

STUDY OF TRACE ELEMENTS IN MEDICINAL PLANTS (*AACHILLEA WILHELMSII*, *FORSKOHLEA TENACISSIMA*, *SOLANUM NIGRUM*, *VITEX AGNUS CASTUS* AND *WITHANIA COAGULAM*) IN PANJGOOR, BALOCHISTAN, PAKISTAN. (A CASE STUDY)

Mida Asad¹, Manzoor Iqbal Khattak*, Rukhsana Jabeen² & Mohammad Hussain³

^{1,*3}-Chemistry Department, Balochistan University, Quetta.Pakistan.

²-Sardar Bahdur Khan Women University, Quetta.

Email:manzoor_iqbal@yahoo.com

ABSTRACT: The prime objective of this work is to determine the concentration of toxic trace elements, such as As, Ca, Cd, K, Mn, Ni, Se, Mg, Cr, Fe, Cu, Zn, Pb and Na in medicinal plant species (*Achillea Wilhelmsii*, *Forskohlii Tenacissima*, *Solanum Nigrum*, *Vitex Agnus Castus* and *Withania Coagulan*), qualitatively and quantitatively. The species were collected from Punjgur in the region of Balochistan, the area not explored in depth in this respect. The technique used was Atomic Absorption Spectroscopy. Further, the amount of heavy metals found in these plants substantiated that these plants are very good accumulators and can play a significant role of detoxification agents. In addition, these plants serve as a potential source of medicinal products, extracted for the treatment of various ailments.

Keywords: Medicinal plants, Trace Elements, Atomic Absorption Spectroscopy & Environmental Pollution

1. INTRODUCTION

Around eighty percent of the World is influenced by different herbal medicines based systems. Excluding homeopathy, the actions of these medicinal herbs are valued, based on their chemical constituents. Probably seventy thousand plants are used in medicine; about 2,000 plants are used by Indians (Ayurwadiq) for the treatment of various disorders. The 5757 Chinese scheme rests on these plants to cure various ailments. Japanese and Korean treatment structures also include a great amount of therapeutic plants, where these plants are used. Herbal treatment is increasing at an impressive speed because of the countless contributions of ethno-medicinal practice that are grouped around the World.

Every useful plant owes to the ability of its ingredients, whether diet, dress or housing. Likewise, any therapeutic herb covers a range of vigorous components that facilitate their healing activity materials. Some of these complexes such as asthma, taxon, and vincristine were secluded on huge scales and used in drug insulation system.

It is well known plants as per an organism proficient of manufacturing its foodstuff and additional substances

required to serve that embraces defense against microorganisms, animal hassle and wound healing and infection of these plants that apply to all to making those functions alone and for that extra kick. This trust must be the reasoning of herbal medication or philosophy.

Achillea (Asteraceae) is a perennial plant that has about hundred types, out of which around 19 types are originated in Iran. *Achillea* species are used in folk medicine as a sedative, anti-inflammatories, analgesics, anthelmintics, and to relieve the symptoms of premenstrual syndrome [1]. Studies show that different *Achillea* spp need a extensive variety of pharmacy-logical properties such as antioxidant, antimicrobial activity, anxiolytic and cytotoxic [2-4]. *Achillea Wilhelmsii* C. Koch (*A. Wilhelmsii*) is one of the most common class of *Achilles* in Iran. Plentiful readings require conducting to estimate the pharmacological properties of various necessary oil and sources of this aromatic plant. It has been exposed that this plant has antibacterial [5], anti-tumor [6], immunomodulators [7], antihypertensives [8] and vagolitics [9].

The general image of the plant of *Achillea Wilhelmsii* (Local Name, *Boe Madran*) is given below in Figure-1



Figure-1. The general image of the plant of *Achillea Wilhelmsii*.

A. Wilhelmsii is used as anxiolytic plant in folk medicine to support the anxiolytic properties of volatile oil. The essential oil components *A. wilhelmsii* and its anti-anxiety effects in rats are investigated.

Forskholea tenacissima is a member of nettles generally not bite *Forskholea* and belongs to the same family *Urticaceae* bites. Designated as a difficult character who does not want or need a caress [10]. *F. tenacissima* marks his home-

grown, wherever they do not live numerous types of plants on rocky ground, along roadsides, in the gravel wades [10] and rocky crevices and receiving water troughs above stone roadways [11]. In medicine, *Forskholea Tenacissima* is also used for the treatment of lung cancer.

The general image of the plant of ***Forskholea Tenacissima*** (Local Name ,*Chaha Mahak*) is given below in Figure-2



Figure-2. The general image of the plant of *Forskholea Tenacissima* .*Solanum Nigrum*.

The composite - also recognized as *Solanum* segment *Solanum* L. - is the cluster of species of shadowy larks categorized by their nonexistence of punctures and stellates' hairs, its snowy florets and emerald fruit or shadowy umbelliforms organized. *Solanum* type of this set can be namely tangled, even by middle systems, and cross-hybridization amongst sorts [12]. Certain of the main

sorts inside the complex of *Solanum Nigrum* are: *Solanum Nigrum*, *Solanum Americanum*, *Solanum Douglasii*, *Solanum Apacum*, *Solanum Ptychanthum*, *Solanum Retroflexum*, *Solanum Sarrachoides*, *Solanum Scabrum* and *Solanum. Villosum*. The overall picture of the plant *Solanum Nigrum* (Local Name, *Tola Angur*) is given below in Figure-3



Figure-3. The general image of the plant of *Solanum nigrum*

Levels of solanicae in *Solanum. Nigrum* may be poisonous. The kids deceased of lethal afterward intake the immature fruits. Nevertheless, once rare herb is deadly, [13] by developed fruitlets that cause mild warning sign of gut pain, nausea and cholera.

Signs of poisoning remain usually stuck for 7 to 13 hours after eating [14]. The primary warning sign of harmfulness consists of temperature, perspiring, spewing, intestinal pain, cholera, misunderstanding and sleepiness. The demise by ingestion of huge quantities of installations resulting from heart arrhythmias and breathing insufficiency. animals also have been infected by the harmfulness of the feedstuff of *S. Nigrum* vegetation [12]. Entire varieties of faunas could be depicted after swallowing the Morel, plus

cows, lambs, fowl and horses.

Vitex agnus-castus (local name, Gwanik), similarly named vitex, uncorrupted tree, *Abraham's balm*, lilac Chaste tree [15] or pepper monaco, is native to the Mediterranean area. One of the insufficient temperate kind of *Vitex*, which as a whole is a kind of hot and subtropical floras [16].

Theophrastus mentioned many times Bush, as *Agnos*, the research in flora. *Vitex*, its title in "Pliny the Elder", vieo comes as of *Latin*, to knit or bond, a mention to the usage of wicker *Vitex Agnus-Castus* [17]. Repeats its specific name macarrónico "caste" in *Greek* and *Latin*, and reflected sacred to the divinity Hestia / Vesta. The general image of the plant of *Vitex Agnus-Castus* is given below in Figure-4



Figure-4. The general image of the plant of *Vitex Agnus-Castus*.

Vitex confusion with the early colonizers of the West Indies might have taken a *Ricinus communis* as the "castor oil plant" term castor oil may derive after its usage for example an additional for *cestrum*. *Vitex* has long been supposed to be a an aphrodisiac, nonetheless its efficacy rests debatable.

Withania Coagulans (local name, Panerbad / Kapai) is one of the Solanaceae or dreamed of the plant family, originally

from Afghanistan and the Indian subcontinent [15]. Within the genus *Withania*, *Withania Somnifera* (*Ashwagandha*) and *Withania. Coagulans* (*Booti of Ashutosh*) are carefully noteworthy, and are mature in different areas for its therapeutic practices.

The general image of the plant of *Withania Coagulans* is given below in Figure-5



Figure-5. The general image of the plant of *Withania Coagulans*.

Contamination and pollution of top soil, water and air have turned out to be unavoidable as an outcome of human accomplishments. Environmental contamination by lethal metals has risen progressively, meanwhile the industrialized revolt, producing serious environmental harms persists [18,11]. Important quantities of heavy metals besides further substances are made pollution of environment, in particular by productions, mines, agronomy, remnant fuel burning and traffic flow [19].

Phytoremediation is a comparatively first-hand technique to remove pollutants as of the atmosphere. It can be well-defined as the usage of floras to eliminate, abolish or confiscate dangerous ingredients into the atmosphere. It becomes a field of research of relevance in recent years, by way of it is harmless and possibly more economical than outdated remediation skills [20-23].

Phytotherapy practices the extraordinary capacity of the floras to quintessence like components and mixtures of the atmosphere and digest many particles in their soft tissue looks exact hopeful for the elimination of environmental contaminants [24]. Meanwhile utmost of the herbal roots are inside the earth, this can show a significant part in the elimination of metals by percolation, absorption and positive ion exchange, and by means of organic variations persuaded by the plant rhizosphere [25-26].

In several means, active floras can remain related with solar thrusts are able to abstract and distillate dissimilar elements of their environment. As of earth and aquatic, entire shrubberies have the capability to store heavyweight metals that are necessary for growing and enlargement. These metals are Cu, Fe Mo, Mn, Zn, Mg and Ni [27]. Some floras also have the capability to store heavyweight metals that have not recognized by biotic purpose. These comprise Ag, Co, Pb, Cd, Cr, Se and Hg [28-29]. Though, excess amassing of these heavyweight metals may be poisonous to utmost floras. The capability to bear high levels of heavyweight metals and collect in very great amount has developed autonomously and jointly in several herbal kinds [30].

Therefore, biologically planned approaches are considered to advance the usage of Phytotherapy to decrease the quantity of heavy metals in polluted earths. Such a procedure has been castoff to clear-out heavyweight metals, poisonous scented contaminants, acid, mine drainage, insecticides and xenobiotic and carbon-based complexes. Phytotherapy is a biologically pleasant, harmless and inexpensive practice to eradicate the contaminants. Phytotherapy of lethal metals from the dirty earth ultimately includes the removal or inactivation of these metals in muds. Phytotherapy is a unique fresh methodology that deals extra ecological remunerations and charge current substitute. While it is inexpensive technique but needs practical approach, skilled project inventors with playing field knowledge that select the appropriate types and cultivars for a specific metals and areas. Through numerous investigates main effort is to comprehend the functional instruments of metal immersion, transference and accommodation, but slight is known concerning the inherent foundation of hyper accumulation [31]. The herbal castoff in the Phytotherapy performance have needed a substantial ability of metal adsorption, its amassing and power to lessening the management period. Various relations of vascular floras must have been recognized as metal hyper accumulator

[32-33]. And several of them have its place to *Brassicaceae*. Those hyper-collector are metal choosy, taking sluggish development rate, yield minor quantities of biotic mass and could be cast-off in their regular habitations merely [34]. A hyper-accumulator is well-defined as an herb that can store: 1 g/kg of “Cu, Co, Cr, Ni and Pb”, or 10 g/kg of Fe, Mn and Zn in their bud waterless stuff. and in collector plants, the metal quantities in sprouts are consistently superior than that in roots, presenting a unusual capability of the herbal to engage and transference metals and stock them in their upstairs portion [35-37]. In the meantime, a hyper-accumulator is observed as herbal in which the quantities of heavyweight metal in sprouts are superior than that in earths, sense advanced heavyweight metal amount in the herbal than in the earth, which give emphasis to the gradation of vegetable metal uptake [38-39].

The main ecological features that mark metal acceptance by floras are earth sharpness, its positive ion exchange capability, the content of carbon-based and emerald and the deliberation of macro and micro nutrients.

Somewhat, it resolves valuable to catch certain floras that have gathering capability to of trace elements. In this learning, we examined the quantities, translocation and supplementation features of As, Ca, Cd, K, Mn, Ni, Se, Mg, Cr, Fe, Cu, Zn, Pb and Na at plant species at Punjgur in region of Balochistan, Pakistan grow enhanced information of the storing ability in plant species to As, Ca, Cd, K, Mn, Ni, Se, Mg, Cr, Fe, Cu, Zn, Pb and Na and select hyper-accumulator that could be castoff for the cure of agronomic areas contaminated by weighty metals and metal source area soils.

EXPERIMENTAL

The most common/dominant species [*Achillea wilhelmsii* from Punjgur (Aap Shaped), *Forskohlea Tenacissima* from Punjgur (Tasp), *Solanum Nigrum* from Punjgoor (Tasp), *Vitex Agnus Castus* from Pungur (Tasp) and *Withania Coagulan* from Pungur (Tasp) were collected during September-October 2016. An aggregate of 7-11 floras plus sprouts of each kind remained together from both spot and variegated to form a complex taster, positioned in regarded as bags and conveyed to our research test center for additional examination. Beforehand examination, from each herbal stalks and leaves were cautiously detached and washed (for 2-3 minutes approximately) with tap aquatic and with deionized aquatic to eliminate the earth and superficial powder. The mineral composition was determined for different parts of plants at different phenological stages following Sucman *et al.*, [40] and AOAC [41] techniques. The data was statistically analyzed using ANOVA to see the significance levels among the phenological stages and plant parts [42].

RESULTS AND DISCUSSION

The heavy metals are released into the atmosphere from different sources. Most of the readings have used tasters of plants to control their levels of metal [43-45]. The ecological contamination with trace elements and xenobiotic is a worldwide problematic, so the expansion of Phytotherapy skill for the cleaning of dirty soil herbal is of significant importance [45].

The idea of phytoremediation has been offered by Chaney [46] that involves the use of trace elements hyper-

accumulators plant for the elimination of contaminants as of soil or water. The hyper-accumulators collect significant amounts of metallic in their muscle, irrespective of quantity of the metallic in the earth. Extra than 4 hundred floras are recognized as hyperactive - accumulators of heavyweight metals that can amass great concentrations of metallic ions in above-ground biomass. These floras are grasses, herbal crops, trees and unwanted plant [48].

Collectors are kinds of accomplished collecting metallic ions at stages hundred fold superior than classically restrained in sprouts of communal non-collector floras. Thus, collector will concentrate additional than the required standards [28,48] at dissimilar circumstances. By the natural plants is an motivating plan to attain this purpose.

Agreeing to the outcomes of the subsequent study, the plants stated beneath can be considered as trace elements collectors while they are unlike as regards their gathering capability for each element (Table-1 and Figure-6). Built on the results, the finest arsenic collector is but *Achillea Wilhelmsii*, should be considered as good arsenic accumulators. The data shows that the plants under

observations are very good accumulators of trace elements. This one capability to eliminate heavyweight metals as of contaminated territories has been calculated as which is proposed for eliminating and cleansing of trace elements (especially Se, As,Mn, Cr and Ni) from polluted soils. Further, the results show that these plants are good accumulator plant for Mn, too,

The present-day learning reveals that certain floras can settle a widespread variety of metal quantities on the surface of the earth. The argument will be focused on acceptance of As, Cd, Ni, Cr, Mn and Se the special of hyper-accumulator plants. About diverse surroundings are required a wide variability of collecting floras for phytotherapy in unlike circumstances. Consuming the instinctive floras is a motivating plan to attain this goal.

Agreeing to the outcome of the next reading, the plants stated below can be observed as trace elements collectors although they are different regarding their absorbing capability for each metal (Table -1 and Figure-6) . It was observed that the level of trace elements in *Forskohlea Tenacissima* and *Vitex Agnus Castus* was found within the range of the standard of WHO.

Table-1. Trace elements level (ppm) in samples of medicinal plants collected from Pungur.

S. NO	Name of Plant	As	Ca	Cd	K	Mn	Ni	Se	Mg	Cr	Fe	Cu	Zn	Pb	Na
1	<i>Achillea wilhelmsii</i>	0.06	1.89	0.006	5.58	0.05	0.004	0.117	7.64	0.23	0.98	0.062	0.01	0.02	30.2
2	<i>Forskohlea Tenacissima</i>	0.04	0.92	0.004	4.31	0.04	0.003	0.01	5.9	0.16	0.63	0.045	0.01	0.04	30.1
3	<i>Solanum Nigrum</i>	0.07	2.03	0.008	5.41	1	0.004	0.115	15.36	0.36	1.995	0.084	0.109	0.16	42.1
4	<i>Vitex Agnus Castus</i>	0.03	0.01	0.001	0.01	0.01	0.02	0.0001	5.4	0.01	1.74	0.01	0.05	0.001	28.3
5	<i>Withania Coagulan</i>	0.04	1.95	0.005	6.94	0.05	0.01	0.1	8.65	0.057	2.899	0.095	0.135	0.12	47.7

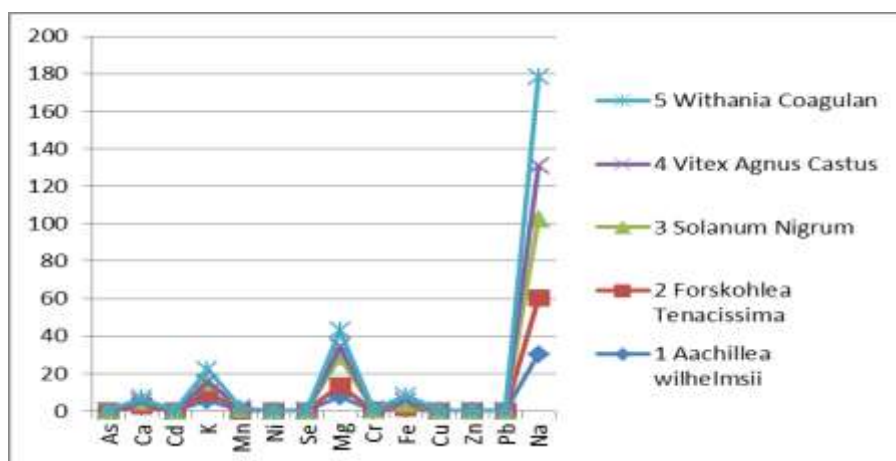


Figure-6. Trace elements level (ppm) in samples of collected medicinal plants from Pungur.

Akguc *et al.*, [18] revealed that the order of mineral contents lie in order of $Zn < Mn < Na < Fe < Ca < K < Mg$ in the leaves of *Solanum Nigrum*. ANOVA indicated non-significant differences among the plant parts. However, the differences were highly significant among the phenological stages *Solanum Nigrum* and *Withania Coagulans* as shown in Table-1.

The tested species had higher level of Mg than recommended values and therefore these forages are good for lactating cattle, goat and sheep. Dua and Care [49] stated that availability of Mg to cattle is affected by other dietary components like K, N, Ca contents. Sodium is associated with body fluid and regulates acid base balance.

CONCLUSION

This research has revealed that similar species of therapeutic plants, rising in dissimilar geographical sites, collect unlike stages of trace elements. Amount of heavy metals also varied for dissimilar plant species together from the same geographical site. Concentrations of trace elements noticed in some plants submitted from Punjgur are inside allowable boundaries, while the stages in other ecological sites surpassed the suggested standards. Therefore, therapeutic plants for the preparation of herbal medicines ought to be collected from contamination-free normal territory. Supplementary, it is concluded that these therapeutic plants, castoff for resident or pharmaceutical determinations, should be collected from zones not polluted with heavyweight metals. It is, consequently, recommended that the metal contented in therapeutic plants be tested for levels of trace elements beforehand their usage for resident and medicinal purposes. Moreover, heavyweight metals are the utmost valuable and poisonous metallic ion, i.e. scattering altogether above the earth. Therefore, for good physical shape energetic lifespan, it is precedence currently to eliminate the heavyweight metallic ions as of our atmosphere. This research was accompanied to monitor floras mounting on a polluted spot to conclude their possible for metal buildup. Generally, tested plants are almost yearly and periodic herbaceous types that persistent species with comparatively huge biomass are more appropriate for phytoremediation purpose. Additional inquiries below measured ecological circumstances are essential for assessing the worth of these species in modern technologies. Assessment of its capability in decontamination metallic contaminated soils is valuable for cleansing of large contaminated region. This is the chief report on its capability as essential trace elements collector. The data revealed that each plant characterized by specific tolerance to a definite metal and no trends in the heavy metal content in plant parts.

RECOMMENDATIONS

- The great importance of collecting good herbarium plants for taxonomic identification of collected species must be stressed. There is a need for conservation of all useful plant species. And also cultivation and assessment of germ plasm for future use, since among the most vulnerable plant species in Pakistan, the most over exploited are the medicinal plants.
- In recent medication also, plants inhabit a very important place as raw material for some significant medicines, though synthetic medicines and antibiotic brought about

a uprising in monitoring different sicknesses but in remote places depend upon traditional dealers.

- Data of organic constituents of herbal is vital for superiority regular examines of the shrub, abstract or any preparation holding theme. A complex or a group of complexes containing or current can serve as a bio marker and the occurrence of the concentration of the same can be fallowed to choose on the generousness of the medicine. Any part other than for these indicators, knowledge of these complexes and these specific approaches will enable the herbal manufacturing in examination thus raise it values.
- As the various chemical parameters either increased or decreased with growing age of plant and seasonal changes. It is therefore recommended that harvesting of these plants might be more beneficial at proper stage to get maximum medicinal benefits.

REFERENCES

1. Zargari A. Medicinal plants. 6th ed. Vol. 3. Tehran: Tehran university publications; 1996. pp. 107–117.
2. Radulovic NS, Dekic MS, Rand-elovic JP, Stojanovic NM, Zarubica AR, Stojanovic ZZ. Toxic essential oils: Anxiolytic, antinociceptive and antimicrobial properties of the yarrow *Achillea umbellata* Sibth. et Sm. (Asteraceae) volatiles. *Food Chem Toxicol.* 2012;50:2016–2026.
3. Baretta IP, Felizardo RA, Bimbato VF, dos Santos MGJ, Kassuya CAL, Junior AG, et al. Anxiolytic-like effects of acute and chronic treatment with *Achillea millefolium* L. extract. *J Ethnopharmacol.* 2012;40:46–54.
4. Maggi F, Bramucci M, Cecchini C, Coman MM, Cresci A, Cristalli G, et al. Composition and biological activity of essential oil of *Achillea ligustica* All.(Asteraceae) naturalized in central Italy: Ideal candidate for anti-cariogenic formulations. *Fitoterapia.* 2009;80:313–319.
5. Tosun F, Kizilay CA, Sener B, Vural M, Palittapongarnpim P. Antimycobacterial screening of some Turkish plants. *J Ethnopharmacol.* 2004;95:273–275.
6. Ali N, Shah SW, Shah I, Ahmed G, Ghias M, Khan I. Cytotoxic and anthelmintic potential of crude saponins isolated from *Achillea Wilhelmsii* C. Koch and *Teucrium Stocksianum* boiss. *BMC Complement Altern Med.* 2011;11:106–112.
7. Sharififar F, Pournourmohammadi S, Arabnejad M. Immunomodulatory activity of aqueous extract of *Achillea wilhelmsii* C. Koch in mice. *Indian J Exp Biol.* 2009;47:668–671.
8. Asgary GH, Naderi N, Sarrafzadegan N, Mohammadifard, Mostafavi S, Vakili R. Antihypertensive and antihyperlipidemic effects of *Achillea wilhelmsii*. *Drugs Exp Clin Res.* 2000;2689–2693.
9. Niazmand S, Khooshnood E, Derakhshan M. Effects of *Achillea wilhelmsii* on rat's gastric acid output at basal, vagotomized, and vagal-stimulated conditions. *Pharmacogn Mag.* 2010;6:282–285.
10. Retrieved 2008-04-24, "endemismos". *Flora endémica, rara o amenazada de Almería (in Spanish)*.

- 11.Huseyinova R, Kutbay HG, Bilgin A, Kilic D, Horuz A, Kirmanoglu C .2009. Sulphur and Some Heavy Metal Contents in Foliage of *Corylus avellana* and Some Roadside Native Plants in Ordu Province, Turkey. *Ekoloji* 18 (70): 10-16.
- 12.Edmonds, J. M., Chewya, J. A., *Black Nightshades, Solanum nigrum L. and related species*, International Plant Genetic Resources Institute, 1997.
13. North, P., (1977) *Poisonous Plants and Fungi in Colour*, Blandford Press, pp140-141.
- 14.Schep LJ, Slaughter RJ, Temple WA (April 3, 2009). [<http://www.nzma.org.nz/journal/122-1292/3547/>] "Contaminant berries in frozen vegetables" Check value. *The New Zealand Medical Journal* 122 (1292): 95-6.
- 15.Retrieved 6 August 2015. "*Vitex agnus-castus*". *Natural Resources Conservation Service PLANTS Database, USDA*.
- 16.David J. Mabberley. 2008. *Mabberley's Plant-Book* third edition (2008). Cambridge University Press: UK.
17. Silvestre A¹, Humbert JF. A molecular tool for species identification and benzimidazole resistance diagnosis in larval communities of small ruminant parasites. *Exp Parasitol.* 2000 Aug;95(4):271-6.
18. Retrieved 3 February 2015, "*USDA GRIN Taxonomy*".
19. Akguc N, Ozyigit II, Yarci C.2008. *Pyracantha coccinea* Roem. (Rosaceae) as a biomonitor for Cd, Pb and Zn in Mugla Province (Turkey). *Pakistan Journal of Botany* 40 (4): 1767-1776.
20. Yilmaz R, Akcali S, Yarci C, Aksoy A, Ozturk M. 2006. Use of *Aesculus hippocastanum* L. as a biomonitor of heavy metal pollution. *Pak. J. Bot.*, 38(5): 1519-1527.
21. Salt DE, Smith RD, Raskin I.1998. Phytoremediation. *Ann Rev Plant Physiol Plant Mol Bio.* 49, 3-668.
21. Mitch ML. 2002. Phytoextraction of toxic metals: a review of biological mechanism. *J. environ. Qual.* 3: 1109-1120.
- 23.Glick, B. R. 2003. Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* 21, 383-393.
- 24.Gurbisu C, Alkorta I .2003. Basic concepts on heavy metal soil bioremediation. *Eur J Min Process Environ Prot* 3(1):58-66.
- 25.Dunbabin JS, Bowmer KH. 1992. Potential use of constructed wetlands for treatment of industrial wastewaters containing metals. *Science of Total Environment* 111, 151-168.
- 26.Wright DJ, Otte ML.1999. Wetland plant effects on the biogeochemistry of metals beyond the rhizosphere. *Biology and Environment: Proceedings of the Royal Irish Academy* 99B (1), 3-10
27. Langille WM, MacLean KS. 1976. Some essential nutrient elements in forest plants as related to species, plant part, season and location. *Plant Soil*, 45: 17-26.
- 28.Hanna WJ, Grant CL .1962. Spectrochemical analysis of the foliage of certain trees and ornamentals for 23 elements. *Bull Torrey Bot Club* 89:293-302.
- 29.Baker AJM, Reeves RD, Hajar ASM. 1994. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. and C. Presl (Brassicaceae). *New Phytol*;127:61- 8.
- 30.Service.2007.Science for Environment Policy., European Commission Dg., J. Env.NEWS Alert.<http://ec.europa.eu/environment/integration/research/newsalert/pdf/69na1.pdf>.
- 31.Pollard AJ, Powell KD, Harper FA, Smith JAC. 2002. The genetic basis of metal hyperaccumulation in plants. *Crit. Rev. Plant Sci.* 21:539-566.
- 32.Reeves RD, Baker AJM. 2000. Metal-accumulating plants. In: Raskin I, Ensley BD (eds), *Phytoremediation of Toxic Metals*, pp. 193-229. John Wiley, NY, USA.
- 33.Prasad MNV, Freitas H. 2003. Metal hyperaccumulation in plants - Biodiversity prospecting for phytoremediation technology. *Electronic J.Biotechnol.* 6:275-321.
34. Kamnev AA, van der Lelie D. 2000. Chemical and biological parameters as tools to evaluate and improve heavy metal phytoremediation. *Bioscience Rep.* 20:239- 258.
- 35.Baker AJM, Brooks RR. 1989.Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. *Biorecovery*;1:81-126.
- 36.Brown SL, Chaney RL, Angle JS, Baker AJM. 1995.Zinc and cadmium uptake by hyperaccumulator *Thlaspi caerulescens* and metal tolerant *Silene vulgaris* grown on sludge-amended soils. *Environ Sci Technol* 29: 1581- 5
- 37.Wei CY, Chen TB, Huang ZC. 2002.Cretan brake (*Pteris cretica* L.). an Arsenicaccumulating plant. *Acta Ecol Sin* 22:777- 82..
- 38.McGrath SP, Zhao FJ. 2003. Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin Biotechnol.* 14, 277-282.
- 39.Yanqun Z, Yuan L, Jianjun C, Haiyan C, Li Q, Schwartz C. 2005. Hyperaccumulation of Pb, Zn and Cd in herbaceous grown on lead-zinc mining area in Yunnan, China. *Environ. Int.* 31, 755-762.
40. Sucman E, Mahrova M, Pac J and Vavrova M (2007). Microwave assisted digestion method for the determination of cadmium, copper, lead and zinc in biological materials. *Electroanalysis*, 20: 386-389.
41. AOAC (2000). 17th edition. Association of official analytical chemists, Gaithersburg, MD, USA, pp. 87.
42. Choudhary SM and Kamal S (2004). Introduction to Statistical THEORY Part 1&2 and 250. Murkazi Kutub Khana, URDU Bazar Lahore,pp62,102,109.
43. Luilo GB, Othman OC .2006. Lead Pollution in urban roadside environments of Dares Salaam city. *Tanz. J. Sci.* 32(2):61-67.
44. Alam S, Ahmad IK, Din ZU, Fazlullahkhan FK. 2008. Variations of Contaminants in the Road Side Agricultural Soil of Thana Malakand Agency. *J. Chem. Soc. Pak.* 30(6):800-804.
45. Kramer U .2005. Phytoremediation: Novel Approaches to Cleaning Up Polluted Soils Current Opinions in Biotechnology. 16: 133-141.
- 46.Chaney RL. 1983. Plant uptake of inorganic waste constituents. In: J.F. Parr, P.B. Marsh and J.M. Kla (eds.) *Land Treatment of Hazardous Wastes*. Noyes Data Corp., Park Ridge, 50-76.
47. Yoon J, Cao X, Zhou Q, Ma LQ. 2006. Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. *Sci. Tot. Environ.* 368, 456-464.

48. Dahmani-Muller H, van Oort F, Gélise B, Balabane M. 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environ. Pollut.* 109:231–238.
49. Dua K and Care AD (1995). Impaired absorption of magnesium in the etiology of grass tetany. *Brit. Vet. J.*, **151**: 413-426.