Pak. J. Bot., 47(4): 1511-1516, 2015.

A STUDY OF SOME HEAVY METALS FOUND IN MEDICINAL PLANTS (*EUPHORBIA CORNIGERA, RHAZYA STRICTA AND CITRULLUS COLOCYNTHIS*) IN TURBAT REGION OF BALOCHISTAN WITH REFERENCE TO PREVENTION OF ENVIRONMENTAL POLLUTION

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Abstract

The aim of this paper is to present the accumulation content of toxic heavy metals such as Cd, Zn, Cu, Ni and Pb in the shoots of three plants species (*Euphorbia cornigera*, *Rhazya stricta* and *Citrullus colocynthis*) collected from Turbat (Balochistan). These therapeutic plants play an important role in the traditional medication system that has been existed for several years and continuously provide new and novel remedies to the human beings. Moreover, these medicinal plants are used throughout the world for the purpose of treatment of many diseases and also a significant source of raw material for pharmaceutical and agricultural industries. Further, the various concentrations of heavy metals in medicinal plants was recorded as Pb > Zn > Cd > Cu > Ni. In the present research the results were related with the safety standards recommended by the World Health Organization (WHO).

Key words: Medicinal plants, Heavy metals, Metal toxicity and atomic absorption spectroscopy.

Introduction

Pakistan largest province is Balochistan covering 43.6% of the total land. The climate of Balochistan is the largest province representing 43.6 percent of the land mass is arid to semi-arid area, start cool temperate area in North extent in tropical costal area.

The six ecological zones are found in Balochistan ranging from tropical dry desert, dry temperate forest, Desert and mangroves Sub Tropical Forest ,Alpine Pasture tropical Dry Mixed Deciduous Forest, annual rain fall in Zob 350mm, in Makran Coastal area f 100mm ranging from100-350 mm Annually (Anon., 2011)

In Balochistan, precious wild medicinal plants grow, abundantly, local community collect and sold in local and international markets (Qasim *et al*., 2010). For checking their demands and utilization and also vulnerability of medicinal herb, scientist give special attention and take step to explore, to test their efficacy against microbes and preserve these species (Shinwari & Qaiser, 2011). In Balochistan desert vinyl plant *Citrullus colocynthis* is usually grows in sandy uncultivated soils. It usually grows and spreading over along the coast of Makran and Turbat, and also grow on Mediterranean region of Balochistan. *Citrullus colocynthis* grow from above sea level 1500 m.a.s.l. Annual rain fall reached 250-100 and tolerate temperature reached 14.8 to 27.8°C on costal area and plants easily grow with pH between 5.0-7.8. *Citrullus colocynthis* is known as Gonage in local language (Fig. 1).

Euphorbia cornigera (**horned spurge**) of the family Euphorbiaceae is native to Pakistan. It is a perennial herbaceous rhizome, growing to 75cm (30 in) tall, rarely 1m (3 ft 3 in), having narrow green leaves with a green spine. In summer they give flower head (Cyathi). All parts of the plant contained toxic compounds, if it ingested and the sap may cause skin irritation. *Euphorbia cornigera* is known as Lanto in local language (Fig. 2).

Family Euphorbiaceae has a largest Genus *Euphorbia cornigera* consist of 2,000 known species (Jassbi, 2006). *Euphorbia* distributed both hemisphere ranging from small herbaceous plants and large succulent tree in desert. Researchers used roots, seeds, leaves, stem bark, stem wood, latex. *Euphorbia* has therapeutic importance but they also display toxicity against cell lines (Hohmann & Molnár, 2004). *Euphorbia* species show antitumor activity due to presence of derivative of abietane diterpene, which contain lactone reported to possess strong anti-neoplastic activity towards many different cell lines (Yan *et al.* 2008; Liu *et al.*, 2002; Luo & Wang, 2006; Duarte *et al.*, 2008; Kigoshi *et al.*, 2001). *Euphorbia* spp. also effective for curing of intestinal parasites, migraines, gonorrhea, have been also utilized as of skin diseases, warts and for mediating pain perception (Shi *et al.*, 2008; Singla & Pathak, 1990; Appendino & Szallasi, 1997).

Some earlier workers have indicated that *Euphorbia* spp. also have anti-proliferative activity (Xu *et al*., 1998), cytotoxicity (Fatope *et al*., 1996), antimicrobial activity (Murugan *et al*., 2007), antipyretic-analgesic activity (Liu *et al*., 1997), inhibitory action on the mammalian mitochondrial respiratory chain (Betancur *et al*., 2003), inhibition of HIV-1 viral infection (Hezareh, 2005, etc.

Rhazya stricta is a poisonous plant distributed all over deteriorated rangelands in the Turbat area. *R. stricta* is an evergreen dawaf shrubs belongs to family Apocyanaceae, there are no report of live stock keepers, heavy grazing puts the *R. stricta* in a competitive advantage over valuable range plants. So, rangelands sometimes *R. stricta* forms pure stand vegetation (Gilani *et al*., 2007a). Plants have pharmaceutical importance and have also capability to act as potential pesticides, Plants anti microbial activity also evaluated against pathogen. Earlier researchers have to be yielded valuable information towards management of rangelands infested with *R. stricta*. *Rhazya stricta* also known as Ashrik in local language Fig. 3.

Fig. 1. *Citrullus colocynthis*.

Fig. 2. *Euphorbia cornigera*.

Fig. 3. *Rhazya stricta*.

Rhazya stricta Decne belongs to family Apocynaceae, is also common in the Peninsula of Arabia, which is use in local medicine to cure, certain inflammatory conditions, helminthiasis, mellitus and diabetes. Leaves of *R. stricta* contain alkaloids with tetrahydrosecamine, rhaziminine and ab-carboline nucleus akuammidine (Jassbi, 2006).

Heavy metals environmental pollution is global disaster/epidemic which is connected to human actions and functions such as mining, intensive agriculture, power transmission, smelting, sludge dumping, electroplating, energy and fuel production and melting operations (Igwe & Abia, 2006; Malik *et al*., 2012). Heavy metals have produce serious toxic effects on environmental pollutants as discussed Chehregani *et al.* (2005) and Sawidis (2008). As the wastewater volume is increasing day-to-day over population and spreading of industrialization and the global volume of wastewater is more than 1,500 km^3 per year (Anon., 2007). The pollution ratio of heavy metals from soil transfer to forage is highly toxic for grazing ruminants during any time of the year as described Asia *et al.* (2011) and Khadijeh *et al.* (2011), heavy metals Pb, Cr, Hg, Cu, Cd, Zn and Ni cause hazardous, various researchers try to adopt various method removing heavy metals from soil, such as phytoremediation previous studies Antonkiewicz & Jasiewicz (2002) and Horsfall & Spiff (2005). Phytoremediation is promising method for reclamation of toxic soil contaminated by heavy metals, has recently caught attention and become a subject of public and scientists as earlier studied (Baker *et al*., 2000; La´zaro *et al*., 2006). Many scientist play key roll to investigate a method to wash heavy metals, which absorbed by root and deposit at their marix. The mentioned plants are considered very convenient for the sustainability of soil reference to stabilization and extraction on the regard of the pollution of heavy metals (Madejon *et al*., 2002). Hyperaccumulator species of plants may play an important role in the fate of the matrixes of contaminated pollutants through their root system. After the introduction of metals hyperaccumulators it has been proved that plants have potentiality of the deposition of extraordinary high heavy metal concentrations which substantiates that many plants have genetic capability to remove pollution of soil by contamination due to said properties of metals reference to plants these plants are used as medicinal plants too.

The selecting strains of plants have capability to resist against heavy metals and have ability to adsorb high amounts of them, would certainly facilitate recovery of contaminated lands as studied by Bizly *et al*. (2000), Zulfqar *et al*. (2012) and Lasat (2002). Thus, the process of phytoremediation is very safe friendly environment, inexpensive and can be carried out in polluted places (remediation in situ), plants are in one site control heavy metal while on other site they have medicinal properties too plus the product of decomposition does not require further utilization.

As the utilizing of plant species can minimize and remediate polluted soil with heavy metals is nonexistence in Pakistan (Zulfqar *et al*., 2012). In this study 03 plant species were investigated for metals such as Cd, Zn, Cu, Ni and Pb from the area of Turbat in region of Balochistan in order to evaluate the total metal accumulation in the plant parts in connection of friendly environment. Many species of plants that have the great effectiveness for phytoremediation against different heavy metals through phytoextraction and phytostabilization process and this work will be base line data for other new young researchers in future.

Table 1. pH of plant samples Species .

	Citrullus colocynthis	*Euphorbia cornigera*	Rhazya stricta
pH of species	6.9 ± 0.06	7.3 ± 0.07	7.3 ± 0.07
%	88.6	92.5	93.2

Table 2. The elemental mean values mg Kg^{-1} in shoots of three samples plant species.

Plant species	Cd	Zn	Cu	Ni	Pb
Citrullus colocynthis	4.78	79.8	68.7	2.71	9.34
Euphorbia cornigera	29.9	0.06	1.04	0.02	30.02
Rhazya stricta	18.29	41.8	78.9	3.13	28.63

Experimental: The samples of plants species were collected from the mostly common/dominant species at the area of Turbat Balochistan. Three plant species such as *E. cornigera, C. colocynthis* and *R. stricta* were collected in November and December in 2013. A total of 8-10 plants including shoots of each species were collected from each site and mixed to form a composite sample, placed in labeled bags and transported to our Laboratory for further analysis. Before analysis, from each plant stems and leaves were carefully removed and washed (for 2-3 minutes approximately) with tap water then washed with deionized water to eliminate any surface dust and soil particles.

Plant species were chosen after taking into account their abundant existence in the field, which shows that that the plants tolerance at various concentrations of heavy metals.The pH and heavy metals viz., Cd, Zn, Cu, Ni and Pb were measured as given in Table 1. Plant species pH was determined using portable pH meter (Instruments, Padova, Italy) in a 1: 2.5 plant species extracted solution suspension. Subsequently, the plant fractions (shoots), were washed very carefully with deionized water. After drying for 48 h at 105^{o}C in air force oven the dry biomass of the plant fractions were measured.

Heavy metals determination: The plant species analysis procedures used in the present study have been described in more detail elsewhere Westerman (Westerman, 1990). Total amount of heavy metals of Cd, Zn, Cu, Ni and Pb in topsoil and in the plant tissues were found by an Inductively Coupled Plasma Emission Spectroscopy (ICP ICP-AES (Perkin Elmer, 4300 DV) of a more comprehensive range of 12-20 elements. During analytical Procedure by ICP, the standard solutions were used for the purpose of accuracy. The levels of heavy metals were determined according to detailed procedure as discussed by Sawidis (2008). Further, volumetric 100 ml Pyrex conical flasks for the samples plant species (shoots) solutions were used after drying of the plants samples at 105^{o}C for 24 h and washing by acid. All the samples were digested by acid in microwave oven (CHEM, MARSX press, USA), in light of EPA procedure / method 3051 (Anon., 1995a). After the digestion the samples were filtered by (0.45mm PTEF) and reference to the requirement of the Atomic Absorption, the concerned solutions were diluted. The treatment of the plant samples with acid was in light of EPA procedure/ method 3052 (Anon., 1995b). And then the samples were analyzed by an ICP-OES. Five replicates were taken and each determination of the samples level of heavy metals for the average value. For analytical accuracy, the relative standard Deviation (RSD) method was followed in regard of 5-10% and the calculations were made by standard deviation divided by mean. All chemicals and reagents used were from Sigma/ Fluka/Merck as analytical grade. Similarly, the glassware used were washed by distilled and water and acid as per requirement of laboratories procedure.

Results and Discussion

Heavy metals accumulation in tested plants: Table 2 indicates the levels of the heavy metals determined in the some parts (shoots and roots) of the plants of *C. colocynthis, E. cornigera* and *R. stricta* the study region of Turbat in Balochistan

Cadmium: Significant differences among the studied plants in Cd concentrations and the pattern of Cd concentrations in studied plants were: *C. colocynthis, E. cornigera* and *R. stricta* reveal important effect of plant type on Bio-Accumulation Coefficient (BAC) and Biological Concentration Factor (BCF) of the concerned plant. The shoots of most studied herbs showed higher values of Cd and Pb oncentrations in following order *Euphorbia cornigera* > *Rhazya stricta*> *Citrullus colocynthis* as observed and the concentration of heavy metals were important from the perception of phytoextraction (Ibrahim *et al*., 2013).

In case of *Euphorbia cornigera*, many earlier researchers have proposed that there is close relation between structures and biological activities of the structure's explanation is necessary for these diterpenes diterpenes. The three significant diterpene skeleton types of *Euphorbia* species, covering 42 abietanes, 51 ingenanes and 30 tiglianes. The structure-activity relationship and the features on the chemical shifts (Qi-Cheng *et al*., 2009).

Zinc: Zinc level in the roots was found very higher in the samples of 0plants species ($p<0.05$). Their levels were found in the following hierarchy *Citrullus colocynthis* > *Rhazya stricta*> *Euphorbia cornigera*.

Copper: With reference to the level of Cu in samples of plant species (shoots) and the highest concentration was noted in case of *Rhazya stricta*.

Nickel: Variable concentrations of Ni were observed in our experiment and found that *Rhazya stricta* had the highest concentrations of Ni in comparison to the other studied plants.

Lead: The concentration of Pb was higher in the *Euphorbia cornigera* as compare to cadmium element.The shoots of plant showed high concentration of Pb as compare to other parts of plant.

Heavy metals Lead and cadmium are not very necessary trace elements having functions neither in human body nor in plants which causes various different toxic in human body at low level doses. The typical symptoms of Pb poisoning are colic, anemia; headache, convulsions and chronic nephritis of the kidneys, brain damage, vascular and immune system and central nervous system disorders, aluminum may cause strong effects including, discomfort, dysuria, neurotoxicity and cataract, if consumption is more than established values of WHO. Cadmium deposition in the human body could damage mainly the liver and kidneys, causing both chronic and acute poisoning. In light of the recommendation of WHO that the medicinal plants can form raw material for the ended product which may be monitored for the presence of heavy metals . Moreover , the monitored highest allowed limits of toxic metals like lead, cadmium and arsenic, and which amount to 10, 0.3 and 1.0 ppm, simultaneously (Ibrahim *et al*., 2013).

Moreover, the established European Commission has declared the standard for lead and Cadmium for food supplement since 2001. Also, there are other acts which provide drugs and healthy food like the Food and Drug Administration (FDA, 2003) suggested the rules and regulations which would make nutritional supplement packaging, manufacturing and storage in acquiescence with Current Good Manufacturing Practicing (cGMP). The recommended levels of heavy metals changes for babies from adult during the preparation of food materials and medicines discussed limit for lead (Pb) amount in herbal medicines in 10 ppm but the dietary dose limit of Pb is 3mg/week. Similarly, the minimum concentration of Cd due to which yield reduction takes place in 5-30 ppm but the maximum recommended levels for food material is around 1-90 ppm. So, the finding may be considered on the regard of the using of the herbs for human consumptions. Then the data of the heavy metals analysis proposed that the plants may be used for the consumption of human on the basis of the preparation of herbal products and standardized extracts must be collected from the un polluted natural habitats human consumption or for preparation of herbal products and standard extracts must be collected from un polluted natural habitats. Generally, as public use herbal medicines for prolong period for getting the desirable effects. Therefore, prolong using of such herbal medicines might less chronic or subtle health hazards. So, nevertheless, using of the plants growing in contaminated areas have high risk of in taking of heavy metals level beyond the allowed level due to which health hazard caused.

The interspecies comparison of the heavy metals concentrations: In order to analyze the interspecies differences of plant heavy metal accumulation capacity, the comparison of the elemental mean concentrations among three plants was shown in Table 2 and Fig. 4. The concentration of elements in *Citrullus colocynthis*, *Euphorbia cornigera* and *Rhazya stricta* were higher and were not according to WHO standards.

Among our studied samples of plant species screened for Cd, Zn,Cu, Ni and Pb, in which most of the species were enough to take up and translocate more than one heavy metal from shoots as shown in (Table. 2 and Fig. 4).The analysis of data showed that the interspecies concentrations of most elements were significantly different. According to the biological characters for each species, such as living form, morphology, in addition , the climatic condition had a great effect on the accumulation capacity of the element in plant. So , it showed that, even, plants were collected from the same region, the uptake efficiency of the elements was significantly varied, this could be regarded as the effect of plant species, climatic condition, as well as, the nature of metal element on its efficiency. The mean of the total concentration of heavy metals are all higher as compared to the value reported by Ruling, 1994 in European countries and other countries (Pignata *et al.*, 2002).

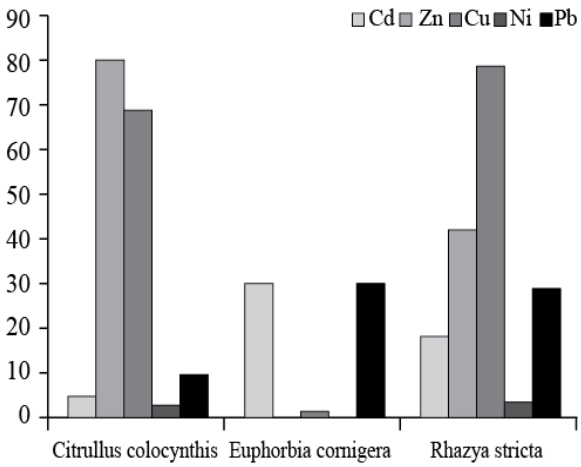

Fig.4. Concentration of metals in plant species.

In light of the data of this study, the plants presented above can be regarded as heavy metal accumulators but different capabilities of accumulation (Table 2 and Fig. 4) which also concludes that *Euphorbia cornigera* could be considered as Cd accumulator plant reference to its amount in their shoot reached the highest concentration in the three studied plants. Also and *Rhazya stricta* could be regarded as Cd accumulator plants (Table 2 and Fig. 4). High shoot concentration of these heavy metal showed that these plants have vital properties to be used in phyto-extraction of these heavy metals. However, heavy metals deposition may be established to well develop extoxification adsorption of heavy metal ions in vacuoles, by binding them on suitable ligands such as organic acids, proteins and peptides in the presence of enzymes that can function at high level of metallic ions (Cui *et al.*, 2007) and metal exclusion strategies of plant species. Generally the data shows that none of these plants species were substantiated as hyperaccumulator because all the samples plant species deposited Cd, Zn, Cu, Ni and Pb are less than 100mg/kg. However, on the regard of highest levels of metals in samples of plant species may have been the capability for phytostabilization and phytoextration.

Citrullus colocynthis may be utilized for various aims *colocynthis* which can either be eaten or explained for other uses in medicines and as well as source of energy. The extracts of *colocynthis* (aqueou and methanol) indicated that high antimicrobial action against *Escherichia coli*, *Staphylococcus aureus* and other bacteria. Extracts of fruits, leaves, roots and stems were also found to be potentially usable against many gram positive bacilli and fungi as *Aspergillus fumigatus*, *Aspergillus flavus* and *Mucor* sp. Moreover, some of these extracts are found to have insulin tropic effect and therefore, an antidiabetic effect, which can prove these related to the cure of diabetes mellitus described Katsaridis *et al.* (2012) and Patel *et al.* (2012).

This study shows that *Euphorbia cornigera* may be considered as depositor plants for most studied heavy metals which is the first information focusing on their capability as metal depositor. Moreover, *Rhazya stricta*, is latest/current plants in the polluted area, were specified as friendly metal depositor, particularly friendly for Cd and Pb.

As cancer is one of the serious and is very severe dangerous which is propagating very fast with high mortality speed diseases of the present era even in the highly developed World but the situation is even worse in the developing countries due to lack of the knowledge of awareness, poverty and non-availability of quality drugs. So, majority of the people rely over folk/indigenous medicines derived from plants etc. plants of Euphorbiaceae have been used for remedy against cancer since ancient time. Therefore, variety of antibacterial, anti-viral, molluscicidal and tumor-promoting to tumor-inhibiting compounds have been isolated from these plants (Baloch *et al.*, 2005).

The toxicity of *Rhazya stricta* for 10 mg leaf leachates are in line with data as discussed Gilani *et al.* (2007a.) and Gilani *et al.* (2007b.), reported that *Rhazya stricta* indicated toxic effect against the seed of lettuce. *Rhazya stricta* contains 89 indole alkaloids. Some of these indole alkaloids have indicated pharmacological charactertics including antibacterial activity. Most probably, some or one of these alkaloids may also be responsible for allelopathic and for antibacterial activities also (Shinwari, 2010).

Conclusion

This study concluded that the growth performance of biomass and metal accumulation in contaminated soils need mitigation management and conservation. Further, it is concluded that among the selected plants showed the higher capacity in Cd, Zn, Cu, Ni, and Pb accumulation. Moreover, this study documented that the selected plants could be considered as bio-indicator and accumulator for soil pollution. Data of this research work shows that the three plants namely *Citrullus colocynthis* , *Euphorbia cornigera* and *Rhazia stricta* become a source for accumulating and the level of heavy metals follows of hierarchy i.e., (Pb > Zn > Cd > Cu > Ni) and the level of heavy metals was found high as compared to the standard of WHO. Also, these studied plants are also used for medicinal purposes too reference to the toxicity of the metals as in case of *Euphorbia cornigera* where the cadmium and cyanide which substantiates that the plant may be used for the treatment of cancer and anti tumor because some elements are essentials for man while the most important thing is that the studied species of plants on the regard of accumulation of metals are environment friendly (Ibrahim *et al.*, 2013).

Recommendations

- Medicinal plants need mitigation, preservation management for good quality of soil any concentration of heavy toxic metals. Medicinal plants should be carefully planted in environment free from pollutants. WHO recommendations for planting medicinal plants in a clean environment should be taken care of, and indeed soil is to be monitored regularly
- Prolong consumption of medicinal plants may be discouraged because it might be chronic or subtle health hazard.
- The Govt. of Pakistan should stress on the developing of education for the eradication of illiteracy and ignorance of the Balochistan backward areas for the purpose of the development of prosperous Pakistan.
- The funds are needed to make the soil fertile for medicinal plants and this can be accomplished with mitigation, cultivation and harvesting strategies. Indigenous resources for soil chemistry and its regular monitoring are needed. Further, the sustainability of the habitants is to be regulate with basic principles of management Sciences.

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(Received for publication 14 June 2014)