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Detection of heavy metals and elements via SEM technique in green leafy vegetables

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Abstract

Randomly collected green leafy vegetable samples were from Lucknow city in Alambagh local area. The experiment was conducted in the research laboratory of the Department of Food Science and Technology, USIC lab BBAU, Luck now. The heavy metals and elements detect by SEM (Scan Electron Microscope) technique. Present study explains the extent of heavy metal contamination in two leafy vegetables spinach (Spinacia oleracea) and coriander (Coriandrum sativum). Results showed that, spinach leaves contain some nontoxic elements are found e.g. C, O, Na, Si, S, Cl, K, The sample of spinach in present traces amount Silica (Si) was found that level weight 0.24% and atomic 0.13%, that level was not toxic, result of second sample coriander found nontoxic heavy metal and elements e.g. C, O, Na, Mg, S, K, Ca, Zr, Pt. Present study describe that present time sample of vegetable safe. In this Bothe sample no anyone heavy metals and toxic substance are not founding that samples.my research main aims to provided is awareness to peoples about toxic substances and heavy metals in vegetables and provided food safety.

Keywords: Heavy metals, leafy vegetables, spinach, coriander, SEM

1. Introduction

Vegetables are important part of human diet. They provide many essential nutrients to human body for normal growth and development. It can also prevent various toxic substances during digestion and support better health such as minimizing the risk of colorectal cancer and other diseases. However, nutritional quality and food safety measures should be kept in mind when recommending vegetables for human consumption because they contain both essential and non-essential minerals (Gupta N, Khan DK,2007) [1]. Present day scenario suggests that majority of population suffer from malnutrition and therefore, noticeable nutrient deficient syndromes are visible in human beings. Variety of leafy vegetables used in balanced diet (116g/day) as they are rich in minerals and vitamins. Implication associated with heavy metal contamination is of great concern, particularly in agricultural production. These metals can pose a significant health risk to humans, particularly in elevated concentrations (Gupta & Gupta, 1998) [2] Dietary exposure to heavy metals like, cadmium, lead, zinc and copper has been identified as a risk to human health through the vegetables consumption (Kachenko & Singh, 2006) [3] Main sources of heavy metals to vegetable crops are their growth media (soil, air, nutrient solutions) from which these are taken up by roots or foliage. Soil gets polluted due to waste water irrigation and absorbed minerals settle in edible tissue of the vegetables (Lokeshwari & Chandrappa, 2006) [4] Heavy metals can accumulate and migrate in soil environments. Due to their cumulative effects and long-term interactions, accumulation of heavy metals in soil negatively affects regional eco-safety and poses a threat to relevant animals and plants. Additionally, heavy metals can enter human bodies through the food chain, leading to an increased incidence of chronic diseases such as deformity and cancer (Müller and Anke 1994; Ramadan and Al-Ashkar 2007; Tembo *et al*. 2006) [5] The behaviors of metal in soils are very complex, which involve adsorption–desorption, complexation– dissociation, oxidation–reduction, ion exchange, and other carrier transport role. The former two reactions mainly affect metal activity in soil, while oxidation–reduction can also change metal valence (Swartjes *et al*. 2007) [6]. These chemical mechanisms can work together and affect one another, which formed a complex interaction system controlling metal fates. Heavy metals enter the vegetable tissues mainly through the roots and foliage, of which root uptake was the dominant pathway. Metals can be transferred from soil pore water into the plants though the roots in the form of dissolved ions (e.g., Cd2+) (McLaughlin *et al*. 2011) [7] Although certain heavy metals (Cr, Mn, Ni, Zn, Cu, and Fe) are essential components for various biological activities within the human body, elevated levels of them can cause numerous health consequences to mankind. In contrast, Pb, Cd, Hg and as are non-essential, toxic elements which are associated with many chronic diseases in human being. (Chen Y., Wu P., Shao Y., 2014) [8]. Heavy metals are detrimental to human health because they have the capability to accumulate in various parts of the human body. They may have adverse health effects even in low concentrations, because they are persistent in nature and cannot be degraded (Duruibe JO, Ogwuegbu MDC 2007) [9]. Many of the food plants can easily accumulate heavy metals and result in an increased quantity of these metals in the farm's produce. Contaminated soils are one of the major sources for food chain translocation of heavy metals and further their intake through consumption of contaminated vegetables and other crop plants which ultimately poses health risks for human health (Khan S, Cao Q 2007) [10]. Furthermore, the intake of metals (Cu, Cd, Ni, Pb, Zn, etc.) can have antagonistic effect on some of the essential nutrients inside the body, which results in the depleted immunological defenses, intrauterine growth retardation (caused by Al, Cd, Mn and Pb), psychosocial dysfunctions, and abnormalities such as malnutrition and gastrointestinal cancer. (Khan S, Rehman S 2010) [11]. Lead and cadmium are among the most abundant heavy metals and are particularly toxic. They are reported to cause several cardiovascular, kidneys, nervous and bone diseases (Kawatra and Bakhetia, 2008; INCHEM, 1993) [12] Heavy metals are mobile and easily taken up by the plants in the environment (Khairiah *et al*. 2004) [13]. Metals accumulation in vegetables may pose a direct threat to human health. Different substances occur naturally in our environment as a consequence of natural events.Many diseases are caused by the inability of environment to support the mineral needs of human, plants and animals or man. Untreated sewage and industrial water is commonly used for the cultivation of vegetables around farm lands (Razaal., 2005) [14]. Heavy metals are non-biodegradable and persistent environmental contaminants and thus readily accumulate to toxic levels. Implication associated with heavy metal contamination is of great concern, particularly in agricultural production. Dietary exposure to heavy metals like cadmium, lead, zinc and copper in elevated concentrations has been identified as a risk to human health through vegetable consumption (Kachenko & Singh 2006) [15] Furthermore, consumption of heavy metals-contaminated food can seriously deplete some essential nutrients in the body causing a decrease in immunological defenses, intrauterine growth retardation, impaired psychosocial behavior, disabilities associated with malnutrition and a high prevalence of upper gastrointestinal cancer (Arora *et al*. 2008) [16] Toxicological significance of these metals has been recognized several decades ago in developed countries.

Main sources of heavy metals to vegetable crops are their growth media (soil, air, nutrient solutions) from which these are taken up by roots or foliage. Soil gets polluted due to waste water irrigation and absorbed minerals settle in edible tissues of the vegetables (Lokeshwari & Chandrappa 2006) [17]. Food safety issues and potential health risks make this as one of the serious environmental concerns. Though, metals are indispensable part of our environment and play positive role in various biological processes such as signaling, homeostasis and enzyme catalysis, higher concentration of metals tend to toxic effects since they are prone to bioaccumulation and bio-magnification along the food chain. Industrialization and urbanization as well as anthropogenic activities are main source for heavy metal contamination (Begum & Harikrishna 2010) [18].

2. Materials and Methods

The experiment was conducted in the research laboratory of the Department of Food Science and Technology, USIC lab BBAU, Lucknow & in the analysis laboratory of RFARC (Regional Food Analysis & Research Centre) situated in Lucknow. The different tools & techniques used during experimental process were broadly described in this research. The samples taken at the Lucknow area. The Lucknow city stands at an elevation of approximately 123 meters (404fit) above sea level and covers an area of 2528 square kilometers (976 sq mi). The coordinates for the study area are 26.80 N, 80.90 E. Collect the vegetables samples from market area. The heavy metals and elements detect by SEM (Scan Electron Microscope) technique.

3. Sample preparation protocol for SEM

Specimen collection

Collect the sample (sample size 2-4mm)

Fixation (primary fixation)

Sample will be fixed in 2.5% Glutaraldehyde/karnovsky's fixative for 2-6 hours at 4oC.

Washing

Sample will be washed in 0.1 M Phosphate buffer, for 3 changes each of 15 min at 4oC.

Post fixation

1% Osmium tetroxide will be used as a post fixation for 2 hours at 4oC

Washing and dehydration

Sample will be wash in 0.1 M Phosphate Buffer for 3 changes each of 15 minutes at 4o^{0}C to remove the unreacted fixative.

Specimen will be dehydrate using increasing concentration of Acetone to remove water

30% Acetone	- 30 min
50% Acetone	- 30 min
70% Acetone	- 30 min
90% Acetone	- 30 min
95% Acetone	- 30 min
100% (Dry Acetone)	- 30 min (30% copper sulphate and 70% absolute acetone)

All steps will be carried out at 4o^{0}C

Drying

Specimen will be drying by air drying and critical point drying (critical point i.e. 31.5o ^{0}C at 1100 p.s.i)

Note

Do not allow the specimen to expose to air (avoid air drying). While transferring from one medium to another.

After drying protect the sample from excessive fluctuation in relative humidity. Keep the specimen in dry and dust free environment. Dessicatore can be used to store the dried sample.

Specimen mounting

Specimen will be mounting on to the aluminums stubs with carbon tape

Coating

Sample will be coat using sputter coater to make the sample conductive.

Specimen will be observing in scanning electron microscope**.**

Required chemicals

- 25% Gluteraldehyde
- Sodium di hydrogen orthophosphate (monobasic)
- Di sodium hydrogen phosphate anhydrous (dibasic)
- Osmium tetroxide
- Absolute acetone
- Anhydrous copper
- Distilled water
- Double distilled water
- Paraformaldehyde (optional)

0.2 M Phosphate buffer (Stock Solution)-

A. 5.93 g sodium di hydrogen orthophosphate will be added in 190 ml dH2O.

B. 23 g Disodium hydrogen phosphate anhydrous will be added to 810 ml of dH2O

Solution A and B will be added to make 1000ml of 0.2 ml phosphate buffer.

For 0.1 M phosphate buffer equal volumes of dH2O will be added to 0.2 M phosphate buffer.

2% Osmium tetroxide (stock solution to be stored at 4oC)

- Supplied as 1 g ampoules
- Dissolve 1 g OSO4 in 50 ml of double distilled water and allow overnight to completely dissolve.
- Store at 4C in tightly stopper, brown color bottle.
- Working solution is 1% OSO4. It is prepared by adding equal volumes of 0.2 M phosphate buffer in 2% OSO4 solution.

4. Tools

Fig 1: SEM (Scan Electron Microscope)

Fig 2: Aluminum stub

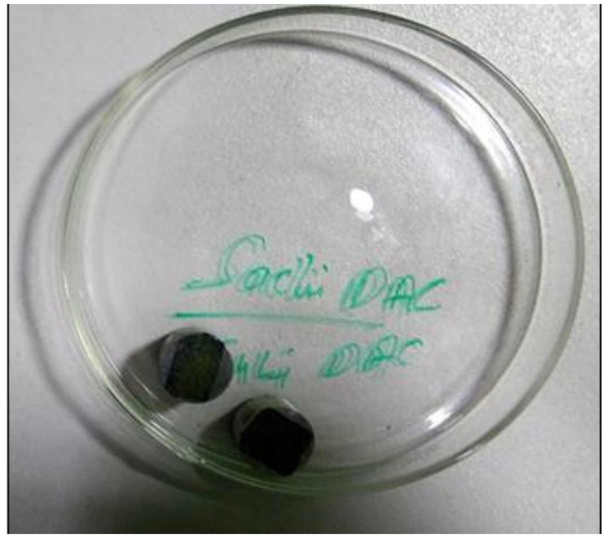

Fig 3: Poetry disc

5. Results and Discussion

The result are described properly. In this testing method use SEM.

Sample 1 coriander

The first sample coriander that have no any heavy metals (pb and As) are founding but some elements are found in this samples that are properly describe.

Table 1: Heavy metals and Elements in coriander

Element	Weight%	Atomic%
C K	56.72	70.42
O K	22.16	20.66
Na K	3.02	1.96
Mg K	1.13	0.69
S K	1.34	0.62
Cl K	3.34	1.40
K K	1.83	0.70
Ca K	6.96	2.59
Zr L	7.97	1.30
Pt M	-4.46	-0.34
Totals	100.00	

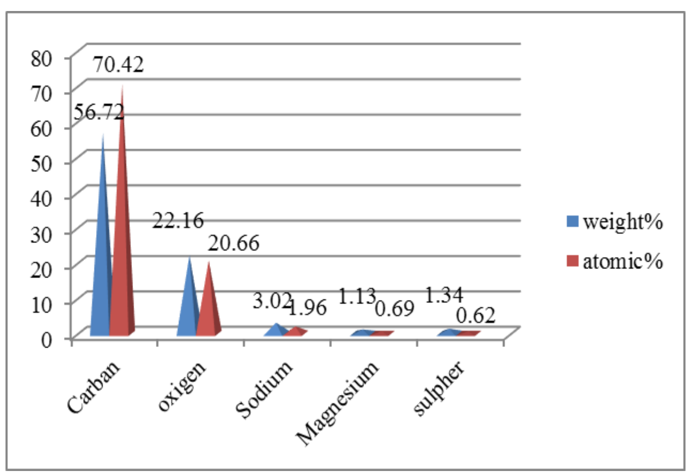

Fig 4: Present heavy metals and elements percentage graphical representation

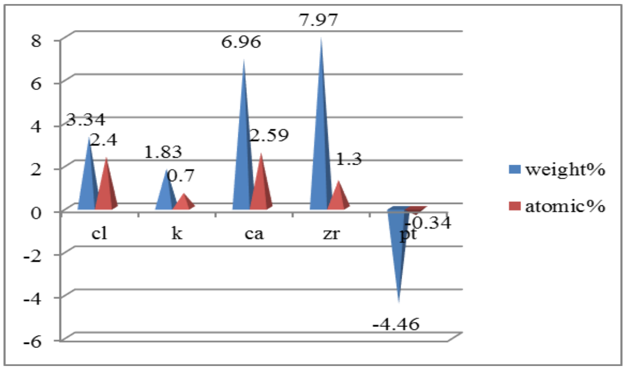

Fig 5: Present elements percentage graphical representation

Sample 2 Spinach

The second sample spinach after detection by SEM technique, no anyone heavy metals detect in the sample but some elements are detect. In this sample some silica particle are found but they are not toxic and harmful.

Table 2: Heavy metals and elements in spinach

Element	Weight%	Atomic%
C K	46.15	56.24
O K	39.94	36.54
Na K	6.07	3.86
Mg K	0.72	0.43
Si K	0.44	0.23
P K	0.91	0.43
S K	0.94	0.43
Cl K	0.76	0.31
K K	4.08	1.53
Totals	100.00	

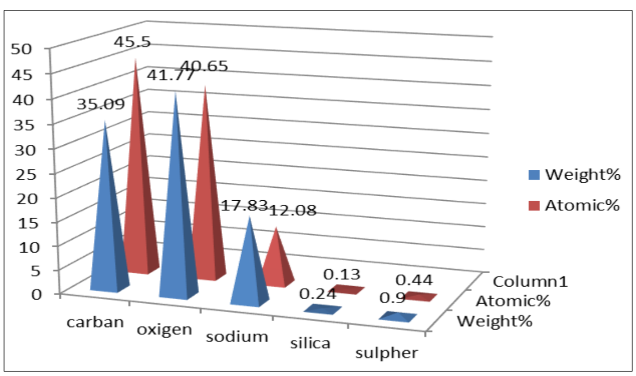

Fig 6: Graphical representation of elements and heavy metals

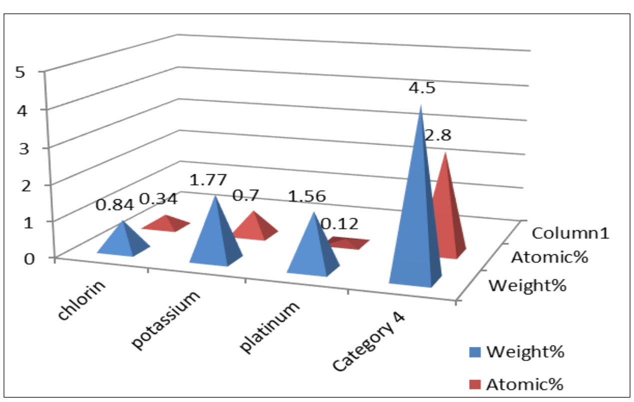

Fig 7: Graphical representation of elements and heavy metals

Present study describe that present time sample of vegetable safe. In this Bothe sample no anyone heavy metal (Pb and As) and toxic substance are not founding that samples. So the vegetable is safe, in this testing some elements are founding eg. C, O, Na, Mg, S, K, Ca, Zr, Pt, these samples are present in sample one coriander. Present these sample pt. are show but they are not natural, they are part of SEM. Pt. are used in testing.

The present second result spinach shows that no anyone heavy metals are fund, and no any toxic substance, after testing some result show elements eg. C, O, Na, Si, S, Cl, K. In this sample found the silica are naturally they are found all green leafs. Si main work to maintain the moisture in leafs.

6. Summary and Conclusion

Air pollution of the natural environment by heavy metals is a universal problem because these metals are indestructible and most of them have toxic effect on living organisms, when permissible concentration levels are exceeded. Heavy metals frequently reported in literature with regards to potential hazards and occurrences in contaminated soil are cadmium, copper, zinc and lead. The vehicular exhausts, as well as several industrial activities emit these heavy metals so that soils, plants and even residents along roads with heavy traffic loads are subjected to increasing levels of contamination with heavy metals. However, heavy metals are natural components of the earth's crust and cannot be degraded nor destroyed. The metal concentration in the green leafy vegetables from the market that is safe and no any toxic effect on health. The testing of both sample spinach and coriander was tested then, no anyone heavy metal (Pb, as) was found. In this sample some nontoxic elements are found eg. C, O, Na, Mg, S, K, Ca, Zr, Pt, these was founding in coriander sample. And second sample elements was found eg. C, O, Na, Si, S, Cl, K. The sample of spinach in present traces amount Silica (Si) was found that level weight 0.24% and atomic 0.13%, that level was not toxic. They have no any effect on health's are founding in green leafs naturally. The main work of silica to maintain the moisture of leafs. But high amount of silica causes cancer.

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