STUDY OF HEAVY METALS IN PLANT OF COUSINIA (NIRIANBAND) FROM KHUZDAR IN REGION OF BALOCHISTAN, PAKISTAN

Sadia Baqa[1], Manzoor Iqbal Khattak[1], Mahmood Iqbal Khattak[2], Rukhsana Jabeen[3]& Saddullah Laghari[4]

[1]Chemistry Department, University of Balochistan, Quetta.

[2]PCSIR Laboratories, Peshawar.

[3]Botany Department, Sardar Bahadur Khan Women University, Quetta.

[4]Botany Department, University of Balochistan, Quetta.

ABSTRACT*: The objective of this study is to determine the level of heavy toxic metals Fe, Zn, Cu, and Mn in Cousinia (local name Nirianband) plant species grown in the surrounding area of Khuzdar in Balochistan, Pakistan. The metals (Fe, Zn, Cu, and Mn) constituents in the adjacent parts of excavation significantly surpass the assortment which plays a very important role in the treatment of hepatitis C. The results were discussed and concluded along with a recommendation in light of the earlier studies.*

Keywords: Heavy Metals, Medicinal plants, Atomic Absorption Spectroscopy & Pollution.

1. INTRODUCTION

Heavy metals make a significant contribution to the environment, as a result of human activities, such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumping, and melting operations [1]. All heavy metals at high concentrations have strong toxic effects and are regarded as environmental pollutants [2]. Phytoremediation that uses the remarkable ability of plants to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues appears very promising for the removal of pollutants from the environment [3]. Since most of the plant roots are located in the soil, they can play an important role in metal removal via filtration, adsorption and cation exchange, and through plant-induced chemical changes in the rhizosphere[5-6]. There is evidence that plants can accumulate heavy metals in their tissues such as Sebera acuminate and Thlaspi caerulescens [7], Arabidopsis thaliana [8], Typha latifolia, and Phragmites Australis [9]. T. Latifolia and P. Australis have been successfully used for phytoremediation of Pb/Zn mine [10]. Metal accumulation by plants is affected by many factors. In general, variations in plant species, the growth stage of the plants, and element characteristics control absorption, accumulation, and translocation of metals. Furthermore, physiological adaptations also control toxic metal accumulations by sequestering metals in the roots [11]. As a result, metal removal by vegetation can be greatly enhanced by the judicious selection of plant species.

A large number of species have been placed in the genus *Arctium* at one time or another, but most of them are now classified in the related genus *Cousinia*(Local name Nirianband). The precise limits between *Arctium* and *Cousinia* are hard to define; there is an exact correlation between their molecular phylogeny. The *Cousinias* are occasionally muddled with the "cockleburs" and "rhubarb".The general image of the plant of *Cousinia* (Local name Irian Band) *is given below in Figure-1:*

Foliage belongs to the genus_*Arctium* possesses a murky greenish appearance that can develop up to 28 inches elongated. Usually, these plants have a huge, bristly, and ellipsoid appearance and the plants grow in small size possess heart-like form. They are crocheted on the lower portion.

Figure-1. The general image of the plant of *Cousinia* (Local name Nirianband).

The leaf-straws are commonly resonating. *Arctium*_species usually undergo flowering from July-October. *Cousinia* florets deliver vigorous pollen and nectar for honey-bees post month of July when clover is on the decline and afore the golden-rod twitches to blossom [12].

Flowers' sprouts that are not fully developed and matured, perhaps get roped in late spring, at that time when fluorescence hasn't completed; they savor bear a resemblance to that of "artichoke", to which the *Cousinia* is associated. The sprouts are methodically peeled, and whichever consumed unprocessed or simmered in saline H_2O [13]. In Japan when the spring season is commenced the floral leaves are also getting used for consumption at the time of the maturation phase of the plant and leaves are softly in touch. Fewer *A.lappa*_cultivars are specified for this task. A widely known famous Japanese food item is kinpira-gobō, julienned or threadbare burdock-root and carrot, casseroled with soy sauce, white-sugar, mirin/sake, and sesame oil.

After 1950, the global acknowledgment was being started giving to burdock for its cookery application because of the increments in the eminence of the whole grain food, which promotes its intake. It comprises a nondiscriminatory aggregate of dietetic fiber (G.D.F;6g/100g), proteins, vitamins, and minerals [14], and is lessen in energy for consumption. It comprises a poly-phenol-oxidase [15], due to which its surface gets murky, opaque, and rugged by developing tannin-iron complexities.

Basilar metalloid forbearance is ostensibly occurring in entire species of flora. In this manner, they operate a

sequence of intricate coordinated comprising of intake/outflow, transportation/confiscation, and sequestration (Fig:2). These determining factors comprise compactly in the metabolic equilibrium of vital metallic micro-elements. The approximate characteristic of these fundamentals diverges the Kingdom_Plantae into 2 categories: hyper-accumulating and non-accumulating ones.

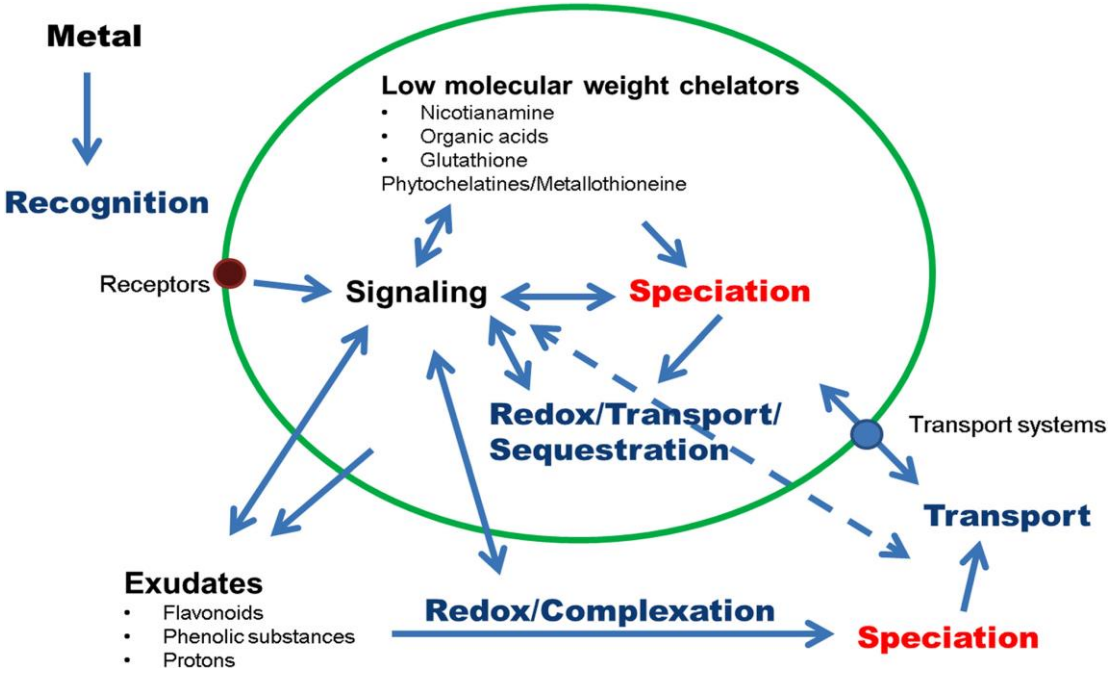

Figure-2. Label diagram for the accumulation level of metals by plants.

This appraisal will deliver a synopsis regarding these forbearance systemic procedures with emphasis on the cellular point and gesticulating alleyways persuaded by metallurgic alloys. The conversation of few instances will accentuate the horde and intricacy of indications and reactions. It will activate additional effort on responses in the direction of metallurgic alloys in florae especially in the track of diminutive, ecologically correlated metalloid applications.

A great number of plants can be categorized as hyper-accumulating and non-accumulating. However, entirely have to deal with heavy metalloids for dietetic aims and developing in metallurgic and ferric topsoil, correspondingly.

Therefore, they must retain terrifically modified systemic approaches for existing with noxious metallurgies [16]. The much-uncomplicated stratagem is to elude metallic consumption from topsoil or to eliminate it by averting metalloids' undertaking into sprouts. Furthermore, essential components for the procurement and confiscation of vital metalloids are repeatedly applied. On the other hand, this can produce interventions with the plants' metallurgic internal and metabolic equilibrium and perhaps persuade poisonous symptoms. These symptoms are multifold, for an assessment perceives [17]. They encompass losses of chlorophyll production subsequently in chlorate leaves, altered proportions of chlorophyll a&b photosynthetically [18-20, 21], pycnodysostosis of foliage, or influences on fine-structural root [22].

On the other hand, yet it's not evident if these effects whichever are the basis or resultant of metabolized trepidations in heavy metalloids disclosed foliage. Henceforth, it is indispensable to inspect metallurgic lenience and noxiousness in cells and molecules.

An idiosyncratic circumstance of metallic forbearance is hyper-tolerance. Metallic hyper-tolerant plants excluding hyper-accumulators are capable to eliminate metalloids from their cellular fluids with the purpose of reducing metallurgic accretion particularly in their surface tissues [23]. This is the fundamental distinction to hyper-accumulating floras. However, metalloids' hyper-accumulation is connected with metalloids' hyper-tolerance disclosing an additional approach of purification.

Metallic alloys in hyper-accumulating plants are categorized by the quotient of metallic accrual in plants' shoots and roots>1[24]. This exceptional metallic accrual is accomplished via: (1) Over-production of transportation coordination necessary for improved confiscation, (2) Systematic polypeptides' production in cellular tissues, (3) Increased levels of heterocyclic compounds in metalloids.

Proteomic research works disclosed that metallurgic hyper-accumulation in Arabidopsis-halleri has been linked with exceeding 30 entrant genes which are excessively communicated in comparison with the non-accumulator A. thaliana [25-26]. Pence et al. [26] could demonstrate that the Zn^{2+} carrier ZnT1 is over-produced in the hyper-accumulator Noccaea-caerulescens in comparison with the non-accumulator_N.arvense. Protuberant illustrations are metallic thrusts associating to the P1B-adenosine-triphosphatase (heavy-metalloid-ATPases, H.M.A) carrier group [27]. These carriers are capable to transport various metalloids. If metalloids get in the cytoplasm consequently they will be attached by an applicable cellular compound instantaneously. This eludes the management of probably poisonous, free-cellular metallic electrolytes and delivers

an organized contribution in metabolized paths for instance particular integration in metalloproteins or purification. Binding agents for metallic electrolytes are frequently reduced molar mass compounds, an inclusive assessment was presented by [28].

Widely known cellular metalloid chelators are nicotine-amine (N.A) and carbon-based acidic components e.g. citriodora [29-30]. Nicotiana-amine demonstrates extreme constancy coefficients for the fusion of transfiguration metallic +ve ions [31] and is necessary in A.thaliana to sustain homeostatic equilibrium by essential minerals and vitamins [32]. Formerly published chapters though stated the amounts of these chelated compounds are greater in hyper-accumulators compared to closely interrelated non-accumulators [33]. Citriodora is the prime binding material for Zn^{+} in leaves of N.caerulescens [34], however, additional carbon-based acidic compounds e.g. malic acid ($C_4H_6O_5$) are too allied with metallic toleration. In recent times it was published that citriodora accelerates uraninite (green salt) displacement from root to shoot being intrusive in Fe^{+} and Zn^{+} transport [35]. This emphasizes the significance of carbon-based acids for transportation and confiscation of metallic electrolytes in various tissues and cellular sections in plants such as the vacuole [36-37] intermingle metabolically and consequently are capable to produce indicators. Thus, the correspondence of biochemical aspects and impacts of various metalloids produce influences crucially and particularly amid vital and non-vital metallurgies. Though, this sort of communication doesn't inexorably deliver an undesirable influence for the floras at every stage. For example, Arabidopsis or carrot foliage demonstrated an observable healthier root extension when developing and rising with uraninite's modification [38-39] perhaps because of the evacuation of chemical compounds based on phenolic acids into the rhizosphere which can excite root extension [40]. This illustration demonstrates additionally the prominence of examinations vis-à-vis entire communication linkages of metallic metabolism [41-42]. Henceforth, it appears to be noticeable, that essentials of octadecanoid passageway intermingle in metallic induction of indications and even though perform in potential defense reactions. It accentuates the straight directive of metallic homeostasis in an extensive channel comprising of several fundamental particles.

The awareness regarding the capabilities of various types of plants to captivate and transfer metallic alloys in the influence of various circumstances will deliver awareness while selecting suitable floras for phytoremediation of the contaminated areas. In Baluchistan, there are several metallic excavations and the procedure of metallurgic excavating has produced serious metallic effluence. Our initial inquiry at metalloid's excavation found that a high number of foliage may perhaps prosper in the crippling effluence of metallic ores in the earth. Therefore, the quantities of Fe, Zn, Cu, and Mn were inspected in many varieties of Cousinia grownup in the adjoining area of Khuzdar, Balochistan.

The principal goals were:

(a) To assess the capability of various plants' species of wild-life developed in such locations that are full of affluence to accrue and endure Fe^{+}, Zn^{+}, Cu^{+}, and Mn^{+} in their cells. (b) To discover the variance of metallic accretion through the identical plant species when developing on metallic effluent places and unsanitary places; (c) To determine the aspects inducing the metallic accrual via foliage (d) To make conservation on the prospective applications of plants for metallic elimination in unhygienic parts. The aforementioned facts may possibly be referred for selection of suitable foliage inbuilt topsoil management arrangements to accomplish the excluding prospective of metalloids by plants joint with additional biochemical applications.

2. EXPERIMENTAL

The assemblage of specimens and Sampling:

The assortments of plants were assembled in the adjacent area of Khuzdar mine in Balochistan, Pakistan in Oct-2015. The verified samplings were placed at the Chemistry Department of Balochistan University, Quetta, and were labeled. The studied species consisted of various plants' varieties associated with Compositae, forming the extremely prevailing factor in places having maximum effluence. Plants' specimens were systematically bathed with consecutive tap water and cleaned with demineralized H_2O to confiscate the entire topsoil constituent part having an attachment to the exteriors. Over and beneath the ground-level of protoplasm were then detached and incubated in the oven for drying at 158°F at consistent mass. The dehydrated tissue specimens were evaluated and pulverized into triturate for metallic proportion assessments. Metallic alloys (Fe, Zn, Cu, Mn) of the trial specimens were hauled out via acidified assimilation ensued by evaluation of entire applications of whole essential components of interest via applying A.A.S (Spectro-Analytical applications) [43].

3. RESULTS AND DISCUSSION

Though plant's resistance approaches occur to manage with metalloids' toxic effects via decreased incorporation into the cellular sap, impounding in the vacuoles via the production of complex structures, fused by Phyto-chelators, production of Amphiphiles, initiation of several deoxidizing biochemical to battle R.O.S, transformed enzymatic production, overproduction of existing genetic material, [44] applications via sprouting seeds fight metallurgic pressure remainders broadly unidentified. The prospect opportunity of this evaluation persists in consideration of the biochemistry of heavy metallurgic toxic effects in sprouting seeds. Comprehending such approaches in seeds to overwhelmed such pressure and operation of alleyways and bio-molecules included will lead to healthier agronomic productions regardless of heavy metalloids' toxic impacts from polluted earth.

Amounts of metallic alloys (Fe, Zn, Cu, Mn) in plants' tissue assembled from the contaminated parts (shoots&roots, distinctly) are publicized in Tab:-1 and Fig:-3. The statistics demonstrate that the metallic particles in the cellular tissues fluctuated amid various plants at the contaminated place demonstrating their diverse dimensions for metallic incorporation.

Table-1. Heavy metals level (mg/kg) in the plant of Cousinia

S.NO	Name of Plant	Fe	Mn	Zn	Cu
1	Cousinia	0.76	1.09	0.14	1.44

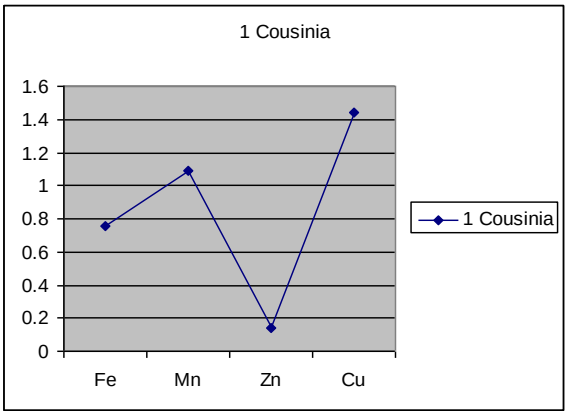

Figure-3. Heavy metals level (mg/kg) in the plant of Cousinia

The displacement aspect, the proportion of metallic incorporation in roots&shoots, specifies interior metalloids' transference. The records displayed designate that metallic accumulation via the anticipated foliage was widely reserved in the plant of Cousinia. While the same foliage' species were developed, and in the substrate, there was a noticeable difference in E.C figures, p.H, aggregate P, and metallic constituents.

The alteration in metallic incorporation b/w plants' kinds echoed the position of their accompanying earth which is connected to the metallic application that could possibly offer impacts by several factors that will be discussed far ahead. Metallic proportions in plants' sorts tracked the direction of Mn>Cu>Zn>Fe for Cousinia plant.

Plants' sorts fluctuate extensively in their capability to accrue metallic alloys. Fig:3 demonstrates that the root's cells accrue greater applications of metallurgies than sprouts, which specified exceeding plant accessibility of the metallic substratum, in addition to the internal restricted movement of the plants. This is constant with erstwhile interpretations [45-46].

Fitzgerald et al. [45] experimented that mono-cotyledonous foliage possessed excessive applications of Pb^{+} in the roots related to shoots. In contrast to the assortments of metallic proportions in the piles of earth and in the under-leveled tissues, the amounts of Fe, Zn, Cu, and Mn in shoots were preserved at decreased intensities. The resultant figures publicized here to recommend that this metallic toleration approach is broadly progressed and occurs in foliage when they propagate in the areas high in metallic effluence. The raised metallic levels in the under-leveled tissues and little displacement to the above-leveled tissues in the species observed may perhaps correspondingly recommend that they are proficient in somewhat well-proportioned incorporation and displacement of metalloids underneath comprehensively metallurgic contaminated situations. It was specified that a proportionated and largely autonomous electrolytic financial plan is characteristic of several grass-like types and is hypothetically somewhat generate their extensive environmental generosity [45]. The intensities of electrolyte magnitude lethal to florae are beyond 500lg/g ([48]. The resultant facts and figures specified that foliage developed in Fe^{+} polluted sites frequently preserved greater applications. Zn^{+} is an indispensable constituent to entire plants the average proportion of which in conventional florae (above-leveled tissues) is 66l.g/g [45] and the toxicity point is exceeding 230l.g/g [49].

The assortments of Zn+ in plant species demonstrated here were normally greater than the intensities recounted for additional evolving foliage. Cu^{+} is likewise crucial to the development of plants; however, will deliver lethal influences when shoots/leaves accrue Cu^{+} levels exceeding 20l.g/gCu^{+} [49]. In this analytical research work, excessive Cu^{+} figures were recorded. The maximum Cu^{+} proportions in the above-ground of C.botrys were 185l.g/g (Fig.2b). Mn2+ in traditional standard florae is b/w 200-300l.g/g and poisonous ranks to foliage are b/w 300-500 l.g/g [48]. The statistics for Mn2+ in plants attained in the present research-based activity were analogous to this assessment and disclosed huge modification amongst plants and cellular sap. The metallic quotient greater than a lethal point in fewer species specifies that inner metallic disintoxication forbearance approaches may perhaps occur in these kinds of plants, additionally to their elimination stratagems [50].

Plants fully-grown in substrates having metallurgic amelioration consume metallic electrolytes in fluctuating gradations. This incorporation is widely affected by the bio-availability of the metalloids which is consecutively assessed by both exterior (soil-linked) and inner (plant-linked) aspects. In accordance with the analytical findings of this research, the Pearson correlation coefficient was measured b/w metallic proportions in the above-leveled and under-leveled tissue. Maximum former investigational works have displayed merely weak associations' b/w metallic incorporation by foliage and topsoil applications [51-52].

Keller et al. [52] conducted an inspective work on metallic incorporation, both via roots& leaves. It was evaluated that this wasn't correlated in a linear arrangement with the increments in outer metallic levels. In this analytical work, entire Cu+ and Zn+ on earth had an affirmative and progressive association with any portion of Cousinia species. This association may perhaps interpret in rapports of the chemical specification of the metals in the soil and competition between metal ions and protons at the plant–soil-water interface. Momentous undesirable associations are found b/w P^{-} and Fe+ matters in every fragment of Cousinia_sp.

Orthodox herbalists' consideration was found for the seeds of *A.lappa* and dehydrated Cousinia to be a suitable treatment for kidney impairments, diaphoretic, and a plasma refining mediator. Cousinia is a customary therapeutic herbal medication applied to treat several disorders. Cousinia's root-oil excerpt, correspondingly known as Bur_oil, is consumed for scalp treatment in many European countries. Up-to-date research-based works specify that burdock's root_oil excerpt is enriched with phytosterols and indispensable fatty-acids [53].

Undeniably, it may perhaps the discrepancy and collaboration of all these aspects and above arrangements of heavier metalloids [54] and biological proportion [6]. Phyto-remediation is stated as the application of green florae to eliminate, enclose, or reduce conservational pollutants innocuous [7]. Phyto-remediation of metallurgic alloys can be categorized into 3 categories: the i) Phyto-extraction: metallic accruing foliage are formed on polluted earth and far along reaped so as to eradicate metalloids from the topsoil; ii) Rhizofiltration: roots of metallic accruing foliage assimilate metallurgic from contaminated

emissions and are well ahead reaped to weaken the metalloids in the waste and iii) Phyto-stabilization: metallic alloys are structurally formed in the substrate [34]. Another motive for utilizing florae for therapeutic and remedial influences associates the comparatively less budget and conservation necessities [7].

Phyto-remediation has been applied in excavated earth refurbishment from the time when these soils have become the bases of affluence in atmosphere and H_2O through phytostabilization and phytoextraction methods to produce stability in lethal excavated indulge and eradicate poisonous metalloids from the hauls, correspondingly [55].

In accordance with the variations in metallic incorporations, species become capable to amass comparatively huge metallic quantities in the above-ground tissues might perhaps righteous contenders for phytoextraction. In accordance with the demonstrated results as displayed in Tab-1. In the context of toxicological science, garnering plants could be an appropriate asset, as metalloids wouldn't get inside the food-chain through plant consumers and consequently evade probable threat to the environs. Reaping plants won't be an operational cradle of metallic alloys' exclusion in metalloid-contamination coordination and amongst the aforementioned phytoremediation systematic approaches; phytostabilization possibly be the utmost applicable to treat therapeutically excavation investigations and additional metallurgies polluted soils. Moreover, for examination, the subjected plants' species in this research work cultivated excellently and advanced rapidly in a substrate having diminutive nutritional status which would be a huge benefit in the re-vegetation of excavation investigations as the budget would be condensed deprived of manure and compost.

In this research, study monitoring was carried out on foliage emerging in a polluted place to evaluate their prospective for metallic accretion. The outcomes illustrated that maximum specimens can produce colonization in the places having high metallic effluence, engross an extensive assortment of metallic alloys present in the earth (Fe, Zn, Cu, and Mn), not be exaggerated by extreme metallic constituents, and may perhaps retain metallic resistance competences or greater toleration than additional susceptible plants. As metalloids' proportions in sprouts are generally sustained at diminutive points, metallurgic toleration in forest foliage may primarily rely on their metallic eliminating capability. Though, the metallic applications greater than poisonous intensity in fewer plants specifies that inner decontamination metallic toleration practical approaches correspondingly occur; consequently, their effectiveness for phytoremediation is potential.

Findings among variants exposed the aspects of persuading metallic incorporation in the field. This indicates that by monitoring and regulating the earth's micro-nutrients, macro-nutrients, and additional associated aspects, metallic elimination through foliage would be dissimilar and could be operated for both phytostabilization and phytoextraction. It also recommends that complete contemplation of collaborations between soil and plants should be taken into explanation while selecting plants and appropriate situations for emerging bio-remediation approaches for instance phytoremediation.

4. CONCLUSION

Virtually entire floral kinds display organic metallic toleration when fronting metalloids. Though, usual forbearance approaches are centered on elimination, chelating agents, requisitioning techniques, metallic accretion, and toleration in plant species. In the current investigation, it was publicized that some foliage can generate colonization in a comprehensive assortment of metallic applications. The metals (Fe, Zn, Cu, and Mn) constituents in the adjacent part of excavation significantly surpass these assortments (Fig:-3); consequently, the Cousinia_species developed in these polluted places have displayed greater metallic toleration. Plants that are genetically contrived possess an enriched accretion of metalloids that are worthy of nutritive determinations (bio-fortification).

5. RECOMMENDATIONS

- It's occasionally suggested to circumvent the gestation period in research findings and investigations on specific fauna that illustrate constituents of Cousinia-species to produce incitement in the uterus [56].
- Distinctive emphasis should emphasize on less, ecologically pertinent heavy metalloids' applications. Consequently, the additional progress of delicate recognition approaches and the mixture of various methodologies are obligatory.
- Consideration should be made on indicator transmission paths persuaded by metalloids as they apply common indication factors that are possibly be correspondingly stimulated by other ecological impediments.
- Clean-up of metallic alloys that are enriched with effluence in soils (phytoremediation) and withdrawal of infrequent metallurgies that are accrued in plants' tissues (phytomining).

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