Chapter-6

SUMMARY

Food is a basic physiological requirement for the existence of life. Population explosion, advent of urbanization, crunch of resources, changes in social outlook and improper distribution of food have elicited a situation where every one in nine people i.e. about 795 million people on the planet earth are deprived of sufficient food. The efforts and policies are being made worldwide to combat the hunger stress and provide the basic fundamental right to every individual alive. Food security is not just about sufficient food but it also refers to safe and nutritious food. Vegetables are important portion of platter because of nutritional benefits that are attained from all sorts of vegetables. Though, according to WHO/FAO, consumption of 400 g of vegetables by an individual per day is essential to remain healthy and prevent various diseases including those associated with heart, and even cancer and obesity but the average consumption of vegetables in developing countries is much less than required due to lack of sufficiency. The availability of a crop is dependent mainly upon the production which in turn is dependent upon the environmental factors i.e., natural resource availability and climatic conditions. Climatic factors are micro-scale conditions and thus their effect is generally not of large spectrum. The major factor responsible to increase the production is resource availability i.e., availability of arable land and water for irrigation. Globally, per capita availability of arable land has decreased drastically from 0.45 ha (approx.) in 1960 to 0.25 ha (approx.) in 2009. Decline in fresh water availability due to increased demand, injudicious use and climate change has also affected the per capita agricultural yield. But, in spite of lack of availability of resources, United Nations admit that the implementation of Millennium Development Goals has resulted in 50% decrease in the undernourished population in the developing nations from 1990 to 2015. Use of improper agricultural practices, excessive use of fertilizers, cultivation on land not suitable for food crops and use of wastewater for irrigation were the steps that helped in managing the food production and availability. Wastewater usage for irrigation is considered as a process that can recycle nutrients and water to the soil, but untreated wastewater can contain pathogens, toxic chemicals, drugs, heavy metals etc. Thus, the risk associated with consumption of food growing in areas irrigated with wastewater is extremely high. Heavy metals are of major concern among all the pollutants owing to global reports documenting their high content in food crops and toxic effects in plants and animals. International organizations (USEPA, FAO and WHO) are continuously making efforts to develop protocols and standards for the production of safe food. Agricultural organizations are trying to develop the practices to reduce heavy metal uptake in food crops growing on contaminated sites or irrigated with wastewater. Heavy metal uptake by plants is dependent on the bioavailability or mobility of metal ions. Metal-immobilizing amendments when added to soil reduce metal availability by forming stable complexes or by acting as sorbents for the adsorption of metal ions. Improving soil quality with organic or inorganic amendments is a cost effective strategy to prevent the transfer of heavy metals to food chain.

Amritsar, being part of food bowl state of India, is amongst major producers of vegetables in the country. The average annual rainfall of Amritsar district is 541.9 mm leading to semi-arid conditions throughout the year. The average depth of ground water table is 302-450 m and the annual draft from ground water exceeds much more than annual replenishment of groundwater resources in the district. To manage the scarcity of water, irrigation in many parts of the district is carried out using wastewater. Two main drains in the district, Tung-Dhab drain and Municipal wastewater drain, receive wastewater from industries, household and other commercial areas through a network of sub-drains.

Considering the alarming consequences of cultivation of food crops along the wastewater drains and/or irrigating with wastewater, the present study was planned to monitor the soil and vegetable samples from agricultural fields along wastewater drains of Amritsar. Also, an effort was made to find a solution to reduce heavy metal uptake in a hyperaccumulator vegetable. The objectives of the present study are:

- Survey and selection of agricultural fields along wastewater drains in Amritsar
- Monitoring of soil samples from selected sites for genotoxicity and various physicochemical parameters including heavy metal content.
- Monitoring of vegetable crops for heavy metal content and estimation of Metal Pollution Index (MPI) and Hazard Quotient (HQ).

- Identification of suitable low cost soil amendments which can reduce heavy metal uptake by food crops.

Extensive survey was carried out along the drains of Amritsar and 3 agricultural fields were selected for the present study, 2 agricultural fields along Tung-Dhab drain and irrigated with groundwater (site 1 and 2), and one agricultural field along municipal wastewater drain and irrigated with wastewater (site 3). Soil and vegetable samples from three selected sites were collected during both summer and winter seasons for different kinds of analyses.

During monitoring studies, genotoxicity assessment of soil samples was carried out using *Allium cepa* root chromosomal aberration assay. The study revealed that soil samples collected from selected sites induced significantly higher percentage of chromosomal aberrations in root tip cells of *Allium cepa* as compared to control but there was no significant variation amongst different samples. Among samples collected during winter season, maximum percentage of aberrant cells (24.24 %) was induced by a sample (SW-1) collected from site along Tung-Dhab drain while among summer season samples, the sample collected from site irrigated with wastewater across municipal waste water drain (Site 3) induced maximum percent aberrant cells (25.63%).

The physicochemical parameters were assessed following the standard protocols. Soil texture was analyzed using sieving method and various portions were classified as sand, silt and clay. The analysis revealed that all the samples collected had higher percentage of sand, followed by clay and silt. Thus, the samples collected in the present study fall under the category of sandy clay. pH was measured using pH meter (Systronics; model μ pH system 361). pH of soil samples from all sites and for both seasons was found to be slightly alkaline and ranged from 7.29 to 7.9. Like pH, alkalinity of soil also influences the movement of ions in soil and it was measured using titrimetric method. Alkalinity of samples collected in winter season was relatively less (0.67-1.07 meq/100 g) than those collected in summer season (1.08-1.29 meq/100 g). Nitrates and phosphates were analyzed using UV-Visible spectrophotometer (Systronics; model GS5701A). For both winter and summer seasons, the nitrate content was maximum in samples from site irrigated with wastewater (2.01 mg/g; 2.12 mg/g, respectively) while minimum was found to be in sample from site 1 (0.95 mg/g; 1.11

mg/g, respectively). Maximum content of phosphates (2.12 mg/g) was observed in a soil sample collected in summer season from wastewater irrigated site while minimum content (1.0 mg/kg) was found in samples collected in winter season from site 2 located along Tung-Dhab drain.

Various elements *viz.* Ca, Mg, Na, K, Fe, Zn, Mn, Cu, Cr, Co, Cd and Pb were analyzed using Atomic Absorption Spectrophotometer (Model: 240FSAA Make: Agilent Technologies). The order of macronutrients on the basis of their content in soil samples was: Ca > Mg > Na > K. Among macronutrients, content of calcium ranged from 7558.33 –13585.26 mg/kg in all samples. The content of magnesium was found to be minimum in samples collected during winter season from site 2 (2060.33 mg/kg) and was maximum in samples collected in same season from site 3 (5481.67 mg/kg). The content of sodium ranged from 92.65 mg/kg in summer season sample of site 3 to 2114 mg/kg in winter season sample from site 1. Potassium content ranged from 732.67 mg/kg (winter season sample of site 3) - 877 mg/kg (winter seasons sample of site 2).

Among heavy metals, iron was the most abundant metal and for both winter and summer samples, maximum content was found in sample from site 3 i.e., 1630 mg/kg and 1465 mg/kg respectively. Content of zinc ranged from 215.25 mg/kg (site1) to 892.2 mg/kg (site 3). Maximum content of copper (280 mg/kg) was found in sample collected from site irrigated with wastewater. The content of chromium ranged from 22.23 – 38.78 mg/kg. The content of cobalt ranged from 14 mg/kg (site 3) to 27.2 mg/g (site 2). Maximum content of cadmium was found in sample from site irrigated with wastewater (3.6 mg/kg). Content of lead was below detection limits for all samples. Content of manganese ranged from 62.5 mg/kg (site 3) to 86.96 mg/kg (site 1).

The percentage of carbon, hydrogen, nitrogen and sulphur in soil samples was estimated using CHNS/O analyzer (Make: Thermoflesh; Model: Thermoflesh 2000). The percentage of carbon varied from 1.25% (Site 1) to 2.54% (Site 3) whereas the hydrogen percentage ranged from 0.31% (Site 1) to 0.48% (Site 3). Percentage of nitrogen varied from 0 (site 2) to 0.018 % (site 3).

The vegetable samples collected from various sites were analyzed for the content of heavy metals like Cd, Co, Cu, Fe and Pb using AAS. Among the vegetable samples from site 1, content of cadmium was found to be maximum in fenugreek (0.80

mg/kg) and minimum in bottle gourd and onion (0.07 mg/kg). Among vegetable samples collected from site 2, content of cadmium was found to be maximum in raddish (1.20 mg/kg) and minimum in fenugreek (0.07 mg/kg). Amongst vegetable samples collected from site 3, content of cadmium was found to be maximum in turnip (1.07 mg/kg) and minimum was in green chilli (0.20 mg/kg). The cobalt content in vegetable samples collected from site 1 was maximum in spinach (92 mg/kg) while onion samples exhibited minimum cobalt content (9.20 mg/kg). Among vegetable samples collected from site 2, cobalt content varied from 17.20 mg/kg (onion) to 68 mg/kg (coriander). Among vegetable samples from site 3, content of cobalt ranged from 37.33 mg/kg (garlic) to 130.67 mg/kg (spinach). Amongst all vegetable samples collected from selected sites, the content of copper was observed to be maximum in spinach samples from site 1 (81.33 mg/kg) while it was minimum in green chilli samples from the same site (3.73 mg/kg). In vegetable samples collected from site 2, copper concentration was found to be maximum (49.33 mg/kg) in spinach samples followed by fenugreek (34 mg/kg) while it was minimum (4.33 mg/kg) in green chilli. Like other two sites, among vegetable samples from site 3 too, copper concentration was found to be maximum in spinach (36.73 mg/kg) while it was minimum in bottle gourd (6.20 mg/kg). Content of iron was found to be maximum (740 mg/kg) in fenugreek samples from site 3 while minimum in garlic samples (16.80 mg/kg) from site 2. Among vegetable samples collected from site 1, mean iron content ranged from 21.60 mg/kg (garlic) to 624 mg/kg (bottle gourd). And, in vegetable samples from site 2, minimum iron content was found in garlic (16.80 mg/kg) and maximum was shown by fenugreek (618.87 mg/kg). Among vegetable samples from site 3 also, minimum content was found in garlic (21.27 mg/kg) while maximum was found in fenugreek (740 mg/kg). In different vegetable samples studied, concentration of lead ranged from 0.07 mg/kg to 0.33 mg/kg. Maximum content of lead was observed in coriander from site 3 followed by coriander samples from sites 1 and 2 and mint from site 1.

Metal pollution index (MPI) and Hazard Quotient (HQ) of vegetables with respect to heavy metal content were calculated using standard procedures. Metal Pollution Index is the geometric mean of concentration of all metals in edible part of the plant MPI of vegetable samples from site-3 which is irrigated with wastewater was higher than that of vegetables from other sites which were not irrigated with wastewater (except raddish and turnip). In general, Metal Pollution Index of leafy vegetables like fenugreek, coriander, mint and spinach was found to be more than that calculated for tuberous vegetables like, raddish and turnip and minimum MPI was found for bulb vegetables.

Hazard quotient (HQ) the ratio of concentration of metal consumed through food intake to reference oral dose. HQ associated with a food if is more than 1, the consumption of food can be hazardous for humans. Hazard quotient (HQ) was very high for vegetables having high content of both copper and cobalt but copper is considered to be more toxic than cobalt. Hence, we selected copper for the mitigation studies, also the content of copper in one of the soil samples was very high (280 mg/kg). Among all the vegetables, content of copper was maximum in spinach samples from all the three sites selected for the present study. Hence, spinach being hyperaccumulator was selected for mitigation studies.

For mitigation studies, a preliminary experiment was carried out to assess the effect of various amendments on the growth of spinach as well as on the uptake of copper by spinach. The preliminary experiment was set up in field conditions using 7 amendments *viz.* compost, onion peels, mausami peels, groundnut peels, bagasse, used tea leaves and calcium carbonate. Seeds of spinach were sown in two sets of pots; one set containing soil amended with each amendment at the rate of 10 g/kg of soil and another set containing soil + each amendment (10g/kg) + copper (250 mg/kg). Non-amended soil was used as control.The percentage germination varied from 0% in spinach seedlings grown on control soil amended with $CaCO_3$ to 75.6% in those grown on control soil amended with onion peels and groundnut peels. The germination rate of spinach seeds observed in different amendments in the presence of Cu_250 was observed in the following order: Soil amended with $CaCO_3$ (0%) < used tea leaves < bagasse < onion peels < mausami peels < non-amended soil < compost < groundnut peels (60%). Content of copper in spinach grown on soil containing various amendments with and without Cu_250 during preliminary experiment ranged from 34.83 mg/kg for control soil amended with groundnut peels to 164.8 mg/kg for non-amended soil with Cu_250. The order of reduction in uptake was onion peels > bagasse > groundnut peels > mausami peels > compost. Used tea leaves and calcium carbonate were phased out as they hindered the growth of spinach.

The selected amendments *viz.* compost, onion peels, mausami, groundnut peels and bagasse were used to carry out detailed experimentation with each of the different concentrations of copper i.e., 250 mg/kg (Cu_250); 500 mg/kg (Cu_500); 1000 mg/kg (Cu_2000) and 2000 mg/kg of soil (Cu_2000). The non-amended soil was used as control. The background concentration of copper in soil was 17.93 mg/kg. The spinach samples from these treatments were analyzed for growth parameters (germination rate, root/shoot length), copper content, other trace elements content, biochemical parameters *viz.* chlorophyll, carotenoids, proteins and ascorbic acid as well as CHNS content. Chlorophyll, carotenoids, proteins and ascorbic acid content were assessed using UV-Visible spectrophotometer (Systronics; model GS5701A) following standard protocols. The CHNS content in spinach was estimated using CHNS/O analyzer (Make: Thermoflesh; Model: Thermoflesh 2000).

In laboratory conditions, the experiment was carried out in petri-dishes and the content of copper in seedlings grown on different treatments was estimated after 7 days. In control soil, the content of copper in seedlings ranged from 41.23 mg/kg (soil amended with groundnut peels) to 79.5 mg/kg (non-amended soil). For Cu_250, it ranged from 65.3 mg/kg in seedlings grown on soil amended with groundnut peels to 174.53 mg/kg in those grown on non-amended soil; for Cu_500 it varied from 217.67 mg/kg for spinach from soil amended with onion peels to 333.47 mg/kg for spinach from non-amended soil. Maximum content of copper in spinach seedlings was found in those grown on non-amended soil (286.43 mg/kg) followed by soil amended with mausami peels (282.37 mg/kg) for Cu_1000 and those grown on soil amended with groundnut peels (596.9 mg/kg) for Cu_2000. Minimum content of copper was found in spinach seedlings grown on soil amended with groundnut peels (232.03 mg/kg) for Cu_1000 and soil amended with onion peels (310.4 mg/kg) for Cu_2000.

In field experiment, the spinach samples were harvested after 30 days for various analyses. Generally, seed germination rate increased with the use of amendments. No specific trend was observed in the length of spinach as a result of various treatments. Copper content in spinach grown on control soil was found to be maximum in samples grown on non-amended soil (76.35 mg/kg) followed by those growing on soil amended with mausami peels (72.35 mg/kg) and was minimum in samples grown on soil amended with groundnut peels (44.25 mg/kg). With Cu_250, order of uptake of copper by spinach was: non-amended soil (183.4 mg/kg) > soil + compost > soil + mausami peels > soil + bagasse > soil + onion peels > soil + groundnut peels (76.58 mg/kg). In spinach samples from pots having 500 mg/kg copper in soil (Cu_500), maximum copper content was found in samples from non-amended soils (340.5 mg/kg) while minimum was found in samples from soil amended with onion peels (222.9 mg/kg). In spinach samples grown on Cu_1000, maximum reduction in copper uptake by spinach was observed in case of soil amended with groundnut peels followed by compost, onion peels and bagasse. In case of highest concentration of copper in soil (Cu_2000), minimum content of copper was found in samples grown on soil amended with mausami peels (274.45 mg/kg) followed by onion peels, compost, non-amended soil, bagasse and groundnut peels (482.4 mg/kg).

Generally, content of chromium decreased in plants with increase in concentration of copper. All amendments resulted in reduction in uptake of chromium from soil at lower concentration of copper (Cu_250) while at higher concentrations of copper, onion peels were found to be most effective amendment while compost was least effective. Among all the amendments, mausami peels, enhanced uptake of cobalt by spinach at various concentrations of copper. Only onion peels reduced the uptake of cobalt in spinach as compared to non-amended soil at all concentrations of copper. Use of all amendments (except mausami peels) reduced the uptake of iron in spinach grown on soil having various concentrations of copper. Manganese content in spinach showed a strong positive correlation with concentration of copper in soil and at lower concentrations of copper (Cu_250 and Cu_500), all amendments led to reduction in uptake of manganese by spinach. At Cu_1000, use of mausami peels, enhanced the uptake of manganese while at Cu_2000, groundnut peels and bagasse enhanced the uptake. At higher concentration of copper (Cu_500 and above) no amendment resulted in significant reduction in uptake of zinc. At Cu_250, use of compost, onion peels and groundnut peels resulted in reduction in uptake of zinc by spinach.

Total chlorophyll content in spinach grown on soil with various treatments and amendments varied from 0.663 mg/g to 1.663 mg/g. No definite pattern of variation in content of chlorophyll was observed with reference to various concentrations of copper or amendments. In general, all amendments led to increase in chlorophyll content in spinach with reference to control. No correlation of chlorophyll with any other parameter was observed in the present study. No significant difference in carotenoid content was also observed with respect to different treatments.

No significant difference in the protein content of spinach was observed in comparison to various amendments. But concentration of proteins increased significantly with increase in concentration of copper in soil amended with mausami peels and bagasse. Of all the spinach samples, maximum content of ascorbic acid was found in spinach grown on non-amended soil with 2000 mg/kg copper concentration (33.379 mg/g) while minimum content was found in spinach grown in soil having 500 mg/kg copper concentration and amended with bagasse (8.927 mg/g). At higher concentration of copper, ascorbic acid content was significantly higher in spinach grown on non-amended soil as compared to those grown on amended soils.

Carbon content in spinach samples varied from 20.56% to 36.876%. Content of carbon increased with increase in concentration of copper in soil. Also, it increased with the use of amendments in most of the treatments. The range of hydrogen in all spinach samples was found to be 2.49% to 4.99%. Use of compost in control, Cu_250 and Cu_500 resulted in decrease in hydrogen percentage in spinach while all other amendments and treatments led to increase in hydrogen content. The range of nitrogen content in spinach samples was found to be 2.05% to 3.85%. In general, use of amendments increased the nitrogen content in spinach but at Cu_500, use of amendments (except compost) decreased the nitrogen content in spinach. Percentage of sulphur was found to range from 0% to 0.33%. Content of sulphur in spinach decreased with increase in concentration of copper in all treatments.

The study reveals that use of food byproducts as soil amendments can help in reducing heavy metal uptake by food crops, hence mitigating the harmful effects of toxic metals. These low-cost, rather free of cost available sorbents can be used directly after sun-drying. Though, onion peels proved to be most efficient but also, use of other amendments can be effective. Apart from amendments discussed, we can also use other food byproducts like vegetable waste, rice husk, etc. This type of mitigation strategy can reduce the cost of land reclamation and cultivation of safe food crops can be carried out at sites contaminated with heavy metals.