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COMPARATIVE STUDY OF HEAVY METALS IN PLANT AND SOIL OF SOME SELECTED MEDICINAL PLANT

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ABSTRACT
Essential heavy metals like Iron, Nickel, Manganese, Zinc, Copper, Cobalt and non-essential like Cadmium, Lead, and Chromium were analyzed in five selected medicinal plants (Newbouldia leaveis, Chromolaena odorata, Chrysophyllum albidum, Azadirachta indica and Morinda lucida) as well as the soil of each medicinal plants for their metal ion concentration using atomic absorption spectrophotometeric method (AAS). The concentration level of heavy metals in the selected plants were found in the order of Fe (829mg/kg) > Mn (531 mg/kg) > Zn (111 mg/kg) > Pb (88 mg/kg) > Cu (36.5 mg/kg) > Co (15 mg/kg) > Ni (15 mg/kg) > Cr (12 mg/kg) > Cd (9mg/kg). The concentrations found in the soil are in the order; Fe (2167.5 mg/kg) > Mn (1380 mg/kg) > Cr (445 mg/kg) > Pb (207 mg/kg) > Ni (197.5 mg/kg) > Co (188.75 mg/kg) > Zn (141.25 mg/kg) > Cu (81.25 mg/kg)> Cd (12.5 mg/kg). Iron, Cobalt, Cadmium and Nickel were found above the permissible limit in both plant and soil samples. The concentration of Cu (1.0 mg/kg) Pb (1.0 mg/kg) and Cr (0.5 mg/kg) were found to be below the permissible limit in Morinda lucida plant. Other metals below the permissible limits include Zn (12.5 mg/kg) in the plant of Chromolaena odorata and (26.5mg/kg) in Chrysophyllum albidium. Mn was also found to be (113.5mg/kg) in Newbouldia leaveis and (1.0mg/kg) in Morinda lucida plant respectively.

Keywords: essential heavy metals, non-essential heavy metals, medicinal plants, atomic absorption spectroscopy

INTRODUCTION

Medicinal plants are commonly used in a large number of medicines. They have worldwide applications in the treatment of different types of diseases. According to the survey reported by World Health Organization (WHO), about 80% of the world's population consumes indigenous medicinal plants in direct and indirect ways to treat their diseases. Due to limited medical facilities in the rural areas of the developing countries, many medicinal plants are traditionally used for the treatment of diseases like skin infections, diarrhea, diabetes, malaria, respiratory problems, fungal and bacterial infections, (Obiajunwa *et al*., 2002). These plants have significant role in the regulation of various body systems. The nutritious value as well as the toxicity of the medicinal plants is due to their chemical composition. The trace heavy metals like Fe, Cu, Zn, Mn, and Ni are essential nutrients but they become harmful and toxic when their concentration exceeds the recommended standards. Lead and cadmium are nonessential heavy metals. They are extremely toxic even in very minute amounts (Khan, and Tullah 2008). Heavy or toxic metals are trace metals which are detrimental to human health and having a density at least five times that of water. Once liberated into the environment through the air, drinking water, food, or countless varieties of man-made chemicals and products, heavy metals are taken into the body via inhalation, ingestion and skin absorption. If heavy metals enter and accumulate in body tissues faster than the body's detoxification pathways can dispose of, then a gradual build-up of these toxins occurs.

In plants, heavy metals such as cadmium (Cd), lead (Pb) and nickel (Ni) are greatly toxic at low amount. Heavy metal poisoning is the product of multifaceted interaction of chief noxious ions with other vital or non-essential ions. The metals can be a source of decrease in the hydrolysis products viz., α-amylase, Phosphatase, RNAs and proteins. They disturb the enzyme activities by substituting metal ions from the metalo-enzymes and prevent various physiological developments of plants (Agarwal, 1999). High concentration exposure is not a necessity to produce a state of toxicity in the body, as heavy metal accumulation occurs in body tissues gradually and, over time, can reach toxic concentration levels, much beyond the permissible limits (Suruchi *et al.*, 2012).

Most medicinal plants are rich in minerals and metals and are usually consumed in low doses as nonprescription herbal drugs for debility prevention (Hay 1984; Obiajunwa *et al*., 2002). Both the deficiency and excess of essential micronutrients and trace of toxic metals may cause serious effects on human health. The purpose of the current study was to quantify heavy metals concentration in some important medicinal plants. The subject

plants are thoroughly consumed by the general public and it is the utmost need of the day to explore the chemical compositions of these plants and make known their impact on public health. The undertaken studies will facilitate physician, health care professional, planners, technicians, and general public to use these plants in a significant manner (afzal *et al.,* 2013).

MATERIALS AND METHODS

The soil samples of *Newbouldia leaveis* (Akoko), *Chrysophyllum albidum* (Agbalumo), *Chromolaena odorata* (Akintola), *Morinda lucida* (oruwo) and *Azadirachta indica* (Dongoyaro) were collected from some designated farm site within Egbeda local Government area of Oyo State using a plastic dust pan at a depth of 2cm, between each sampling, the plastic pan was thoroughly cleaned. Samples collected were stored in a plastic bag with appropriate labeling codes. The leave samples was also picked from different plant with the aid of a lynon glove and stored in a plastic bag with proper labeling codes. The leave samples were properly identified by a certified botanist at the department of Laboratory Technology Federal polytechnic Offa, Kwara State. The samples are coded using the following acronyms for leave samples:

AKL – Akoko Leave (Neubouidia leaveis)
ATL – Akintola Leave (chromolena odorata)
AGL – Agbalumon Leave (chrysophyllum albidum)
DGL – Dongoyano Leave (Azadirachta indica)
ORL – Oruwo Leave (Morinda Lucida)
While the soil samples are presented as AKS – Akoko Soil (Neubouidia leaveis), ATS – Akintola Soil (chromolena odorata) AGS – Agbalumon Soil (chrysophyllum albidum), DGS – Dongoyano Soil (Azadirachta indica) ORS – Oruwo Soil (Morinda Lucida).

Sample Pretreatment

Moisture Content Determination

The leaves were sun dried for one week, and re- weighed until a constant weight of the leaves was obtained. The dried leave sample was cut into smaller pieces and it was crushed using mortal and pestle. The moisture content was also carried out on the soil samples. It was found that the soil contains 0.24±0.042%. This affirms that the soil is less hydrated.

Leave Samples Ashing and Digestion

The leaves samples were digested using wet digestion procedure. 2g of powdered leaves of each sample was weighed inside crucible (small size) and was subjected into muffle furnace for an hour at 500 ^{0}C. After an hour the crucible was then removed and kept inside desiccators for 30minutes for cooling. After cooling, the ash sample was removed for digestion. The ashes sample was digested with 30ml of dilute HCl in a beaker; this was allowed to stand for 30minutes. The samples were then filtered into sample bottle and 20ml of distilled water was added to made up 50ml.

Digestion Procedure for Soil Samples

This was carried out using Aqua regia, i.e 3:1 of HNO_3 and HCl. 2g of the soil sample was weighed in a beaker and the aqua regia was added and subjected to heating until clear solution to a near dryness in a fume cupboard was obtained. 50ml of distilled water was added and the resulting solution was filtered into sample bottles, the procedure was carried out for the other soil samples and the sample containers were labeled. The samples were analyzed using (AAS) Atomic Absorption Spectrophotometer.

RESULTS AND DISCUSSION

Table 1: Heavy Metals Concentration in mg/kg in different medicinal plant species ND: Not Detected

Samples	Fe	Cu	Ni	Co	Cd	Pb	Cr	Mn	Zn
AKL	213.50	24.00	4.00	4.00	3.50	33.50	4.50	113.5	59.00
ATL	538.00	32.5	15.5	15.0	9.00	88.00	8.50	211.00	12.50
AGL	262.00	36.50	6.50	6.50	5.50	39.30	2.50	221.00	26.50
DGL	829.00	25.00	6.50	13.00	6.50	81.00	12.00	531.00	111.00
ORL	N D	1.00	7.50	10.50	4.50	40.00	0.50	1.00	52.00

Values are given as mean ± standard deviation of triplicates

Table 2: Heavy Metals Concentration in mg/kg in soil samples

Samples	Fe	Cu	Ni	Co	Cd	Pb	Cr	Mn	Zn
AKS	25.0	2.50	41.25	37.50	10.00	207.50	147.5	137.50	6.25
ATS	436.25	81.25	13.75	17.50	11.25	81.25	67.5	301.25	52.50
AGS	106.25	10.00	12.50	27.50	12.50	85.00	25.00	156.25	83.75
DGS	487.5	13.75	7.50	23.75	12.50	91.25	36.25	326.25	37.50
ORS	2167.50	53.75	197.50	188.75	10.00	237.5	445.00	1380.00	141.25

IRON (Fe)

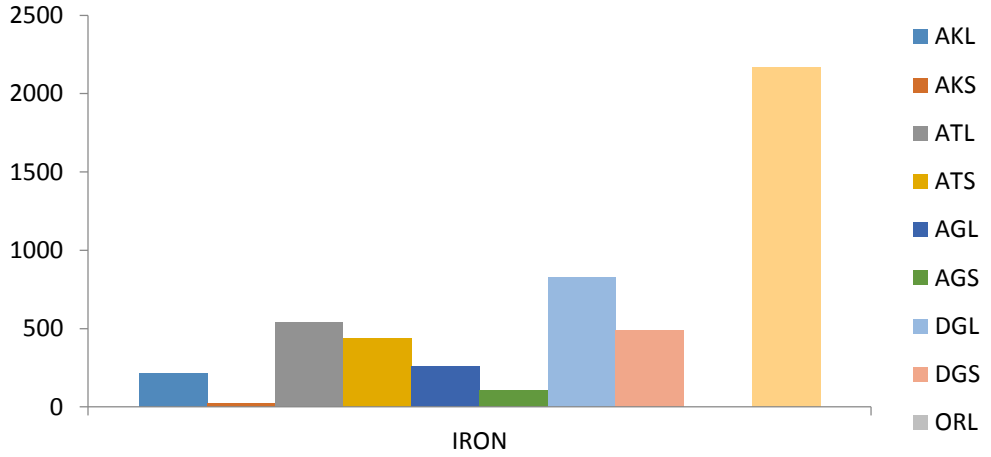

Fig 1: Concentration of Iron (Fe) in leaves and soil samples

Iron (Fe) Determination: It is the most abundant and essential constituents for all plants and soils, on the other hand, at high concentration it causes tissue damage and some other diseases in human. It is responsible for anemia. Excess amount of iron causes rapid increase in pulse rate and of blood in blood vessels, and hypertension. The WHO permissible level of iron in plants is 20 mg/kg (Afzal *et al.*, 2013) .In the leaves of AKL, ATL, AGL , DGL and ORL the concentration of iron was recorded as 213.50mg/kg in *Neubouldia leavis*, 538.00mg/kg *chromolena odorata*, 262.00mg/kg in *chrysophyllum albidum*, 829.00mg/kg in *azadirachta indica*, all were above the permissible limit, while in ORL (*morinda lucida*) iron is Not determined. Concentration of Iron in all the collected soil samples were recorded to be 25.0mg/kg, 436.25mg/kg, 106.25mg/kg, 487.5mg/kg and 2167.5mg/kg , in all the soil samples, concentration of iron were found above the permissible limit set by WHO. Out of the soil samples, the soil for Azadirachta indica was found to have the highest level of iron concentration, while the soil of Neubouldia Leavis has the lowest concentration.

COPPER (Cu)

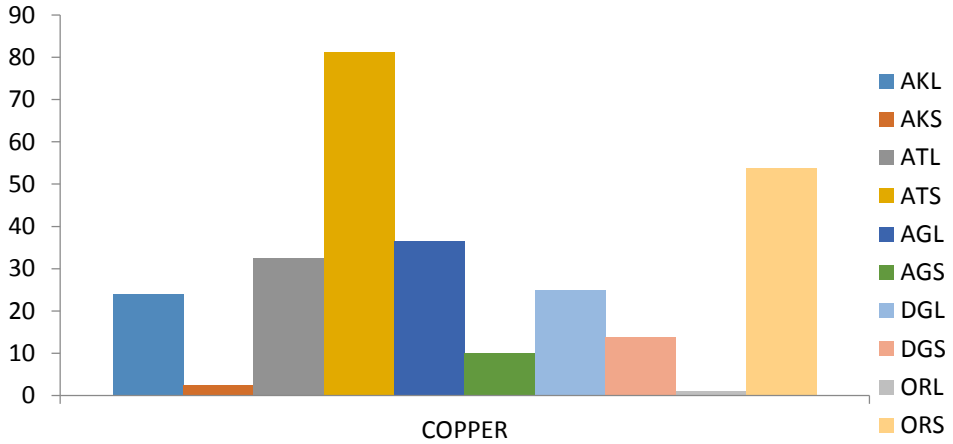

Fig 2: Concentration of Copper (Cu) in leaves and soil samples

Copper (Cu): Copper particulates are released into the atmosphere by windblown dust; volcanic eruptions; and anthropogenic sources, primarily copper smelters and ore processing facilities. The fate of elemental copper in water and soil is complex and influenced by pH, dissolved oxygen and the presence of oxidizing agents and chelating compounds or ions. The permissible limit of copper for plants is 10mg/kg as recommended by WHO (Zigham *et al.*, 2012). In all the collected plant samples concentration of copper was recorded to be 24 mg/kg in *Neubouldia leavis*, 32 mg/kg *chromolena odorata*, 36.5mg/kg in *chrysophyllum albidum*, 25 mg/kg in *azadirachta indica*, and 1mg/kg (*morinda lucida*). The

concentration of copper in morinda Lucida is below the permissible limit. The maximum permissible limit for Cu in soil samples is 36.5 mg/kg. Contamination of plant with high level of copper may lead to chronic anemia (Asma *et al.*, 2011). Copper accumulates in liver and brain and it is a fundamental cause of Wilson's disease (Samuel *et al.*, 2011). Concentration of copper in all the soil samples was recorded as 2.5mg/kg in *Neubouldia leavis*, 81.25 mg/kg in *chromolena odorata*, 10 mg/kg in *chrysophyllum albidum*, 13.75mg/kg in *azadirachta indica* and 53.75mg/kg in (*morinda lucida*).

NICKEL (Ni)

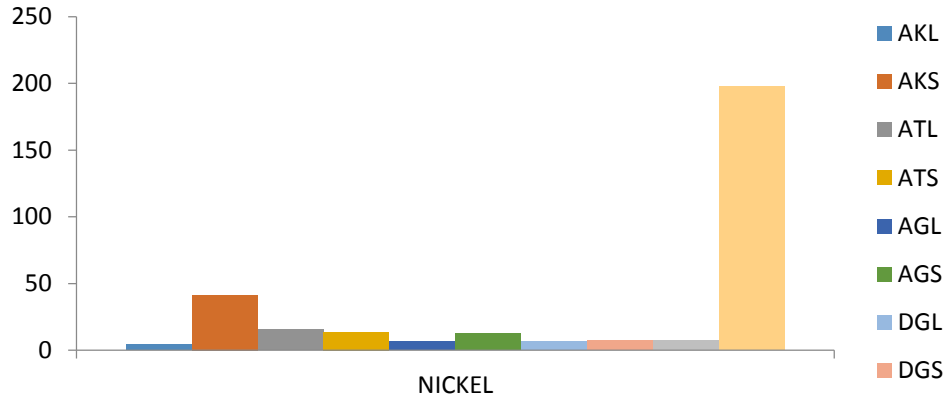

Fig 3: Concentration of Nickel (Ni) in leaves and soil samples

NICKEL (Ni): Nickel has been considered to be an essential trace element for human, plants and animals health. In small quantity, nickel is necessary for the regulation of lipid content in tissues and for the formation red blood cells. But at high level, it become toxic and causes several diseases like loss of body weight, loss of vision, and heart and liver failure, as well as skin irritation. The most common ailment arising from Ni is an allergic dermatitis known as nickel itch, which usually occurs when skin is moist, further more Ni has been identified as a suspected carcinogen and adversely affects lungs and nasal cavities. Although Ni is required in minute quantity for body as it is mostly present in the pancreas and hence plays an important role in the production of insulin. Its deficiency results in the disorder of liver (Pendias & Pendias, 1992), the permissible limit of Nickel in plants recommended by WHO is 10mg/kg. The experimental data revealed Ni to be 4mg/kg in *Neubouldia leavis*, 15mg/kg in *chromolena odorata*, 6.5mg/kg in *chrysophyllum albidum*, 6.5mg/kg in *azadirachta indica* and 7.50mg/kg in (*morinda lucida*). In all the collected plant samples there was low concentration of nickel found in all samples, except chromolena odorata which is above the permissible limit of Ni by WHO. In the collected soil samples concentration of nickel are 41.25mg/kg in *Neubouldia leavis*, 13.75mg/kg in *chromolena odorata*, 12.50mg/kg in *chrysophyllum albidum*, 7.50mg/kg in *azadirachta indica* and 197.50 in *morinda lucida*. In soil sample ORS (morinda lucida) concentration of nickel was recorded above the maximum permissible limit set by WHO.

COBALT (Co)

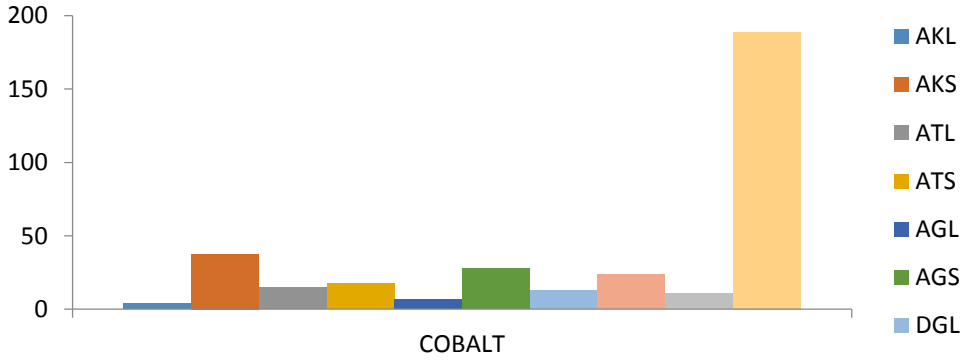

Fig 4: Concentration of Cobalt (Co) in leaves and soil samples

Cobalt: Cobalt has been long been known as essential for animal, cobalt place a role in the production of ethylene by plants. Higher level of cobalt can reduce the amount of cadmium that is up taken by plant. Concentration of 1.2mg/kg was set as the maximum permissible level in plant. In the plant samples the concentration of cobalt was recorded as 4mg/kg in *Neubouldia leavis*, 15 mg/kg in *chromolena odorata*, 6.5mg/kg in *chrysophyllum albidum*, 13mg/kg in *azadirachta indica* and 10.5mg/kg in *morinda lucida*. The concentration of leave samples were above the permissible limit, in the leaves of Neubouldia Leavis the concentration is 4mg/kg which is the lowest value compare with others. The Concentration of cadmium in all the collected soil samples ranged 37.5mg/kg in *Neubouldia leavis*, 17.5mg/kg in *chromolena odorata*, 27.5mg/kg in *chrysophyllum albidum*, 23.75mg/kg in *azadirachta indica* and 188.75mg/kg in *morinda lucida*. In all the collected soil samples, concentration of cobalt was recorded above the maximum permissible limit set by WHO.

CADMIUM (Cd)

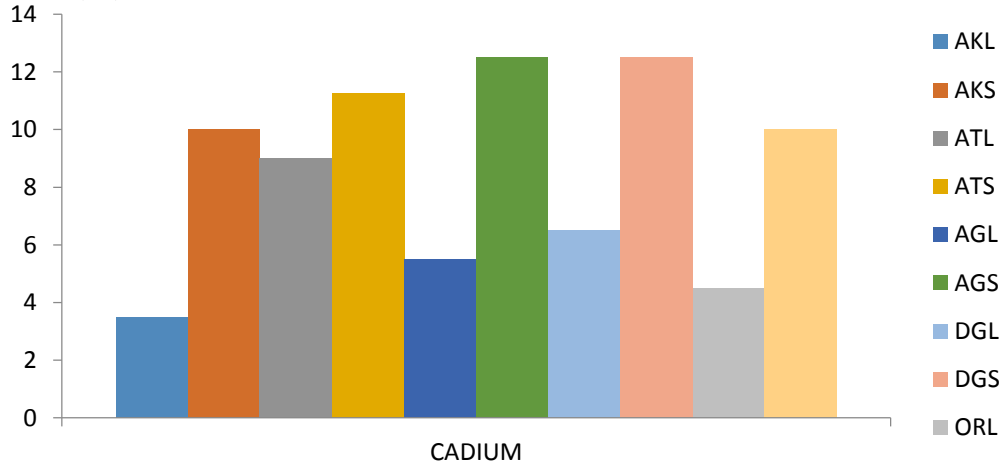

Fig 5: Concentration of Cadium (Cd) in leaves and soil samples

Cadmium (Cd): Cadmium (Cd): It is also a non-essential heavy metal. It is extremely toxic even at low concentration. It causes learning disability and hyperactivity in children. The permissible limit of cadmium in plants as recommended by WHO is 0.03 mg/kg. In the plant samples the concentration of cadmium was recorded as 3.5 mg/kg in *Neubouldia leavis*, 9 mg/kg in *chromolena odorata*, 5.5mg/kg in *chrysophyllum albidum*, 6.5mg/kg in *azadirachta indica* and 4.5mg/kg in *morinda lucida*. The concentration of leave samples were above the permissible limit, in the leaves of Neubouldia Leavis the concentration is 3.5mg/kg which is the lowest value compare with others. The Concentration of cadmium in all the collected soil samples ranged 10mg/kg in *Neubouldia leavis*, 11.25mg/kg in *chromolena odorata*, 12.50mg/kg in *chrysophyllum albidum*, 12.50mg/kg in *azadirachta indica* and 10mg/kg in *morinda lucida*. In all the collected soil samples, concentration of cadmium was recorded above the maximum permissible limit set by WHO.

LEAD (Pb)

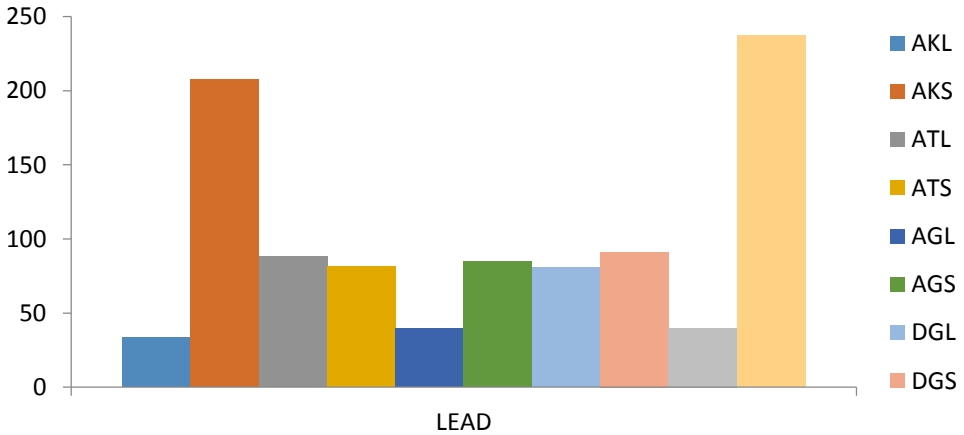

Fig 6: Concentration of Lead (Pb) in leaves and soil samples

Lead (Pb): It is a non-essential heavy metal. Pb causes oxidative stress and contributes to the pathogenesis of lead poisoning by disrupting the delicate antioxidant balance of the mammalian cells. High level accumulation of Pb in body causes anemia, colic, headache, brain damage, and central nervous system disorder (Rehman *et al*., 2013). Lead (Pb) toxicity results in decrease of hemoglobin production, disorder in the working of kidney,reproductive system, joints and cardiovascular systems and causes long-lasting injury to the central and peripheral nervous systems (Nolan, 2003). The permissible limit in plants recommended by WHO is 2mg/kg, in the leave samples the concentration of lead was recorded to be 24mg/kg in *Neubouldia leavis*, 32.5mg/kg in *chromolena odorata*, 36.5mg/kg in *chrysophyllum albidum* , 25mg/kg in *azadirachta indica* and 1.0mg/kg in *morinda lucida*. Concentration of morinda lucida is below the permissible limit while in its soil lead was recorded to be high, in the concentration of other plant samples lead was above the permissible limit of concentration set by WHO. The Concentration of lead in soil samples was recorded to be 207.5mg in *Neubouldia leavis*, 81.25mg/kg in *chromolena odorata*, 85mg/kg in *chrysophyllum albidum*, 91.25mg/kg in *azadirachta indica* , 237.5mg/kg in *morinda lucida* . In all the collected soil samples concentration of lead was recorded above the permissible limit.

CHROMIUM (Cr)

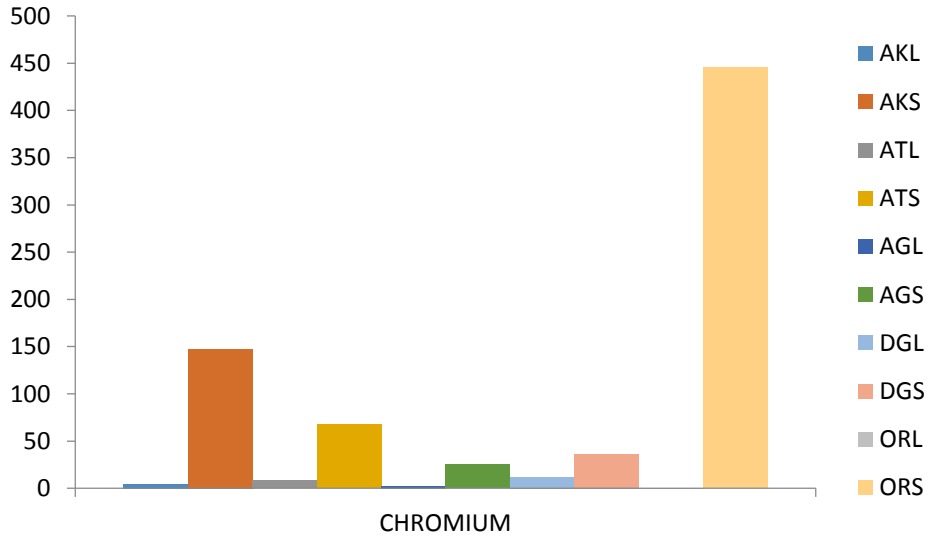

Fig 7: Concentration of Chromium (Cr) in leaves and soil samples

Chromium (Cr): It play a vital roles in the metabolism of cholesterol, fat, and glucose. Its deficiency causes hyperglycemia, elevated body fat ,and decreased sperm count, while at high concentration it is toxic and carcinogenic (Chishti *et al*., 2011). The permissible limit of Chromium for plants is 1.30mg/kg recommended by WHO. The level of Chromium concentration in plants samples were recorded to be 4.5mg/kg in *Neubouldia leavis*, 8.5mg/kg in *chromolena odorata*, 2.5mg/kg in *chrysophyllum albidum*, 12mg /kg in *azadirachta indica* and 0.50mg/kg in *morinda lucida* . Concentration of Morinda Lucida was below the permissible limit of chromium set by WHO while other samples concentration of chromium was above the permissible limit, samples concentration of chromium was recorded for the soil samples to be 147.5mg/kg in *Neubouldia leavis*, 67.5mg/kg in *chromolena odorata*, 25mg/kg in *chrysophyllum albidum*, 36.25mg/kg in *azadirachta indica* and 4.5mg/kg in *morinda lucida* .The maximum Concentration of chromium in soil samples is in morinda lucida which is leave sample is the lowest in all the collected leave samples.

MANGANESE (Mg)

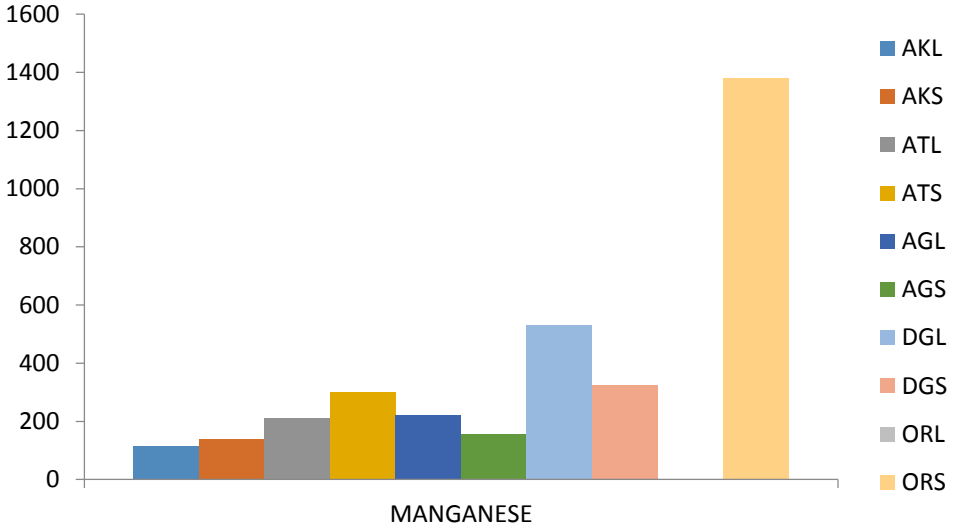

Fig 8: Concentration of Manganese (Mg) in leaves and soil samples

Manganese (Mn) determination: It is a very essential trace heavy metal for plants and animal growth. Its deficiency produces severe skeletal and reproductive abnormalities in mammals. High concentration of Mn causes hazardous effects on lungs and brains of humans (J¨arup 2003). WHO maximum permissible limit of Mn in medicinal plant is 200mg/kg, while its daily intake is 11mg. The concentration of manganese in plants was recorded to be 113.5 in mg/kg in *Neubouldia leavis*, 211mg/kg *chromolena odorata*, 221mg/kg in *chrysophyllum albidum*, 531mg/kg in *indica* and 1 mg/kg in *morinda lucida* , concentration of Manganese present in Azadirachta indica is above the permissible limit of Mn recommended by WHO while that of Morinda lucida concentration was found below the permissible limit of Mn recommended by WHO. Soil values are 137.5 mg/kg in *Neubouldia leavis*, 301.25 mg/kg in *chromolena odorata*, 156.25mg/kg in *chrysophyllum albidum*, 326.25 mg/kg in *azadirachta indica* and 1380mg/kg in *morinda lucida*. The concentration of Mn present in Morinda lucida soil is above the permissible limit of Mn recommended by WHO.

ZINC (Zn)

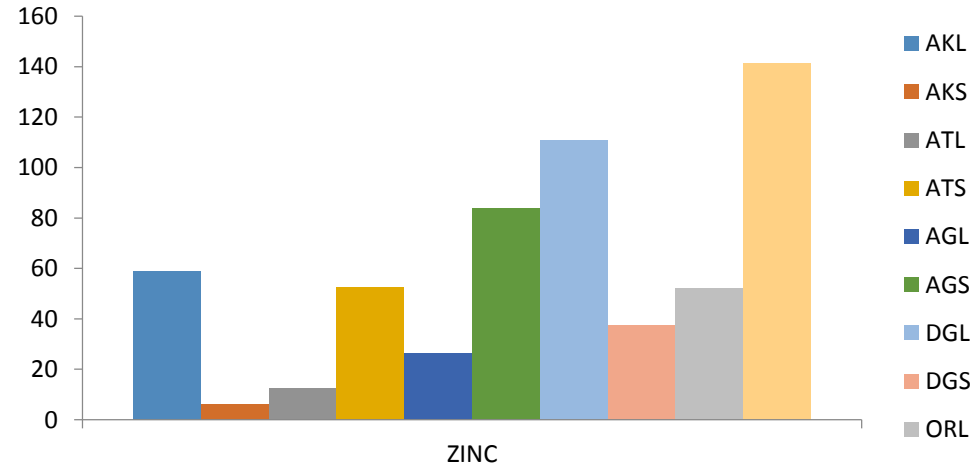

Fig 9: Concentration of Manganese (Mg) in leaves and soil samples

Zinc (Zn): It is the basic components of different enzymes and plays structural, regulatory, and catalytic functions. It also has very important role in DNA synthesis, normal growth, brain development, bone formation, and wound healing. At high level,

zinc is neurotoxin. Zinc is one of the important trace elements that play a vital role in the physiological and metabolic process of many organisms. Nevertheless, higher concentrations of zinc can be toxic to the organism. It plays an important role in protein synthesis and is a metal which shows fairly low concentration in surface water due to its restricted mobility from the place of rock weathering or from the natural source. Concentration of Zinc was determined and compare between the selected medicinal plants and soil WHO's recommended limit of Zinc in medicinal plant is 50mg/kg, while its intake in food is 11mg/day, concentration recorded to be 59mg/kg in *Neubouldia leavis*, 12.5mg/kg in *chromolena odorata*, 26.5 mg/kg in *chrysophyllum albidum*, 111mg/kg in *azadirachta indica* and 52mg/kg in *morinda lucida*, concentration of Zinc recorded in Azadirachta indica is above the permissible limit of Zinc recommended by WHO, while soil values are 6.25mg/kg in *Neubouldia leavis*, 52.5mg/kg in *chromolena odorata*, 83.75mg/kg in *chrysophyllum albidum*, 37.5 mg/kg in *azadirachta indica* and 141mg/kg in *morinda lucida*. Concentration of all soil samples meet up with the Zn permissible limit for soil recommended by WHO except morinda Lucida which is above the permissible limit.

CONCLUSION

For long periods, the medicinal plants are traditionally used by local people for treatment of various ailments, but in the process some heavy metals are ingested to human body because of the environment in which the plants are grown. The assays of heavy metals varied from soil to soil, plant to plant as carried out in this study. Thus, it is essentially required that every medicinal plant should be checked for contaminant load before processing it for further pharmaceutical purposes or for local human consumption. The main goal of this research work was to assess the concentration of some heavy metals in some selected medicinal plants and its soil. Also the collected medicinal plants were analyzed for heavy metals. A total of 5 medicinal plants and its soil samples were collected and were analyzed (AKL, ATL, AGL, DGL, ORL, AKS, ATS, AGS, DGS, ORS) and nine heavy metals (Zn, Fe, Cr, Cd, Pb, Cu, Mg, Co and Ni) using WHO standard for comparative. The results show that some of the samples were within, above and below the permissible limits set by WHO. The concentrations of these metals (Fe, Co, Cd and Ni) were found above the permissible limit in both plant and soil samples. However, the concentration of Cu (1.0 mg/kg) Pb (1.0 mg/kg) and Cr (0.5 mg/kg) was found to be below the permissible limit in *Morinda lucida plant.* Other metals found below the permissible limits include Zn (12.5 mg/kg) in the plant of *Chromolaena odorata* and (26.5mg/kg) in *Chrysophyllum albidium*. Mn was also found to be (113.5mg/kg) in *Newbouldia leaveis* and (1.0mg/kg) in *Morinda lucida plant* respectively. Hence the need to properly screen these plants before consumption for any form of ailment.

REFERENCES

Agarwal S.K., (1999). Studies on the effect of the auto exhaust emission on the Mitragynapatriflora. Ajmeer, India: MDS University; Master Thesis

Khan I. Ali J. and Tullah H. (2008). Heavy metals determination in medicinal plant Withania somnifera growing in various areas of peshawar, NWFP, Pakistan," *Journal of the Chemical Society of Pakistan*, vol. 30, no. 1, pp. 69–74, 2008.

Hay R.W., (1984) Bio-inorganic chemistry, Ellis Horwood Ltd, pp 1–397.

Obiajunwa E.I, Adebajo C.A., Omobuwajo O.R., (2002). Essential and trace element contents of some Nigerian medicinal plants. J Radioanal Nucl Ch 252:473–476.

Ivey M., and Elmen, G., (1986). Nutritional supplements, mineral and vitamin production in Handbook of nonprescription Drugs, 8th Ed. American Pharmaceutical Association, The National Professional Society of Pharmacists, Washington DC pp 1–215.

Afzal S., Abdul N., Nazeef U., Ali R. , Muhammad A., Muhammad Z., and Muhammad S., (2013). Comparative Study of Heavy Metals in Soil and Selected Medicinal Plants Journal of Chemistry, Article ID 621265, http://dx.doi.org/10.1155/2013/621265

Rehman A., Ullah H., Khan R.U., and Ahmad I.,(2013) Population based study of heavy metals in medicinal plant Capparis decidua, *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 5, no. 1, pp. 108–113,.

Chishti K. A., Khan F. A., Hassan, S. M..,(2011) Estimation of heavy metals in the seeds of blue and whitecapitulum's of silybum marianum grown in various districts of Pakistan, *Journal of Basic and Applied Science*, vol. 7, no. 1, pp. 45–49,

Pendias A.K., and Pendias K., (1992). Trace Elements in Soils and Plants. 2nd ed. Boca Raton F1: CRC Press, p. 365.

J¨arup L., (2003). Hazards of heavy metal contamination,*British Medical Bulletin*, vol. 68, pp. 167–182,.

Suruchi and Khanna P., (2011). Assessment of heavy metal contamination in different vegetables grown in and around urban areas. Research Journal of Environmental Toxicology 5:162–179.

Nolan K., (2003). Copper toxicity syndrome. Journal of Orthomolecular Psychiatry 12: 270-282.