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A multivariate study: Variation in uptake of trace and toxic elements by various varieties of *Sorghum bicolor* L.

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Abstract

The aim of present study was to evaluate the variation in uptake of elements (As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) by different varieties of *Sorghum bicolor* L., plants grown in soil amended with untreated industrial waste water sewage sludge (SUIS), on same experimental plots. The power of chemometrics was also used in exploring the potential natural and/or anthropogenic sources responsible for elemental contents in different varieties of sorghum. Hierarchical cluster analysis was used to explore the different variety of sorghum grouping according to corresponding their SUIS samples as additional information to the output obtained by principal component analysis. Significant genotypic variation was detected in the fourteen elements concentrations in sorghum grains, indicating the possibility to reduce the concentration of toxic elements in grains through breeding approach. It was observed that high tolerance limit of toxic elements was observed in sorghum variety PARC-SV-1.

Keywords: Micronutrient; Sorghum; Toxic elements; Untreated industrial sewage sludge

1. Introduction

The beneficial effects of using waste water sludge on agriculture have been proved by numerous researchers. The presence of toxic elements in the applied sludge can result in phytotoxic effects, soil and water contamination and accumulation of toxic elements in food supplies [1,2]. Moreover, toxic elements enter the food chain due to uptake and accumulation by crops, posing a potential threat to human health [3,4].

Metals accumulation in crops is a function of complex interaction among soil, plant and environmental factors. It has been well documented that the contents of elements in crop plants are closely associated with their levels in soil. Moreover, the uptake and accumulation of elements by plants are largely dependent on the available rather than total level of an element in soil [5,6]. In recent years, there has been increasing awareness and concern over toxic elements contamination of soils and the effects; this may be having on the food chain. Furthermore, the location of soil where the plants were cultivated should be also subjected to investigation as far as the content of major trace and toxic elements in plants is affected both by the geochemical characteristics of soil and by the ability of plants to uptake, transport and accumulate elements selectively [7].

The uptake of some toxic elements varies in different plant species [8]. It was investigated that the accumulation of Cd in rice was reduced by means of genetic improvement [9,10]. Breeding for low Cd accumulating cultivars has been undertaken in sunflower and durum wheat [11]. This practice depends on understanding genetic and environmental variation in toxic elements concentrations of crops. However, little is known about genotypic and environmental variation in toxic elements concentration, including As, Cd, Cr, Ni and Pb.

Complex and high dimensional quantitative relationships between plants and soil as environmental compartments require additional approaches and chemometry could provide complementary information in this sense. Thus, it appeared as very

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useful to apply pattern recognition techniques such as principal component analysis (PCA) and cluster analysis (CA) for determining elemental patterns and visualizing rather complex relationships in different matrices [12–14] and in plants and soils [15,16].

Uncontrolled disposal of sludge on abandoned land, marine, and river are common in developing countries such as Pakistan, because of the expensive cost in providing proper treatment for sludge. In Pakistan, currently, there is still a lack of central policy on untreated sewage waste application on agricultural land, which is currently the major option for sewage sludge disposals in nearby the cities, where mostly grain crops and vegetables are grown. Unacceptable health and aesthetic problems are associated with untreated sewage pooling on the ground surface or being directly discharged into receiving waterways. For selecting different varieties of grain crops having high tolerance limit for toxic elements, to safely grown in soil dressed with untreated sewage sludge. In present work, the variation in uptake of elemental contents (As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) by different varieties of sorghum grains, grown in same experimental plots contains soil amended with untreated industrial sewage sludge (SUIS) were studied. The all elements were measured by spectroscopic techniques (FAAS/ETAAS), after the complete dissolution of their matrices with microwave assisted acid digestion [17]. The results were statistically evaluated by multivariate methods of analysis to identify some investigated varieties of sorghum plants have high tolerance limit for toxic elements.

2. Materials and methods

2.1. Reagents and glassware

Ultrapure water obtained form ELGA labwater system (Bucks, UK) was used throughout the work. Hydrochloric acid (37%, s.g. 1.4), nitric acid (65% s.g. 1.19), hydrogen peroxide (30%), were analytical reagent-grade E. Merck (Darmstadt, Germany). Standard solutions of As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn were prepared by dilution of 1000 ppm certified standard solutions, Fluka Kamica (Buchs, Switzerland) of corresponding metal ions. $Mg(NO_3)_2$ stock standard solution, $5.00\,g\,L^{-1}$, used as a chemical modifier, was prepared from $Mg(NO_3)_2$ Merck (Darmstadt, Germany). Pd stock standard solution, $3.00\,g\,L^{-1}$, used as a chemical modifier, was prepared from Pd 99.999% Sigma–Aldrich (Milwaukee, WI, USA). The certified reference material BCR 483 and BCR 189 was purchased from the Bureau of References of European Communities. All glassware and plastic material used was previously treated for a 24 h in 2N nitric acid and rinsed with double distilled water and then with ultrapure water.

2.2. Apparatus

A domestic microwave oven (PEL PM023 Japan), programmable for time and microwave power from 100 to 900 W, was used for digestion of samples (soil amended with sewage sludge and grains of sorghum). A WTW pH meter was used to determine pH of SUIS (SUIS:water ratio 1:2.5, w/v). The determination of elements in digests of grains and SUIS were carried out by means of atomic absorption spectrometer of Hitachi Ltd., Model 180–50, S.N.5721-2, with a deuterium lamp back corrector equipped with 10 cm burner head and graphite furnace GA-03. Hitachi Model 056 recorder was used for recording analytical data of the metals under investigation. Hollow cathode lamps were used as radiation sources. Lamp intensity and band pass width and other measurement conditions were used according to the manufacturer's recommendations. The Ca, Cu, Fe, K, Mg, Na and Zn were measured under optimized operating conditions by FAAS with air-acetylene flame [18], while As, Cd, Co, Cr, Mn, Ni and Pb were determined by ETAAS [19].

2.3. Sampling of soil and untreated industrial sewage sludge

Soil samples were collected from agricultural site in Hyderabad division (South Western Pakistan), from the surface layer (0–25 cm). The soil samples placed in plastic containers, and transported to the laboratory. The untreated industrial sewage sludge samples were collected from industrial catchment areas of Hyderabad having tanneries, electroplating and glass industries. Twenty-four sewage sludge samples were collected in 2004–2005. All twenty-four samples were mixed together thoroughly with hand blender and made a representative sample for pot experiment. The soil and composite sewage sludge samples were air dried for eight days, and ground with an Agate mortar and sieved through a nylon sieve <75 μm mesh screen. The final samples were kept in labeled polypropylene containers at ambient temperature before analysis.

2.4. Plant growth experiment

In the green house of the National Centre of Excellence in Analytical Chemistry, Sindh University Jamshoro, Pakistan, twenty plots of $45\,cm \times 35\,cm \times 35\,cm$ (length × width × height) were prepared. Plots were filled with soil amended with industrial sewage sludge in the ratio of (3:1 w/w), used to planting different varieties of sorghum.

The experimental design consisted of randomized complete blocks, in split-lots with four replications. During the sorghum growing season (June to August) in 2 years, 2004–2005, five sorghum varieties, named (PARC-SV-1, PARC-SS-1, PARC-SS-2, 95-CS-002BARI, ICRICHAT-CSV13) which are commonly grown in different areas of Pakistan, issued by Federal Seed Certification and Registration Department Pakistan, were cultivated under the same conditions. Throughout the experimental period, the humidity of all plots was maintained at a uniform level by daily application of deionized water. After maturity the sorghum plants grown in the plots were harvested, and the edible and nonedible parts were separated. The edible parts (grains) were washed thoroughly with tap water, dilute acid solution (0.01N HCl) and ultrapure water for removing the mechanically adhering impurities. The sorghum grain samples were oven dried for 72 h at 70 °C and then ground to pass a 1.0 mm sieve for determining of elemental contents.

2.5. Microwave acid digestion procedure

A procedure based on an acid digestion induced by microwave energy was optimized in order to measure the total elemental content in SUIS and grain samples [20]. The acid digestion was carried out in a domestic microwave oven. About 200 mg of replicate five samples of SUIS, BCR 483, BCR 189 and grains of each variety were weighed in the 25 mL flasks separately and placed in a polytetrafluoroethylene container. The 2 mL of acid oxidant mixture (HNO_3:H_2O_2 (2:1) ratio) were used for sorghum grains and BCR 189, where as 3 mL of the aqua regia (HNO_3:HCl (3:1) was used for SUIS and BCR 483 samples. All flasks were kept at room temperature for 2 h. Then the container was placed in domestic microwave oven (PEL PM 023) programmable for time, with microwave power from 100 to 900 W, and heated at 80% of total power, 15 min for BCR 483, BCR 189, SUIS samples, where as 5 min was required for the total dissolution of sorghum grains. After cooling, the sample digests were diluted with ultrapure water and filtered through a Whatman 42 filter paper, transferred into a 25 mL flask, and brought to volume with ultrapure water. Analytical blanks were prepared in the same way, without addition of any sample.

3. Data processing

3.1. Univariate analysis

Univariate characterization was carried out previously to check the distribution of data for each element by applying Ryan–Joiner's test and to detect potential outliers with Grubbs test as well.

3.2. Multivariate analysis

A data matrix, which rows have different varieties of Sorghum and SUIS, the columns contain elements under study As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn. A data pretreatment was made in order to avoid the differences in measurement units. Auto scaling is the most widely used scaling technique. The result is a variable with zero mean and a unit standard deviation. The data set of concentration measurements was subjected to a principal component analysis in order to decrease the number of descriptors responsible for the highest percentage of a total variance of the experimental data. It allows the relationship between variables and observations to be studied, as well as recognizing the data structure.

3.3. Cluster analysis

This technique comprises an unsupervised procedure that involves measuring either the distance or the similarity between objects to be clustered. Objects are grouped in clusters in terms of their similarity. The initial assumption is that nearness of objects in the space defined by the variables reflects the similarity of their properties. In our study, the Ward's method as the amalgamation rule and squared Euclidean distance as metric were used.

3.4. Software

Univariate and multivariate analysis were performed by means of software packages: XLSTAT (version 2007) and Minitab (release ver. 13.20).

3.5. Quality control

The linear range of the calibration curve reached from the detection limit up to 0.0–0.015, 0.0–2.0, 0.0–0.025, 0.0–0.2, 0.0–0.2, 0.0–1.0, 0.0–2.0, 0.0–1.0, 0.0–0.125, 0.0–1.0, 0.0–0.5, 0.0–0.2, 0.0–0.1, 0.0–1.0 $\mu g\,mL^{-1}$ for As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn, respectively.

The limits of detection (LOD) for elements were calculated as under,

$$LOD = 3 \times (S/m)$$

respectively, where "*S*" is the standard deviation of 10 measurements of the blank and "*m*" is the slope of the calibration graph obtained for each case, the LODs; 0.126, 164.3, 0.327, 6.9, 4.7, 17.3, 69.2, 14.0, 2.46, 17.7, 5.52, 6.67, 3.38, 10.0 $\mu g\,L^{-1}$ for As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn, respectively. Accuracy of the method employed was documented by using certified reference materials, BCR 483 and BCR 189 as reported in previous work [21].

4. Results and discussion

4.1. Elements concentration in SUIS and grains

To understand the mobility and transport of micronutrient, trace and toxic elements to sorghum grains of different varieties were grown in the same experimental plots containing SUIS. The mean pH values of 24 batches of the SUIS were found in the 7.33–7.53, the results indicated that SUIS have weakly basic characteristics. The variations in accumulation of elements in SUIS and its subsequent uptake by different varieties of sorghum grains represent a direct pathway of these elements into the animal and human food chains, which is a major concern.

The basic source of essential elements for humans is the food chain and beneficial levels do not cause any disorders and have no harmful effect. However, the levels of toxic elements are not beneficial for all living beings. Bearing in mind complex chemical, physical and biological processes between environmental compartments one should definitely consider the other sources of influence and contamination as well (contaminated water and sewage sludge as fertilizer) that is of special importance for toxic elements. In that order, the chemometric approach was recognized as a useful way in providing additional and complementary information [22,23].

In the present work, essential micronutrients, trace and toxic elements (As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) were determined in soil amended with untreated industrial sewage sludge and sorghum grains by means of common spectroscopic techniques (FAAS/ETAAS) after the complete dissolution of their matrices with microwave assisted digestion. Reduced time required for sample preparation and reduced

Table 1
Variations of elemental contents in different varieties of sorghum grown in soil amended with untreated industrial sewage sludge (SUIS) [mg kg^{-1} dried basis]

	PARC-SV-1	PARC-SS-1	PARC-SS-2	95 CS-002 BARI	ICRICHAT-CSV-13	SUIS*
As	1.11 ± 0.218	1.24 ± 0.125	1.52 ± 0.0791	1.21 ± 0.115	0.941 ± 0.321	4.71 ± 0.321
Ca	133 ± 10.7	95.2 ± 5.51	93.1 ± 5.51	71.5 ± 4.62	91.1 ± 5.32	49900 ± 1990
Cd	1.36 ± 0.0321	1.50 ± 0.0952	2.61 ± 0.294	2.32 ± 0.202	1.91 ± 0.105	27.1 ± 2.52
Co	1.85 ± 0.0511	3.82 ± 0.312	5.21 ± 0.421	5.42 ± 0.291	3.61 ± 0.281	37.9 ± 4.97
Cr	0.252 ± 0.0221	0.281 ± 0.0162	0.293 ± 0.0131	0.271 ± 0.0191	0.441 ± 0.0322	76.4 ± 6.43
Cu	7.21 ± 0.412	8.64 ± 0.671	8.91 ± 0.712	7.52 ± 0.451	9.94 ± 0.691	180 ± 17.5
Fe	186 ± 8.31	147 ± 8.22	123 ± 7.61	165 ± 8.23	106 ± 7.43	10200 ± 729
K	914 ± 22.7	973 ± 45.4	1150 ± 78.4	996 ± 37.6	1050 ± 80.2	1510 ± 199
Mg	1350 ± 47.9	1390 ± 76.4	1400 ± 76.8	1770 ± 75.1	1450 ± 71.5	7640 ± 240
Mn	4.51 ± 0.361	5.73 ± 0.401	5.31 ± 0.351	5.92 ± 0.321	6.24 ± 0.491	204 ± 4.57
Na	1360 ± 83.4	1020 ± 66.4	598 ± 31.8	446 ± 18.0	504 ± 18.7	8650 ± 225
Ni	3.65 ± 0.317	8.51 ± 0.515	14.0 ± 1.31	10.9 ± 1.23	8.31 ± 0.522	82.3 ± 5.92
Pb	3.12 ± 0.291	3.51 ± 0.192	5.33 ± 0.304	4.34 ± 0.311	5.85 ± 0.402	104 ± 7.21
Zn	88.4 ± 6.42	102 ± 8.11	88.0 ± 6.61	91.5 ± 7.42	71.3 ± 5.81	410 ± 31.7

* Soil amended with untreated industrial sewage sludge.

amounts of acids and oxidants used, minimal contamination within the laboratory, reduced the loss of more volatile analytes and consequently better detection limits and accuracy of the method are advantageous over the numerous preparation procedures [24,25]. For trace elements determination by ETAAS different modifiers were used to enhance the sensitivity and stability of volatile elements. The concentrations of all elements in different varieties of sorghum grains, on the basis of dry weight, are presented in Table 1. Each result is average of ten-grain samples measurement of each variety. It is noteworthy that elemental contents in SUIS samples is in accordance with the maximum allowable concentrations (MACs) ranges considered

Table 2
Descriptive statistics of elemental concentrations for different varieties of sorghum [mg kg^{-1} dried basis]

Variable	Mean	S.D.[a]	Minimum[b]	Maximum[c]	Maximum/minimum
Total data set of 5 varieties of sorghum (5SV)					
As	1.20	0.184	0.941	1.52	1.60
Ca	96.7	19.9	71.5	133	1.86
Cd	1.93	0.468	1.36	2.61	1.91
Co	3.97	1.28	1.85	5.42	2.92
Cr	0.306	0.0681	0.252	0.441	1.76
Cu	8.42	0.979	7.21	9.93	1.38
Fe	145	28.7	106	186	1.75
K	1020	79.4	914	1150	1.26
Mg	1470	151	1350	1770	1.31
Mn	5.52	0.588	4.50	6.24	1.38
Na	785	350	446	1360	3.05
Ni	9.07	3.41	3.65	14.0	3.84
Pb	4.41	1.04	3.10	5.85	1.89
Zn	88.2	9.84	71.3	102	1.43
Total data set of (5SV) and soil amended with untreated industrial sewage sludge					
As	1.78	1.32	0.921	4.71	5.00
Ca	8390	18550	71.5	49900	697
Cd	6.13	9.40	1.41	27.1	19.9
Co	9.63	12.7	1.92	37.9	20.5
Cr	13.0	28.4	0.312	76.4	306
Cu	37.0	63.9	7.22	180	25.0
Fe	1820	3740	106	10200	95.9
K	1100	196	914	1510	1.65
Mg	2500	2300	1350	7640	5.64
Mn	38.7	74.1	4.53	204	45.4
Na	2100	2950	446	8650	19.4
Ni	21.3	27.5	3.62	82.3	22.6
Pb	21.0	37.2	3.11	104	33.6
Zn	142	120	71.3	410	5.75

[a] Standard deviation.
[b] Minimum value.
[c] Maximum value.

for the European Union except in the case of Cu and Zn were found to be higher than permissible limit [26]. Weak alkaline reaction of SUIS favors the strong binding of toxic elements in soil and on the other hand optimal bioavailability of essential elements [27,28]. The toxic elements in sorghum grains were high (Tables 1 and 2), as compared to maximum allowed amounts of these elements in a dried plant material, e.g. 10 and 0.3 mg kg^{-1} for Pb and Cd, respectively.

4.2. Basic statistics

Table 2 shows the concentrations of elements in sorghum grains vary by a factor of up to 1.60, 1.86, 1.91, 2.92, 1.76, 1.38, 1.75, 1.26, 1.31, 1.38, 3.05, 3.84, 1.89 and 1.43 for As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn, respectively. The standard deviation is the greatest for Na (350) and the smallest for Cr (0.0681). The arithmetic mean and standard deviation of elements, for all samples, were used to describe the variation of the data.

All data obtained were subjected to chemometric methods of analysis to check firstly, the distribution of elements in different varieties of sorghum grains, to compress relatively large data set(s), to eliminate the redundancy and noise to visualize complex and high dimensional quantitative relationships, and finally to perform patterns recognition.

Parametric statistical tests require the data to be normally distributed. Normality of the data was checked, if the data came from a population with normal distribution by applying Ryan–Joiner's test. Significance level α was 0.05. Testing firstly the total data set (sorghum grains and SUIS), relatively high degree of deviation from normal distribution was observed for majority of elements. The high deviation was observed for all elements. Correlation coefficients were improved in a Log-transformed data set. The Normal probability values for elements (correlation coefficients and approximate probability levels) are shown in Table 3. One can observe clearly separated two groups of data that refer to different variety of sorghum grains and SUIS samples. Generally, all the data obtained are positively skewed and with less deviation from normal distribution after logarithmic transformation, with majority of elements, which is also in agreement with literature data [29]. Furthermore, data within plants data set only, generally fit better to a normal population. Before further modeling one should test the data matrix in order to detect outliers, if there are any. Application of Grubb's test resulted in the detection of several outliers. With critical value for $\alpha = 0.05$ and $n = 6$ (five varieties of sorghum and SUIS) is 1.89 outliers were detected as follows: in original data set: Ca = 49866, K = 1506, Na = 8653 and Mg = 7940 are high in SUIS. In Log-transformed data: PARC-SV-1 Mg = 3.132 and 95-CS-002BARI Ca = 1.854 (low). Applying the same test for five varieties of sorghum grains data set only, with critical value ($\alpha = 0.05$, $n = 5$) of 1.71, two outliers for Log-transformed data set were identified as follows 95-CS-002BARI Ca = 1.854 (low) and ICRICHAT-CSV13 Fe = 2.026 (low).

4.3. Principal component analysis

The data set of uptake of elements by different varieties of sorghum from SUIS was subjected to a principal component analysis in order to decrease the number of descriptors responsible for the highest percentage of a total variance of the experimental data. Chemometric evaluation within sorghum varieties data set (without SUIS) will be presented as follows. When PCA was applied to the auto scaled data matrix, with eigen analysis as initial (Fig. 1), four principal components (PCs) were extracted according to the Kaiser criterion which explain up to 100% of variance.

Loading plot of correlated elements is presented in Fig. 2. The loading plot indicates the similarities and correlations between elements. The elements with small loadings located near the origin have only little influence on data structure, whereas the elements with high loadings represent those elements with the greatest influence on the grouping and separation of sorghum varieties. A close relation (covariation) was observed between

Table 3
Correlation coefficients and the corresponding approximate probability values, obtained in Ryan–Joiner's test within the original and Log-transformed data set

Elements	Sorghum samples and SUIS		Sorghum samples	
	Original	Log	Original	Log
As	0.770(<0.01)*	0.849(0.0165)	0.980(>0.1)	0.985(>0.1)
Ca	0.686(<0.01)	0.735(<0.01)	0.922(>0.1)	0.941(>0.1)
Cd	0.717(<0.01)	0.826(<0.01)	0.981(>0.1)	0.983(>0.1)
Co	0.748(<0.01)	0.895(0.0637)	0.960(>0.1)	0.929(>0.1)
Cr	0.687(<0.01)	0.736(<0.01)	0.847(0.0222)	0.874(0.0451)
Cu	0.695(<0.01)	0.748(<0.01)	0.980(>0.1)	0.980(>0.1)
Fe	0.691(<0.01)	0.764(<0.01)	0.995(>0.1)	0.993(>0.1)
K	0.898(0.0687)	0.925(>0.1)	0.984(>0.1)	0.989(>0.1)
Mg	0.719(<0.01)	0.763(<0.01)	0.846(0.0213)	0.858(0.0313)
Mn	0.691(<0.01)	0.737(<0.01)	0.968(>0.1)	0.956(>0.1)
Na	0.754(<0.01)	0.899(0.0715)	0.940(>0.1)	0.961(>0.1)
Ni	0.762(<0.01)	0.919(>0.1)	0.980(>0.1)	0.940(>0.1)
Pb	0.704(<0.01)	0.804(<0.01)	0.982(>0.1)	0.984(>0.1)
Zn	0.735(<0.01)	0.799(0.0577)	0.950(>0.1)	0.939(>0.1)

* Values in parentheses (probability values).

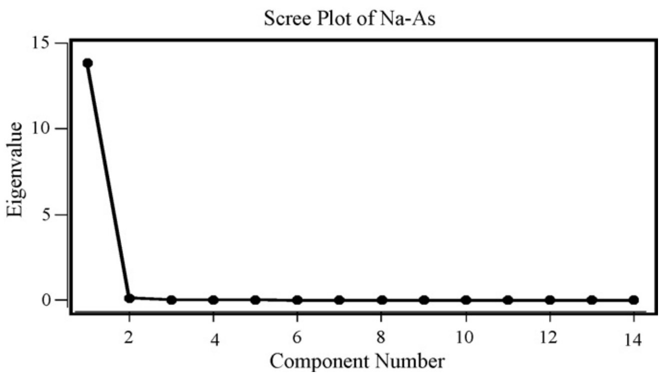

Fig. 1. Screen plot of eigen values for the principal components of SV with SUIS.

the concentrations of: (a) Mg, Co, Ni, K and Cd, (b) Zn, Fe and As, (c) Na and Ca, and (d) Cu, Mn, Pb and Cr. Thus, a high concentration of Co is expected to be associated with high concentrations of Ni (Fig. 2). It can also be seen that there is a general connection between Cr, Cu and Pb.

According to the Kaiser criterion [30], only the first four PCs are retained because subsequent eigen values are all less than one. Hence, reduced dimensionality of the descriptor space is four. Table 4 shows four significant factors obtained, these factors are related to the sources of the elements in the studied sorghum varieties. The first factor with 55.9% of variance comprises Cd. Co, Cu, K, Mn, Ni and Pb with high loadings (see also Fig. 2) and Cr and Mg with relatively low loadings, while As, Ca, Fe, Na and Zn have negative loadings in this factor. The positive loading was observed for As, Cd, Co, Fe, Mg, Ni and Zn, while for Zn have high loading factor than other in the second factor, which is responsible for 22.8% of total variance. The As, Cd and Ni are attributed to the high level in industrial sewage sludge as potential anthropogenic contaminant for the crops grown in SUIS. The other variables have relatively low loadings and are highly negative for Ca, Cr, Cu and Pb. It was observed that in sorghum varieties concentration of Ca, essential elements are low. The third factor with 15.1% of variance

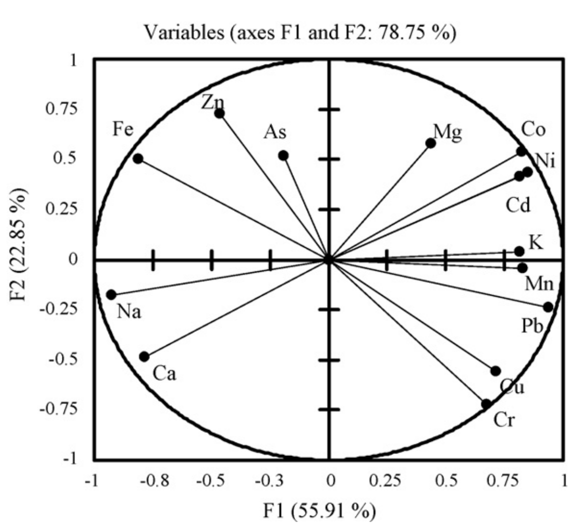

Fig. 2. Loading plot.

Table 4
Factor loadings eigen values for different varieties of sorghum

	F1	F2	F3	F4
As	−0.189	0.515	**0.835**	−0.0381
Ca	−0.787	−0.486	0.350	−0.150
Cd	**0.814**	0.416	0.204	−0.351
Co	**0.825**	0.539	0.071	0.156
Cr	0.673	−0.725	−0.136	0.060
Cu	**0.712**	−0.557	0.273	0.328
Fe	−0.810	0.499	−0.277	−0.132
K	**0.815**	0.0401	0.559	−0.144
Mg	0.436	0.578	−0.666	−0.179
Mn	**0.829**	−0.043	−0.379	0.410
Na	−0.926	−0.177	0.255	0.214
Ni	**0.849**	0.437	0.256	0.152
Pb	**0.937**	−0.239	0.132	−0.217
Zn	−0.463	**0.729**	0.164	0.476
Eigen value	7.827	3.198	2.110	0.864
Variability (%)	55.9	22.8	15.1	6.17
Cumulative (%)	55.9	78.8	93.8	100

Bold values show significant correlation

was composed of As with high loadings. In addition, K has positive, but medium-low loadings, while Cr, Fe, Mg and Mn have negative loading. The high uptake of As by sorghum varieties is attributed to anthropogenic effect of industrial sewage sludge. The fourth factor with 6.17% of variance comprises for Cu, Mn and Zn shows low positive loading. These elements present in SUIS are essential for plants, constituents of co-enzymes.

4.4. Cluster analysis (CA)

In order to identify relatively similar i.e. homogenous groups of objects in the space of measured features a hierarchical agglomerative cluster analysis (HCA) was performed. Ward's method, as an amalgamation rule, was applied on standardized concentrations of the elements and the Euclidian distance as a measure of the uptake of trace and toxic elements from SUIS. The dendrogram results form cluster analysis according to the Ward's method is represented in Fig. 3. Two well-differentiated clusters can be seen, one found by single sorghum variety PARC-SV-1 at minimum similarity from other varieties (−9.36%), shows that this variety have minimum uptake of toxic elements while high accumulation of essential elements as compared to the other varieties grown on same agricultural containing SUIS

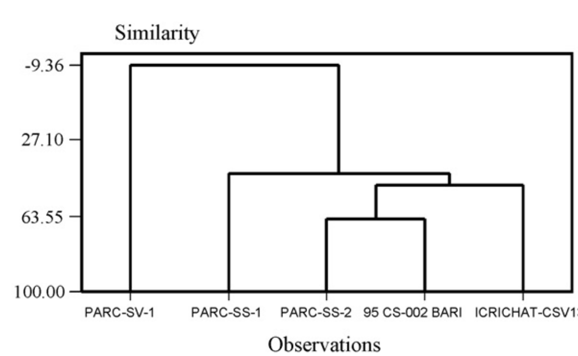

Fig. 3. Dendrogram of cluster analysis (sorghum varieties).

(Table 1). The other cluster has three sub groups according to the similarity in accumulation of trace and toxic elements from SUIS. A subgroup has two varieties PARC-SS-2 and 95-CS-002 BARI have similarity level (64.96%), uptake higher level of all trace and toxic elements as shown in Table 1. While other two varieties have medium uptake of trace and toxic elements but difference between these groups are not significant $p > 0.05$. Cluster analysis shows the variation in uptake of trace and toxic elements from SUIS, although they are grown in same experimental plot. So CA demonstrates that the variables have sufficient explanatory power to detect the suitable variety for growing on SUIS.

5. Conclusion

The uptake of elemental contents (As, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Pb and Zn) by five sorghum varieties growing on same experimental plots containing soil amended with untreated sewage sludge of industrial origin was studied. The variations in uptake of elemental contents by different varieties were evaluated to check higher tolerance limit for toxic elements present in SUIS. Multivariate methods, such as PCA and CA were employed to obtain possible correlations between investigated uptakes of elements by different varieties in order to identify influences of sewage sludge used as agricultural fertilizer. Consequently, individual elemental contents seem to be appropriate descriptors for identifying suitable variety of sorghum, safely grown in SUIS. This is additionally significant in the food crops research field because of their special importance for human as well as animal health. CA showed that among five varieties of sorghum, PARC-SV-1 is best due to minimum accumulation of toxic elements as compared to other varieties. The farmer should prefer this sorghum variety to cultivate safely in soil dressed with untreated industrial sewage sludge.

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