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Investigation of Some Trace Elements and Heavy Metals Concentrations in Selected Sudanese Medicinal Plants

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Abstract

In Sudan, medicinal plants represent the primary source of health care. Therefore, determining the content of trace elements and heavy metals are of high importance. Thus, the purpose of this research is to investigate the content of trace elements (Fe, Mn, Zn and Cu) and heavy metals (Pb, As, Cd and Hg) in 30 selected Sudanese medicinal plants using Energy Dispersive X-ray fluorescence technique (EDXRF). Twelve elements K, Ca, Mn, Fe, Cu, Zn, Pb, Br, Rb, Sr, Ti and Zr were determined, and the concentrations of As, Cd and Hg below the detection limits. Using SPSS, the correlation between elements was performed; also cluster analysis checked the similarity between the samples results. The obtained results showed that the contents of the trace elements and heavy metals in the investigated medicinal plants species within the allowable safety limits except for Fe. The consumption of traditional Sudanese medicinal plants does not appear to pose a health risk factor by metals.

Keywords: EDXRF; heavy metals; Sudan medicinal plants; trace elements.

1. Introduction

Medicinal plants have been used as remedies for various diseases across the world from ancient times [1]. As it was reported by the World Health organization (WHO), about 80% of people in the developing countries use only medicinal plants for the treatment of many diseases [2].

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In the past decade they were used increasingly in medicinal plants due to their minimal side effects, availability and acceptability to the majority especially in the third world countries [3]. Also, there are a lot of scientific interests for the development of plant products as dietary supplements and to understand their pharmacological actions [2].

The efficacy of medicinal plants for curative purposes is often accounted for in terms of their organic constituents, but inorganic constituents (minerals) are partially responsible for their medicinal and nutritional properties, as well as their toxic ones [4]. These elements play an important role in plant metabolism and biosynthesis and as cofactors for enzymes [5], and in human health as catalysts or parts of prosthetic groups for enzymes, and, consequently, insufficient supply leads to element specific deficiency symptoms [6]. In some cases, plants may be contaminated with toxic concentrations of heavy metals which may cause serious health problems, such as renal failure, symptoms of chronic toxicity and liver damage [7]. The concentration of trace elements and heavy metals in collected medicinal plants is a matter of public safety because they are easily contaminated during growth, development and processing [8].

The different concentrations of elements in plants vary according to the geographical region, geochemical soil characteristics, and the ability of plants to selectively accumulate some of these elements. Generally, these elements are absorbed through the root systems and dispersed throughout the plant body [5]. Other factors responsible for a variation in elemental content are preferential absorbability of the plant use of fertilizers, irrigation water and climatological conditions [9]. Determination of metals in medicinal plants is a part of quality control to establish their purity, safety and efficacy according to the World Health Organization (WHO) [10].

The main objectives of this research are to determine levels of trace elements Fe, Mn, Zn and Cu and heavy metals Pb, As, Cd and Hg in some Sudanese medicinal plants that are used as a traditional medicines using Energy Dispersive X-ray fluorescence technique (EDXRF).

2. Material and Methods

2.1. Sample Collection

Thirty medicinal plants were purchased from Al-Teyman Herb Store, one of the oldest and major herbalists in Omdurman local market, Sudan. Most Sudanese citizens purchase their medicinal plants from this shop. Depending on their grown location in Sudan, the samples are divided in three locations (Central Location Loc1, Northern Location Loc2 and Western Location Lco3). Code, local name, scientific names, locations and used part of medicinal plants samples are presented in Table 1.

2.2. Sample Preparation

The samples were washed with distilled water and air-dried at room temperature. The dried samples were grinded to fine homogeneous powder and stored in plastic bags prior to analysis. For analysis, 1g of each dried sample was accurately weighed and pressed using manual hydraulic pressing machine (20 tons). The pellet diameter was 2.5 cm. Pellets of certified reference materials Hey powder (IAEA-V-10) was also prepared in similar way to check the accuracy of the technique.

Table 1: Code, local name, scientific names, locations and used part of medicinal plants samples:

Code	Local Name	Scientific Name	Locations	Used Part
MP1	Shamar	*Foeniculum vulgare*	Loc1	fruit
MP2	Kazbara	*Coriandrum sativum*	Loc1	fruit
MP3	Garad	*Acacia nilotica*	Loc1	fruit
MP4	Limone	*Cymbogon citratus*	Loc1	fruit
MP5	Fol Sudaney	*Arachish hypogaea*	Loc1	fruit
MP6	Karah	*Cucurbita moschata*	Loc1	seed
MP7	Nanaa	*Mentha spicata*	Loc1	leave
MP8	Gerger	*Eruca sativa*	Loc1	leave
MP9	Sidr	*Zizyphus vulgris*	Loc1	leave
MP10	Sanamaka	*Senria italic*	Loc1	leave
MP11	Doom	*Hyphaene thebaica*	Loc2	fruit
MP12	Shaata	*Solanum capcicum*	Loc2	fruit
MP13	Tormos	*Lupinus luteus*	Loc2	Seed
MP14	Helpa	*Trigonella foenum-graecum*	Loc2	Seed
MP15	Hargal	*Solenostemma argel*	Loc2	Leave
MP16	Hena	*Lawsonia inermis*	Loc2	Leave
MP17	Mhaaraib	*Acorus calamus*	Loc2	Leave
MP18	Zangabel	*Zingiber officinale*	Loc2	Root
MP19	Basal	*Allium cepa*	Loc2	Root
MP20	Toome	*Allium sativum*	Loc2	Root
MP21	Kmpa	*Xylopia aethiopica*	Loc3	Fruit
MP22	Nkhwa Hendia	*Tracys permummammi*	Loc3	Fruit
MP23	Tabaldi	*Adansonia digitate*	Loc3	Seed
MP24	Karawia	*Carum carvi*	Loc3	Seed
MP25	Hap Alrashad	*Lepidium sativum*	Loc3	Seed
MP26	Semaeem	*Sesamum indicum*	Loc3	Seed
MP27	Hazaa	*Dycrosia ismales*	Loc3	Leave
MP28	Sheh	*Artemisia absinthium*	Loc3	Flower
MP29	Karkade'	*Hibiscus subdariffa*	Loc3	Flower
MP30	Samog Arabey	*Acacia Senegal*	Loc3	Stem

2.3. Sample Analysis

The measurements were carried out using ED-XRF spectrometer with 109Cd radioactive source as source of excitation. The characteristic X-rays emitted from the sample were detected using Si(Li) detector with Full Width at Half Maximum intensity (FWHM) equal 170 eV at 5.96 K eV, each spectrum was collected for a life time of 1000s. Quantitative calculations were made by the QAES-Radioisotopes version 1995 method.

The accuracy of the technique was checked by analyzing Hey powder (IAEA-V-10). The measured values, the certified values along with the accuracy are given in Table 2. The measured values are in good agreement with the certified values [11].

Table 2: XRF Accuracy results using Standard Reference Material Hay powder (IAEA-V-10)

Element	Certified value (ppm) [11]	Measured value (ppm)	Accuracy (%)
K	21000	22000	4.76
Ca	21600	22500	4.17
Mn	47	49	4.25
Fe	186	194	4.30
Cu	9.4	9.81	4.36
Zn	24	25	4.17
Pb	1.6	1.66	3.75
Br	8	8.25	3.13
Rb	7.6	7.80	2.63
Sr	40	41.0	2.50

3. Results and Discussion

3.1. Elemental concentrations in medicinal plants samples

Sudanese medicinal plants (non-controlled growth) samples have been analyzed by EDXRF. Twelve elements K, Ca, Mn, Fe, Cu, Zn, Pb, Br, Rb, Sr, Ti and Zr were determined and the elements As, Cd and Hg were below detection limit (BDL) in all samples. The elemental concentrations in the Sudanese Medicinal plants samples are listed in Table 3.

The highest element concentration in most of the studied plants was K, followed by Ca. Both K and Ca are present in plants in large amounts; they are nutrient elements as major elements [12]. The mean concentration of K was 23567 ppm, Shamar (MP1) showed the highest value while Samog Arabey (MP30) showed the lowest value. Whereas, the mean concentration of Ca was 17834 ppm, Sidr (MP9) showed the highest concentration while Karah (MP20) showed the lowest value (below detection limits in 3.3% of the samples).

Fe and Mn are considered essential trace elements nutrients [12]. Fe is an essential for human body in the production of hemoglobin and in the oxygenation of red blood cells. It is needed for healthy immune system and energy production since the deficiency of Fe the most common nutritional deficiency in the world [13,3]. The concentrations of Fe in studied plants were high and came second after those of Ca. The mean concentration of

Fe was 1983 ppm, Zangabel (MP18) recorded the highest concentration value, while Samog Arabey (MP30) recorded the lowest value. The high concentrations of Fe in the analyzed Sudanese medicinal plant samples may help in checking cross deficiency of Fe [3]. In the seed and the root of medicinal plant samples we found that the concentration of Fe is low. The high concentration of Fe in the investigated plants may partially be derived from foliar absorption from the surroundings air (air pollution) [5]. The permissible limit set by WHO in medicinal plants for Fe has not yet been established [14]. The range of Fe in selective medicinal plants of Lebanon in the study carried out was between 77 ppm to 3643 ppm [5]. The concentration of all the selected medicinal plants under this study is in the range, indicating that all the concentration of Fe in the plants studied is around the normal range when compared with the medicinal plants from Lebanon.

Mn is also important in regulation of immune responses of the body, production of energy and by activation of various enzymes important for proper digestion and utilization of foods [13]. Mn can be correlated with maintenance of blood sugar level, formation of normal bone structure and in utilization of fats [3]. The mean concentration of Mn was 366 ppm, Tormos (MP13) recorded the highest value that represent an anomaly high most probably due to Tormos phytoextraction plant for Mn [15], while the Kazbara (MP2), Garad (MP3), Limone (MP4), Fol Sudaney (MP5), Gerger (MP8), Doom (MP11), Shaata (MP12), Bsal (MP19), Toome (MP20), Tabaldi (MP23) and Samog Arabey (MP30) recorded the lowest values (below detection limits in 36.7% of the samples). The permissible limit set by WHO in medicinal plants for Mn has not yet been established [14]. The range of Mn concentration in selective medicinal plants of Sudan was between 1.3 ppm to 517.5 ppm [7]. The concentration of all the selected medicinal plants under the present study is in the range, indicating that all the concentration of Mn in the plants studied is around the normal range except in Tormos (MP13) which shows abnormally high concentration of Mn when compared with the medicinal plants from Sudan.

Zn and Cu are essential trace elements micronutrients [12]. They are essential trace metals for plant growth and development [5]. Phytotoxicity can occur if its concentration in plants is higher than 20 - 100 ppm DW (dry weight) [16]. All Cu concentrations of samples fell within this range or below range. The mean concentration of Cu was 16.22 ppm, Shamar (MP1) showed the highest concentration while Samog Arabey (MP30) showed the lowest value. Cu is essential to normal red blood cell formation, help store and release Fe, works with vitamin C to keep blood vessels elastic and lower LDL and raise HDL cholesterol in the blood [17].The permissible limit set by WHO for medicinal plants limit has not yet been established for Cu. In medicinal plants the permissible limit of Cu set by China and Singapur were 20 ppm and 150 ppm respectively [14]. The selected plants were found to have permissible limits around the normal range set by China and Singapur. The range of Cu concentration in selective medicinal plants of India was between 5 ppm to 22.6 ppm [6]. The concentration of all the selected medicinal plants under this study is in the range, indicating that all the concentration of FCu in the plants studied is around the normal range when compared with the medicinal plants from India. Zn is not considered to be highly phytotoxic, but high contents of Zn in plants may cause the loss of leaves production. Zn is plays an important role in various cell processes including normal growth, brain development, behavioral response, bone formation and wound healing [16]. The mean concentration of Zn was 17.93 ppm, Karah (MP6) showed the highest concentration (36.4 ppm) while Tabaldi (MP23) and Samog Arabey (MP30) showed the lowest values (below detection limits). The permissible limit of Zn set by WHO in medicinal plants have not yet been established [14]. The range of Zn concentration in selective medicinal plants of Sudan was between 0.2 ppm to 81.4 ppm [7]. The concentration of all the selected medicinal plants under this study is in the range, indicating that all the concentration of Zn in the plants studied is around the normal range when compared with the medicinal plants from Sudan.

Table 3: Elemental concentration of Sudanese medicinal plants (ppm = mg/Kg) except as specified by *(mg/g)

Code	K*	Ca*	Mn	Fe*	Cu	Zn	Pb	Br	Rb	Sr	Ti	Zr	As	Cd	Hg
MP1	٤٨.٨	٢٧.٣	٢٤٦	٣.٨٧	٢٤.٥	١٩.٧	١.٨٩	٣٩.١	٤٣.٥	١٥٣	BDL	BDL	BDL	BDL	BDL
MP2	٣٥.٧	١٦.٤	BDL	٢.٢١	١٨.٨	٣٠.٤	١.٤	٦٠.٦	٤٠.٩	١٢٧	BDL	BDL	BDL	BDL	BDL
MP3	٢٢.٠	١٢.٨	BDL	٢.٩٨	١٥.٩	٧.٤٩	١.٦٧	١٠٥	٤٧.٢	٥٩.٥	BDL	BDL	BDL	BDL	BDL
MP4	٢٦.٩	٢٠.٠	BDL	0.٧١	١٨.٧	٤٣.٤	١.٨٧	BDL	٢٣.٥	١١٣	BDL	BDL	BDL	BDL	BDL
MP5	١٩.٣	٥.٨٨	BDL	١.٨١	١٧	٢٤.٢	١.٨٥	BDL	١٩.٣	٦٧.٩	BDL	BDL	BDL	BDL	BDL
MP6	١٤.٦	٢.٧٩	٦٧.٣	0.٢٣	١٥.١	٤٨.٨	١.٣٣	BDL	١٦.٦	٦.٢٩	BDL	BDL	BDL	BDL	BDL
MP7	١٥.٩	١٩.٩	١٠٢	١.٨٨	١١.٦	٧.٣٣	١.١	٢٧.٨	٤٨.٣	١٥٩	BDL	BDL	BDL	BDL	BDL
MP8	٢٥.١	٣٧.٦	BDL	0.٩٣	١٠.٨	٨.٣٥	١.٠١	١٢٢	٩٩.٣	١٧٥	BDL	BDL	BDL	BDL	BDL
MP9	١٤.٢	٥٥.٦	٢٢٤	١.٢٦	٢٢.٥	٨.٩١	١.٦٣	١٦١	١٧.٧	٣٨٦	BDL	BDL	BDL	BDL	BDL
MP10	٢٢.٩	١٦.١	٥٧.٣	٤.٠١	٩.٧	٤.٧	٠.٩٩	٣٥.١	٢٩.٣	١٤١	BDL	BDL	BDL	BDL	BDL
MP11	٣٨.٦	٦.٨٣	BDL	١.٠٧	١٥.٤	٥.٨٤	١.٥٢	٧٨.٤	٤٠.٨	٤٦.٥	BDL	BDL	BDL	BDL	BDL
MP12	٣٤.٦	٨.٥٥	BDL	١.٠٥	٢١.٩	١٠.٣	٢.٠٣	٢٥	٢٧.٣	٦١.٢	BDL	BDL	BDL	BDL	BDL
MP13	١٤.١	٥.٧٣	٣٢١٠	0.٣٤	١٣.٦	٣٠.٩	١.٣٩	١٥	٨٣.٨	٢٧.٥	BDL	BDL	BDL	BDL	BDL
MP14	١٥.٣	٣.٥٥	٦١.٨	0.٩٣	١٥.١	٢٤.٩	١.١٥	٥١.٢	٣١.١	٣٩.٦	BDL	BDL	BDL	BDL	BDL
MP15	٢٠.٠	٩.٦١	٣٦٢	٣.٨٦	٢٤.٧	٢٦.٤	٢.٠٨	BDL	٢٨.٦	٦٤	٥١٨	٤٨	BDL	BDL	BDL
MP16	٣٠.٨	٣١.٧	٣٩٤	٣.٨٥	٢٠	١٠.٧	١.٨٥	٩٠.٩	٣٨.١	٢٣٥	BDL	BDL	BDL	BDL	BDL
MP17	١٠.٩	٣٩.٧	٢٣٥	٧.٥٤	٢١.٥	١٦.٥	١.٦٩	٤٤.٧	١٨.١	٤١٢	BDL	BDL	BDL	BDL	BDL
MP18	٣٧.٤	١٢.٥	٥٦١	0.٩٦	١٩.٩	١٠.٢	٢.٦٤	٧.١	١٨٠	٧٣.٣	١١٨٠	٢٢٩	BDL	BDL	BDL
MP19	٢٢.٣	٦.٤٢	BDL	0.٢٢	١٨.٢	١٥.١	٢.٢	٤٥.٥	١٩.٣	٦٥.١	BDL	BDL	BDL	BDL	BDL
MP20	٢٧.٨	BDL	BDL	0.0٨	١٧.١	١٣.٣	١.٧٥	BDL	١٠.٧	١١.٢	BDL	BDL	BDL	BDL	BDL
MP21	١٣.3	٦.٥٨	١٤٨	0.٣1	١٠.٨	٣.٥٤	٠.٨٨	١٢.٢	١٢١	٣٠	BDL	BDL	BDL	BDL	BDL
MP22	١٩.٤	٢.٢٢	٩٤.٧	٣.٠٤	١١.٩	١٢.٥	٠.٩٤	١٨	١٩	٣١٢	BDL	BDL	BDL	BDL	BDL
MP23	١٥.١	٢٦.٢	BDL	0.0٦	١٢.٩	BDL	١.٣١	BDL	٢٥.٢	١٨.٤	BDL	BDL	BDL	BDL	BDL
MP24	٣١.٠	١١.٨	٨٢.٤	0.50	٩.٧٦	١٣.٤	١.١٨	٥٧.٦	٢٩.١	٨٥	BDL	BDL	BDL	BDL	BDL
MP25	٢٩.٣	١٠.٧	٧١.٧	0.20	٢٠	٢٩.٢	١.٥٣	١٦.٨	١٠.٤	٧٣.٢	BDL	BDL	BDL	BDL	BDL
MP26	١٣.٦	٣٦.٩	٧٢.٤	0.٥2	١٧.٣	٣٦.٤	١.١٧	BDL	٢٩.٢	٨٣	BDL	BDL	BDL	BDL	BDL
MP27	٢٣.٩	١٦.٦	٧٧.١	٢.١٠	١٠.٣	٧.٣٢	٠.٩٣	١٤٣	٢٨.٤	١٢٢	BDL	BDL	BDL	BDL	BDL
MP28	١٦.٦	١٢.٢	١٠٧	٣.٦٢	٩.٦٨	١١.٣	١.١٢	٢١.٥	٤٢.٤	١١٣	BDL	BDL	BDL	BDL	BDL
MP29	٣٧.٨	٢٧.٤	٧٨٨	0.٦5	٢٢.٩	٢٠.٩	٢.١١	١٢٤	٦٨.٣	١٧٧	BDL	BDL	BDL	BDL	BDL
MP30	٩.٨	٧.٦٤	BDL	0.05	٩.١٧	BDL	٠.٩١	BDL	٢١	١٠٢	BDL	BDL	BDL	BDL	BDL

BDL = Below Detectable Limit

Pb, Br, As, Cd and Hg are heavy metals of no nutrient value for either humans or plants, while could easily induce toxic effects in humans at low concentrations [2,12]. The maximum permissible levels in medicinal plants for Pb, As, Cd and Hg are respectively 10, 5, 0.3 and 0.5 ppm [14].

The mean concentration of Pb was 1.51 ppm, kmpa (MP21) showed the highest concentration while Zangabel (MP18) showed the lowest value. The concentrations Pb were within the permissible limits in all samples. Cd, As and Hg were below the detection limits in all samples. The range of Cd concentration in selective medicinal plants of Sudan was between BDL ppm to 0.19 ppm and of Hg was between BDL ppm to 0.02 ppm [7]. That mean that the undetectably limits of As, Cd and Hg in tested Sudanese medicinal plants in this study is highly acceptable which is known to be highly toxic to both humans and animals (within the permissible limits in all samples). And It means that the plants grown in the pollution free areas of such elements [2].

The mean level of Br was 59.16 ppm, Sidr (MP9) recorded the highest concentration value, while the Limone (MP4), Fol Sudaney (MP5), Karah (MP6), Hargal (MP15), Toome (MP20), Tabaldi (MP23), Semaeem (MP26) and Samog Arabey (MP30) recorded the lowest values (below detection limits in 26.67% of the samples). The permissible limit of Br set by WHO in medicinal plants have not yet been established [14]. The range of Br concentration in selective medicinal plants of India was between 2.7 ppm to 320.5 ppm [6]. The concentration of all the selected medicinal plants under this study is in the range, indicating that all the concentration of Br in the plants studied is around the normal range when compared with the medicinal plants from India.

Rb, Sr, Zr and Ti are not clear so far their effect on the plants, animal and human health [12]. The mean concentration of Rb was 41.91 ppm, Gerger (MP18) recorded the highest concentration value while Karah (MP25) recorded the lowest value. The mean concentration of Sr in Loc1 was 117.96 ppm, Mhaaraib (MP17) showed the highest concentration while Karah (MP6) showed the lowest value. Ti and Zr only found in two samples Hargal (MP15) and Zangabel (MP18). The detection of Ti and Zr lead to the fact that the plants are grown in the pollution areas of such elements [5].

The concentrations of elements in the samples from Location1 are compared with those, from Location2 and Location3 (Figures 1 and 2). It is evident that the samples from Loc2 had high contents of K, Mn, Fe, Pb, Rb and Cu, and the samples from Loc1 had high contents of Ca, Sr, Zn and Br. While the samples from Location3 have had low contents of K, Cu, Fe, Zn and Pb, the samples from Location2 have had low contents of Ca, Br and Sr and the samples from Location1 have had low contents of Mn and Rb. Generally, Loc3 are low polluted area and Loc2 are high polluted most probably due to minor source associated with industrial air pollution in Loc2 [5].

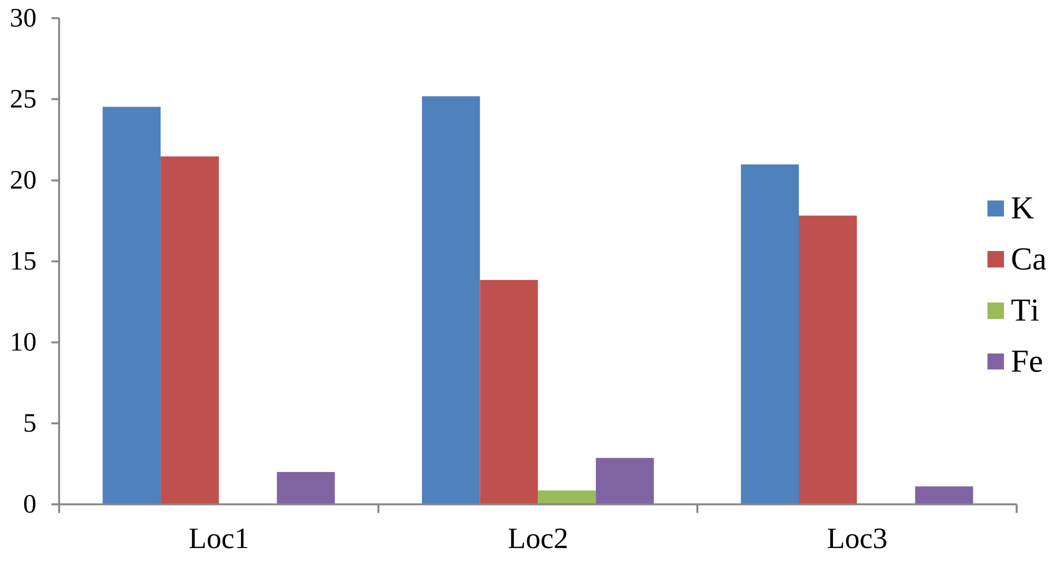

Figure 1: Mean elemental mean concentrations (ppm) in different locations

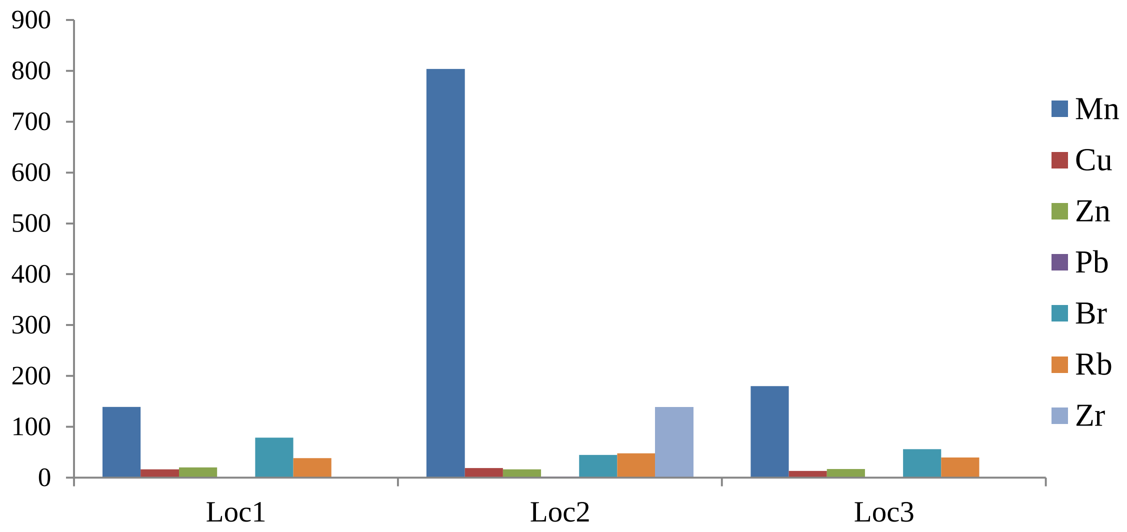

Figure 2: Mean elemental mean concentrations (ppm) in different locations

3.2. Correlation Analysis

In order to detect the interrelationship between the elements, correlation coefficients analysis was applied [18]. The results of calculations are shown in Table 5. In the study, only positive statistically significant relationship was obtained, and some of them were strong, with the correlation coefficient higher than 0.7, the other relations were lower. In two cases, between Cu and Pb, as well as between Ca and Sr, the correlation was strong. This may indicate that these elements take part in mutual biochemical reactions [5].

Table 5: Correlation coefficients for each element in different location

	K	Ca	Mn	Fe	Cu	Zn	Pb	Br	Rb	Sr
K	1									
Ca	-.025	1								
Mn	-.047	-.167	1							
Fe	0.188	0.183	-.099	1						
Cu	0.431	0.286	0.052	0.268	1					
Zn	-.059	-.150	0.226	-.272	0.296	1				
Pb	0.504	0.011	0.197	0.378	**0.812**	-.125	1			
Br	0.056	0.575	-.134	-.216	0.129	-.215	-.013	1		
Rb	0.204	-.066	0.344	0.391	-.070	-.274	0.633	-.189	1	
Sr	-.085	**0.778**	-.184	0.408	0.221	0.221	-.023	0.339	-.143	1

3.3. Cluster Analysis

It is a task of grouping a set of objects in such a way that objects in the same group are more similar to each other [5]. The clustering procedure generated three groups of samples in high independency for each cluster. The cluster 1 includes Cu, Zn, Fe, Ca, Mn, K and Pb that all those elements have a physiological function in plants except of Pb have the potential to cause toxic symptoms. Cluster 2 includes Br and Rb which reflects the similarity in the concentration in plant samples. Cluster 3 includes Sr which has no nutrient value [12].

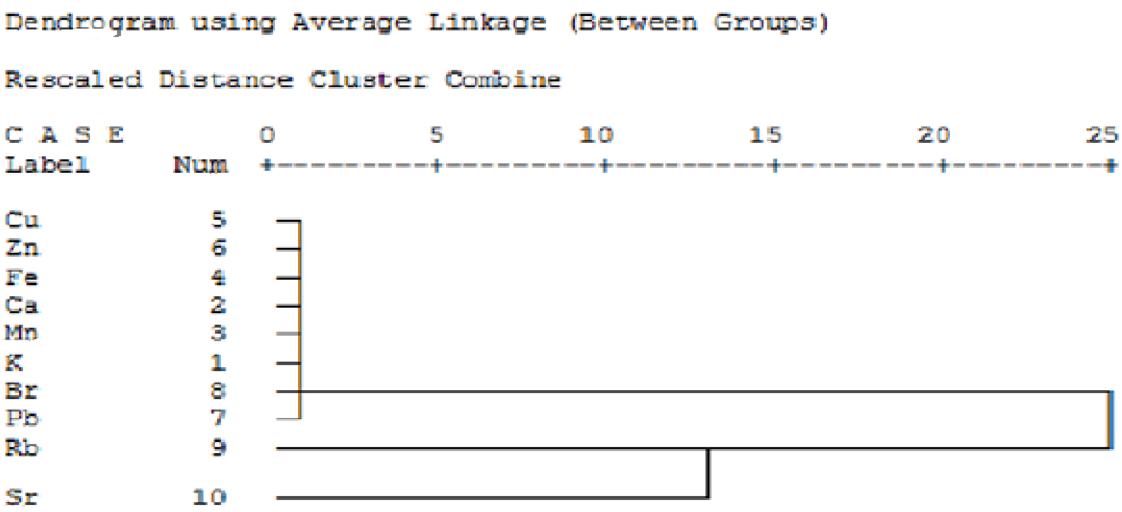

Figure 3: Cluster grouping by elements for sediment samples

4. Conclusions and Recommendations

The results obtained in this study show that the concentration of the studied elements was similar in all locations, but slightly higher in Loc2. Correlation analysis shows relationship between (Ca, Sr) and (Cu, Pb).

Analyzed medicinal plants divided all the elements in three high independency clusters. The detected trace elements and heavy metals were within the allowable safety limits except for Fe. The data showed that many Sudanese medicinal plants can be considered as sources of a reasonable amount of the trace elements to patients of different health disorders. The values of some Sudanese medicinal plants support their integration in modern therapy and may also be of importance for the development of new medical supplements. The consumption of traditional medicinal plants infusion in Sudan does not appear to pose a health risk factor by trace elements and heavy metals. However, we may not assume these plants do not pose health risk that might result from fungicides, pesticides, fertilizers, and organics in dumped wastes and irrigation by contaminated wastewater. It was also not possible to study other essential and non-essential elements due to time and financial limitations. Further assessment of these should be conducted to assess the quality control.

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