Profile of Heavy Metals in medicinal plants collected from different areas of Karak, Khyber Pakhtunkhwa, Pakistan

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Abstract: Medicinal plants have got important hold up in the current time for their curative potentials. The present study deals with the evolution of the elemental analysis of ten medicinal plants namely *Arabidopsis arenosa, Cistanche tubulosa, Nonea micrantha, Ornithopus compressus, Orobanche ramosa, Ifloga spicata, Ballota pseudodicatmnus, Cryptantha crassisepala, Farsetia aegyptia* and *Heliotropium bacciferum.* All these plants were collected from different areas of District Karak. Out of the nine heavy metals analyzed, Iron, Zinc, Cobalt, Copper and Manganese are considered as essential for human health and the remaining four elements including Chromium, Nickel, Cadmium and Lead are considered non essential. The heavy metals were analyzed using Flame Atomic Absorption Spectrophotometer (FAAS). The highest concentrations of Fe, 161.55 mg/kg in *Cryptantha crassisepala,* Zn, 52.07 mg/kg in *Cistanche tubulosa,* Co, 28.35 mg/kg in *Orobanche ramosa,* Cu, 35.37 mg/kg in *Ifloga spicata,* Mn, 36.75 mg/kg in *Orobanche ramose,* Cr, 1.00 mg/kg in *Ornithopus compressus,* Ni, 7.05 mg/kg in *Heliotropium bacciferum,* Cd 1.12 mg/kg in *Ballota pseudodicatmnus* and Pb, 19.81 mg/kg in *Cryptantha crassisepala* were noted

[Nasser M. Abd EI-Salam, Shabir Ahmad, Waheed Murad, Tahir Iqbal, Laiq Zuman, Ahteram Bibi, Ali Rehman, Riaz Ullah, Zia Mohammad, Anwar Ali Shad. **Profile of Heavy Metals in medicinal plants collected from different areas of Karak, Khyber Pakhtunkhwa, Pakistan**. *Life Sci J* 2013;10(7s):914-921] (ISSN:1097-8135). http://www.lifesciencesite.com. 146

Keywords: Heavy metals, Medicinal plants, Atomic Absorption Spectrophotometer

Introduction

In most of the developing countries, approximately 70–80% of the population relies on herbal drugs. [1] *Orobanche ramosa* (Hemp broomrape) which live as a parasite on other plants is used for treating whooping cough and increases fatness of milk in cattles. [2] *Arabidopsis arenosa* (Sand rock crees) is used for treating asthma, throat and chest complaints. [3] *Cistanche tubulosa* (Desert hyacinth) is used to cure whooping cough and jaundice. [4] *Nonea micrantha, Ornithopus compressus, Ifloga spicata* and *Cryptantha crassisepala* are of fodder value they act as galactagogues. [5] *Farsetia aegyptia* is also of fodder value for cattles. [6] *Ballota pseudodicatmnus* is analgesic and posses wound healing, antioxidant and antimicrobial properties. [7] *Heliotropium bacciferum* is cytotoxic and causes poisoning to live stock. [8]

Despite of being confident on the use of various traditional medicines, there is a lack of scientific research on safety and efficacy. The herbal plants are contaminated with heavy metals which may cause dangerous effect on human health. [9] Due to high importance of herbal drugs, it is crucial for researchers to aware people about the safety of traditional medical drugs. There are few research groups published papers regarding the usefulness of herbal drug, but certain things are uncertain for example negative or dangerous effects can appear by the inappropriate use of certain medicinal plant. [10] The main problem associated with phytotherapy is the contamination of plant by heavy metal. Usually polluted irrigation water, soil and unsuitable storage condition contaminated the plants with heavy metals. Large quantity of research has been done on phytotherapeutic properties but scant attention has been given to analytical methodology to determine heavy metals in medicinal plant. [11] Due to the poisonous nature of some heavy metals present in herbal plants, it is of great importance to know their concentration in the particular specie.

Heavy metals are not harmful when they are present in appropriate concentration but unsuitable concentration of heavy metals may negatively affect living organisms. For example Iron is an important

element it helps in the oxidation of carbohydrates, fats and proteins but beyond it's allowed upper or lower limit it may cause metabolic disturbance such as Iron (Fe) deficiency is associated with myocardial infraction. People having increased amount of iron have greater tendency to bacterial infections. [12, 13]

Zn is important in wound healing, numerous body function and also act as an antioxidant. Various diseases such as mental lethargy and emotional disorder may be linked with the unbalanced use of Zn. [13] It is an important component of various plant enzymes such as proteinase, phospohydrolases, proteinases, and dehydrogenase and hence play a vital role in the metabolic process of plants. [14] Nickel is another heavy metal essential for human body in small quantity as it plays an important role in the production of insulin, and its deficiency in body adversely affects the liver. On the other side Ni has been recognized as carcinogen and badly affects lungs and nasal cavity. The most common disease related to Ni is nickel itch, which is commonly happen on moist skin. According to environmental protection agency (EPA) the human body daily required less than 1 mg of Ni. [15] Copper is also required for the perfect human body functioning in specific quantity and its deficiency or excees can cause a syndrome of anemia and central nervous system. The inappropriate intake of Copper can also cause hyperactivity and hypertension. [13] The human body did not require lead and cadmium at all and its intake in very small concentration adversely effects various part of the body. [16]

MATERIALS AND METHODS

Sample Collection

Samples of different plants namely *Arabidopsis arenosa*, *Cistanche tubulosa, Nonea micrantha, Ornithopus compressus, Orobanche ramosa, Ifloga spicata, Ballota pseudodicatmnus, Cryptantha crassisepala, Farsetia aegyptia* and *Heliotropium bacciferum* were collected from different areas of Karak, Khyber Pakhtunkhwa during appropriate season and were identified by Dr. Waheed Murad, Department of Botany, Kohat University of Science & Technology Kohat, Pakistan. Aerial parts of the plants were analyzed for heavy metals. The samples were washed thoroughly with tape water to eliminate dust, dirt, and other possible parasites and then were properly rinsed with deionized water. The rinsed plants were dried under shade at room temperature (25^0C). The dried samples were powdered by mortar and pestle and then stored in clean, dried plastic bottles for further processing.

Analysis of Plant Samples

Weighed quantity of powdered plant samples were taken in China dishes for heating in an oven at 110^0C for several hours to remove moisture. Then the moister free samples were placed in furnace. The furnace temperature was gradually increased from room temperature to 550^0C in 1 hr. The samples were ashed for about 5 hr until a grey or white ash residue was obtained from each sample. All the china dishes and their contents were cooled to room temperature (25^0C) in desiccators and 2.5 mL of 6 M HNO_3 solution was added into each China dish. The solutions were filtered through whatman (#42) filter paper into 25 mL flask and were diluted to the mark. The solutions were then stored in clean and dry plastic bottles [5]. The solutions were analyzed for the elements of interest like Fe, Ni, Zn, Co, Cu, Mn, Cr, Cd and Pb utilizing Flame Atomic Absorption Spectrophotometer (Parkin elmer 400) with suitable hollow cathode lamps.

Calibration of Instrument

For the elements under investigation, the following sensitivity and detection limits respectively were established for the calibration of FAAS apparatus.

Fe 0.5 and 8 ppm, Zn 0.5 and 5.0 ppm, Ni 0.5 and 4.0 ppm, Co 1.0 and 5.0 ppm , Cu 0.5 and 3.0 ppm, Mn 0.5 and 4.0 ppm, Pb 0.5 and 5.0 ppm, Cr 0.5 and 3.0 ppm, Cd 0.2 and 2.0 ppm,

Results and Discussion

Due to high importance of medicinal plants, all over the world the researchers have great concern to aware public about the permissible limits of certain heavy metals in these species. The problem is rather more serious in Pakistan, because medicinal plants which are used for curative purposes are not properly regulated by quality assurance parameters. The selected medicinal plants under investigation namely *Arabidopsis arenosa, Cistanche tubulosa, Nonea micrantha, Ornithopus compressus, Orobanche ramosa, Ifloga spicata, Ballota pseudodicatmnus, Cryptantha crassisepala, Farsetia aegyptia* and *Heliotropium bacciferum* were analyzed for heavy metals load. In this respect concentration levels of Mn, Zn Co, Cu, Fe, Pb, Ni, Cd and Cr in units of mg/kg have been determined. The results of our investigation are being presented by table 2 and also have been shown through figures 1-8. In general the concentration level of heavy metals in the selected plants was found to decrease in the order of Fe > Mn > Cu > Zn > Co >Pb > Ni> Cd > Cr.

Table-2. Concentration of Heavy metals (mg/kg) in different Medicinal Plants

S. No	Plant Name	Fe	Zn	Ni	Co	Cu	Mn	Cd	Cr	Pb
1	*Arabidopsis arenosa*	35.73±0.91	18.57±1.01	4.77±0.45	3.22±0.22	19.8±1.00	6.48±0.56	0.65±0.04	0.50±0.11	14.93±0.91
2	*Cistanche tubulosa*	63.45±0.85	52.07±1.23	3.04±0.32	2.19±0.19	32.43±1.23	28.97±1.23	0.83±0.09	0.31±0.09	11.72±0.88
3	*Nonea micrantha*	43.05±0.83	26.70±1.16	1.92±0.21	2.96±0.34	32.52±1.45	28.20±1.34	0.43±0.01	0.12±0.04	15.01±0.85
4	*Ornithopus compressus*	48.45±0.80	18.95±1.30	2.67±0.20	16.70±1.00	27.86±1.2	30.26±1.67	1.08±0.12	1.00±0.21	15.28±0.52
5	*Orobanche ramosa*	82.2±0.76	26.85±1.62	3.21±0.50	28.35±1.90	29.85±1.21	36.75±1.23	1.11±0.02	0.92±0.13	10.12±0.67
6	*Ifloga spicata*	92.68±0.98	26.41±1.90	3.47±0.51	6.95±1.31	35.37±1.29	20.41±1.22	0.80±0.13	0.38±0.10	13.97±0.39
7	*Ballota pseudodicatmnus*	65.40±1.04	29.85±1.80	6.22±0.34	13.13±1.29	14.80±1.00	21.84±1.20	1.12±0.01	0.46±0.02	15.61±0.92
8	*Cryptantha crassisepala*	161.55±1.78	30.45±1.56	1.06±0.12	7.74±1.23	15.01±1.11	19.93±1.00	0.54±0.03	0.98±0.09	19.81±0.78
9	*Farsetia aegyptia*	125.66±1.34	32.85±1.34	1.50±0.11	4.46±0.8	25.96±1.29	30.67±1.45	1.00±0.08	0.56±0.12	19.80±0.86
10	*Heliotropium bacciferum*	156.00±1.89	23.25±1.48	7.05±1.20	14.80±1.30	32.46±1.78	30.00±1.21	0.48±0.20	0.44±0.04	14.78±0.96

± = standard Deviation

Iron

The Figure 1 shows that the highest Iron concentration 161.55 mg/kg, was recorded in *Cryptantha crassisepala* followed by *Heliotropium bacciferum* 156.00 mg/kg and *Farsetia aegyptica* 125.66 mg/kg while the lowest concentration was found in *A. arenosa* 35.73 mg/kg. The permissible limit of iron in food is generally 30-150 mg/kg. [17]

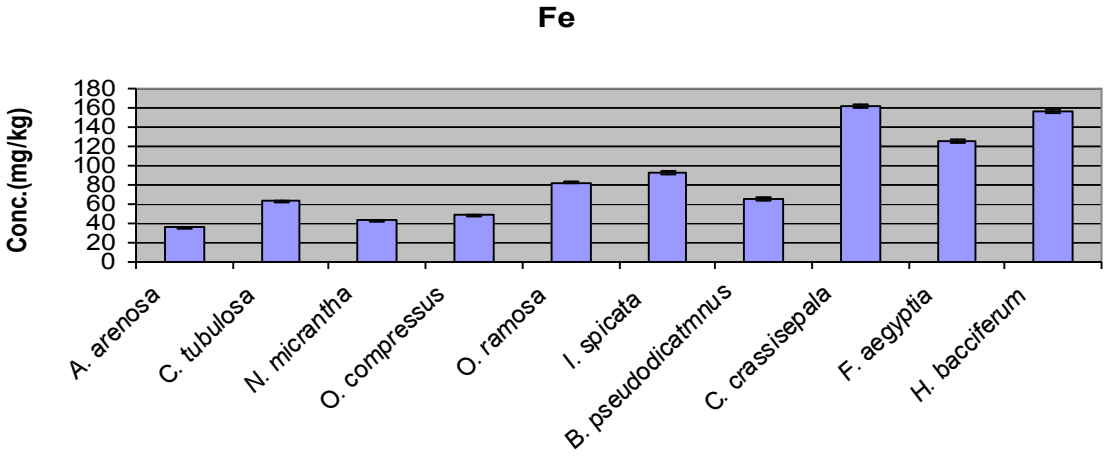

Figure 1. Comparative concentration of Iron in different medicinal plants

Zinc

It is clear from the figure 2 that Zinc ranges from 18.57- 52.07 mg/kg. Highest concentration was found in *Cistanche tubulosa* 52.07 mg/kg followed by *Farsetia aegyptia* 32.85mg/kg. Nearly same concentration of zinc 18.57 and 18.95mg/kg was found in *Arabidopsis arenosa* and *Ornithopus compressus* respectively, similarly *Nonea micrantha, Orobanche ramosa* and *Ifloga spicata* contains nearly the same concentration while lowest concentration 18.57 mg/kg was recorded in *Arabidopsis arenosa.* The dietary limit of Zn is 100 mg/day. [15]

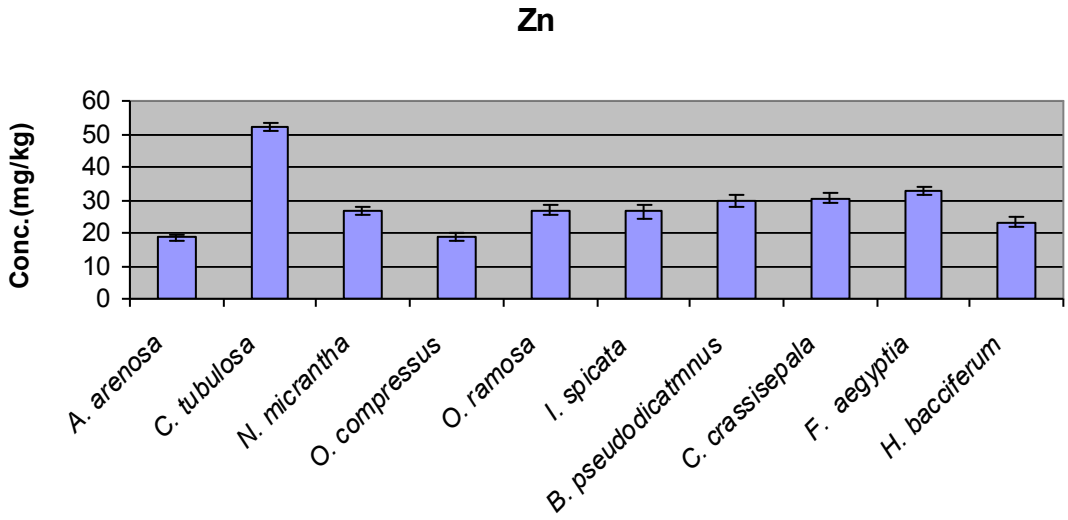

Figure 2. Comparative concentration of Zinc in different medicinal plants

Nickel

In case of nickel the concentration range in different analyzed plant samples was 1.06-7.05 mg/kg and it was in the tune of *Heliotropium bacciferum 7.05 mg/kg, Ballota pseudodicatmnus* 6.22 mg/kg, *Arabidopsis arenosa* 4.77 mg/kg, *Ifloga spicata* 3.47 mg/kg, *Orobanche ramosa* 3.21 mg/kg, *Cistanche tubulosa* 3.04 mg/kg, *Ornithopus compressus* 2.67 mg/kg, *Nonea micrantha* 1.92 mg/kg, *Farsetia aegyptia* 1.50 mg/kg and *Cryptantha crassisepala* 1.06 mg/kg.

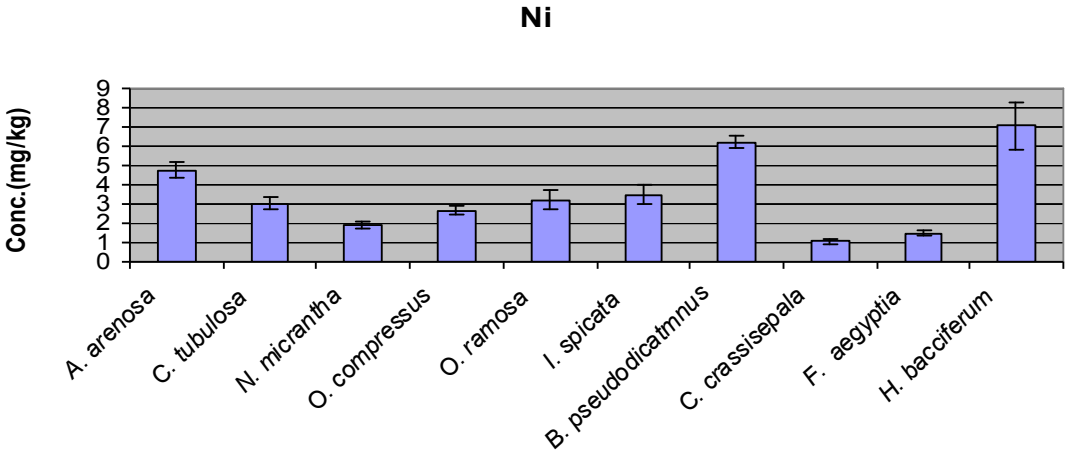

Figure 3. Comparative concentration of Nickel in different medicinal plants

Cobalt

The concentration range of Cobalt in different plant samples analysed was 2.19-28.35 mg/kg. Highest concentration of Cobalt 28.35 mg/kg was noted in *Orobanche ramosa*. While its lowest concentration 2.19 mg/kg was noted in case of *Cistanche tubulosa*. The concentrations values of other plant samples are shown in table # 2.

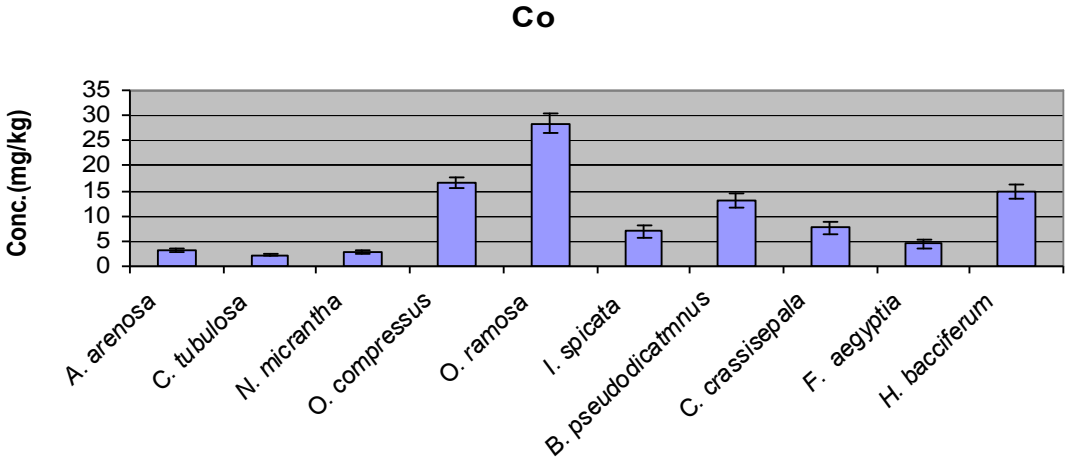

Figure 4. Comparative concentration of Cobalt in different medicinal plants

Copper

As evident from table -2 and figure -5, Highest concentration of copper was found in *Ifloga spicata* 35.37 mg/kg while its concentration was lowest in *Ballota pseudodicatmnus* 14.80 mg/kg. Nearly same concentration 32.43mg/kg , 32.52mg/kg and 32.46 mg/kg was recorded in *Cistanche tubulosa, Nonea micrantha and Heliotropium bacciferum* respectively. The permissible limit of 200 ppm [18].

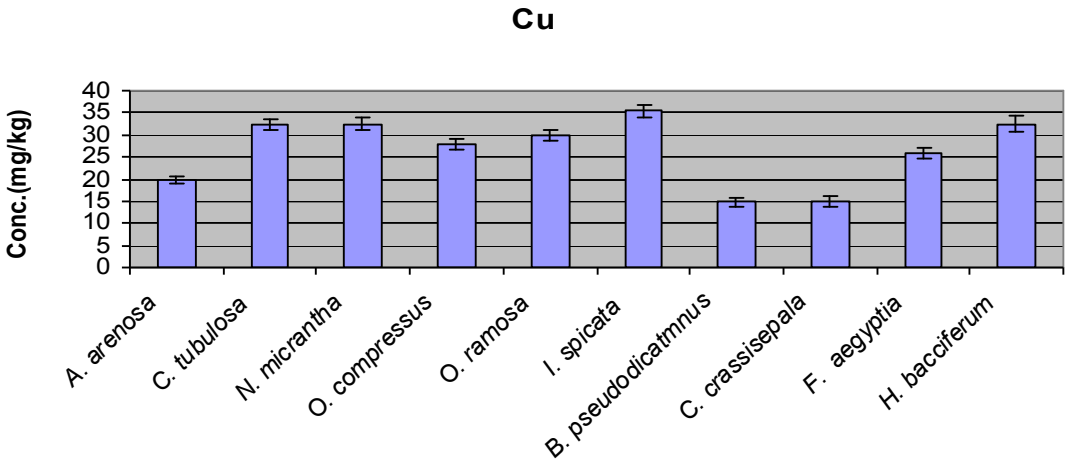

Figure 5. Comparative concentration of Copper in different medicinal plants

Manganese

The mean values for Manganese have been presented in table -2 and also have been illustrated by figure-6. The concentration of manganese ranges from 6.48-36.75 mg/kg, in which high concentration was found in *Orobanche ramosa* 36.75mg/kg, while low content of manganese 6.48 mg/kg was found in *Arabidopsis arenosa*. The difference of manganese in *Cistanche tubulosa* 28.97 mg/kg and *Nonea micrantha* 28.20 mg/kg was non-significant. The results further substantiated that variation in plant samples for Mn in *Ornithopus compressus* 30.26 mg/kg, *Farsetia aegyptia* 30.67 and *Heliotropium bacciferum* 30.00 was non-significant. The estimated safe andadequate daily dietary intake in adults is 11 mg/day. [19]

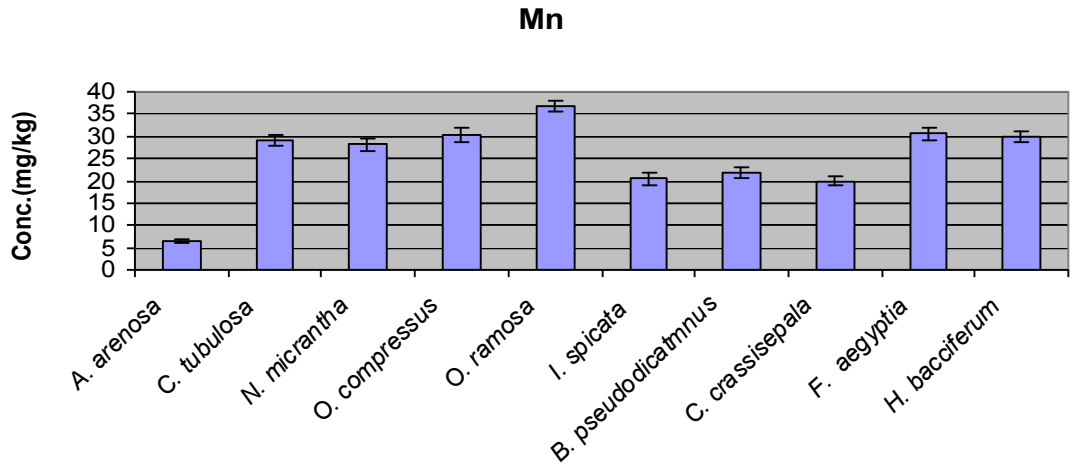

Figure 6. Comparative concentration of Manganese in different medicinal plants

Chromium

The concentration of Chromium was the highest 1.00 mg/kg in case of *Ornithopus compressus*. It was 0.56 mg/kg in *Farsetia aegyptia,* 0.44 mg/kg in *Heliotropium bacciferum,* 0.50 mg/kg in *Arabidopsis arenosa,* 0.92 mg/kg in *Orobanche ramosa* 0.46 mg/kg in *Ballota pseudodicatmnus,* 0.98 mg/kg in *Cryptantha crassisepala,* 0.31 mg/kg in *Cistanche tubulosa,* 0.38 mg/kg in *Ifloga spicata* and the lowest concentration of Cr 0.12 mg/kg was noted in case of *Nonea micrantha.* The permissible for Cr in food is generally 0.5 ppm. [17]

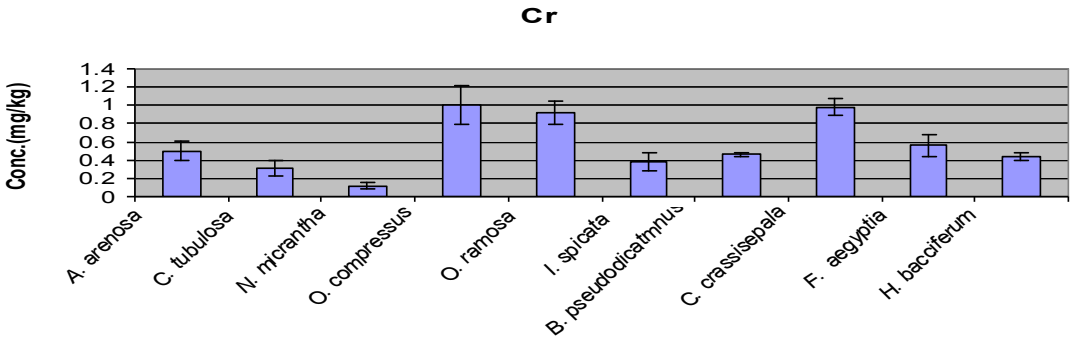

Figure 7. Comparative concentration of Chromium in different medicinal plants

Cadmium

The concentration range of Cadmium in different plant samples was 0.43-1.12 and was the highest 1.12 mg/kg in case of *Ballota pseudodicatmnus.* It was 1.08 mg/kg in *Ornithopus compressus*, 1.00 mg/kg in *Farsetia aegyptia,* 0.48 mg/kg in *Heliotropium bacciferum,* 0.65 mg/kg in *Arabidopsis arenosa,* 1.11 mg/kg in *Orobanche ramosa,* 0.54 mg/kg in *Cryptantha crassisepala,* 0.83 mg/kg in *Cistanche tubulosa,* 0.80 mg/kg in *Ifloga spicata* and the lowest concentration of Cd 0.43 mg/kg was noted in case of *Nonea micrantha.* The permissible limit for cadmium in liver has been reported as 0.5 ppm. [20]

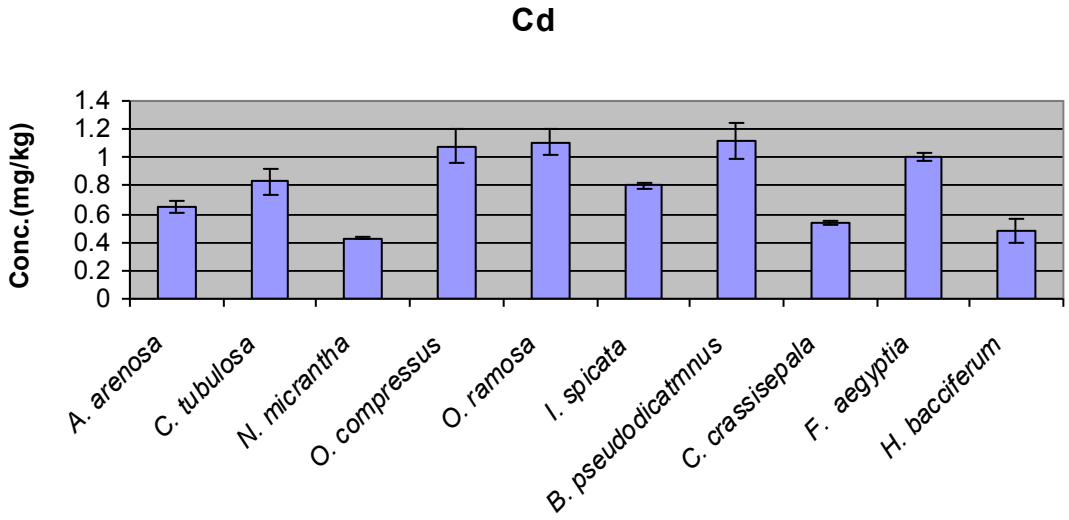

Figure 8. Comparative concentration of Cadmium in different medicinal plants

Lead

The concentration values of Pb for different plant samples are given in Table # 2. As clear from the graph below Lead content was the highest in *Cryptantha crassisepala.* The difference of Mn content for *Cryptantha crassisepala* and *Farsetia agyptia* was non-significant. Pb concentration was the lowest 10.12 mg/kg in case of *Orobanche ramosa.* WHO (1998) prescribed limit for Pb contents in herbal medicine is 10 ppm while the dietary intake limit for Pb is 3 mg/week. [21]

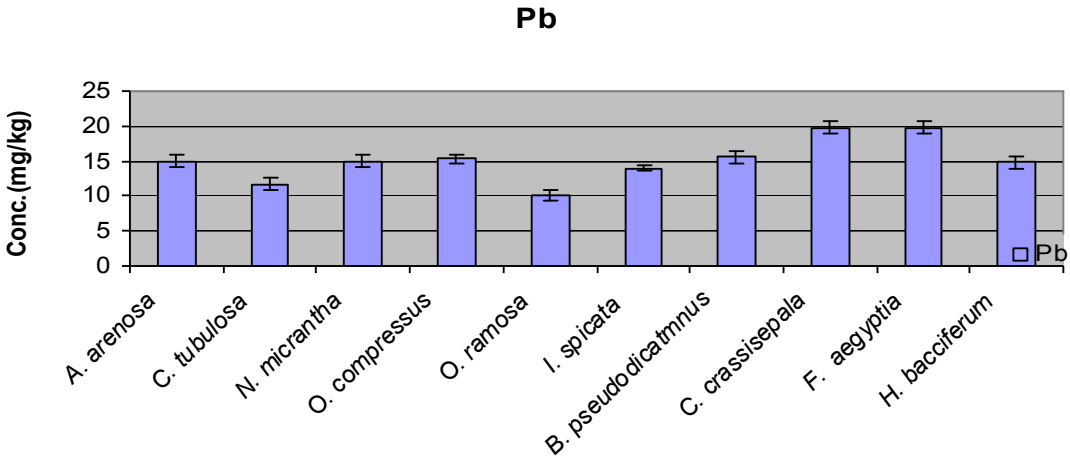

Figure 9. Comparative concentration of Lead in different medicinal plants

Acknowledgements:

This research was supported by King Saud University, Deanship of Scientific Research and College of Science Research center.

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5/22/2013