La raison d'être of in situ electro-mobilization, phyto-extraction and phyto-stabilization of lithium tailing in heterogeneous rhizosphere by *Brassica juncea* and the monocotyledonous plants

Zohrab Keropian

A Thesis

In

The Department of Building, Civil and Environmental Engineering

Presented in Partial Fulfillment of the Requirements for the

Degree of Master of Applied Sciences (Civil) at Concordia University

Montreal, Quebec, Canada

August 2012

© Zohrab Keropian, 2012

CONCORDIA UNIVERSITY

School of Graduate Studies

This is to certify that the thesis prepared

By: Zohrab Keropian

Entitled: La raison d'être of in situ electro-mobilization, phyto-extraction and phyto- stabilization of lithium tailing in heterogeneous rhizosphere by *Brassica juncea* and the monocotyledonous plants

and submitted in partial fulfillment of the requirements for the degree of

Master of Applied Sciences (Civil) at the Department of Building, Civil and Environmental Engineering

complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the final Examining Committee:

_____Dr. A.M. Hanna_____ Chair

_____Dr. S. Williamson_____ Examiner

_____Dr. S. Rahaman_____ Examiner

_____Dr. M. Elektorowicz_____ Supervisor

Approved by _____

Chair of Department or Graduate Program Director

_____ 2012 _____

Dean of Faculty

Abstract

The research tested the *Brassica juncea* ability, to phytoextract and phytostabilize lithium from mine tailings in lieu with vanadium and chromium, sown in a heterogeneous acidic rhizosphere. Five different heterogeneous growth media formulations were prepared from lithium mine tailings, homogenized peat and dewatered municipal biosolids. The *Brassica juncea* was grown for eighty six days, under homogeneous growing conditions, irrigated bi-daily with organic fertilizer, amended with LiCl, harvested and chemically analyzed. The phytoextraction and phytostabilization data revealed that the *Brassica juncea* was capable of absorbing more vanadium in its physiological parts rather than lithium and chromium. Likewise the monocotyledonous plant was grown homogeneously on the most favorable growth media, amended with lithium chloride and was able to phytoharness and phytostabilize more lithium rather than chromium per dry weight basis. In botanical efficiency parameters the monocotyledonous plant was ten times more efficient than the *Brassica juncea* in the bioaccumulation and efficiency removal rates for lithium and twice as much as for chromium. The relative growth rate of the monocotyledonous plant was twice as much as the *Brassica juncea*. Moreover, it surpassed the monocotyledonous plant in translocation indexes for chromium more than six times and twenty times for lithium. The findings revealed the possibility of a three way symbiosis formed between the hyperaccumulant plant grown in a heterogeneous rhizosphere and coupled with EK system at certain growth periods that will result in an increased electromigration and electrophoresis of heavy metals in the growth media solution.

Keywords: *Brassica juncea*, monocotyledonous plant, lithium, vanadium, chromium, phytoharness phytostabilization, phytoextraction, growth media, organic fertilizer, peat, biosolids, lithium

mine tailing, relative growth rate, bioaccumulation ratio, translocation index, efficiency of removal, electrokinetics, electromigration, electrophoresis.

Acknowledgement

First and foremost, I would like to thank my academic advisor and mentor at Concordia University, the Department of Building, Civil and Environmental Engineering, Prof. Maria Elektorowicz. This thesis could not have been written without who not only served as my supervisor but also encouraged and challenged me throughout my academic program, never accepting less than my upmost efforts. In addition, this study was supported graciously by the NSERC Discovery Grant awarded to Dr Elektorowicz. I owe a sincere and earnest thankfulness to Prof. Dallas Kessler, Chady Stephan, Claude Devreau, Ritch Nally and the "Agriculture and Agri-Food Canada" research branch in Saskatoon, SK. for the provision of the *Brassica juncea Czern* var. Cutlass accession CN: 46238 seeds.

Finally, I would like to dedicate my thesis to my parents and especially to my Mom for her endless love and dedication towards my graduate studies. It is a great pleasure to express my heartfelt gratitude to everyone who helped me achieve my goals specially to the members of my 'special forces' team including my brother Dr. Chant and my two lovely sisters Ani and Jasmine.

LIST OF TABLES

LIST OF FIGURES

The Acronyms

The Acronyms	The complete terminology
Temp	Temperature
Det of M	Determination of moisture
Det of OM	Determination of organic matter
AAS	Atomic absorption spectrometer
ORP	Oxidation reduction potential
TPB	Tailing Peat Biosolids
DNA	Deoxyribonucleic acid,
rRNA	ribosomal RNA,
NADPH	Nicotinamide adenine dinucleotide
tRNA	Transfer RNA.
df	Dilution factor.
PEP	Phospho-enol-pyruvate.
Ini	Initial
Lea Or L	Leachate,
GM	Growth Media.
Homo	Homogenous
Di	Digestion
LMT	Lithium mine tailing.
A	Amperage.
EK	Electrokinetics.
DC	Direct Current.

V	Volts.
Ref	Reference.
DW	Dry Weight.
SD	Standard Deviation.
CL	Cathode Leachate.
AL	Anode Leachate.
M-GM	Middle Growth Media.
A-GM	Anode Growth Media.
C-GM	Cathode Growth Media.
MC	Monocotyledonous.
DW	Dry Weight.
N/S	Nitrogen to sulphur ratio.
ppb	Parts per billion.
ppm	Parts per million.
De-B	Dewatered biosolids.
CWP	Column wash procedure
E of R	Efficiency of removal
BAR	Bioaccumulation ratio
RGR	Relative growth rate
TI	Translocation index
AMD	Acid mine drainage
PPE	Polypropylene
EK-GM	Elektrokinetics-Growth Media

Table of Contents

Chapter One: Statement of the problem

Excessive anthropogenic activities resulted in the degradation of the biosphere. Mining industrial discharge, smelting of metalliferous ores, domestic sewage and agricultural runoffs aggravated the situation producing toxic pollutants. Counterbalancing this situation enabled the enactment of strict environmental policy guidelines and regulations. Fulfilling theses guidelines paved the way for the development of novel green technologies.

Lithium as a cornerstone component of green technologies, its usage and production skyrocketed. It is mined from brine or rock sources and rendered in chlorite or carbonate form. Its annual increase rate reached 5.1% or around 25 tons in 2008. It is projected to reach 7.7 tons in year 2020. Moreover, its predicted increase will come from its usage in electrical vehicles (EV-s). According to Mohr *et al.* 2012 an EV battery of capacity 20kWh encloses 3 kg of lithium.

As a result the environmental flow of lithium will dramatically increase, added upon secondary sources like pharmaceuticals products, lubricants, certain chemical and dyes. As a result it will have its negative impacts on human quality of life and ecosystem biodiversity alike. Moreover, in some lithium dissipative applications its recovery is extremely difficult due to the absence of technical or economic reasons. This might lead to bioaccumulation in nature and end up in human food chain.

According to Fellet *et al.* 2011 and Grangera *et al*. 2011, lithium extraction process produces hazardous heavy metals in its tailings. As an example elevated amounts of vanadium, chromium, iron, etc. In addition, under certain environmental conditions they might get weathered producing toxic effluents. This will have detrimental impacts on ecosystem biodiversity.

Having stated the negative impacts of lithium mine tailings as a major source of toxic heavy metals. Added upon its very low fertility, minimum water holding capacity and unfavorable physical structure, urged a novel way of approach in rendering it suitable for reclamation and vegetative growth

purposes. Through the addition of one or more additives or ingredients that will improve its stability, fertility and water holding capacity.

Objectives

The research was centered on the hypothesis that a hyperaccumulator plant has in vivo ability to phytoextract and phytostabilize lithium, inside its physiological parts. It was supposedly grown homogeneously, in a heterogeneous growth media. In lieu with heavy metals that might have been present in its rhizosphere.

Detailed objectives

- ✓ To investigate the hyperaccumulator plant (e.g. *Brassica juncea*) ability to phytoextract and phytostabilize lithium in a wide pH spectrum.
- ✓ To evaluate the effectiveness of *B. juncea*'s phytoextraction and phytostabilization of lithium, in lieu with vanadium and chromium present in the growth media.
- ✓ To analyze the vanadium, chromium and lithium phytoaccumulation in different plant physiological parts of the *Brassica juncea*.
- ✓ To analyze the different heterogeneous rhizosphere formulated in terms of macro/micronutrient storage and availability to the hyperaccumulant plant uptake.
- ✓ To verify the suitability and effectiveness of the different growth media for phytoextraction and phytostabilization purposes.
- ✓ To evaluate the monocotyledonous plant ability to phytoextract and phytostabilize lithium and chromium in the most favorable growth media.
- ✓ To compare between the monocotyledonous and the *Brassica juncea* hyperaccumulant plants, in terms of botanical efficiency parameters in the most favorable growth media.

Phase one: literature review

1.1.1 Introduction to phytoremediation

The importance of detoxification of the contaminated sites is taking the front seat of action nowadays. The traditional environmental engineering measures are very costly and impacts negatively on environmental biodiversity. It is roughly estimated that the cost of cleaning an acre of soil in the United States costs over a million dollars. Regarding this astrological cost and biodiversity loss, favors phytoremediation as a viable alternative. [1,2,3]

The process of extraction heavy metals or organic pollutants from soil, water and air media through the usage of green plants defined as phytoremediation. In common terms it is referred to as environmental restoration. However, biologists have classified certain plant families that have special abilities that entitle them suitable for remediation. The science of phytoremediation is very recent and evolving very fast. Moreover, the phytoremediation process is a 'marriage' between numerous multidisciplinary approaches like molecular chemistry, biology, soil science, plant physiology, genetics, plant selection, etc. [4,5]

According to Marchiol *et al*. [6] the ideal hyperaccumulant plant should have the following traits. First it must be able to hyperaccumulate the desired metallic element more than one percent of its dry weight basis. Second it must tolerate the desired heavy metals without showing clear toxicity symptoms. Third it must be a fast grower, high biomass producer and easily harvestable. [6] Hyperaccumulant plants are capable of phytoextraction of heavy metals like Cd, Cr, Pb, Co, As, Se, Hg and U. They might have no biological benefits to the plant itself. These plants are also capable of storing heavy metals inside their upper or lower harvestable physiological sites, leading to the removal of the targeted heavy metals from biosphere. The harvested plants are dried and ashed. The recovered

heavy metals are reused or recycled if the technology permits; otherwise its ashes are buried in a landfill. [4]

The hyperaccumulant plant ability is limited to the rhizosphere level of the contaminated soil profile, which is determined by the root penetration depth. However, its advantages are reflected in its ability to preserve the environment and surrounding ecosystem in comparison with different in or ex-situ engineering procedures. Phytoextraction typically costs 40% to 50 % less than any other engineering interventions, like precipitation, flocculation, sedimentation, ion exchange, reverse osmosis and microfiltration. Consequently altering and degrading the biodiversity at the expense of contaminant removal [7]. On the contrary, phytoremediation is easier to manage, it is an autotrophic system that produces large biomass that requires little attention, nutrient input and is generally approved by the public perception primarily to its aesthetic and eco friendly approach [8]. Some common hyper-accumulators or phytoextractors are *Brassica juncea* (Indian mustard), *Pelargonium* (geranium) and *Helianthus annuus* (sunflower); and many others.

Phytoremediation includes phytoextraction which is the extraction of the pollutant or the heavy metal from soil. Phytodegradation or phytotransformation refers to the symbiotic relationship between the rhizospheric microorganism and the hyperaccumulant plant roots. That decomposes the organic pollutant to water and carbon containing compounds that are later used as plants nutrient. Rhizofiltration is referred to plant root uptake of pollutants from aquatic environments. Phytostabilization is the usage of the extensive root network of the hyperaccumulant plant to stabilize the soil profile and render the pollutants immobile. Thus decreasing the effects of erosion and leaching possibilities of the pollutant in the environment. According to Salt *et al*. [9] they proved that phytovolatilization is also possible. It is defined as the absorbance of pollutants or heavy metals from soil or aquatic media and its direct volatilization towards the atmosphere.

The transport cycle starts from the roots of the hyperaccumulant plant. The pollutant passes through different channels and membrane gates towards the upper physiological parts like the leaves, flowers and the seeds. According to Baker [10], a few of the plants posses what is referred to as plant-mineral barrier, which enables it to distinguish between different metals for uptake or not. [10]

Sometimes chemical similarities play a pivotal role in mineral uptake and storage. Besides absorbing different micro or macro nutrients for growth, maintenance and reproduction purposes, the hyper accumulator plants posses the ability to accumulate more than one percent of its dry weight basis the toxicant concerned. [10,11]

According to Chaney *et al*. [12], it is possible to accelerate the whole process of phytoextraction through different plant breeding programs. Selection, cross breeding and genetic engineering processes, which might pave the way for commercialization of the naturally occurring hyper accumulator plants.

Hyperaccumulator plants mostly belong to *Brassicaceae* (ex. Indian mustard), *Euphorbiaceae* (ex. Phyllanthus), *Asteraceae* (ex.pantaclia) and *Fabaceae* families. Presently at least forty five families are classified as phytoextractant. [12,13,14,15]

Phytoextraction is accomplished by two methods. First chelate assisted phytoextraction is applied to heavy metals of upmost toxicity like chromium, arsenic, uranium, plutonium etc. According to Huang *et al*. [16, 17] and Jørgensen [18], they showed that there are not suitable plants to extract Pb, Cd and U from soil in sufficient amounts. They are usually accumulated around 0.01% to 0.06 % of their dry biomass. Moreover, synthetic chelates like EDTA (Ethylenediaminetetracetic acid) was applied to lead its phytoaccumulation increased 100 %, likewise to EGTA to cadmium and citrate to uranium. The procedure of chelate application is done when the plant has reached its optimal growth stage.

However; a chelate is added to soil and left for optimal metal extraction to occur. Afterwards the plants are harvested, dried, burned and its ashes stored. [16,17,18]

It is estimated that a specific hyperaccumulator plants can extract between 180 to 530 $kg \cdot ha^{-1}$ of lead per year, making a 2500 $mg \cdot kg^{-1}$ contaminated site, decontaminated achieved in less than ten years of continuous planting. [17,19]

Besides its numerous advantages the chelate assisted phytoextraction, has numerous disadvantages. As an example it increases the mobility of the contaminant in the soil profile, which might lead to increased leachate leading to ground water contamination. Therefore, a careful examination and assessment of the proposed site is of upmost importance. The second approach is the continuous phytoextraction method which was followed throughout the research. Basically it is based on the specific hyperaccumulant plant to extract the desired amount of the toxicant throughout its life cycle from early germination to full maturity and early senescence. The continuous phytoextraction process appear to be cost effective, non intrusive, socially accepted, aesthetically pleasing phytotechnology with great potential for remediation of heavy metal polluted soils [20]. Thus the accumulation of more than one percent of its dry mass basis was regarded as a benchmark of success. [21]

Besides being based on its genetics and physiological traits, hyperaccumulator plants are slow growing, with somehow low biomass producing and the lack of specificity for a specific heavy metal is a major drawback. In order to overcome the previously mentioned shortfalls breeding, selection and other genetic engineering procedures are applied. [22]

Up to now, the assessment of success of a designated phytoextraction process was placed on the pollutant removal. However, the ultimate objective of a phytoextraction process must be not only to remove the pollutant from the soil profile but also to restore soil vigor. Thus rendering the soil profile capacity to re-function again as a vital living ecosystem, to sustain biological growth, promote the

quality of air and water environments, and maintain plant, animal sustainability and overall husbandry. [23]

Hence, indicators of soil health must be routinely assessed in terms of botanical efficiency parameters. Its activity, size and biodiversity of microbial communities must also be taken under careful consideration. This paves the way for a truthful assessment of success or failure of a phyto-reclamation and extraction approach and its implications as a whole. [24,20]

1.1.2 Prerequisites of successful ecological restoration

Successful ecological restoration of mine tailings depends on many factors. It must first prevent its erosion and further degradation. However, many mining sites are characterized by increased acidity. Second it is nutrient deficient and has a poor textural structure is a major drawback, which impedes proper vegetative growth [263].

A noval approach was tailored in the form of addition of additives that were mixed with the lithium mine tailing mass. They were characterizes as being cheap, abundant and readily available. The added additives were the municipal biosolids and peat that compensated the shortcomings of the LMT. The latter improved its texture and water holding capacity and the prior improved its living biota.

1.1.3 *Brassica juncea* " hyperaccumulant plant"

The *Brassica juncea* Czern variety was chosen for the study because of its high phytoremediation potential [28,9]. It has a high efficiency for accumulating numerous heavy metals, in its different physiological parts [9]. Furthermore, the *Brassica* species are adaptable to a wide range of environmental conditions and to different cultivation processes. [11, 28, 19, 29]

On the plant side, the hyperaccumulant plant has a high resistance to heavy metal toxicity. On the rhizosphere level it is known to form different symbiotic relationships within the growth media biota that facilitates metal uptake and proper storage inside its harvestable biomass. [294]

Genetically the *Brassica juncea* family is tetraploid, thus having the double number of chromosomes, which makes it very suitable for genetic breeding and selection procedures. For example the *B. juncea* (AABB genome, 2n = 36), *B. rapa* (AA genome, 2n = 20) and *B. nigra* (BB genome, 2n = 16).

Finally China is considered the original region and varietal homeland with its highest level of differentiation around the province of Sichuan. [27]

1.1.3.1 General description

Brassica juncea belongs to the family *Brassicaceae* and genus *Brassica* or commonly recognized as the mustard family. Despite the variance between the brassicas and the mustards, but throughout the research thesis the umbrella term of Brassica as a general term for all species will be used. The family name reflects the shape of its yellow flowers that have four diagonally opposed petals forming the shape of a cross. Its lower leaves are deeply lobbed and upper leaves are narrow and entire in pale green color, with hairy like outgrowth from it. The leaves and leaf blades ends with a petiole. It is worth mentioning that *Brassica juncea* is an annual crop, cool season, high biomass producer with edible leaves. In addition, the genus *Brassica* makes a major contribution to human diet and to livestock feeds. Moreover its oil is appreciated throughout the world. [25]

Mature plant can reach up to three feet high and produce seed pods that enclose 2.5 cm to 5 cm in length yellow or brown sickle shaped seeds in sac like outgrowths. [26]

The US cultivars are called Southern giant curled, Florida's as Broadleaf with leaf length reaches up to 0.6m wide. Asian cultivars are called green in snow, like the Osaka purple leaf with distinctive red

leaves with white veins, Tai Tau Choi which is very popular in china mostly grown for its roots, which resembles the turnip. In India the very well known varieties are Laha, Lahi, Lahta and Desi Rai. The Abyssinian mustard (*B. carinata*) is planted widely in Ethiopia's highlands and Eritrea. European cultivars are French brown, Bargonde, Tilney, etc.

1.1.3.2 Origin and cultivation

According to Woods *et al*. [28] *Brassica* family plants domesticated were first a few centuries ago in the far eastern Himalayan region. Later on it was spread to Europe and the Americas. Its edible parts are above and below ground like its leaves, flowers and seeds as well as its roots. In the western world it is regarded as a spice crop while in some parts of Asia it is considered as an essential source for cooking oil.

In Canada its cultivation fields are mostly located in the Prairie Provinces like Manitoba, Saskatchewan and Alberta reaching up to 41000 ha of yield range from 900 to 1235 $kg \cdot ha^{-1}$. The principal growing countries are Bangladesh, Central Africa, China, India, Japan, Nepal and Pakistan, as well as some parts of southern Russia. [28]

Brassica plants family cultivation is considered as one of the easiest and less time and effort consuming. Planted usually in early spring or in autumn like done in Florida during the months of September or October and as a secondary crop in January. Major pests and diseases that affect its yield are aphids, cabbage worms, etc. It is considered as a 'hardy' plant, as it can withstand extreme temperature fluctuations as low as – 4 ^{o}C up to 29 ^{o}C. Likewise to extreme rain precipitations of 500 to 4200 mm and to a wide pH spectrum of 4.3 to 8.3.

Like any other oil and glucosinolate producing plant the *Brassica* family needs proper nitrogen to sulfur of 7:1 N/S fertilizer application. It is usually in the form of potash applied 50 to 75 $kg \cdot ha^{-1}$, which is regarded as its comfort zone needs. [29]

Gastronomically, its leaves are consumed in raw salads or cooked like spinach. In Kashmiri and Bengali cooking it is usually pickled and usually is referred to as 'Hum Choy and Sajur Asia'. Its seeds are used as a primarily cooking oil source in some parts of India, Nepal and Pakistan. Moreover, low glucosinolate and erucic acid content varieties are used as additives for cattle, pig and chicken feeds. [26, 28, 30]

Finally, the genus *Brassica* engulfs fascinating varieties of sometimes distinct and complex species like, *B. hirta* the white flowered mustard, *B. juncea*, or the brown mustard, *B. napus* as the rutabaga, Siberian kale, rape seed (canola oil plant), *B. narinosa* as the broad beaked mustard, *B. nigra* as the black mustard grown for, *B. oleracea*, as the cauliflower, the broccoli, the Brussels sprouts, the cabbage, the collards, the kale and the kohlrabi and finally the *B. rapa* the turnip, broccoli raab, Chinese cabbage and the Chinese mustard. [26]

1.1.3.3 Harvesting

The *Brassica juncea*'s growing period stretches for sixty days, depending on the variety and weather conditions. The plant usually harvested before full maturity and fruiting stage, in order to avoid fully ripe and seed shattering. Its harvesting usually achieved early morning, when the entire plant is pulled manually in regions where labor is cheap but otherwise it is achieved mechanically. Entire plants are tied together and left to dry for 4 to 10 days. Extraction of the oil from its seeds is done through rotary mill, expeller or hydraulic processes. Its green leaves are immediately refrigerated for freshness and quality purposes, packed and used as an ingredient in fresh salads and other edible dishes. [31]

1.1.3.4 Benefits

The *Brassica* plants are sometimes used as a cover crop for their rapid growth, very efficient nutrient uptake and storage inside its biomass and fast canopy closure [32]. Lately some species of *Brassicas* have gained renewed interest in their ability for pest management purposes as biofumigant. Its root exudates biotoxins or metabolic byproducts that are believed to contain active ingredient of sulfur like thiocyanates that are regarded as repellent and even toxic to soil borne pathogens and pests, like nematodes, fungi based pests, insects and some types of weeds [33, 34, 35]. Thus it is usually incorporated in soil during tillage and other agricultural activities, usually on the onset of the pest life cycle. [31]

*Brassica*s are also used as winter or even as a rotational crop in vegetable or mixed planted with orchard trees. Moreover, it increases soil fertility through the increase of soil nitrogen content.

As a fast grower and a big biomass producer, it prevents soil erosion, if used as a cover crop. It is estimated that *Brassica*s under normal circumstances can produce up to 8000 lb of biomass per acre, but under stress conditions the biomass production mass decreases considerably.

*Brassica*s are very well suited for nutrient capture specially nitrogen, because of its dense upper physiology [29]. Its roots may reach six feet deep, which plays a pivotal role in soil aeration, aggregate formation and stabilization. [36, 29, 31]

1.1.3.5 Diseases

Brassica family plants are effected from Rhizoctonia (canker and black scurf), Verticillium (common wilt), *Spongospora subterranea* (powdery scab) and *Streptomyces scabiei* (common scab) are its common “enemies”, inflicting serious damage and even plant biomass loss. Nematodes affect greatly its rhyzosphere like *Meloidyne chitwoodi* (Columbia root knot nematode) and *Meloidogyne hapla* (northern root knot nematode) [37, 38].

As for weed infestation that might result in nutrient loss are mostly annual like the pigweed, shepherd purse, green foxtail, kochia, long spine sandbur and barnyard grass. [39]

1.1.3.6 Folk medicine

It is believed to be recognized as a diuretic, aperitif, anodyne, emetic, rube facient and stimulant. Indian mustard varieties are remedies for arthritis, foot ache and rheumatism [40]. Its seeds are used for treatment of tumors in China, also in Africa it is used as galactogogue. Its sun dried leaves and flowers are smoked in Tanganyika in order to pave the wave for spiritual connection. Its ingestion in Africa and in other parts of the tropics imparts mosquitoes through body odor [41].

Its oil produce is used as a counter irritant and also as a stimulant. In Java the plant is used as an anti syphilitic agent. Its leaves if applied on the forehead are believed to relieve headache [41].

In the Korean peninsula its seeds are used against cold, lumbago, rheumatism and stomach disorders and as in regard to the Chinese traditional medicine its fresh leaves are consumed as a cure for bladder inflammation or hemorrhage and its oil produce against skin eruptions and ulcers. [42]

1.1.3.7 Chemical constituents

Brassica family plants are extremely rich in vitamins, minerals, oils and other important constituents which are vital to human life; thus its leaves are high in Vitamin A and C as well as Iron. A 140 g of its leaves provide to an adult human being with sixty percent of his or hers daily recommendation of vitamin A and all the vitamin C requirements as well as the one fifth of the iron daily need; moreover, It contains 24 calories, 91.8 g of water, 2.4 g of protein, 0.4 g of protein, 0.4 g of fat, 4.3 g of carbohydrate, 1 g of fiber, 1.1 g of ash, 160 mg of Ca, 48 mg of phosphorus, 2.7 mg of iron, 24 mg of sodium, 297 mg of potassium, 1825 μg of β carotene equivalent, 0.06 mg of thiamine, 0.14 mg riboflavin, 0.8 mg niacin and 73 mg ascorbic acid. [44, 45, 46]

According to Pryde *et al*. [46] and Knowless *et al*. [44] a 100 g of its root sample is reported to contain 38 calories, 85.2 g of water, 1.9 g of protein, 0.3 g of fat, 8.8 g of total carbohydrate, 2.0 g of fiber, 3.8 g of ash, 111 mg of calcium, 65 mg of P, 1.6 mg of Iron, 447 mg of K, 45 μg of β carotene equivalent, 0.05 mg thiamine, 0.12 mg of riboflavin, 0.7 mg niacin and 21 mg of ascorbic acid.

Moreover, according to Leung [43], a 100 g of its seed is tested to contain 6.2 g of water, 24.6 g of protein, 35.5 g of fat, 28.4 g of total carbohydrate, 8.0 g of fiber and 5.3 g of ash. Seed sterols contain 19.2 % brassicasterol (9.1 % esterified), 23.6 % free campesterol (34% esterified), 57.2 % sitosterol (55.2% esterified), 1.7% esterified Δ-5-avenasterol and a trace of Δ-7- stigmasterol. It also contains the glucosinolate sinigrin (potassium myronate) and the enzyme myrosin (myrosinae), sinapic acid, sinaprine (sinapic acid choline ester), fixed oils 25 to 37 % consisting mainly of glycerides of erucic, eicosenoic, arachidic, nonadecanoic, behenic, oleic and palmitic acids, among others proteins like globulins and mucilage.

Brassica juncea also contains volatile components such as methyl, isopropyl, sec-butyl, butyl, 3-butenyl, 4- pentyl, phenyl, 3-methyl thiopropyl, benzyl and β-phenylethyl isothiocyanates. [44, 45, 36, 46]

Phase Two: Mechanisms of mineral uptake and storage in hyperaccumulator plant

1.2.1 Phyto-uptake and accumulation mechanism

Certain hyperaccumulant plant varieties have naturally developed tendencies to hyperaccumulate certain metals. According to Reeves [47], the elevated amount of metal accumulation is due to their distinct traits of tolerance, genetic composition and adaptation that distance them from the ordinary plant species. These kinds of plants are getting much attention lately and regarded as stepping stone for upcoming value added plants. The hyperaccumulant plants have evolved unique physical traits and distinct enzymatic secretions that enable them to detoxify and precipitate them in benign forms inside their different physiological resting compartments. [47]

It is widely accepted that a few of the hyperaccumulant plants have developed special tissue ligands that chelate with the heavy metal present in the rhizosphere area. According to Reeves [47] this trait is so strong that it enables the leaves of *Alyssum* plant to concentrate high amounts of organic acids like citrate, malate and malonate. Moreover, according to Andrew *et al.* [48], suggested that the hyperaccumulant plant *Alyssum* possess an increased amount of histidine as a ligand that gets complexed with Ni and subsequently translocated it throughout its physiology. [48]

According to Boyd *et al*. [49], the *Straptanthus polygaloides* (grey Brassica) has the tendency to hyperaccumulate Ni in considerable amounts. Thus it can tolerate up to 500 µm of nickel without showing any toxicity symptoms, inside its roots. Furthermore, it was verified that the high concentration of Ni in plant roots behaved as a defense mechanism against pathogenic microorganisms that might cause damage to its roots.

According to Rauser [50], the chelating compounds are mostly composed of peptides with a general structure of […….-GloCys] $_n$-Gly; where n > 1. Thus the “marriage” between the chelating agent and the metal enables the newly formed compound to leave the hyperaccumulant plant root system and concentrate inside its different physiological compartments.

1.2.2 Toxic metal resistance mechanism

The continuous phytoextraction process that the research was anchored upon raised numerous challenges to the hyperaccumulant plant. The plant has genetically developed certain traits and abilities to concentrate the excess toxic metals inside its physiology without affecting it much. It is assumed to be detoxified first and then stored. According to Rugh *et al.* [51], the mercury resistant *Arabidopsis thaliana* hyperaccumulant plant oozes certain types of reductases that efficiently detoxify mercury in a hydroponically grown system.

On the biochemical level different enzymes that interact each other with numerous triggering and blocking mechanisms in the form of reductases, such as superoxide dismutase (SOD) and antioxidant catalase, which detoxify heavy metals. According to Tomsett and Thurman [52], Jackson *et al*. [53], Ernst *et al*. [54], Gwozdz *et al.* [55], proved that heavy metal concentration increased the SOD activity to a certain plateau and decreased thereafter, leading to a kind of toxification symptoms to appear on the plant tissues.

1.2.2.1 Chelating process

Chelating agents like metallothionein (MT) and phytochelatin play a pivotal role in reducing the concentration of free toxic metals in soil solution through the formation of aggregates. The most common type of chelating agent is the MT-s, which is mostly gene coded with lower molecular weight and cysteine rich polypeptides [56]. According to Murphy and Taiz [57], the MT production levels in *A. thaliana* hyperaccumulator plant increased considerably due to the extensive cupper presence. As for the phytochelatin, they possess a low molecular weight. It is enzymatically synthesized with rich in cysteine peptides. It is well documented to bind to Cd and Cu [58, 59, 60]. These peptides are responsible for cadmium detoxification and proper precipitation in different physiological parts of the *A. thaliana* hyperaccumulant plant. [61]

1.2.2.2 Biotransformation and compartmentalization processes

It is the incorporation of toxic heavy metals into special storage sites in plant cellular level. However, rendering them non toxic and non harmful to the hyperaccumulant plant physiology. A vivid example is the detoxification and biotransformation process of selenium. According to Läuchli [62]; *Astragalus* plant is able to withstand high selenium concentration due to its ability, to get the selenium metabolized to seleno-cyteine and seleno-methionine products. Then gradually replacing cysteine and methionine in protein biosynthesis cycle, as a result the funneling of selenium out of the methionine biosynthesis pathway into non protein amino acids like methyl-seleno-cysteine and seleno-cystathionine, which is regarded as the main reason for selenium detoxicity.

Arsenic is another toxic metal to plants which is the main ingredient of several organo-arsenical based herbicides. According to Francesconi [63], marine macro algae incorporates arsenic inside its leaf

vacuoles through differentiating it into different dimethyl-arsinyl-ribosides and certain types of lipids depriving its presence in ecosystem thus rendering arsenic benign.

Cr is detoxified in some hypertolerant plants through reducing the toxic Cr (VI) to a lesser toxic form of Cr (III). According to Storage [65] demonstrated that the uptake of Cr (VI) was an active one in contrast to Cr (III) uptake. Moreover, it is verified that the hyperaccumulant plant spends energy in the form of ATP in order to uptake Cr (VI) rather than Cr (III). This process paves the way for the hyperaccumulant like *Leptospernum scoperium* to withstand high chromium concentration in soil through passive absorption of Cr (III) rather than Cr (VI).

1.2.2.3 Cellular repair mechanism

It is believed that the plasma membrane repairs are done as the direct result of absorption of toxic heavy metals. The repairs are performed by MT-s [64, 65]. According to Murphy and Taiz [57] in *A. thaliana* plant, the Acyl carrier protein (ACP) and Acyl CoA binding protein (ACBP) are involved in lipid metabolism, which are primary constituent of the plasma membrane. It is primarily responsible for the repair of the damaged membrane. In addition, they are very specific to the presence of elevated amounts of Cu in rhizosphere area. However high concentrations of metal tolerance are not enough to entitle the designated plant varieties as phytoextractant but its ability to successfully uptake, translocate and store them efficiently.

1.2.2.4 Root and shoot uptake and accumulation

In the continuous phytoextraction approach of phytoremediation, the chelate assisted uptake of metals play an essential role in the whole process. Most of the heavy metals are strongly bound to fine particles of the soil, rendering unavailable to plant uptake. However, the increased chelate production such as mugenic and avenic acids by the plant root tips increased their soil bioavailability [66]. For example iron triggers their secretion like root ferric reductases reducing chelated Fe (III) to Fe (II) which is readily absorbed by the root [67] [68] [69].

According to Crowley *et al.* [71] and Welch *et al*. [70], certain plants have the ability to acidify their rhizosphere region by pumping excessive protons from their roots, to absorb heavily bound metals like Cu and Mn. Decreasing soil pH renders strongly bound heavy metals; that are otherwise unavailable, to become available for phytoextraction. On the plasma membrane level some transporters like Cu-COPTI and Fe-(IRT4) chelate compounds are responsible for cupper and iron translocation like in *Arabidopsis thaliana* plant forming what is referred to as phytosederophore complexes. [71, 72]

Once the metal phytosederophore complex enters the root system, it gets transported to the shoots and further reaching to its leaves through its xylem vessels. According to Salt *et al*. [73], cupper is transported via the xylem sap of the *Brassica juncea* plant by displaying biphasic saturation kinetics, suggesting it was accomplished by specialized and metal specific transport processes. It is known that xylem walls have a high cation exchange capacity [73]. This may impede the free metal movement through it. Therefore the non ionic chelate complexes assist towards their transport and final translocation, mostly in the form of Cu-citrate complexes [74].

According to Salt *et al*. [73], in the xylem system of *Brassica juncea* plant, the Cd is translocated through its complexation with a chelating agent like organic acids. Moreover, according to Stephan *et al*. [75] clearly proved that the presence of non protein based amino acids in certain hyperaccumulant

plants that have the ability to form chelating like complexes with divalent metal ions like Cu, Ni, Co, Zn, Fe and Mn. [75]

1.2.2.5 Phytovolatilization

Phytovolatilization is a unique ability that the phytoextractant plants possess, as a means of detoxification of the heavy metal absorbed through volatilizing it to the atmosphere. According to Lewis *et al*. [76], proved the postulate that selenium hyperaccumulant plant species were able to phyto-volatilize it as a dimethyl diselenate. According to Lewis *et al.* [76,77] proved that even a non hyperaccumulator plant like the alfalfa was able to volatilize selenium in a similar way.

Zayed and Terry [78] demonstrated that when antibiotic penicillin was added to hydroponically grown *Brassica juncea* (Indian mustard), it inhibited selenium volatilization by ninety percent. They suggested that certain types of root-bacteria symbiosis activities in the rhizosphere area assisted in reducing selenium into volatile forms.

However, this said it is of immense importance to take under careful consideration the different environmental regulations, restrictions and impacts of such a process on human life and ecosystem biodiversity.

Phase Three: Overview of metals in lithium mine tailing

1.3.0 Geological formation

The parent rock pegmatite belongs to the superior geological province in the Canadian Shield. It is composed of meta-volcanic and consequently derived from meta-sedimentary rocks and synvolcanic to late tectonic intrusive rocks. The original pegmatite is one of the numerous sub-horizontal pegmatite sheets, which belongs to the well famous Bernie lake pegmatite group. It is hosted by a synvolcanic metagrabbro intrusive. It is composed of eight discrete mineralogical zones with different ores of great economic importance, such as tantalum, spodumene $LiAl(SiO_6)$, cesium and rubidium, each occurring in different zones. Since its discovery different geologists have proven that the pegmatite is the host of more than eighty different minerals, some of which being rare earth minerals, such as Rb, Ta, Sn, Ti, Nb, Li, F, Cs, etc.

For simplicity reasons the sequence of metal analysis in the lithium mine tailing were divided into two groups. First the heavy metals containing metals like Cr, V and to a certain extent Fe. Second the remaining metals that are found in it and regarded as beneficial for hyperaccumulant growth and development like Ca, Mg and Na.

1.3.1 Chromium

1.3.1.1 Introduction

Chromium is present in environment abundantly. However, in soil it ranges between 10 to 50 $mg \cdot kg^{-1}$. Depending on the parent bedrock material, like in serpentine soils its concentration can peek up to 125 $g \cdot Kg^{-1}$ [80].

Chromium belongs to group VI-B and its electronic configuration is Ar. $3d^5\ 4s^1$. In nature the stable forms of chromium is Cr (III) which is a primary constituent of ores like ferrochromite $\{FeCr_2O_4\}$. Cr (VI) is found in ores like $\{K_2Cr_2O_7\}$, though its haxavalent form is more toxic. Even though, the divalent chromium is relatively unstable and readily oxidizable to its trivalent form. The tetravalent chromium form does not occur naturally, but represents an important intermediate state as a rate determinant for the pentavalent form of chromium $(CrO_4)^{-3}$. Its half-life duration is very small and usually defies detection. [79]

1.3.1.2 Chromium as a contaminant

Chromium is a heavily exploited mineral. It is used in numerous industries like leather processing, finishing, electroplating, as a cleaning agent and in the production of chromic acid. The haxavalent form of chromium is usually used in numerous industries, like metal plating, wood preserving and in tanning products. As a result to its wide spread use, the haxavalent chromium easily leaches into the environment and threatens the well being of its surrounding ecosystem. As an example in India it is estimated that annually 2000 to 3200 tons of elemental chromium 'escapes' into environment mostly as a byproduct from different tanning industries. [82]

In plants with high chromium toxicity is expressed as stunted growth, which renders the root inability to elongate growth and differentiate, causing cell collapse and death due to the absence of water and nutrients in sufficient amounts.

According to Vasquez *et al*. [83], Mishra *et al*. [84] and Jain *et al*. [85] the high toxicity of chromium decrease cell turgor pressure leading to plasymolysis. In epidermal and cortical cells of the bush bean plants. They also proved that in neutral pH levels of Cr (VI) which is a strong oxidizer, might cause oxidative damage to cells leading to cell death. Moreover, the Cr (VI), which is less water soluble compared to Cr (III). In hydrated forms it penetrates cell walls and gets precipitated as a metal chelating agent form.

1.3.1.3 Phytoextraction of chromium

It is known that the toxic chromium forms have detrimental effects on plant growth and development, especially in acidic rhizosphere conditions, which increases its mobility. According to Huffman and Allaway [86] and Huffman *et al*. [87] proved that chromium is not regarded as an essential element to plant growth. They proved that as low as $0.38 \cdot 10^{-6}$ mM of chromium content did not have any effect on plant growth. It gets absorbed through the plant root system through specific carriers due to chromium's structural similarity with essential plant minerals like K, Mg, P, Fe and Mn.

According to Cervantes *et al*. [88], Cr (VI) gets actively transported, through cellular carriers. On the plant tissue surface level, it competes against essential anions such as sulfates. Furthermore, due to its structural similarity, it competes for the binding sites of essential plant growth metals like Fe, S and P, but opposite to its trivalent form, whose uptake is passive.

According to Shanker *et al*. [89], chromium gets immobilized in plant root vacuole cells. Only 0.1 % of the total chromium uptake gets translocated to shoots and seeds. On the contrary over 98% of the absorbed chromium stays in plant roots.

According to Skeffington *et al*. [91], and Zayed *et al*. [90], used radioactive tracers to study the Cr^{51}. They reported that chromium moves mainly in plant xylem system. According to Rout *et al*. [92], different concentrations of 2 ppm, 10 ppm and 25 ppm of chromium in sandy soil samples observed 11%, 22% and 41 % reduction in plant height with respect to the control. Moreover, they interpreted their findings as high concentrations of chromium successfully competes with essential plant nutrients and sometimes takes their place on membrane carriers, resulted in less nutrients and water available for plant growth. Affecting plant overall reduction in size and height, especially around its leaves where reduction was as much as 50 %. Finally major symptoms of chromium toxicity in plants are burned like leaf tips or alongside its margins.

Chromium uptake by *Brassicaceae* family plants like cauliflower, kale and cabbage are noted due to their Sulfur loving properties. They are reflected in their 'hunger' for chromium, especially if it is supplied as CrO_4^{-2} form, due to the structural similarity with sulfate. It is absorbed, concentrated and stored mostly in plant roots, but only a minute amount gets anchored in its upper parts. [90] *Brassica* species as a whole and the Indian mustard in particular have an unusual 'appetite' for heavy metal like Pb, Cr, Cd, Zn and Cu storage in its roots and translocating lesser amounts towards its vegetative parts. Finally phytoextraction of chromium is performed usually in contaminated soil but virtually nonexistent on tailing amended samples.

1.3.2 Vanadium

1.3.2.1 Introduction

Vanadium is ranked as the twenty third element in the Mendeleev's periodic table preceding niobium and tantalum. It has a silvery metallic appearance of symbol "V", has an atomic weight of 50.9415 with a specific gravity of 5.96 and a melting point of 1929 ^{o}C [94,95].

Vanadium as a metal is recognized as one of the hardest of the metallic elements. It is very soluble in nitric and sulphuric acid and lesser in hydrochloric acid. Vanadium is rust resistant; in addition, to its toughness, it is greatly exploited in steel production. As example a half percent increase in steel content raises the tensile strength of steal from 7½ to 13 tons per square inch. [95]

Vanadium is also added to non ferrous alloys used as tools in different machinery. As a vivid example the ball bearings and the crank shafts of the world famous Ford T-Model were partially made from vanadium [95]. Aluminum added to vanadium provides additional strength in titanium alloys in missile cases production, nuclear reactors and jet engine compartments [96].

Vanadium is the twenty second most abundant element found in the earth's crust at a mean concentration of 150 g·ton^{-1}, similar to zinc, copper and nickel [93]. It is found as a major constituent in over fifty different mineral ores with variable concentration in petroleum products. [97]

Major world suppliers of vanadium are China, South Africa, Russia, United States and Canada. The latter deposits are in the form of titaniferous magnetite form mostly concentrated in Pipestone, Manitoba, Lac Dore' and Bell River in Quebec provinces. [98]

Generally vanadium consists of two isotopes of 0.24 % V^{50} and 99.76% of V^{51}. The first isotope is slightly radioactive with a half life of $3.9 \cdot 10^{17}$ years (94).

In nature vanadium has extremely complex and ever changing chemistry. It can readily change and evolve anionicly or cationicly under different physiological conditions. It has been verified that the VO^{+2} is more prevalent in acidic soil solution rather than the VO_3^- and VO_4^{-2} species, which are prevalent in more neutral or alkaline soils. [99]

Vanadium is a ubiquitous element present in higher plants in trace amounts and in animals and humans in ultra trace amounts. In human physiology the vanadium concentrate ranges between 100 to 200 μg [100]. As early as the 19th century vanadium was recommended in dire human pathological cases like pneumonia, malnutrition, anemia, diabetes and tuberculosis. [101, 102]

Finally the vanadate (+5) has a special ability to mimic the cellular insulin functions which paves the way as being regarded as a viable alternative. In brief the insulin-mimicking actions of vanadate are related to its ability to block several liver metabolic enzymes. In addition, to several muscle and adipose tissue enzymes, which all united decrease cellular glucose level. Moreover, it forces several anti insulin enzymes to cease action, thus decreasing bodily glucose levels. [100, 103, 104, 105].

1.3.2.2 Vanadium as a contaminant

Vanadium has an extremely complex chemistry and is regarded as extremely toxic. The above mentioned complexity might be attributed to vanadium's multiple oxidation, hydrolysis and polymerization states. The oxidation states of biological interest are the ($^{+3}$), ($^{+4}$) and the ($^{+5}$) states. The first is stable in extremely acidic media {pH<2} and in complete absence of oxygen, yet in vivo it is rare to occur. The second ($^{+4}$) state is stable in acidic media too like its predecessor as blue vanadyl cation. As for the ($^{+5}$) state which has wide range presence is mostly present in acidic pH as well as in physiological pH. It tends to aggregate into polymer complexes. At lower pH, V^{+5} is a powerful oxidant and pre dominates as an orange colored decavanadate {$V_{10}O_{28}H_5$}. [106]

Vanadium pentoxide is one of the most toxic forms to human physiology. It can cause inflammation of the bronchi and trachea, severe irritation to the eyes, skin, pulmonary edema, systemic poisoning (MSDS, 2010). It elevates heart beat, causes skin rush, cough, labored breathing, loss of body weight, reproduction and developmental toxicity, if untreated might eventually lead to death. [107]

At human cellular level it is documented that vanadium inhibits phosphate metabolizing enzymes, such as phosphohydrolases, phosphotransferases, DNA-polymerases, thymidilate synthetase and glyceraldehydes-3-phosphate dehydrogenase. [108, 109, 110]

According to Fay and De Vasconcelos [109], increased concentrations of vanadium effects nitrogen metabolism in higher plants by inhibiting nitrate reductases. In sugar beets it inhibits phosphatases, glutamic-pyruvic transaminases and invertases from secretion completely. Vanadate inhibits plasma membrane hydrogen $\{H^{+}\}$ translocating ATPase, which impairs nutrient uptake on the plant membrane, deprieving its ability to maintain its cell turgor pressure and stomata opening, leading to cell collapse and eventual death. According to Beffagna *et al.* [116] and Kasai *et al*. [117] vanadate inhibits phosphatases, phosphoenolpyruvate carboxylase, fructose 2, 6 bisphosphatase and many other on the plant cellular level.

Vanadium is a common element in the lithosphere. Its average concentration is estimated around 150 $\mu g \cdot g^{-1}$. It is a common component of alkaline and argillaceous rocks. Soils close to industrial areas or near coal or fuel generating power stations are usually highly contaminated with vanadium [111]. Thus said the V^{+5} valency is considered extremely toxic, but little attention is shed on it. The V^{+5} $\{VO_4^{-3}\}$ and Cr^{+6} {as CrO_4^{-2}} have similar chemical properties [112], indeed they both can exist under similar environmental conditions. Therefore, if plant vanadium $\{V^{+5}\}$ concentration exceeds more than 2 $\mu g \cdot g^{-1}$ it may cause chlorosis and stunts its growth [113]. According to Rehder [114] and Panichev *et al*. [115] it reduces the amount of phosphates in plant physiology, when V^{+5} rich grass is consumed by the

grazing cattle it will transform into $H_2VO_4^{-2}$ which replaces PO_4^{-3} from cattle bones leading to numerous forms of deformities, deficiencies and eventual herd loss.

1.3.2.3 Phytoextraction of vanadium

Vanadium is regarded by botanists and soil scientists as an essential but trace plant nutritional metal but, its function is still somehow obscure. According to Cantley et al. [118], Macara [119] and Biggs and Swinehart [120] *Amarita musaria,* mushroom specie is one of handful of plants that does accumulate elevated amounts of vanadium in their physiology.

Vanadium hyperextractant plants are scarce and virtually nonexistent or unknown under field testing conditions. Thus revealing and documenting vanadium hyper-accumulating plants are extremely important, especially inside mine tailing containing growth media circumstances.

1.3.2 Iron

1.3.3.1 Mineral and availability

Iron weights around 5% of earth's crust and it is present in all soil types without exception. Fe is present in minerals like olivine, biotite, etc. located in the centre of the octahedral in di or trivalent form [164]. The Fe^{+3} easily oxides and has a low solubility due to its fast precipitation as $Fe^{+3} + 3OH \leftrightarrow Fe(OH)_3$ which is a solid [165].

Iron influences soil color from yellowish brown to fully brown in tropical well drained soils as hematite form {α-Fe_2O_3} [166]. On the other hand, Ferrihydrate {$HFe_5O_8.4H_20$} is regarded as a major source for plant iron uptake. According to Chen and Barak [167], the solubility of iron oxides/hydroxides decreases in the following order; $Fe(OH)_3$ amorphous > $Fe(OH)_3$ in soils > γ-Fe_2O_3 maghaemite > γ-FeOOH lepidocrocide > α-Fe_2O_3 haematite > α-FeOOH goethite.

In alkaline soil Fe^{+3} solubility decreases as much as a thousand fold time for each unit of pH rise. Forming $Fe(OH)_2^+$, $Fe(OH)_3$ and $Fe(OH)_4$. On the contrary in acidic soils iron solubility and availability to plant needs increases. [168]

In anaerobic soil conditions, Fe^{+3} is reduced to Fe^{+2}. Furthermore, the whole process is governed by the presence of anaerobic bacteria, which uses Fe oxides as electron acceptors in their respiration pathways [169], thus reducing the ferric {$^{+3}$} to ferrous {$^{+2}$} as follows;

$$Fe(OH)_3 + e^- + 3H^+ \leftrightarrow Fe^{+2} + 3H_2O \qquad [170]$$

One obvious outcome from the above mentioned equation is that the reduction of Fe^{+3} to Fe^{+2} is associated with the consumption of H^+, leading to an oxidation process of Fe^{+2} to Fe^{+3}.

The most important characteristic of iron in botany science is its ability to form organic complexes called siderophores. They are formed as an interaction between the bacteria as well as fungi and

plants; which are sometimes referred to as phyto-siderophores, which aid in numerous element transport and availability. [171,172]

According to Masalha *et al*. [172] and Becker et al. [173] more than hundred distinct siderophores are known, which are stable at various pH ranges. As an example a very well known siderophores is the hydroxamic acid {R-CO-NH-OH} that binds with Fe^{+3} forming an ionic bond which is referred to as ferric mono hydroxamate. [173]

1.3.3.2 Iron in plant physiology

The transport of Fe^{+2} is generally accomplished into the roots by simple diffusion or by mass flow phenomenon. It is translocated through the plasmalemma membrane, bound to Fe^{+3} reductases. Moreover, the reductase accepts e^- from the siderophores and disintegrates releasing Fe^{+2}. Later on it passes through special channels that lead it into the cytosol. The whole process takes place alongside with the reduction of NADPH to $NADP^+$ and H^+ ions [174].

According to Takaji [175] and Takaji *et al*. [176], rice and barley roots excrete phyto-siderophores like mugineic and avenic acid which are capable of mobilizing Fe^{+3}, rendering it available to plant uptake.

Iron containing enzymes have an immense importance in rDNA synthesis. Furthermore, without which the growth is depressed and senescence follows soon. Despite its immense abundance and importance to plant growth and development iron toxicity and deficiency is characterized by failure of chlorophyll production, chlorosis of younger leaves and further reduction of growth [177]. Maintaining a good humus levels in soil is a way of optimizing the availability of iron. Finally iron toxicity is a serious problem in rice plantations which might range between 300 to 1000 $\mu g \cdot gr^{-1}$ of iron per dry weight. As a result its leaves will be covered by tiny brown spots and chlorosis, the whole phenomenon is referred to as bronzing. [178]

1.3.4 Potassium

1.3.4.1 Mineral and availability

It is estimated that the mean potassium availability in earth's crust is around 23 $g \cdot kg^{-1}$. It is largely bound to primary minerals and secondary clay particles. Its availability and absence depends on the type of parent material. Weathering, time, topography and other soil formation parameters, play a vital role in its presence or absence.

According to Laves [121], soil K^+ content is closely related to illites, aluminum bearing chlorites and to a lesser amount to smectites. The potassium element is a basic constituent inside the feldspars forming its tetrahedral form. Thus the K^+ is sandwiched between the Si-Al-O lines of the crystal lattice and held tightly by covalent bonds [122, 123]. Weathering and presence of weak organic acids make potassium available in lithosphere solution. It is also present abundantly in different types of micas which differ from feldspars due to its Si-Al-O tetrahedral sheets, which contains a M-O,OH octahedral sheet in which potassium occupy the hexagonal spaces.

According to Farmer and Wilson [124], the weathering process converts micas to secondary two to one clay minerals by the following order; Micas (10% K) to hydro micas (6-8% K) to illite (4-6% K) to transitional minerals (3%K) and finally vermiculites or montmorinolite (<2% K).

In mobility and fixation terms clay minerals fix potassium in dry and moist conditions like in micas and vermiculites. Other smectites fix potassium under dry conditions only [125]. The increase of the planar surface (p-position) and the interlayer (i-position) increases potassium fixation ability on a clay surface. According to Schuffelen [126] and Sparks [127], the three different K^+ binding positions on illite have the following Gapon coefficients; for p-position 2.21 $(mol \cdot m^{-3})^{-½}$, for the e-position 102 $(mol \cdot m^{-3})^{-½}$ and for i-position as infinite per $(mol.m^{-3})^{-½}$.

Potassium is a major constituent in numerous soil structural elements, which is regarded as unavailable for plant use. Secondly it is bound or adsorbed on the exchange sites of the soil colloids, which might be available to plant uptake and use under appropriate circumstances. Thirdly dissolved potassium in soil solution is regarded as the readily available source for plant use. According to Martin and Sparks [128], in sandy loamy soils the exchangeable K^+ ions are estimated around 1.72 mol·kg^{-1} and the non exchangeable portion around 2.20 mol· kg^{-1}.

1.3.4.2 Potassium in plant physiology

Potassium is an essential macro nutrient in plant growth, flowering and fruit bearing stage. It plays a pivotal role in different enzymatic secretions. In addition, potassium is an essential ingredient in starch synthesis and the development of chlorophyll. Unlike phosphorus and nitrogen, which are regarded as structural metals, potassium plays a catalytic role in different chemical processes. Potassium is taken up by the root plasma membrane and transported through different membrane based transporters.

According to Matthuis and Sanders [129], Fox and Guerinot [130] and Schachtman and Shroeder [131] on the plasma membrane, there are two main types of transporters related to potassium uptake and translocation. The high and low affinity transporters, the former being very selective for K^+ and is characterized by low Km which ranges between a few mM per m^{-3}. The latter being less selective with a Km of 1 mM per m^{-3}, thus the potassium ion passes from the plasmalemma into cytosol and further inside the cell. Moreover, it gets transported through the xylem and phloem transport cells. The whole process is governed by the difference in electrochemical gradient and saturation kinetics.

The benefit of potassium in plant growth is defined by its broad spectrum functions. It initiates meristematic growth, water uptake, photosynthetates translocation, enzymatic activation in starch synthase inside sweet corn [133] and ribulose biophosphate carboxylase in polypeptide synthesis in the ribosome, paving the way for protein synthesis in different plant tissues. [134]

On the other hand potassium deficiency in plants does not produce immediate or instant symptoms, but gradually reduces its growth and necrosis of older leaves. It decreases the turgor pressure, leading to poor resistance to drought, salinity, and frost. It increases susceptibility to fungal, microbial and viral diseases leading to premature cell death. [135]

On the cellular level its deficiency leads to the collapse of chloroplast [135], mitochondria [136] and retards cuticle development [137], leading to an overall low yield. According to Glynne [138] and Goss [139] its deficiency symptoms can be easily corrected through proper fertilizer combinations that might prevent lodging of maize and wheat. It is observed that a relative increase in K^+ will increase plant resistant to *Fusarium* wilt in banana, brown wilt (*Puccinia hordei*) in barley and brown spot (*Ophiobolus miyabeanus*) in rice.

1.3.5 Calcium

1.3.5.1 Mineral and availability

Calcium (Ca) concentration in earth's lithosphere is estimates around 36.4 $g.kg^{-1}$. It is one of the most abundant minerals ever found. Calcium occurs as Ca bearing Al-silicates like feldspars and amphiboles. As well as in calcium phosphates and calcium carbonates [140] moreover, in calcite {$CaCO_3$} or dolomite Ca Mg $(CO_3)_2$. The weathering process is the major supplier of calcium into soil solution thus its solubility and availability is as follows;

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca(HCO_3) \rightarrow Ca^{+2} + 2HCO_3^-$$

Calcium is recognized for its unmatched abilities in soil buffering and rendering it suitable for agricultural crops growth and development [140]. As an example plant roots excrete increased amounts of H^+ ions that acidify the rhizosphere and renders heavily bound trace elements soluble and available for plant uptake. The presence of calcium based soils have the ability to quickly reverse the direct effects of acidification and render the pH at the optimum growth level.

1.3.5.2 Calcium in plant physiology

In dried plant calcium content ranges between 5 to 30 $mg \cdot g^{-1}$. Generally calcium concentration is ten times higher that the potassium. It is absorbed by the endodermis of the young developing root tip. [141]

Despite its considerable presence in plants, calcium concentration differs in monocots in lesser amounts rather than in dicots in greater amounts. According to Lonergan and Snowball [142], proved that the rye grass needs are estimated around 2.5 $mM \cdot m^{-1}$, where 100 $mM \cdot m^{-1}$ to tomato (a dicot) of available calcium.

The reason behind such a difference was presumed to be due to the higher cation exchange ability in the tissue cells and elevated amounts of carboxylic radicals in their cell walls. [143]

The Ca^{+2} pathway in plant entry and translocation starts from the non suberized apoplast of the root cell tips. Then through the process of facilitated diffusion it enters plasmalemma and furthermore, through electro chemical gradient difference between the apoplast and the cytosol gets translocated throughout the plant physiology. [143]

In plants, calcium is present as a free ion Ca^{+2} or bound to radicals like carboxylic, phosphorylic, hydroxylic groups. Its role is mainly to counter the cationic or anionic inorganic and organic compounds. In seeds calcium is present in the form of salt inositol hexakisphosphate (usually referred to as phytate). Calcium is often applied to the soil to release other nutrients by altering the soil acidity (pH). Calcium is essential for the proliferation of soil bacteria. Calcium displaces sodium attached to clay particles and holds clay particles further apart leading to its friability.

On cellular level calcium ions plays a regulating and stabilizing role for different membrane bound ionic pumps like the Ca^{+2}-ATPase and Ca^{+2}/nH^{+} antiporters. They drive ions back and forth in the plant cell vacuoles [144,145].

Calcium plays a pivotal role in different plant growth and development processes, through preserving the integrity of the cell wall. Its deficiency on plants is reflected in reduced growth, browning of root tips and early senescence [146]. It is also required in cell division, elongation, extension and differentiation [147,148]. In addition calcium is responsible for fruiting and ripening processes through the increased secretion of ethylene, which is secreted from cell wall membrane complex, outside the cytoplasm [149].

Poovaiah and Leopold [150], Bush [143] and Lerchl *et al*. [151] showed that the Ca^{+2} absence from the maize leaves render it susceptible to early senescence and abscission of its leaves.

On the cellular processes level calcium is regarded as an ionic balance, gene expression, mitosis and secretion of numerous enzymatic secretions as well as an ion safeguarding the cellular homeostasis situation.

Similarly, calcium deficiency reduces growth of the meristematic tissue, resulting in chlorosis and necrosis of leaf margins. In Brussels sprouts (*Brassica oleracea*) calcium deficiency is categorized as internal browning leading to internal rot [152]. Its deficiency causes the 'black heart' nutritional symptom on the celery core [153].

1.3.6 Magnesium

1.3.6.1 Mineral and availability

Magnesium concentration in the earth crust ranges between 0.5 to 5 $g \cdot kg^{-1}$. Magnesium is as an easily weatherable element usually from biotite, serpentine and olivine minerals. In which its concentration might reach up to 130, 250 or 240 $g \cdot kg^{-1}$ of soil [154].

The plant beneficiary magnesium, constitute 5% of total magnesium present in soil solution. It constitutes normally 4 to 20 % magnesium on total CEC available sites, presumably the average Ca^{+2} ranges on CEC sites is between 60 to 80 % [155,156].

Mg^{+2} is not highly bound to clay minerals like K^{+} which is found between the 1:1 silica layers and therefore resistant to leaching and plant availability. On the contrary magnesium is prone to leaching and rapid loss from rhizosphere, which might reach up 30 $kg \cdot ha^{-1} \cdot year^{-2}$ [157].

Finally soil texture and parent material formation play an important role in magnesium storage or loss. Sandy textured soils are prone to excessive leaching and loss. While the clayey based soils like basaltic, petidolitic and dolomitic supply ample amounts of magnesium for plant uptake and usage.

According to Grimme *et al*. [159] and Schimansky [160] magnesium uptake by plant roots occur due to concentration difference gradient between the roots and the rhizosphere. Its entry and passage is accomplished through the tonoplast and mediated by facilitated diffusion.

1.3.6.2 Magnesium in plant physiology

A well known location of magnesium in plants is in the centre of chlorophyll molecule. One of the major tasks of Mg^{+2} in plant physiology is the formation of bridge between the ATP and numerous enzymes. As a result phosphorylation is catalyzed and transfer of energy occurs [161]. Likewise in dephosphorylation v-pyrophosphatase based processes as well as in chlorophyll synthesis [145].

According to Travers [162], one of the fundamental functions of Mg^{+2} in the nucleic acids is safeguarding its structural and conformational integrity. In addition it is a major constituent in DNA and rRNA which requires metallo-enzymes for synthesis alongside the divalent cation Mg^{+2}.

In particular most ribozymes require high concentration of Mg^{+2}, which is a prerequisite for efficient binding of tRNA strand to the ribosome, but its absence deprives the polypeptide chain from synthesis [163]. In addition to its numerous benefits, magnesium deficiency reveals chlorosis on older leaves and spreads to younger ones. Magnesium deficient leaves fall prematurely, in celery its deficient leaves show small dark spots on pale colored background [144]. Finally a 2 $mg.g^{-1}$ of magnesium is regarded as a threshold level for proper growth, development and fruit bearing stage. [106]

1.3.7 Sodium

1.3.7.1 Mineral and availability

Sodium (Na) is derived from the Latin word sodanum, meaning a headache remedy, has an atomic mass of 22.99 $g \cdot mol^{-1}$ and a valence of ($^{+1}$). It is the sixth most abundant element comprising about 2.6 % of its crust [179]. It is found in different salt formation, which is usually easily dissolvable in water. Therefore, its hydrated radius is 0.38 nm and its crystal ionic radius is 0.097 nm [180]. Sodium is not considered as an essential plant nutrient except for those using the C4 pathway and halophyte plants. [181]

Sodium has a similarity with potassium chemically and structurally. The potassium hydrated ratio is 0.33nm, which is very close to sodium. Its primary source is from irrigated water especially in arid or semi arid areas. These types of textured soils contain considerable amounts of soluble salts like sodium chloride, which hinders plant growth and development. [182]

Even though it is regarded as a non essential mineral to plant physiology, but it is extremely essential to human as well as animal growth and development. It is regarded as the principal electrolyte in their physiology and plays a pivotal role in maintaining the ionic balance of the body fluids and safeguards against excessive loss of water from cell content [183]. The excessive sodium levels are mostly associated with high salinity effects which deprive large areas of land for agricultural practices, leading to fertility loss and desertion. [184]

1.3.7.2 Sodium in plant physiology

According to Arnon and Stout's [186] and Epstein [185] a mineral is considered essential when the plant can not complete its life cycle, starting from its germination and ending by its seed production and senescence. Second the desired mineral must have a specific and targeted action on the organism. Third if it is a constituent of an essential compound, it is regarded as an essential element.

Based on the above mentioned definition, sodium is regarded essential for C4 plants like *Atriplex vesicarra, Kochia childsii* etc. Without which chlorosis and failure to form flowers follow. Sodium is needed in micro amounts (100 μm) to alleviate the above mentioned symptoms.

Sodium is generally recognized to promote growth on asparagus, barley, sprout, carrot, radish, rape and Swiss chard [187,188]. However it is not detected directly in their metabolic processes, thus remaining 'discrete or hidden'. Sodium is suggested to get absorbed on plant root sites by the process referred to as selective ion transport. It depends on metabolic energy derived from ATP breakdown [189]. The overall absorbance of sodium depends on the presence of potassium in solution, leading to a difference in gradient ratio. [190]

The absorbed sodium is translocated freely and stored in plant shoots in different concentrations. The whole process of storage and translocation is referred to as natrophilic [191]. According to Greenway and Osmond [192], the natrophilic plants avoid sodium toxicity due to their ability to efficiently compartmentalize the excess sodium into their cellular vacuoles and use it as an inorganic osmoticum for overall cellular ionic regulation. Even though, it is assumed that the reasoning behind sodium storage in cellular vacuoles is due to the inability of the cytoplasmic 'entourage' to tolerate elevated levels of more than 20 mM. Its excess might interference with the proper functioning of the homeostatic process. [193,194]

On the cellular level, sodium is proven to be beneficial to C4 plants. It plays a critical role in regenerating the phosphoenolpyruvate (PEP) in the mesophyll chloroplasts of *Amaranthus tricolor* plant [195]. Sodium takes part in chlorophyll synthesis [196]. According to Marschner [196], sodium deficiency in C4 plants result in excessive accumulation of pyruvate in their mesophyll chloroplast and inability to get it transformed to PEP.

On the enzymatic level sodium is generally regarded as a less effective mineral in activating various enzymes, in comparison to other minerals like potassium and magnesium [197]. On the contrary at elevated concentrations it triggers protein synthesis and oxidative phosphorylation to occur in vitro experiments. [134,197]

Regardless of sodium concentration in rhizosphere that might be regarded as a potential toxicant to plants roots. Most crops translocate it in minute concentrations and store it in their reproductive sites like the seeds, fruits or even in other physiological compartments like inside its roots which might sometimes be regarded as its edible part [198]. Like in wheat, rice, fruits, vegetables, tubers, carrots, etc. The main reason behind it is its translocation system which is usually achieved through its xylem, rather than phloem. [199, 200]

1.3.8 Lithium: 'as the target metal'

1.3.8.1 Introduction

Lithium is an ultra microelement, relatively little known and investigated. It is the third element in the periodic table in group 1 A. Lithium does not occur freely in nature but it is bound in merely trace amounts to igneous rocks like leidolite, pedalite, spodumene, amblygonite and many others. It is also found in aquatic media.

Nowadays, lithium production and pricing sky rocketed once again. It is an integral part in the production of rechargeable batteries in the form of lithium carbonate. Nowadays lithium based batteries are found in human heart pacemakers buried deep inside the human chest, in television sets, calculators, airframes and other structural components in the aerospace industry which consists of Al-Cu- Li alloy of 2 to 3 % lithium by weight. [201, 202]

According to the United States Geological survey (USGS), in 2007 Chile was the leading producer of lithium with 43% of worldwide production followed by Australia, 27%, China 12%, Argentina 11%, Russia, U.S, Canada and Zimbabwe. Finally it is estimated that a pound of lithium carbonate is worth around three Canadian dollars.

1.3.8.2 Lithium metallurgy

Lithium is strongly bound to rocky samples in minute amounts. The strategy of exploitation and removal of lithium involves its conversion to carbonate, then to chloride followed by salt electrolysis. [203]

In Canada lithium is specially mined around Lac du Bonet area in the province of Manitoba, in Quebec there are numerous findings associated around Baie James, Eastman, Pontiac and around the Preissac and Lac Corne areas. [204,205]

1.3.8.3 Properties

The lightest of all metals, soft, silvery white in color. Its atomic number is 3 and its electronic configuration is $1s^2$ and $2s^1$. A single atom of lithium consists of a nucleus in its innermost core, surrounded by 2 electrons orbiting around it, which is inert. On the outer layer a single electron is present which is readily given in order to obtain a state of stability, thus a ($^{+1}$) sign is designated.

Its geometrical arrangement represents a cubical lattice shape that renders it a perfect conductor of electricity. Its atomic weight is 6.941 $g \cdot mol^{-1}$ and has one of the lowest densities a mere of 0.53 $g \cdot cm^3$ at 20 ^oC. [206]

Chemically, lithium is denoted as the first metallic element found in group IA; the alkali metals group, followed by sodium, potassium, rubidium and cesium. Lithium is slightly harder than sodium but much softer than lead. It readily reacts with water and even is able to absorb moisture from its surrounding forming LiOH and hydrogen gas. Lithium's melting point is 180 ^oC and its boiling point is 1347 ^oC. [207]

Lithium is stable in dry air, when the dew point is maintained below – 38 ^oC. The lithium element isotopes occurs as ^6Li (7%) and ^7Li (93%) forms. According to Sephton *et al*. [210], Suzuki *et al*. [209] and Meneguzzi *et al*. [208], ^7Li was produced during the cosmic big bang rather than the ^6Li. Hence according to them the ratio of ^7Li to ^6Li is an important indicator of galactic evolution in relation to carbonates and phyllosilicates in rocky chondrites formations.

In a recent study that was conducted on different lithium isotopes revealed that a moderate temperature of ~ 300°C enabled the retention of ^6Li isotope in its solid phase. While on the contrary the ^7Li passed into the aqueous solution. However, lithium at room temperature reacts readily with nitrogen forming Li_3N. [211, 210]

1.3.8.4 Occurrence

Lithium in nature is in a combined form but in minute quantities. As an example it is associated inside igneous rock formations like lepidolite (3.84 % wt), pedalite (2.09% wt), spodumene (3.73% wt), amblygonite (3.44 % wt) and zinnwaldite (1.59 % wt). Lithium is also found in seawater, mineral springs and sandy formations. It is estimated that lithium soil content is estimated around 13 million tons while in sea its content is estimated at 230 billion tons.

Lithium is found in freshwater at different concentrations ranging from 0.1 ppb to 100 ppm, usually higher concentration of lithium is accompanied with higher concentration of sodium. Hence it is common to find lithium in fresh water content up to 10 ppb. In irrigated water its presence varies between 2 to 5 ppm. At higher concentrations it may hinder plant growth and fruiting process.

Medically it is proven that elevated concentration of lithium in drinking water has a balneological effect by decreasing the risks of coronary illnesses. Furthermore, a higher concentration of lithium in drinking water has a positive influence on human nervous system, in disguising aggressive manners. [212,213]

During human embryonic development, lithium levels reach its maximal values during the first trimester of gestation period and subside afterwards. In animal studies lithium plays a significant role in expansion of pluri-potential stem cell pool to new progenitor cells, leading to the formation of blood cells. [214]

Moreover, an average daily intake of a 70 kg adult person in the United States ranges between 650 to 3100 µg of lithium. The green vegetables and drinking water are considered as its major sources. [214] In human physiology, numerous trials have proven the presence of high concentration of lithium in cerebellum, followed by cerebrum, kidneys, lungs, ribs, thyroid and liver.

Astonishingly women physique surpasses men in lithium content by merely 10% to 20 %. [214] Lithium is regarded as a non physiological cation. It has numerous in vivo effects. It became a "household" medicine starting from the early 1950-s, when its carbonated form was believed to be beneficial in treating different manic depression forms. The current hypothesis attributes lithium to its ability to inhibit the enzyme inositol mono phosphate phosphomonoesterase. Under certain circumstances it increases the stimulation of phospholipase C (PLC) coupled receptor systems in cells. Eventually it may lead to the reduction of intracellular free inositol concentration, which may be sufficient to diminish inositol phospholipid resynthesis and therefore the availability of the substrate for PLC synthesis. [215, 216]

According to Del Rio *et al*. [217], assumed that the absence of PLC synthesis leads to inositol phospholipids which generate a ubiquitous second messengers like inositol 1, 4, 5-triphosphate [Ins (1, 4, 5) P_3] and diacylglycerol, which effects human brain function. Nowadays, lithium bearing medicine is widely prescribed. It plays a vital role against different dermatological and oncological diseases. [214, 215]

On inter or intra cellular level lithium biochemical effects appear to be extraordinary complex and partly unknown. It is presumed that lithium is involved in numerous biological actions like inhibition of adenacylate cyclase and the increases of GABA activity. It is also assumed to simulate parathyroid activity, blocks vanadate bonding, and inhibits PGEI synthesis. On the other chemical level lithium is linked with the increase in MAO (monoamine oxidase) enzyme synthesis which elevates mood [218]. However, it is medically proven that lithium enhances folate and vitamin B_{12} transport and distribution into L1210 cells. It is assumed that lithium might stimulate the production of new brain cells, which might lead for a suitable cure against Alzheimer's disease. [214]

1.3.8.5 Toxicity of lithium

The effects of lithium toxicity on cultivated plants were first investigated by Kent in 1941. He was able to prove its increased resistance led to disease resistance and stimulation of growth. A lithium toxicity symptom varies in different plant genera. In fruiting trees above 0.05 ppm might be is considered toxic. However, the main source of plant toxicity is the presence of lithium in irrigated water. Lithium toxicity symptoms are difficult to recognize and differentiate from other type of necrotic spots, leaf curling or chlorotic symptoms.

Generally speaking lithium injury reveals necrosis along the leaf margins, subsequent intervienal chlorosis and leaf abscission [221]. It is well documented that lithium inhibits rhythmic movements of pulvini and petals [222], disrupts normal pollen development by inducing symmetrical mitosis in the microspores [223], blocking pollen germination to take place. [224]

According to Berridge [225], lithium affects the inositol depletion form cells which lead to the inhibition of inositol monophosphatase synthesis. The stoppage of the inositol cycle leads to de-signaling of cellular calcium and proper cellular membrane carrier activity. [223,224,226]

According to Boller [227] and Conegero *et al*. [228], higher concentrations of lithium stimulate increased production and secretion of ethylene, salicylic and gentistic acids in tobacco plants. Its excessive presence mimics the presence of a pathogen, triggering the start of the defense mechanism, through induction of the 1-aminocyclopropane-1-carboxylate synthesis genes.

According to Smith and Blair [229], wheat powdery mildew (*Erysiphe graminis*) severity was reduced as much as 11 % when wheat leaf area was fed with 8 $g \cdot l^{-1}$ lithium chloride amended in fertilizer. In addition Carter and Wain [230] analyzed the lithium sulphate increased the resistance of wheat root seedlings against the *E. graminis.*

In humans lithium overdose effects depend on its concentration especially when it is taken in 'concert' with alcohol or other illegal drugs. Moreover, lithium is used as a catalyst in ammonia/ alkali synthesis method of the methamphetamines or commonly known as meth, from ephedrine. [231]

Its overdose symptoms are characterized by shakiness, vomiting and excessive thirst, frequent urination, muscle weakness, seizures, blurred vision which might lead to an eventual coma. It is also proven that the abuse of legal medications like Eskalith® and Lithobid®; which are used to treat bipolar disorder or manic depression, might lead to lithium overdose.

However, lithium deficiency in humans might give rise to altered behaviors, like aggressiveness, schizophrenic behaviors and homicide. After four weeks of clinical trials its supplementation resulted in general happiness, friendliness and energy. [214]

Finally lithium's ability to cause tetratogenicity (birth defects) in developing fetus is also a medically proven condition. On a similar study of lithium carbonate on pregnant mice [232], numerous scientists have reported that excess lithium might cause congenital defects and Ebstein's anomaly; a rare cardiac defect. [233]

1.3.9 Discussion

According to the mining company data, the lithium mine tailing (LMT) sample was the produce, of the direct result of the lithium extraction from different pegmatite sheets composed of meta-volcanic and derived meta-sedimentary rocks and synvolcanic to late tectonic intrusive rocks. Internally the pegmatite was composed of eight discrete mineralogical zones with different ores of economic interest. The pegmatite rock was the host of more that eighty different minerals. The important ones were stated as, spodumene {$LiAl(SiO_6$}, quartz {SiO_2}, feldspar {$KAlSi_3O_8$-$NaAlSi_3O_8$-$CaAl_2Si_2O_8$}, montebrasite {$LiAlPO_4(F_9OH$}, wodgonite {$Mn_4(Sn>Ta,Ti,Fe)_4(Ta>Nb)_8O_{32}$, microlite {$(Na,Ca)_2Ta_2O_6(O.OH.F)$}, pollucite {$Cs,Na)(AlSiO_6)H_20$}, lepidolite {$(K,Rb)\ (LiAl)_2\ (Al,Si)_4O_{10}$ and feldspark {$AlSi_30_8$}.

The richness of the metal content enabled to divide them into two distinct and antagonist groups. First group contained heavy and toxic metals like vanadium, chromium and to certain extent iron. The second group contained the beneficial macronutrients like iron, calcium, sodium, potassium, magnesium and lithium.

Chapter Two: Experimental

Methodological approach

The reclamation of the lithium mine tailings (LMT) was achieved through five different formulation mixes, which were referred to as growth media. Moreover, phytostabilization and phytoextraction processes were achieved through direct sowing of the hyperaccumulant plant seeds in the five different growth media of four subsamples each. Phytomining of lithium in lieu with heavy metals like chromium and vanadium was accomplished inside different physiological parts of the hyperaccumulant plant.

Each growth media had a unique physical or chemical characteristic like pore volume, metal adsorption capacity, CEC, AEC, salinity, pH, and ORP. The five different formulations were composed of LMT, homogenized peat and the dewatered municipal biosolids mixed on weight per weight basis (w: w).

The growth media ingredients were assembled in a sustainable way such that the preference was given to local and readily available ingredients. The peat was brought from northern Quebec, as well as the municipal biosolids was obtained from local wastewater treatment plant. Moreover, no major transport expenses and special handling procedures were needed. The five different growth media were vigorously tested for their different physical characteristics. The physical characteristics of the three growth media ingredients were determined separately. First the lithium mine tailing sample was homogenized, dried, its organic matter content was determined, classified and its texture, pH, salinity were measured. Likewise for the second and the third ingredients, were peat and municipal biosolids.

In addition, the five different growth media mixes were tested for their pH, ORP, salinity and metal mass content, through the usage of Perkin Elmer Analyst 100™ atomic absorption spectroscopy (AAS).

The digestive process was achieved through the addition of concentrated (12N) hydrochloric acid following the cold extraction procedure. The three ingredients; homogenized peat, lithium mine tailing and dewatered municipal biosolids were analyzed for the eight metals (V, Cr, Fe, Mg, Na, K, Ca, Li) mass content, followed by the five different growth media, the organic fertilizer and the leachates that were generated throughout this research.

As for the metal adsorption and release characteristics of the five distinct growth media, two distinct experiments were conducted. First the column leachate test, in which one pore volume of DI water was added specific to each growth media and a pressure of 5 psi units (~35kPa) of pressure was applied under ambient testing conditions, which generated a leachate. The second experiment was the Electro-Kinetically metal mass determination. Likewise a one pore volume of DI water was added specific to each growth media and a DC field of 1 $mV \cdot cm^{-1}$ was applied on it, which generated a leachate. Finally the leachates and growth media samples were taken and chemically analyzed for their metal mass content.

The *Brassica juncea* 'the hyperaccumulant' plant seeds were sown directly on the growth media. They were germinated, grown, harvested, dried and stored properly. In addition, its leaves, stems, roots and rhizospheric growth media samples were also analyzed for the eight metals mass content in $mg \cdot kg^{-1}$ of dry weight basis. In addition, the monocotyledonous plant seeds were sown on the most favorite growth media. Finally both of the hyperaccumulant plants were compared based upon their distinct botanical efficiency parameters.

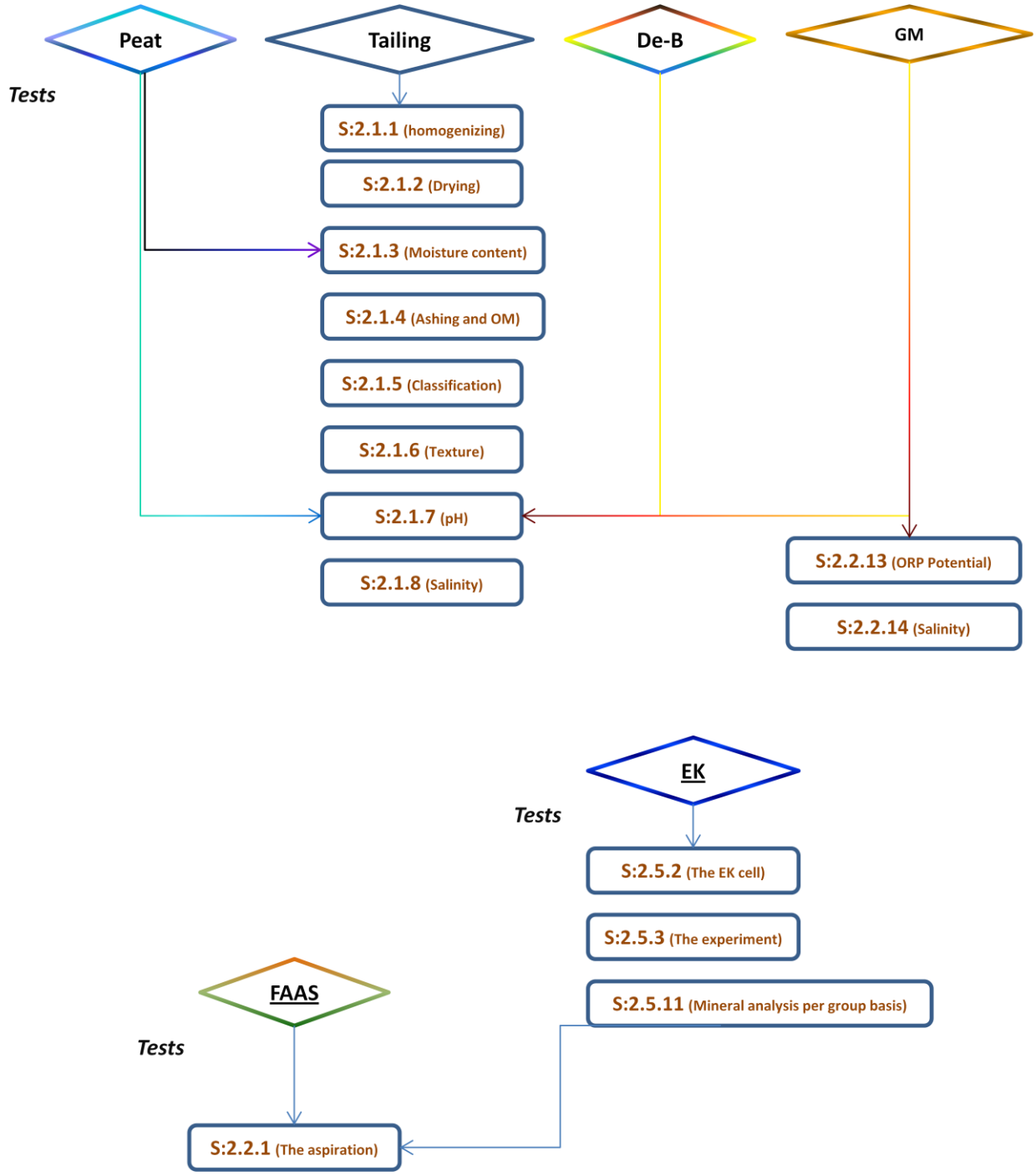


Figure 2.1: The methodological flowchart approach [1]

[1]: S, the abbreviation for section.

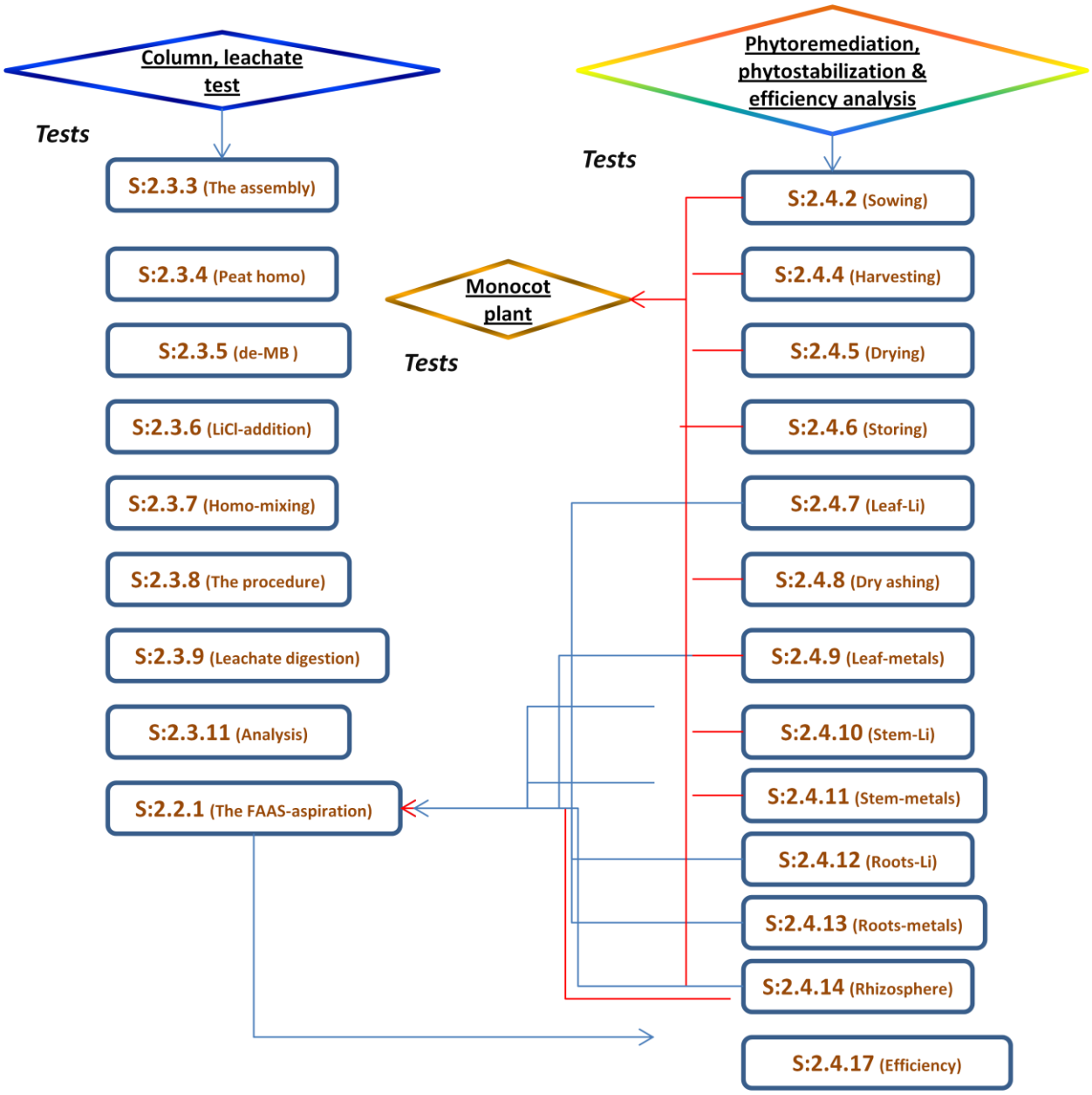


Figure 2.1: The methodological flowchart approach, cont'd

Phase one: Formulation of growth media

2.1.1 Lithium mine tailings (LMT) homogenous mixing

The LMT sample was spread on a flat surface in a square manner. The square was divided into eight equal sections. The mixing was done through the addition of sections 1 on top to 5, 2 to 6, 3 to 7 and 4 to 8 respectively, later on the sections of 1, 5 on top of 3, 7 and 4, 8 on 2 and 6 accordingly. Finally homogenous mixing was achieved through homogeneous mixing of sections 1, 5 and 3, 7 on top of 2, 6 and 4, 8. [234]

2.1.2 Lithium mine tailings drying process

The lithium mine tailing (LMT) sample was exposed to room temperature until it was thoroughly dried. Furthermore, its aggregates were crushed and its particle-size analysis was done through the usage of No.10 (2.00 mm) sieve. (In accordance to ASTM-D421, 2010)

2.1.3 Lithium mine tailings moisture content

This method enabled the laboratory determination of the moisture content on mass basis. The reduction in mass after drying was regarded as a result of direct evaporation of its moisture content. Thus a 200 g of LMT sample was dried in an oven at 110 ⁺⁄₋ 5 ˚c for 16 hours until a constant weight was achieved. The dried sample was removed and weighted directly. Its moisture content was measured to be 7.008%. Finally the excess samples were stored in a sealed envelope and were kept in a dry place. (In accordance to ASTM-D2216, 2010)

2.1.4 Lithium mine tailings organic matter determination

A 25 g of the LMT sample was taken from the oven dried sample. It was placed in a high silica covered dish inside a muffle furnace. The sample was ashed gradually by increasing the temperature of the muffle furnace to 440 ˚C. Consequently the sample was ashed for an hour until a constant weight was obtained to the nearest 0.01 g. Thus the organic carbon content was determined to be 0.29%, which was multiplied by 1.4, resulted in 0.4% as its total organic matter content.

(In accordance to ASTM-D2974, 2010)

2.1.5 Lithium mine tailings classification

The US standard sieves were arranged on top of each other in a decreasing order such that, the mesh number 4 (4.76mm), on top of 8 (2.36mm), then 10 (2.00mm) and likewise to the remaining ones, 12 (1.68mm), 16 (1.19mm), 30 (600 µm), 50 (247 µm), 100 (150 µm) and No.200 (75 µm) sieves. After sieving was achieved the data obtained enabled to construct the “Cumulative grading curve” which was plotted in figure 2.3. Finally the LMT sample was overwhelmingly classified as sandy (figure 2.2).

(In accordance to ASTM-D2487 and ASTM-D2488, 2010)

Figure 2.2: Lithium mine tailing (LMT)

2.1.6 Lithium mine tailings texture determination

The LMT sample was passed through the sieve No.4 and retained on sieve number 10 was considered as coarse sand. Likewise its retained sample on the sieve number 30 was regarded as medium texture sand and on sieve number 200 as fine textured sandy sample.

The sieves were arranged on top of each other according to their size in a decreasing order such that the sieve number 4 was on top and the sieve number 200 was on the bottom. A 1000 g of the LMT sample was passed through the above mentioned sieve complex. The sieving operation was conducted mechanically by means of lateral and vertical motions, accompanied by jarring action that kept the LMT samples moving continuously over the surface of the sieve for twenty four minutes. Finally the mass of the LMT fraction left on each sieve was weighed on the balance to the nearest ± 0.01 g. The retained mass on each sieve size was summed for accuracy and precision purposes.

(In accordance to ASTM-D422, 2010)

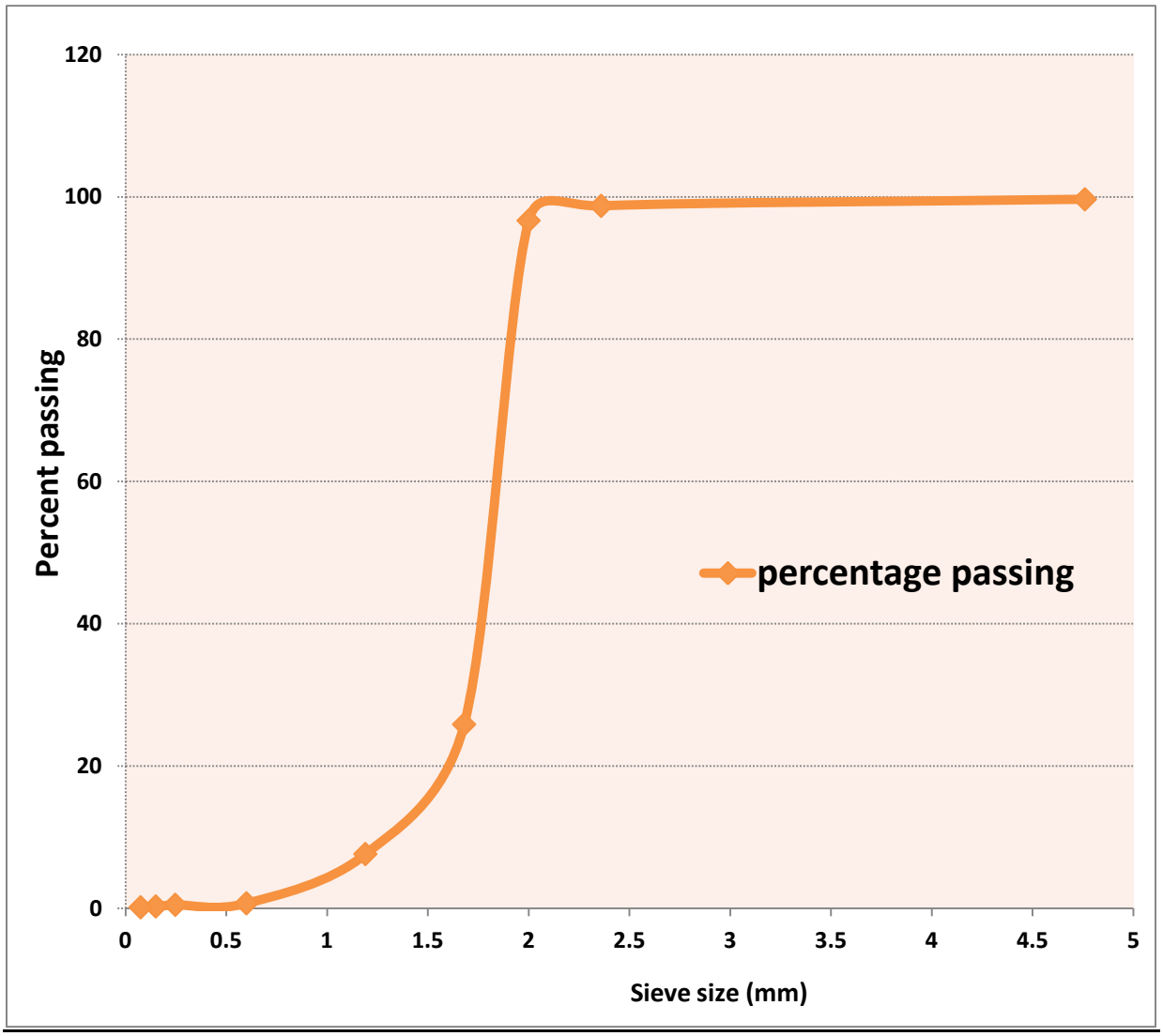


Figure 2.3: LMT cumulative grading curve

2.1.7 Lithium mine tailings pH determination

The test enabled the determination of the H+ ions in LMT solution. It was the determination of the degree of acidity of the LMT sample, suspended in water and in a 0.01 M calcium chloride solution. Measurements in both liquids were essential to constitute the full status of the LMT sample. The pH measurement of the LMT in both water and calcium chloride suspensions were done using the Hach™ electrode. The pH was measured in a calcium chloride solution because calcium displaces some of the exchangeable aluminum. The low ionic strength counters the dilution effect on the exchange equilibrium by settling salt. Finally, the pH values obtained in the solution of calcium chloride was slightly lower, in comparison to the DI water suspension due to the release of more aluminum ions, which consequently got hydrolysed.

The DI water used had a resistance of 10.0 MΩ cm TC at 11.5°C milli-Q-water, Millipore USA. The Calcium chloride stock solution (1.0 M) was prepared by dissolving 147 g of $Ca\ Cl_2 \cdot 2H_2O$ in a 1 L of DI water. The calcium chloride solution (0.01 M) was prepared through the dilution of 20.0 ml of stock 1.0 M $CaCl_2$ solution in 2 L of water. The pH of this solution ranged between 5 and 7.

The electrodes of the pH meter were placed into the partially settled suspension phase and recorded its reading. For both solutions, the measurements were taken from an air-dried soil samples that had been sieved through a No.10 (92 mm) sieve. The pH of the LMT sample was 6.58.

(In accordance to ASTM-D4972, 2010)

2.1.8 Lithium mine tailings salinity determination

The salinity test revealed the total amount of soluble salts bound on the LMT surface. A 10 g of room dried LMT sample was taken and added 50 ml of deionized water. The sample solution was shacked on the BURRELL- Wrist Action™ Pittsburgh, PA-USA vigorous shaker for 5 minutes. The suspended solution was left to settle for one minute before measuring its salinity. Its salinity was measured through the salinity meter electrodes suspended in the solution. Finally, the result was rendered to an actual salinity (EC_e) by multiplying the value by a conversion factor based on the values stated in table 2.1. [235]

The test for salinity was referred to as $EC_{(1:5)}$; which reflected the ratio of one part of LMT sample added on top of five parts of distilled water in weight per volume basis (wt: v). Finally salinity of 2 dS/m was considered a low value that does not impede hyperaccumulant plant growth and survival. On the contrary a value of 6 dS/m was considered an elevated concentration that impedes its growth and development. The results were tabulated in table 2.1. [235,236]

Table 2.1: EC (1:5) to EC (e) Conversion Factors

Soil Texture	**EC (1:5) 'Multiplication factor'** ($dS \cdot m^{-1}$)	**EC (e)** ($dS \cdot m^{-1}$)
Sand	17	0.3638
Sandy loams	13.8	
Loams	9.5	
Clay loams and light clays	8.6	
Medium and heavy clays	7	

(Source: Salinity notes, number 8, October 2000)

2.1.9 Homogenized peat moisture content

The moisture content was expressed as a percent of the oven dry mass. An empty high silica evaporating dish was weighed under room temperature. A 50.0 g of the homogenized peat (figure 2.4), sample was weighted. It was oven dried for 1 hour at 105 ^{o}C. The oven dried sample was weighed and recorded its weight to the nearest 0.01 g. For geotechnical purposes the result was referred to as the moisture content as a percentage value of the oven dried mass, which was measured to be 9 %.

(In accordance to ASTM-D2974, 2010)

Figure 2.4: A homogenized peat sample

2.1.10 Homogenized peat pH determination

A 3 g of well grounded air dried homogenized peat sample was used. The sample was placed in a 100 ml beaker. A 50 ml of DI water was added in a weigh per volume ratio (w: v). It was occasionally stirred for 30 min. Afterwards the calibrated Hach™ pH meter electrode was introduced and its pH measurement was 3.08. (In accordance to ASTM-2976, 2010)

2.1.11 Municipal biosolids pH determination

A 10 ml of freshly obtained municipal biosolids; figure 2.5, mixture was taken. The sample was centrifuged for 5 min at 3.000 rpm and was left to settle for 10 min. The calibrated Hach™ pH meter was introduced and its pH reading was recorded as 6.33.

Table 2.2: Biosolids contents

Content	**Concentration** ($mg \cdot l^{-1}$)
Total and reactive phosphorus	7.52
Phosphorus	8.98
Nitrate	1.08
Chemical oxygen demand	2393
Nitrite	0.732
Volatile acids	4742

Figure 2.5: Fresh municipal biosolids sample

2.1.12 Growth media pH determination

A 10.0 g of the air dried sample was weighed from of each of the five different growth media sample. Furthermore, a 10.0 ml of deionized water was added on top of each. The solution was shacked on a BURRELL- Wrist Action™ Pittsburgh, PA-USA vigorous shaker for 5 min and was left to equilibrate for 1 hour. The calibrated Hach™ pH meter electrode was introduced and its measurements were summarized in table 2.3.

2.1.13 ORP (Eh) determination of different GM formulations

2.1.13.1 Introduction

The oxidation reduction potential is an electrical measurement that shows the tendency of a certain growth media solution to transfer electrons to or from a reference electrode. The measured reading enabled to estimate whether the five different growth media formulations were aerobic (oxidative state) or otherwise anaerobic (reductive state). [237]

2.1.13.2 Procedure

The Hach™ pH meter was attached to a platinum based electrode. The instrument was standardized before usage. A 10.0 g of the growth media sample was placed in a glass container and a 10 ml of DI water was added. The sample was shaken for 5 min on a BURRELL- Wrist Action™ Pittsburgh, PA-USA vigorous shaker. The platinum based electrodes were introduced and recorded its reading. Generally the ORP (Eh) value was represented in milli-volts (mV). It ranges from 800 mV, which reflects an oxidative state to – 500 mV for a strongly reductive state; the full results were summarized in table 2.3. [238]

2.1.14 Salinity (EC) levels of different GM formulations

2.1.14.1 Introduction

Metal salts like lithium chloride are serious threat to the environment and ecosystem biodiversity. It has potential health risks on humans as well as on plant toxicity and loss. As much as 25 % of world's cultivated land and more than the half of the irrigated land are affected by salinity, including lithium chloride salts.

2.1.14.2 Procedure

A 10 g from each of every five different growth media formulations were taken. A 50 ml of deionized water was added. The suspension was shaken on a BURRELL- Wrist Action™ Pittsburgh, PA-USA for 5 minutes and was left to rest for a few minutes. The calibrated salinity probe was introduced and recorded the measurement. It was worth mentioning that an EC range between, 0 to 800 $\mu S \cdot cm^{-1}$ was regarded as suitable for drinking, irrigation and livestock feeding purposes.

On the other hand a range between, 800 to 2500 $\mu S \cdot cm^{-1}$ was regarded as a borderline and used under special management procedures like application of proper drainage and plantation procedures. [241,242]

Finally, a measurement of EC exceeding 10,000 $\mu S \cdot cm^{-1}$ was regarded as non suitable for human consumption, livestock and for irrigation purposes. Moreover, the total dissolved salts (TDS), was measured by multiplying it with a constant value of 0.6. Thus the results of the five different growth media were tabulated in table 2.3.

$$TDS\ (mg.L^{-1}) = EC \cdot 0.6 \qquad [243]$$

Table 2.3: ORP, pH, salinity and TDS characteristics per group basis

Group №	pH	ORP (mV)	EC ($\mu S \cdot cm^{-1}$)	TDS ($mg \cdot l^{-1}$)
Group 1 (100% T)	7.92	-33.4	1264	758.4
Group 2 (50% T, P)	3.74	197.5	538	322.8
Group 3 (33.3% T,P,B)	4.04	170.7	893	535.8
Group 4 (25% T,P, 50% B)	4.64	139.3	728	436.8
Group 5 (50% T and B)	7.16	-5.5	2103	1261.8

2.1.15 Discussion

The different tests described throughout phase one enabled to determine the physical characteristics of the lithium mine tailing (LMT) like its texture, classification, color, pH, moisture content, ORP and salinity. The same was achieved for peat and municipal dewatered biosolids, which were the three main ingredients of the growth media. The research was cornered around the five different growth media mixes that were formulated and engineered as a means of supporting the hyperaccumulant plant seed germination and sustainment of growth. Moreover its ability to successfully reclaim the lithium mine tailings.

First the lithium mine tailings sample was homogenized dried and its initial moisture content was determined to be 7 %, with a 96% sandy textured sample (table 5.1). In addition, its organic matter

content was measured to be 0.4 %. Moreover, its pH was 6.58 and was regarded as suitable for plant germination and survival. Its salinity was 0.363 $dS \cdot m^{-1}$ which was regarded as non saline, therefore suitable for the survival of the hyperaccumulant plant seed germination and growth.

The peat sample was one of the ingredients besides the municipal biosolids sample that was amended to the LMT sample forming the growth media. It was grinded and homogenized its moisture content was determined to be 9.221% and its pH 3.08. It was considered as an extremely acidic sample, due to the abundance of organic acids like, humic (HA) and fulvic acids (FA) in its content. [269]

The second ingredient in the growth media was the addition of the dewatered municipal biosolids sample. It was centrifuged, its pH was measured to be 6.33 and its different characteristics were summarized in table 2.2.

The different components of the growth media, peat, dewatered municipal biosolids and lithium mine tailings (LMT) were mixed differently into five different groups. The mixes were expressed in percentage value, such that the Group 1 had 100% (LMT) which was regarded as the control. The Group 2 had 50 % (LMT) and 50 % homogenized peat, the Group 3 had 33.3 % of (LMT), homogenized peat and dewatered biosolids. The Group 4 had 25 % of (LMT) and homogenized peat and 50% dewatered biosolids and finally the Group 5 had 50 % (LMT) and 50 % dewatered biosolids. Moreover each of the five different growth media formulations pH, ORP, EC as well as their total dissolved salts were measured and summarized in table 2.3. The TDS content in each growth media was considered suitable, for hyperaccumulant plant seed germination and subsequent growth and development despite its relatively elevated contents in Groups one and five.

Phase two: Chemical analysis of growth media

2.2.1 Introduction

The chemical analysis of the three growth media ingredients peat, dewatered biosolids and lithium mine tailing were analyzed for the eight metals; lithium, magnesium, iron, calcium, sodium, chromium, potassium and vanadium mass content. The extraction process was achieved through the implementation of the cold extraction procedure. The usage of concentrated (12N) hydrochloric acid was added on peat dewatered municipal biosolids and lithium mine tailing. They were subjected to concentrated hydrochloric acid digestion. Later on the samples were shaken on a BURRELL- Wrist Action™ Pittsburgh, PA-USA wrist action shaker. Then filtered, subsequently diluted and aspired on Perkin Elmer's Analyst 100™ flame AAS machine.

Finally the results were tabulated in table 2.4 and 2.5 as well as the content of the organic, ESSF™ Earth Solutions organic fertilizer.

2.2.2 Atomic absorption spectroscopy (AAS)

The flame AAS analysis was used. It is based on the principle of atoms at ground state energy status, absorbs electromagnetic radiation at specific wavelengths. The radiation was generated from the hollow cathode lamp. The photons pass through the flame containing atoms of the element. The degree of absorption is proportional to the concentration of the element in the flame. Therefore the concentration of the metal was determined in $mg \cdot l^{-1}$. [245]

Chemical interferences were corrected through the addition of the proper chemical. It was usually in a salt form that was added in equal proportions mixed with the standard and the prepared solution samples [244].

Certified primary elemental standards were used that reduced technical workload and errors. The metallic element aspiration was done through flame atomic absorption spectroscopy, using the Perkin Elmer's, Analyst 100 ™ machine. The main parts of the flame AAS were the hollow cathode lamp, the nebulizer, the burner system and the radiant detection system [245]. The sample was nebulised on the spray which was injected into the flame where it was volatilized. Moreover, an atomic gas was produced into the flame through which its absorbance was measured.

The atomisation was the critical step of atomic absorption. The flame generated was executed under optimum conditions, depending on each of the specific metallic species specificity. The temperature of the flame was adjusted accordingly, which was done by using the appropriate fuel/oxidant combination. The light beam had passed from a hollow cathode lamp onto a mono-chromator and ended up on a detector that measured the amount of light absorbed. Each and every of the eight metals had a specific wavelength and operational guidelines.

Finally the light energy was absorbed by the flame through the detector. In addition the amplifier measured the concentration of that metal in the prepared sample solution. [244,245]

2.2.3 Metal extraction procedure from LMT, peat and de-B samples and results

The process was achieved through the usage of the cold extraction procedure, or the addition of concentrated (12N) hydrochloric acid.

2.2.3.1 Hydrochloric acid extraction procedure for eight metals and results

A 2 g oven dried samples of lithium mine tailings and homogenized peat samples were taken. A 20 ml of concentrated hydrochloric acid, was added with a solid to solution ratio of 1:10 (m: v) [246].

The suspended solution was shaken on a BURRELL- Wrist Action™ Pittsburgh, PA-USA wrist action shaker for 1 hour and was left overnight to settle [247, 248]. The suspended solution was centrifuged at 2.000 rpm, for 10 min and was filtered through Whatman No.1 filter paper (particle retention of 11 μm) [247]. Finally the extraction procedure was repeated twice on the above mentioned samples. The supernatants were then taken and the precipitates were stored. [248]

2.2.4 Lithium measurement

The optimum concentration range was 22 $mg \cdot l^{-1}$ using a wavelength of 670.8 nm and a sensitivity of 0.035 $mg \cdot l^{-1}$, fuel Acetylene and oxidant air. The Standard solution was prepared by dissolving 5.324 g of lithium carbonate (Li_2CO_3) in a minimum volume of (1+1) HCL and diluted to 1 L with deionised water. 1 ml = 1.00 mg Li (1000$mg \cdot l^{-1}$). Furthermore, the dilution of the stock lithium solution was prepared and used as calibration standards at the time of the experimental analysis. The calibration standards were prepared using the same type of acid and the concentration as that of sample being analysed. For the analytical purposes, the manufacturer's manual and recommendations were followed.

2.2.5 Magnesium measurement

The optimum concentration range was 8.5 $mg \cdot l^{-1}$, with a wavelength of 285.2 nm, sensitivity of 0.007 $mg \cdot l^{-1}$, and detection limit of 0.001 $mg \cdot l^{-1}$. The fuels used were acetylene and oxidant air. The standard solution was prepared from dissolving 0.829 g of magnesium oxide (MgO) in 10 ml of redistilled HNO_3 and was diluted to 1 l mark, using deionised or distilled water, such that 1 mL = 0.5 mg of Mg (500 $mg \cdot l^{-1}$). A 0.1% of Lanthanum chloride was added to samples and standards to overcome the interference of aluminum, titanium, silica and phosphorus on magnesia signal. Furthermore, the dilutions of the magnesium solution stock solution were prepared and were used as calibration standards at the time of the experimental analysis. The calibration standards were prepared using the same type of acid at the time of concentration as that of sample that was being analysed. For the analytical purposes, the manufacturer's manual and recommendations were followed.

2.2.6 Iron measurement

The optimum concentration range was 30 $mg \cdot l^{-1}$ with a wavelength of 248.3 nm, sensitivity of 0.12 mg/l and detection limit of 0.03 $mg \cdot l^{-1}$. The fuel used was acetylene and oxidant air. The standard solution was prepared by mixing 1000 g of pure iron wire dissolved in a 5 ml of redistilled hydrochloric acid and was made up to 1 L mark with deionised water. 1 ml = 1 mg Fe (1000 mg/l). The stock solution was used as calibration standards with the prepared dilutions at the time of the experimental analysis. The calibration standards were prepared using the same type of acid and at the time of concentration as that of sample being analysed. Finally 0.2% calcium chloride was added on the samples and standards solutions to overcome the interferences of silica, cobalt, copper and nickel. For the analytical purposes, the manufacturer's manual and recommendations were followed.

2.2.7 Calcium measurement

The optimum concentration range was 17 $mg.l^{-1}$, with the wavelength of 422.7 nm, sensitivity of 0.08 mg/l and detection limit of 0.01 mg/l. The fuel used was acetylene and oxidant air. The stock solution was prepared in hydrochloric acid and used in standards. A 0.1% potassium chloride was added to the samples and standards equally to overcome the interferences of Al, Be, P, Si, Ti, V and Zr. Finally for the analytical purposes, the manufacturer's manual and recommendations were followed.

2.2.8 Sodium measurement

The optimum concentration range was 6.5 $mg.l^{-1}$, with the wavelength of 589.6 nm, sensitivity of 0.015 mg/l and a detection limit of 0.002 mg/l. The fuel used was acetylene and oxidant air. A 0.1% of potassium chloride was added to the samples and standards equally to overcome the interferences of high concentration of mineral acids. For the analytical purposes, the manufacturer's manual and recommendations were followed.

2.2.9 Chromium measurement

The optimum concentration range was 9 $mg{\cdot}l^{-1}$, at the wavelength 357.9 nm. Sensitivity limit of 0.25 mg/l and the detection limit of 0.05 mg/l. The fuel used was acetylene. The stock solution was prepared by dissolving 1.923 gr of chromium trioxide (CrO_3) in deionised water. The solution was acidified with HCL acid and diluted to 1 L mark with deionised water such that 1 ml = 1 mg of Cr (1000 mg/l). A 2% ammonium chloride was added to the samples and the standards equally to overcome the interferences of iron and excess phosphates that might have been present. For the analytical purposes, the manufacturer's manual and recommendations were followed.

2.2.10 Potassium measurement

The optimum concentration was 22 $mg \cdot l^{-1}$, at a wavelength of 766.5 nm, sensitivity of 0.04 mg/l and the detection limit was 0.01 $mg \cdot l^{-1}$. The stock solution was prepared by dissolving 0.1907g of KCL in hydrochloric acid and diluted in deionised water to the 1 litre mark. 1 ml = 0.10 mg K (100 mg/l). Finally a 0.1% of lanthanum chloride was added to the samples and the standards to depress the interference of mineral acids that might have been present. For the analytical purposes, the manufacturer's manual and recommendations were followed.

2.2.11 Vanadium measurement

The optimum concentration was 250 $mg \cdot l^{-1}$, at the wavelength of 318.4 nm, with a sensitivity of 0.8 mg/l and a detection limit of 0.2 mg/l. The preparation of the stock solution was done through dissolving 1.7854 gr of vanadium pentoxide V_2O_5, in 10 ml of concentrated in hydrochloric acid. It was diluted to 1 litre level with deionised distilled water additions. 1 ml = 1 mg V (1000 mg/l). Finally a 0.1% of potassium chloride was added to the samples as well as the standards to depress the interferences of Fe, Al, Ti and H_3PO_4. For the analytical purposes, the manufacturer's manual and recommendations were followed.

(In accordance with ASTM-E885 and D1971, 2010)

Table 2.4: Metal mass content in peat, de-B, LMT and organic fertilizer [1-2-3]

	Peat [2] Mean ± SD	De-B [3] Mean ± SD	LMT [2] Mean ± SD	Organic Fertilizer [3] Mean ± SD
V	17.043 ± 0.516	33.89 ± 0.774	16.967 ± 0.305	5.147 ± 0.478
Li	*<LLD*	*<LLD*	*<LLD*	*<LLD*
Cr	0.180 ± 0.031	0.361 ± 0.041	0.171 ± 0.047	0.054 ± 0.018
Fe	0.475 ± 0.005	1.064 ± 0.048	0.494 ± 0.005	0.148 ± 0.034
Mg	0.76 ± 0.745	1.387 ± 0.710	0.009 ± 0.003	0.327 ± 1.744
Ca	0.446 ± 0.064	0.342 ± 0.008	0.266 ± 0.014	0.082 ± 0.006
Na	0.047 ± 0.002	0.095 ± 0.002	0.009 ± 0.003	0.014 ± 0.001
K	*NIL* ± 0.002	*NIL* ± 0.001	0.009 ± 0.001	30.999 ± 30.041

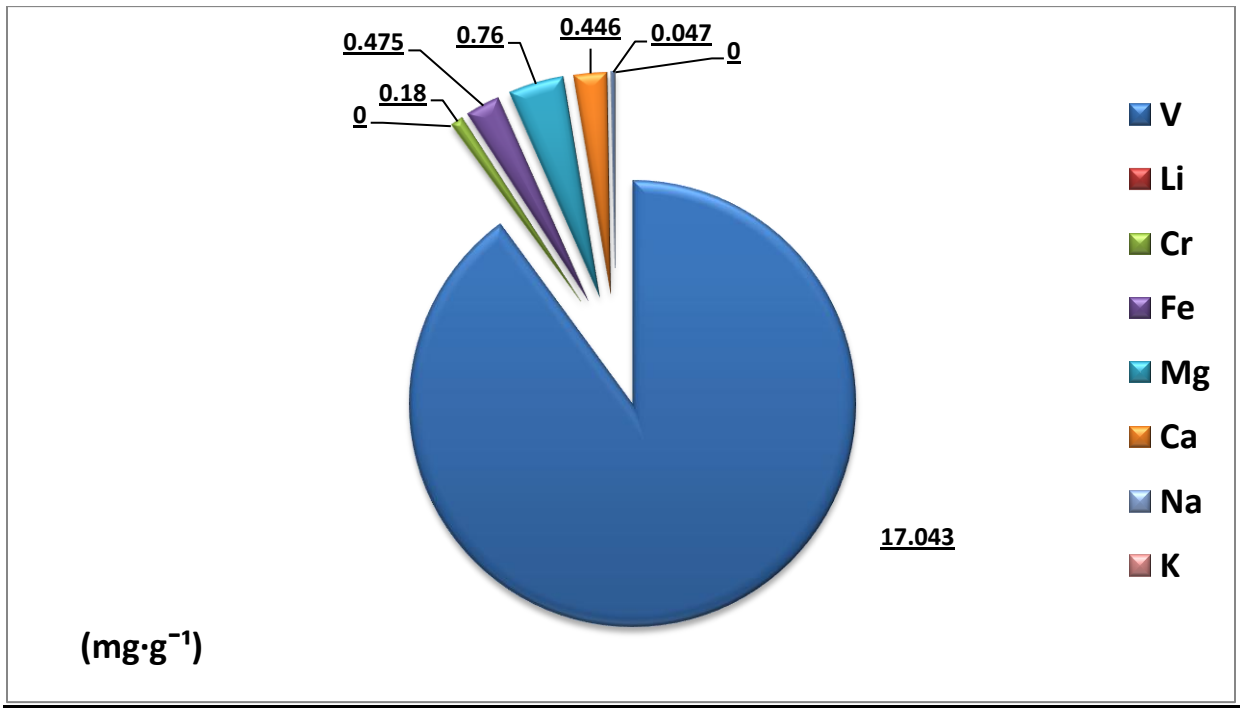
[1]: In hydrochloric acid matrix

[2]: Metals mass content in mg per g of peat and LMT taken assuming uniformity

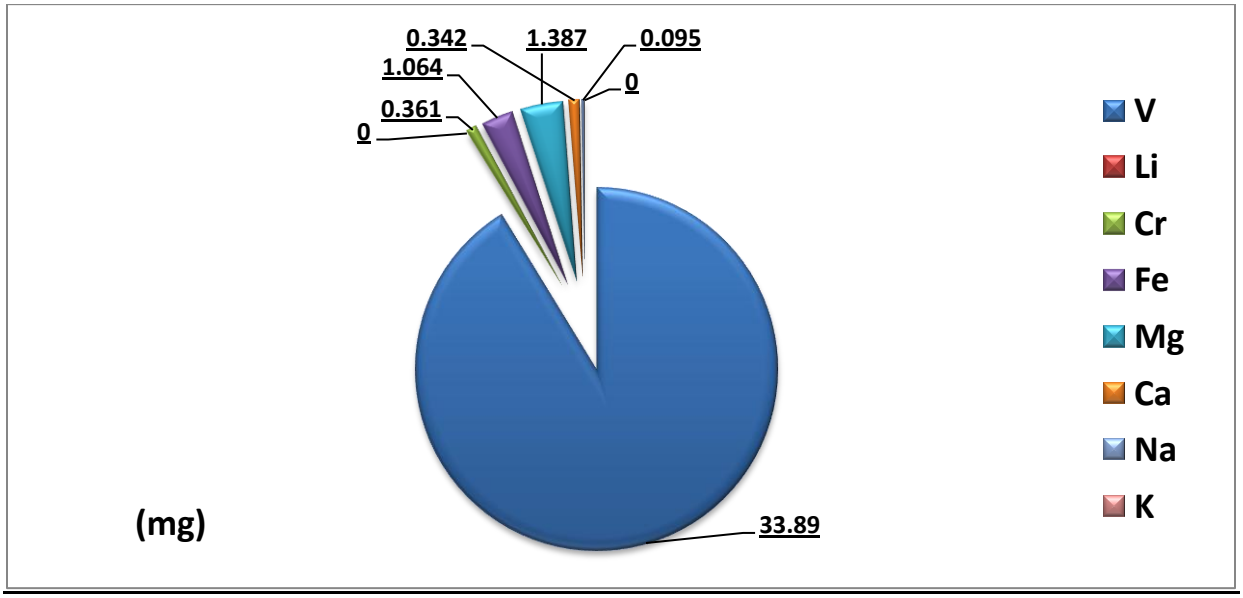
[3]: Metals mass content in mg

<LLD: Lower level of detection

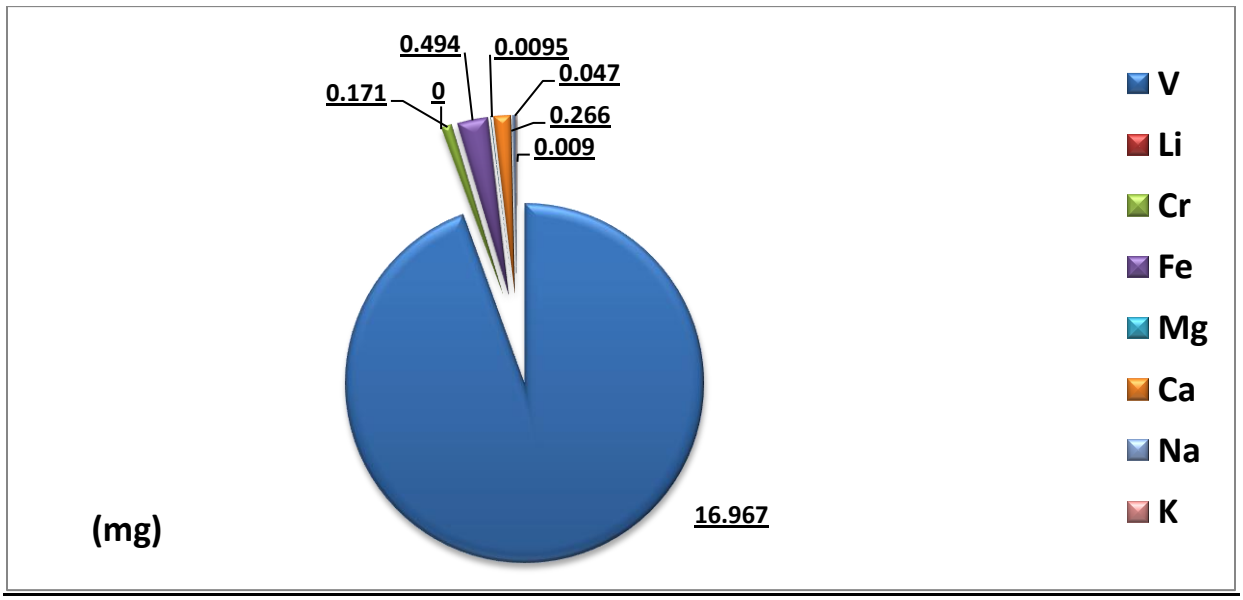
a) ***The peat***



b) ***The dewatered municipal biosolids***



c) ***The lithium mine tailing***



d) ***The organic fertilizer***

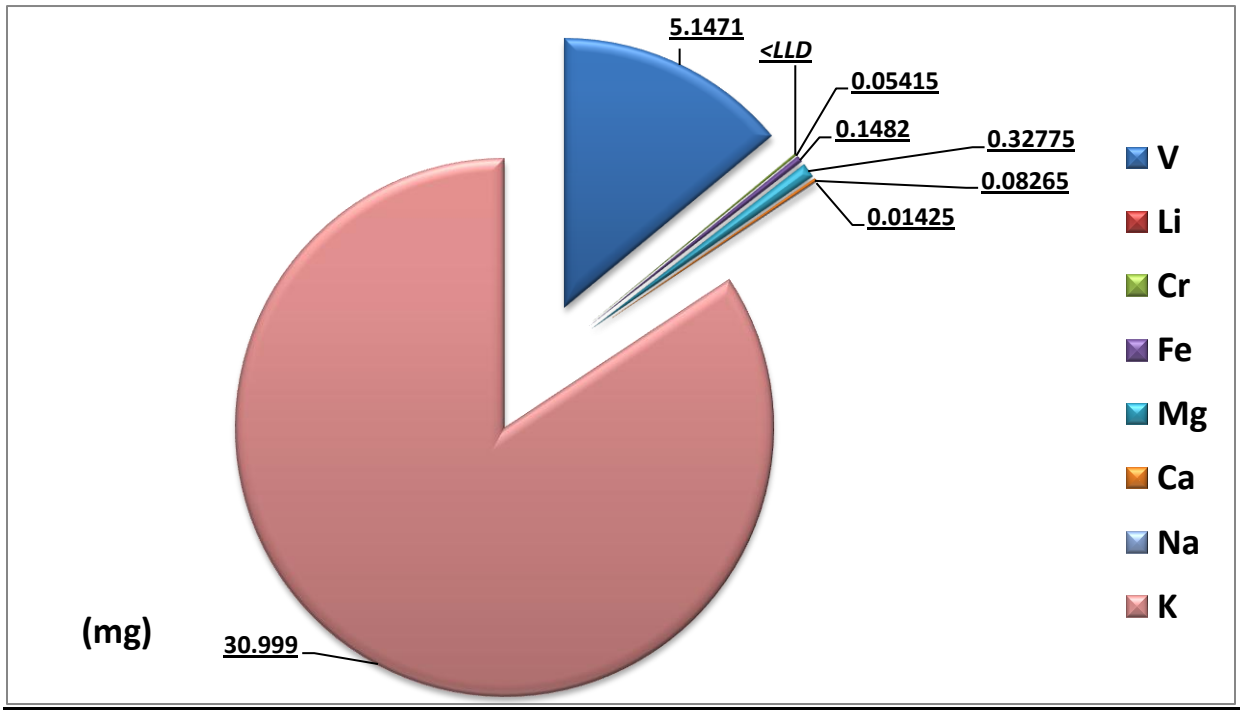


Figure 2.6: Metal mass content in a) peat, b) de-MB, c) LMT and d) organic fertilizer

Table 2.5: Initial metal concentration in five different growth media [1-2-3]

	Group 1 (100% T) Mean ± SD	**Group 2** (50% T and P) Mean ± SD	**Group 3** (33.3% T,P and B) Mean ± SD	**Group 4** (25% T,P and 50% B) Mean ± SD	**Group 5** (50% T and B) Mean ± SD
V	17.024 ± 0.168	17.176 ± 0.567	16.986 ± 0.359	17.005 ± 0.044	17.043 ± 0.391
Li	0.361 ± 0.191	0.418 ± 0.248	0.247 ± 0.651	0.380 ± 0.358	0.342 ± 0.181
Cr	0.171 ± 0.037	0.171 ± 0.007	0.171 ± 0.013	0.171 ± 0.031	0.171 ± 0.014
Fe	0.475 ± 0.069	0.465 ± 0.066	0.465 ± 0.059	0.465 ± 0.049	0.484 ± 0.034
Mg	0.019 ± 0.003	0.589 ± 1.292	0.484 ± 0.312	0.427 ± 0.287	0.152 ± 0.049
Ca	0.285 ± 0.009	0.408 ± 0.003	0.180 ± 0.007	0.142 ± 0.004	0.142 ± 0.020
Na	0.047 ± 0.003	0.038 ± 0.000	0.047 ± 0.000	0.047 ± 0.002	0.047 ± 0.001
K	0.009 ± 0.013	0.009 ± 0.003	0.009 ± 0.004	0.019 ± 0.022	0.009 ± 0.010

[1]: In concentrated hydrochloric acid digestion matrix.

[2]: Metal mass content in $mg \cdot g^{-1}$ homogenized samples taken, assuming uniformity.

[3]: Per different group basis.

2.2.12 Discussion

The chemical analysis of the three ingredients of the growth media was done through the addition of the concentrated (12N) HCl acid. It enabled the measurement of the eight different metals mass content as well as the organic fertilizer. The flame AAS procedure verified the presence of elevated amounts of vanadium and chromium inside the peat and the lithium mine tailing samples (table 2.4 and figure 2.6).

According to Shotyk [270], peat belongs to the *Sphagnum* plant family. It has an organic origin composed of partially decomposed mosses, bryophytes, sedges, grasses, trees, etc. Peat is usually brownish to blackish in color and it is very susceptible to decomposition and form change with time, but slowly due to the presence of high concentration of lignin that withstand decay.

When it is taken out of its biosphere and used in horticultural and reclamation purposes, its rate of decomposition accelerates due to the increase oxygen exposure. As a result its redox conditions are changed favoring the enhancement of growth and flourishing of the microbial colonies numbers, which use it as a major nutritional source for their growth and reproduction. [269,271].

Natural peat has a low mechanical strength and high affinity to water [272]. As a result it was primarily incorporated into the growth media Groups of 2, 3 and 4.

According to Spedding [273], he defined peat as the first stage of coal formation. However, under increased pressure and temperature it eventually forms coal. The whole process takes over forty million years. Therefore peat is formed in ecosystems with poor nutrient availability like in waterlogged areas. Under anoxic conditions, the microbial activity is at its minimum due to the presence of humic acid (HA), fulvic acid (FA), cellulose based and low molecular weight compounds. These are generally amphipathic in nature especially in acidic entourage, which increases the availability of heavy metals like V, Cr, Cd and Fe. These metals might also be bound to the above mentioned compounds forming

stable organo-mineral complexes due to their chemical and biological bond stability [274,275,276]. These organo metallic complexes hinder heavy metals percolation in ecosystems. However, the HA is widely present in peat. It is characterized by oxygen containing functional groups like COOH, phenolic, alcoholic (OH), ketonic and quinonoid (C=O) endings. According to Kendorff and Schnitzer [277] the humic acid successfully interacts with inorganic minerals forming stable compounds.

According to Chaney and Hundemann [278], Coupal and Lalancette [279], the polar characteristics of the peat enables it to bind and successfully adsorb and retain organic molecules as well as inorganic metals like Cd, Zn, Pb, Cu, Hg, Fe, Ni, Cr (VI), Cr (III), Ag and Sb.

According to Crist *et al*. [280], verified that numerous ion exchange mechanisms takes place on the peat surface, due to the presence of natural acids like the HA and the FA. It reacts rapidly with heavy metals, releasing protons at acidic pH, which enables the displacement of metals by others.

According to Wolf *et al*. [281], Ong and Swanson [282] succeeded proving that increasing calcium concentration in peat enhanced the sorption of heavy metals like Pb, Cd, Cu and Zn. As an outcome different peat surface adsorption mechanisms occurs between the positively charged metals and the negatively charged radicals, forming bonds and stable entities, that eventually increases its surface area. Moreover, Chen *et al*. [283], proved after frequent additions of $Cu(NO_3)_2$ under certain pH conditions, adsorption-complexation interactions occurred between the cations and the anion entities.

Sharma and Forster [284] were able to prove the strong sorption of peat surface which was possible in acidic pH. Thus the Cr (VI) on the peat surface was strongly adsorbed but only a negligible amount was released of the total Cr (VI). It was verified that the Group 4 growth media, which was composed of 25% LMT, 25% homogenized peat and 50 % of dewatered municipal biosolids at a pH of 4.64. The initially measured chromium content was 0.171 $mg \cdot gr^{-1}$. However, the hyperaccumulant plant *Brassica juncea*, managed to phytoextract from the Group 4 growth media and translocate 0.0655 mg in its

roots 0.296 mg in its stem, 0.1595 mg in its leaves and phyto-stabilize 0.256 $mg \cdot gr^{-1}$ around its rhizosphere, totaling 0.5212 $mg \cdot gr^{-1}$. Comparing the two totals revealed a considerable difference between them, which is a clear indication that the above mentioned sorption processes had surely occurred. It was speculated that the roots of the hyperaccumulant plant was able to extract the hard bound chromium from the growth media of the Group 4.

Finally as a source for the elevated concentrations of vanadium 17.043 $mg \cdot gr^{-1}$, chromium 0.1805 $mg \cdot gr^{-1}$ and iron 0.475 $mg \cdot gr^{-1}$ in the peat content, as measured in table 2.4 and figure 2.6, was speculated to two main sources.

The first source was speculated to be the direct result of the parent material formation. Moreover, the second source might be due to the different anthropogenic and industrial activities that might have occurred or still occurring near or around its vicinity. Like fossil fuel combustion or coal burning in which the content of vanadium in fuel might range between 60 to 1000 $mg \cdot kg^{-1}$ and from 2 to 100 $mg \cdot kg^{-1}$ in coal combustion. [285,286]

The purpose behind the usage of dewatered municipal biosolids was to enhance in the different growth media Groups with a potential source of viable microorganism colonies for different symbiotic interactions that might occur with the hyperaccumulant plant root system. Eventually this interactions will enhance the nutrient availability specially nitrogen and phosphorus to the hyperaccumulant plant uptake. It will also render the hard bound metals, like vanadium, chromium, iron and lithium more accessible to plant uptake translocation and storage into its different physiological parts. [287]

Chapter three: Column and leachate test

3.3.1 Introduction

The column leaching procedure is a standard method for generating aqueous leachate from the five different growth media formulations. A 0.3 g of lithium chloride was amended in every 100 g of growth media mix. Each growth media was placed inside the column (figure 3.7). A one pore volume of deionized water was added specific to each group. Furthermore, a 5 psi (~ 35kPa) of pressure was exerted on the column, which resulted in leachate generation. It provided the proper means to analyse the solute metal mass content solubility in the solvent. The effluent collected was aspired by the flame atomic absorption spectroscopy (AAS). It enabled the measurement of the lithium and the remaining seven metals mass content. The column leachate procedure enabled to conclude the metal absorbing and releasing abilities of the five different growth media formulations for the eight metallic elements.

The maximum permissible particle size was 10mm; larger particles were removed through sieving that ensured adequate compaction. Organic immiscible materials were removed from the column since it might have lead to plugging and fouling. Volatilization was a big problem to column test procedure. Its effect was negligible due to the implementation of proper handling and testing procedures.

3.3.2 General assembly

Each of the circular cylindrical sections of the column apparatus were thoroughly washed, cleaned and consequently dried. After each repetitive experiment they were placed properly on top of each other, starting from the bottom cap, the middle cylindrical rings and ending up with the upper cap. They were fitted and fastened with outer screws, bolts and metallic rings. Throughout the column test experiment, the applied pressure was synchronized by the pressure tower, alongside the column apparatus. It regulated the inflow of air pressure that was exerted on the deionized water. The applied pressure was able to wash the different growth media mix profiles and dissolve the solute within the solvent effluent.

3.3.3 Peat homogenization process

A 180 g of the peat sample was taken; its lumps and formations were crushed with a spatula. Furthermore the sample was homogenized with a blender. It was covered with an opaque cap to deprive it for absorbing moisture and light penetration from its surrounding.

3.3.4 Municipal biosolids dewatering procedure

A 250 ml of raw sludge or municipal biosolids suspension was taken. It was centrifuged for 5 minutes at 3,000 rpm speed. The supernatant was decanted and the concentrate was used as an ingredient in the growth media formulations.

3.3.5 LiCl amending procedure

The five different growth media formulations or groups were amended with a 0.3 g of lithium chloride, per 100 g of the growth media mix.

3.3.6 Homogenous mixing

The different growth media formulations or Groups were spread on a flat surface in a square manner. The square was divided into eight equal divisions. Furthermore, the primary mixing was done by the addition of divisions 1 on top 5, 2 on top 6, 3 on top 7 and 4 on top 8. The process was continued by mixing the divisions of (1, 5) on top of (3, 7) and (4, 8) on top of (2 and 6) respectively. Finally a complete homogenization was achieved through mixing divisions (1, 5) and (3, 7) on top of (2, 6) and (4, 8). [234]

3.3.7 Experimental procedure

The five different growth media mixes were amended with 3 $g \cdot kg^{-1}$ of LiCl. It was left to react for ten consecutive days. Furthermore, the five different media formulations were introduced into the column apparatus one after the completion of the other. The void volume was calculated for each growth media using the equation stated beneath. Moreover, a one pore volume of deionized water was added specific to each growth media. In addition, a pressure of 5 psi (~35kPa) was applied on the column apparatus; as a result it generated effluent.

$$Vp = Vc \{M/ [(1+W) \cdot S \cdot D]\}$$

Where; Vp = void volume in column in cm^3

Vc = volume of column in cm^3

M= as packed mass of the material including its moisture, contained in the column in g.

W= moisture content of the material inside the column in g- water/g-solids.

S= Specific gravity of the material, unit less.

D= density of water in g/cm^3.

The degree of saturation was determined through the calculation of porosity (n) by using the following relationship: $n = Vp/Vc$

(In accordance with ASTM-D4874, 2010)

The column apparatus was filled with a unique growth medium mix. A pressure of 5 psi units (~35 kPa) was adjusted and exerted by the pressure tower. Furthermore, the same growth medium profile was washed for three consecutive times. Every wash consisted one pore volume of deionized water specific to each growth media. In addition, the column and the pressure tower were run under ambient temperature and under normal testing protocols of room temperature 22 °C and humidity 40 %. The collected leachate samples were stored and refrigerated (at 7˚C) in sealed containers.

3.3.8 Leachate digestion procedure in concentrated hydrochloric acid

The collected leachate samples were tested for their salinity, pH and oxidation reduction potential. Moreover, a 3 ml of the generated leachate was digested with 3 ml of concentrated (12N) hydrochloric acid. The digestion was left to proceed for 48 hrs. Its metal mass content was analyzed through the atomic absorption spectrometry (AAS). Finally the concentrations of the eight metallic elements were calculated using the following formula;

$$\text{Metallic element concentration (mg/l)} = (C) \cdot (d.f.)$$

Where;

C= concentration of element in sample solution in ml.

df = is the dilution factor.

3.3.9 Organic fertilizer analysis

A 3 ml of the organic fertilizer was taken. It was digested by the addition of 3 ml concentrated (12N) hydrochloric acid on volume per volume basis (v: v). The digestion process was proceeded for 72 hrs and its metal mass content was analyzed through the flame atomic absorption spectrometry (AAS).

2.3.10 Metal mass content analysis per group basis and results

A 10 g from different growth media formulations was taken. Furthermore, the samples were oven dried at 105 ±5°C for one hour. Their moisture content was measured and was each sample was digested in a 100 ml of (12N) concentrated hydrochloric acid.

Finally the eight metallic element concentrations were measured using the flame AAS. The results were summarized in tables 3.1, 3.2 and 3.3.

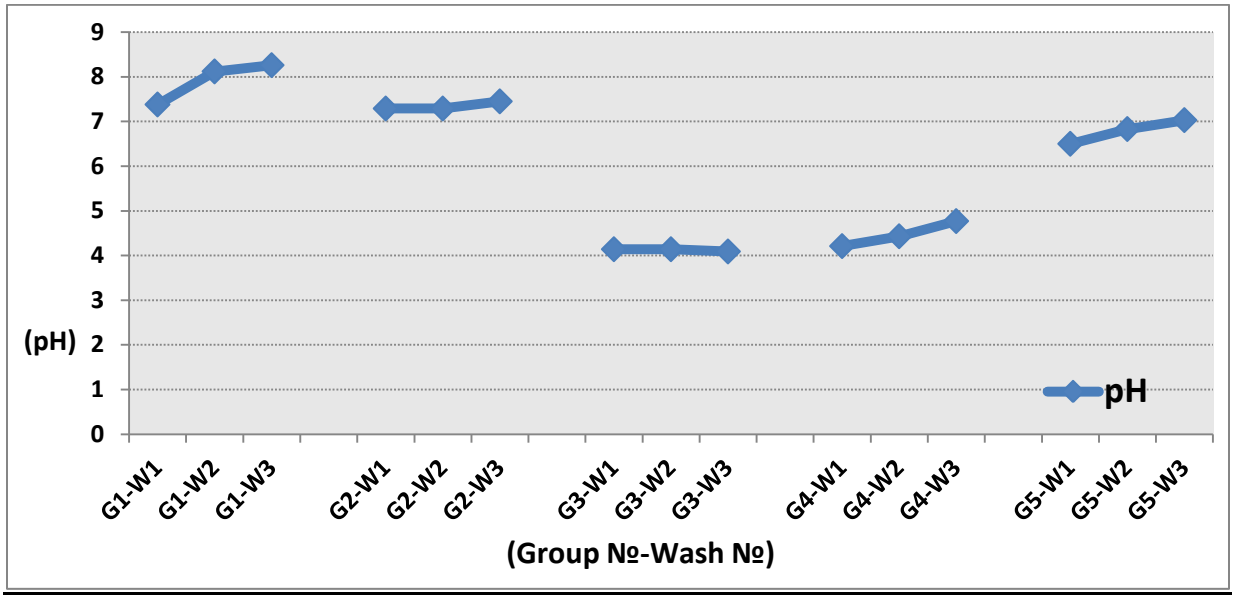
Table 3.1: Leachate characteristics per different group and wash basis [1-2]

Group №	**Washes** №	**pH**	**ORP** (mV)	**EC** ($mS \cdot cm^{-1}$)
1 100% lithium mine tailing (LMT)	**1**	7.38	-52.8	11.99
	2	8.12	-60.7	5.79
	3	8.26	-68.9	2.53
2 50% (LMT) and peat (P)	**1**	7.29	-13.2	0.662
	2	7.29	-13.0	0.531
	3	7.45	-22.4	0.390
3 33.3% (LMT),(P) and biosolids (B)	**1**	4.14	167.1	5.80
	2	4.14	166.5	3.36
	3	4.09	169.1	2.030
4 25% (LMT),(P) and 50% (B)	**1**	4.21	163.4	4.69
	2	4.43	151.1	2.171
	3	4.77	131.4	1.704
5 50% (LMT) and (B)	**1**	6.50	32.4	7.13
	2	6.83	13.2	3.21
	3	7.03	1.9	1.840

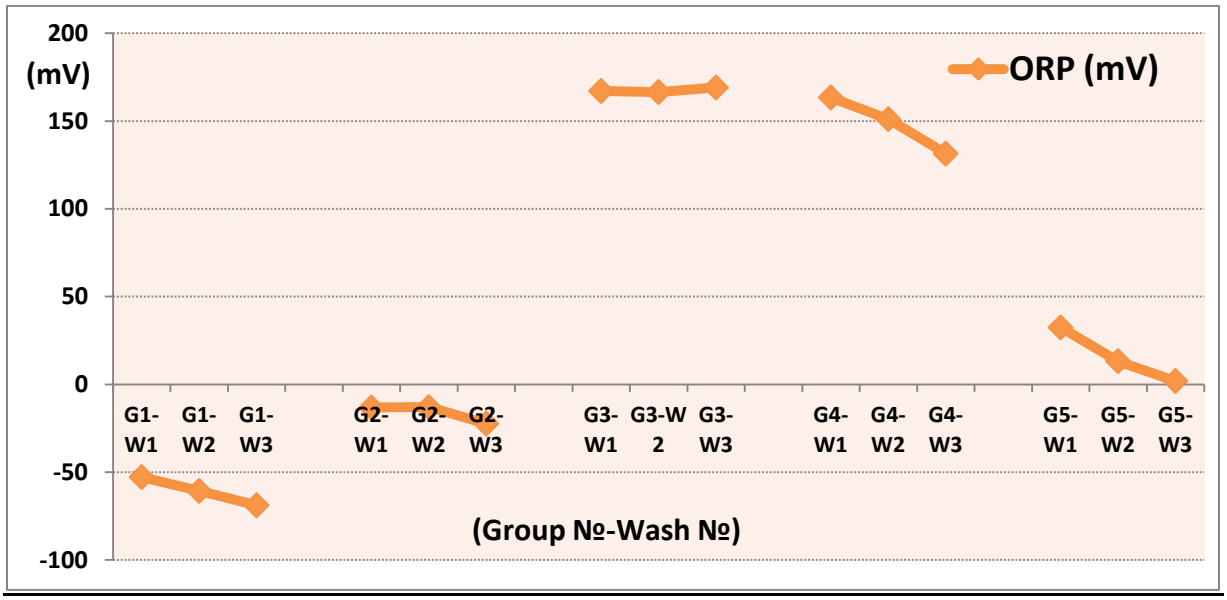
[1]: The EC was measured at 21 °c non linear NaCl

[2]: LMT stands for lithium mine tailing, P stands for homogenized peat and B stands for dewatered biosolids

a) pH


9
8
7
6
5
4
3
2
1
0
(pH)
pH
G1-W1
G1-W2
G1-W3
G2-W1
G2-W2
G2-W3
G3-W1
G3-W2
G3-W3
G4-W1
G4-W2
G4-W3
G5-W1
G5-W2
G5-W3
(Group №-Wash №)

b) ORP


200
(mV)
150
100
50
0
-50
-100
ORP (mV)
G1-W1
G1-W2
G1-W3
G2-W1
G2-W2
G2-W3
G3-W1
G3-W2
G3-W3
G4-W1
G4-W2
G4-W3
G5-W1
G5-W2
G5-W3
(Group №-Wash №)

*c) **EC***

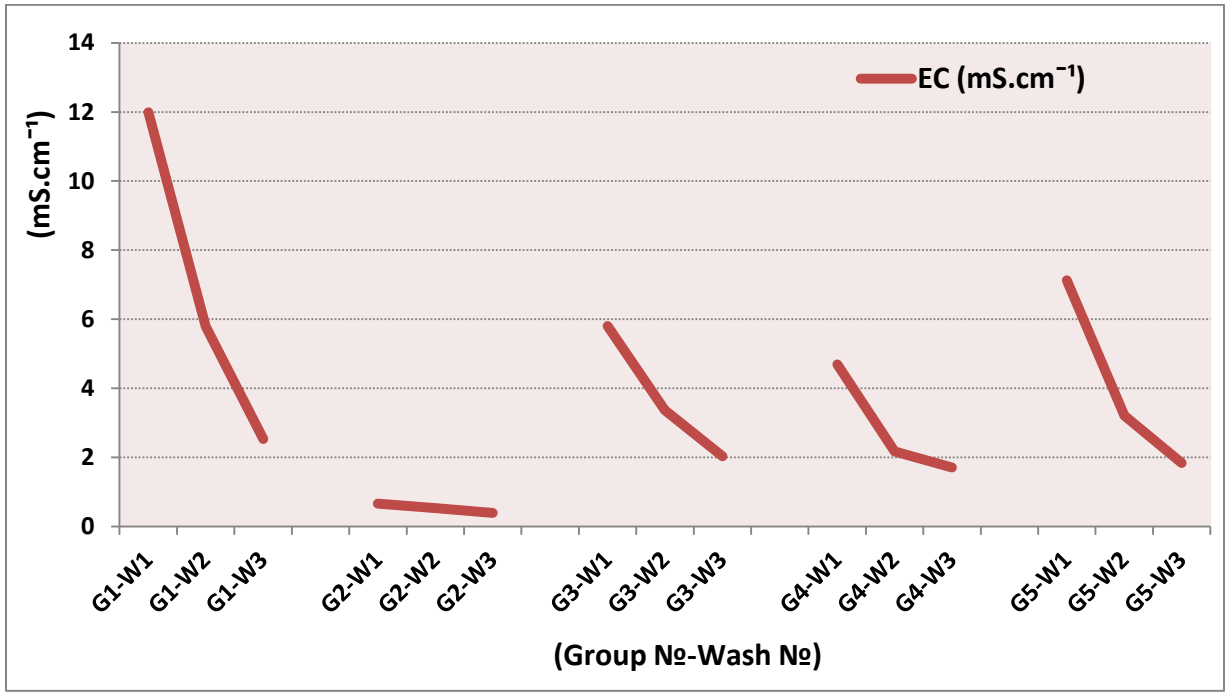


Figure 3.1: pH, ORP and EC per group and wash basis [1]

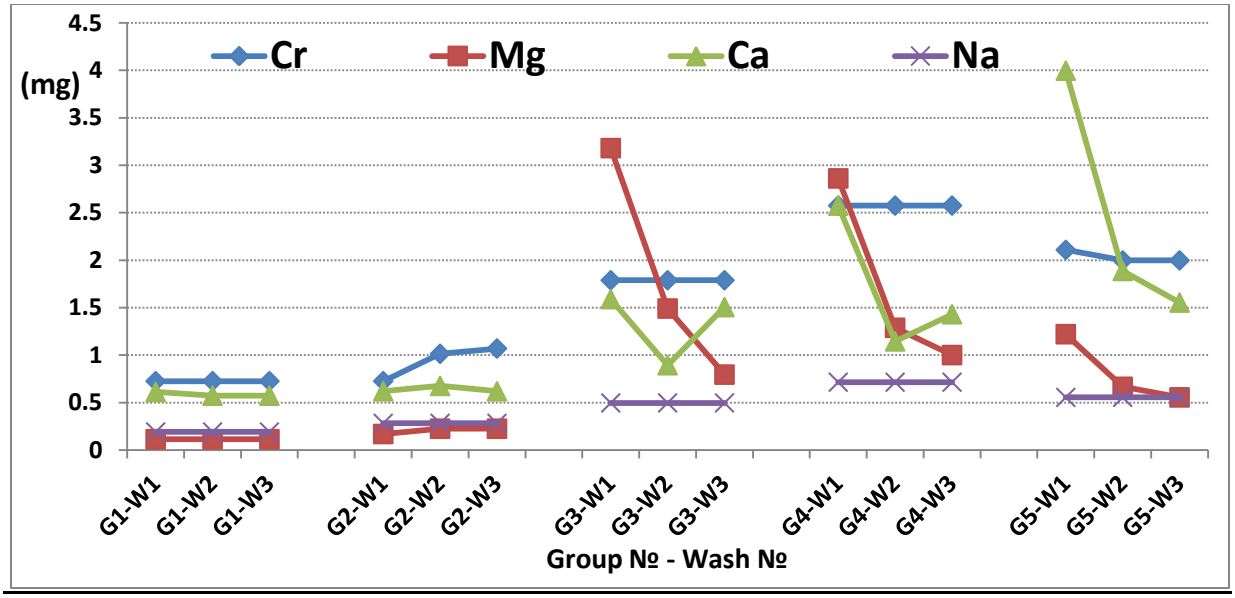
[1]: The leachate generated after three consecutive washes

Table 3.2: Leachate metal mass content per Group and wash basis [1]

GM	Wash №	V Mean ± SD	Li Mean ± SD	Cr Mean ± SD	Fe Mean ± SD	Mg Mean ± SD	Ca Mean ± SD	Na Mean ± SD	K Mean ± SD
1 (100 % T)	1	67.719 ±0.668	12.632 ±1.001	0.726 ±0.031	1.912 ±0.049	0.114 ±0.004	0.612 ±0.010	0.191 ±0.004	0.038 ±0.006
	2	68.249 ±0.485	6.733 ±0.095	0.726 ±0.025	1.874 ±0.044	0.114 ±0.005	0.573 ±0.006	0.191 ±0.003	0.038 ±0.009
	3	67.867 ±0.293	1.606 ±0.748	0.726 ±0.016	1.874 ±0.018	0.114 ±0.001	0.573 ±0.027	0.191 ±0.000	0.267 ±0.913
2 (50% T and P)	1	100.66 ±0.090	*<LLD*	1.014 ±0.032	2.761 ±0.021	0.169 ±0.006	0.619 ±0.018	0.2818 ±0.001	0.056 ±0.003
	2	100.19 ±0.178	*<LLD*	1.07 ±0.016	2.761 ±0.146	0.225 ±0.007	0.676 ±0.032	0.2818 ±0.001	0.056 ±0.008
	3	100.21 ±0.684	*<LLD*	1.07 ±0.012	2.761 ±0.028	0.225 ±0.007	0.619 ±0.012	0.2818 ±0.002	0.056 ±0.003
3 (33.3 % T,P and B)	1	176.61 ±0.480	*<LLD*	1.789 ±0.039	4.871 ±0.095	3.181 ±0.184	1.59 ±0.021	0.497 ±0.001	0.894 ±0.161
	2	177.40 ±0.678	*<LLD*	1.789 ±0.037	4.871 ±0.027	1.491 ±0.035	0.894 ±0.009	0.497 ±0.002	0.596 ±0.213
	3	177.20 ±0.611	*<LLD*	1.789 ±0.009	4.871 ±0.016	0.795 ±0.007	1.506 ±0.016	0.497 ±0.003	0.198 ±0.045
4 (25% T,P and 50% B)	1	253.90 ± 0.075	*<LLD*	2.576 ±0.019	7.013 ±0.005	2.862 ±0.019	2.576 ±0.011	0.715 ±0.001	2.29 ±0.003
	2	255.91 ±0.228	*<LLD*	2.576 ±0.043	7.013 ±0.043	1.288 ±0.020	1.145 ±0.006	0.715 ±0.002	0.429 ±0.017
	3	255.05 ±0.519	*<LLD*	2.576 ±0.019	7.013 ±0.054	1.001 ±0.016	1.431 ±0.018	0.715 ±0.002	6.154 ±1.868
5 (50% T, and B)	1	199.19 ±0.356	22.651 ±0.241	2.109 ±0.023	5.44 ±0.089	1.221 ±0.008	3.997 ±0.008	0.555 ±0.001	0.222 ±0.014
	2	198.08 ±0.514	7.550 ±0.113	1.998 ±0.047	5.44 ±0.099	0.666 ±0.004	1.887 ±0.013	0.555 ±0.001	0.111 ±0.004
	3	199.198 ±0.413	3.775 ±0.002	1.998 ±0.062	5.329 ±0.033	0.555 ±0.011	1.554 ±0.023	0.555 ±0.001	0.111 ±0.007

[1]: Metallic element concentration in mg

<LLD: Lower level of detection



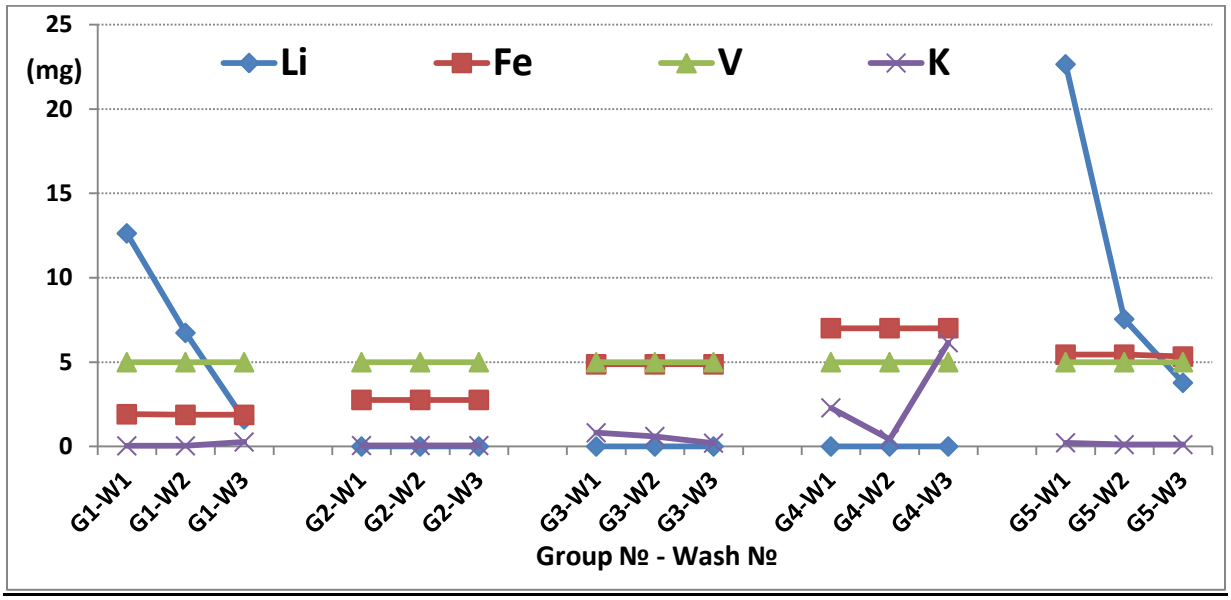


Figure 3.2: Variation of metal mass content per Group and wash basis [1-2-3]

[1]: In concentrated hydrochloric acid matrix

[2]: Metal mass content in mg

[3]: The vanadium concentrations were capped at 5 mg, below are its actual values

G1-W1	G1-W2	G1-W3	G2-W1	G2-W2	G2-W3	G3-W1	G3-W2	G3-W3	G4-W1	G4-W2	G4-W3	G5-W1	G5-W2	G5-W3
67.714	68.249	67.869	100.66	100.19	100.21	176.612	177.4	177.2	253.9	255.91	255.05	199.19	198.08	199.19

Table 3.3: Metal mass left in Groups after CWP [1-2]

	Group 1 (100% T) **Mean ± SD**	**Group 2** (50% T and P) **Mean ± SD**	**Group 3** (33.3% T,P and B) **Mean ± SD**	**Group 4** (25% T,P and 50% B) **Mean ± SD**	**Group 5** (50% T and B) **Mean ± SD**
V	17.024 ± 0.483	17.024 ± 0.166	17.119 ± 0.070	16.986 ± 0.253	17.119 ± 0.894
Li	*<LLD*	0.304 ± 0.122	*<LLD*	*<LLD*	0.019 ± 0.004
Cr	0.171 ± 0.021	0.171 ± 0.043	0.171 ± 0.031	0.171 ± 0.036	0.171 ± 0.008
Fe	0.475 ± 0.044	0.465 ± 0.046	0.465 ± 0.026	0.465 ± 0.031	0.475 ± 0.046
Mg	0.001 ± 0.003	0.465 ± 0.413	0.161 ± 0.047	0.142 ± 0.012	0.171 ± 0.039
Ca	0.266 ± 0.021	0.427 ± 0.032	0.123 ± 0.014	0.133 ± 0.013	0.133 ± 0.008
Na	0.047 ± 0.001	0.0475 ± 0.002	0.047 ± 0.001	0.047 ± 0.004	0.047 ± 0.002
K	*NIL* ± 0.002	0.0095 ± 0.004	0.0095 ± 0.005	0.0095 ± 0.007	0.009 ± 0.003

[1]: In concentrated hydrochloric acid matrix

[2]: Metal mass in $mg \cdot g^{-1}$, assuming uniformity

<LLD: Lower level of detection

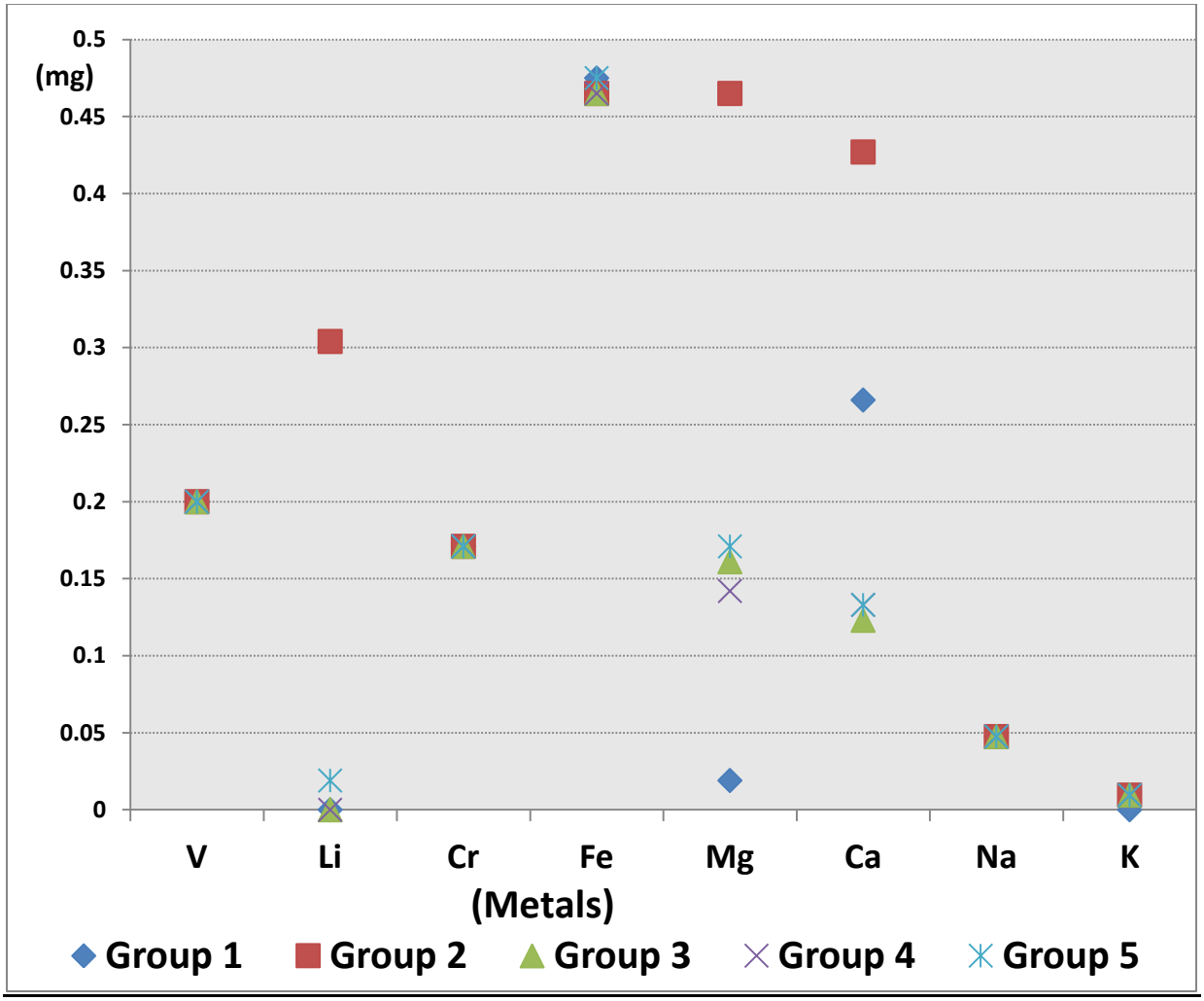


Figure 3.3: Metal mass left in Groups after column washing procedure [1-2]

[1]: Vanadium concentration was capped at 0.2 mg, below are its actual values

[2]: Metals mass content in $mg \cdot g^{-1}$, assuming uniformity

	Group 1	Group 2	Group 3	Group 4	Group 5
V (mg.)	17.024	17.024	17.119	16.986	17.119

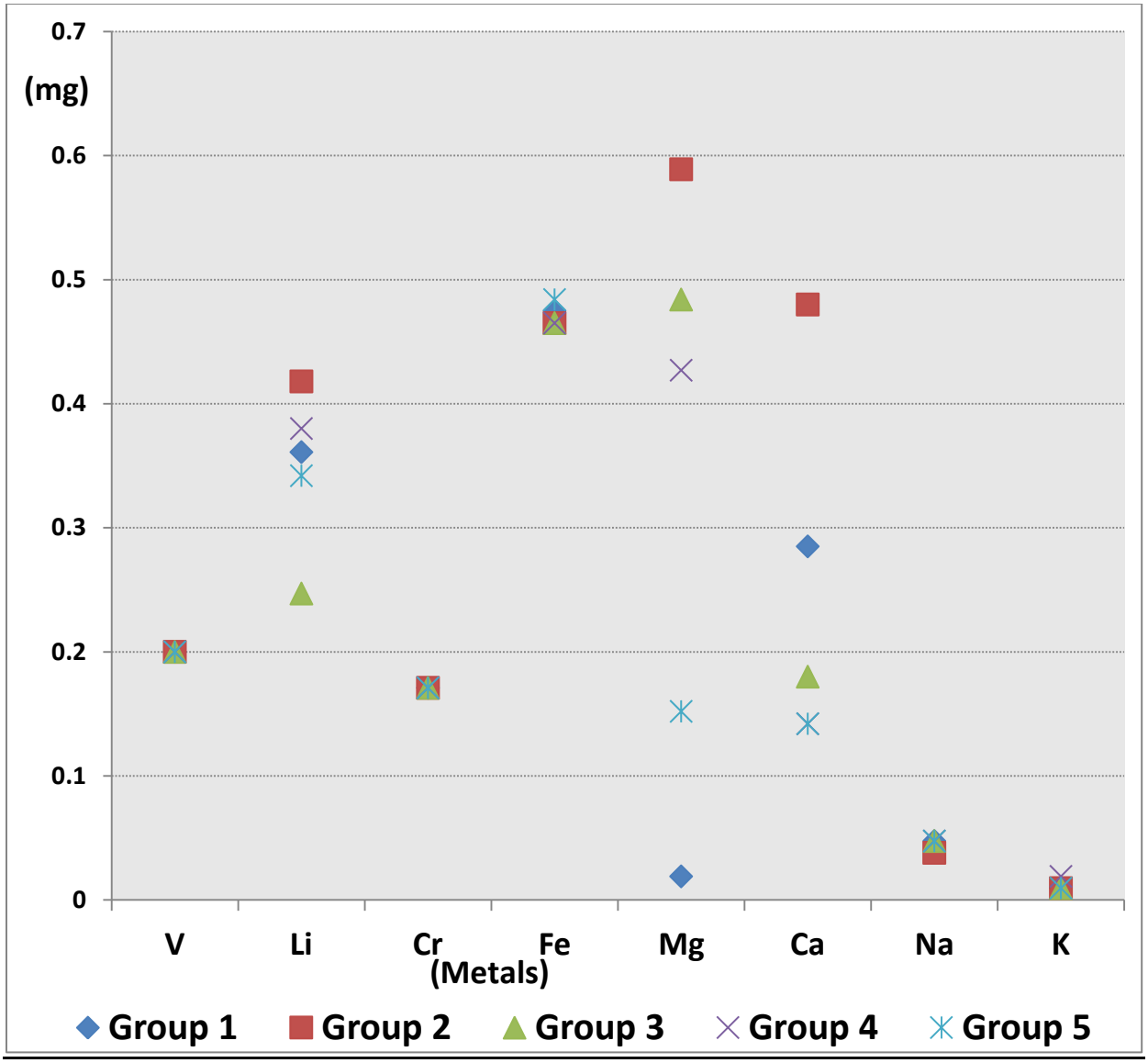


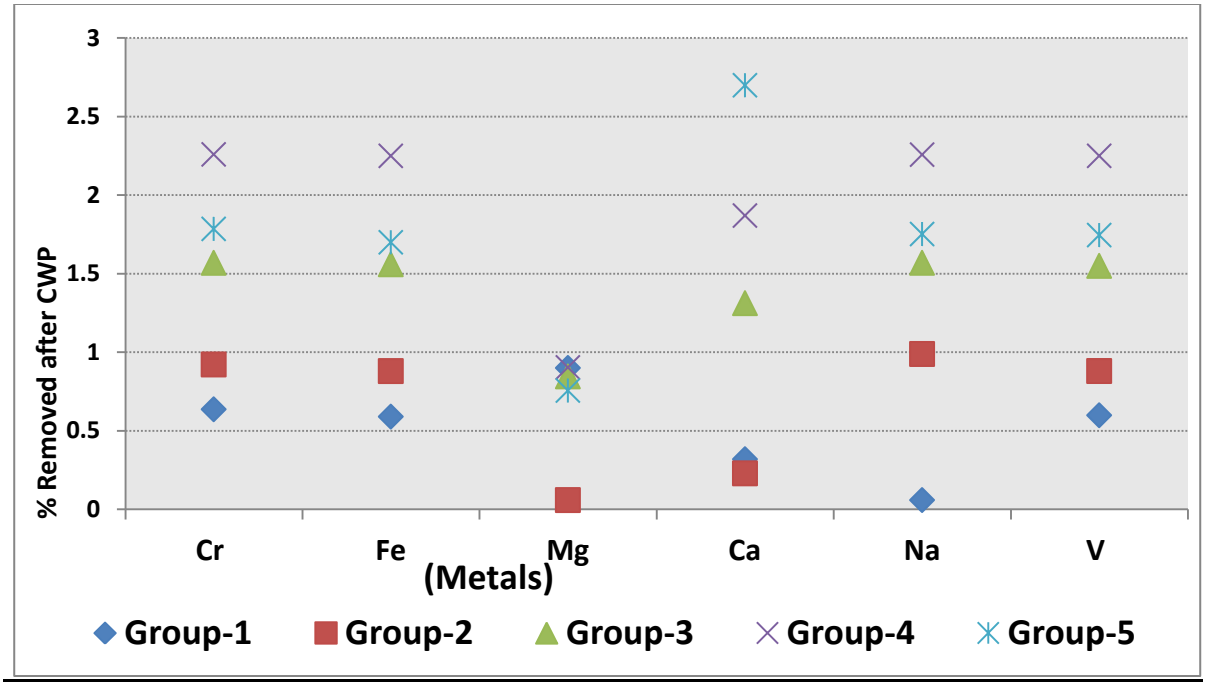
Figure 3.4: Initial metal mass content in different Groups [1-2-3]

[1] Vanadium, mass content was capped at 0.2 mg below are its real

[2]: Metals mass content in $mg \cdot g^{-1}$, assuming uniformity

[3]: Prior to CWP

	Group 1	Group 2	Group 3	Group 4	Group 5
$V_{(mg)}$	17.024	17.176	16.986	17.005	17.043



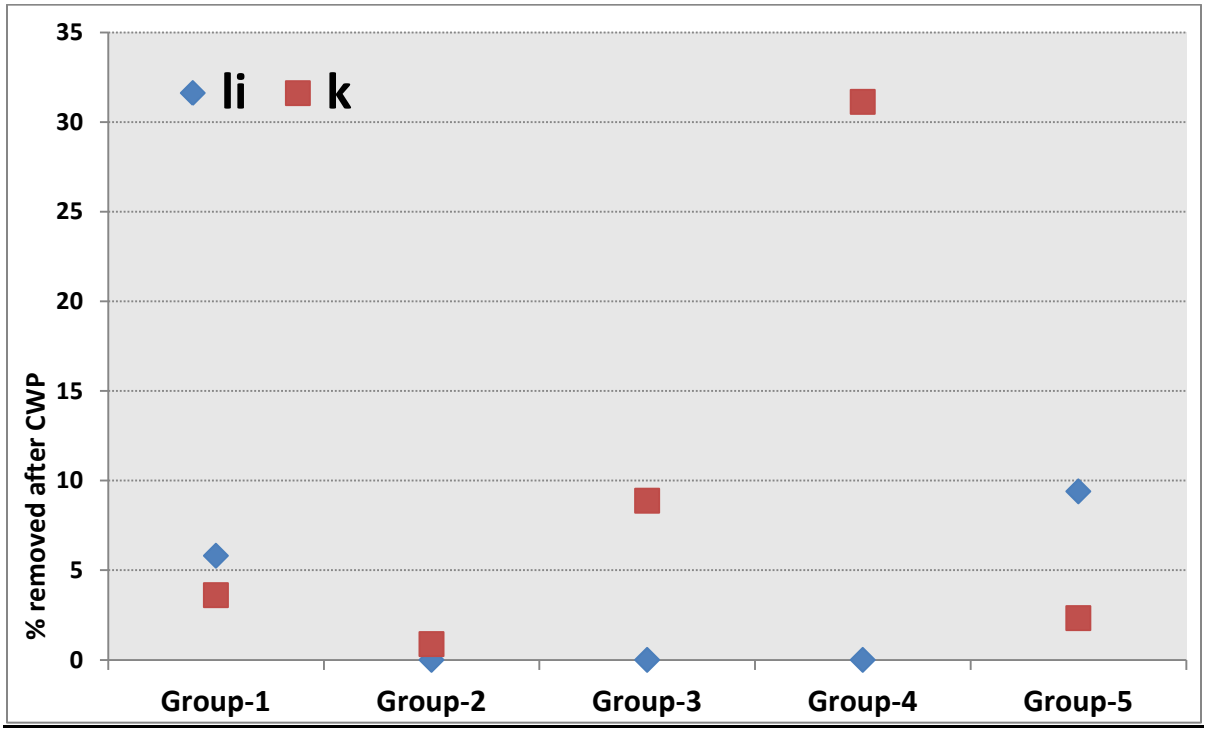
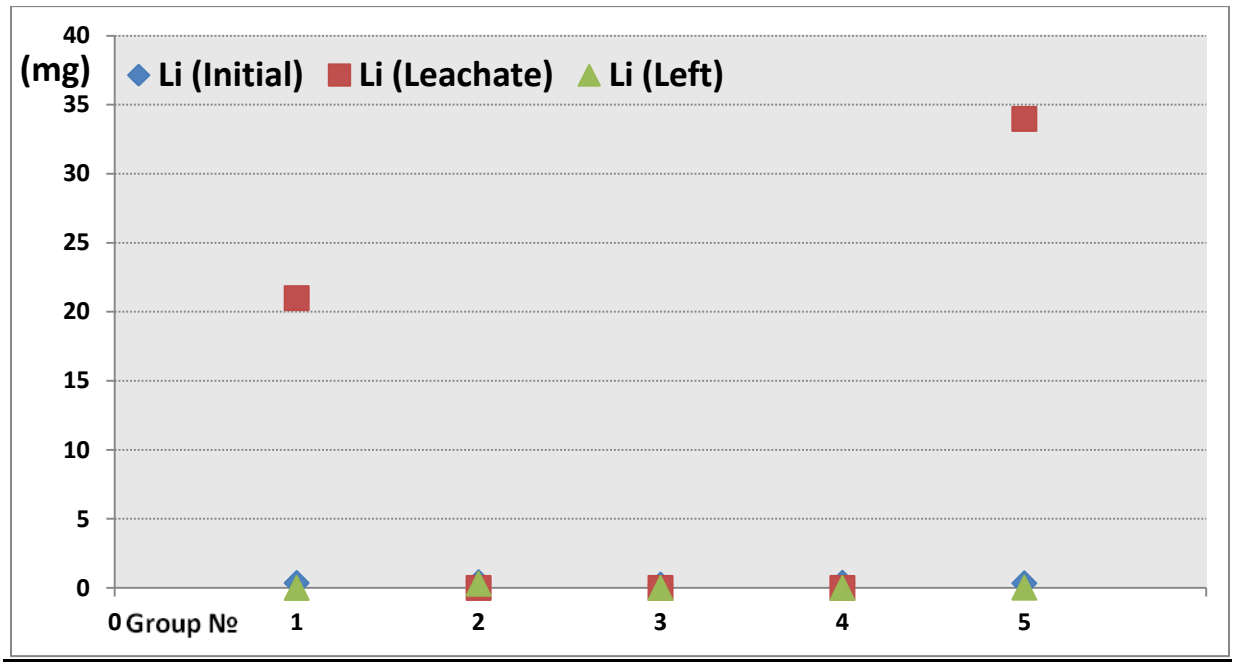


Figure 3.5: Metal mass removed [1]

[1]: In percentage basis

a) Lithium



b) Vanadium

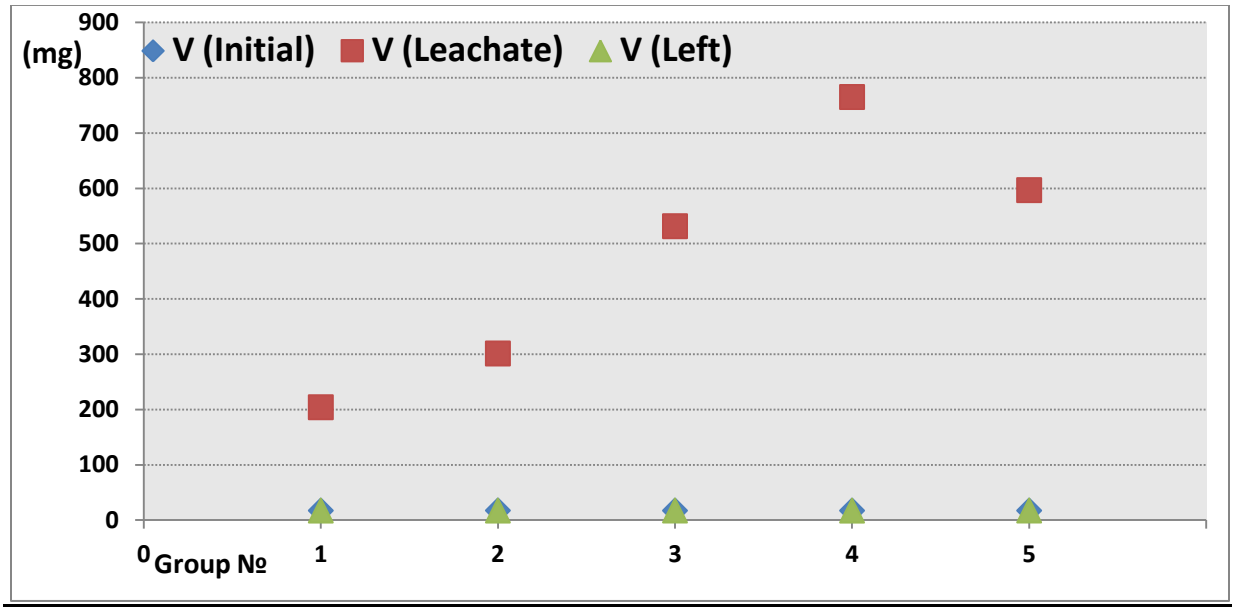
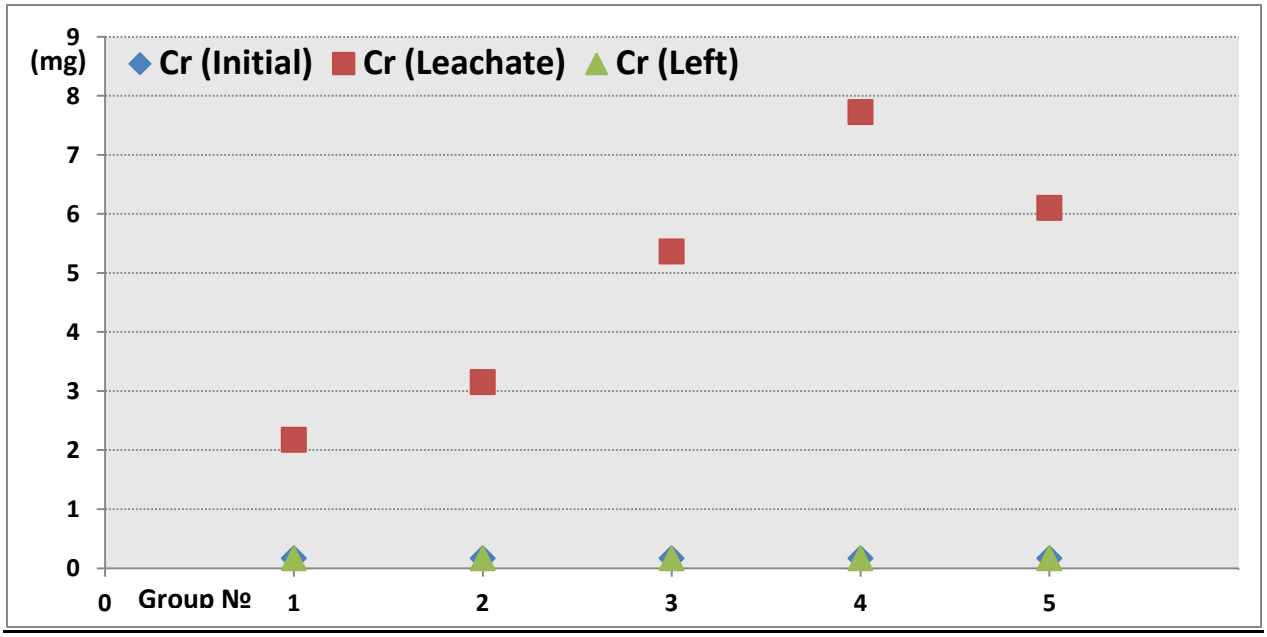
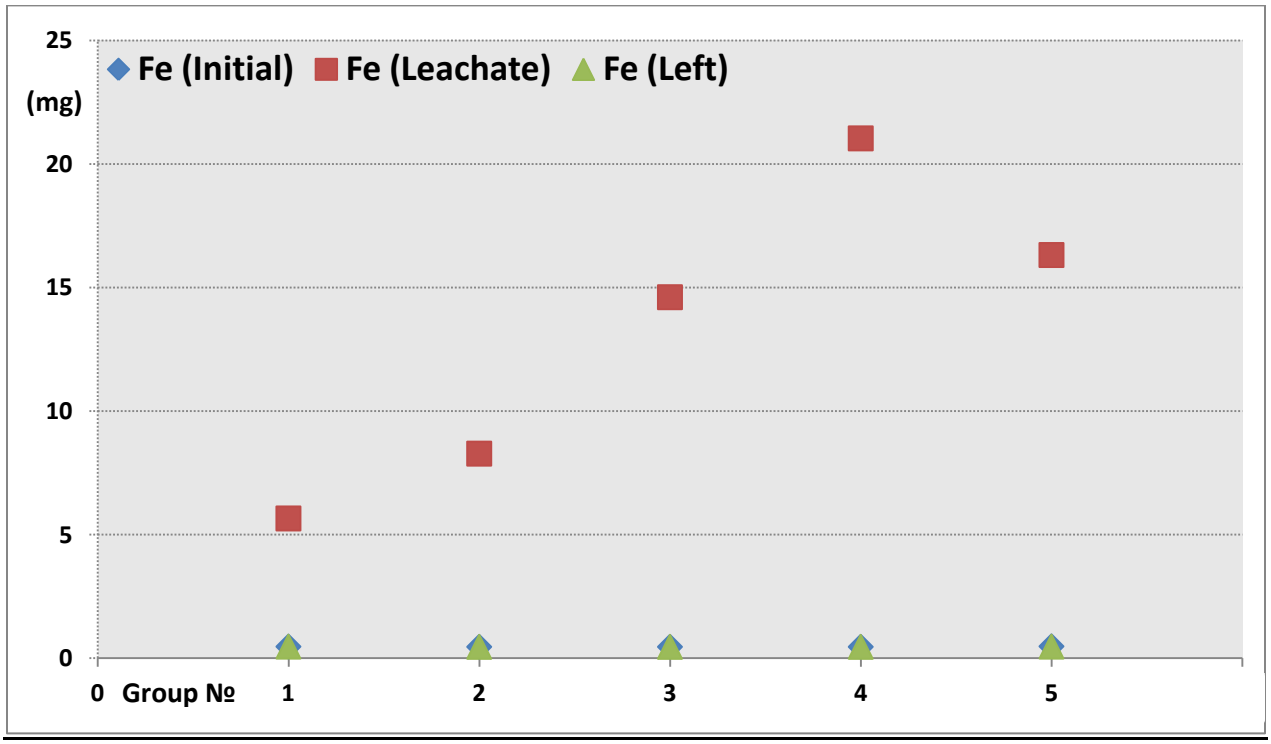


Figure 3.6: Initial vs. left vs. leachate of metal mass content per group basis

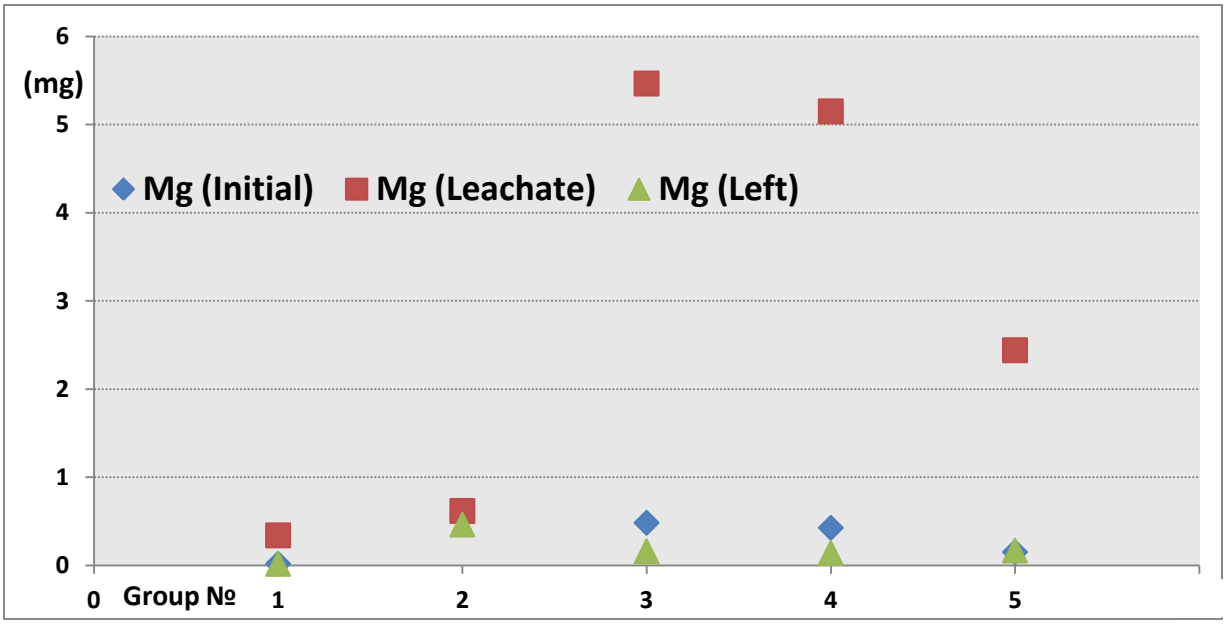
c) Chromium



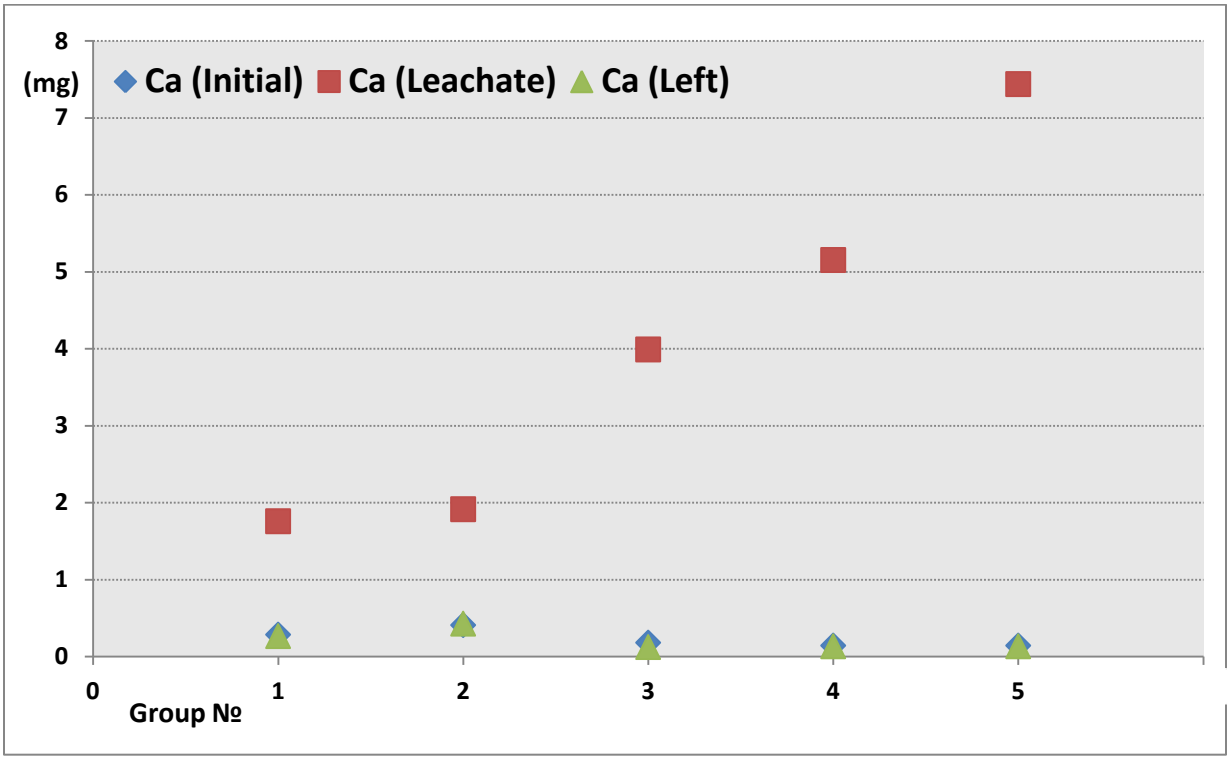
d) Iron



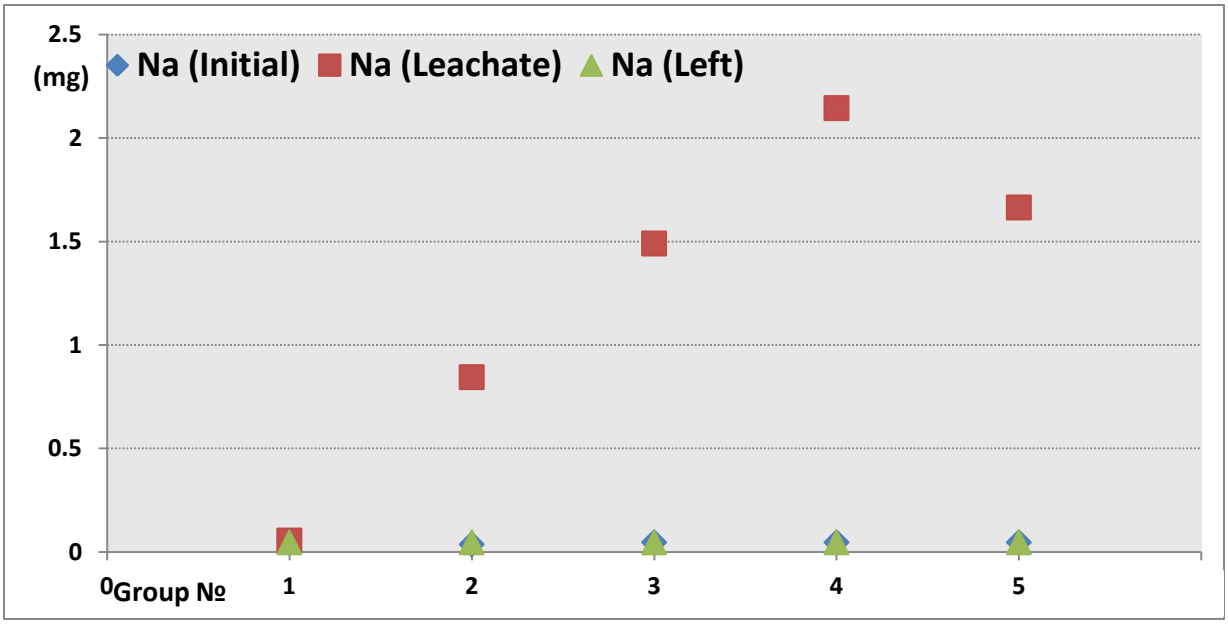
e) Magnesium



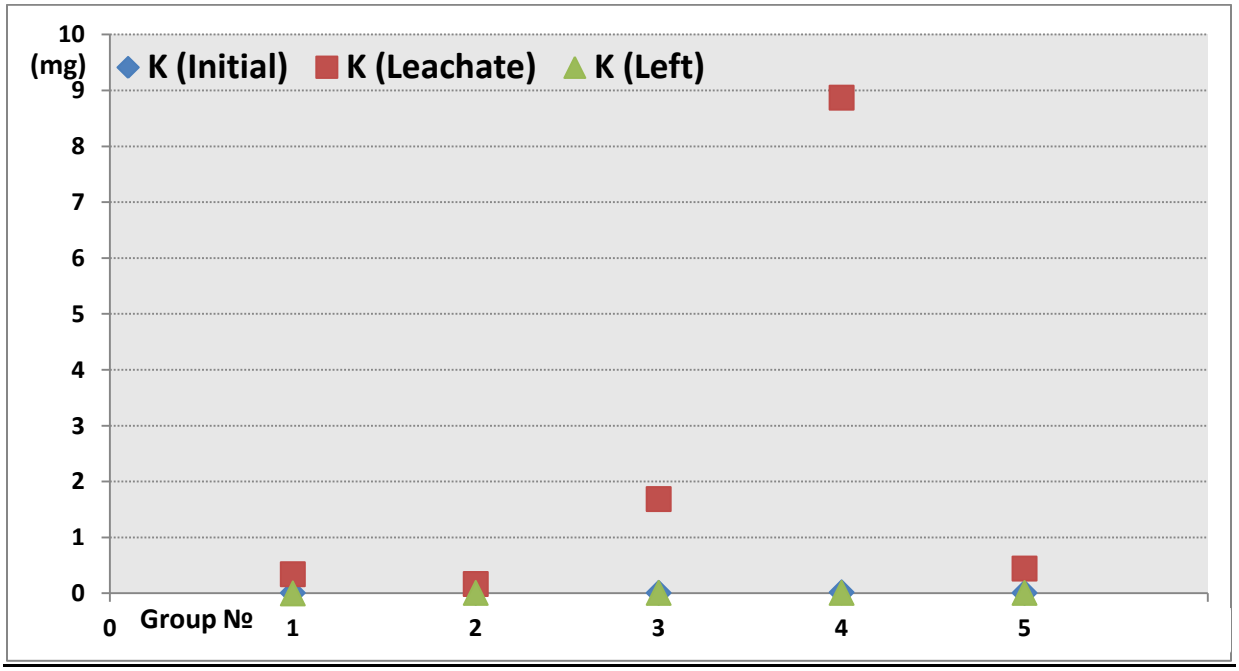
f) Calcium



g) Sodium



h) Potassium



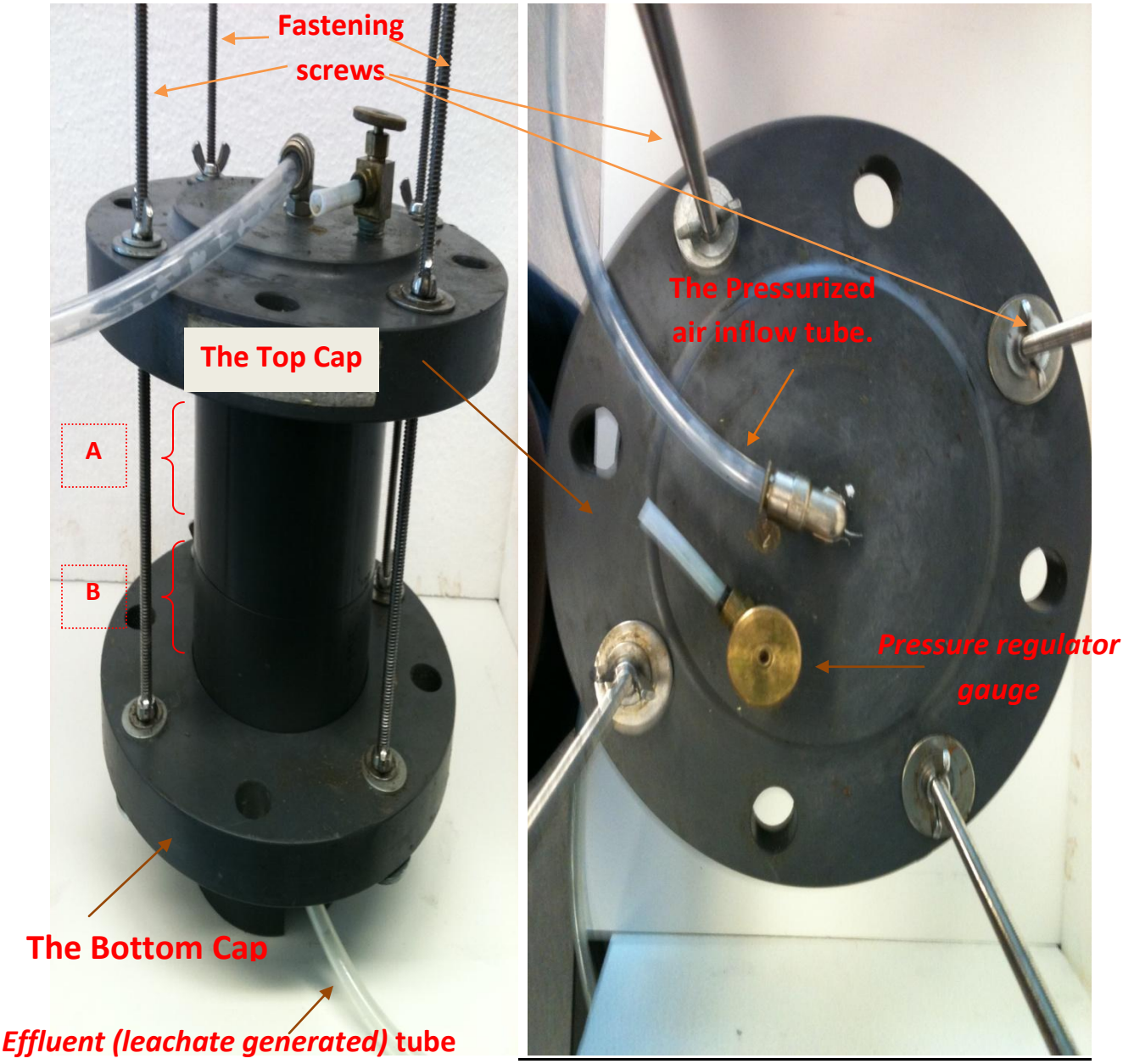


Figure: 3.7: Column apparatus (front vs. aerial views)

Where; the "A" is the DI water compartment and the "B" is the GM compartment

2.3.11 Discussion

The column wash process (CWP - figure 3.7) enabled to measure the metal holding capacity of the five different growth media Groups. The generated leachates were digested by concentrated (12N) hydrochloric acid. The solution was filtered, diluted and was aspired through the Perkin Elmer's Analyst 100™ flame atomic absorption spectroscopy machine (AAS). Moreover, the total mass balance was calculated for each metal. The total mass was rendered in milligram by multiplying its concentration (ppm) by its leachate volume [261, 262], the full results were summarized in table 3.2.

The pH, ORP and the EC of the generated leachate was measured after consecutive wash. The results were summarized in table 3.1 and figure 3.1. The decreasing trend of ORP and EC values after each wash was assumed to be due to the decrease concentration of soluble salt ions like sodium or chlorine in the growth media. As well as the decrease of oxygen content due to the prevalence of anoxic or waterlogging condition (figure 3.1-b & c).

As for the slight pH increase after each consecutive wash was assumed to be due to the increased presence of water molecules.

It was observed that lithium was strongly adsorbed in acidic growth media Groups of 2, 3 and 4, rather than vanadium, chromium and the rest of the metals which were easily leached. On the contrary lithium was easily leached under neutral pH conditions like in Groups 1 and 5, likewise, to the remaining metals. Finally, the strong adsorption of lithium in Groups 2, 3 and 4 was assumed to be due to the abundance of peat ingredient in their formulation. The peat in these Groups played the role of a buffer and a storage reservoir, hindering lithium leachate into the lithosphere.

Phase four: Impact of electrokinetics (EK) on growth media and metal content determination

3.4.1 Introduction

The electrokinetic application on growth media (EK-GM) technique was tailored and applied according to the outcome needs. It was a viable alternative approach to the mobilization of the hard bound eight metals and their mass content determination. They were presumably present in abundance within the growth media Groups.

The EK-GM process consisted of DC source applied as $1V \cdot cm^{-1}$. It was related to the EK cell via inert electrodes immersed in the growth media (figure 3.9, 3.10). As a result the applied DC generated an electrical potential gradient, which via the electrodes enabled the migration of the charged ions enabling electroosmotic, electrophoretic and electromigratory movements to respective electrodes the anode and cathode. [265, 288, 289, 290]

The electromigratory, electrophoretic and electroosmotic movement was made possible due to the one pore volume of deionized water supplemented specific to each growth media group. It was dragged towards the cathode electrode and throughout the course it was dissociated to oxygen and hydroxide ions. These ions were an important source for electron transport on both electrodes such that;

$H_2O \rightarrow 2H^+ + ½\ O_2\ (gas) + 2e^-$ (at the anode) and

$2H_2O + 2e^- \rightarrow 2OH^- + H_2\ (gas)$ (at the cathode)

The DC supplied throughout the growth media, might have lead to the formation of two distinct fronts around each confronting electrodes. The different fronts that were created and the radicals generated were carried towards the respective electrode resulting in electro-migration, electrophoresis and electro-osmosis and enhancement of diffusion process. [265, 266, 302]

The generated effluents on both electrodes were collected in PPE bottles. Their pH, ORP, EC and moisture content was also measured (tables 3.4, 3.7 and figures 3.11, 3.13). The samples were chemically digested by concentrated (12N) hydrochloric acid. Then they were filtered and diluted. Finally the samples were aspired and the eight metals mass content was determined (tables 3.5, 3.6 and figure 3.12).

3.4.2 Electrokinetics cell description

The experimental cell was made from a transparent Plexiglas material of 24 cm by 8 cm and 6 cm in size. Its cap contained 18 stainless steel rods of 1cm apart, figure 3.8.

Figure 3.8: Cap of the electrokinetics cell and the stainless steel rods

The bottom plate had two distinct holes 20 cm apart. It was fastened onto the anode and the cathode, figure 3.9 through stainless steel screws.

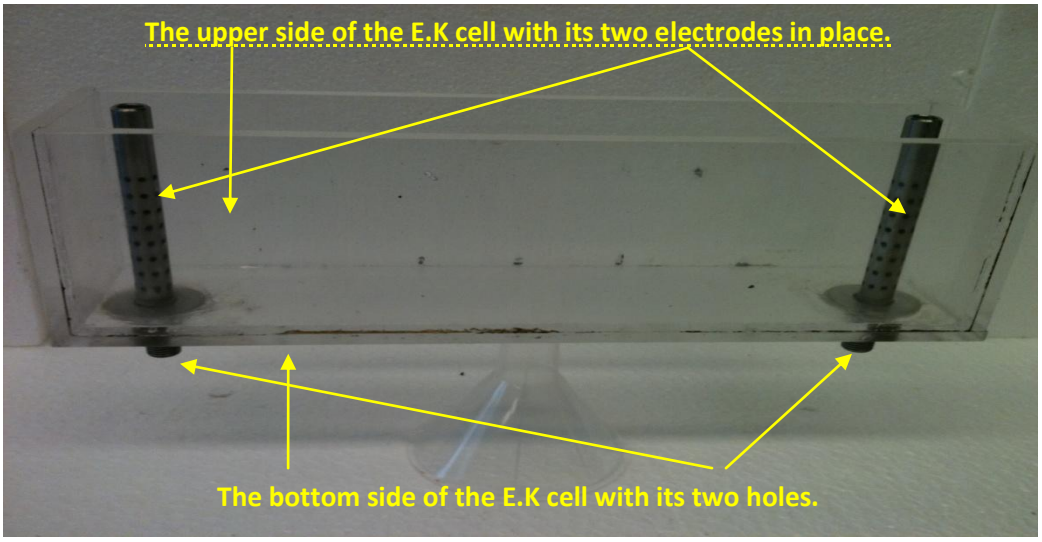


Figure 3.9: Bottom plate of electrokinetics cell in an upright position

The electrodes were made of stainless steel material. They were hollow inside perforated on its sides that allowed the generated leachate to percolate (figure 3.9). The low DC field deprived electrode corrosion and fouling. The growth media was added inside the cell and a one pore volume of deionized water was added specific to each growth media group. The cell was properly sealed by its cap that prevented water loss due to evaporation. The whole unit was linked to the DC power supply generator, figures 3.10.

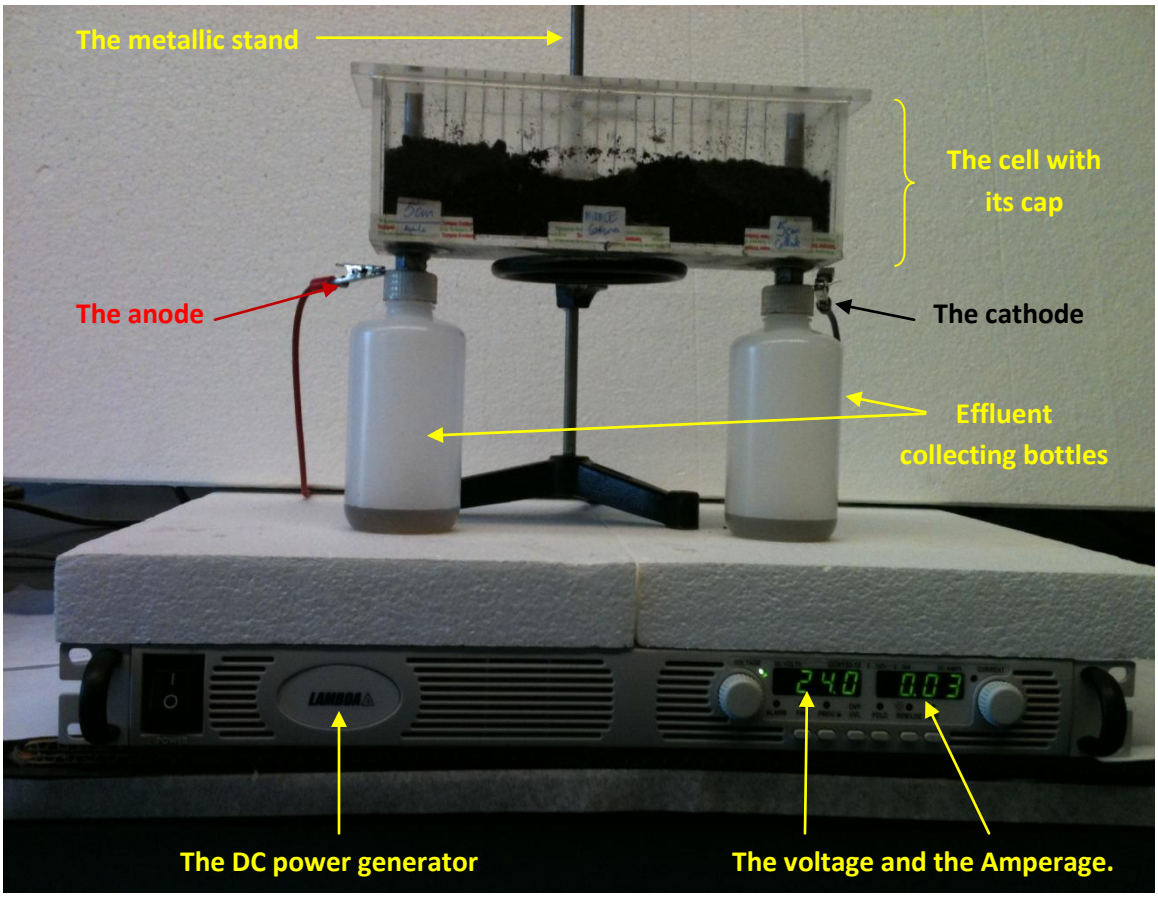


Figure 3.10: Electrokinetics setup and the DC power generator in process

3.4.3 Experimental process and results

The each of the five different growth media groups was amended with 3 gr·kg^{-1} lithium chloride. Moreover, one pore volume of deionized water was added specific to each growth media holding capacity. The DC power generator was turned on for two consecutive days. It was kept on a constant voltage gradient of 1V·cm^{-1} of the EK cell. The amperage decreased with time due to the electroosmotic transport of water towards opposing electrodes. However due to the very low DC field of 1V.cm^{-1} electrode corrosion and fouling was not observed. Moreover the electric power consumption was calculated by multiplying the amps with the voltage divided by a thousand. Thus the power consumption was recorded around 0.1584 KWh.

The pH, ORP and salinity characteristics of the collected leachate from the anode and the cathode was measured and refrigerated at 4°C. Moreover, the growth media in the EK cell was divided into three distinct parts (or slices). The first slice was around the cathode. The second slice was around the anode and the third slice was the middle part of the EK cell. Thus representative samples were taken from the three distinct slices. The samples were oven dried at 105 °C for one hour. Moreover, their pH, ORP and salinity characteristics were also measured. The eight metal mass concentrations were determined through the application of cold digestion procedure (12N HCL). The samples were digested and then diluted with deionized water of resistance 10.0 Ω cm TC at 11.5°C –milli-Q-water, Millipore USA. Finally the samples were aspired on Perkin Elmer's Analyst 100™ flame AAS. The full results were summarized in tables 3.4, 3.5, 3.6 and expressed graphically in figures 3.11, 3.12 and 3.13.

Table 3.4: pH, ORP and EC in five different groups [1-2]

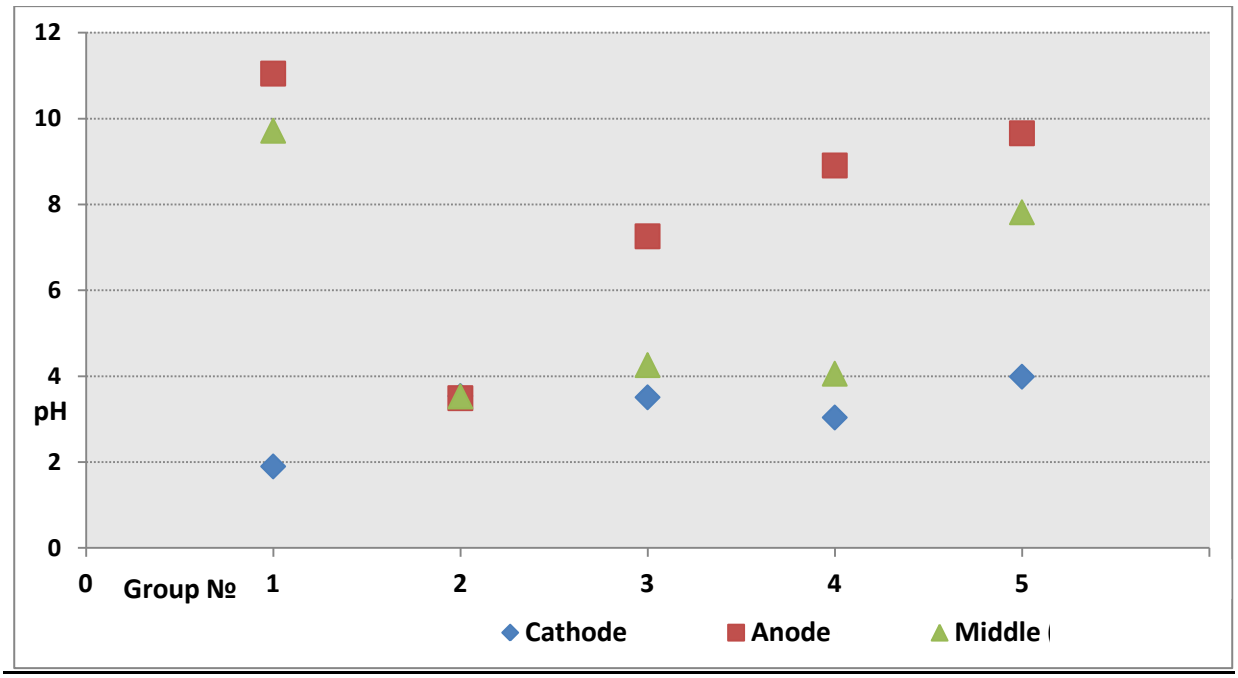
	Group 1 (100% T)	Group 2 (50% T and P)	Group 3 (33.3% TP and B)	Group 4 (25% TP and 50% B)	Group 5 (50% T and B)
Cathode (L)	pH 3.34	7.90	5.30	3.28	2.32
	ORP 214	-48.1	101.5	217.3	273.1
	EC 7.63	0.474	1.474	6.12	29.7
Anode (L)	pH 11.97	[3]	7.02	8.85	12.33
	ORP - 282.2	[3]	2.4	-102.8	-303.0
	EC 31.2	[3]	1.396	3.79	17.01
Cathode (GM)	pH 1.9	3.54	3.51	3.04	3.99
	ORP 296.0	202.4	204.2	231.4	176.3
	EC 6.61	0.888	1.159	1.124	2.057
Anode (GM)	pH 11.05	3.49	7.26	8.91	9.66
	ORP -228.9	205.3	-11.2	-106.4	-149.5
	EC 1.984	0.782	0.235	1.009	0.729
Middle cell (GM)	pH 9.72	3.53	4.26	4.06	7.82
	ORP -152.8	202.7	161.0	172.7	-43.3
	EC 0.29	0.923	0.597	0.00014	0.2014

[1] ORP expressed in mV and EC expressed in $mS \cdot cm^{-1}$

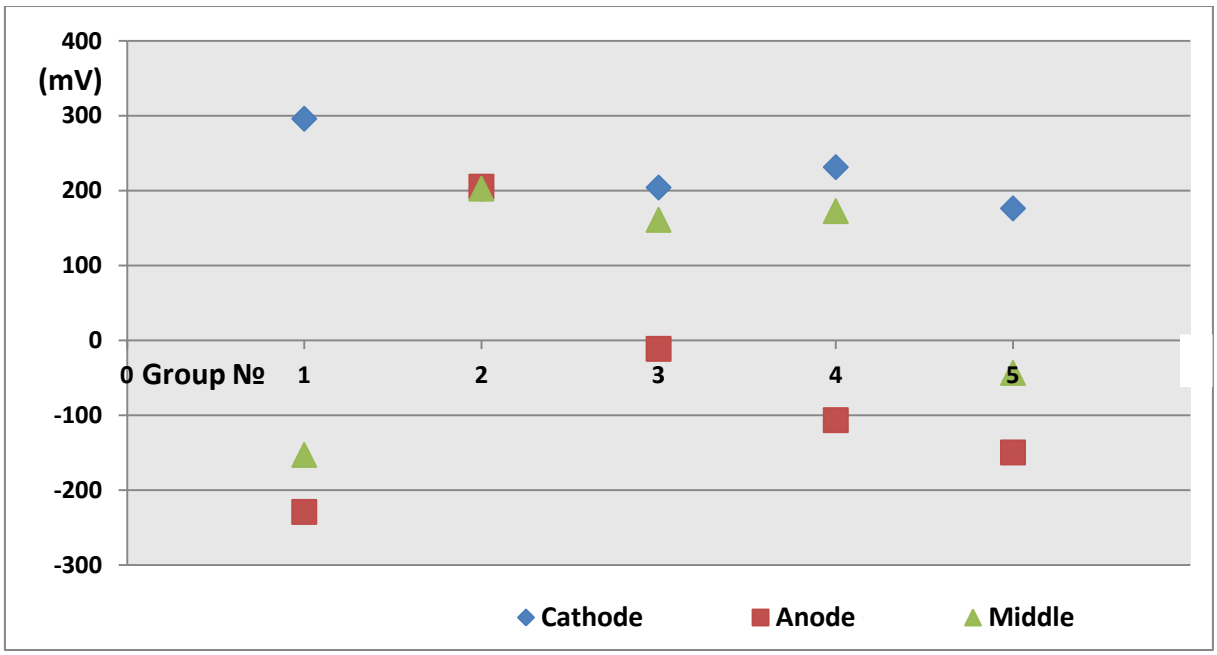
[2] L, GM are referred to leachate and growth media respectively

[3] Absence of sample

a) pH in per group basis



b) ORP per group basis



c) EC per group basis

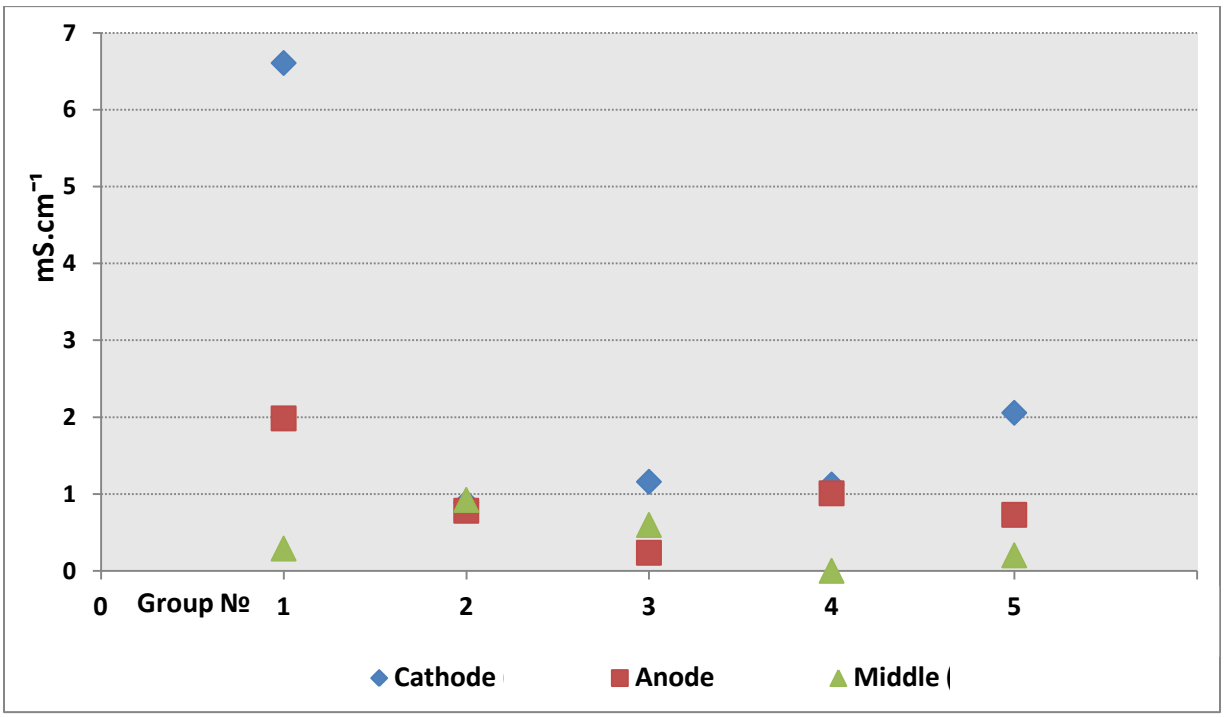


Figure 3.11: pH vs. ORP vs. EC per group basis

Table 3.5: Metal mass content in five different Groups [1–2–3]

		Li Mean ± SD	V Mean ± SD	Fe Mean ± SD	Cr Mean ± SD	Mg Mean ± SD	Ca Mean ± SD	Na Mean ± SD	K Mean ± SD
Group1 (100% LMT)	**CL**	0.2832 ± 6.175	1.202 ± 0.028	0.041 ± 0.260	0.019 ± 0.030	0.0039 ± 0.005	0.032 ± 0.087	0.0006 ± 0.008	0.665 ± 0.036
	AL	0.328 ± 3.005	1.200 ± 0.087	0.030 ± 0.095	0.015 ± 0.049	0.0006 ± 0.001	0.002 ± 0.003	0.0006 ± 0.010	0.021 ± 0.070
	C-GM	0.019 ± 0.001	17.157 ± 0.374	*<LLD*	*<LLD*	0.009 ± 0.001	0.399 ± 0.097	0.0285 ± 0.049	0.066 ± 0.030
	A-GM	0.741 ± 0.033	17.062 ± 0.453	0.541 ± 0.281	0.237 ± 0.031	0.019 ± 0.000	0.665 ± 0.057	0.019 ± 0.026	0.104 ± 0.035
	M-GM	0.152 ± 0.007	17.195 ± 0.461	0.589 ± 0.617	0.247 ± 0.040	0.019 ± 0.002	0.655 ± 0.119	0.019 ± 0.012	0.095 ± 0.039
Group2 (50% LMT and P)	**CL**	*NIL*	6.908 ± 0.146	0.178 ± 0.266	0.091 ± 0.020	0.030 ± 0.014	0.060 ± 0.022	0.003 ± 0.008	0.201 ± 0.397
	AL [3]								
	C-GM	0.304 ± 0.018	17.328 ± 0.251	0.456 ± 0.335	0.228 ± 0.032	0.693 ± 0.181	0.855 ± 0.166	0.009 ± 0.010	0.076 ± 0.027
	A-GM	0.342 ± 0.020	17.309 ± 0.346	0.446 ± 0.039	0.228 ± 0.055	0.731 ± 0.371	1.035 ± 0.093	0.009 ± 0.004	0.104 ± 0.005
	M-GM	0.285 ± 0.012	17.271 ± 0.086	0.446 ± 0.103	0.228 ± 0.024	0.684 ± 0.303	0.836 ± 0.150	0.019 ± 0.016	0.171 ± 0.068
Group3 (33.3% LMT,P and B)	**CL**	0.029 ± 0.009	2.936 ± 0.223	0.077 ± 0.534	0.0371 ± 0.030	0.016 ± 0.021	0.016 ± 0.011	0.0016 ± 0.009	0.009 ± 0.010
	AL	0.034 ± 0.012	3.849 ± 0.124	0.109 ± 0.293	0.052 ± 0.016	0.019 ± 0.013	0.021 ± 0.014	0.002 ± 0.003	0.017 ± 0.025
	C-GM	0.247 ± 0.028	17.366 ± 0.713	0.693 ± 0.467	0.304 ± 0.061	0.712 ± 0.694	0.608 ± 0.153	0.009 ± 0.004	0.104 ± 0.034
	A-GM	0.418 ± 0.041	17.385 ± 0.687	0.503 ± 0.491	0.228 ± 0.050	0.722 ± 1.044	0.750 ± 0.059	0.0095 ± 0.015	0.104 ± 0.006
	M-GM	0.285 ± 0.018	17.404 ± 0.243	0.484 ± 0.223	0.228 ± 0.019	0.760 ± 1.800	0.456 ± 0.021	0.009 ± 0.009	0.085 ± 0.011

		Li Mean ± SD	V Mean ± SD	Fe Mean ± SD	Cr Mean ± SD	Mg Mean ± SD	Ca Mean ± SD	Na Mean ± SD	K Mean ± SD
Group4 (25% LMT,P and 50% B)	**CL**	0.1672 ± 0.041	3.686 ± 0.479	0.146 ± 0.359	0.060 ± 0.033	0.062 ± 0.044	0.035 ± 0.074	0.002 ± 0.023	0.064 ± 0.083
	AL	0.156 ± 0.053	4.180 ± 0.123	0.111 ± 0.380	0.054 ± 0.024	0.026 ± 0.012	0.016 ± 0.026	0.004 ± 0.020	0.026 ± 0.030
	C-GM	0.133 ± 0.001	16.739 ± 0.508	1.035 ± 1.599	0.399 ± 8.105	0.437 ± 0.077	0.617 ± 0.103	0.009 ± 0.005	0.114 ± 0.038
	A-GM	0.760 ± 0.063	16.758 ± 0.162	0.456 ± 0.029	0.228 ± 0.032	0.494 ± 0.077	0.494 ± 0.081	0.009 ± 0.003	0.294 ± 0.079
	M-GM	*NIL*	16.777 ± 0.302	0.589 ± 0.292	0.237 ± 0.019	0.408 ± 0.138	0.731 ± 0.187	0.009 ± 0.023	0.057 ± 0.035
Group5 (50% LMT and B)	**CL**	0.003 ± 0.001	3.006 ± 0.289	*<LLD*	*<LLD*	0.003 ± 0.003	0.006 ± 0.015	0.011 ± 1.859	0.034 ± 0.066
	AL	1.882 ± 1.949	12.937 ± 0.424	0.355 ± 0.251	0.170 ± 0.012	0.029 ± 0.005	0.007 ± 0.010	*<LLD*	0.800 ± 1.558
	C-GM	0.038 ± 0.003	16.758 ± 0.431	*<LLD*	*<LLD*	0.304 ± 0.020	0.085 ± 0.021	0.009 ± 0.003	0.266 ± 0.024
	A-GM	0.304 ± 0.003	16.739 ± 0.116	0.722 ± 0.735	0.228 ± 0.027	0.437 ± 0.084	0.408 ± 0.056	0.009 ± 0.020	0.104 ± 0.033
	M-GM	0.057 ± 0.004	16.739 ± 0.186	4.512 ± 7.669	0.228 ± 0.019	0.361 ± 0.093	0.494 ± 0.060	*<LLD*	0.095 ± 0.010

[1]: In hydrochloric acid matrix

[2]: Metal mass content in $mg \cdot g^{-1}$

[3]: Absence of sample

<LLD: Lower level of detection

Table 3.6: Metal mass difference between column and EK leachate [1-2-3]

Group №		Li	V	Cr	Fe	Ca	Na	K	Mg
1 (100% LMT)	EK	874	3433.3	50.35	103.55	50.35	1.9	981.35	6.65
	CL	313.7	1681.5	18.05	47.5	15.2	4.75	0.95	2.85
2 (50% LMT and P)	EK	*NIL*	1721.1	22.8	44.65	15.2	0.95	50.35	7.6
	CL	*<LLD*	1696.7	17.1	46.55	10.45	4.75	0.95	2.85
3 (33.3% LMT, P and B)	EK	32.3	3401	44.65	93.1	19	1.9	13.3	18.05
	CL	*<LLD*	1689.1	17.1	46.55	15.2	4.75	8.55	30.4
4 (25% LMT, P and 50% B)	EK	138.7	3347.8	49.4	111.15	22.8	2.85	39.9	38.95
	CL	*<LLD*	1685.3	17.1	46.55	17.1	4.75	15.2	19
5 (50% LMT and B)	EK	243.2	3328.8	21.85	45.6	4.75	6.65	121.6	5.7
	CL	193.8	1704.3	18.05	46.55	34.2	4.75	1.9	10.45

[1]: Eight metal concentrations in $mg \cdot l^{-1}$

[2]: EK and CL abbreviations to electrokinetics and column leachate respectively

[3]: First wash effluent metal concentration was considered from column leachate procedure

<LLD: Lower level of detection

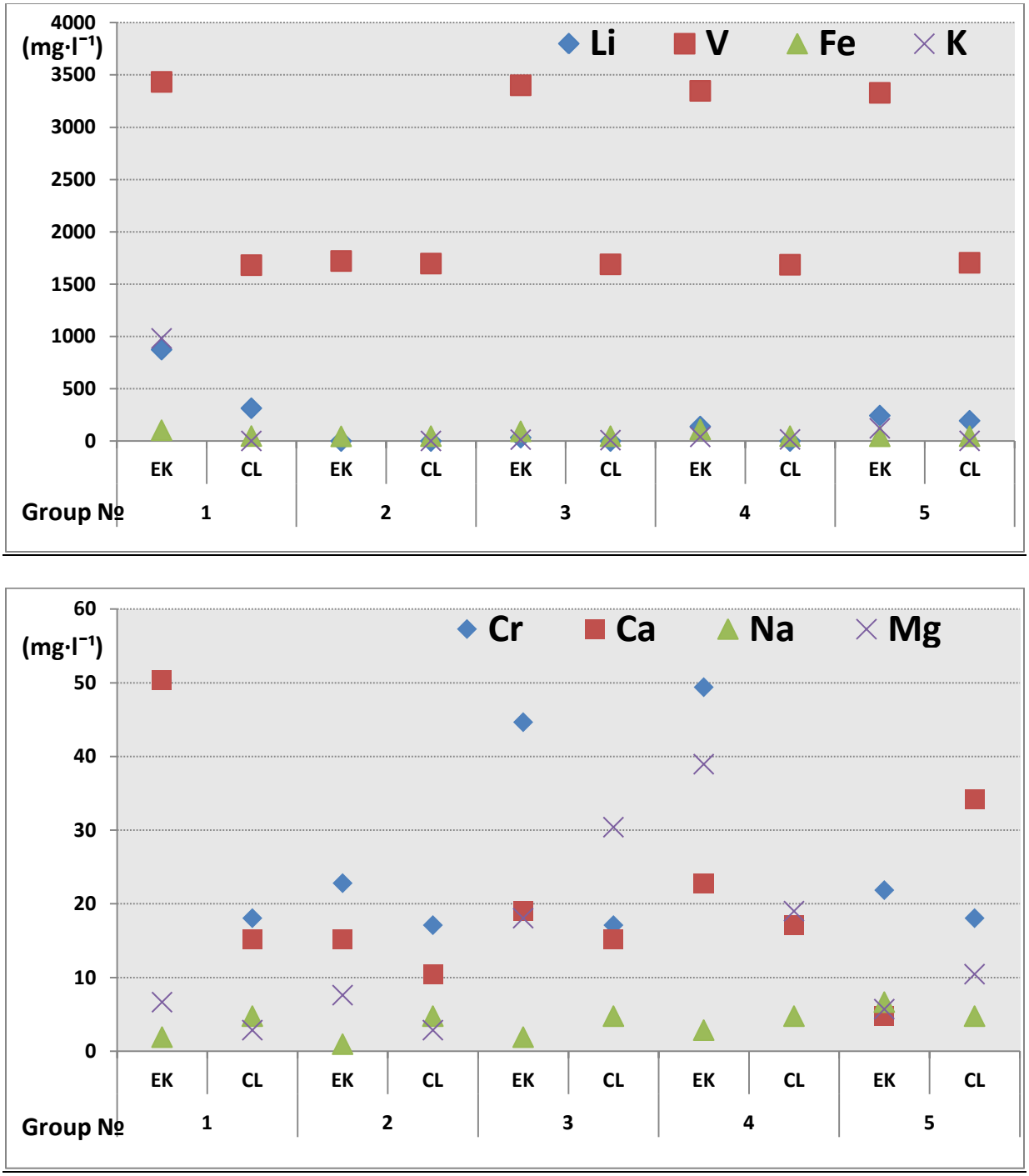


Figure 3.12: Metal mass difference between EK vs. column leachate [1]

[1]: Metal concentrations in $mg \cdot l^{-1}$

Table 3.7: Moisture content difference per slices and group basis [1]

	Group 1 (100% LMT)	Group 2 (50% LMT and P)	Group 3 (33.3% LMT, P, B)	Group 4 (25% LMT,P, 50% B)	Group 5 (50% LM T and B)
Cathode (GM)	17.95	10.02	10.82	9.22	28.24
Middle (GM)	10.91	9.14	10.98	6.20	13.13
Anode (GM)	17.11	10.54	13.59	9.97	31.71

[1]: In percentage values (%)

GM and B are abbreviations for growth media and biosolids

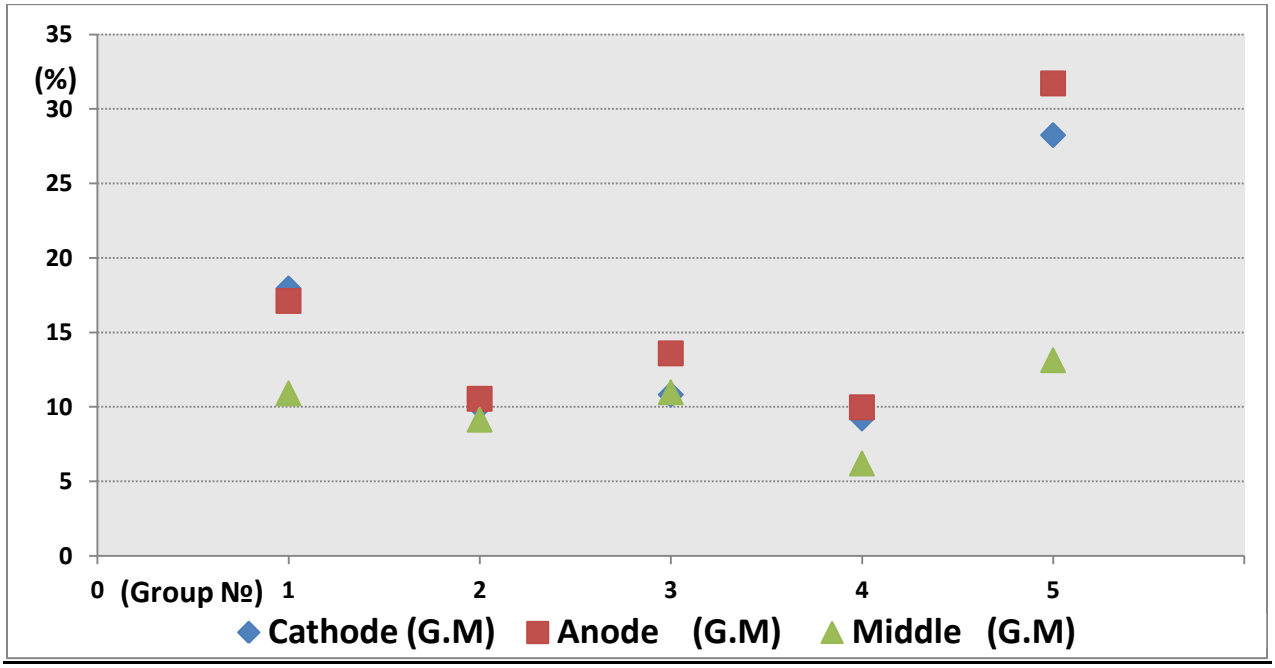


Figure 3.13: Moisture content difference in EK cell per group basis

3.4.4 Discussion

The EK-GM procedure enabled the strongly bound metals specially the heavy metals to detach through the introduction of 1 $mV \cdot cm^{-1}$ of DC field. The process enabled electromigratory, electrophoretic and different desorption transport reactions to occur throughout the growth media. As a result effluent was generated that contained the eight metallic elements, especially in the high cation exchange capacity growth media Groups of 2, 3 and 4. The direct current field enabled the flow of water towards the cathode. On the other hand peat was assumed to be amphotheric in its nature, enabling it to change radical valency throughout the experimental procedure.

The application of the DC field on the growth media enhanced the availability of the hard bound metals like V, Cr, Fe and Li in comparison to the column wash procedure in the generated leachate. The migration of the subsurface contaminants as a result of different processes like hydrolysis, electromigration and metal harvesting might be a useful approach for the enhancement of the phytoextraction and phytostabilization processes by the hyperaccumulant plant. It might also be applied at certain growth periods of the hyperaccumulant plant. Hence it might increase the overall efficiency of metal uptake and subsequent physiological storage.

The metal content difference between the EK and Cl was significant. The metal mass content in the EK leachate was at least three times more than the column leachate. The difference in metal mass content was assumed to be the result of the direct current of 1 $mV \cdot cm^{-1}$ application. It initiated electromigratory and electrophoretic displacements of the hard bound metals. The whole process was assumed to be due to the disintegration of the lignin coating of the peat ingredient and exposing its metal content pool. This dissociation enabled the detachment of the hard bound metals from its sub surface radicals and rendering them detectable in the generated effluent.

Chapter four: Phyto-sequestration/stabilization

Phase one: Phytoremediation

4.1.1 Introduction

The process of phyto-sequestration (phytoextraction) and phytostabilization evaluation was accomplished by direct sowing of the *Brassica juncea* Czern var. Cutlass (accession CN: 46238) seeds. The five different growth media formulation mixes were placed in a completely random order of subsamples of four (n=4). After germination the seedlings were grown for eighty six consecutive days. They were harvested, dried, ashed, and stored. The different physiological parts of the hyperaccumulant plant were digested in concentrated (12N) hydrochloric acid. Likewise the growth media surrounding the rhizosphere for the eight metals mass content. The results were summarized in tables 4.2 and 4.3.

Finally, the efficiency of *Brassica juncea* hyperaccumulate plant was evaluated based on its efficiency of removal (E of R), bioaccumulation ratio (BAR), translocation index (TI) and the relative growth rate (RGR) illustrated in table 4.4.

4.1.2 Sowing plant seeds in different growth media

The seeds of *Brassica juncea* Czern var. Cutlass (accession CN: 46238) or commonly known as Indian mustard was brought from “Agriculture and Agri-Food Canada”, research branch in Saskatoon, Saskatchewan. The seeds were spherical in shape, approximately 1 mm in diameter with pale white in color. The seeds were sown directly on the five different growth media. They were irrigated once in

every other day with 10 ml of the organic fertilizer (figure 4.2) amended with 3 g of lithium chloride diluted in 1 litre of tap water on volume per volume basis (v:v).

After germination the seedlings were grown for eighty six consecutive days. The temperature and moisture were kept constant as in standard testing and measurement requirements. The lightning duration was 18 by 6 (18 light and 6 dark) photoperiod hours [249, 250]. The Woods TIM 1000™ light period regulator was used, (figure 4.1) with four 65 Watts Sylvana spot-grow™ light bulbs. Moreover, the hyperaccumulant plants were harvested, cleaned, dried, digested and analyzed for their metal mass content.

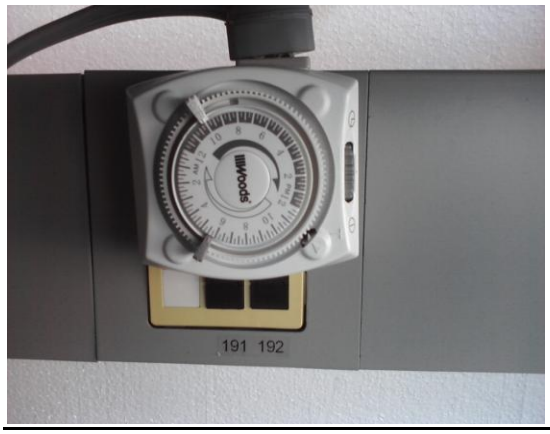


Figure 4.1: Woods TIM 1000™ timer

Figure 4.2: Organic fertilizer

4.1.3 Seeding on the five different growth media mixes

The seedlings were grown in pots of 10 cm by 9 cm in size. The five different soil media misses were arranged in subsamples of four pots (n=4) in a completely random order. Later on they were thinned to one plant per every pot. [249,106]

The plants were grown for eighty six days. Furthermore, they were irrigated by organic fertilizer once in two consecutive days. It was composed of 10 ml of the organic fertilizer (figure 4.2) amended with 3

g of lithium chloride diluted in one litre of water on volume per volume basis (v: v). The irrigated volume for every pot was specific to each growth media's water holding capacity.

Table 4.1: Void volume and porosity per group basis

Treatment Group №	**LMT, Peat and de-B mixture** (% content)	**Void Volume** (ml)	**Porosity** (%)
1	100 % LMT	40.27	2.5
2	50 % LMT and 50 % Peat	59.33	3.77
3	33.33 % LMT, Peat and de-B	104.65	6.66
4	25 % LMT, 25 % Peat and 50 % de-B	150.66	9.59
5	50 % LMT and 50 % de-B	116.88	7.44

4.1.4 Harvesting process

The hyperaccumulant plants were harvested after eighty six days of growth (figure 4.3). The lower and the upper harvestable physiological parts of the hyperaccumulant plants were separated and visually checked. They were washed and rinsed by tap water for the shortest possible period by a soft sponge. The washing procedure removed the impurities that were present on its leaves, stem and especially around its roots. [250, 251]

After the washing process the plants were dried immediately. As a result it stabilized the tissue and stopped enzymatic reactions to occur [252]. Finally the fresh and the dried weights were recorded of the different physiological parts of the hyperaccumulant plant. It included its below ground and upper physiological parts like the leaves, stems, roots which were packed and refrigerated.

→ The *Brassica juncea* plant seedling germinated on the first week.

→ The *Brassica juncea plant* side view.

Figure 4.3: Chronology of germination of *Brassica juncea* in Group 4

→ Aerial view of *Brassica juncea* plant

→ *Brassica juncea* plant ready to harvest

Figure 4.3: Chronology of growth of *Brassica juncea* in Group 4, Cont'd

4.1.5 Drying process

The drying process removed the water content that was bound inside the plant tissue. It effectively stopped the enzymatic reactions that might have led to premature decomposition and sample loss. In addition water removal facilitated particle size reduction process and homogenization, thus resulted in a precise and accurate weighting [252].

The pre drying process was achieved after the plant samples were harvested and were placed inside paper bags. Afterwards, the plant samples were placed in an oven at 80 ^{o}C for two consecutive days [253]. As a note, a temperature below 80 ^{o}C wouldn't have removed the combined water totally from the tissue sample. Otherwise above 80 ^{o}C would have decomposed the plant sample totally. [254]

The dried samples were placed inside sealed bags. It prevented the samples from absorbing moisture from its surrounding. The dried plant samples were grinded and passed through the 1mm (mesh 20 sieve) screen. Finally the samples were thoroughly mixed and a 5g of aliquot withdrawn for analysis. The remaining samples were carefully stored and refrigerated.

4.1.6 Storing process

The dried samples were stored in sealed envelopes. It preserved the samples throughout the experimental duration. In addition, the grinded and homogenized samples were also placed in sealed envelopes. It preserved its integrity and guaranteed its longevity. Finally the samples were refrigerated under 4 ^{o}C. [253]

4.1.7 Plant leaf analysis for lithium

Two fully developed leaves were selected randomly. Subsequently, the leaves were washed with deionized water. The leaves were air dried for one day. Moreover, the leaves were oven dried at 50 ^{o}C for two consecutive days which avoided the volatilization of lithium. The leaves were grinded in a mill and were passed through a 40 size mesh sieve. In addition a 0.25 g of finely grinded material was placed in a beaker and was added upon 3 ml of concentrated hydrochloric acid in mass per volume (m: v) basis. The digestion was allowed to proceed for two consecutive days. Finally the solution was filtered through Whatman No.1 filter paper of particle retention size of 11 µm. It was transformed to a 25 ml volumetric flask and was bulked by deionized water.

4.1.8 Plant leaf ashing process

The high temperature oxidation method (ashing) was regarded suitable for quantitative analysis of magnesium, chromium, iron, calcium, sodium, potassium and vanadium. The ashed sample was digested with concentrated (12N) hydrochloric acid. The filtered sample was aspired by flame atomic absorption spectrometry (AAS). [255]

Thus a 500 +/- 5 g of the oven dried plant material was weighted and placed into the porcelain crucible. The crucible was placed in a muffle furnace for 4 hours at 500 ^{o}C. A one gram of the ashed sample was digested in a 10 ml of concentrated (12N) hydrochloric acid for two days. Moreover, the suspended solution was transferred into a 50 ml volumetric flask and diluted to the volume with deionized water. Finally the content was analyzed and metal mass content was achieved through the flame AAS. [252, 254]

4.1.9 Analysis for the remaining metals in leaf tissue samples

4.1.9.1 Introduction

The Perkin Elmer Analyst 100™ flame AAS was used as stated earlier for the detection of metal mass contents in different plant physiological parts.

4.1.9.2 Procedure

A 1 g of ashed plant sample was weighted. A 2 ml of concentrated (12N) hydrochloric acid solution was added and the suspension was left to react for 2 days. Afterwards the suspension was filtered and diluted with deionized water into a 100 ml volumetric flask. [256]

In plant sciences the concentration of calcium, iron, magnesium, potassium and sodium in plant tissue solution are expressed as a mass per volume basis. A concentration of 1 to 10 μg/ml of detection limit 0.002 for calcium, 2 to 10 μg/ml of detection limit 0.005 for iron, 0.1 to 0.2 μg/ml of detection limit 0.0003 for magnesium and 1 to 10 μg/ml of detection limit 0.005 for sodium is found usually. However, chromium and vanadium concentrations in plant tissues range between 0.006 to 18 mg/kg and 0.16 to 1 mg/kg respectively. [258, 254,253,257,259]

4.1.10 Plant stem analysis procedure for extraction of lithium

The stems were separated from the leaves and the roots. They were washed carefully with deionized water which removed its surface impurities. The stems were left to dry under room conditions for one day. Afterwards, the samples were oven dried for 12 hours at 50 ^{o}C. Furthermore, they were grinded and passed through a 40 size mesh sieve.

A 0.25 g of the finely grounded material was added upon a 3 ml of concentrated (12N) hydrochloric acid. The digestion was allowed to continue for two consecutive days. Finally the solution was filtered

through Whatman No.1 filter paper with particle retention size of 11 µm. The concentrate was transformed to a 25 ml volumetric flask and was bulked by deionized water.

4.1.11 Analysis for the remaining minerals in plant stem tissue samples

A 1 g of ashed plant stem sample was taken. A 2 ml of concentrated (12N) hydrochloric acid was added. The concentrate was diluted into a 100 ml volumetric flask [256]. The seven metallic element concentrations were measured through the flame AAS method as stated earlier.

4.1.12 Plant root analysis procedure for extraction of lithium

The roots were separated from the hyperaccumulant plant stem. Its integrity and unity was preserved as much as possible. The roots were thoroughly washed and cleaned. They were oven dried for 2 consecutive days at 50 ^{o}C. [260]

The roots were grinded in a mill and were sieved through a 40 size mesh size sieve. Furthermore, a 0.25 g of the finely grounded material was placed in a beaker. A 3 ml of concentrated (12N) hydrochloric acid was added. The digestion was allowed to continue for two consecutive days. Finally the solution was filtered through Whatman No.1 filter paper of particle retention size 11 µm. The concentrate was transformed to a 25 ml volumetric flask and was diluted by deionized water. The lithium metal content concentration was analyzed by the flame AAS.

4.1.13 Analysis for the remaining metals in the plant root samples

A 1 g of ashed plant stem sample was weighted. A 2 ml of concentrated (12N) hydrochloric acid was added and the digestion was allowed to proceed for 2 days. The concentrate was diluted into a 100 ml volumetric flask [256]. Finally its metal mass content was measured through the flame AAS.

4.1.14 Metal concentration analysis in rhizosphere area

A 2 g of oven dried sample was taken. A 20 ml of concentrated hydrochloric (12N) acid was added; in accordance to ASTM-D3974, 2010. The growth media mass to volume ratio was 1:10. The suspension was shacked on a BURRELL- Wrist Action™ Pittsburgh, PA USA shaker and was kept to equilibrate overnight. Furthermore, the samples were centrifuged for 10 minutes at 3,000 rpm and the supernatant was filtered through Whatman No.1 filter paper of particle retention size 11 µm. [247,249,246]

Finally the metal mass content was measured through the flame AAS system.

Table 4.2: Metal mass content in *Brassica juncea* physiological parts and rhizosphere [1–2]

	Rhizosphere Mean ± SD	Leaves [2] Mean ± SD	Stem [2] Mean ± SD	Roots [2] Mean ± SD
V	34.168 ± 0.370	10.111 ± 0.263	18.858 ± 0.280	5.090 ± 0.206
Li	0.988 ± 0.328	0.319 ± 0.044	0.084 ± 0.005	0.005 ± 0.001
Cr	0.513 ± 0.090	0.159 ± 0.097	0.296 ± 0.053	0.065 ± 0.050
Fe	1.235 ± 1.047	0.142 ± 0.658	0.042 ± 0.834	0.014 ± 0.702
Mg	1.083 ± 0.204	0.148 ± 0.985	0.306 ± 0.052	0.014 ± 0.002
Ca	0.855 ± 0.046	0.336 ± 0.093	0.190 ± 0.033	0.031 ± 0.013
Na	0.076 ± 0.727	0.011 ± 0.031	0.031 ± 0.089	0.005 ± 0.049
K	2.641 ± 0.543	1.590 ± 2.425	1.555 ± 1.255	0.035 ± 0.029

[1]: In hydrochloric acid matrix

[2]: Metal mass content in $mg \cdot g^{-1}$ per dry weight basis

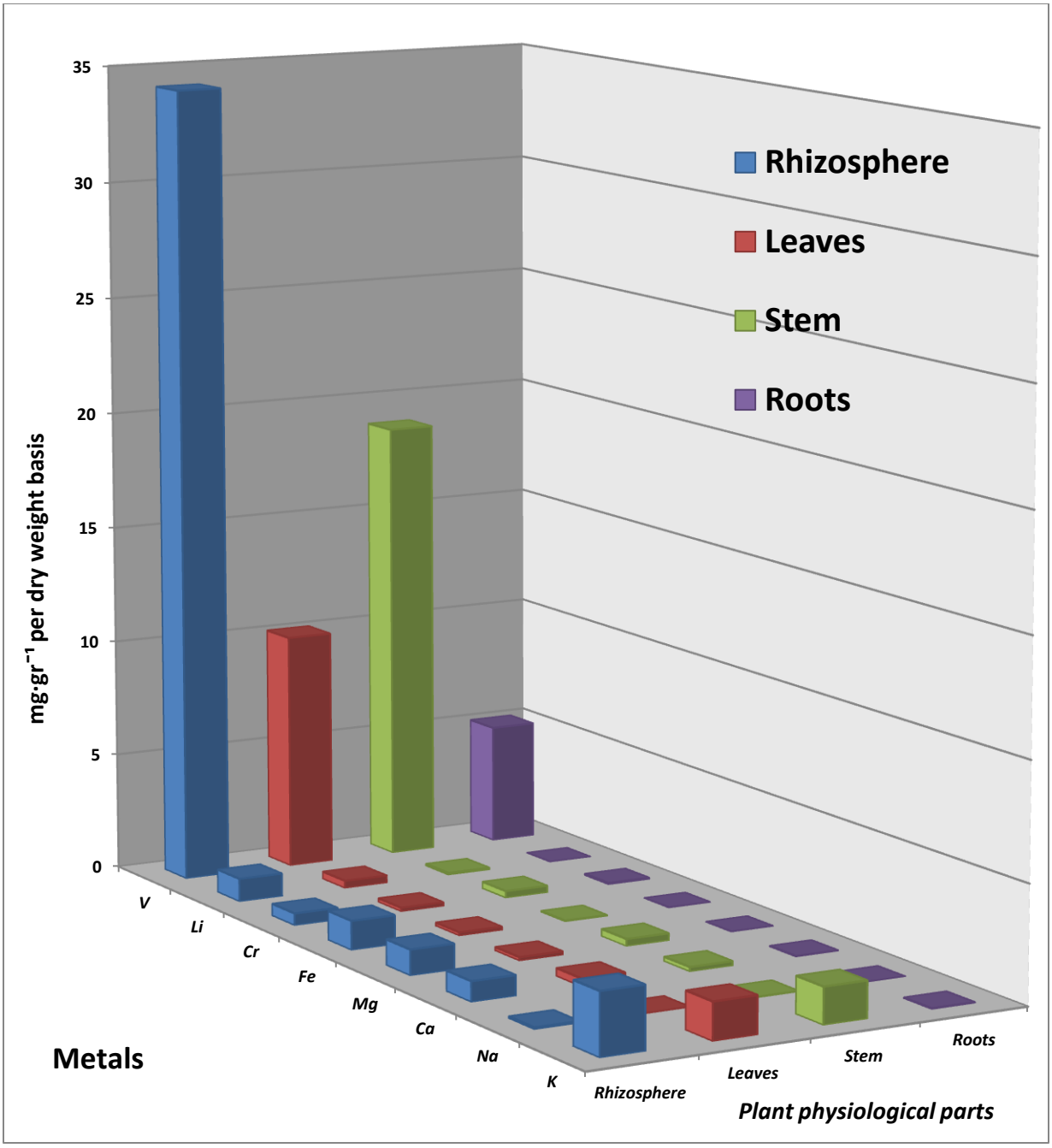


Figure 4.4: Metal mass content in *Brassica juncea* physiological parts [1]

[1]: Metal mass content in $mg \cdot g^{-1}$ per dry weight basis

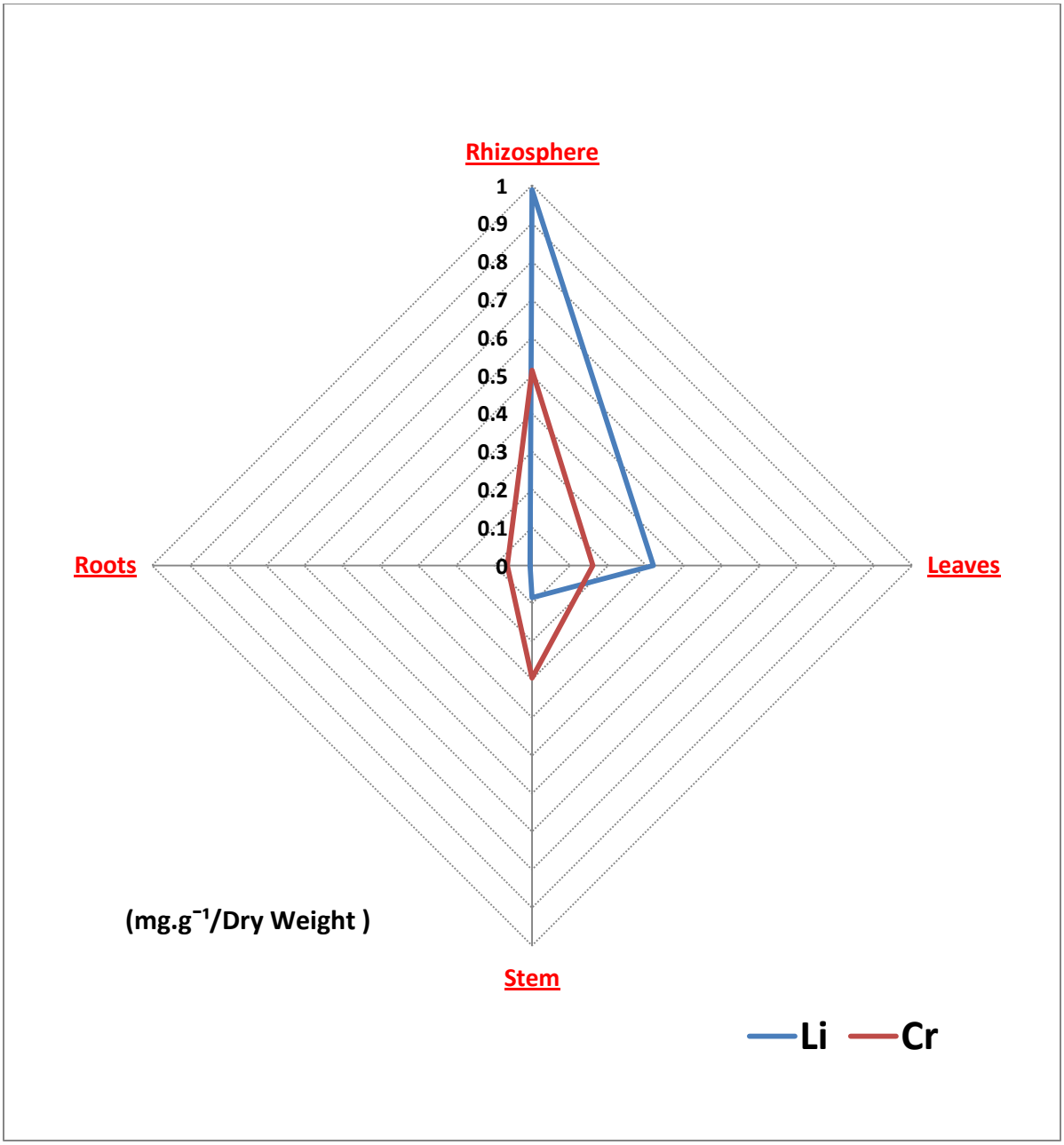


Figure 4.5: Li and Cr phytoextraction and phytostabilization in different *Brassica juncea* physiological parts [1]

[1]: Metal mass content in $mg \cdot g^{-1}$ per plant dry weight basis

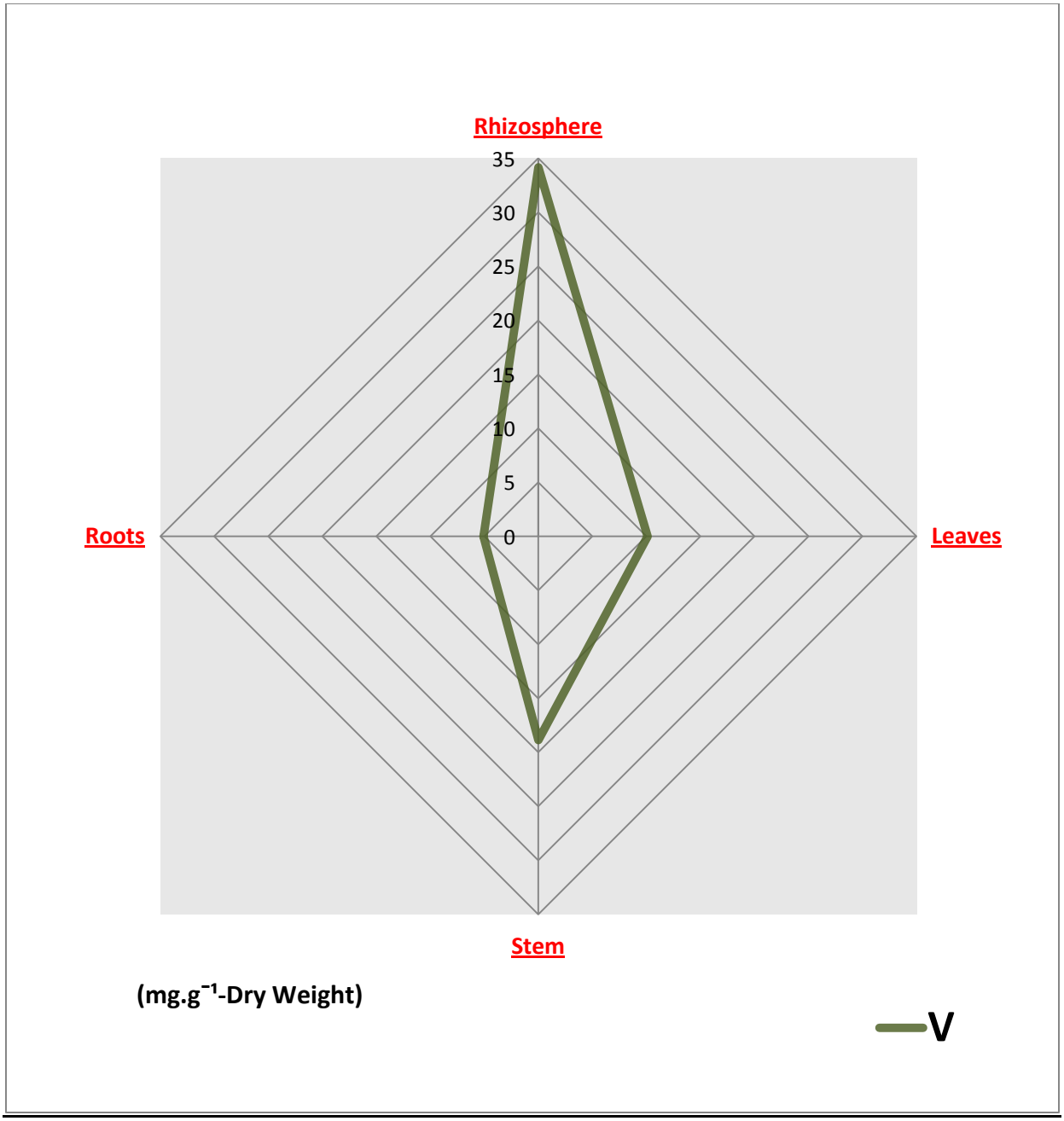


Figure 4.6: Vanadium phytoextraction and phytostabilization in different *Brassica juncea* plant physiological parts [1]

[1]: Metal mass content in $mg \cdot g^{-1}$ per plant dry weight basis

Table 4.3: Remaining metal mass content, after phytoextraction [1-2-3]

Concentration [2] (mean± SD)	**Metal**
0.513 ± 0.154	**Li**
1.140 ± 25.859	**V**
0.276 ± 0.084	**Fe**
0.352 ± 0.028	**Cr**
0.333 ± 0.127	**Mg**
0.342 ± 0.132	**Ca**
0.447 ± 0.057	**Na**
0.941 ± 1.520	**K**

[1]: In Group 4 growth media only

[2]: Metal mass content in $mg \cdot g^{-1}$, assuming uniformity

[3]: Cold digestion procedure (12N HCL-matrix)

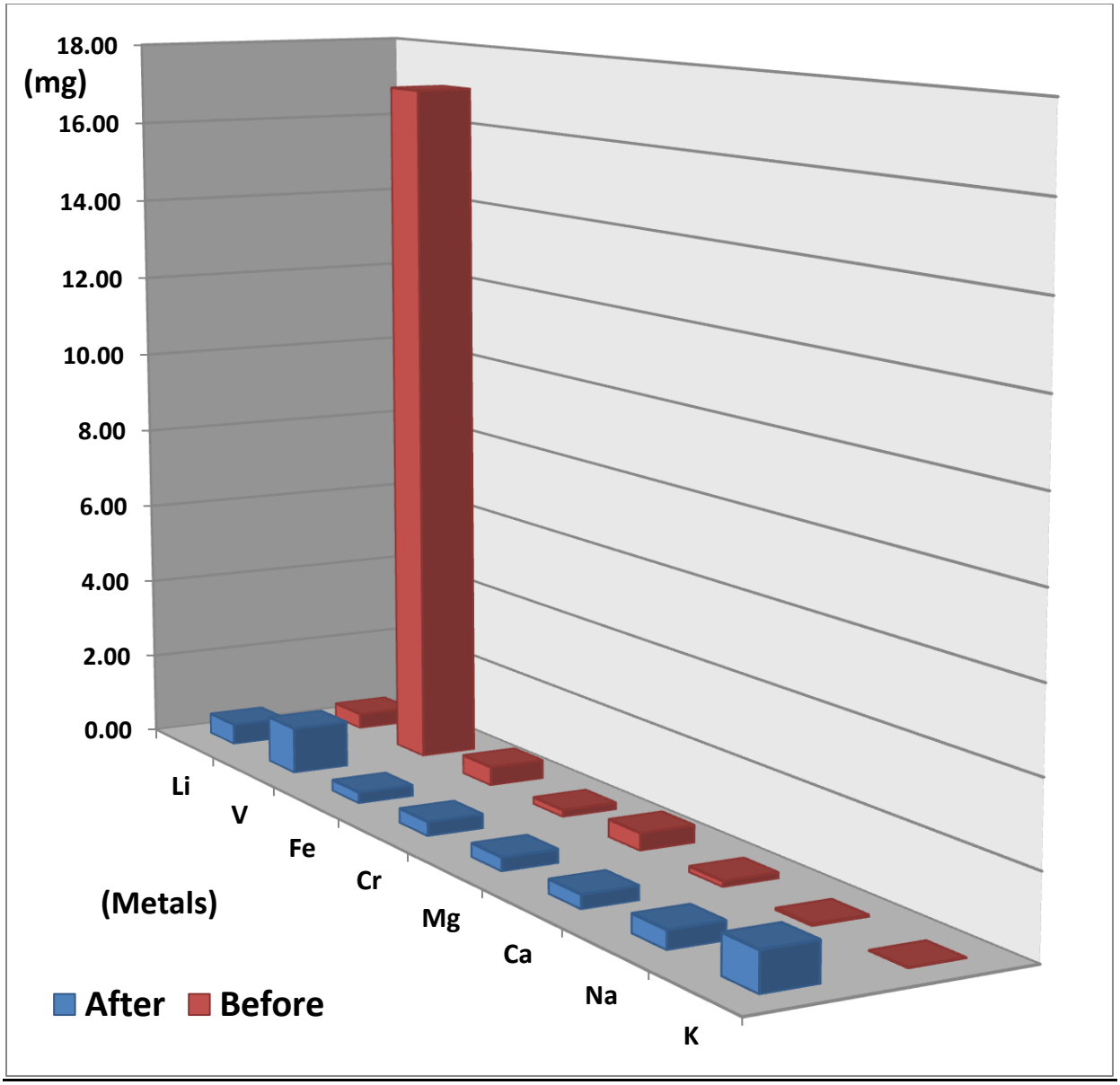


Figure 4.7: Comparison between prior and after phytoextraction of metals mass content [1-2]

[1]: Metal mass content in $mg \cdot g^{-1}$, assuming uniformity

[2]: In Group 4 growth media

4.1.15 Mass balance calculation

It was achieved through the computation of the following formula;

$$C' = C \cdot Vn \cdot (df)/W)\}$$

Where; C'= metal mass content in the sample in $mg.kg^{-1}$

C= measured metal concentration in the solution in $mg.l^{-1}$

Vn= acid volume in l.

W= mass of the initial sample in g.

df = dilution factor.

[261,262]

4.1.16 Effectiveness of different GM mixes

The total metal mass content in the hyperaccumulant plant physiological parts was measured. Furthermore, it was compared with the remaining metal mass content in each growth media. The efficiency and percentage removal of the metal mass was calculated in terms of botanical efficiency parameters (section 4.1.17). [7]

4.1.17 Parameters of efficiency

Different botanical efficiency parameters were used to assess the phytosequestration and phytoextraction efficiency of the hyperaccumulant plant. The efficiency of removal was expressed in percentage value. The botanical efficiency parameters of metal removal were represented in efficiency of removal, bioaccumulation ratio, the relative growth rate and the translocation index.

→ **Efficiency of metal removal (E of R)** in (%) = (weight of metal in shoots + weight of metal in roots (g) /Total metal weight in pot (g) · 100

{Equation: 1}, [249]

→**Bioaccumulation ratio of a metal (BAR)** = Concentration found in plant tissue / Conc. in soil i.e. on dry weight basis (DW) {Equation: 2}, [7]

→**Relative growth rate (RGR)** = $\{\ln(X_2) - \ln(X_1)\}/ (T_2 - T_1)$ Where;

X_1 = the height of the plant at time T_1 in (cm) at the start of the experiment.

X_2 = the height of the plant at time T_2 in (cm) at the harvest period of the experiment.

{Equation: 3}, [263]

→**Translocation Index (TI)** = weight of metal in plant upper physiology (DW)/ weight of metal in plant roots (DW)

{Equation: 4}, [264]

i.e per plant dry weight basis (DW)

Table 4.4: *Brassica juncea* efficiency parameters [1]

Metal	Li	Cr	V
Efficiency of removal (%) (E of R)	35	71	161
Bioaccumulation ratio (BAR)	0.345	0.714	1.612
Translocation index (TI)	70.931	6.954	5.691
Relative growth rate (RGR)	0.032		

[1]: Unit less numbers

→The Group 1 growth media composed of 100% LMT

→The group 5 growth media composed of 50% LMT and 50% dewatered municipal biosolids.

i.e. note the formation of the rigid on top.

Figure 4.8: Groups 1 and 5 growth media formulations

4.1.18 Discussion

The determination of the metal mass content inside the different physiological parts of the hyperaccumulant plant was the cornerstone of the research. Thus the *Brassica juncea* was able to phytoextract and phytostabilize lithium in lieu with chromium and vanadium in a heterogeneous acidic rhizosphere of pH 4.64 (table 4.4). The outcome paved the way for a possible phytoremediation under natural circumstances.

The hyperaccumulant plant seeds were planted in the five different growth media formulations of subsamples of four (n=4). The success of germination, phytoextraction and stabilization was made possible only in Group 4 pots. It was composed of 25% LMT, 25% homogenized peat and 50% dewatered municipal biosolids. Its pH was 4.64, oxidation reduction potential was 139.3mV and its EC was 728 $\mu S \cdot cm^{-1}$ However it was regarded as the suitable formulation under such growth circumstances.

The other remaining groups utterly failed to sustain growth. Consequently they failed to provide a suitable growth media for phytosequestration/extraction and phytostabilization processes to occur. As a result, the hyperaccumulant plant failed to germinate, therefore phyto-harness lithium in lieu with chromium and vanadium.

It was presumed that the reason behind such an outcome was related to the interaction between the pH, the ORP (oxidation reduction potential) and the EC of the five different growth media groups. It tended to influence the *Brassica juncea* plant germination, growth and maturity stages [293].

According to Ernst [294], heavy metals compete with essential macronutrients in acidic medium. They sometimes can be taken in excess amounts that might hinder early seed germination and plant growth periods. As an example in Groups 2 (pH= 3.74, ORP= 197.5 mV and EC= 538 $\mu S \cdot cm^{-1}$) and 3 (pH= 4.04, ORP= 170.7 mV and EC= 893 $\mu S \cdot cm^{-1}$). Their pH was below the physiological need of the *Brassica*

juncea hyperaccumulant plant (pH between 4.3 and 8.3). The relatively low pH that was recorded in Group 4 (pH 4.64) prolonged hyperaccumulant plant seed germination time stunted its growth and extended its phytosequestration/extraction period. As a proof, its the very low relative growth rate parameter of 0.0325. Moreover, it prolonged the phytoextraction and phytostabilization period of the hyperaccumulant plant to 86 days. The acidic growth media impacts were clearly observed on the plant surface. It was characterized by stunted growth, discoloration on the older leaves and premature senescence. It was also reflected through the low values of its bioaccumulation ratios (BAR), of 0.3456, 0.7142 and 1.6128 for lithium, chromium and vanadium respectively.

According to Belouchi *et al.* [295], the further acidification of the rhizosphere was a direct result of root exudates. The process enhanced metal bioavailability and increased its mobility around the plant root cells. Facilitating its uptake through the secondary pumps on the root cellular level and translocating it throughout the hyperaccumulant plant physiology. These pumps are made up of proteins that get triggered by the presence of abundance of H^+ ions.

In addition, the oxidation reduction potential in Group 4 growth media was 139.3 mV. According to Belouchi *et al*. [295], the root plasma membrane potential around the epidermal cell layer might exceed – 200 mV. This gradient difference generates a strong suction force on both sides.

The difference between the outside and the inner epidermal cell enabled the suction force to occur. This lead to the subsequent uptake of the heavy metals bound to nitrates or phosphates into its vascular system. The metals thereafter were transported throughout the xylem system to the upper physiological parts of the hyperaccumulant plant. These metals were presumably stored as precipitates in different forms, like carbonates, sulphates and phosphates inside plant vacuoles, Golgi bodies and endoplasmic reticulum. [295,264,296]

The translocated index (TI) of lithium, chromium and vanadium was 70.931, 6.954 and 5.6913 (table 4.4) respectively. The closeness of the last two values between the chromium and the vanadium was presumably due to the closeness of their hydrated Van der Waals (VdW) radii length. Furthermore, the chemical forms of V (V) and Cr (VI), were suspected to be present in Group 4 growth media. These forms exist under natural environmental conditions in anionic forms of VO_4^{-3} and CrO_4^{-2}. These forms are readily accessible to plant uptake and subsequent storage.

As for Group 1 (100% LMT), which was regarded as the control, with pH= 7.92, ORP= -33mV and an EC= 1264 $\mu S \cdot cm^{-1}$ and the Group 5 (50% LMT and 50% dewatered biosolids), with a pH= 7.16, ORP= -5.5mV and an EC= 2103 $\mu S \cdot cm^{-1}$. The failure of hyperaccumulant seed germination and subsequent growth was assumed to be due to the inability of the above mentioned two growth media to hold and store water sufficiently. This was assumed to be due to the absence of the homogenized peat ingredient from their formulations. Moreover, a quick visual inspection upon Group 5 (figure 4.8), showed a formation of a rigid opaque layer on its upper surface which was due to the drying of the dewatered biosolids with its surrounding lithium mine tailing. The rigid layer formed deprived the seed from light and water contact.

In Group 1 (figure 4.8-b), the absence of hyperaccumulant seed germination was due to the absence of water holding capacity. The irrigated water (amended with organic fertilizer and LiCl) was easily lost due to evaporation and percolation processes.

The first botanical parameter was the efficiency of metal removal (E of R). It was defined as the percentage of the metal mass in shoots divided by the total metal mass remaining in the growth media [249]. It was represented by a percentage value. The *Brassica juncea*'s efficiency of removal for lithium, chromium and vanadium were 35%, 71% and 161% respectively.

The second parameter was bioaccumulation ratio (BAR). It was defines as the metal mass content in the plant tissue divided by the metal mass remaining in the pot. The BAR for lithium, chromium and vanadium were 0.3456, 0.7142 and 1.6128. It reflected the hyperaccumulant plant ability to translocate twice amount of vanadium rather than chromium and lithium into its different physiological parts.

The third criterion was the relative growth rate (RGR) of the hyperaccumulant plant. It showed the relative growth rate of the hyperaccumulant plant under specific growth circumstances at two different time and height intervals of the hyperaccumulant plant. The RGR criterion was based on the growth media characteristics of pH, ORP and EC. The germination and growth of the hyperaccumulant plant occurred solely in the heterogeneous acidic rhizosphere of Group 4. Therefore, the RGR value was 0.0325 which was regarded as very low due to the acidic nature of the rhizosphere.

The forth plant efficiency parameter was the translocation index (TI) of the three metals, lithium, chromium and vanadium in the hyperaccumulant plant physiology. Thus the TI represented a comparison of metal mass content in upper versus the lower physiology of the hyperaccumulant plant. In the continuous phytoremediation process the higher the value of translocation index, the more successful the plant was regarded. This is because the precipitated metals in the shoots are more easily harvested, recycled or stored as opposed to its roots. As a result, the translocation index for lithium was 70.931, for chromium 6.954 and for vanadium 5.6913. Therefore, the hyperaccumulant plant was extremely successful in translocating lithium, rather than chromium and vanadium and precipitating it inside its upper physiology.

On the rhizosphere level of Group 4, it was assumed that due to the abundant content of dewatered municipal biosolids (50% of the total growth medium weight) was successful in improving the metal uptake specially lithium from the growth medium. However, it was speculated that the different

organisms like mycorrhizal fungi, strains of *Bacillus* and *pseudomonas* formed symbiotic relationships with the *Brassica juncea* hyperaccumulant plant roots. [264]

Moreover, these root colonizing bacterial organisms might have played a pivotal role in the sequestration and mobilization of these metals to the hyperaccumulant plant roots system [1].

Phase two: Monocotyledonous plant phytoextraction and phytostabilization analysis

4.2.1 Introduction

The monocotyledonous (grass) plant belongs to the *Poaceae* or *Gramineae* families. It encloses more than eleven thousand species and over 800 genera in total, mostly annuals but few perennials. The grasses are essential food producers like rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), barley (*Hordeus vulgare* L.) and many others. [267]

Physiologically, the grasses possess fibrous, abundant and very dense root system. They are regarded as suitable for phytoextraction and phytostabilization of soil pollutants. Their leaves are linear with parallel veins vertically pointed. The grasses are extremely successful in tolerance to various ecosystems due to their varied means of reproduction and photosynthesis. Moreover; grasses produce seeds through cross pollination, self fertilization, and asexual reproduction through vegetative parts like rhizomes and stolons. [268]

Finally the aim of planting the monocotyledonous plant seeds in the most favourable growth media (Group 4) was to quantify its efficiency to phytoextract/stabilize under acidic heterogeneous rhizosphere and compared it with the dicotyledonous plant (*Brassica juncea*) results.

Figure 4.9: Monocotyledonous plant growth and harvest

4.2.2 Experimental process and results

The monocotyledonous plant seeds were directly sown on the most successful growth media Group 4 of subsamples of four (n= 4). They were left to grow for eighty six days. They were irrigated once every two days by a 10 ml of the organic fertilizer, amended with 3 g of lithium chloride on weight per volume basis (w/v) diluted in a liter of water. The irrigated volume of water mixed fertilizer was specific to its one pore volume.

The hyperaccumulant plants were harvested, dried, stored and its different physiological parts were digested in concentrated (12N) hydrochloric acid matrix. The metal mass content of lithium and chromium was analyzed through the Perkin Elmer Analyst 100 ™ flame AAS.

Finally the excess samples were properly stored and refrigerated in caped containers.

Table 4.5: Metal mass content in monocotyledonous plant [1–2–3]

Element	Rhizosphere mean ± SD	Roots mean ± SD	Leaves mean ± SD	Remaining GM mean ± SD
Cr	0.275 ± 0.038	0.319 ± 0.063	0.342 ± 0.092	0.266 ± 0.046
V	*<LLD*	*<LLD*	*<LLD*	*<LLD*
Na	0.256 ± 0.057	1.014 ± 0.684	0.741 ± 0.073	0.313 ± 0.129
K	0.779 ± 0.451	1.721 ± 2.872	8.094 ± 1.682	0.807 ± 0.988
Li	0.228 ± 0.037	0.592 ± 0.058	2.211 ± 0.644	0.551 ± 0.032
Fe	0.456 ± 0.439	0.535 ± 0.326	0.433 ± 0.125	0.456 ± 0.148
Ca	2.536 ± 0.084	0.946 ± 0.228	0.250 ± 0.074	1.263 ± 0.109
Mg	0.427 ± 0.093	0.877 ± 0.065	1.197 ± 0.408	0.589 ± 0.321

[1]: Concentrations in $mg \cdot g^{-1}$ per dry weight basis

[2]: *<LLD* as low level of detection

[3]: The GM as the growth media

Table 4.6: Monocotyledonous plant efficiency parameters [1]

Metal	Cr	Li
Efficiency of removal (%) (E of R)	122	360
Bioaccumulation ratio (BAR)	1.22	3.6
Translocation index (TI)	1.071	3.730
Relative growth rate (RGR)	0.052	

[1]: Unit less numbers

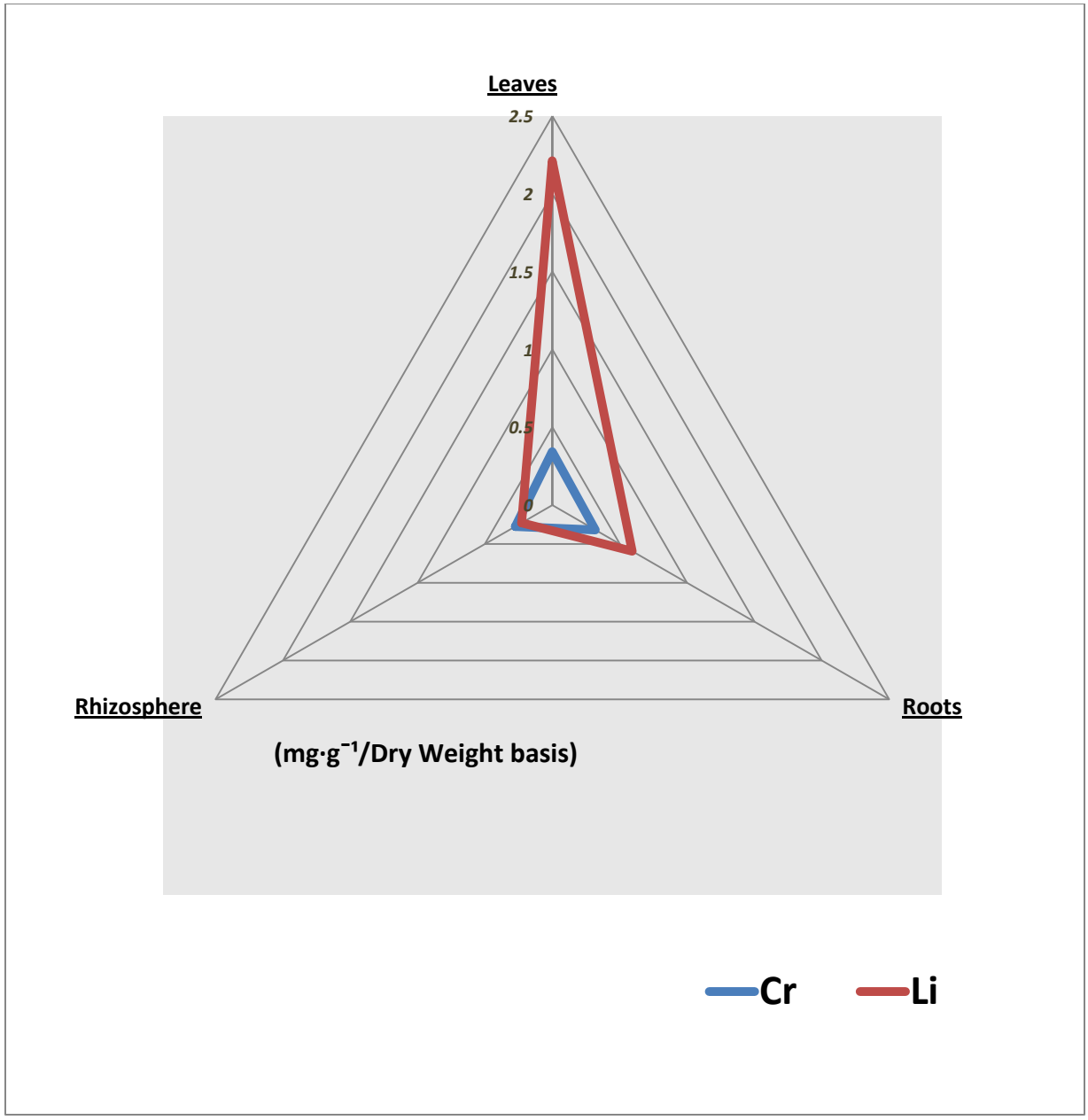


Figure 4.10: Li and Cr phytoextraction and phytostabilization in monocotyledonous plant [1]

[1]: Metal mass content in $mg \cdot g^{-1}$ per plant dry weight basis

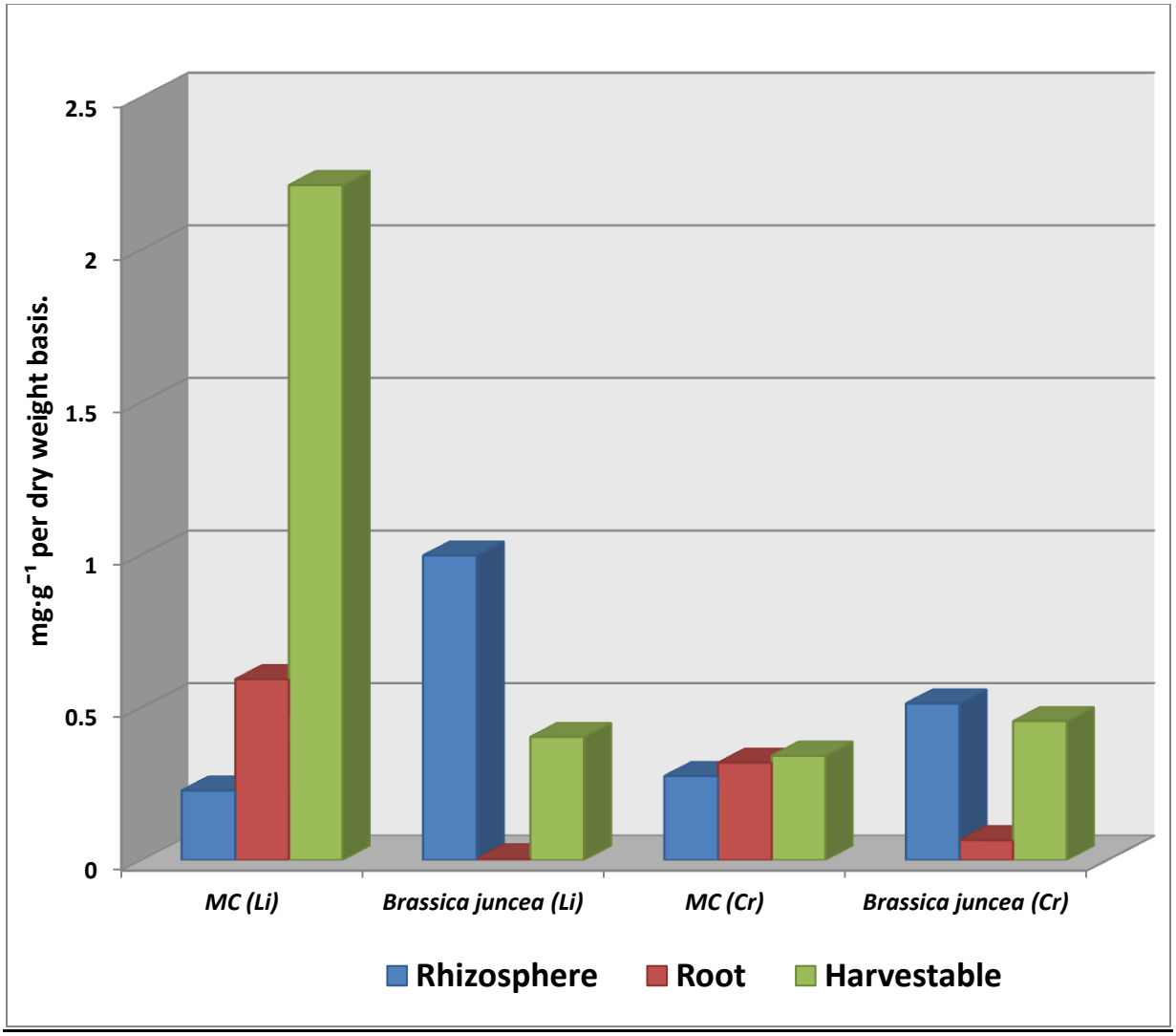


Figure 4.11: *Brassica juncea* vs. monocotyledonous plant phytoaccumulation of Li and Cr [1]

[1] Harvestable weights constituted the added leaf and the stem metal masses

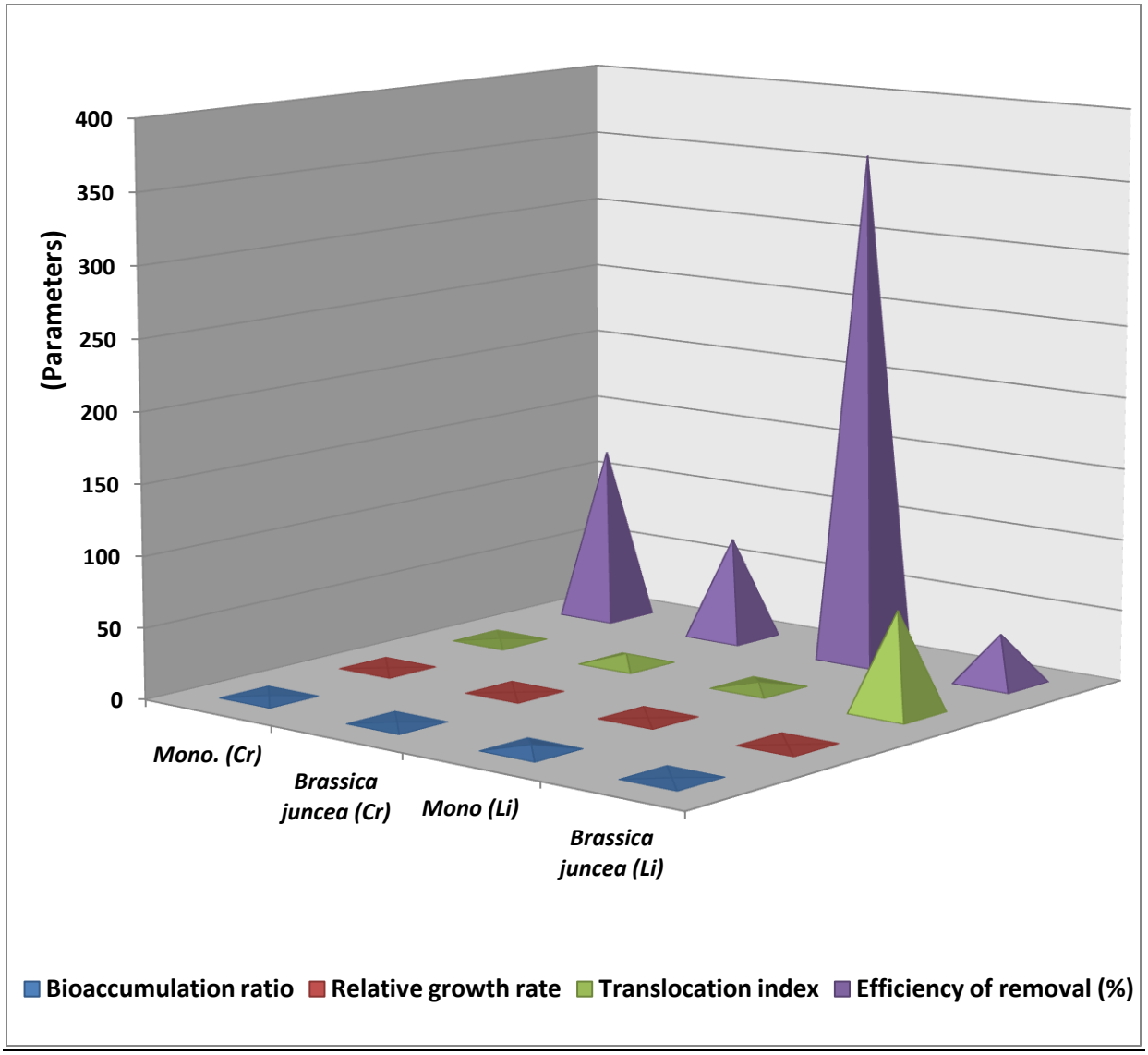


Figure 4.12: *Brassica juncea* vs. monocotyledonous plant in botanical efficiency terms for Li and Cr [1]

[1] Mono: The abbreviation for the monocotyledonous plant

4.2.3 Discussion

The aim of sowing the monocotyledonous plant seeds, directly on the most favorable growth media was to compare it, with the dicotyledonous plant the *Brassica juncea*. It was sown under homogeneous growth conditions. It was compared with the dicotyledonous plant based on the botany wise efficiency parameters (tables 4.4 and 4.6).

The monocotyledonous surpassed the *Brassica juncea* in efficiency of removal, relative growth rate, and bioaccumulation ratios but lagged behind in translocation index. The monocotyledonous plant was more than two times successful in its relative growth rate. Moreover, it was twice more efficient in removal and in bioaccumulation ratios for chromium and more than ten times for lithium, rather than the *Brassica juncea* hyperaccumulant plant.

On the other hand the *Brassica juncea* was six times more efficient in translocating chromium and more than twenty times for lithium inside its upper harvestable physiological parts rather than the monocotyledonous plants.

Chapter five: Conclusion

- ✓ This research showed the capability of *Brassica juncea* to phytoharness 0.408 $mg \cdot g^{-1}$ of lithium in its different physiological parts per dry weight basis. Also it phytostabilized 0.988 mg inside its heterogeneous acidic of Group 4.
- ✓ *Brassica juncea*'s efficiency of lithium in lieu with chromium and vanadium of removal were 35%, 71% and 161%, but with an extremely low relative growth rate of 0.0325.
- ✓ *Brassica juncea* bioaccumulation ratio for lithium, chromium and vanadium were 0.345, 0.714 and 1.61 and their translocation indexes were 71, 7 and 5.6 respectively.
- ✓ Five different growth media groups were formulated and tested. The most favourable one was the Group 4 (pH 4.64, ORP 139.3 mV, EC 728 $\mu S \cdot cm^{-1}$ and TDS 436.8 $mg \cdot l^{-1}$). It supported hyperaccumulant plant seed germination, growth and permitted phytostabilization and phytosequestration/extraction to occur.
- ✓ Macronutrient presence throughout the five different groups ranged between 0.142 mg to 0.589 mg, which were regarded as very low but were supplemented through the mixed water and organic fertilizer irrigations.
- ✓ Monocotyledonous plant was able to phytoextract 2.803 $mg \cdot g^{-1}$ and 0.561 $mg \cdot g^{-1}$ of lithium and chromium per its dry weight basis. Similarly it phytostabilized 0.228 mg and 0.275 mg of lithium and chromium inside its heterogeneous acidic rhizosphere.
- ✓ The comparison between the two hyperaccumulant plants; the *Brassica juncea* and the monocotyledonous were achieved through the botany wise efficiency parameters. The relative growth rate of the monocotyledonous plant was twice that of the dicot plant. Moreover, the efficiency of removal and bioaccumulation ratios was more than twice and ten times that for

chromium and lithium. On the contrary the *Brassica juncea*'s translocation index was more than six and twenty times for chromium and lithium, compared with the monocotyledonous plant.

- ✓ A novel proposal for an efficient phytosequestration/stabilization and proper reclamation for mine tailing. It included the incorporation of ingredients, such as peat and dewatered municipal biosolids incorporated with the tailing mass. However, for optimal phytoharnessing results an integrated mixed planting pattern between the monocot and the dicot hyperaccumulating plants might be a viable alternative. Maximizing the overall process efficiency might include the coupling of an EK field at the vigorous growth stage of the hyperaccumulant plants. In addition, the engineered ecosystem might mimic the role of a natural buffer and a reservoir minimizing the metal mass runoffs in the lithosphere.

Future work recommendations

- ✓ Determination of lithium allelopathic symptoms and effects on plant growth and reproduction.
- ✓ Determination different lithium precipitation forms and sink sites on the hyperaccumulant plant cellular level.
- ✓ An insitu trial of the novel integrated approach for mine tailing reclamation, phytosequestration /stabilization under natural circumstances.
- ✓ Assessing new techniques like; heat treatment, electrorefining and other conventional metal recovery procedures for the recovery of the phytomined metals specially lithium.

Appendix

Table 5.1: LMT texture data in detail [1-2]

Mesh Sieve № (mm)	Empty Weight (g)	Final Weight (g)	Remaining Soil (g)	Percentage Retained (%)	Percentage Passed (%)
4 (4.76 mm)	490.37	493.78	3.41	0.341	99.65
8 (2.36 mm)	497.41	506.61	9.2	0.920	98.739
10 (2.00 mm)	438.10	458.80	20.7	2.070	96.669
12 (1.68 mm)	493.39	1201.45	708.06	70.814	25.855
16 (1.19 mm)	442.39	624.65	182.26	18.228	7.627
30 (600 µm)	442.63	511.84	69.21	6.922	0.705
50 (247 µm)	361.02	363.15	2.13	0.213	0.492
100 (150 µm)	360.48	362.75	2.27	0.227	0.265
200 (75 µm)	342.70	343.96	1.26	0.126	0.139
Collecting Pen	369.80	371.19	1.39	0.139	
Total			**999.89**	**100**	

[1]: The size of sand was divided into three types coarse; that retained on sieve № 10, medium on sieve № 30 and finally fine retained on sieve № 200.

[2]: SD ± 0.1 mg.

References

1. **Raskin I, Smith RD, Salt DE. 1997.** Phytoremediation of metals: using plants to remove pollutants from the environment. Curr. Opin. Biotechnol. 8: 221-226.

2. **Cunningham SD, Anderson TA, Schwab AP, Hsu FC. 1996.** Phytoremediation of soils contaminated with organic pollutants. Adv. Agron. 56: 55-114.

3. **Cunningham SD, Ow DW. 1996.** Promises and prospects of phytoremediation. Plant Physiol. 110: 715-719.

4. **Cunningham SD, Berti WR. 1993.** Remediation of contaminated soils with green plants: an overview In Vitro. Cell Dev.Biol. 29:207-212.

5. **Raskin I, Kumar PBAN, Dushenkov S, Salt DE. 1994.** Bioaconcentraton of heavy metals by plants. Curr.Opin.Biotechnol. 5:285-290.

6. **Marchiol L, Assolari S, Sacco P, Zerbi G. 2004.** Phytoextraction of heavy metals by canola (Brassica napus) and radish (Raphanus sativus) grown on multicontaminated soil. Environmental Pollution. 132:21-27.

7. **Lasat MM,Norvell WA, Kochian LV. 1997.** Potential for phytoextraction of 137 Cs from a contaminated soil. Kluwer Academic Publishers. The Netherlands : Plant and Soil 195:99-106.

8. **Kramer U, Cotter-Howells JD, Charnock JM, Baker AJM, Smith JC. 1996.** Free histidine as a metal chelator in plants that accumulate nickel. Nature. 379: 635-638.

9. **Salt DE, Smith RD, Raskin I. 1998.** Phytoremediation. Ann.Rev.Plant Physiol.Plant Mol. Biol. 49: 643-668.

10. **Baker AJM. 1981.** Accumulators and excluders:Strategies in the responce of plants to heavy metals. J.Plant Nutr. 3: 643-654.

11. **Blaylcok MJ, Salt DE, Dushenkov S, Zakharova O,Gussman C. 1997.** Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents.Enviro. Sci. Technol. 31: 860-865.

12. **Chaney, R.L.,Li,Y.M.,Brown,S.L.,Angle,J.S & Baker,A.J.M. 1995.** Hyperaccumulator beased phytoremediation of metal rich soils. Columbia,Missouri : Fourteenth Annual symposium. 33-34.

13. **Boyajian GE, Carreira LH. 1997.** Phytoremediation: a clean transition from laboratory to marketplace. Nat. Biotechnol. 15:127-128.

14. **Boyd RS, Martens SN. 1992.** The raison d'etre for metal hyperaccumulation by plants. Soils. 11a.pp. 279-289.

15. **Reeves RD, Brooks RR, Macfarlane RM. 1981.** Nickel update by Californian Streptanthus and Caulanthus with particular reference to the hyperaccumulator S. polygaloids Gray (Brassicaceae). Am. J. of Bot. 68: 708-712.

16. **Huang JWW, Chen JJ, Berti WR, Cuningham SD. 1997.** Phytoremediation of lead contaminated soils:role of synthetic chelates in lead phytoextraction. Environ.Sci. Technol. 31: 800-805.

17. **Huang JW, Cuningham SD. 1996.** Lead phytoextraction: species variation in leaduptake and translocation. New Phytol. 134: 75-84.

18. **JØrgensen SE. 1993.** Removal of heavy metals from compost and soil by ecotechnological methods. Ecol.Eng. 2:89-100.

19. **Blaylock MJ, Salt DE, Dushenkov S, Zakharova O, Gussman C. 1997.** Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ. Sci. Technol. 31: 860-865.

20. **Epelde L, Becerril JM, Hernandez-Allica J, Barrutia O, Garbisu C. 2008.** Functional diversity as indicator of the recovery of soil health derived from Thlaspi caerulescens growth and metal phytoextraction. Applied Soil Ecology. 39: 299-310.

21. **Chaney, R.L. 1983.** Plany uptake of inorganic waste.In land treatment of hazardous waste. s.l. : JE.Parr ed.Park Ridge,Il. pp.50-76.

22. **Morrey DR, Balkwill K, Balkwill MJ, Williamson S. 1992.** A review of some studies of the serpentine flora of Southern Africa.12: 147-157.

23. **Doran JW, Safley M. 1997.** Defining and assessing soil health and sustainable productivity.Biological Indicators of Soil Health. Wellingford, UK. : CAB International. pp.1-28..

24. **Alkorta I, Aizupua A, Riga P, Albizu I, Amezaga I, Garbisu C. 2003.** Soil enzyme activities as biological indicators of soil health. Rev.Environ.Health. 18: 65-73.

25. **Terry N, Carlson C, Raab TK, Zayed A. 1992.** Rates of Se volatilization among crop species. J.Environ.Qual. 21: 341-344.

26. **Hemingway JS. 1995.** The mustard species: Condiment and food ingredient use and potential as oilseed crops.In brassica oilseeds production and utilization. CAB.International,Wallingford. pp. 373-383.

27. **Gulden RH, Shirtliffe SJ, Thomas AG. 2003a.** Harvest lossed of canola cause large seedbank inputs. Weed Sci. 51:83-86.

28. **Woods DL, Capcara JJ, Downey RR. 1991.** The potential of mustard (Brassica juncea (L.) Coss) as an edible crop on the Canadian Prairies. Can. J. Plant Sci. 71: 195-198.

29. **Palmer CE, Warwick S, Keller W. 2001.** Brassicaceae (Cruciferae) Family, Plant Biotechnology, and Phytoremediation. Int. Jour. of Phyto.: Vol.3, No.3, Pp.245-287.

30. **Reed CF. 1976.** Information summaries on 1000 econimic plants. USDA.

31. **Williams SM, Weil RR. 2004.** Crop cover root channels may alleviate soil compaction effects on soybean crop.Soil Sci. Soc. Am.J. 68: 1403-1409.

32. **Haramoto ER, Gallandt ER. 2004.** Brassica cover cropping for weed management: A review. Ren. Ag. and Food Sys. 19: 187-198.

33. **Boydson RA, Al-Khatib K. 2005.** Utilizing Brassica cover crops for weed suppression in aanual cropping systems. Binghampton, NY. : Handbook of sustainable weed management. Haworth Press. pp.77-94.

34. **Gardiner JB. 1999.** Allelachemicals released in soil following incorporation of rapeseed (Brassica napus) green manures. J. Agric. Food Chem. 47: 3837-3842.

35. **Peterson J et al. 2001.** Weed supression by release of isothiocyanates from turnip rape mulch. Agron. J. 93: 37-43.

36. **Patel JR, Parmar MT, Patel JC. 1980.** Effect of different sowing dates,spacing and plant populations on yiels of mustard. Indian J. Agron. 25 (3): 526-527.

37. **Larkin RP, Griffin TS. 2007.** Control of soilborne potato diseases with Brassica green manures. Crop Protection. 26: 1067-1077.

38. **Larkin RP, Griffin TS, Honeycutt CW. 2006.** Crop rotation and cover crop effects on soilborne diseases of potato. Phytopath. 96: S48.

39. **Munoz FN, Graves W. 2000.** Medics General cover crops. Sac. California: Univ. of Calif.Sarep.

40. **Duke JA, Wain KK. 1981.** Medicinal plants of the world. Com. In. 3 volumes.

41. **Burkill JH. 1996.** A dictionary of economic products of the Malay pennisula. Art printing works, Kuala Lumpur. 2 vols.

42. **Perry LM. 1980.** Medicinal plants of East and Southeast Asia. MIT press, Cambridge.

43. **Leung AY. 1980.** Encyclopedia of common natural ingredients used in food, drugs, and cosmetics. John Wiley and Sons, New York.

44. **Knowless PF, Kearney TE, Cohen DB. 1981.** Species of rapeseed and mustard as oil crops in California. Pryde EH, Princen LH and Mukherjee KD. ed. p. 255-268.

45. **Maity PK, Sengupta AK, Jana PK. 1980.** Responce of mustard variety varuna (Brassica juncea) to levels of irrigation and nitrogen. Indian Agriculturist. 24 (1): 43-47.

46. **Pryde EH, Doty HO. Jr. 1981.** World fats and oils situation. American Oil Chemists Society. Champaign, Il. pp.3-14.

47. **Reeves RD. 1992.** The hyperaccumulation of nickel by serpentine plants. In.Backer AJM, Proctor J, RDReeves (ed). The ecology of ultramafic (serpentine) soils.Intercept, Andover.

48. **Andrew J, Smith C, Kramer U, Baker JM. 1995.** Role of metal transport and chelation in nickel hyperaccumulation in genus Alyssum. 14th annual symposium,University of Missouri-Columbia. pp 9-10.

49. **Boyd RS, Shaw JJ, Martens SC. 1994.** Nickel hyperaccumulation defends Streptanthus polygaloides (Brassicaceae) against pathogens. Am.J. Bot. 81: 294-300.

50. **Rauser RD. 1990.** Phytochelatins. Annu.Rev.Biochem. 59: 61-86.

51. **Rugh CL, Wilde HD, Stack NM, Thompson DM, Summers, AO. Meagher RB. 1996.** Mercuric ion reduction and resistance in transgenic Arabidopsis thaliana plants expressing a amodified bacterial merA gene. USA : Proc. Natl. Acad. Sci. 93: 3182:87.

52. **Tomsett AB, Thurman DA. 1988.** Molecular biology of metal tolerances of plants. Plant Cell. Environ. 11: 383-94..

53. **Jackson PJ, Unkefer PF, Delhaize E, Robinson NJ. 1990.** Mechanisms of trace metal tolerance in plants. San Diego : In Environmental Injury to Plants, ed F. Katterman. 10: 231-55.

54. **Ernst WHO, Verkleij JAC, Schat H. 1992.** Metal tolerance in plants. Acta Bot.Neerl. 41: 229-248.

55. **Gwozdz EA, Przymusinski R, Rucinska R, Decker J. 1997.** Plant cell responces to heavy metals:molecular and physiological aspects. Acta Physiol. Plant. 19: 459-65..

56. **Robinson NJ, Tommey AM, Kuske C, Jackson PJ. 1993.** Plant metallothioneins.Biochem. J. 295: 1-10.

57. **Murphy AS, Taiz L. 1995.** Comparison of metallothionein gene expression and non protein thiols in ten Arabidosis ecotypes. Plant Physiol. 109: 1-10.

58. **Rauser WE. 1990.** Phytochelatins. Ann.Rev. Biochem. 59: 61-86.

59. **Rauser WE. 1995.** Phytochelatins and related peptides.Structure biosynthesis and function. Plant Physiol. 109: 1141-1149.

60. **Stepan UW, Scholz G. 1993.** Nicotianamine: mediator of transport of iron and heavy metals in the phloem ? Physiol. Plant. 88: 522-29.

61. **Howden R, Goldsbrough PB, Andersen CR, Cobbett CS. 1995.** Cadmium sensitive , cad1 mutants of Arabidopsis thaliana are phytochelatin deficient. Plant Physiol. 107: 1059-1066.

62. **Lauchli A. 1993.** Selenium in plants: uptake, functions and environmental toxicity. Bot. Acta. 106: 455-468.

63. **Francesconi KA, Edmonds JS. 1994.** Biotransformation of arsenic in the marine environment. New York:Wiley : In Arsenic in the Environment. Part 1, Cycling and characterization. pp. 430.

64. **Murphy A, Zhou JM, Goldsbrough PB,Taiz L. 1997.** Purification and immunological identification of metallothioneins 1 and 2 from Arabidopsis thaliana. Plant Physiol. 113: 1293-1301.

65. **Storage J, Macnair MR. 1991.** Evidence for a role for the cell membrane in copper tolerance of Mimulus guttatus. Fisher ex. DC. New Phytol. 119: 383-388.

66. **Kinnersely AM. 1993.** The role of phytochelates in plant growth and productivity. Plant Growth Regul. 12: 207-17.

67. **Kanazawa K, Higushi K, Nishizawa, NK, Fushiya S, Chino M, Mori S. 1994.** Nicotianamine aminotransferase activities are correlated to the phytosiderophore secretion under Fe-deficient condotions in Gramineae. J.Exp.Bot. 45: 1903-6.

68. **Moog PR, Bruggeman W. 1994.** Iron reductase systems on the plant plasma membrane-a review. Plant Soil. 165: 241-60.

69. **Yi Y, Guerinot ML. 1996.** Genetic evidence that induction of root Fe(II) chelate reductase activity is necessary for iron uptake under iron deficiency. Plant J. 10: 835-844.

70. **Welch RM, Norvell WA, Schaefer SC, Shaff JE, Kochian LV. 1993.** Induction of iron(II) and cupper(II) reduction in pea (Pisum sativum L.) roots by Fe and Cu status:does the root-cell plasmalemma Fe(III)-chelatereductase perform a general role in regulated cation uptake. Planta. 190: 555-561.

71. **Crowley DE, Wang YC, Reid CPP, Szanislo PJ. 1991.** Mechanisms of iron acquisition from siderophores by microorganisms and plants.Plant Soil. 130: 179-98.

72. **Guerinot ML. 1997.** Metal uptake in Arabidopsis thaliana. J.Exp. Bot. S 48: 96.

73. **Salt DE, Prince RC, Pickering IJ, Raskin I. 1995.** Mechanisms of cadmium mobility and accumulation in Indian mustard. Plant Physiol. 109: 427-33.

74. **Senden MHMN, Van Paassen FJM, Van Der Meer AJGM, Wolterbeek A. 1990.** Cadmium-citric acid-xylem cell wall interactions in tomato plants. Plant Cell Environ. 15: 71-79.

75. **Stephan UW, Shmidke I, Stephan VW, Scholz G. 1996.** The nicotianamin molecule is made to measure for complexation of metal micronutrients in plants. Biometals. 9: 84-90.

76. **Lewis BG, Johnson CM, Broyer TC. 1974.** Volatile selenium in higher plants. The production of dimethyl selenide in cabbage leaves by enzymatic cleavage of Se-methyl selenomethionine selenonium salt. Plant Soil. 40: 107-18.

77. **Lewis BG, Johnson CM, Delwiche CC. 1966.** Release of volatile selenium compounds by plants.Collection procedures and preliminary observations. J.Agric.Food Chem. 14: 638-40.

78. **Zayed AM, Terry N. 1994.** Selenium volatilizationin roots and shoots:effects of shoot removal and sulfate level. J.Plant Physiol. 143: 8-14.

79. **Zayed AM, Terry N. 2003.** Chromium in the environment: factors affecting biological remediation. Kluwer Academic Publishers.The Netherlands. 249: 139-156.

80. **Adriano DC. 1986.** Trace elements in the Terrestrial Environment. New York : Springer Verlag. p.105-123.

81. **Nriagu JO. 1988.** Production and uses of chromium. Chromium in natural and human environment. New York,U.S.A : John Wiley & Sons. p.81-105.

82. **Chandra P, Sinha S, Rai UN. 1997.** Bioremediation of Cr. from water and soil by vascular aquatic plants. American Chemical Society,Wasington, DC. p.274-82.

83. **Vazquez MD, Poschenrieder CH, Barcelo J. 1987.** Chromium VI induced structural and ultrastructural changes in bushbean plants ([haseolus vulgaris L.). Annals Bot. 59: 427-438..

84. **Mishra S, Singh V, Srivastava S, Srivastava R, Srivastava M, Dass S, Satang G, Prakash S. 1995.** Studies on uptake of trivalent and hexavalent Cr by maize (Zea mays). Fd. Chem.Toxic. 33(5): 393-397.

85. **Jain R, Srivastava S, Madan VK, Jain R. 2000.** Influence of chromium on growth and cell division of sugarcane. Indian J. Plant Physiol. 5: 228-31.

86. **Huffman J, Allaway WH. 1973.** Chromium in plants:distribution in tissues, organelles and extracts and availability of bean leaf Cr to animals. J. Agric. Food Chem. 21: 982-986.

87. **Barcelo J, Poschenriender C, Ruano A, Gunse B. 1985.** Leaf water potential in Cr(VI) treated bean plants (Phaseolus vulgaris L.). Plant Physiol. Suppl. 77: 163-4.

88. **Cervantes C, Garcia JC, Devars S, Corona FG, Tavera,H.L. 2001.** Interactions of chromium with micro-organisms and plants. FEMS Microbiol. Rev. 25: 335-47.

89. **Shanker AK, Djanaguirman M, Sudhagar R, Chandrashekar CN, Pathmanabhan G. 2004a.** Differential antioxidative responce of ascorbate glutathionepathway enzymes and metabolitesto chromium speciation stress in green gram (Vigna radiata (L) R Wilczek,cv CO 4) roots. Plant Sci. 166:1035-43.

90. **Zayed A, Lytle CM, Jin-Hong Q, Terry N, Qian JH. 1998.** Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta. 206: 293-9.

91. **Skeffington RA, Shewry PR, Petersen PJ. 1976.** Chromium uptake and transport in barley seedlings Hordeum vulgare. Planta. 132:209-14.

92. **Rout GR, Samantaray S, Das P. 1997.** Differential chromium tolerance among eight mungbean cultivars grown in nutrient culture. J.Plant Nutr. 20: 473-483.

93. **Habashi F.2002a.** Two hundred years of Vanadium In: Vanadium, geology, processing and applications. Montreal,Canada : Proceeding of the International Symposium on Vanadium, Conference on metallurgies. August,11-14; pp.3-15.

94. **Bauer G, Guther V, Hess H, Otto A, Reidl O, Roller H, Sattelberger S. 2002.** Vanadium and vanadium compounds. Weinheim, Germany. : Wiley-VCH Verlag Gmbh. Excerpt from Ullmann's.

95. **Barcelaux DG.1999.** Vanadium. Clin. Toxicol. 37:265-278..

96. **Vanadium Statistics and Information.** 2003. Government minerals publications website,mineral community summaries.

97. **Reese RG Jr. 2001.** Vanadium. Domestic survey tables by A.A.Wario and world data. : R.G.Coleman. pp.81.1-81.2.

98. **Sawarin J. 2002.** "The Lac Dore" vanadium project:Current and future trends. Montreal,Canada. Proceedings of the International symposium on Vanadium,Conference of metallurgies. August,11-14,pp.105-112.

99. **Brichard SM. Henquin JC. 1995.** Trends. Pharmacol. Sci. 16:265.

100. **Shechter Y, Goldwaser I, Mironchik M, Fridkin M. Gefel D. 2003.** Historic perspective and recent developments on the insulin-like actions of vanadium, towards developing vanadium based drugs for diabetes. Coord.Chemistry Reviews. 237:3-11.

101. **Schechter Y. Shisheva A. 1993.** Endevour. 17:23.

102. **Lyonett BM, Martin E. 1999.** La Presse Medicale. 1: 191.

103. **Dubyak GR, Klientzeller A. 1980.** Vanadium. J.Biol.Chem. 255: 5306.

104. **Rider MH, Bartons R, Hue L. 1990.** Vanadium as insulin alternative. Eur.J.Biochem. 190: 53.

105. **Mirapeix M, Deceux JF, Kahn A, Bartrons R. 1991.** Diabetes. Diab.J. 40: 462.

106. **Mengel K, Kirkby EA. 1987.** Principles of plant nutrition. Bern,Switzerland: International Potash Institude. 4th Ed.

107. **Sanchez JS, Colomina MT, Domingo JL. 1998.** Effects of vanadium on activity and learning in rats. Phys.Behav. 63: 345-350.

108. **Sing B, Wort DJ. 1970.** Sugar. Jour. 19-24.

109. **Fay P, De Vasconcelos L. 1974.** Vanadium. Arch. Mikrobiol. 99: 221.

110. **Serra MA, Sabbioni E, Marchesini A, Pintar A, Valoti M. 1989.** Vanadate as an inhibitor of plant and mammalian peroxidases. Biol.Trace Element Research. Vol.23: 151-164.

111. **Kabata-Pendias A, Pendias H. 1979.** Rare elements in biological environments. Warsaw.Poland. Geological Publishers.

112. **Greenwood NN, Earnshaw A.1984.** Chemistry of Elements. Oxford. Pergamon Press.

113. **Kabata-Pendias A, Pendias H. 1993.** Biogeochemistry of Trace Elements. Warsaw,Poland. PWN.

114. **Rehder D. 1999.** The coordination chemistry of vanadium as related to its biological functions. Coord.Chem.Rev. 182: 297-322.

115. **Panichev N, Mandiwana K, Moema D, Molatlhegi F, Ngobeni P. 2006.** Distribution of Vanadium (V) species between soil and plants in the vicinity of Vanadium mine. Jour.of Hazardous Materials. A317:649-653.

116. **Beffagna N, Romani G, Lovadina S. 1993.** Inhibition of malate synthesis by vanadate:Implications for the cytosolic alkalinization induced by vanadate concentrations partially inhibiting plasmalemma H+ pump. J.Exp.Bot. 44: 1535-1542.

117. **Kasai M, Yamazaki J, Kikuchi M, Iwaya M, Sawada S. 1999.** Concentrations of vanadium in soil water and its effects on growth and metabolism of rye and wheat plants. Comm. in Soil Science and Plant Analysis. 30: 7,971-982.

118. **Cantley LC Jr, Cantley LG, Josephson L. 1978.** Vanadium in plants ecosystem. J.Biol.Chem. 253: 7361-7368.

119. **Macara IG. 1978.** Vanadium-an element in search of a role. J.Biol.Chem. 44: 15-19.

120. **Biggs WR, Swinehart JH. 1976.** Vanadium in selected biological systems. New York : Metal ions in biological systems. Vol 6. pp 142.

121. **Laves D. 1978.** Potassium transformation in soil. Arch.Acker-u. Pflanzenbau u. Bodenk. 22(8) pp. 521-528.

122. **Sparks DL. 1987.** Potassium dynamics in soils. Adv.Soil Sci. 6: pp. 1-63.

123. **Sparks DL. 1986.** Potassium release from sandy soils.In: Nutrient balances and the need for potassium. Int. Potash Institude,Bern: Int. Potash Insitude. pp. 93-107.

124. **Farmer VC, Wilson MJ. 1970.** Experimental conversion of biotite to hydrobiotite. Nature. 226: pp.841-842.

125. **Shroeder D. 1955.** Potassium fixation and release of loess soils. Landw. Forsch. 8: pp. 1-7.

126. **Schuffelen AC. 1971.** Nutrient content and nutrient release in soils. Wien : Land. Chem. Bundes. pp.27-42.

127. **Sparks DL. 1988.** Kinetics of soil chemical processes. London : Academic Press. pp. 166-167.

128. **Martin HW, Sparks DL. 1985.** On the behaviour of nonexchangeable potassium in soils. Comm.Soil Sci. Plant Anal. 16(2): pp. 133-162.

129. **Matthuis FJM, Sanders D. 1997.** Regulation of K + absorption in plant root cells by external potassium: Interplay of different plasma membrane potassium transporters. J.Exp.Bot. 48: pp.451-458.

130. **Fox TC, Guerinot ML. 1998.** Molecular biology of cation transport in plants. Annu. Rev. Plant. Mol. Biol. 49: pp. 669-696.

131. **Schachtman DP, Schroeder JI. 1994.** Structure and transport mechanism of a high affinity potassium uptake transporter from higher plants. Nature. 370: pp. 655-658.

132. **Rubio F, Gassman W, Schroeder JI. 1995.** Sodium driven potassium uptake by the plant potassium transporter HKTI and mutations conderring salt tolerance. Science. 270: 1660-1663.

133. **El-Sheikh AM, Ulrich A. 1970.** Interactions of rubidium, sodium, and potassium on the nutrition of sugar beet plants. Plant Physiol. 46: 645-649.

134. **Wyn Jones RG, Pollard A. 1983.** Proteins, enzymes and inorganic ions. Inor. Plant Nut. Encyc. Vol.15B. pp. 528-562.

135. **Pissarek HP. 1973.** The development of potassium deficiency sumpyoms in spring rape. Z. Pflanze. Bodenk. 136: pp.1-96.

136. **Kursano AL, Vyskrebentzewa E. 1966.** The role of potassium in plant metabolism and the biosynthesis of compounds important for the quality of agricultural products. Bern : 8th Congr. Intern. Potash Institude. pp.401-420.

137. **Bergmann W. 1992.** Nutritional disorder of plants, development visual and analytical diagnosis. Jena: Gustav Fischer.

138. **Glynne MD. 1959.** Effect of potash on powdery mildew in wheat. Plant Path. 8:15-16.

139. **Goss RL. 1968.** The effects of potassium on diseases resistance, In: The role of potassium in agriculture. Madisson, USA. pp.221-241.

140. **Donner HE, Lynn WC. 1989.** Carbonate,halide,sulfate and sulfide minerals, in: Minerals in Soil Environments. Madison,Wisconsin. Soil Soc. of America. p.279-330.

141. **Clarkson DT, Sanderson J. 1978.** Sites of absorption and translocation of iron in barely roots, tracer and microautoradiographic studies. Plant Physiol. 61: 731-736.

142. **Loneragan JF, Snowball K. 1969.** Calcium requirements of plants. Aust. J. Agric.Res. 20: 465-478.

143. **Bush D.S. 1995.** Calcium regulation in plant cells and its role in signaling. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46: 95-122.

144. **Mengel K, Kirkby EA. 2001.** Principles of plant nutrition. Kluwer Academic Press. 5th ed. pp 513-541.

145. **Barkla BJ, Pantoja O. 1996.** Physiology of iron transport across the tonoplast of higher plants. Annu. Rev, Plant Physiol.Plant Mol. Biol. 47: 159-184.

146. **Burstrom HG. 1968.** Calcium and plant growth. Biol.Rev. 43: 287-316.

147. **Cleland RE, Virk SS, Taylor D, Bjorkman T. 1990.** Calcium cell walls and growth, In: Calcium in plant growth and development. American Society of Plant Physiology. Symposium Series, Vol.4. pp. 9-16.

148. **Marme D. 1983.** Calcium transport and function.In: Inorganic plant nutrition. Encyc. Plant Nutrition. Vol.15B, pp. 599-625.

149. **Faust M, Shear CB. 1969.** Biochemical changes during the development of cork spot of apples. Qual. Plant Mater. Veg. 19: 255-265.

150. **Poovaiah BW, Leopold AC. 1973.** Inhibition of abscission by calcium. Plant Physiol. 51: 848-851.

151. **Lerchl D, Hillmer S, Grotha R, Robinson DG. 1989.** Ultrastructural observations on CTC-induced callose formation in Riella helicophylla. Bot. Acta. 102: 62-70.

152. **Maynard DN. 1979.** Nutritional disoders of vegetable crops. A review. J. Plant Nutrition. 1:1-23.

153. **Secer M, Unal A. 1990.** Nutrient concentrations in leaf lamina and petioles of sugar melons and their relationships to yield and quality. Gartenbauwiss. 55: 37-41.

154. **Dejou J. 1992.** Review of minerals and rocks containing magnesium. Paris. IRNA. p. 35-49.

155. **Fried M, Shapiro RE. 1961.** Soil plant relationships in ion uptake. Annu.Rev. Plant Physiol. 12:91-112.

156. **Schnitzer M, Skinner SIM. 1967.** Organo metallic interactions in soils:Stability constants of Pb-2, Ni-2, Mn-2, Co-2, Ca-2 and Md-2 fulvic acid complex. Soil Sci. 103: 247-252.

157. **Ballif JL. 1992.** Loss of magnesium by leaching from chalk soil: Eleven years of lysimeter measurements. Paris. INRA. p.86-92.

158. **Hallbacken L. 1992.** Long term changes of base cation pools in soil and biomass in a beech and in a spruce forest of southern Sweden. Bodenk. 155: 51-60.

159. **Grimme H, Braunschweig LC, Nemeth K. 1974.** Potassium, calcium and magnesium interactions as related to cation uptake and yield. Landw. Forsch. 30/II. Sonderth, 93-100.

160. **Schimansky C. 1981.** The influence of certain experimental parameters on the flux characteristics of Mg-28 ib the case of barley seedlings grown in hydroculture. Landw. Forsch. 34:154-165.

161. **Cohen P. 1989.** The structure and regulation of protein phosphatases. Annu.Rev.Biochem. 58: 453-508.

162. **Travers AA. 1989.** DNA conformation and protein binding. Annu.Rev.Biochem. 58: 427-452.

163. **Ahsen Von U, Noller HF. 1995.** Identification of bases in the 16S rRNA essential for tRNA binding at the 30S ribosomal P site. Science. 267: 234-237.

164. **Fanning DS, Kermidas VZ, El-Desoky MA. 1989.** Micas in minerals and environments. Madison, USA. Soil Science Soc. of America. p.551-634.

165. **Schwertmann U. 1991.** Solubility and dissolution of iron oxides, In: Iron nutritionand interactions in plants. Kluwer Academic Publishers. pp. 3-27.

166. **Allen BL, Hajek BF. 1989.** Mineral occurence in soil environments. Madison,USA. Soil Soc. of America. p. 199-278.

167. **Chen Y, Barak P. 1982.** Iron nutrition of plants in calcareous soils. Adv. Agron. 35:217-240.

168. **Lindsay WL, Schwab AP. 1982.** The chemistry of iron in soils and its availability to plants. Jour. Plant Nutrition. 5: 821-840.

169. **Munch JC, Ottow JC. 1983.** Bacterial reductions of amorphous and crystallineiron oxides. Science du Sol. 3/4. Pp. 125-205.

170. **Ponnamperuma FN. 1972.** The chemistry of submerged soils. Adv. Agron. 24:29-69.

171. **Crowley DE, Wang YC, Reid CPP, Szaniszlo PJ. 1991.** Mechanisms of iron acquisition from siderophores by microorganisms and plants. Kluwer Academic Pubishers. Pp. 213-232.

172. **Masalha J, Kosegarten H, Elmaci O, Mengel K. 2000.** The central role of microbial activity for iron acquisition in maize and sunflower. Biol.Fertil. Soils. 30: 433-439.

173. **Becker R, Grun M, Scholz G. 1992.** Nicotianamine and the distribution of iron in the apoplasm and symplasm of tomato (*Lycopersicon esculentum* Mill.). Planta. 187: 48-52.

174. **Rubinstein B, Luster DG. 1993.** Plasma membrane redox activity: Components and role in the plant processes. Annu.Rev. Plant Physiol. Plant Mol. Biol. 44: 131-155.

Related bibliography

175. **Takagi S. 1976.** Naturally occuring iron chelating compounds in oat and rice root washings. Soil Sci. Plant Nutr. 22: 423-433.

176. **Takagi S, Nomoto K, Takemoto T. 1984.** Physiological aspects of mugeneic acid,a possible phytosiderophore of graminaceous plants. J. Plant Nutr. 7: 469-477.

177. **Kosegarten H, Hoffmann B, Mengel K. 1999b.** Apoplastic pH and Fe +3 reduction in intact sunflower leaves. Plant Physiol. 121: 1069-1079.

178. **Ottow JCG, Benckiser G, Watanabe I, Santiago S. 1983.** Multiple nutritional soil stress as the prerequisite for iron toxicity of wetland rice (*Oryza sativa L*.). Trop. Agric. 60: 102-106.

179. **Flowers TJ, Lauchli A. 1983.** Sodium versus potassium: Substitution and compartmentation. Spring-Verlag, Berlin. Vol. 15B,pp. 651-681.

180. **Cramer GR, Lauchli A. 1986.** Ion activities in solution in relation Na+ & Ca+2 interactions at the plasmalemma. J. Exp. Bot. 37: 321-330.

181. **Marchner H. 1995.** Mineral nutrition of higher plants. San Diego. Academic Press Inc. 2nd ed.

182. **Greenway H, Munns R. 1980.** Mechanisms of salt tolerance in nonhalophytes. Annu.Rev. Plant Physiol. Plant Mol. Biol. 31: 149-190.

183. **Harrison TR. 1991.** Harrison's Principles of Internal Medicine. McGrow-Hill,Inc. New York. 12th ed.

184. **Wheeler R, Stutte G, Subbarao GV, Yorio M. 2002.** Plant growth and human life support for space travel.In handbook of plant and crop physiology. New York. : Pessarakli,M. ed. Maercel Dekker Inc. pp. 925-941.

185. **Epstein E. 1965.** Mineral metabolism. Academic Press, New York. Plant Biochemistry. pp. 438-466.

186. **Arnon DL, Stout PR. 1939.** The essentiality of certain elements in minute quantity for plants for special reference to copper. Plant Physiol. 14: 371-375.

187. **Harmer PM, Benne EJ. 1945.** Sodium as a crop nutrient. Soil Sci.Soc.Am.J. 60: 137-148.

188. **Larson WE, Pierre WH. 1953.** Interaction of sodium and potassium on yield and cation composition of selected crops. Soil Sci. Soc.Am.J. 76: 51-64.

189. **Rains DW, Epstein E. 1965.** Transport of sodium in plant tissue. Science. 148: 1611.

190. **Epstein E. 1961.** The essential role of calcium in selective cation transport by plant cells. Plant Physiol. 36: 437-444.

191. **Smith GS, Middleton KR, Edmonds AS. 1980.** Sodium nutrition of pasture plants. Translocation of sodium and potassium in relation to transpiration rates. New Phytol. 84:603-612.

192. **Greenway H, Osmond CB. 1972.** Salt responses of enzymes from species differing in salt tolerance. Plant Physiol. 49: 256-259.

193. **Walker DJ, Leigh RA, Miller AJ. 1996a.** Potassium homeostasis in vacuolate plant cells. USA : Proc. Natl. Acad.Sci. 93: 10510-10514.

194. **Blumwald E, Aharon GS, Apse MP. 2000.** Sodium transport in plant cells. Biochem.Biophys.Acta. 1465: 140-151.

195. **Murata S, Kobayashi M, Matoh T, Sekiya J. 1992.** Sodium stimulates regeneration of phosphoenol pyruvate in mesophyll chloroplasts of Amaranthus tricolor. Plant Cell Physiol. 33: 1247-1250.

196. **Marschner H. 1995.** Mineral nutrition of higher plants. London. Academic Press.

197. **Wyn Jones RG. 1999.** Cytoplasmic potassium homeostasis: Review of the evidence and its implications. SK. Potash and Phosphate Institude of Canada. pp.13-22.

198. **Watt BK, Merrill AL. 1975.** Composition of foods. USDA. Handbook No.8.

199. **Subbarao GV,Wheeler RM, Stutte GW. 2000a.** Feasibility of substituting sodium for potassium in crop plants for advanced life support systems. Life support and Biosphere Sci. 7:225-232.

200. **Subbarao GV, Wheeler RM, Stutte GW, Levine LH. 1999a.** How far can sodium substitude for potassium in redbeet ? J.Pl.Nutr. 22: 1745-1761.

201. **Kszos LA, Stewart AJ. 2003.** Review of the Lithium in the aquatic environment:Distribution in the United States, toxicity and case example of groundwater contamination. Ecotoxicology.12: 439-447.

202. **Nishihama S, Onishi K, Yoshizuka K. 2011.** Selective recovery process of Lithium from seawater using integrated ion exchange methods. Kitakyushu, Japan: Solvent extraction & ion exchange. 29: 421- 431.

203. **Kipouros GJ, Sadoway DR. 1998.** Toward new technologies for the production of lithium. JOM.

204. **Boily M, Gosselin C. 2004.** Les principaux types de minerales en metaux rares (Y-Zr-Nb-Ta-Be-Li-ETR) du Quebec. s.l. : Ministeres des resources naturelles et de la faune du Quebec. Et 2004-01,46 pages..

205. **Ste-Croix L, Doucet P.2001.** Potentiel en metaux rares dans les sous-provinces de L'abitibi et du Pontiac. Ministeres des Ressources naturelles et de la Faune du Quebec. PRO.2001-08,14 pages.

206. **Lopez T, Arriaga S. 2000.** Geochemical evolutions of the Los Azufres, Mexico. Geothermal Reservoir. Vol.Part 1.

207. **Roy P, Gosselin C. 2009.** Rare earth elements and Lithium: Quebec's new wave of mineral exploration. Quebec. MNRF.2009.

208. **Meneguzzi M, Audouze J, Reeves H. 1971.** Lithium isotopes differences. A&A. 15.337.

209. **Suzuki TK, Yoshii Y, Beers TC. 2000.** Rare earth element Lithium. Ap. J. 9: 540.

210. **Sephton AM, James RH, Bland PA. 2004.** Lithium isotope analyses of inorganic constituents from the Murchison meteorite. s.l. : The Astrophysical Journal. The American Astronomical Society. U.S.A. 612: 588-591.

211. **Heslop RB, Jones K. 1976.** Inorganic chmistry.A guide to advanced study. New York. : Elsevier scientific publishing company.

212. **Bardet J, Tchakirian A, Lagrange R. 1937.** Dosage du Lithium darts l'eau de mer. Comptes rendus de l'academie des sciences. 204: 443-445.

213. **Hall JM, Chan LH, McDonough WF, Turekian KK. 2005.** Determination of the lithium isotopic composition of planktic forminifera and its application as a paleo-seawater proxy. Marine Geology. 217:255-265.

214. **schrauzer N.G.2002.** Lithium: Occurence, dietary intakes, nutritional essentiality. Jornal of the American College of Nutrition. Vol.21 No.1:14-21.

215. **Nahorski SR, Ragan CI, Challiss RAJ. 1991.** Lithium and the phosphoinositide cycle:an example of uncompetitive inhibition and its pharmacological consequences.Trends Pharmacol. Sci. 12: 297-303.

216. **Berridge MJ, Downes CP, Hanley MR. 1989.** Neutral and developmental actions of lithium: A unifying hypothesis. Cell. 411-419.

217. **Del Rio E, Nicholls DG, Downes CP. 1996.** Characterization of the effects of lithium and Inositol on phosphoinositide turnover in cerebellar granule cells in primary culture. Journal of Neurochemistry. Vol.66,No.2, pp. 517-524.

218. **Anke M, Arnhold W, Groppel U, Krause U. 1991.** The biological importance of lithium. Weinheim: VCH Verlag. pp. 149-167.

219. **Mohr S H, Mudd GM, Giurco D. 2012.** Lithium resources and production: Critical assessment and global projections. Minerals. 2: 65-84.

220. **Kent NL. 1941.** The influence of Lithium salts on certain cultivated plants and their parasitic diseases. Annals. of Applied Biology. 289: 189-209.

221. **Bingham FT, Bradford GR, Page AL. 1964.** Toxicity of Lithium. California Agriculture.

222. **Birch MJ. 1991.** Lithium and the cell: Pharmacology and biochemistry. San Diego. Academic Press.

223. **Zonia LE, Tupy J. 1995a.** Lithium treatment of Nicotiana tabucum microspores blocks polar nuclear migration,disrupts the partitioning of membrane-associated Ca+2 and induces symmetrical mitosis. Sex Plant Reprod. 8:152-160.

224. **Zonia L, Tupy J. 1995b.** Lithium sensitive calcium activity in the germination of apple (*Malus vs. domestica Borkh*.) tobacco (*Nicotiana tabacum L*.) and patato (*Solanum tuberosum L*.) pollen. J.Exp.Bot. 46: 973-979.

225. **Berridge MJ. 1993.** Inositol triphosphate and calcium signalling. Nature. 361: 315-325.

226. **Gillaspy GE, Keddie JS, Oda K, Gruissem W. 1995.** Plant inositol monophosphatase is a lithium sensitive enzyme encoded by a multigene family. Plant Cell. 7: 2175-2185.

227. **Boller T. 1990.** Ethylene and plant pathogen interaction in biochemistry, physiology and interactions. Rcokville,MD. : American Society of Plant Physiologists. pp. 138-145.

228. **Congero V, Belles JM, Garcia-Breijo F, Garro R, Hernandez-Yago J, Rodrigo I, Vera P. 1990.** Signaling in viroid pathogenesis,recognition and responces in plant virus interactions.Springer. pp. 1883-1886.

229. **Smith HC, Blair DI. 1950.** Wheat powdery mildew investigation. Annals. of Applied Biology. 37: 570-583.

230. **Carter GA, Wain RL. 1964.** Investigation of fungicides IX. The fungitoxicity, phytotoxicity and systemic fungicidal activity of some inorganic salts. Annals. of Applied Biology. 53: 291-309.

231. **Salocks C, Kaley BK. 2003.** Toxicology clandestine drug labs: Methamphetamine. Sacramento : Office of Environmental health hazard assessment. Volume 1, Number 4.

232. **Smithberg M, Dixit PK. 1982.** Tetratogenic effects of lithium in mice. Tetratology. 26: 239-246.

233. **Becker RW, Tyobeka EM. 1990.** Lithium enhances proliferation of HL60 promyelocytic leakemia cells. Leakemia Res. 14: 879-884.

234. **Gee GW, Bauder JW.** 1986. Methods of soil analysis.Partcle size analysis, Part 1. Wisconsin, U.S.A

235. **Ellis B.** 1998. Recommended Chemical Soil Test Procedures. Rec. Chemical Soil Test Procedure for the North Central Region. 14, Vol. I, Missouri Agri.Exp.Station SB101.

236. **Anderson H, Cummings D.1999.** Landcare notes, Measuring the salinity of water. Victoria. Australia : Department of sustainability and Environment.

237. **DeLaune RD, Reddy KR. 2005.** Redox potential. Baton Rouge, LA.USA : Louisiana State University.

238. **Fiedler S, Vepraskas MJ, Richardson JL. 2007.** Soil Redox Potential: Importance, Field Measurements & Obsevations. Lincoln,Nebraska : USDA-NRCS National Soil Survey Center.

239. **Naranjo MA, Romero C, Belles JM, Montesinos C, Vicente O, Serrano R. 2003.** Lithium treatment induces a hypersensitive like responce in tobacco. Planta. 217: 417-424.

240. **Li X, Gao P, Gjetvaj B, Westcott N, Gruber MY. 2009.** Analysis of the metabolome and transcriptome of *Brassica carinata* seedlings after lithium chloride exposure. Plant Science. 177: 68-80.

241. **Chaffey B. 1992.** Principles of sustainable agriculture-dryland salinity. Victoria. Dept. of Agriculture. 1st ed.

242. **Committee Victorian irrigation Research & Advisory Services.** 1980. Quality aspects of farm water supplies. Melbourne. Government printers. 2nd ed.

243. **Andersen H, Cummings D. 1999.** Measuring the salinity of water. Victoria. : Dept. of Sus. and Environment. ISSN 1323-1693.

244. **Baker DL, Suhr NH. 1982.** Atomic absorption and flame emission spectrometry. pp 13-27. Madison,WI. : American Society of Agronomy.

245. **Ure AM. 1991.** Atomic absorption and flame emission spectrometry, pp. 1-62. Soil Analysis: Modern instrumental techniques.Second edition.Marcel Dekker, New York.

246. **Haddadin MSY, Khattari S, Caretto D, Robinson RK. 2002.** Potential Intake of Lithium by the inhabitants of different regions in Jordan. Pak. Journal of Nutrition.1 (1): 39-40.

247. **Sinex SA, Cantillo AY, Heiz GR. 1980.** Accuracy of acid extraction methods for trace metals in sediments. University of Maryland,College Park. Maryland : Anal.Chem: 52: 2342-2346.

248. **Knudson D, Peterson GA, Pratt PF.** 1982. Lithium, Sodium and Potassium.In: Methods of soil analysis. Madisson. Wisconsin : Soil Science Society of America.

249. **Ariyakanon N, Winaipanich B. 2006.** Phytoremediation of copper contaminated soil by *Brassica juncea (l.) Czern* and *Bidens alba* (L.) DC.var radiata. Bangkok,Thailand : J.Sci.Res.Chula. Univ.Vol. 31,No.1.

250. **Nanda Kumar PBA, Dushenkov V, Motto H, Raskin I.1995.** Phytoextraction:The use of plants to remove heavy metals from soils. Environmental Science and Technology.Vol 29,No.5.

251. **Sonneveld C, van Dijk PA. 1982.** The effectiveness of some washing procedures on the removal of contaminants from plant tissue of glasshouse crops. Commun.Soil Sci. Plant Anal.13: 487-496.

252. **Karla PY. 1998.** Handbook of Reference methods for plant analysis. Boca Raton, FL. : Soil and plant analysis council, Inc.

253. **Jones Jr. JB, Wolf B, Mills HA. 1991.** Plant analysis handbook,pp.23-26. Micro-Macro Publishing Athens. Ga.

254. **Jones Jr. JB, Case VW. 1990.** Sampling, handling and analyzing plant tissue samples. pp.389-427. R.L.Westerman, Soil testing and Plant analysis.Soil Science Society of America.

255. **Munter RC, Grande RA. 1981.** Plant analysis and soil extracts by ICP-atomic emission spectrometry, pp.653-672. Heyden and son, Ltd. London England.

256. **Corp. 1976, Perkin Elmer.** Analytical methods for atomic absorption spectrophotometry. Norwalk, CT. Perkin-Elmer Corp.

257. **Hanlon EA, Gonzales JS, Bartos JM. 1994.** IFAS extension soil testing laboratory chemical procedure and training manual. Gainsville. FL. : Florida Coop.Ext. Service. Institude Food Agricultural Science. University of Florida.

258. **Pawlisz AV. 1997.** Canadian water quality guidelines for Cr. Envir.Toxicol.Water Qual. 12(2) 123-161.

259. **Shankler AK, Cervantes C, Loza-Tavera H, Avudainayagam S. 2005.** Chromium toxicity in plants. Environment International 31: 739-753.

260. **Fuliang L, Shan X, Zhang S. 1998.** Evaluation of plant availability of rare earth elements in soils by chemical fractionation and multiple regression analysis. Environmental Pollution 102: 269-277.

261. **Oygard JK, Mage A, Gjengedal E. 2004.** Estimation of the mass-balance of selected metals in four sanitary landfills in Western Norway, with emphasos on the heavy metal content of the deposited waste and the meachate. Water Research. 38: 2851-2858.

262. **Seth CS, Misra V, Chauhan KS. 2011.** Accumulation, Detoxofocation and Genotoxicity of heavy metals in Indian Mustard (*Brassica Juncea L.*). International Journal of Phytoremediation. 14:1,1-13.

263. **Han SH, Lee JC, Jang SS, Kim PG. 2004.** Composted sewage sludge can improve the physiological properties of Betula schmidtii grown in tailings. Journal of plant biology 47(2): 99-104.

264. **Salt ED, Prince RC, Pickering IJ, Raskin I. 1995.** Mechanisms of Cadmium mobility & accumulation in Indian mustard. Plant Physiol. 109: 1427-1433.

265. **Acar YB, Alshawabkeh AN. 1993.** Principles of elektrokinetic remediation. Environ. Sci. Technol. Vol. 27,No. 13.

266. **Elektorowicz M, Boeva V. 1996.** Elektrokinetic supply of nutirnets in soil bioremediation. Environ. Technology. Vol. 17. Pp 1339-1349.

267. **Watson L, Dallwitz MJ. 1992.** The grass Genera of the world. Cambridge. University Press.

268. **Jacobs WL, Everett,J. 2000.** Grasses: Systematics and Evolution. Collingwood : CSIRO.

269. **Kalmykova Y, Stromvall AM, Rauch S, Morrison G. 2009.** Peat filter performanceunder changing environmental conditions. Gothenburg.Sweden. Journal of Hazardous Materials. 166: Pp. 389-393.

270. **Shotyk W. 1988.** Review of the inorganic geochemistry of peats and peatland waters. Earth-Sci. Rev. 25: 95-176.

271. **Johnson LC, Damman AWH. 1990.** Decay and its regulation in *Sphagnum* peatlands. Adv. Bryol. 5: Pp. 249-296.

272. **Couillard D.** The use of peat in wastewater treatment. 1994.Water Research. 28:6, Pp. 1261-1274.

273. **Spedding PJ. 1988.** Peat. Fuel. 67: Pp. 883-899.

274. **Mrtinez-Cortizas A, Portevedra-Pombal X, Garcia-Rodeja E, Novoa-Munoz JC, Shotyk JC. 1999.** Mercury in a Spanish peat bog:archive of climate change and atmospheric metal deposition. Science. 284: 939-942.

275. **Murphy EM, Zachara JM. 1995.** The role of sorbed humic substances on the distribution of organic and inorganic contanminants in groundwater. Geoderma. 67: 103-124.

276. **Zaccone Z, Cocozza C, Cheburkin AK, Shotyk W, Miano TM. 2008.** Distribution of As, Cr, Ni, Rb, Ti and Zr between peat and its humic fraction alomg an undisturbed ombrotrophic bog profile (N.W. Switzerland). Applied Geochemistry. 23:Pp. 25-33.

277. **Kerndorff H, Schnitzer M. 1980.** Sorption of metals on humic acid. Geochimica et Cosmochimica Acta. Vol.44, Pp 1701-1708.

278. **Chaney RL, Hundermann PT. 1979.** Use of peat moss columns to remove cadmium from wastewaters. Journal of the Water pollution Control Federation. 51(1): Pp. 17-21.

279. **Coupal B, Lalancette JM. 1976.** The treatment of waste waters with peat moss.Water Research. 10: 1071-1076.

280. **Crist RH, Martin JR, Chonko J, Crist DR. 1996.** Uptake of metals on peat moss: An ion exchange process. Environmental Science and Technology. 30: 2456-2461.

281. **Wolf A, Bunzl K, Dietl F, Schmidt WF. 1977.** Effect of Ca(+2) ions on the adsorption of Pb (+2), Cu(+2) and Zn(+2) by humic substances. Chemosphere. 5: 207-213.

282. **Ong LH, Swanson VE. 1966.** Adsorption of copper by peat, lignite and bituminous coal. Economic Geology. 61: 1214-1231.

283. **Chen XH, Gosset T, Thevenot DR. 1990.** Batch copper ion binding and exchange properties of peat. Water Research. 24:12. Pp. 1463-1471.

284. **Sharma DC, Forster CF. 1993.** Removal of hexavalent chromium using sphagnum moss peat. Water Research. 27:7. Pp. 1201-1208.

285. **Wallstedt T, Bjorkvald L, Gustafsson JP. 2010.** Vanadium. Appl. Geochem. 25: 1162-1175.

286. **Cloy JM, Farmer JG, Graham MC, MacKenzie AB. 2011.** Scottish peat bog records of atmospheric vanadium deposition over the past 150 years:comparison with other ecords and emission trends. J. Environ. Monit. 13: 58-65.

287. **Schnoor, J,L., Licht,L,A., McCutcheon,S,C., Wolfe,N,L. & Carreira,L,H. 1995.** Phytoremediation of organic and nutrient contaminants. s.l. : Env. Science & Technology. Vol.29, No 7, Pp. 319-323..

288. **Virkutyte J, Sillanpaa M, Latostenmaa P. 2001.** Electrokinetic soil remediation-critical overview. The Science of the Total Environment. 289: 97-121.

289. **Li Z, Yu JW, Neretnieks I. 1995.** A new approach to electrokinetic remediation of soils polluted by heavy metals. Journal of Contaminant Hydrology. 22: 241-253.

290. **Lee HH, Yang JJ. 2000.** A new method to control electrolytes pH by circulation system in electrokinetic soil remediation. Journal of Hazardous Materials. B77: 227-240.

291. **McBride MG. 1998.** Soluble trace metals in alkaline stabilized sludge products. Journal of Environmental Quality. 27:578-584.

292. **Tyler G, Olsson T. 2001.** Concentration of 60 elements in the soil solution as related to the soil acidity. European Journal of Soil Science. 52: 151-165.

293. **Blaylock MJ, Huang JW. 2000.** Phytoextraction of metals In: Phytoremediation of toxic metals: Using plants to clean up the environment. New York. : John Wiley. Pp: 53-70.

294. **Ernst WHO. 1996.** Bioavailability of heavy metals and decontamination of soil by plants. Appl. Geochem. 11: 163-167.

295. **Belouchi A, Kwan T, Gros P. 1997.** Cloning and characterization of the OsNramp family from Aryza sativa, a new family of membrane proteins possibly implicated in the transport of metal ions. Plant Molecular Biology. 33: 1085-1092..

296. **Williams LE, Pittman JK, Hall JL. 2000.** Emerging mechanisms for heavy metal transport in plants. Biochem Biophys Acta. 1465: 104-126.

297. **Diwan H, Ahmad A, Iqbal M. 2010.** Uptake related parameters as indices of phytoremediation potential. Biologia. 65: 1004-1011.

298. **Zu Y, Q Li Y, Chen JJ, Qin L, Schvartz C. 2005.** Hyperaccumulation of Pb, Zn and Cd in herbacious grown on lead-zinc mining area in Yunnan, China. Environ. Int. 31: 755-762.

299. **Smith M, Storey JB. 1976.** The influence of washing procedure on surface removal and leaching of certain elements from trees. Hort.Sci.14: 718-719.

300. **Fellet G, Marchiol L, Delle Vedove G, Peressotti A. 2011**. Application of biochar on mine tailings: Effects and perspectives for lan reclamation. Chemosphere. 83: 1262-1267.

301. **Grangeia C, Avila P, Matias M, Ferreira da Silva E. 2011.** Mine tailings integrated investigations: The case of Rio tailings (Panasqueira Mine, Central Portugal). Engi. Geology. 123: 359-372.

302. **Ottosen LM, Christensen IV. 2011.** Electro-kinetic desalinization of sandstones for NaCl removal-test of different clay poultices at the electrodes. Electrochimica Acta.