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MULTIVARIATE ANALYSIS OF THE HEAVY METAL CONCENTRATIONS IN THE VEGETABLE AND SOIL SAMPLES–A CASE STUDY FROM DISTRICT CHARSADDA AND DISTRICT MARDAN

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Abstract

Multivariate statistical methods like cluster analysis, principal component analysis (PCA) and regression analysis were applied on the metals concentration of both the vegetables and soil samples collected from the districts Charsada and Mardan to classify them in different groups. The concentrations of seven heavy metals including Cu, Cr, Co, Ni, Ag, Pb, Sb were investigated using atomic absorption spectrophotometer (AAS) in the leaves, fruits, extracts, and soil samples of potato, colocasia, turnip, radish, cabbage, angular loofah, cucumber, bitter gourd, round melon, and pumpkin. The concentration of copper, chromium, cobalt, nickel, silver, lead and antimony were found in the range of 6.133-72.933, 32.233-92.5, 2.25-8.083, 0.366-143.53, 0.4-4.467, 11.916-157.96, 28.75-165.367 mgKg^{-1} respectively.

Key words: Heavy metals, Multivariate analysis, Vegetables and soil samples, AAS.

Introduction

Vegetables constitute important components of the diet, by contributing vitamins, proteins, calcium, iron and other nutrients. These nutrients are essential for plants, animals, and human growth (Bigdeli & Seilsepour, 2008).Heavy metals including Zn, Mn, Ni, and Cu etc., have been reported to have negative and positive roles in human life. Some of these heavy metals at lower concentrations act as micro-nutrients. However, at higher concentrations, Zn, Ni, and Cu become toxic and the other metals like Pb, Cr, Cd, Ag, Sb become cumulative poisons. These metals can cause the environmental hazards as well as the detrimental effects on human health (Chen *et al*., 1999; Anthemidis *et al*., 2005).

Waste water irrigation, atmospheric deposition, climatic conditions, concentrations of heavy metals in soil, degree of maturity on harvesting, and nature of soil are some key factors affecting the uptake and bioaccumulation of heavy metals in both the non-edible and the edible parts of vegetables (Jan *et al*., 2010; Singh *et al*., 2004; Sharma *et al*., 2009; Jassir *et al*., 2005). Vegetables and Crops grown-up in soils contaminated with heavy metals have greater accumulation of heavy metals than those grown in uncontaminated soil (Marshall *et al*., 2007). Intake of vegetables is an important path of heavy metal toxicity to human being and the health risk due to heavy metal contamination of soil has been widely reported (Eriyamremu *et al*., 2005; Muchuweti *et al*., 2006; Satarug *et al*., 2000).

The current study was aimed at exploring the presence of seven heavy metals *viz.* Cu, Cr, Co, Ni, Ag, Pb, and Sb in the edible parts of ten vegetables and in the soil samples collected from the places where these vegetables are grown, based on the fact that the intake of these heavy metals poses health hazards to the human beings and animals relying on these metal contaminated nutritious food crops (Tripathi *et al*., 2001).

Materials and Methods

Sampling: Soil, fruit and leaves of radish and potato were collected from Katlang (Khyber Pakhtunkhwa KPK Pakistan), turnip and calocasia were collected from Sardheri, Charsadda district (Fig. 1; No. 17), and cabbage were collected from Mardan district (Fig. 1; No. 13).

Reagents: Analytical grade reagents and double deionized water were used in the study for the sample preparations and making various dilutions. Standard solution for a calibration curve were prepared by diluting a standard solutions containing 1000 mg L^{-1}(PerkinElmer) of given element.

Apparatus: A PerkinElmer Analyst Pin AAcle 900T atomic absorption spectrometer (FAAS) equipped with HGA graphite furnace and with deuterium background corrector was used in the experiment. Analyses were performed on a 10 cm long slot-burner head; a lamp and an air-acetylene flame were used. The metals; Cu, Cr, Co, Ni, Ag, Pb, Sb were determined by Flame AAS. The recommended operating parameters used for FAAS are given in Table 1.

Sample preparation: To remove the dust and other foreign particles, the samples were thoroughly washed after collection by using the distilled water. Roots, stems and leaves of each sample were separated and cut into small pieces with the help of a knife. Soil samples were dried in sun while leaves and fruit samples were dried in an oven at 70˚C for 72 hours. Dried samples were ground with a grinder. All the samples were sieved through 50 mesh sieves. Fine powders were then stored in air tight bottles. 2.0 g of each sample was weighed, dissolved in a mixture of HNO_3, $HClO_4$ and HCl (2:1:1), and 5 mL of H_2O_2 was added to each sample to enhance the oxidation of samples. The samples were kept overnight in air at room temperature and then were heated at 80-85˚C for 3-4 hours till red fumes appeared. After heating the samples were allowed to cool and then were filtered with whatman 42 and diluted with distilled water to 100 mL in a volumetric flask. This clear solution was then analyzed for different heavy metals by FAAS.

Table 1. Instrumental conditions for investigated elements for FAAS.

Element	Acetylene ($Lmin^{-1}$)	Air($Lmin^{-1}$)	Wavelength (nm)	Slit width(nm)
Cr	2.50	10	357.9	0.7
Co	2.50	10	240. 7	0.2
Ni	2.50	10	232.0	0.2
Ag	2.50	10	328.1	0.7
Pb	2.50	10	283.3	0.7
Sb	2.50	10	217.6	0.2
Cu	2.50	10	324.8	0.7

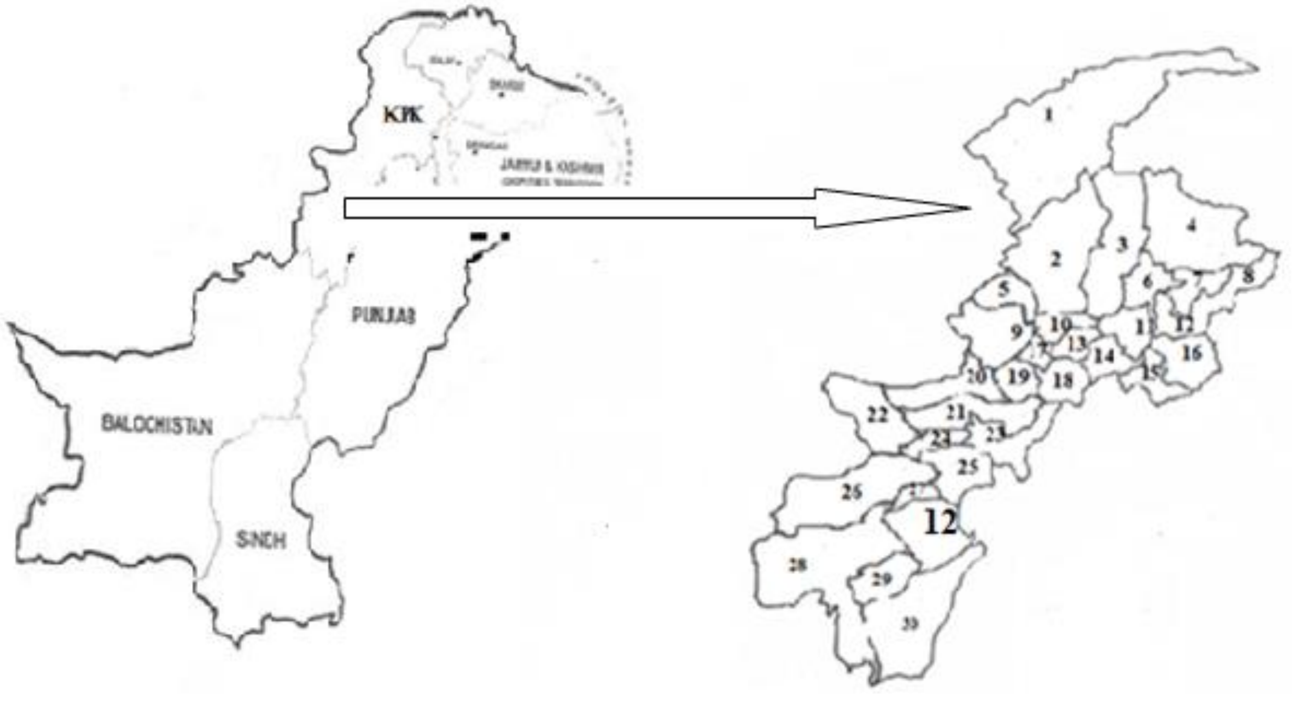

Fig. 1. Location map of the sample collection points in the polluted area.1: Chitral; 2: Dir; 3: Swat; 4: Kohistan; 5: Bajaur agency; 6: Shangla; 7: Battagram; 8: Naran; 9: Mohmand agency; 10: Malakand agency; 11: Buner; 12: Lakki Marwat; 13: Mardan; 14: Swabi; 15: Haripur; 16: Abbottabad; 17: Charsadda; 18: Nowshehra; 19: Peshawar; 20: Khyber agency; 21: Orakzai agency; 22: Kurram agency; 23: Kohat; 24: Hangu; 25: Karak; 26: North Waziristan; 27: Bannu; 28: South Waziristan; 29: Tank; 30: Dera ismail Khan.

Results and Discussion

The concentrations of seven heavy metals (Cu, Cr, Co, Ni, Ag, Pb, Sb) in various edible parts of the selected vegetables and fruits as well as in the soil samples where these are grown is determined by the established methods. The results of the current study indicated the concentrations of copper, chromium, cobalt, nickel, silver, lead and antimony in the range of 6.133–72.933, 32.233–92.5, 2.25–8.083, 0.366–143.53, 0.4–4.467, 11.916–157.96, and 28.75–165.367 $mgKg^{1-}$of the dry weight respectively (Table 2).

Copper is essential element for human body but very high intake can cause adverse health problems such as nausea, vomiting, central nervous system inhibition, blood cells, and liver and kidney damage (Eftekhari *et al*., 2014; Vadiraj & Belagali, 2014). Osteoporosis, leukopenia, and hypochromic anaemia are some of the diseases due to copper deficiency in children (Kanumakala *et al*., 2002). High concentration of copper was found in cabbage soil (72.933 $mgKg^{-1}$) while its lower concentration (6.133 $mgKg^{-1}$) was found in bitter gourd fruit.

High concentration of cobalt was found in potato soil (8.083 $mgKg^{-1}$) while its lower concentration (2.25 $mgKg^{-1}$) was found in red cabbage. Cobalt is a component of vitamin B12 (cyanocobalamin), and plays an important role in the production of erythropoietin. It has been used medically for the treatment of anemia (Barceloux, 1999); however, exposure to high levels of cobalt can result in thyroid gland damage and the disorders of lungs and heart (Stojanovic *et al*., 2014).

High concentration of chromium was found in round melon soil (92.5 $mgKg^{-1}$) while its lower concentration (32.233 $mgKg^{-1}$) was found in radish leaves. Chromium is among one of the important component of diet and considered an essential trace element. It is involved in the function of insulin and metabolism of lipids (Anderson, 1997; Bratakos *et al*., 2002) in living organisms. However its high concentration can cause renal tubular necrosis, dermatitis, lung cancer, and perforation of the nasal system (Gad, 1989).

Table 2. Metals concentration in vegetables and soil samples (N = 3).

Sample	Cu*	Co*	Cr*	Ag*	Ni*	Pb*	Sb*
Potato soil	62.07±0.02	8.08±0.01	60.80±0.03	4.47±0.01	104.45±0.001	157.95±0.001	165.37±0.035
Potato extract	22.133±0.000	4.05±0.01	66.67±0.02	2.77±0.00	8.02±0.001	27.283±0.017	84.583±0.019
Potato leaves	14.72±0.00	3.217±0.005	65.67±0.01	2.32±0.00	7.283±0.0020	27.45±0.003	83.567±0.024
Colocasia soil	62.467±0.003	5.167±0.006	75.27±0.05	2.67±0.005	113.067±0.005	52.00±0.007	123.217±0.008
Colocasia extract	37.95±0.001	2.35±0.00	51.08±0.01	1.50±0.00	2.417±0.007	23.63±0.001	86.92±0.01
Colocasia leaves	32.150±0.001	2.750±0.008	52.083±0.007	1.367±0.001	6.083±0.001	26.417±0.010	90.633±0.009
Turnip soil	67.117±0.002	5.567±0.003	67.267±0.007	2.60±0.001	102.20±0.003	61.33±0.015	140.783±0.026
Turnip extract	12.050±0.003	3.150±0.003	53.717±0.016	1.717±0.001	4.067±0.001	36.633±0.003	99.633±0.0095
Turnip leaves	10.100±0.001	3.850±0.001	48.167±0.005	1.950±0.001	6.500±0.001	43.85±0.004	105.267±0.026
Radish soil	53.433±0.002	5.983±0.012	50.400±0.344	2.650±0.001	93.60±0.006	64.833±0.005	159.35±0.017
Radish extract	7.0167±0.001	3.517±0.014	43.017±0.021	0.933±0.000	6.483±0.0010	19.133±0.005	123.033±0.021
Radish leaves	29.533±0.004	4.133±0.006	32.233±0.039	1.617±0.001	11.967±0.001	38.933±0.005	132.733±0.003
Cabbage soil	72.933±0.002	4.817±0.015	69.30±0.02	2.367±0.001	143.53±0.01	52.45±0.01	113.483±0.01
Red cabbage	9.183±0.001	2.250±0.003	49.30±0.01	1.27±0.00	2.767±0.001	27.683±0.001	80.47±0.01
White cabbage	7.933±0.001	2.950±0.004	45.50±0.01	1.15±0.01	4.08±0.00	30.083±0.001	90.15±0.019
Angular loofah soil	23.200±0.007	6.633±0.008	61.05±0.014	1.083±0.001	48.667±0.007	27.983±0.003	63.983±0.015
Angular loofah fruit	8.166±0.003	2.383±0.010	48.617±0.020	0.5±0.001	0.45±0.001	11.916±0.007	28.75±0.115
Angular loofah leaves	7.383±0.004	4.533±0.004	47.967±0.016	0.783±0.002	3.7±0.003	20.65±0.01	50.216±0.023
Cucumber soil	45.70±0.01	6.40±0.013	74.05±0.013	1.283±0.001	77.733±0.006	34.016±0.007	55.3±0.031
Cucumber fruit	18.666±0.004	4.00±0.005	48.067±0.001	0.950±0.002	3.267±0.002	18.3±0.011	38.85±0.019
Cucumber leaves	9.383±0.003	7.417±0.006	49.117±0.019	1.083±0.001	23.733±0.002	22.083±0.008	68.75±0.022
Bitter gourd soil	22.716±0.005	6.867±0.004	62.85±0.010	1.350±0.001	45.65±0.011	29.55±0.014	68.283±0.005
Bitter gourd fruit	6.133±0.003	3.083±0.009	48.05±0.005	0.483±0.001	0.366±0.003	14.283±0.005	33.467±0.017
Bitter gourd leaves	7.333±0.001	5.00±0.006	45.667±0.035	0.85±0.0002	3.183±0.001	17.616±0.005	50.2±0.002
Round melon soil	52.183±0.0097	7.35±0.006	92.50±0.004	1.350±0.001	124.833±0.013	38.55±0.008	64.35±0.015
Round melon fruit	6.283±0.001	3.35±0.007	45.483±0.016	0.65±0.001	1.350±0.026	16.116±0.004	36.283±0.009
Round melon leaves	6.15±0.001	2.933±0.007	46.133±0.012	0.667±0.001	1.033±0.001	13.85±0.002	34.3±0.023
Pumpkin soil	24.15±0.003	5.216±0.011	62.667±0.018	0.817±0.001	41.417±0.008	25.70±0.005	50.433±0.019
Pumpkin fruit	10.367±0.002	2.867±0.006	45.65±0.013	0.4±0.001	1.25±0.001	14.933±0.007	31.967±0.004
Pumpkin leaves	12.567±0.002	6.567±0.003	49.167±0.016	0.75±0.001	5.317±0.001	18.816±0.009	47.8±0.029

*Concentrations are reported in $mgKg^{-1}$ of the dry matter

Table 3. Linear correlation coefficient matrix for selected metals in vegetable and soil samples.

Sample	Ag	Co	Cr	Cu	Ni	Pb	Sb
Ag							
Co	0.297						
Cr	0.549	0.484					
Cu	0.417	0.432	0.735				
Ni	0.357	0.626	0.798	0.884			
Pb	0.674	0.495	0.305	0.388	0.433		
Sb	0.856	-0.109	-0.417	0.124	0.044	0.093	

High concentration of silver was found in potato soil (4.467 $mgKg^{-1}$) while its lower concentration (0.4 $mgKg^{-1}$) was found in pumpkin fruit.

Nickel (Ni) plays important role in lipid metabolism, hormonal activity, stabilization of RNA and DNA, and enzyme activation (Grembecka & Szefer, 2011; Nabrzyski, 2007). Although the inhalation of Ni and its compounds poses moderate toxicity, they can cause serious problems to the respiratory system including cancer (Silva *et al*., 2009). High concentration of nickel was found in cabbage soil (143.53 $mgKg^{-1}$) while its lower concentration (0.366 $mgKg^{-1}$) was found in bitter gourd fruit.

Lead has carcinogenic properties, which affect the digestive system and the respiratory system. The immune system is also affected by this metal. Children are affected particularly, where it affects the nervous system and the intelligence (Borges *et al*., 2003). High concentration of lead was found in potato soil (157.96 $mgKg^{-1}$) while its lower concentration (11.916 $mgKg^{-1}$) was found in angular loofah fruit.

Antimony is a toxic element, and excess intake results in many diseases in humans, such as cancers, cardiovascular disease, liver and respiratory disease (Anon., 2003). Long term exposure to Sb could cause liver cirrhosis (Feng *et al*., 2013). High concentration of antimony was found in potato soil (165.367 $mgKg^{-1}$) while its lower concentration (28. 75 $mgKg^{-1}$) was found in angular loofah fruit.

The metal-to-metal correlation study (Table 3) in the vegetables and soil samples showed that the mean concentration of Ag, Sb, Cu, Ni, Cr mutually depend on each other. A strong correlation was observed between Ni and Cu (r = 0.884), Ag-Sb (r = 0.856), Ni-Cr (r = 0. 798), Pb and Ag (r = 0. 674), Ni-Co (r = 0. 626).

Multivariate and univariate techniques have been proven to be the effective statistical tool in analyzing the food and environmental samples for the evaluation of analytical quality. Cluster analysis using average linkage method classified various samples into two small groups, three medium groups and a large group. BGF, RML, RMF, PF, AlF, CuF, ALL, BGL, PL were placed in one small group, second small group comprised of RC, WC, TF, TL, PoF, PoL, CF, CL First medium group comprised of CuF, ALL, BGL, PL, ALS, BGS, PS, second medium group comprised of PL, ALS, BGS, PS, CuL, RC, WC, TE, TL, PoE, PoL while third medium group comprised of CuS, RMS, CS, TS and RS. Large group comprised of RC, WC, TE, TL, PoE, PoL, CE, CL, RE, RL, CuS, RMS, CS, TS and RS (Fig. 2).

The PCA reduces a large number of variables into a new set of variables based on their mutual dependence. PCA using varimax normalized rotation was used for factor loading in vegetables and soil samples. PCA extracted two factors, embodying 82.77 of total variance. The contribution of first factors was 64.430 with the maximum loading for Cu, N, Ag, Pb, Sb, Cr, Co (Cu>Ni>Ag>Pb>Sb>Cr>Co), the contribution of second factor was 18.340 with the maximum loading for Cr, Co and Ni (Cr>Co>Ni) and a reasonable loading for Cu (Table 4).

Table 4. Regression and correlation.

Regression equation	Correlation (r)
Co = Ag[0.687] + 3.532	0.297
Cr = Ag[5.578] + 47.002	0.549
Cu = Ag[16.443] + 1.059	0.417
Ni = Ag[29.621] - 10.516	0.357
Pb = Ag[26.068] - 4.746	0.674
Sb = Ag[35.469] + 27.625	0.856
Cr = Co[3.579] + 38.974	0.484
Cu = Co[5.910] - 1.507	0.432
Ni = Co[16.971] - 43.901	0.626
Pb = Co[7.965] - 2.424	0.495
Sb = Co[5.511] + 55.007	-0.109
Cu = Cr[1.103] - 35.552)	0.735
Ni = Cr[2.709] - 116.407	0.798
Pb = Cr[0.647] - 1.967	0.305
Sb = Cr[0.388] + 58.648	-0.417
Ni = Cu[1.942] - 15.978	0.884
Sb = Cu[1.159] + 50.676	0.124
Pb = Ni[0.380] + 21.153	0.433
Sb = Ni[0.442] + 65.366	0.044
Sb = Pb[1.062] + 44.166	0.093

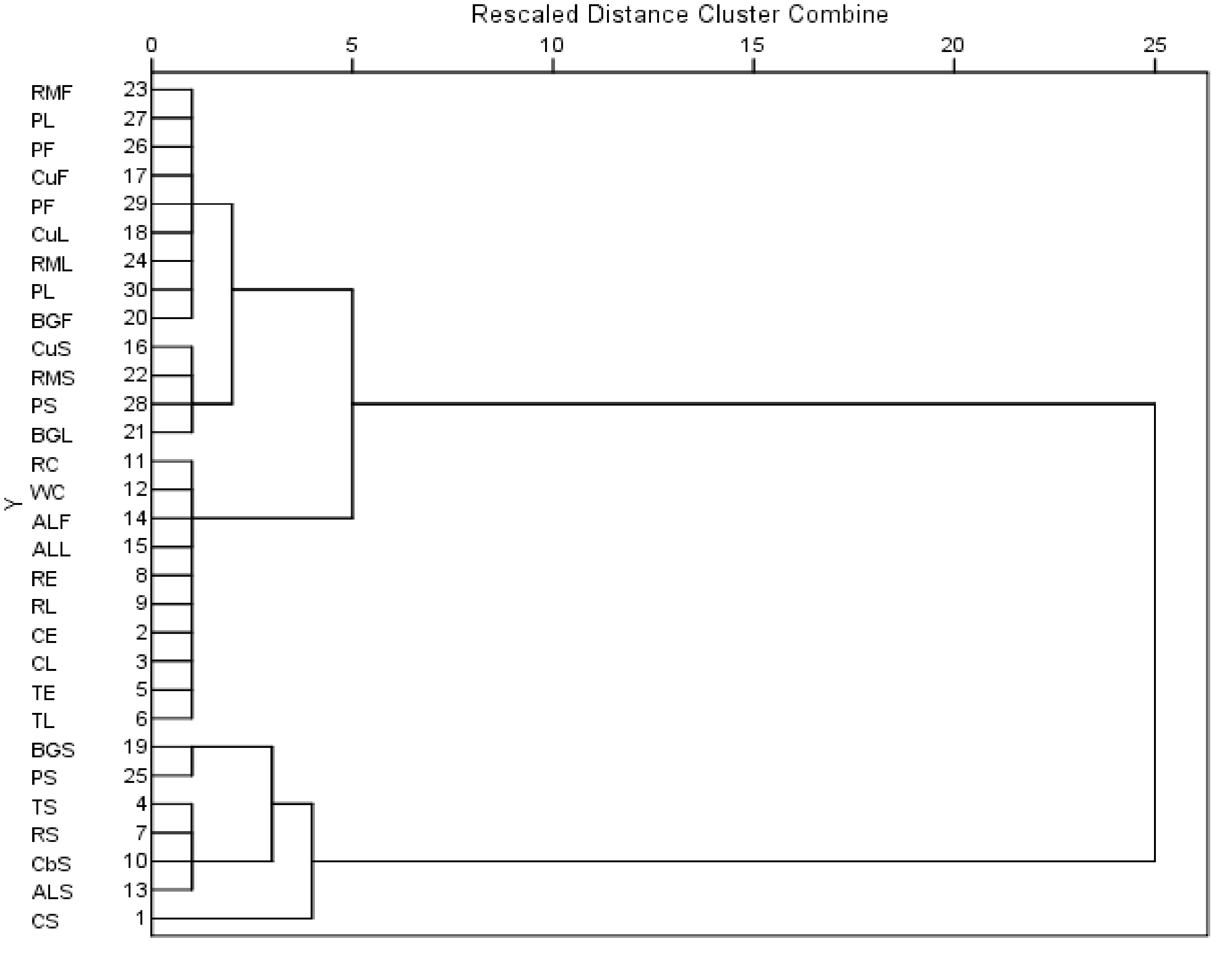

Fig. 2. Dendogram of selected metals in vegetables and soil using ward's method. PoS-Potato soil, PoE-Potato extract, PoL-Potato leaves, CS-Colocasia soil, CE-Colocasia extract, CL-Colocasia leaves, TS-Turnip Soil, TE-Turnip extract, TL-Turnip Leaves, RS-Radish Soil, RE-Radish extract, RL-Radish leaves, CbS-Cabbage soil, RC-Red cabbage, WC-White Cabbage, ALS-Angular loofah Soil, ALF-Angular loofah fruit, ALL-Angular loofah leaves, CuS-Cucumber soil, CuF-Cucumber fruit, CuL-Cucumber leaves, BGS-Bitter gourd soil, BGF-Bitter gourd fruit, BGL-Bitter gourd leaves, RMS-Round melon soil, RMF-Round melon fruit, RML-Round melon leaves, PS-Pumpkin soil, PF-Pumpkin fruit , PL-Pumpkin leaves.

Multivariate and univariate statistical analysis are useful for classification of samples into new groups, correlation of metals in different samples. Principal component analysis reduces a large number of variables to a new set of variables based on mutual dependence. Thus the multivariate analysis helps in the interpretation of complex data. Cluster analysis using average linkage method divided the samples into three broad groups. From the statistical analysis it is clear that Zn, Sb and Fe are the predominant elements. The results of the studied soil and vegetable samples produced in Pakistan were acceptable to human consumption at nutritional and toxic levels. The levels of Cr, Co, Ag, Pb, Sb, and Zn in the studied samples were found within the recommended legal limits for human consumption.

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