vegetable samples were added to 50 ml Butterfield's Phosphate Buffer and washed in it for about 5 minutes further serially diluted the sample in Butterfield's Phosphate Buffer, appropriate dilutions were spread on Tryptone Soya Agar plates in triplicates. All plates were incubated at 37^oC for 24-48 hrs. Colonies in each plate were counted, averaged and expressed as cfu/g and converted to log.
2. Total faecal coliform was enumerated using Violet Red Bile Agar medium: For enumerating the total coliforms, appropriate dilutions were, prepared from samples as above, were spread plated on to Violet Red Bile Agar plates in triplicates. All plates were incubated at 37^oC for 24-48 hrs. Violet coloured colonies were counted and results were expressed as cfu/g and converted to log.

1. Butterfield phosphate buffer

Stock solution
KH_2PO_4 = 34 gm/L
Distilled water = 500ml
Adjusted the pH = 7.2 with 1N NaOH and made the volume to 1 L with distilled water and autoclaved for 15 minutes at 121^oC.

2. Tryptone Soya Agar

Composition:

Ingredients	g/L
Casein enzymatic hydrolysate	17.00
Papaic digest of soybean meal	3.00
NaCl	5
Dipotassium phosphate	2.50
Dextrose	2.50
Agar	15.00
Distilled water	1L
Final pH (at 25^oC)	7.2

3. Violet Bile Salt Agar

Composition:

Ingredients	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 (before adding agar). The contents were heated in a microwave oven with continuous agitation until boiling started and agar was melted properly.

4. RESULT AND DISCUSSION

4.1 Metals in random samples of green leafy vegetables

In randomly collected samples of palak (*Beta vulgaris L*), coriander (*Coriandrum sativum*) and fenugreek (*Trigonella foenum-graecum*) from 34 different vendors within the city of Patiala different heavy metals Fe, Zn, Pb, Cr, Cd and Ni were analysed. Leafy vegetables were preferred for sampling since past research indicates that they accumulate heavy metals at a greater capacity than other vegetables (Jinadasa *et al.,* 1997). The study showed that the concentration of metals greatly varied in palak collected from different vendors (Table 1). Among various elements iron ranged from 24.7 to 291.5 mg/Kg. Zn varied from 6.4 to 64.5 mg/Kg, Pb from less then 0.06 to 60.4 mg/Kg, Cr from less then 0.05 to 17.8 mg/Kg, Cd from less then 0.009 to 1.8 mg/Kg and Ni from less then 0.004 to 6.7 mg/Kg. Statistical analysis showed that the trend for the heavy metals in palak samples was in the order of Fe>Zn> Pb> Cr> Ni> Cd.

In coriander iron ranged from 28.0 to 372.5 mg/Kg and Zn from 3.3 to 65.9 mg/Kg (Table 2). Lead ranged from less then 0.06 to 47.8 mg/Kg, Cr from less then 0.05 to 72.5 mg/Kg, Cd from less then 0.009 to 1.7 mg/Kg and Ni from less then 0.04 to 33.5 mg/Kg. The trend observed from the mean values was Fe> Zn> Pb> Cr> Ni> Cd.

In fenugreek samples Fe concentration varied from 23.3 to 449.5mg/Kg, Zn from 9.6 to 79.7 mg/Kg, Pb from less then 0.006 to 49.2 mg/Kg, Cr from 0.60 to 53.4 mg/Kg, Ni from less then 0.004 to 21.5 mg/Kg and Cd from less then 0.009 to 8.00 mg/Kg (Table 3). The statistical analysis indicated that the various metals were in the order of Fe> Zn> Pb> Cr> Ni> Cd.

In all the three green leafy vegetables (Table1 3) a similar trend in metal content was observed i.e., Fe>Zn>Pb>Cr>Ni>Cd however their mean values in all vegetables were different. Iron was found to be highest in fenugreek with a mean of 262.1 mg/Kg followed by coriander with a mean of 211.0 mg/Kg and palak 198.1mg/Kg. But Zn concentration was highest in palak with a mean of 41.7 mg/Kg followed by coriander 37.3 mg/Kg and fenugreek 35.9 mg/Kg.

The order of vegetables according to their metal content along with their mean was

Fe: Fenugreek (262.1mg/Kg)> Coriander (211 mg/Kg)>Palak (198.1mg/Kg)

Zn: Palak (41.7 mg/Kg)> Coriander (37.3)>Fenugreek (35.9mg/Kg)

Pb: Palak (23.8mg/Kg)> Coriander (16.6mg/Kg) >Fenugreek (14.8 mg/Kg)

Cr: Coriander (14.2 mg/Kg)> Fenugreek (8.5mg/Kg) > Palak (7.5mg/Kg)

Cd: Fenugreek (1.3 mg/Kg) > Palak (1.02 mg/Kg) >Coriander (0.49 mg/Kg)

Ni: Palak (2.5 mg/Kg)> Fenugreek (3.4 mg/Kg)> Coriander (5.8 mg/Kg)

The metal content in all the vegetables was above the concentration as defined by nutritiondata.com and elook.org. The Fe concentration in palak ranged from 24.7 mg/Kg to 291.5 mg/Kg in our studies whereas the actual concentration is 26 mg/Kg as reported by nutritiondata.com.

In coriander iron concentration varied from 28.05 mg/Kg to 372.5 mg/Kg whereas the actual concentration as reported by elook.org was 0.425 mg/Kg. In fenugreek samples iron ranged between 23 to 449.5 mg/Kg and the actual concentration as reported by elook.org is 333.5 mg/Kg. The daily intake of Fe by human body ranges between 0.08 to 0.18 μg 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).

In palak concentration of Zn has been reported to be 5.3 mg/Kg (nutrition.com) whereas in these random samples it was found to be in the range of 6.4 to 59.6 mg/Kg. Coriander normally had Zn in the concentration of 0.047 mg/Kg (elook.org) but in random samples of coriander it was been observed to be in the range of 3.3 mg/Kg to 65.9 mg/Kg. In fenugreek also the normal concentration of Zn was 0.025 mg/Kg (elook.org) but the studies showed the range to be as low as 9.6 mg/Kg to as high as 63.3 mg/Kg. As reported by Genova Diagnostics, concentration above 9.0 to 11.0 mg/Kg per day can cause kidney disease, hair loss and skin rash. Variation in metal content in random samples indicates that the vegetables were grown in soils irrigated with metal contaminated water or may be due to high metal content in ground water and soil. Since many samples at serial no. 26-28, the Pb, Cr, Ni were below detection limit indicating that they were grown in soils having no contamination with heavy metals and thus are safe for human consumption.

The maximum level allowed by Prevention of Food Adulteration Act (PFA), India for Pb, Zn, Cd is 2.5, 50 and 1.5 mg/Kg. The study showed that only 32.2 % of palak 26.4

% of coriander and 23.5 % of fenugreek samples had Pb below 2.5 mg/Kg as recommended by PFA. In 61.6 % of palak, 82.3 % of coriander and 82.5 % of fenugreek samples Zn was found to be lower then 50 mg/Kg. All samples of palak and 94.1 % of coriander and 91.1 % of fenugreek had Cd less then 1.5 mg/Kg as recommended by PFA. Over all almost 68-74% of green leafy vegetables were found to have high Pb content much beyond the safe limits, nearly 18-39 % samples had high Zn whereas 6-9% samples had high Cd as per PFA limits.

4.2 Heavy metals in vegetables collected from selected villages around Patiala City

Vegetables were collected from different agricultural fields around Patiala City to study the metal concentration in the fresh farm produce. The concentration of iron in palak (Table 4 a) was found to be as high as 378.4 mg/Kg in Main village (S3) to as low as 266 mg/Kg in samples from Khusropur village (S5). Zn ranged from 52.2 mg/Kg in Khusropur (S5) to 75.5 mg/Kg in samples from Main village (S3), Pb varied from 1.1 mg/Kg in samples from Main village (S4) to 17.9 mg/Kg in samples from Bahadurgarh (S1) and Cr from 1.6 mg/Kg in samples collected from Thapar campus (S8) to 15.6 mg/Kg in samples from Bahadurgarh (S1), Ni from 2.8 mg/Kg in Main village samples (S4) to 42.0 mg/Kg in Sullar road samples (S6) and Cd from 0.5 mg/Kg in Khusropur village samples (S5) to 1.3 mg/Kg in samples collected from Main village (S3) and Khusropur (S5). Statistical analyses showed that the order of content in palak was Fe > Zn> Cr > Pb = Ni > Cd.

Coriander samples showed iron concentration in the range of 168 mg/Kg in samples from at Thapar campus (S8) to 399.5 mg/Kg in samples from Main village (S3) and Khusropur village (S5), Zn between 52.65 mg/Kg in Thapar campus samples (S8) to 75.0 mg/Kg in Khusropur samples (S5), Pb between 2.5 mg/Kg in Bahadurgarh samples (S1) to 16.2 mg/Kg in Thapar Campus samples (S8), Cr between 11.3 mg/Kg in Khusropur samples (S5) to 26.1 mg/Kg in Thapar Campus sample (S8). Cd between 0.6 mg/Kg at Khusropur (S5) to 1.5 mg/Kg at Thapar Campus (S8) and Ni between 1.2 mg/Kg in Thapar Campus samples (S8) to 5.4 mg/Kg in Bahadurgarh samples (S1) (Table 4.c). Trend in metal content was Fe> Zn> Cr> Pb> Ni> Cd.

Fenugreek had an iron varying between 197.9 mg/Kg in Thapar Campus samples (S8) to 408.4 mg/Kg in samples from Sullar road (S6), Zn was between 42.1 mg/Kg in Sullar road samples (S6) to 59.4 mg/Kg in Thapar campus samples (S8), Pb between 5.0 mg/Kg in Bhanri samples (S7) to 10.6 mg/Kg in Sullar road samples (S5), Cr between 10.5 mg/Kg in samples from Bhanri (S7) to 12 mg/Kg in Khusropur samples (S5), Ni was 4 mg/Kg in Khusropur (S5) and Bhanari (S7) and 1.8 mg/Kg in Sullar road (S6) and Cd was 1.6 mg/Kg in Bhanri (S7) and 0.6 mg/Kg at Khusropur (S5) (Table 4 b). According to their mean values the metal content in fenugreek was in the order of Fe> Zn> Cr > Pb > Ni > 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 Sullar road samples had high concentration of Fe and Ni. Samples from Main village (S3) had high concentration of Zn whereas Cd and Pb were high at Thapar Campus (S8) and Bahadurgarh (S1) and Cr was high in samples collected from Bahadurgarh (S1). 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 predominantly 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 emissions has also been identified as an important pathway of heavy metal contamination in vegetable crops (Bassuke 1986; Salim *et al.* 1992).

4.3 Pot culture experiment

In pot culture experiment leafy vegetables i.e., palak, coriander and fenugreek were grown to study the uptake potential for different metals Fe, Zn, Pb, Cu, Ni, Cd, Cr (Table 5 a). These plants were grown in alkaline soils, which were irrigated with metal containing water (treated) and normal water (control).

In palak (*Beta vulgaris L)* uptake of iron was found to be highest. In control, which was irrigated with normal water, the shoot had 160.5 mg/Kg of Fe and root 400.2

mg/Kg. In treated ones irrigated with metal containing synthetic effluent, Fe was 185.8 mg/Kg in shoot while in root was 711.5 mg/Kg showing significant increase in metal uptake. Zn was found to be 133.5 mg/Kg in shoot and 225.1 mg/Kg in root in control. In treated plants Zn was 267.0 mg/Kg in shoot and 30.4 mg/Kg in root. Pb was observed to be 7.1 mg/Kg in shoot and 6.2 mg/Kg in root in control and 15.2 mg/Kg in shoot and 6.3 mg/Kg in root in treated ones. Cu was 7.65 mg/Kg in shoot and 7.62 mg/Kg in root in control. Shoot and root in treated plants showed Cu concentration of 9.9 mg/Kg and 7.2 mg/Kg respectively. Cr in case of control in shoot was 3.5 mg/Kg and 7.4 mg/Kg in root as against 18.2 mg/Kg in shoot and 52.1 mg/Kg in root in treated ones. Cd in control plants was found to be 1.2 mg/Kg in shoot and 1.1mg/Kg in root. In treated Cd in shoot was 6.9 mg/Kg and in root was 9.2 mg/Kg. Heavy metals in the shoot of control plants was in the order of Fe> Zn> Cu> Pb > Cr > Ni > Cd and in treated ones the trend was Zn>Fe>Cr>Pb>Cu>Cd>Ni. Heavy metals in root followed the following order for control Fe> Zn>Cu >Cr >Pb >Cd >Ni and for treated plants the trend was Fe > Zn >Ni > Cr > Cd > Cu > Pb (Table 5 b).

In coriander (*Coriandrum sativum)* (Table 6 a) iron in shoot was 295.8 mg/Kg while in root it was 866 mg/Kg in control. Treated plants had 317.2 mg/Kg Fe in shoot and 858.0 mg/Kg in root. Zn in control showed 158.2 mg/Kg in shoot and 515.5mg/Kg in root while treated plants were found to be 237.1 mg/Kg in shoot and 516.8 mg/Kg in root. Cu in shoot and root in control was 4.02 mg/Kg and 9.9 mg/Kg respectively while in treated ones in shoot and root it was 14.07 mg/Kg and 10.8 mg/Kg. Cr was 6.7 mg/Kg in shoot and 6.1mg/Kg in root in control and 17.7 mg/Kg and 44.4 mg/Kg in shoot and root in treated. Pb was 7.2 mg/Kg and 3.3 mg/Kg in shoot and root in control, while 14.7 mg/Kg and 21.4 mg/Kg in shoot and root of treated plants respectively. Cd in control was 1.2 mg/Kg in shoot and 1.5 mg/Kg in root. Treated plants had 6.7 mg/Kg and 14.4 mg/Kg of Cd in shoot and root. Controls showed a concentration of 17.9 mg/Kg and less then 0.04 mg/Kg of Ni metal in shoot and root while treated plants had 11.0 mg/Kg and 19.6 mg/Kg of Ni in shoot and root respectively. The order of heavy metals in the shoot of control was in the order of Fe> Zn> Cu> Cr > Pb > Cd > Ni and for treated the trend was Fe > Zn > Cr > Pb > Cd > Ni. Heavy metals in the root were in the order of Fe> Zn> Cu > Cr > Pb > Cd > Ni and for treated plants the trend was Fe > Zn >Cr > Pb > Ni > Cd >Cu (Table 6 b).

Fenugreek (*Triqonella foenum- qraecum)* plants were found to have 168 mg/Kg and 476 mg/Kg of iron in shoot and root in control and 190.6 mg/Kg in shoot and 453.2 mg/Kg in root in treated ones. Zn in shoot was 82.5 mg/Kg and 110.7 mg/Kg in root of control plants while, treated plant root and shoot had 148.7 mg/Kg and 452.2 mg/Kg. Cu in control shoot and root was 6.4 mg/Kg and 16.6 mg/Kg while, 6.2 mg/Kg in shoot and 11.7 mg/Kg in root in treated plants. Cr was found to be 5.7 mg/Kg in shoot and 19.7 mg/Kg in root in control and 13.9 mg/Kg in shoot and 32.0 mg/Kg in root in treated ones. In controls Pb was found to be 4.0 mg/Kg in shoot and 8.6 mg/Kg in root while 11.0 mg/Kg and 11.3 mg/Kg in shoot and root in treated. Cd was 0.8 mg/Kg in shoot and 9.5 mg/Kg in root in control, while treated plants had 4.0 mg/Kg and 10.3 mg/Kg of Cd in shoot and root. Ni was 4.9 mg/Kg and 10.1 mg/Kg in shoot and root in controls and 8.6 mg/Kg and 23.0 mg/Kg in shoot and root in treated ones (Tables7 a). The order of heavy metals in the shoot of control was in the order of Fe>Zn>Cu>Cr>Ni>Pb>Cd and for treated the trend was Fe> Zn>Cr>Pb>Ni>Cu>Cd. Heavy metals in root followed the given order in control Fe>Zn>Ni>Cr>Cu>Cd>Pb and for treated plants the trend was Fe>Zn>Cr>Cu>Pb>Cd >Ni (Table 7 b).

Concentration of all the metals Fe, Zn, Pb, Cr, Cu, Cd and Ni was higher in irrigation with synthetic metal containing effluent. 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).

Uptake of these metals was higher in root in all the vegetables as compared to shoot, also the uptake potential of all the three leafy vegetables varied from each other. Metal content and ratios of nutrients differ in different parts of the plant and also among different plant species (Jarvan and Poldma, 2004). The uptake potential of palak shoot and root for Fe was highest followed by fenugreek and coriander. Zn uptake was found to be high in shoot of palak as compared to other vegetables but root of fenugreek was found to have high uptake potential for Zn than other two. Pb uptake in shoot of all three vegetables was nearly same but uptake by root of coriander was highest followed by fenugreek and palak. Cr uptake was in high concentration in shoot and root of palak followed by coriander and fenugreek. Cu uptake was observed to be highest by palak shoot followed by coriander and fenugreek. But root of coriander showed higher uptake of Cu as compared to palak and fenugreek. Cd concentration was found to be highest in roots than shoots. A large proportion of cadmium was known to accumulate in root tissues, especially when plants were grown in contaminated soil (Pendias, 1992). Coriander shots had highest concentration of Cd as compared to palak and fenugreek. Coriander shoot showed no Ni uptake but root showed highest uptake followed by fenugreek and palak.

4.4 Analysis of soil

Nursery soil was analysed for physico-chemical parameters of the nursery soil were also tested (Table 8). pH of the soil was 7.8 and EC was 146 $\mu S\ cm^{-1}$, organic carbon was 0.006% and clay + silt was 54.3%. Available nitrogen was 0.003%, phosphorous was 1.42 mg/Kg and potassium was 25.5 mg/Kg and sulphur was 0.43 mg/Kg.

Heavy metal analysis was done for soil before (nursery soil) and after pot culture trial i.e. control (irrigated with normal water) and treated (irrigated with synthetic water) (Table 9). Fe concentration in nursery soil was 1975 mg/Kg while in control soil in pots in which fenugreek was grown was 2037.5 mg/Kg, for palak and coriander grown soil it was 2707.5 and 2552.5 mg/Kg. In treated soil in fenugreek grown soil Fe was 2255 mg/Kg and in palak and coriander soil it was 2435 and 2177.5 mg/Kg respectively. Zn in nursery soil was 30.2 mg/Kg while in control soils with fenugreek palak and coriander grown plants Zn was 161.2, 113.0 and 151.7 mg/Kg against the treated soil with 85.0, 92.7,106.2 mg/Kg respectively. In nursery soil Cu was 5.8 mg/Kg and in control for fenugreek grown soil it was 4.4 mg/Kg and 5.5 and 5.1 mg/Kg for palak and coriander grown soil. In treated soil in fenugreek, palak and coriander grown soil Cu was 5.8, 2.3 and 5.1 mg/Kg respectively. Cr in nursery soil was 15.7 mg/Kg, in control in fenugreek, palak and coriander grown soil it is 14.0 mg/Kg, 13.3 mg/Kg and 12.7 mg/Kg respectively. Treated for fenugreek, palak and coriander grown soil Cr was 13.2, 14.3 and 15.5 mg/Kg. Pb in nursery soil was 19.9 mg/Kg and in control fenugreek, palak and coriander grown soil Pb was 8.2 mg/Kg, 6.4 mg/Kg and 0.6 mg/Kg while in treated for same plants it was 9.3 mg/Kg, 9.1 mg/Kg and 1.7 mg/Kg respectively. In nursery soil Cd was 9.0 mg/Kg. Control for fenugreek, palak and coriander grown pot soil Cd was 0.6 mg/Kg, 0.8 mg/Kg and 9.0 mg/Kg. Treated soil for fenugreek, palak and coriander was 1.4, 4.0 and 11.2 mg/Kg. Ni in nursery soil was 22.8 mg/Kg and 11.4, 4.9 and 11.9 mg/Kg in fenugreek, palak and coriander grown control soil in control while in treated fenugreek grown soil Ni was 13.9 mg/Kg and 8.6 mg/Kg and 14.6 mg/Kg in palak and coriander grown soil.

The metal concentration in nursery soil (before trial) was same as in control in pot soil (after trial). But inconsistency was found for Zn and Cd. The treated soil had much more higher concentration then the control soil but in case of Zn same order was not followed in all the three vegetables, similar inconsistency was found for Fe in palak and coriander grown soil. Cu also showed high concentration in control as compared to treated in case of palak grown soil. The uptake of metals by plants depended on the availability of metals from the soil and, the amount of metals in plants would reveal the availability of metals in 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).

4.5 Bacterial pathogen in randomly collected samples of green leafy vegetables

Randomly collected samples of palak, coriander and fenugreek from 34 different vendors located in the city were directly enumerated for bacterial pathogen 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 10).

In coriander the log cfu/g of total bacterial count varied from 4.4 to too numerous to count (TNTC), while coliforms were observed to be present in negligible number to TNTC. The cfu /g value of coriander samples was in the range of 3 to TNTC for total bacterial count and between nil to TNTC for coliforms. Fenugreek also showed total bacterial count in terms of log cfu/g in between 4.3 to TNTC for total bacteria present and between nil to TNTC for coliforms, the cfu/g value ranged between 2 to TNTC for total bacterial count and between nil to 80 for coliforms. In terms of log cfu/g palak showed a variation between 3.6 to TNTC for total bacterial count and between nil to TNTC for coliforms. The cfu/g values of palak ranged between 0.4 -3900 for total bacterial count and between 0.08 -520 for coliforms.

The mean values followed following order for coliforms and total bacterial count for the three vegetables. Total bacterial count was highest in fenugreek followed by palak and coriander and coliforms were highest in coriander followed by palak and fenugreek.

The number of pathogens found on the surface of palak, fenugreek and coriander showed a great variation. The count does not depend upon the vegetable type rather it depends on the survival and growth of pathogen 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 (Blakeman, 1981, Andrews 1992, Beattie and Lindow 1995, 1999). 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. On harvesting, contamination could be caused by improperly cleaned harvesting equipment, wash water and cross contamination from other vegetables. Proper storage, control of temperature and hygiene are important in limiting contamination and growth of pathogens. High total bacterial count and coliforms in some samples especially coriander indicates that they were irrigated by domestic sewage and wastewater.

Table 1. Heavy metals (mg/Kg) in random samples of palak

S.No	Pb	Zn	Cd	Cr	Ni	Fe
1	19.95	42.25	<0.009	0.15	<0.004	191.45
2	32.10	53.75	<0.009	0.05	<0.004	230.90
3	35.20	26.15	0.35	14.55	<0.004	158.35
4	41.75	40.75	0.85	6.75	<0.004	279.65
5	51.75	32.05	0.95	7.15	<0.004	105.45
6	50.05	50.30	1.10	6.60	0.70	162.35
7	47.00	6.60	1.60	6.45	1.35	275.05
8	27.95	6.45	1.50	13.45	1.05	274.50
9	46.30	13.45	1.85	15.95	<0.004	179.65
10	53.30	15.95	1.25	17.80	2.80	178.45
11	58.15	54.25	0.80	10.35	2.30	54.25
12	60.45	63.70	1.25	11.15	2.25	63.70
13	13.30	26.05	0.80	8.25	<0.004	26.05
14	13.95	24.70	1.25	7.20	5.45	24.70
15	13.50	54.00	1.60	15.05	5.75	197.20
16	15.05	42.95	1.45	9.40	6.70	312.50
17	9.60	44.85	1.05	9.95	6.15	199.00
18	16.00	46.55	1.25	10.15	3.60	221.25
19	14.40	56.95	1.50	9.70	3.20	199.45
20	0.45	40.70	1.20	9.75	1.30	247.40
21	<0.06	57.75	1.25	9.85	3.95	247.60
22	<0.06	58.65	1.20	<0.05	5.00	291.50
23	<0.06	50.05	1.50	<0.05	<0.004	67.40
24	2.45	24.35	1.00	<0.05	0.50	242.75
25	1.50	31.40	0.045	1.35	<0.004	271.70
26	<0.06	64.55	<0.009	4.60	<0.004	211.35
27	<0.06	64.55	<0.009	1.60	0.05	251.10
28	<0.06	59.60	<0.009	5.00	0.50	217.00
29	2.40	19.90	<0.009	5.65	1.05	175.40
30	<0.06	48.45	<0.009	<0.05	<0.004	272.05
31	5.65	39.15	<0.009	1.95	0.60	195.10
32	4.55	78.45	<0.009	0.15	0.15	256.25
33	3.00	46.85	<0.009	2.90	3.00	199.85
34	5.25	32.05	<0.009	2.60	2.20	255.55
Mean±SE	23.88±3.36	41.71±2.93	1.02±0.10	7.51±0.86	2.59±0.34	198.11±12.73

Table 2. Heavy metal (mg/Kg) in random samples of coriander

S.No	Pb	Zn	Cr	Cd	Ni	Fe
1	17.55	29.70	72.55	0.35	0.60	286.30
2	28.95	32.00	17.60	0.50	<0.04	177.35
3	33.50	33.50	18.90	0.65	<0.04	169.10
4	41.35	36.25	24.65	0.95	1.65	204.00
5	42.95	29.75	26.15	0.80	<0.04	181.80
6	22.50	41.95	21.15	0.50	4.95	271.55
7	12.60	39.55	19.25	0.20	<0.04	325.30
8	39.65	28.05	6.90	1.70	<0.04	248.30
9	29.35	39.30	9.75	1.40	<0.04	229.70
10	26.30	44.25	11.25	0.85	<0.04	28.05
11	45.70	54.35	14.70	0.65	<0.04	39.30
12	47.85	65.90	7.45	0.90	<0.04	44.25
13	37.50	64.60	12.20	1.25	<0.04	54.35
14	17.45	31.15	43.90	1.60	1.70	65.90
15	11.40	32.20	9.90	1.05	1.25	37.50
16	7.80	24.85	13.90	1.00	3.10	242.30
17	10.15	35.20	9.60	1.00	6.60	206.35
18	5.15	48.80	23.55	0.75	3.65	277.60
19	1.65	51.85	<0.05	<0.009	<0.04	258.00
20	1.90	55.00	<0.05	<0.009	<0.04	174.50
21	3.00	46.65	1.90	<0.009	0.85	239.65
22	<0.06	34.80	0.95	<0.009	0.40	243.65
23	1.45	31.05	0.80	<0.009	0.55	199.85
24	<0.06	55.95	4.75	0.05	22.50	329.05
25	1.15	20.90	4.40	0.30	0.35	372.50
26	2.00	3.30	4.50	0.50	0.45	215.55
27	2.25	31.90	5.85	<0.009	0.15	270.75
28	4.85	28.85	3.85	<0.009	2.60	268.65
29	5.60	22.60	4.10	<0.009	3.10	309.85
30	6.95	34.70	42.40	<0.009	3.85	189.40
31	2.05	37.70	5.40	<0.009	2.750	154.75
32	4.30	44.40	5.80	<0.009	30.50	246.00
33	7.50	33.20	4.05	<0.009	33.50	349.65
34	10.25	25.20	5.05	<0.009	3.10	263.60
Mean±SE	16.64±2.66	37.33±2.20	14.28±2.57	0.49±2.57	5.82±1.40	211.01±16.1

Table 3. Heavy metal (mg/Kg) in random samples of fenugreek

S.No	Pb	Zn	Cr	Cd	Ni	Fe
1	19.80	32.25	11.35	0.40	<0.04	163.80
2	29.55	40.00	10.60	0.65	<0.04	222.00
3	37.45	31.30	5.75	0.80	0.70	284.50
4	42.10	29.75	8.40	0.85	<0.04	242.10
5	49.20	19.85	13.65	0.95	0.90	237.50
6	45.30	29.20	53.45	8.00	1.80	395.00
7	25.10	25.35	17.50	0.45	<0.04	235.20
8	27.50	12.55	10.55	1.30	0.20	27.50
9	23.35	12.40	16.85	1.40	<0.04	23.35
10	26.80	12.35	10.40	1.65	<0.04	26.80
11	35.40	9.65	10.60	1.55	<0.04	35.40
12	14.05	29.55	8.95	0.75	3.65	366.10
13	21.10	79.70	12.25	0.80	5.65	421.10
14	9.80	55.75	9.45	10.00	5.70	290.60
15	7.25	39.75	9.85	0.55	4.60	222.40
16	9.00	50.40	10.25	0.80	3.50	300.50
17	13.05	29.10	10.20	1.00	3.25	257.30
18	11.4	50.90	13.70	1.05	21.50	169.70
19	7.00	59.45	11.40	1.05	3.20	197.90
20	0.65	27.75	3.00	0.15	1.05	304.60
21	2.35	25.50	3.25	0.30	0.70	228.00
22	1.35	39.85	1.95	0.35	1.65	311.50
23	3.35	40.60	2.15	0.45	1.90	364.00
23	2.90	53.15	2.25	0.40	2.90	438.40
24	1.35	34.15	2.50	0.60	1.55	444.00
25	0.90	28.15	1.10	0.45	2.55	310.30
26	3.35	36.90	0.60	0.78	1.85	446.00
27	2.70	32.55	0.60	2.90	1.30	250.60
28	<0.06	42.10	3.25	<0.009	1.40	251.70
29	<0.06	26.95	2.50	<0.009	2.50	269.70
30	6.15	49.80	4.65	<0.009	4.65	296.10
31	4.00	63.35	6.95	<0.009	6.95	216.60
32	2.05	38.70	2.75	<0.009	2.75	449.50
33	3.45	37.10	2.55	<0.009	2.55	187.20
34	2.75	33.70	4.70	<0.009	4.70	286.80
Mean±SE	14.89±2.50	35.99±2.70	8.57±1.56	1.35±0.40	3.41±0.72	262.1±19.89

Table 4 a. Heavy metals in palak samples collected from different villages of Patiala City.

S. No	Pb	Zn	Fe	Ni	Cd	Cr
S1	17.95	74.20	271.35	3.45	0.60	15.63
S2	9.75	52.90	309.00	6.10	0.85	14.15
S3	10.45	75.55	378.45	3.25	1.35	13.10
S4	1.15	67.65	374.00	2.85	1.15	11.60
S5	8.70	52.20	266.00	6.00	0.55	11.40
S6	7.95	56.85	402.10	42.00	0.85	11.60
S8	11.60	70.00	327.10	3.95	1.00	1.65
Mean±SE	9.65±4.89	64.19±10.00	332.57±53.88	9.65±14.32	0.90±.29	12.91±5.12

Table 4 b. Heavy metals in coriander samples collected from different villages of Patiala City.

S. No	Pb	Zn	Fe	Ni	Cd	Cr
S1	2.50	64.15	209.30	5.45	0.7	15.55
S3	5.75	70.25	399.50	2.80	0.95	16.60
S5	12.65	75.00	399.50	3.55	0.60	11.35
S7	7.55	69.05	302.25	2.85	0.65	16.25
S8	16.20	52.65	168.05	1.25	1.50	26.15
Mean	8.93±6.10	66.22±28.08	295.72±172.92	3.18±1.88	0.88±.49	17.18±8.53

Table 4 c. Heavy metals in fenugreek samples collected from different villages of Patiala City.

S.No	Pb	Zn	Fe	Ni	Cd	Cr
S5	10.60	53.70	330.00	4.0	0.615	12
S6	9.0	42.15	408.45	1.8	1.35	11.95
S7	5.0	45.15	295.75	4.0	1.6	10.55
S8	7.0	59.45	197.90	3.2	1.05	11.4
Mean	7.9±1.21	50.11±23.43	308.02±157.12	3.25±1.70	1.15±.63	11.47±5.16

Samples	Place
S1, S2	Bahadurgarh
S3, S4	Main village
S5	Khusropur
S6	Sullar road
S7	Bhanari
S8	Thapar Institute of Engg. And Tech.

Table 5 a. Heavy metals in shoot and root of palak grown in pot culture irrigated with normal water (control) and synthetic metal containing water (treated).

Metals		Shoot	Root
		Mean±SE	Mean±SE
Fe	Control	160.50±16.70	400.25±130.70
	Treated	185.80±17.77	711.5±20.00
Zn	Control	133.5±10.07	225.17+61.80
	Treated	267±24.50	30.40±21.50
Cr	Control	3.55±0.35	7.40±0.06
	Treated	18.2±0.99	52.12±1.87
Ni	Control	3.22±0.37	<0.04
	Treated	5.82±0.07	11.28±0.78
Cu	Control	7.65±0.92	7.625±0.85
	Treated	9.98±0.55	7.2±1.07
Pb	Control	7.12±1.12	6.25±0.00
	Treated	15.27±0.53	6.3±0.00
Cd	Control	1.22±0.03	1.18±0.27
	Treated	6.95±0.05	9.2±0.25

Table 5 b. Trend observed in heavy metal uptake by shoot and root of palak

Shoot	
Control	Fe>Zn>Cu>Pb>Cr>Ni>Cd
Treated	Zn>Fe>Cr>Pb>Cu>Cd>Ni
Root	
Control	Fe>Zn>Cu>Cr>Pb>Cd>Ni
Treated	Fe>Zn>Ni>Cr>Cd>Cu>Pb

Table: 6 a. Heavy metals in shoot and root of coriander in pot culture irrigated with normal water (control) and synthetic metal containing water (treated).

Metals		Shoot	Root
		Mean±SE	**Mean±SE**
Fe	Control	295.87±76.02	866.00+79.51
	Treated	317.25±6.69	858.00+100.00
Zn	Control	158.2±50.36	515.50±0.61
	Treated	237.15±16.64	516.80±90.52
Cu	Control	4.02±0.22	9.90±0.29
	Treated	4.07±0.43	10.85±2.85
Cr	Control	6.75±1.39	6.16±0.69
	Treated	17.75±0.50	44.45±3.30
Pb	Control	7.27±3.27	3.3±0.00
	Treated	14.72±0.92	21.45±6.54
Cd	Control	1.2±0.49	1.57±0.27
	Treated	6.70±1.61	14.45±2.05
Ni	Control	17.97±6.32	<0.04
	Treated	11.02±9.37	19.6±5.99

Table 6 b. Trend observed in heavy metal uptake by shoot and root of Coriander

Shoot	
Control	Fe>Zn>Cu>Cr>Pb>Cd>Ni
Treated	Fe>Zn>Cr>Pb>Ni>Cd>Cu
Root	
Control	Fe>Zn>Cu>Cr>Pb>Cd>Ni
Treated	Fe>Zn>Cr>Pb>Ni>Cd>Cu

Table: 7 a. Heavy metals in shoot and root of fenugreek in pot culture irrigated with normal water (control) and synthetic metal containing water (treated).

Metals		Shoot	Root
		Mean±SE	Mean±SE
Fe	Control	168.00±23.79	476±5.34
	Treated	190.60±2.14	453.2±162.27
Zn	Control	82.50±10.99	110.72±10.22
	Treated	148.75±43.75	452.25±174.77
Cu	Control	6.47±0.02	16.65±0.85
	Treated	6.22±0.12	11.77±0.26
Cr	Control	5.75±0.02	19.7±9.04
	Treated	13.97±1.07	32±26.25
Pb	Control	4.02±0.32	8.6±3.19
	Treated	11.02±1.82	11.35±0.79
Cd	Control	0.85±0.00	9.55±8.39
	Treated	4.00±0.00	10.37±1.01
Ni	Control	4.92±0.31	10.10±4.20
	Treated	8.69±0.44	23.02±1.62

Table 7 b. Trend observed in heavy metal uptake by shoot and root of Fenugreek

Shoot	
Control	Fe>Zn>Cu>Cr>Ni>Pb>Cd
Treated	Fe>Zn>Cr>Pb>Ni>Cu>Cd
Root	
Control	Fe>Zn>Ni>Cr>Cu>Cd>Pb
Treated	Fe>Zn>Cr>Cu>Pb>Cd>Ni

Table 8. Physico-chemical properties of soil used in pot culture experiment

Soil property	
pH (1:2 H_2O)	7.86
EC (μS cm^{-1})	146.00
Organic carbon	0.01%
Bulk density (g/c.cm)	1.092
Clay + silt %	54.30
Available Nitrogen %	0.003
Phosphorous (ppm)	1.42
Potassium (ppm)	25.50
Sulphur (ppm)	0.43

Table 9 Heavy metal (mg/Kg) in the nursery soil before and after pot culture trial irrigated with normal water (control) and synthetic water (treated).

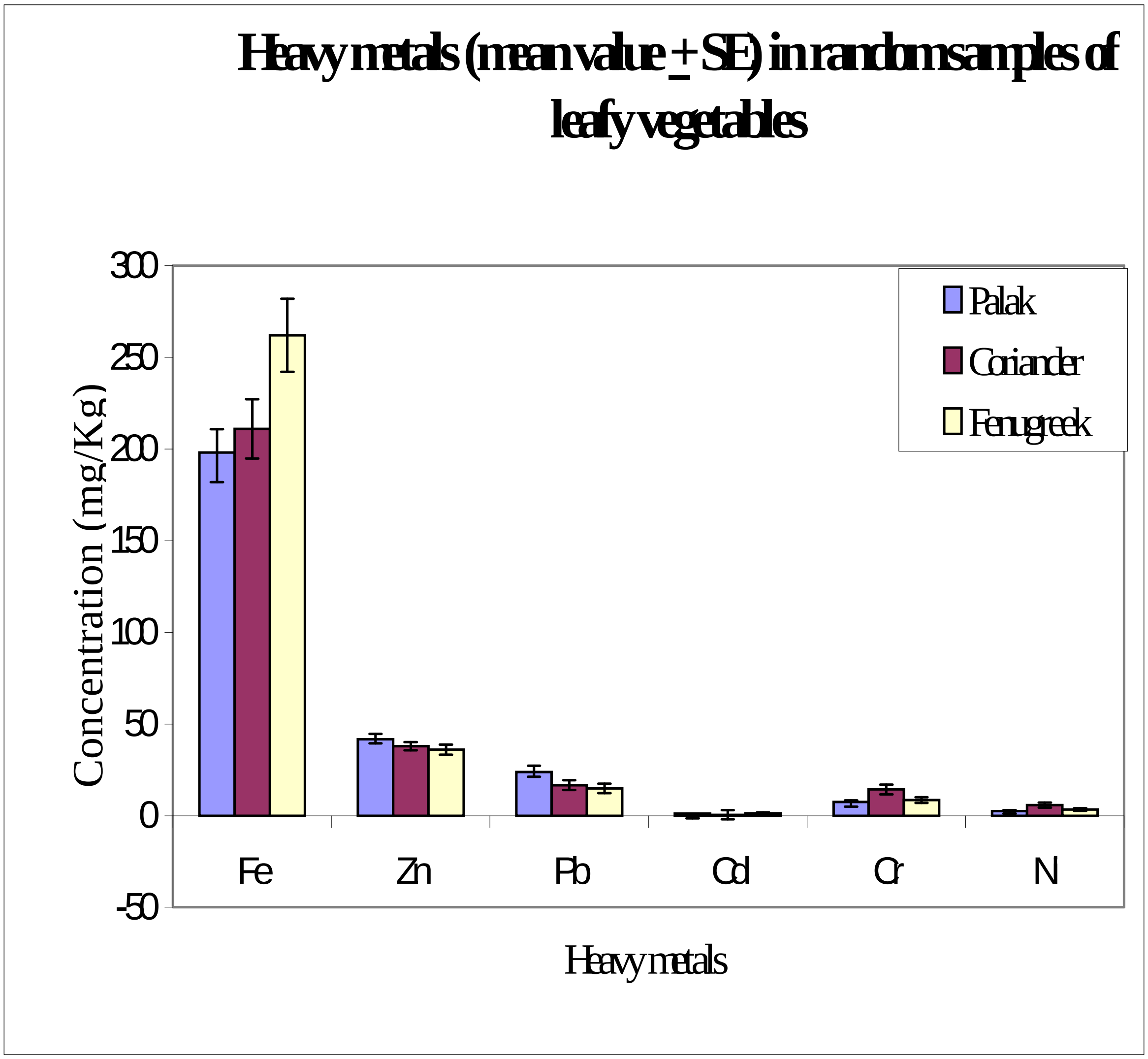
Nursery soil before trial		Nursery soil after trial			
		Fenugreek		Palak	Coriander
Metals	**Mean±SE**	**Mean±SE**		**Mean±SE**	**Mean±SE**
Fe	1975.00±44.84	Control	2037.50±67.50	2707.50±47.50	2552.50±97.51
		Treated	2255.00±92.50	2535.0±145.02	2177.50±32.50
Zn	30.26±0.63	Control	161.25±22.75	113.00±30.00	151.75±38.25
		Treated	85.00±11.00	92.70±22.75	106.25±16.25
Cu	5.81± 0.09	Control	4.48±0.52	5.55±0.00	5.10±0.55
		Treated	5.85±0.20	2.30±1.90	5.13±0.025
Cr	15.70 ± 1.43	Control	14.02±0.97	13.30±0.95	12.75±0.15
		Treated	13.25±0.10	14.30±0.65	15.57±1.47
Pb	19.90±1.01	Control	8.27±2.97	6.42±0.22	0.6±0.05
		Treated	9.30±0.10	9.13±0.87	1.70±0.67
Cd	9.00±0.25	Control	0.63±0.02	0.85±0.00	9.05±1.50
		Treated	1.43±0.27	4.00±0.00	11.27±1.17
Ni	22.86±0.75	Control	11.45±0.05	4.92±0.31	11.95±0.75
		Treated	13.97±2.82	8.60±0.44	14.65±1.40

Table 10. Total bacterial count and coliforms in random samples of green leafy vegetables.

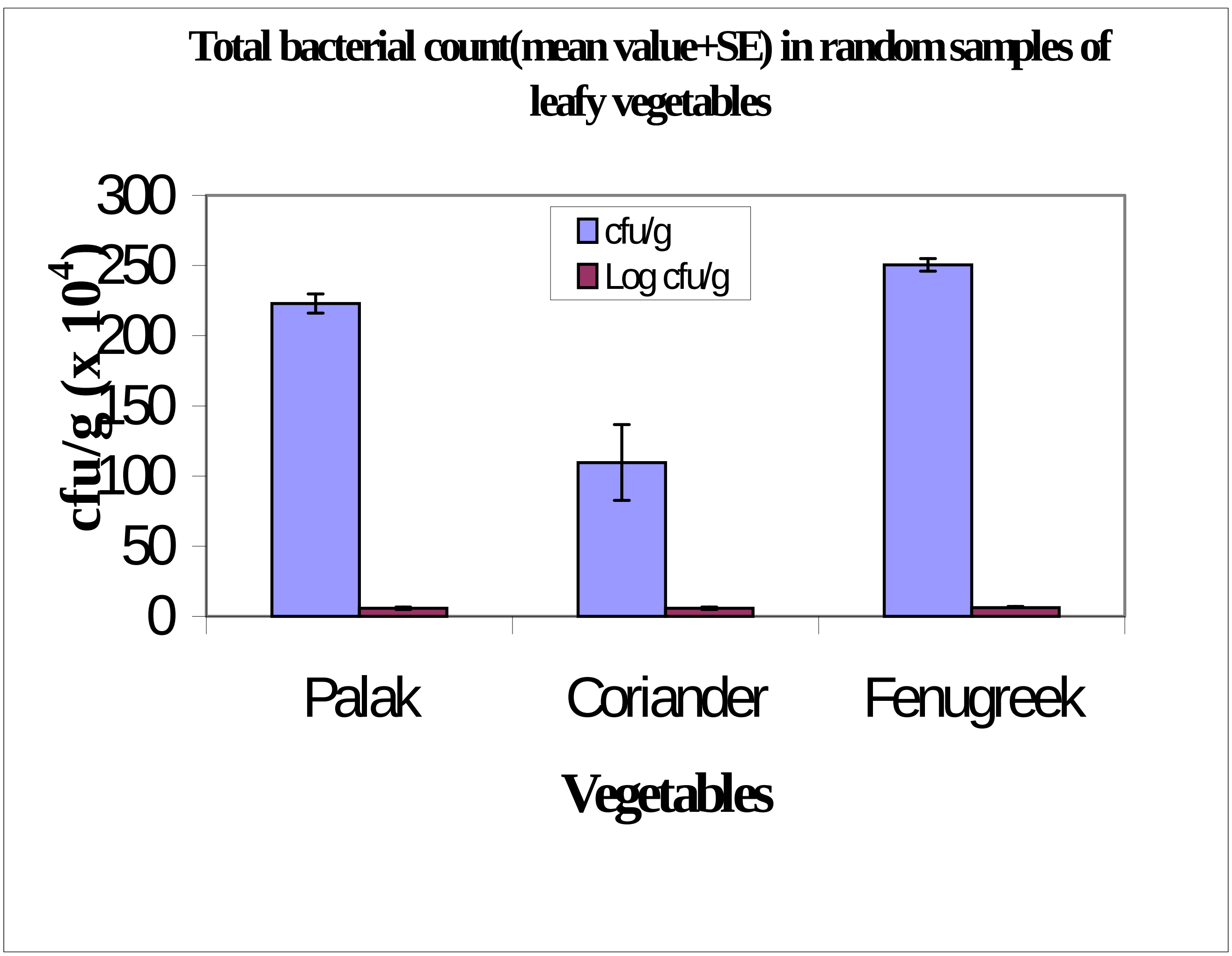
Coriander					Fenugreek				Palak			
S.No	TSA		VRBA		TSA		VRBA		TSA		VRBA	
	cfu x10^4/g	log cfu/g	cfu x10^4/g	log cfu/g	cfu x10^4/g	log cfu/g	cfu x10^4/g	log cfu/g	cfu x10^4/g	log cfu/g	cfu x10^4/g	log cfu/g
1	44	5.64	Nil	Nil	133	6.12	0.3	3.47	400	6.60	Nil	Nil
2	15	5.17	Nil	Nil	2	4.30	40	4.60	0.4	3.60	Nil	Nil
3	400	6.60	5	4.69	6	4.78	4	4.60	30	5.47	0.2	3.3
4	3	4.47	300	6.47	200	4.30	Nil	Nil	20	5.30	0.08	2.9
5	164	6.21	448	6.65	100	6.00	60	5.77	3900	7.59	TNTC	TNTC
6	TNTC	TNTC	TNTC	TNTC	TNTC	TNTC	0.3	3.47	68	5.83	0.4	3.60
7	34	5.53	Nil	Nil	TNTC	TNTC	80	5.9	44	5.64	520	6.71
8	36	5.55	24	5.38	TNTC	TNTC	5.2	4.71	20	5.30	164	6.21
9	TNTC	TNTC	96	5.98	360	6.55	24	5.38	320	6.50	24	5.38
10	16	5.20	28	5.44	TNTC	TNTC	28	5.44	TNTC	TNTC	120	6.07
11	10	6.00	44	5.64	400	6.60	6.8	4.83	TNTC	TNTC	16	5.20
12	28	5.4	160	6.20	48	5.68	56	5.74	40	5.60	12	5.07
13	TNTC	TNTC	112	6.04	120	6.07	32	5.50	120	6.07	8	4.90
14	24	5.3	12	5.07	560	6.74	1.6	5.20	80	5.90	16	5.20
15	480	6.6	26	5.41	170	6.23	94	5.97	0.4	3.60	TNTC	TNTC
16	360	6.5	9	4.95	400	6.60	38	5.58	8	4.90	0.44	3.64
17	TNTC	TNTC	114	6.05	TNTC	TNTC	16	5.20	92	5.96	12	5.07
18	12	5.07	86	5.93	530	6.72	16	5.20	200	6.30	40	5.60
19	640	6.80	122	6.09	280	6.44	64	5.80	240	6.38	0.8	3.90
20	104	6.01	34	5.53	440	6.64	34	5.53	56	5.74	16	5.20
21	116	6.06	58	5.76	200	6.30	36	5.55	32	5.50	120	6.07
22	60	5.77	8	4.90	520	6.71	29	5.46	TNTC	TNTC	3.6	4.55
23	40	5.60	200	6.30	200	6.30	20	5.30	19.4	5.28	2.4	4.38
24	22	5.34	12	5.08	24	5.38	1.6	4.20	120	6.08	1.1	4.04
25	16	5.20	16	5.20	15	5.17	1.2	4.07	34	5.53	34	5.53
26	18	5.25	8	4.90	560	6.74	16	5.20	23	5.36	23	5.36
27	260	6.41	68	5.83	84	5.92	20	5.30	560	6.70	67	5.82
28	32	5.50	480	6.68	120	6.07	52	5.71	125	6.09	0.9	3.95
29	45	5.65	16	5.20	400	6.60	58	5.76	43	5.63	3.4	4.53
30	28	5.45	112	6.02	120	6.07	48	5.68	28	5.44	2.5	4.40
31	102	6.00	15	5.17	TNTC	TNTC	TNTC	TNTC	34	5.53	56	5.74
32	11	5.04	17	5.23	TNTC	TNTC	TNTC	TNTC	89	5.94	43	5.63
33	19	5.28	22	5.34	960	6.98	68	5.83	120	6.07	0.78	3.89
34	59	5.77	21	5.32	37	5.56	2.8	6.44	45	5.65	4.5	4.65
M±SE	109.64±27.06	5.69±.0.94	87.38±20.68	5.61±0.09	250.53±4.46	6.03±0.93	29.88±0.51	5.28±0.98	222.94±6.80	5.71±0.85	43.74±0.75	4.88±0.77

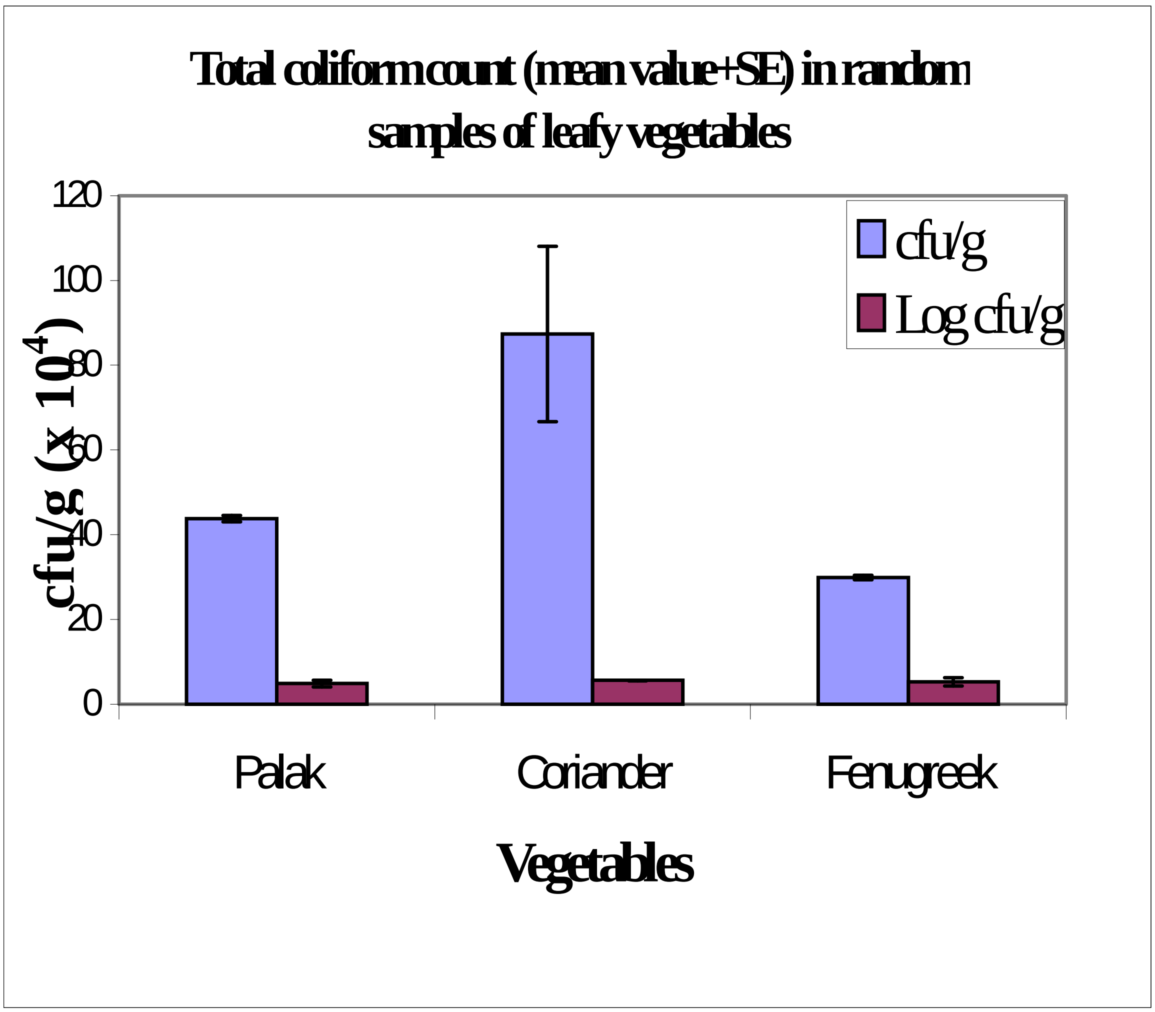
Nil = no growth

TNTC = Too numerous to count



Heavy metals (mean value ± SE) in random samples of leafy vegetables
Concentration (mg/Kg)
300
250
200
150
100
50
0
-50
Fe
Zn
Pb
Cd
Cr
N
Heavy metals
Palak
Coriander
Fenugreek


Total bacterial count(mean value+SE) in random samples of leafy vegetables
cfu/g (x 10⁴)
300
250
200
150
100
50
0
cfu/g
Log cfu/g
Palak
Coriander
Fenugreek
Vegetables


Total coliform count (mean value+SE) in random samples of leafy vegetables
120
100
80
60
40
20
0
cfu/g (x 10^4)
cfu/g
Log cfu/g
Palak
Coriander
Fenugreek
Vegetables

CONCLUSIONS

1. The metal concentration in the green leafy vegetables from in the market was higher than the permissible limits of Indian Prevention of Food adulteration Act (PFA), 1954. 67-76% samples were found to have high Pb which was beyond the limit of PFA of 2.5 mg/Kg, 18-39% samples had high Zn which crossed Indian permissible limit of 50 mg/Kg and 6-9% samples were high in Cd, which was beyond 1.5 mg/Kg. Only 32% palak, 26% of coriander and 23% of fenugreek samples contained Pb concentration below permissible limit. In 61.6 % of palak, 82.3 % of coriander and 82.5 % of fenugreek samples Zn was found to be lower then 50 mg/Kg. All samples of palak and 94.1 % of coriander and 91.1 % of fenugreek had Cd less than 1.5 mg/Kg as recommended by PFA.
2. Different agricultural field soils have different concentration of metals thus the crops (vegetables) grown in such soils have different concentration of metals in them. There is a danger of heavy metal accumulation of Pb and Cd in the vegetables grown in the vicinity of Main Village, Bahadurgarh, Sullar road, Khusropur, Bhanari and Thapar Techno*log*y Campus.
3. The metal content in vegetables irrigated with water containing high concentration of metals was higher than the vegetables irrigated with normal water. Metal content in green leafy vegetables was higher in roots than shoots. Uptake of Fe in palak was highest as compared to coriander and fenugreek whereas uptake of Cr, Cu, Pb, Ni was higher by shoots of palak as compared to that of coriander and fenugreek.
4. Bacterial pathogen contamination in vegetables depends upon pre harvest and post harvest conditions. The total bacterial count in the vegetable samples ranged between 250-109 cfu $x10^4$ and 5.7-6.0 log cfu/g and the coliform count was in the range of 29-87 cfu $x\ 10^4$ and 4.8-5.6 log cfu/g which indicates that the crops were irrigated by waste water from domestic sources.

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