Application of Multivariate Analyses Techniques (PCA and CA) for Evaluation of Trace Elements in the Selected Fruits of Winder Area

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Abstract *- Ninety seven samples of nine different fruits were collected from various agriculture farms of Winder area, cultivated over igneous rocks of Bela Ophiolite (Cretaceous) and sedimentary rocks of Jurassic age (Ferozabad Group).In the present study Principal Component Analysis(PCA) has been employed to discriminate possible sources that influence trace element distribution in different fruits.It (PCA) revealed contribution of Mn and Ni from different segments of Bela Ophiolite, while the source of Zn and Cu is linked with MVT and Sedex-type mineralization present in the area. Cluster Analysis (CA) of different fruits organized sampling sites into two or three statistically significant groups mainly on the basis of Fe, Co, Cu and Zn concentration.*

Trace elements content showed wide variation in concentration with respect to sample sites and fruit type in the study area. The fruits of M. zapota (Chikoo) showed higher concentration of Fe (14mg/kg) among the other trace elements. The trace element assemblage of mango (M. indica) shows wide spread but at low magnitude. Iron (3.3mg/kg), Cr (2.6mg/kg), Zn (1.8mg/kg) and Cu (1.6mg/kg) are the main contributors. Comparatively, fruits of some locations have shown highest intake of trace elements relatively daily recommended.

Key words: *Trace element, fruits, Bela ophiolite, PCA, cluster analysis, Balochistan*

I. INTRODUCTION

Trace elements have significant role on human health. Certain essential micronutrients (Mn, Zn &P) are required for proper biological functions in human body (Adriano et al., 2004). On the other hand certain elements (As, Cd & Pb) are mutagen in character and are injurious for human health (Naseem et al., 2014; Soetan et al., 2010). Trace quantities of Cu, F and Cr are essentially required for healthy growth but are toxic after certain high concentration. There are several sources and pathways of trace elements contamination in the human body (Ritter et al., 2002). They mainly entered in the human body either through food or drinking water. Inhalation and dermal absorption are other minor contributors. Fruits, vegetables and other food stuffs are one of the important pathways in the human body. The level of concentration of trace elements in the pulp of the fruit is mainly communicated from the soil over which they grow. The geochemistry and nature of the rocks is primarily control the composition of the soil (Cohen et al., 1999).Another important factor that controls the enrichment of different trace elements in the fruits tissues is the exclusion mechanism of the plant species that allow or restrict uptake of elements from the soil.

Several fruit farms are present in and around Winder town. It is situated nearly 80km NW of Karachi city along the RCD Highway in the southern Balochistan. In the study area, sedimentary rocks of Jurassic age (Ferozabad Group) and igneous rocks (Bela Ophiolite) of Cretaceous age are widely exposed. The ophiolitic rocks are characterized by high amount of Fe, Mn, Cr, Ni and Co (Naseem et al., 2012); while Ferozabad Group possesses Mississippi Valley-type mineralization in which Zn, Pb, Fe, and Cu mineralization are reported (Ahsan & Mallick, 1999).

During weathering these elements are concentrated in the soils of the study area. The bioavailable trace elements are absorbs by the plants through their root system and are dispersed in the different part of the plant, including fruits. These fruits have vast market in the neighbouring Karachi city. The entry of trace elements through food chain is probably responsible for the spread of diseases related to toxic elements in the inhabitants of the study area (Fig.1).

Sometime raw geochemical data is unable to present full perception of the problem studied. Multivariate statistical analysis has ability to analyze complex data of trace elements having variables (Simunaniemi, et al., 2013;Gergen and Harmanescu, 2012).Principal components (PCA) and cluster analysis (CA) are two significant methods that determine the association of trace elements data of fruits (Patras et al., 2011).Both techniques are capable to reduced huge data of trace elements and elaborate the hidden patterns in trace elements dataset to compare the finding of raw data and signifying natural association including identified their genetic affiliation on fruits samples which were collected from various localities of Winder area (Chen et al., 2007). The CA is also an investigative data analysis method for systematize original data into significant segments based on combinations of independent variance, which helps to recognize groups of trace elements concentrations of fruits considering the composition of soil, type of fruit and exclusion mechanism of plant (PBH, 2012).

Present study aims to assess trace element composition of nine important fruits of Winder area. Principal component analysis and cluster analysis were employed to assess complex phenomenon of trace element concentration in the fruits.

II. MATERIALS AND METHODS

Ninety seven fruits samples of nine different types were collected from Winder agriculture farm area (Fig.1A& B). After peeling, 10 grams of homogenized fruit pulp is treated with 20ml HNO_3 and heated near to dryness. Add a few drops of H_2O_2 (30%) until the residue is colour less or consistent pale yellow (Ranganna, 2008). The residue was then treated with 10ml concentrated HCl and after heating for 30 minutes, 20ml distilled water was added and solution was further heated for further 15 minutes, after that the solution was filtered and made up to 100ml (Hashmi et al., 2007). Trace elements content of fruit samples were measured from above solution, using PerkinElmer (AAnalyst 700) Atomic Absorption Spectrophotometer. A-grade borosilicate glassware (Pyrex) was used during experimental process in order to minimize contamination through containers (Kozmutza & Picó, 2009). Analar grade chemicals were used in all analyses. High grade double deionized water of conductivity <0.5µS was utilized for sample preparation and analyses. Blank and repeated samples were introduced in each batch of the analysis to ensure accuracy and reproducibility of analytical data.

Statistical Analysis

Multivariate analysis was performed using SPSS_20 for Windows. PCA was interpreted in accordance to presence of trace elements combination. Varimax rotation was applied for data reduction. CA was applied to identify different geochemical groups, clustering the samples with similar trace elements content in selected fruits. CA was formulated according to the Ward method. Based on both techniques results are shown in a 3D rotated space of PCA and dendrogram where steps in the hierarchical clustering solution and values of the distances between clusters (squared Euclidean distance) are represented.

III. RESULTS AND DISCUSSION

Distribution of trace elements

Basic statistical data of trace element composition of nine different fruits is given in Table 1. Box-and-whisker plots are most appropriate way of presenting a complex concentration of fruit analysis. Additionally, the diagram explores five imperative statistical parameters (Weiss, 2007). The five-number summary consists of the median, the quartiles, and the smallest and greatest values in the distribution. Total 54 box diagrams are constructed on a single page to demonstrate elemental distribution in fruits of the study area at a glance (Fig. 2).

The fruits of *M. zapota* (Chikoo) show higher concentration of Fe (14mg/kg) among the other trace elements. Box-Whisker plots (Fig. 2a) shows the lowest and highest values of Fe is in the range of 4.43 & 44.5mg/kg, while median is 11.45mg/kg.The trace element assemblage of mango (*M. indica*) shows wide spread but at low magnitude. Iron (3.3mg/kg), Cr (2.6mg/kg), Zn (1.8mg/kg) and Cu (1.6mg/kg) are the main contributors (Table 1; Fig. 2b).In the tamarind (*T. indica*)the level of trace element follows Fe>Cr>Zn>Mn>Cu>Co>Ni trend (Table 1).

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Table 1. Basic statistics of trace elements of fruits of Winder area. (mg/kg)

	Min.	Max.	Mean	Min.	Max.	Mean	Min.	Max.	Mean
	Manilkara zapota (*n* = 33)			*Mangifera indica* (*n* =14)			*Tamarindous indica* (*n* = 15)		
Cu	0.53	4.37	1.70	0.77	2.83	1.63	0.71	6.62	2.05
Zn	0.55	2.06	1.02	0.71	3.02	1.75	1.69	6.40	3.74
Ni	0.32	2.44	1.13	0.36	1.64	1.01	0.10	0.35	0.24
Fe	0.00	44.50	13.95	1.14	7.66	3.33	0.49	33.00	16.17
Co	0.00	13.02	1.91	0.02	1.54	0.73	0.00	1.60	0.92
Mn	0.60	2.86	1.49	0.14	2.02	0.62	1.11	5.90	2.99
Cr	0.75	6.98	2.96	1.84	3.03	2.62	1.51	11.0	5.57
	Syszygium cuminin = 6			*Psidium guajavan* = 5			*Ziziphus jujuben* = 6		
Cu	0.97	4.12	1.62	1.00	3.62	2.59	0.81	1.92	1.11
Zn	0.88	1.80	1.26	0.84	4.38	2.07	0.95	2.15	1.38
Ni	0.98	1.58	1.33	0.19	0.40	0.30	0.14	0.38	0.28
Fe	14.32	91.00	55.19	7.72	8.77	8.20	2.88	106.65	26.73
Co	0.66	2.17	1.71	0.14	1.10	0.71	0.10	1.43	0.84
Mn	1.92	2.90	2.35	1.92	8.17	0.71	1.95	7.75	3.97
Cr	1.34	4.47	3.51	1.24	5.56	3.52	1.46	10.71	5.12
	Annona reticulaten = 8			*Musa acuminaten* = 6			*Carica papayan* = 4		
Cu	0.57	3.78	1.96	0.61	3.62	1.54	0.68	2.29	1.46
Zn	0.92	6.70	2.90	0.90	2.47	1.78	1.01	3.16	1.85
Ni	0.10	0.95	0.51	0.22	0.42	0.34	0.33	2.95	1.00
Fe	6.04	15.77	10.67	3.43	19.57	6.69	3.03	11.00	6.29
Co	0.20	0.56	0.33	0.37	0.49	0.42	0.49	0.55	0.51
Mn	1.01	5.99	2.79	2.08	9.56	5.15	0.44	5.80	2.18
Cr	2.18	3.83	3.04	2.58	4.50	3.77	2.47	4.82	3.76

The Fe shows wide range with distinctive minima, maxima, median and quartiles values (Fig. 2c). The Co which is slightly more than Ni exhibits no clear upper extreme on Box-Whisker plot while in case of Cu the lower extreme is merged with lower quartile (Fig. 2c). The Fe (55.20mg/kg) is the most abundant trace element in jambolan(*S. cumini*)along with minor contents of Cr (3.51mg/kg), Mn (2.35 mg/kg), Co (1.71mg/kg), Cu (1.63mg/kg), Ni (1.34mg/kg) and Zn (1.26mg/kg). The Fe is widespread with median (49.25 mg/kg) and shows lower limit much below than lower quartile in contrast to the upper limit and quartile. The concentration level of all elements decreases many folds of Fe lacking any discrimination of extremities (Table 1). Although on Box-Whisker plot all component of trace elements are merged with each other (Fig. 2d), but Cr and Cu marked distinct lower and upper boundaries respectively.Beside other fruits in the vicinity of Winder, guava (*P. guajava*)reveals contradiction assemblage of trace elements. The Mn, Cr, Zn and Cu show wide distribution rather than Fe (Fig. 2e). The Co and Fe exhibit nearly same range of distribution with different levels of concentrations, while the abundance of Ni is least (0.19-0.4mg/kg). Iron is still relatively high than other elements but its ratio is much lower than in other fruits (Table 1).The diagram (Fig. 2f) shows that Fe is the only promising element found in the edible part of the Jujube(*Z.jujuba*). It has a wide spread in the lower (2.88mg/kg) and upper values (106.65mg/kg), indicating a very variable accumulation in the fruits. Possibly, this distinction in the Fe storage is due to different level of Fe in the soil.The populations of all trace elements exhibit differential accumulation in the soft tissues of custard apple(*A. reticulata*; Fig. 2g). The top leading element is Fe with a mean of 10.67mg/kg (Table 1). In spite of wide spread of Zn, Mn and Cu, the average of Cr is high (3.05mg/kg). The concentration of Co keeps minimum in the fruit.The banana(*M. acuminata*) fruit show wide range of Fe accumulation with an average of 6.7mg/kg (Fig. 2h). The mean of Mn, Cr, Zn and Cu are 5.2, 3.8, 1.8 and 1.5mg/kg respectively. The other elements are less than 0.5mg/kg (Table 1).The elemental concentration in the pulp of the papaya shows Fe as the top leading element (6.32mg/kg). Although, the spread of Mn is high but the mean of Cr (3.76mg/kg) represent it as next most common element (Table 1). The elemental distribution of *C. papaya*indicates that all the elements minimum and maximum values are merged up within the quartile values, which is not common in any fruit (Fig. 2i).

Principal Component Analysis (PCA)

Principal component analysis (PCA) is a powerful technique for recognizing hidden patterns, classification, and interpretation of data clusters (Sellés et al., 2007).The PCA reduces variable analytical data to a small number of newly derived parameters (Micó et al., 2006). Maisuthisakul et al. (2008); Pestana et al. (2005);Jiang et al. (2013) have utilized PCA in the assessment of fruit composition. In the present study, trace element data of different fruits of Winder area were assessed to infer their mutual behavior and genetic association.

Three distinct groups of combinations have been observed in *M. zapota* (Fig.3 a). Chromium (0.95) and Mn (0.93) have made the strongest positive correlation, group 2 exhibits high positive loadings among Ni (0.87), Cu (0.78) and Fe (0.55) while third group consist Zn (0.89) and Co (0.82). Possibly the relation indicates enrichment of these elements from the rocks of Bela Ophiolite.The processing of analytical data through PCA is helpful to render trace element contamination through soil in the fruits (Gergen and Harmanescu, 2012).

M. indica, based on rotated component matrix, demonstrate three separate populations (Fig. 3b). In component 1, Mn (0.88), Cu (0.72) and Fe (0.70) are strongly associated, while Zn (0.82) and Ni (-0.82) have shown inverse trend group. Zinc is commonly found as metalloenzymes in plants and high Ni may replace the Zn (Brooks, 1972). Third combination of trace minerals group has been recorded as consisting of Cr (0.85) and Co (0.69) as members. In *T. indica* (Fig.3c), rotated components matrix reveals Co (0.95), Cr (0.88) and Mn (0.65) correlating positively and forming a group of elements. Based on correlation matrix a second group consists on Zn (0.80), Ni (0.85) and Cu (0.60) while Fe (0.86) and Mn (-0.71) have formed a combination of an inverse nature in *T. indica*. Trace elements in *S. cumini*shows a complex combination, Co (0.98), Cr (0.98) and Fe (0.84) have emerged as revealing a positive nature, but Zn (-0.87) and Cu (-0.86) react a negative attitude in component 1 of rotated space (Fig. 3d). Mn (0.98) and Ni (0.99) emerge as a single group in component 2 and 3 respectively. Probably it indicates that, the source of Zn and Cu is linked with MVT and Sedex-type mineralization, while Mn and Ni are associated from different segmented Bela Ophiolite.

The PCA analysis of *P. guajava* fruit revealed strong correlation among Cr (0.98) and Co (0.851), while Ni (-0.98) exhibits opposite trend (Fig. 3e). Another group, comprising of Mn (0.97), Zn (0.89) and Cu (0.83) also exist in the rotated component space. Iron (0.97) plays an independent role in *P. guajava*, probably due to low and consistence concentration (Fig 3e). A large group, consisting of Mn (0.93), Cr (0.92), Zn (0.91) and Co (0.73) exist in *Z. jujuba* (Fig. 3f). Second group comprised of Ni (0.89) and Cu (0.83), while Fe (0.95) occur independent. Mutual relation of trace elements of *A. reticulata* is quite identical with *P. guajava* (Fig. 3g). First set consists of Cu (0.99), Zn (0.89) and Mn (0.84). Second cluster of elements reveals dual interactions, where Co (0.86) and Cr (0.77) mutually support to each other, while Ni (-0.77) regulates their concentration. High positive loadings of trace elements have emerged for Fe (0.94), Co (0.90) and Mn (0.88) in *M. acuminata*while, Cu (0.92) and Ni (0.74)show negative relation with Cr (-0.91). Zinc (0.99) has emerged as an individual entity (Fig.3h). In the case of *C. papaya* (Fig.3i), the group combination of elements are quite similar to M. acuminate except for Zn(0.82) and Fe(0.93) and the distributions are simple except for Cr(-0.93),Ni(0.86)and Cu(0.84).

Cluster Analysis (CA)

Cluster analysis is an exploratory data analysis tool for organizing observed data into meaningful taxonomies, groups, or clusters, based on combinations of variables (Lua, et al., 2010; Lu, et al., 2012). In the present study, CA was employed for distinguished geochemical groups among sample sites based on content of selected trace elements (Fig. 4). The dendogram obtained, was used to define three geochemical groups based on trace elements (Group 1-3) that illustrate the complexity of the sample site regarding *M.zapota* (Fig.4a). Group 1 consists of sample sites 15 (AR) and 8 (GS) in which high Fe content (44.03 and 44.50 mg/kg) have been found. These sample sites are located near industrial unit (Siddiq Sons) and the north western area along RCD highway.

Group 2 consists of a large number of sample sites SB (2) - AF (4). Abnormal sites in terms of Cu content are GL and HS areas (4.12 & 4.13 mg/kg respectively) while Zn 2.06 mg/kg at AF location. Sample site MB has recorded content of Ni 1.94 mg/kg while, Fe 13.25, 13.74 and 14.32 mg/kg have been registered at CG, GD and HS locations, respectively. Highest content of Co 13.02 mg/kg has been observed at AF sample site. The content of Cr 6.13, 6.32, 6.39, 6.96, and 6.98 mg/kg have been as certain at sample sites of TA, JB, MB, CM and MN respectively. Similarly, a clustering analysis calculated for sample sites AZ (6) – CF (28) in Group 3 revealed anomaly distribution of trace elements corresponding to the location QS where content of Fe, 24.02, Co, 1.31, Mn, 2.76 and Cr, 6.41 mg/kg were recorded.

The obtained dendogram of *M. indica,* CA revealed two major groups in which sample sites AH, GE, QS, KR and GH formed the first group while, the second group consists on rest of the sample sites (Fig.4b). In Group 1, similarity index among locations is high along with high content of Fe. Based on selected traceelements CA classified three Groups of sample sties. Group I composed on UG, SB, GD, CF and RB locations where *T. indica* intake high Fe content. Sample sites WT, MN, QS, SB, NS and GF are part of Group 2 while low content of Cu intake locations HM, AH, KR and HG are formed of congregate in Group 3. The dendogram of *S.cumini* has explained 2 Clusters (Fig.4c). High similarity index has been observed in Group 1 where sample sites RB and GD are Fe rich. Fruits of S.cumini have content of Fe 85 and 91mg/kg, respectively (Fig.4d). There are two groups labeled in *P. guajava* by CA according to trace elements content (Fig.4e). Fruit of *P.guajava* has been found to be rich in Zn and Mn with reference to distribution of trace elements in Group 1 sample sites. The content of Mn was 8.17 and 7.6 mg/kg at QS and GF locations while that of Zn was 4.38 and 2.5 mg/kg, respectively.

The obtained dendrogram of *Z. jujuba* has been classified into 2 groups (Fig.4f). The content of Fe (106.65mg/kg) is very high in Group I. GN site of Group 2 has anomaly distribution of trace elements where level of content Zn, 2.15, Co, 1.43, Mn, 7.75 and Cr, 10.71 are 2.15,1.43, 7.75 and 10.71 mg/kg respectively in *Z. jujuba* fruit.

CA of *A. reticulata* calculated 3 Groups based on studied trace elements (Fig.4g). Group 1 followed the sample sites QS, HC and SB. The content of Cu, Zn and Mn are high in fruits of *A. reticulata*. Group 2 sample sites comprise on MB and UG where trace minerals intake is low in terms of content. Sample sites GX, KR and GD are members of Group 3. The content level has noted as being average among groups of *A.reticulata.* GX location shows anomaly level of trace elements content among members of Group 3 where elements intake in *A. reticulate* is comparatively high.

The obtained dendogram of *M. acuminata* has been classified into 3 major groups (Fig.4h). Group 1 only consists on sample site HG. The content of Fe (19.57 mg/kg) and Mn 9.56 (mg/kg) intake in *M. acuminata* at HG distinguished as separate group. Group 2 are composed on HM and SB locations where most of the studied trace elements content in *M. acuminate* fruit is high while members (UG, AH and AF) of Group 3 display low level accumulation of contents. CA categorized 2 major clusters based on trace elements concentration of C. papaya (Fig.4i). Group 1 consists of sample locations KR and UG in which the content of Fe is high in fruits of *C.papaya*.

IV. CONCLUSION

A Comparative study of selected fruits regarding maximum intake of trace elements have been found different from fruit to fruit. Average trace elements assemblages of edible part of all fruits show Fe (16.35)> Cr (3.76)> Mn (2.47)> Zn (1.97) > Cu (1.74) >Co (0.89)> Ni (0.68mg/kg). The average contents of minerals are relatively high to recommended dietary allowance which is indication of high impacts of the geology of the study area. Regular human intake of these fruits will accumulate these minerals in human body and result serious health hazardous due to excess constituent. *T. indica* shows high intake of Cu and Cr while content of Zn high in *A. reticulate*. Maximum intake of Ni were observed in *C. papaya* while Fe in *Z. jujuba*. In *M. zapota* concentration ofCo content highest among nine fruits along with *M. acuminate* in terms of Mn.

PCA has explained Cr and Mn relation is strongest relatively other trace elements in *M.zapota* while, Mn, Cu and Fe combination are strongest in *M. indica* although Zn resists against Ni concentration. In *T. indica*, Co, Cr and Mn group is much stronger and Fe resists against Mn. Cobalt, Cr and Fe combination in *S. cumini* reacts against Zn and Cu. Probably it indicates that, the source of Zn and Cu is linked with MVT and Sedex-type mineralization, while Mn and Ni are associated from different segmented Bela Ophiolite. *P. guajava* fruit revealed strong correlation among Cr and Co, while Ni exhibits opposite trend. A large group, consisting of Mn, Cr, Zn and Co exists in *Z. jujuba*. Mutual relation of trace elements of *A. reticulata* is quite identical with *P. guajava*. First set consists of Cu, Zn and Mn. High positive loadings of trace elements have emerged for Fe, Co and Mn in *M. acuminata* while, Cu and Ni show negative relation with Cr. Zinc has emerged as an individual entity. In the case of C.

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Papaya the group combination of elements are quite similar except for Zn and Fe and the distributions are simple except for Cr, Ni and Cu. Cluster Analysis was organized groups of sample sites in terms of anomaly content level of trace element concentration. Most of geochemical major groups were classified based on Fe, Co, Cu and Zn.

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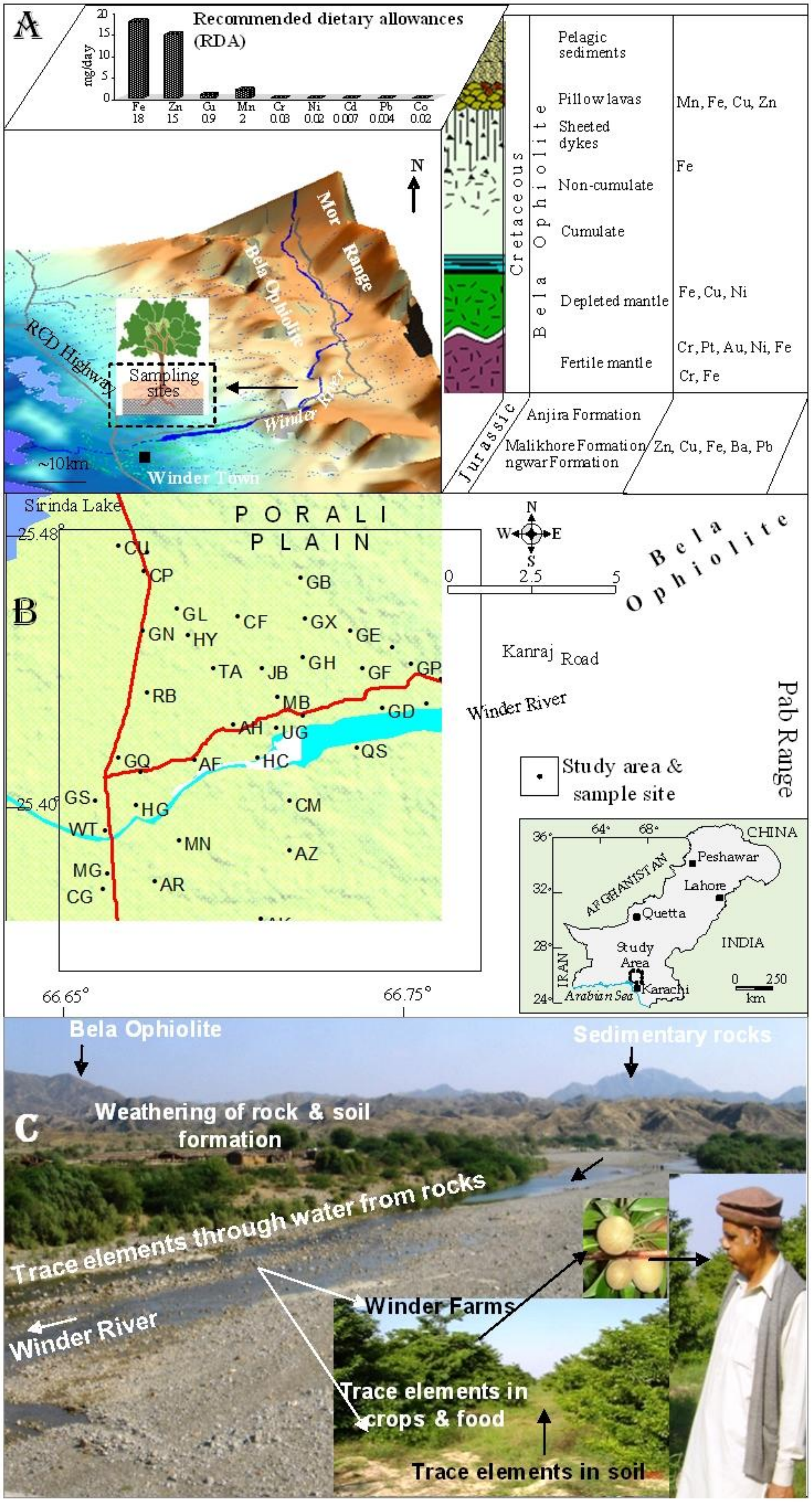
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Recommended dietary allowances (RDA)
mg/day
Fe 18
Zn 15
Cu 0.9
Mn 2
Cr 0.03
Ni 0.02
Cd 0.007
Pb 0.004
Co 0.02
Mor Range
Bela Ophiolite
RCD Highway
Sampling sites
Winder River
~10km
Winder Town
N
Pelagic sediments
Pillow lavas
Mn, Fe, Cu, Zn
Sheeted dykes
Fe
Non-cumulate
Cumulate
Depleted mantle
Fe, Cu, Ni
Fertile mantle
Cr, Pt, Au, Ni, Fe
Cr, Fe
Cretaceous
Bela Ophiolite
Jurassic
Anjira Formation
Malikhore Formation/ Zn, Cu, Fe, Ba, Pb
ngwar Formation
B
Sirinda Lake
PORALI PLAIN
25.48°
25.40°
Bela Ophiolite
Kanraj Road
Winder River
Pab Range
Study area & sample site
66.65°
66.75°
CHINA
AFGHANISTAN
Peshawar
Lahore
Quetta
IRAN
Study Area
INDIA
Karachi
Arabian Sea
km
C
Bela Ophiolite
Sedimentary rocks
Weathering of rock & soil formation
Trace elements through water from rocks
Winder River
Winder Farms
Trace elements in crops & food
Trace elements in soil

Fig.1 (A) shows study area, geology and RDA while (B) pertain to sample sites (C) depicts Geogenic sources of trace elements in the human body through rock-soil-water-vegetation in the study area (A& B).

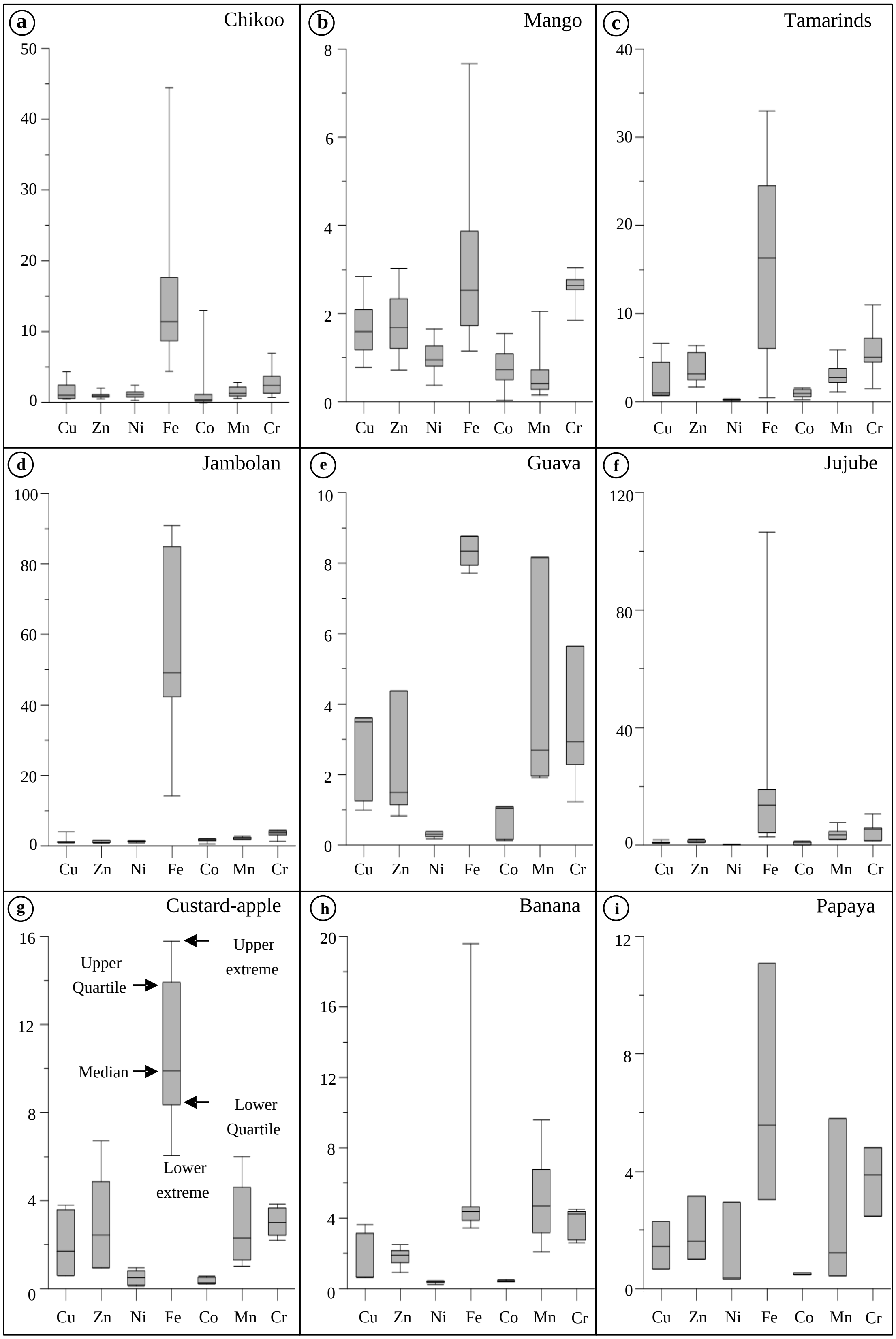

Fig. 2 Box-Whisker Plots illustrating the concentration of selected metals in fruits plump of Winder farms (mg/kg).

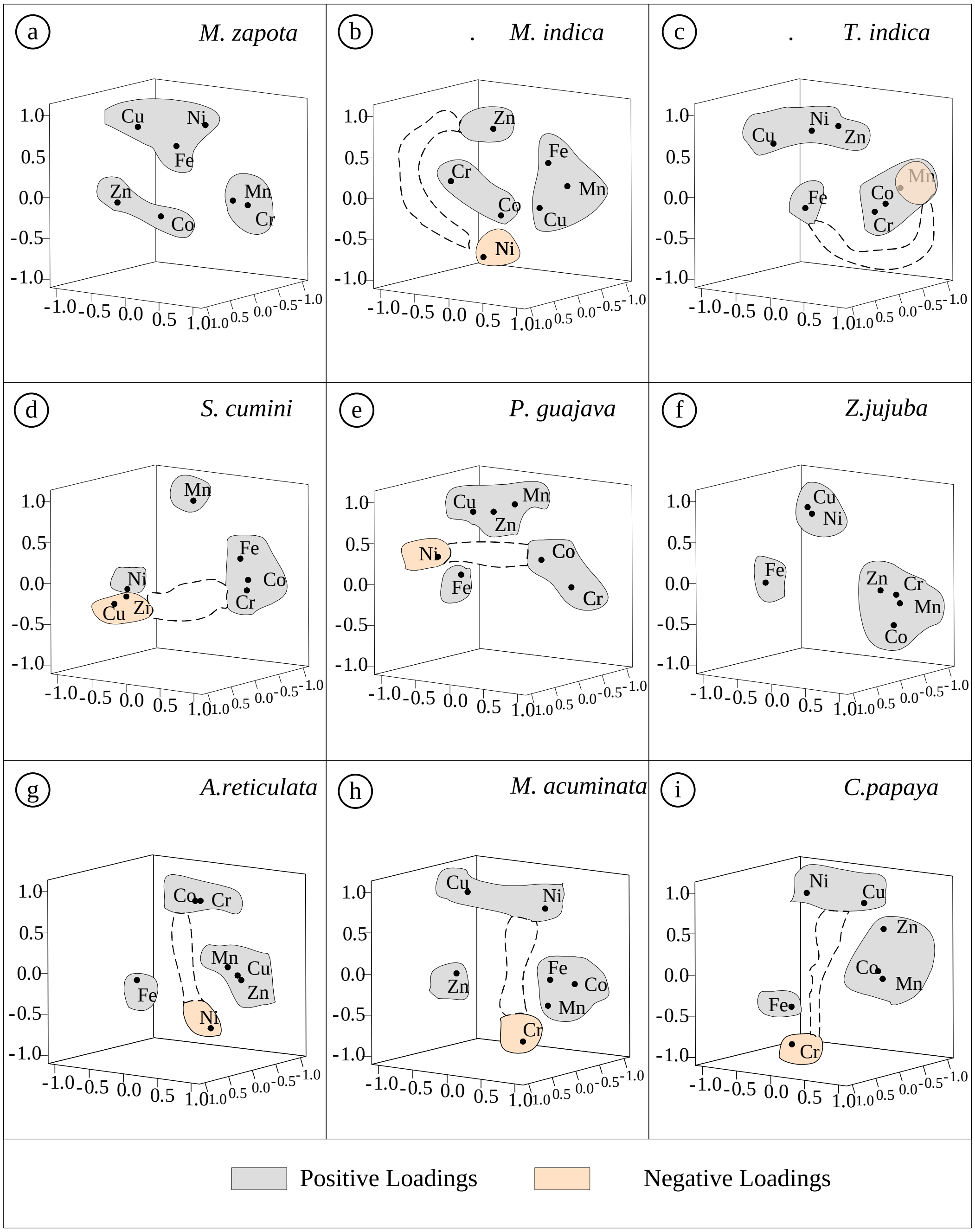

Fig. 3 rotated loadings show correlation among trace metals in fruits plump of Winder farms.

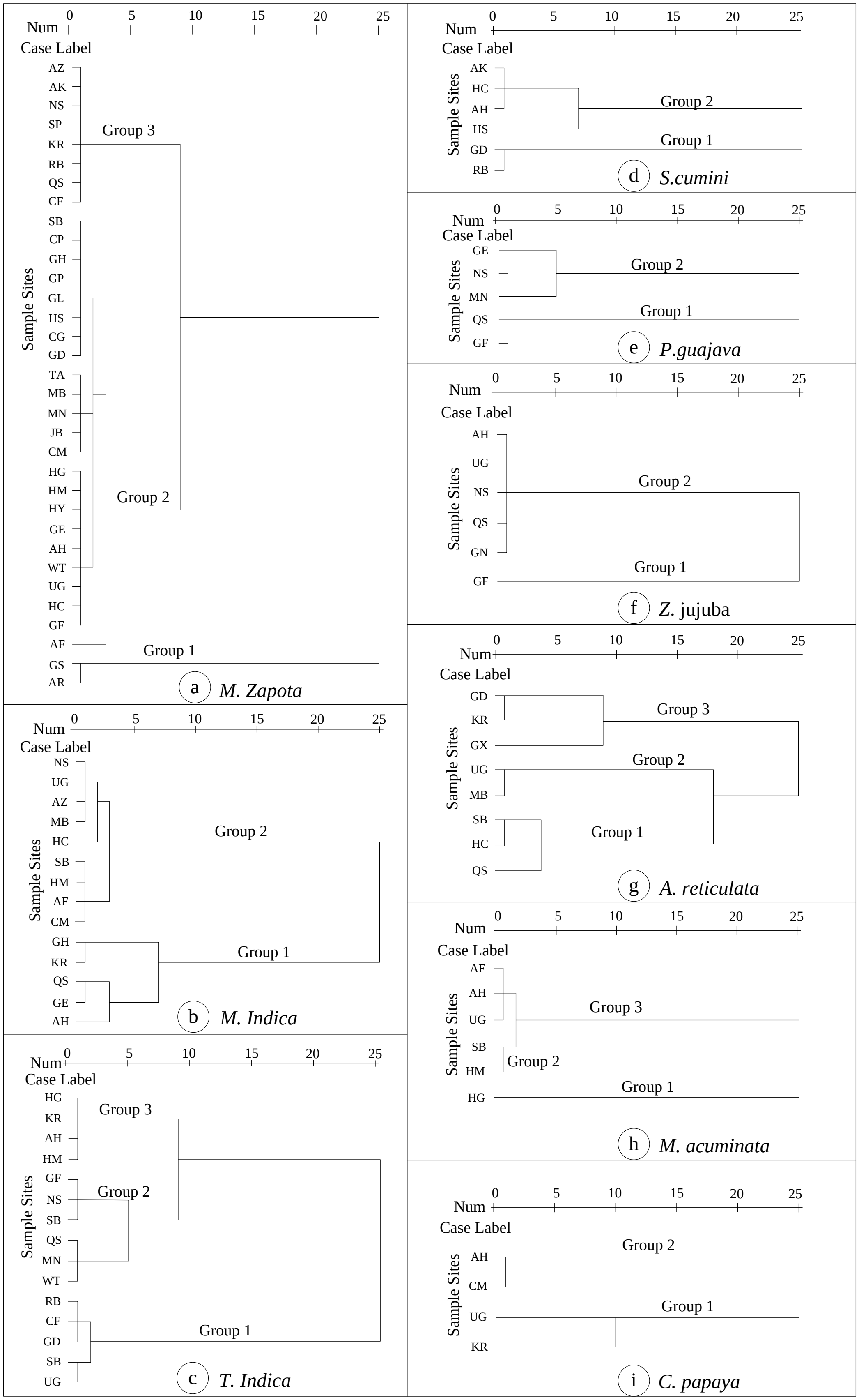

Fig. 4 depicts clusters based on concentration of trace elements in the study area