

A COMPARATIVE ANALYSIS OF HEAVY METALS IN SELECTED MEDICINAL PLANTS (*ACROPTILON REPENS* L. DC, *CITRULLUS COLOCYNTHIS* L. SCHRAD, *FERULA ASSA-FOETIDA* L & *SOLANUM NIGRUM* L) OF MASTUNG, BALOCHISTAN

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ABSTRACT: The contents of some selected toxic heavy metals as Cd, Cu, Ni, and Pb & Zn in ppm are studied by operating flame atomic absorption spectrophotometer in some important medicinal plants (*Acroptilon repens* L. DC), (*Citrullus colocynthis* L. Schrad), (*Ferula assa - foetida* L), (*Solanum nigrum* L) which play very important role in the life of human beings. Furthermore, these therapeutic plants are used all over the world for a substantial basis of natural material for pharmacological and agricultural productions and also the intent medication of various ailments. Moreover, the hierarchy of heavy metals by analysis of these therapeutic plants was testified as Zn > Cu > Ni while Pb and Cd were examined below standard detection limit. In the current analysis, the calculations were correlated with the standardized values mentioned in the record of the World Health Organization (WHO). Also, the data of this research work will be the baseline for preparing of high-quality drug.

Keywords: Flame Atomic Absorption Spectroscopy (FAAS), Medicinal Plants, Heavy metals, & Metal toxicity.

INTRODUCTION

The valuable wild medicinal plants grow profusely, collected by local people and sold-out in native and international markets in the region of Balochistan [1] particularly, Mastung which is the sub-division of Kalat district, Balochistan (Pakistan). The area is mountainous, barren and has rugged terrain with ground elevation varying from 934 - 3414 meters above Mean Sea Level [2]. The district Mastung extent is of 5,896 square kilometers of Balochistan. The geographical position of Mastung lies between 29°20'13"-30°15'8" North latitudes and 66°11'34"-67°25'59" East longitudes. Location of Mastung is at 735 km (above ground distance) south-west (235 DB) of Pakistan's Capital City Islamabad and 45 km (above ground distance) South-West (200 DB) from Quetta City. The map of Mastung is given in Fig. 1

The utilization of herbs, shrubs, and therapeutic plants as essential remedies are a Worldwide phenomenon. Every civilization on planet of Earth, through written or oral tradition, has relied on the enormous variety of natural chemistry found in medicinal plants for their restorative residences. Awareness within remedy herbs all over the world is rising, in the developed world; a lack of hard currency to pay for imported pharmaceuticals is encouraging a reappraisal of traditional folk remedies. This trend toward more natural medicine has gained added impetus from our growing concern with environmental issues, such as the destruction of the rain forests and the loss of rare species [3]. The utilization rehearses that was begun from the most punctual circumstances of human history [4]. In any case, the utilization of plants as a wellspring of the drug is especially critical for people [5]. Restorative plants are the "backbone" of conventional prescription, which implies in excess of 3.3 billion persons in the less fashioned nations use medicinal plants all the time [6] of herbs to treat ailments and diminish sufferings is a typical



Fig-1. The Map shows valley of district Mastung Balochistan

For checking their requests and usage and furthermore powerlessness of therapeutic herb, researcher give extraordinary consideration, what's more, make move to investigate, to test their viability against microorganisms and what's more, to protect these species [7]. Therapeutic plants assume a critical part from the old time as they are utilized as a part of customary pharmaceutical and furthermore as home cures. Condition, contamination, climate, soil, gathering, what's more, taking care of a portion of the components, which assume a noteworthy part in defilement of restorative metallophytes along and furthermore by microbe's development.

Consequently, it is important toward gauge along with set up the grade of metallic components in the homegrown plants as

these components when expended on safe limit amounts will be harmful. The World health organization WHO additionally underscored the need to guarantee the nature of the plant and its items by utilizing present day procedures and applying appropriate principles [8].

Plants can absorb heavy metals in their parts and exchanges it from soils into the evolve means of existence [9]. This gathering is a standout amongst the most genuine natural concerns due to the potential unsafe impact. At high fixations, a few metals like phosphorous, zinc, chromium, copper, and cobalt are harmful whereas others like mercury, lead and cadmium are entirely poisonous [10]. Accumulation of destructive mechanical effluents in water, soil and air is intermittently expanding because of speedy urbanization and broad contaminations of the setting. Amongst these destructive chemical substances, nearness of genuine metals (atomic weights: 63.5- 200.6 g mole) that zone unit ubiquitous in nature, cause genuine unsafe consequences for living creatures [11]. The likelihood that the harmful heavy metals can be transmitted to people and creatures using herbs developed in dirtied regions is a noteworthy worry for customary and natural pharmaceutical [12]. So in nature the heavy metals have very closed linked with medicinal plants reference to environment and treatment of ailments.

***Acroptilon repens* (L.) DC** belong to family Asteraceae local name is Talkha (local language) and in English Russian knapweed is an attractive plant, common in irrigated fields and orchards in Northern Balochistan (1400 -2400m). It exhibits allelopathic characteristics and competes with desirable forage [13]. In lands that have shallow water tables or spare water from irrigation Russian knapweed commonly appears [14]. It has additionally been advised that Russian knapweed by concentrating metal (Zn) within the high soil layers through the buildup of high Zn levels in stems and leaves, that are then discharged into soils with litter deposition and decomposition could interfere with neighboring plants [15]. Interference between plants has been mentioned in terms of the assembly of toxicant organic chemicals [16]. Treatments may be applied to plants and soils to stop or alleviate zinc toxicity because iron (Fe) and Zinc (Zn) are both divalent and have similar radii, extreme Zn availability can result in the replacement of Zn for Fe in critical processes, a follow-on in Fe deficiency. Consequently, enhancing Fe uptake has been found to diminish stress triggered by high Zn to test whether or not the elevation of zinc in soils related to infestations of *A. adversely affects fascinating vegetation* [17]. Even to the purpose of causation Zn deficiency the mechanism is not entirely understood, high amounts of phosphorus (P) found to decrease Zn activity in plant structure, [18]. The plants decrease uptake of all metals by restraint them within the soil Iron oxides, once applied to the soil [19]. The leaves of the plant are granulated and blended with a little measure of flour and water to make a glue. This glue is connected remotely on the tummy for loose bowels. *Acroptilon repens* (L.) DC is well-known as Talkha in native language Fig. 2.



Fig. 2. *Acroptilon repens* (L.) DC

***Citrullus colocynthis* (L.) Schrad**

Citrullus colocynthis (L.) Schrad (Cucurbitaceae family), utilized as a therapeutic plant in the pharmaceutical industry regularly known as bitter apple [20]. Even under crucial circumstances, it can tolerate unforgiving situations without shrinking of the leaves or drying up [21]. This plant is a dry spell tolerant animal varieties with profound root framework, broadly appropriated in the Mediterranean district and the Sahara-Arabian locale in Africa [22]. Fruits are dried and granulated to acquired powder which is utilized for dropsy (Jalandari). *Citrullus colocynthis* is known as Kharengirirhi in native language Fig.3.



Fig. 3. *Citrullus colocynthis*

Ferula assa - foetida L is monoecious, perennial, herbaceous plant of the (UMBELLIFERAE family) local to Mastung region of Balochistan. The social occasion of sap and cutting of the root are reiterated until exudation stops (3 months around after the fundamental cut). The sap is occasionally accumulated from dynamic passage focuses made at the crossing point of the taproots and rhizome [23]. This plant is a prescription for nerving stimulant, stomach related problems and an opiate [24]. Warm water concentration of the got dried out gum is required orally as an (a substance that stimulates or increases menstrual flow) emmenagogue [25], also taken orally as an agent that prevents or relieves flatulence (gas in the gastrointestinal tract) and, in infants, may help in the treatment of colic. The origin of the word "carminative" is particularly curious, an antispasmodic (is a pharmaceutical drug or other agents that suppresses muscle spasms) and an expectorant (a medicine which promotes the secretion of sputum by the air passages, used to treat coughs) perpetual bronchitis. [26]. The mixture is treated with Brassica Alba act as an abortifacient (chiefly of a drug causin

abortion) [27]. Dried gum sap exudates are eaten to turn away (Dracunculiasis) similarly named Guinea-worm disease (GWD), which is an infection caused by the Guinea worm [28]. The mixture of gum and salt with the bark juice of *Moringa pterygosperma* is utilized for stomachaches (John 1984). *Ferula assa - foetida* L is known as Hing in local language Fig. 4.



Fig. 4. *Ferula assa - foetida* L

Solanum nigrum L is a species in the Solanum family [30]. The other vernacular names of *S. nigrum* incorporate dark or dark berry nightshade in Australia, South Africa and West Indies the plant is called Black Nightshade [31]. The *S. nigrum* plant is sub-glabrous to villous yearly which can grow up to 120 cm high, secured with basic multi-cell hairs with glandular or non-glandular heads [31]. The *S. nigrum* is a yearly plant having 21 species of this genus is available in the flora. Certain are weeds crop of country natural habitations. It even has a place with South-Eurasian (Mediterranean) botanical component and nowadays is a diverse species [32]. For great extent used as a vegetable and natural product source in many countries through collecting plants from developing unexpectedly as weeds in developed fields, or in weedy plant networks, under trees, along wall and streets, in shaded territories, closed structures and on wasteland. Sometimes these plants develop as a vegetable in home greenery [33].

The flying bits of *S. nigrum* is acknowledged to have shown up as hostile to ulcer action through corrosive and peptic covering in headache medicine incited ulcerogenesis in rodents (Solanum, 2010). *Solanum nigrum* L is known as Tolungoor in local language Fig. 5.



Fig.5. *Solanum nigrum* L

Tribulus terrestris (*T. terrestris*) is the species of *Zygophyllaceae* family, the plant is widely utilized in conventional Chinese medicine, usually prepared as an infusion and/or dried extract. *T. terrestris* is native from temperate climate regions, its consume is popularized and stimulated due to health claims, such as improvements in symptoms of erectile dysfunction, increase in libido and supposedly promotion of muscle mass gains [34-35]. There are, in scientific literature, case reports in which *T. terrestris* was successfully used to treat renal lithiasis, menorrhagia and rheumatic pain, however, mechanisms of action and phytopharmacological features of *T. terrestris* are poorly understood [35]. Ingestion of *T. terrestris* by physical activity practitioners is stimulated by allegiance that plant metabolites are capable of raising plasma testosterone (TST), consequently inducing greater increases in skeletal muscle mass through its androgenic effects [36]. There are few works exploring the side effects of *T. terrestris* extracts in humans. In a case report from Iran, a young man whom continuously ingested large doses of *T. terrestris* during two days with the aim to treat renal lithiasis was hospitalized for presenting symptoms of hepatitis, neurological disorders and renal lesions. After 22 days of suspending the use of *T. terrestris* and starting specific treatments, the patient has recovered hepatic and kidney functions, also, there was no observable neurological damage. *T. terrestris* is a widespread medical plant used by physical activity practitioners mainly due to commercial claims [37]. *Tribulus terrestris* is known as Gurgunduk in local language Fig. 6.



Fig. 6. *Tribulus terrestris* (*T. terrestris*)

MATERIALS AND METHODS

Five medicinal plants mostly common/dominant species including; (*Acroptilon repens* L. DC), (*Citrullus colocynthis* L. Schrad) , (*Ferula assa - foetida* L), (*Solanum nigrum* L) Plants samples were collected freshly in August and September in 2018 from the field area of Mastung, Balochistan. Mentioned plants were identified by a taxonomist, Dr.Rasool Bakhsh Tareen. All plants samples were first washed, dried and powdered. The samples were utilized for Atomic Absorption Spectrophotometer (AAS) in powdered form. The sample of each plants having (2 g) were taken in 100ml flask and include 32.5 ml in given ratio of Nitric acid (HNO_3), Sulfuric acid (H_2SO_4) and Per chloric acid (HClO_4) (25:05:2.5) These plant samples were immersed for boiling in mixture of acidic media on hot

plate model (JENWAY 1000) under the fuming cupboard to avoid the fumes for direct contact at 80-85 degree Celsius . The digestion sample was observed until white fumes appear from the 250 ml flask. The deionized water added in the running sample and permitted to cool. After that, the extracted sample in 250 ml volumetric flask is shifted and the volume was moved to 100ml for each sample. At that point the extract was filtered with filter paper grade (Whatmann No. 42) and filtrate was gathered in marked plastic containers. The samples were applied to Flame Atomic Absorption Spectrometer (FAAS) PE- an Analyst 700 for elemental detection with suitable hollow cathode standard lamp. The instrumentation Principle of Flame Atomic Absorption Spectrophotometer is shown in Fig.7

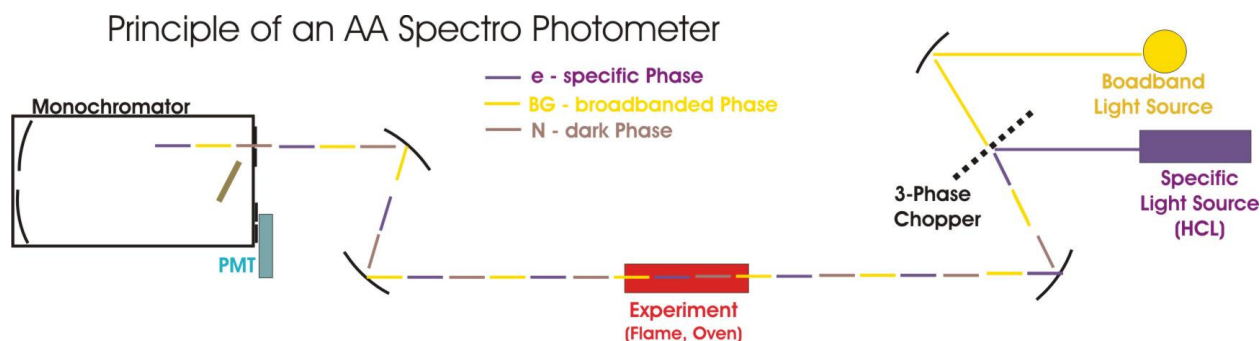


Fig.7. Principle of Atomic Absorption Spectrophotometer

The parameters that were assigned for heavy metals (Cd , Cu , Ni, Pb ,Zn) during the operation on Flame Atomic Absorption Spectrophotometer (FAAS) PE-A Analyst 700 for heavy metal determination in selected medicinal plants

were carried out with AR Grade standards of concern elements shown in Table -1.

Table 1- Parameters used for Metals analysis on Flame Atomic Absorption Spectrophotometer (FAAS) PE-A Analyst 700.

Metal	Wavelength (nm)	Replicates	Fuel flow (L/min)	Flame Type
Cd	228.8	3	10	Air - C_2H_2
Cu	324.8	3	10	Air - C_2H_2
Ni	232.0	3	10	Air - C_2H_2
Pb	283.3	3	10	Air - C_2H_2
Zn	213.9	3	10	Air - C_2H_2

RESULTS AND DISCUSSION

Every element has a significant morphological and physiological impact on biological processes of a living organism. The current research work reflects the concentration of five heavy metals, in the medicinal plants of Mastung region of Balochistan. While doing the quantitative

analysis of selected heavy metals some standard calibration curve was obtained with a prescribed focus range for the standard solution of heavy metals. The unit for the concentration of selected heavy metals was expressed in terms of ppm as shown in Table -2.

TABLE-2.Determination of metals content (ppm) in samples on Flame Atomic Absorption Spectrophotometer (FAAS) PE- AAnalyst 700.

Plant species	Cd Mean $\pm$ SD	Cu Mean $\pm$ SD	Ni Mean $\pm$ SD	Pb Mean $\pm$ SD	Zn Mean $\pm$ SD
<i>Acroptilon repen</i> L. DC)	BDL	0.319 $\pm$ 0.0061	BDL	BDL	0.192 $\pm$ 0.0016
<i>Citrullus colocynthis</i> (L.Schrad)	BDL	0.424 $\pm$ 0.0028	BDL	BDL	0.044 $\pm$ 0.0014
<i>Ferula assa - foetida</i> L	BDL	0.731 $\pm$ 0.0036	0.065 $\pm$ 0.0071	BDL	0.346 $\pm$ 0.0051
<i>Solanum nigrum</i> L	BDL	0.589 $\pm$ 0.0037	BDL	BDL	0.369 $\pm$ 0.0029
<i>Tribulus terrestris</i> L	BDL	0.421 $\pm$ 0.0032	0.024 $\pm$ 0.001	BDL	1.241 $\pm$ 0.0083

BDL = Below Detection Limits SD = Standard deviation

Copper

Cu as an important bio-catalyst role in usual plant development plus growth is, however, at excessive levels, can be toxic. The occurrence of phytotoxicity signals is evident when the concentration is upper than 20- 100 ppm DW (dry weight) in plants. It is recorded in (Table-2). The concentration of Cu was high in *Ferula assa - foetida* L 0.731ppm, followed by *Solanum nigrum* L 0.589 ppm, *Citrullus colocynthis* (L.Schrad 0.424ppm, *Tribulus terrestris* L 0.421 ppm, And *Acroptilon repen* L. DC) 0.319 ppm. The Cu concentration in the chosen therapeutic plants stands low, however, it is elsewhere considered dangerous in herbs [37]. The excess Concentration of Cu may result in indications like discoloration of the skin as well as hair, aggravation of the superior respiratory region, dermatitis and Nausea. World Health Organization [38] has referenced the lower limit of the tolerant range for Cu as per 20 μ g/mg daily [39]. Wilson's ailment (may be a hereditary infection that avoids the body from evacuating additional copper since of copper insufficiency [40].

Nickel

The Ni concentration in different plants was tried within the range of; *Ferula assa - foetida* L 0.065 ppm, *Tribulus terrestris* L 0.421 ppm, while *Acroptilon repens* L. DC), *Citrullus colocynthis* (L.Schrad and *Solanum nigrum* L were found to be below the detection limit of Nickel concentration. Furthermore, Ni has been distinguished as an alleged cancer-causing agent and antagonistically influences lungs and nasal cavities. The deficiency of Ni having serious consequences in the complaint of the liver [41]. EPA has prescribed every day permissible limit of Ni ought to be under 1 mg beyond remains hazardous [42]. Early studies showing beneficial effects of the use of *T. terrestris* were performed by a pharmaceutical company in Bulgaria. Preliminary results have shown that the supplementation raised plasma TST and, consequently, would promote gains in lean mass and strength. Authors proposed that weightlifting athletes would improve performance with *T. terrestris* supplementation and a protein-rich diet, suggesting an important effect caused by the presence of naturally occurring saponins in the plant, which are related to traditional mechanisms and/or androgenic pathways such as the release of LH and TST [43].

Zinc

The Zn was analyzed in excess amount while testing the *Tribulus terrestris* L 1.241 ppm. *Solanum nigrum* L 0.369 ppm, *Ferula assa - foetida* L 0.346 ppm, *Citrullus colocynthis* (L.Schrad) 0.044 ppm, and *Acroptilon repen* L. DC) 0.192

ppm. Furthermore, it is assumed that the zinc act as growth premotor in different cell forms including typical development, mental health, social reaction, and bone arrangement. The Zinc deficiency may cause loss of feeling of touch and smell [44]. The nutritional limit of Zn is 100 ppm [45]. Moreover, the *S. nigrum* has been utilized as hostile to septic, calming, expectorant, cardio-tonic, stomach related, diuretic, purgative, diaphoretic, narcotic, swelling, hack, asthma, in restoring cardio-pathy and hemorrhoids. It is also remarkable in action on cirrhosis related to the liver. This herb squeeze is the best remedy for facilitating torment and reestablishing fever [46]. All the portions of the plant are vital as every one of the parts like seeds, leaves, blooms, have therapeutic value. The juice arranged from the leaves of *S. nigrum* utilized for skin issues and tumors. The seeds are utilized in home restorative, as it helps in evacuating spots by basically scouring the seeds on the cheeks. Decoctions made of stalk, leaves, attaches are known to be viable on the injuries and furthermore on dangerous wounds. An imbue ment arranged by the plant is likewise turned out to be useful in newborn children experiencing stomach issues. The poultice arranged by the leaves is utilized by the neighborhood specialist for the treatment of rheumatic and gouty joints, skin illnesses and furthermore given in the treatment of tuberculosis. The leaves are likewise given in dropsy, queasiness and anxious issue. The juice made of berries of the plant utilized as hostile to the looseness of the bowels. It demonstrates noteworthy outcomes in coronary illness. The berries have tonic and also act as a diuretic and therapeutic stuff. The part like seeds serves as energy and dyspnea. Roots of this medicinal plant additionally similarly critical as they likewise are utilized in the treatment of different infections like hepatitis as well as osteopathy.

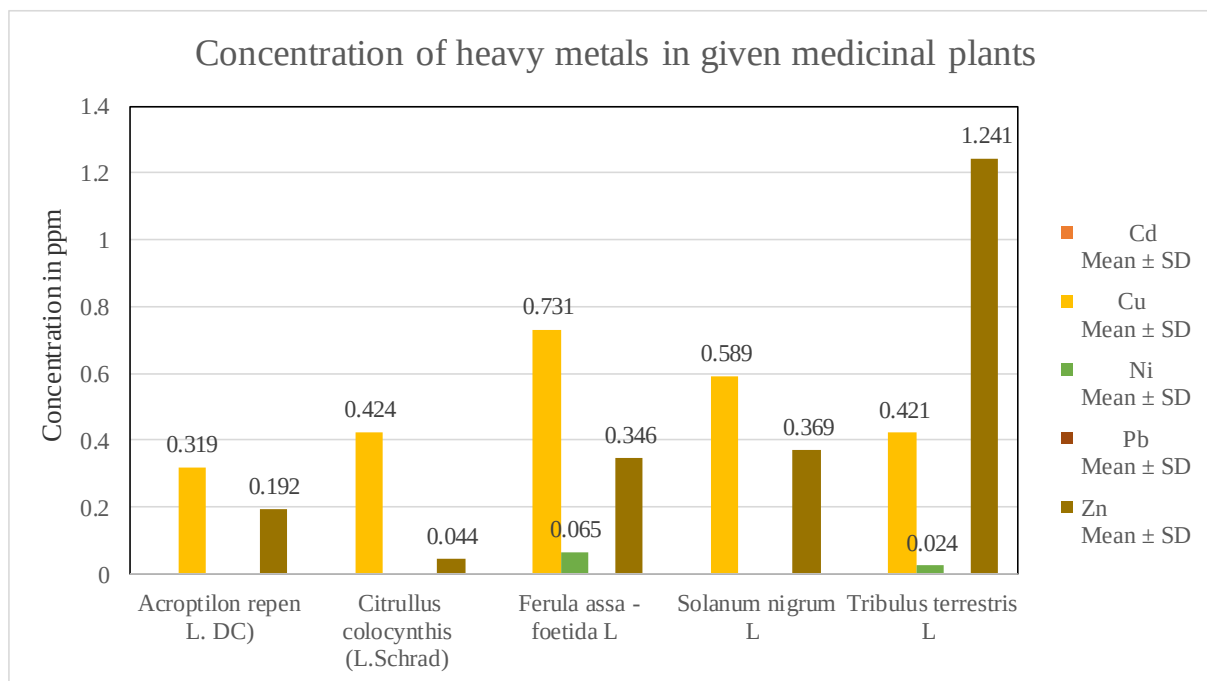
Cadmium and Lead

In the biological process of plants and the human body the lead and cadmium have no significant role while diverse harmless impact in people in low proportions. The common indications of lead harming are pallor, and cerebral pain, seizures and perpetual nephritis of the kidneys, mind harm and focal sensory system issue. WHO [47] recommended limit for Pb substance in homegrown medication is 10 ppm while the Nutritional admission limit for Pb is 3mg/week [48] the Pb and Cd were analyzed in given samples BDL (Below deduction limit). The grouping of substantial metals was essential from the view of phytoextraction. [49].The result of selected medicinal plants for heavy metal determination is shown in (Fig.7)

Fig.7. Shows the concentration of the medicinal herbs in heavy metals in Mastung region of Balochistan.

CONCLUSION

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The therapeutic herbs, as responses to a group of diverse conditions in the regular course of action for medication are suggested. In phototherapy, enormous progression was documented concerning the consistent approximation of therapeutic herbs around the globe. The sensible consequence for changing condition might stand in the WHO profiles, Nationalized Pharmacopeias as well as plants dealing with endeavors. Most prominent merging of Zn (1.241 ppm) in *Tribulus terrestris* L. Cu (0.731ppm) in *Ferula assa-foetida* L, and besides Ni (0.065ppm) in *Ferula assa-foetida* L were dissected in given plant samples Lead and cadmium were poisonous and toxic. The consequences of revelations may be pondered while manipulating the medicinal herbs aimed by human being tradition. The research findings recommend the remedial plants treatment for intended for anthropological practice and provide the baseline for safe limit values of selected heavy metals to sustain the natural environment from hazardous signals as well as a biological indicator.

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