Trace element geochemistry of *Manilkara zapota* (L.) *P. Royen*, fruit from winder, Balochistan, Pakistan in perspective of medical geology

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Abstract: An integrated study of rocks, soils and fruits of *Manilkara zapota* (L.) (Sapotaceae) of Winder area have been carried out to elaborate trace elements relationship between them. The igneous rocks of the study area have elevated amount of certain trace elements, upon weathering these elements are concentrated in the soil of the area. The trace elements concentration in the soil were found in the range of 0.8-197 for Fe, 1.23-140 for Mn, 0.03-16.7 for Zn, 0.07-9.8 for Cr, 0.05-2.0 for Co, 0.52-13.3 for Ni, 0.03-8.8 for Cu, 0.08-10.55 for Pb and 0.13-1.8µg/g for Cd. The distribution pattern of elements in the rocks and soils reflected genetic affiliation. Promising elements of edible part of the fruit were Fe (14.17), Mn (1.49), Cr (2.96), Ni (1.13), Co (0.92), Cu (1.70) and Zn (1.02µg/g). The concentration of these elements in the fruits is above the optimum level of recommended dietary intake, probably due to this, disorder in the human health is suspected in the inhabitants of the area.

Keywords: *Manilkara zapota*, trace elements, winder, Pakistan, medical geology.

INTRODUCTION

The tropical fruit *Manilkara zapota* (Sapodilla), in Pakistan is known as Chiko. It belongs to class Magnoliopsida, order Ericales and family Sapotaceae. It is a perennial mesophyte with numerous branches. Leaves crowded at the end of branches, ovate-elliptic to elliptic-lanceolate, glabrous, subcoriaceous, midrib prominent. Flowers white, 1-1.5 cm across, long pedicelled. Sepals unequal, 3 larger than the others, Fruit usually a globose fleshy berry, 5-10 cm in diameter, epicarp thin, rough rusty brown. Seeds 5-12, shining black, obovate, 2 cm long (Ali and Qaiser, 1967). The coagulated resinous latex is derived from the bark and is used commercially for making chewing gum. Sapodilla flowers are small, inconspicuous and bell-like (CRFG, 1996). The fruit is either rounded or egg shaped and covered with brown skin. Flesh is yellowish-orange to earthy-brown with a grainy texture. The taste is sweet when ripe. The fruits have 3-5 ellipsoidal black seeds (Orwa *et al*., 2009).

The fruit has many health beneficial ingredients in sufficient quantities, such as dietary fiber, fructose, sucrose, vitamins, minerals and antioxidant compounds (USDA, 2000). The *M. zapota* is conventionally used as an important plant in the eastern medicine. Blend of the young fruits and flowers is used to alleviate pulmonary complaints. The boil extracts of the young fruit is exercised/used in the treatment of diarrhea (Kulkarni *et al*., 2007). The seed kernel contains toxic saponin called sapotinin, which upon ingestion causes abdominal pain and vomiting. The crushed seeds have a diuretic action and are claimed to bladder and kidney stones. The seeds are aperients (Laxative), diuretic, tonic and febrifuge. Paste of crushed seed is employed to remove stones from the kidney and urinary bladder and also provide relief if applied on stings and bites from venomous animals (Morton, 1987). The extract of the leaves of *M. zapota* is effective for antibacterial activity against 10 Gram positive, 12 Gram negative bacteria (Nair and Chanda, 2008). The total antioxidant capacity was found to be 10.85µg of ascorbic acid/mg of extract (Jamuna *et al*., 2011). The fruit juice showed potential antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl free radicals and superoxide radicals. It also inhibited the free radical-mediated lipid peroxidation. The nutraceutical components of the fruit has excellent multiple radical-scavenging potential (Kulkarni *et al*., 2007).

Winder is a medium-sized town of district Lasbela, Balochistan, nearly 80km NW of Karachi city along the RCD Highway. Several fruit farms are present and *M. zapota* is the most common widely planted fruit of the area. In the eastern side, small hills are present in which igneous rocks of Cretaceous age are well exposed (Naseem *et al*., 2010). These rocks are known as Bela Ophiolites, which are enriched with appreciable quantities of Fe, Mn, Cr, Ni, Co, Cu, Zn, Pb, and Cd. During weathering these elements are concentrated in the soils of the study area. The bioavailable trace elements are absorbed by the plants through their root system and are dispersed in the different part of the plant, including fruits. The fruits of *M. zapota* have vast market in the neighboring Karachi city. The entry of heavy metals through food chain is probably responsible for the spread of diseases related to toxic elements in the inhabitants of the city. Unfortunately proper statistical data is not

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available to exactly pinpoint their contribution regarding the correlation of diseases in human and geological existence of plant species.

The medical geology discipline deals with the relationships between the geological environment and health problems in human, animals and plants (Bunnell *et al*., 2007; Selinus, 2008) and also to explore preventative methods for ameliorating these problems (Komatina, 2004). This emerging scientific discipline has fascinated both geoscientists and medical professionals due to its highly interdisciplinary nature. The geoborne diseases are known to humans since long time, such as Arsenosis, dental and skeletal fluorosis, iodine deficiency disorders, asbestosis and geophagia (Dissanayake and Chandrajith, 2007). In the Indian subcontinent, diseases related to geology are also common (Dissanayake *et al*., 2010). Similarly, deficiencies and excesses of certain trace elements (Fe, Mn, Zn, Cu, Se, etc.) through the food chain are also responsible for disorders in human beings (Lindh, 2005).

The present study accounts to assess the sources of accumulation of different trace elements through geological environment on the locally grown an ornamental cum edible plant, *M. zapota*. It is also aimed to evaluate the impact of various trace elements assemblage of fruits and potential health hazards in the local communities in the light of geomedical research.

MATERIALS AND METHODS

Sampling

Representative 21 igneous rock samples, 49 soil samples, 33 fruits of *M. zapota* were collected from different sites of Winder town, Balochistan.

Digestions

Rocks: Igneous rocks are decomposed by a mixture of 3:1 Na_2CO_3 and K_2CO_3 flux in a platinum crucible for about one hour in an electric muffle furnace at a ± 1000°C temperature (Gill, 2000). The fused cake was then reacted with HCl (2N) in 150ml china dish and heated over water bath at 70°C till complete dryness. Finally, the solution was made by adding 1ml conc. HCl (Jeffery and Hutchison, 1983). The resulting solution was transferred to 100ml volumetric flask.

Soils: Moisture free pulverized and sieved soil samples (10g) were soaked in double distilled water for overnight, followed by vigorous shaking on an electric shaker for two hours. The contents were filtered through a Whatman No. 42 filter paper in a 100ml volumetric flask ensuring that the filtrate is free of colloidal matter (Gupta, 2004). Volume was made-up to the mark with double distilled water.

Fruits: Homogenized fruit pulp (10g) was treated with 20ml HNO_3 (Conc.) and heated near to dryness. Followed by the addition of H_2O_2 (30%) few drops until the residue was colourless 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 again heated for further 15 minutes, after that the solution was filtered and made up to 100ml (Hashmi *et al*., 2007).

Estimation

The AAS was set according to manufacturer's instructions (AAnalyst 700, PerkinElmer). Working curves were prepared for absorbance values (A) vs. standard solutions of variable strength. The concentration of each element was estimated with reference to standard solution of that element and final calculations were made with reference to weight of the sample and dilution of original solution.

Precision and accuracy

Borosilicate glassware (Pyrex) was used during experimental process in order to minimize contamination through containers (Kozmutza and Picó, 2009). Analar grade Merck/BDH/Fluka chemicals and high grade double distilled water of conductivity <0.5µS were 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.

RESULTS

Basic statistical parameters of trace elements composition of igneous rocks, soils and fruits of *M. zapota* are combinedly presented in table 1. The results of individual samples are displayed in different diagrams. Attention has been paid to the data of fruits analysis.

Rocks

Iron is the most copious element of the igneous rocks and range between 156-11,984µg/g in the studied samples. It is important to note that the mean (5,044) is <50% of the maximum value. The other promising elements showed Mn>Cr>Ni>Cu trend and their average concentration in the rock are in hundreds µg/g. Zinc, Co and Pb are found comparatively in lower amount (table 1) and their mean are 79, 63 and 58 µg/g correspondingly. Cadmium is not a common element of the igneous rocks and it ranges 0.86 to 6 with a mean of 3.8µg/g.

Soils

Analytical data of the water extract of the soils from study area revealed dominance of Fe. Iron is in the range of 0.8-197.6µg/g (av. 28.056µg/g). The average amount of water soluble Mn in the soils from study area was found to be 29.91µg/g (table 1). Lead occupies third position in order of abundance and range between 0.08-10.55µg/g, then Cr which has nearly same range and mean of abundance. The average Ni, Cu and Zn are 3.12, 2.66 and 1.02µg/g respectively. Cobalt and Cd are <1µg/g in the soils of the Winder area (table 1).

Fruits

The fruits of *M. zapota* (Chiko) showed higher concentration of Fe (14µg/g) among the other trace metals, the lowest and highest values of Fe are in the range of 4.43 & 44.5µg/g were mentioned in table 1, while median is 11.45µg/g. The average concentration of Cr (2.96µg/g) and Cu (1.7µg/g) in the edible part of the fruits were found second and third in order of abundance. The rest of the elements were <1.5µg/g and their statistical parameters are very close, their concentrations in the fruits of *M. zapota* were found quite consistence and their standard deviations (SD) were mostly <0.7.

Table 1: Composite statistics of rocks, soils and fruits of *M. zapota*, Winder area (µg/g).

	Min	Max	Mean	SD
Igneous Rocks (n=21)				
Fe	156	11,984	5,044	4,062
Mn	42	2,252	907	511
Cr	12	3,984	694	854
Ni	5.4	1,410	635	372
Co	12.1	103	62.8	24
Cu	2.6	422	245	132
Zn	2.2	141	79	35
Pb	1.2	97	58	36
Cd	0.86	6	3.8	1.66
Soils (n=49)				
Fe	0.8	197	28	46
Mn	1.23	140	30	39
Cr	0.07	9.8	4.7	2.61
Ni	0.52	13.3	3.12	2.90
Co	0.05	2.00	0.97	0.38
Cu	0.03	8.80	2.66	2.73
Zn	0.03	16.70	1.02	2.67
Pb	0.08	10.55	5.12	4.14
Cd	0.13	1.80	0.49	0.46
Fruits (n=33)				
Fe	4.43	44.5	14.17	8.42
Mn	0.60	2.86	1.49	0.68
Cr	0.75	6.98	2.96	2.03
Ni	0.32	2.44	1.13	0.53
Co	0.00	13.02	0.92	0.46
Cu	0.53	4.37	1.70	1.12
Zn	0.55	2.06	1.02	0.32

DISCUSSION

The bioavailability of different trace metals mainly depends upon the exclusion mechanism of the plants, mobility and abundance of elements in the soils over which they grow (Kabata-Pendias, 2004). These elements usually occur in a variety of chemical species that can vary widely according to the basis of solubility, mobility and toxicity.

Rock-soil

In the study area, various trace elements were derived mainly from the rocks. The average abundance of important trace elements of the rocks is displayed in table 1. Majority of them were found in elevated concentrations as compare to world average. The natural concentration of trace elements in soils is a result of weathering that releases trace elements from their host minerals during soil formation (Kabata-Pendias, 1993). A close relationship between the metal content of the parent material and soils has been observed in a number of studies (Singh and Steinnes, 1994). The trace elements assemblage of the soil shows good agreement with the composition of the rocks of the area (fig. 1). It is remarkable to note that the concentration level of these trace elements is lower than the world average of the soils, except Cd. Possibly, the bioavailability of elements decreases in the presence of soil carbonates, organic matter and basic pH (Oluwatosin *et al*., 2008; Irha *et al*., 2009). The mean of Cd in the 49 samples of the soils of the study area is 0.49µg/g, which is slightly higher than the European Mediterranean agricultural soils 0.30µg/g (Micó *et al*., 2006).

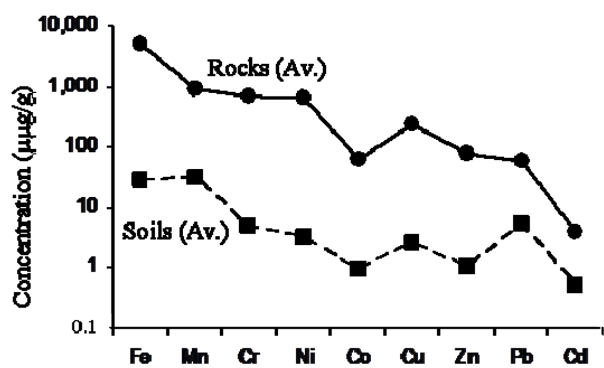

Fig. 1: Average concentration of trace elements in the rocks and its comparison with the soils of the Winder area on log scale.

The speciation of trace metals ultimately determines their bioavailability and their mobility in the soil (Pelfrêne *et al*., 2009). A number of trace elements are contributed through the weathering of igneous rocks. In order to assess the role of rock in dispersing the elements, a graph is constructed to illustrate mutual relationship between rock and soil (fig. 1). It is important to note that concentration trend is quite similar except the level of enrichment. The concentration levels of Cu, Zn, Cr and Co were high in the rocks and nearly 1% is transformed in the soils of the study area. Lead and Cd showed relatively high proportion in the soil while Fe and Ni were found hardly half percent in the soils of the study area.

Fruits

Lead (Pb) and Cd were recorded below the detection limit of the instrument (AAnalyst 700, PerkinElmer) in edible part (pulp) of the fruit. The fruits of *M. zapota* showed higher concentration of Fe (14µg/g) among the other trace metals. Box-Whisker plots (fig. 2) shows the lowest and highest values of Fe which are in the range of 4.43 & 44.5µg/g, while median is 11.45µg/g. The average value of present study is 14.17µg/g; it is close to 14.54µg/g of Zahir *et al*. (2009) for some market available *M. zapota* of Karachi, but much lower to 135µg/g of Indian *M. zapota* (Mahdavian and Somashekar, 2008). USDA (2000) mentioned Fe 8µg/g as mean value in the fruits. The Cu content (av. 1.7µg/g) in the edible part of the fruits is slightly higher than the amount (0.86µg/g) mentioned by (USDA, 2000). In general, all trace elements present in the pulp of the *M. zapota* grown in the Winder area were found high in contrast to USDA (2000); probably this is due to abundantly bioavailable trace elements in the soils of the study area.

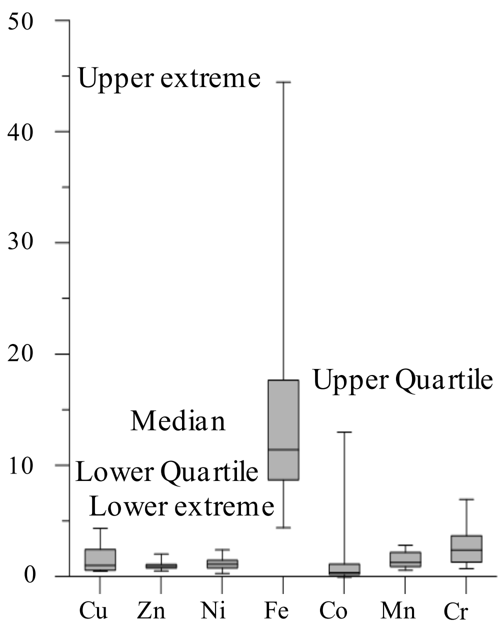

Fig. 2: Box-Whisker Plots showing concentration of elements in fruits pulp of Winder farms (µg/g).

In the edible part of *M. zapota,* the most promising correlation exist among Cr-Mn (*r*=0.82), while moderate relationship was present in Ni-Cu and Zn-Co as shown in fig. 3. Chromium-Cu relation is found negative (*r*=-0.54), indicating that fruit tissues prefer to accumulate Cr at the expanse of Cu, because negative correlation matrix indicates that as one variable increases, the other decreases (Stockburger, 2007). This information can be exploited to alleviate excessive toxic Cr from the fruit, by adding some fertilizer Cu in the soil. Copper on the other hand is beneficial in low quantities and also reduce the uptake of Cr.

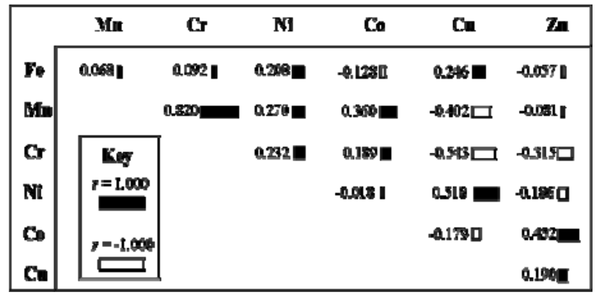

Fig. 3: Correlation matrix of important trace elements of *M. zapota*.

The statistical analysis of data-set of trace elements of fruit pulp is decisive to interpret the communal performance. The x-y scattered and triangulation diagrams were found significant to evaluate accumulation of trace elements in the fruits, grown in the Winder area. The Mn-Cr relationship display strong positive correlation (r=0.82), but this relation is strange in the fruit because Mn is a biophile element while Cr is lithophile and toxic element to plants (fig. 4A). The Cr-Cu relation in *M. zapota* displayed crescent shaped pattern. At the low concentration of Cr (~1µg/g), Cu shows variable absorptions, while in the other part; the content of Cr is high for nearly uniform amount of Cu (fig 4B). A composite ternary plot between Fe-(Cu+Zn)-(Cr+Ni+Co) illustrate bifurcation into two separate trends. One set of fruits are rich in Fe with minor quantities of Cu+Zn, while other set exhibits variable amount of Cu+Zn in the pulp of the fruits (fig. 5). Possibly this variation is due to the availability of Cu in the soils of the study area.

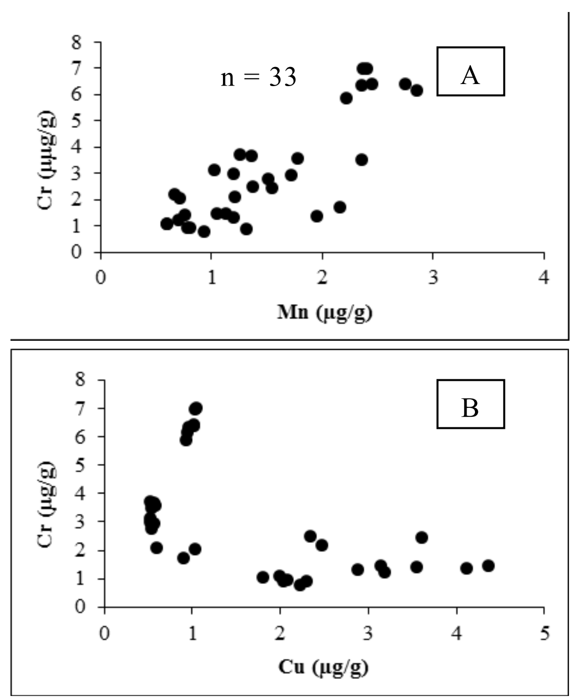

Fig. 4: Degree of correspondence between A. Mn-Cr & B. Cu-Cr in the fruits of *M. zapota* of Winder area.

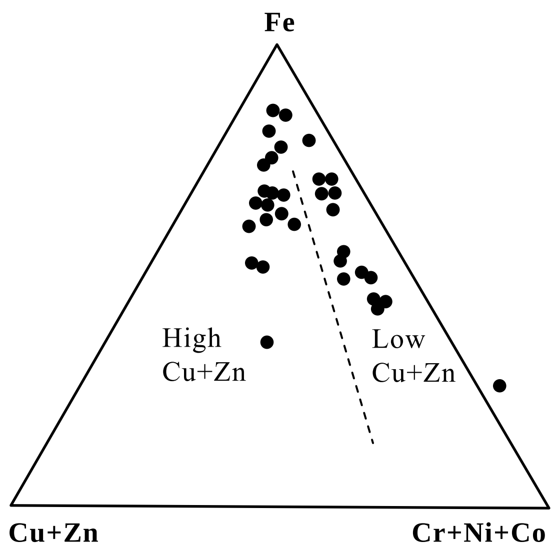

Fig. 5: Ternary relation between Fe-(Cu+Zn)-(Ni-Co-Cr) in the pulp of fruits of *M. zapot*a of Winder area.

The ratio of fruits to soil is termed as Bio-concentration factor (Ebong *et al*., 2007) and presented in form of ratio of accumulation of elements in these two mediums (fig. 6). The *M. zapota* showed high Zn and Co in the fruit pulp, while Mn was transferred least in the fruit from the soil. Present analysis is valuable for selecting better fruits in the perception of local geology. Thus it is helpful to discriminate between the level of enrichment of essential and toxic elements, to mitigate problems related to medical geology in the inhabitants of the area.

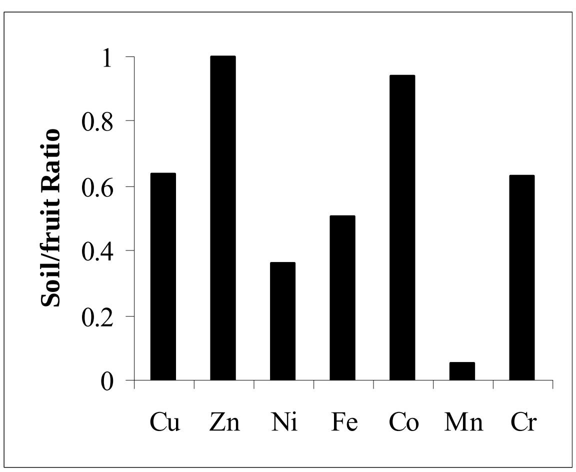

Fig. 6: Soil/fruit ratio of *M. zapota* of Winder area.

In the present study, comparison between trace elements assemblage of *M.* zapota and average values given by USDA (2010) is made and it is assessed in the light of Recommended Dietary Allowance (fig. 7), given by Dietary Guidelines for Americans (DGA, 2005). The amount of Fe in *M. zapota* is much higher (14.17µg/g) than the average value (8µg/g) given by USDA (2010). Excess iron damages cellular macromolecules and promotes cell death, tissue injury leading to malignant condition (Papanikolaou and Pantopoulos, 2005). Dietary intake of high Fe may lead to many blood syndromes, such as leukemia, thalassemia, etc. Such diseases are rapidly increasing in Karachi (Sajid *et al*., 2010 and WHO, 2006). The RDA of Mn is about 2000µg/day (DGA, 2005) and 5000µg/day (Wardlaw, 2000). The edible part of *M. zapota* contains Mn approximately 1,490µg/kg, which is slightly higher than 1000µg/day of USDA (2010). Deficiency of Mn may lead to paralysis, convulsions, blindness and deafness in infants. Manganese can also cause lung embolism, low sex appeal, in-coordination and Parkinson syndrome (Zayad, 2002). The amount of Cr required for healthy growth increases with age from infant (2µg/day) to adult (30µg/day) (Anderson, 2007). It may help to improve type-2 diabetes mellitus and beneficial for blood lipid profiles. The fruits of *M. zapota* of the study area has high amount of Cr (av. 2,960µg/kg) which may cause malignancy in the inhabitants of the area. The function of Ni in the human body is ambiguous and the human requirement for Ni does not exceed 20µg/day (fig. 7). The fruits of *M. zapota* have 1,130µg/kg average Ni (table 1). High levels of Ni may cause stomach aches and adverse effects to the blood and kidneys. Excessive values of Ni also cause alteration of the activity of certain enzymes and suppress immunity. Khan *et al*. (1984) found high Ni in the blood serum of Pakistani patients suffering from acute myocardial infarction. Similarly, Co is also high (920µg/kg) in contrast to RDA of 20µg/day (fig. 7). Elevated amount of Co in the blood serum were noted by the Khan *et al*. (1984) in the Pakistani patients with acute and chronic ischemic heart disease and hypertension. Copper in trace quantities is essentially required for good health, because it is the part of many enzymes. The high concentration of Cu (1,700µg/kg) in the fruits of *M. zapota* is slightly higher than the daily requirement (900µg/day). Excess Cu consumption may lead to liver damage. Entrance of Cu (10,000µg/day) through food chain over several weeks may lead to general weakness, abdominal pain, nausea, vomiting, diarrhea and muscle cramps (Turnlund *et al*., 2005). Siddiqui *et al*. (2006) examined 14 patient of Wilson's disease at the Aga Khan University Hospital, Karachi and found high Cu in their blood serum. The study may be helpful to control Wilson's disease in future with better scientific approach. Despite, the maximum translocation of Zn from soil to fruit, it is very low in the fruit pulp (1,020µg/kg). The amount is low as a healthy person daily required (15,000µg/day). Intake larger than 25,000µg may cause anemia, gastric distress and dizziness. On the other hand, the chronic zinc deficiency in human results in retarded growth, prolonged healing wounds, impaired physical and neuropsy-chological development, white spots on finger nails, fatigue and susceptibility to infections (Hambidge, 2000).

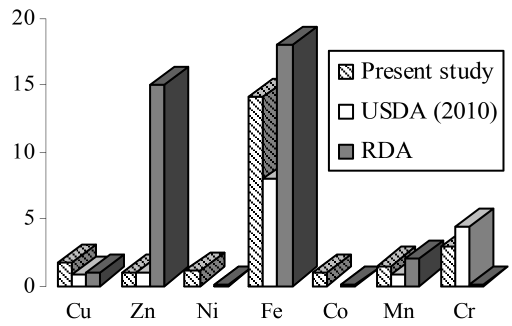

Fig. 7: Abundance of trace elements in *M. zapota* from Winder area (µg/g) and its comparison with USDA (2010) and RDA (DGA, 2005).

CONCLUSIONS

As a result of this research work it could be concluded that the plants growing around the rocky areas, affected differently on their trace elements assemblage. The abundance pattern of elements of the soils signifies contribution from the rocks. The fruits of *M. zapota* are enriched in certain trace elements (Cr, Cu, Ni etc.), which are more than the world average. The transformation ratio from soil to fruit was noted high in case of Zn and Co, while it was least for Mn. The edible part of *M. zapota* shows high concentration of trace elements as compared to recommended dietary intake (RDA). High level of these elements on consumption may lead to several consequences due to biochemical disfunctioning in the body and many diseases are suspected in the inhabitants of the area related to toxic elements. The mutual behavior in form of correlation matrix, scattergrams, and ternary plots signify that elements are contributed through rocks and soils of the study area.

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