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Heavy metal concentration and bacterial load on Spinach (*Spinacia oleracea L.)* phyllosphere under different regions in Lucknow, Uttar Pradesh

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Abstract

Levels of some heavy metals (Cd, Fe and Copper) were investigated in edible portions of Spinach (*Spinacea oleracea*) . Sample of Spinach leafe were collected from 5 different region of Lucknpw (Mohanlalganj, Sarojani nagar Industrial area, Kakori, Bakshi ka talab, and Telco Industrial area Chinhat) The metals were analyzed using atomic absorption spectrophotometer (AAS). Bacterial loads at same time on the plant Spinach (*Spinacea oleracea*) were also determined. Bacterial load find vary in different sapling region, from $1.5x10^6$ to $5.6x10^7$. Biochemical characterization shown the possibility of *Bacillus* and *Streptococcus sp.* Strains. Consumption of these vegetables as food may not pose possible health hazards to humans at the time of the study.

Key-Words: Spinach (*Spinacea oleracea*), Heavy Metals, Microorganisms, AAS, Phyllosphere

Introduction

Environment is defined as the totality of circumstances surrounding an organism or group of organisms especially, the combination of external physical conditions that affect and influence the growth, development and survival of organisms [1].It consists of the flora, fauna and the abiotic, and includes the aquatic, terrestrial and atmospheric habitats. The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic even at low concentration[2], it is a group of metals and metalloids with atomic density greater than 4 g/cm^3,or 5 times or more, greater than water [3]. Every 1000 kg of "normal soil" contains 200 g Chromium, 80 g Nickel, 16 g Lead, 0.5 g Mercury and 0.2 g Cadmium, theoretically [5]. Monitoring the endangerment of soil with heavy metals is of interest due to their influence on groundwater and surface water and also on plants, animals and humans [6]. Spinach plant take up metals by absorbing them from contaminated soils, as well as from deposits on different parts of the vegetables exposed to the air from polluted environments (Zurera-Cosano and Moreno-Rojas, 1984).

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It has been reported that nearly half of the mean ingestion of lead, cadmium and mercury through food is due to plant origin (fruit, vegetables and cereals). Leafy vegetables accumulate much higher contents of heavy metals as compared to other vegetables. This is because "leafy vegetables have higher translocation and transpiration rate as compared to other vegetables in which transfer of metals from root to stem and then to fruit is longer which results in lower accumulation than leafy vegetables" [7]. Several investigations of water, soil and vegetables pollution by waste water are available [8]. Besides contamination of edible plants by heavy metals, there is another problem that arises by colonization of pathogenic bacteria on phyllospheric region (leaf surfaces of plants). The membership of spinach plant phyllosphere communities is dominated by members of a few phylogenetic groups, chiefly α-*proteobacteria*, γ -*proteobacteria* and *Bacteroidetes*. β-*proteobacteria* and *Firmicutes* also are a large part of the bacterial community, while *Acidobacteria*, *Actinobacteria* and *Cyanobacteria* occur infrequently. Study of the phyllosphere of spinach leaves would improve the understanding of how pathogenic microorganisms are able to populate edible plants during pre- and post harvest handling since possible interactions with the members of the bacterial

populations on plants might play a role on their survival. In recent years, spinach consumption was implicated in an *E. coli* O157:H7 outbreak [9]. Since spinach is a leafy vegetable, it accumulates a good amount of heavy metal, if grown on contaminated soil or irrigated with contaminated water. So the present study was undertaken to observe for the presence of heavy metal, if any (iron, copper and cadmium) in spinach leaves from different regions of Lucknow which will be an indicator of soil contamination. Besides this, how the post harvest handling affects the bacterial load in the Phyllosphere was also studied and an attempt was made to establish the correlation between heavy metal presences, if any, with the bacterial load on spinach leaves.

Material and Methods

All the samples of Spinach leaf were collected into sterile sample Collection bags from different regions of Lucknow.

Requirements for Heavy Metal estimation

1- Gram oven dried sample of spinach leaf.

2-Nitric acid (HNO_3) and Perchloric acid ($HClO_4$) in ratio of 3:1.

Isolation of Microbial Cell from Phyllosphere of Spinach

Two types of methods were used:

1. Leaf impression method
2. Serial dilution method

An aliquot of 0.1ml was drawn from 10^{-2} and 10^{-6} dilutions and streaked into sterile petriplates. The plates were incubated at 28-37°C for 24 hours.

Identification of bacteria

Identification of isolates was done on the basis of cell morphology, cultural characteristics and biochemical reactions as described in Bergey's manual of Systematic Bacteriology

Estimation of heavy metal content in the plant sample

The plant samples were washed then after plant material was oven dried separately at 650C. One gram of oven dried plant taken in conical flask having 10 ml of acid mixture kept overnight. Keep the flask containing sample and acid mixture [HNO3: HClO4 (3:1)] digest at 70 to 800C on hotplate. The solution was allowed to evaporate to dryness until all the tissue had been digested and raised the temperature to 1050C to reduce the volume to 0.5-1.0 ml (clear white solution/crystals). The residue was re-dissolved and diluted to 25 ml with 0.1 N HNO3. The solution was filtered through filter paper in a volumetric flask. Metal content was estimated using AAS (Atomic Absorption Spectrophotometer).

Metal concentration (µg g-1 dwt.) = $\frac{(X-Y)\,V}{W}$

Where is Y= Reading in ppm for blank, X = Reading in ppm for test sample, V = Final volume of digested samples (ml) and W = Dry weight of the sample (g).

Results and Discussion

Sample collected from Telco Industrial area Chinhat have highest no. of bacterial colony than others and sample collected from Kakori region having less bacterial colony than others.

Table 1: List of sites at which the spinach leaves samples were collected

Location	Co – ordinate	No. of samples collected	No. of Bacterial colony isolated
Mohanlalganj	26°41' 0"North, 80° 58' 0" East	1	$1.7x10^6$
Sarojani nagar Industrial area	26°44'40"North 80°52'12"East	1	$2.0x10^6$
Kakori	26.880 North 80.80 East	1	$1.5x10^6$
Bakshi ka talab	26059'0" North 800 53'0" East	1	$4.4x10^6$
Telco Industrial area Chinhat	26°53'29"North 81°2'45"East	1	$5.6x10^7$

Bacterial load on sample

Bacterial load was determined using leaf impression and serial dilution method. Bacterial load varied from 1.5x106 to 5.6x107 cfu/g of the leaf sample.

Table 2: Bacterial count of the leaf phyllosphere

Name of Sample	CFU/gm
Sample-1 (Mohanlal Ganj)	$1.7x10^6$
Sample-2 (Bakshi ka Talab)	$2.0x10^6$
Sample-3 (Kakori)	$1.5x10^6$
Sample-4 (Sarojini Nagar market)	$4.4x10^6$
Sample-5 (Telco Industrial area, Chinhat)	$5.6x10^7$

Biochemical characterization of isolates

The colonies that developed on nutrient agar were varied type and some of them were subjected to Gram staining to know the type of microflora dominating.

Table 3: Gram reaction results of bacterial colonies from spinach leaf sample

Test sample	Gram staining Result
Sample – 1	Gram negative, Rod shape
Sample – 2	Gram positive, Rod shape
Sample – 3	Gram positive, Rod shape
Sample – 4	Gram negative, Rod shape
Sample – 5	Gram negative, Spherical

After the biochemical characterization it estimates *Bacillus* and *streptococcus sp*. strains in leaf sample.

Table 4: Biochemical Characterization

Biochemical Characterisation	1	2	3	4	5
Casein hydrolysis	+ve	+ve	+ve	+ve	+ve
Motility Test	Nonmotile	Nonmotile	Motile	Motile	Motile
Catalase Test	+ve	+ve	+ve	-ve	+ve
Methyl Red Test	+ve	-ve	+ve	-ve	-ve
Citrate Utilization	+ve	+ve	+ve	-ve	-ve
Mannitol Fermentation	-ve	-ve	-ve	-ve	-ve
Lactose Fermentation	-ve	-ve	-ve	+ve	+ve
Sucrose Fermentation	-ve	-ve	-ve	+ve	+ve
Amylase Test	-ve	-ve	-ve	-ve	+ve

Estimation of Cadmium (Cd) heavy Metal: All the five samples showed the presence of cadmium indicating that either the soil is contaminated or the irrigation water contained cadmium [11]. Out of 5 spinach plant samples, sample from Telco industrial area, Chinhat had the highest concentration of cadmium in the leaves followed by sample 4 obtained from Sarojini nagar market which was probably grown in soil contaminated with industrial effluent.

These results clearly indicate that the soil was contaminated by the industrial effluents or during rainy season there is runoff from the industrial areas which may be contaminating the soil.

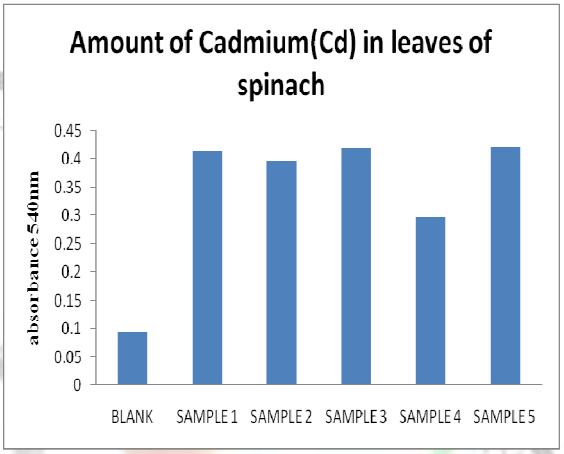

Fig. 1:Cadmium content in different samples of spinach leaf

Estimation of Iron (Fe) heavy Metal

All 5 samples were analyzed by AAS technique for iron content in leaves. Iron is an essential element for the growth of plants and comes under the category of micronutrients. The concentration of ferrous ranged from 3.825 ppm (Sample 3) to 21. 525. ppm (sample 5). Rest of the sample showed iron content in between.

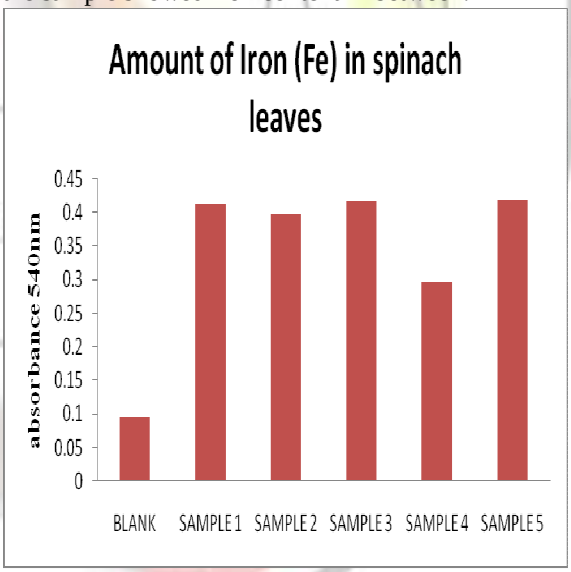

Fig.: 2 Iron content in different samples of spinach leaf

Here, presence of iron in good quantity in the leaves is an indication that other metals could not affect the iron uptake by the spinach leaves.

Estimation of Copper (Cu) heavy Metal

Sample five had the highest copper content followed by other samples Copper (Cu) is required in trace amounts for bacterial growth.

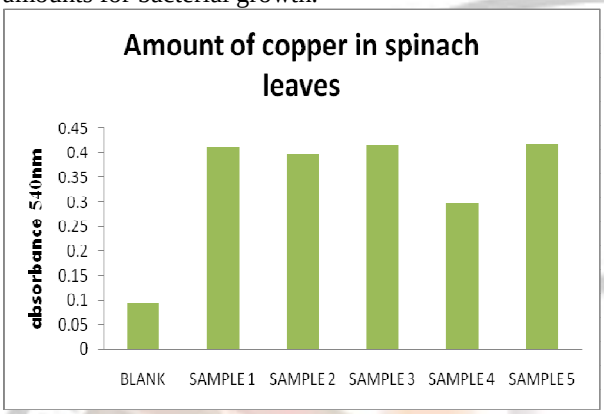

Fig. 3: Copper content in different samples of spinach leaf

Correlation between heavy metal content and bacterial load.

Increase in microbial load was directly correlated to the heavy metal content i.e. more the heavy metal in the sample, more was the microbial load i.e the tissues of the plant sample were more prone to attack by the microbes due to poor health of the leaves. For instance, chronic Cd exposures result in kidney damage, bone deformities, and cardiovascular problems. High levels of Fe can cause vomiting, upper abdominal pain, pallor, cyanosis, diarrhea, dizziness, shock, haemochromatosis, diabetes, diseases of liver, lungs and kidney, haepatoma and cardinomyopathy.

Result suggest that there may be cause different types of deseases which are create by these heavy metals awareness is must for food resources and for their irrigation water which farmer are used for the agriculture crops also. The obtained results coincide with [10], who have reported Toxic Metals Uptake by Spinach (*Spinacea oleracea*).

Conclusion

Higher concentration of heavy metals causes metabolic anomalies. For e.g. Lead inhibit the synthesis of RBC and thus vital transport system may get hamper. Higher concentration of Cadmium is toxic that damage kidney, liver, lungs. Muscular weakness and paralysis causes by Mercury (Hg). The presence of heavy metals in good quantity in the leaf tissues of spinach indicates that spinach easily uptakes heavy metals from the soil or through polluted water and also that post harvest handling is responsible for more microbial flora contribution[4]. The microbial community structure of the spinach Phyllosphere is impacted by the cultivar and environmental conditions during spinach development. Time of harvest and weather conditions before harvest may have important implications for maintaining product quality and potentially influence the establishment of human pathogens on the leaf surface. Consumption of these vegetables as food may not constitute possible health hazards to humans at the time of the study. Taking the health risks encountered in diets' as a result of high levels of heavy metals in vegetables, the maximum allowable levels of these metals in vegetables should not exceed levels that reflect good agricultural practices. Farmers should be educated on the problems associated with excessive usage of fertilizers and other chemicals, as well as irrigating the crops with waste and all sorts of polluted water and the needs to grow crops with safe levels of heavy metals.

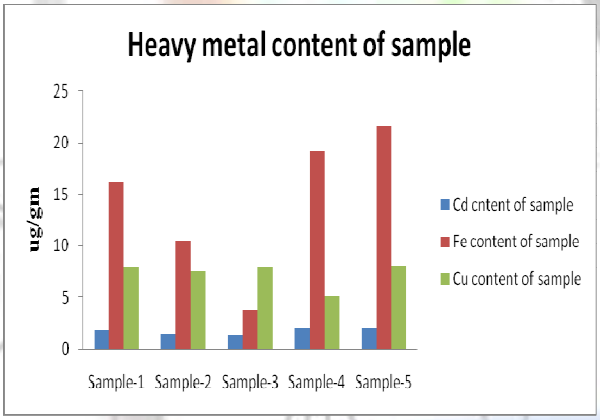

Fig. 4: Correlation between heavy metal presence and bacterial load in spinach

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