PHYTOREMEDIATION OF SOIL CO-CONTAMINATED WITH COPPER, CADMIUM AND ORGANOCHLORINE PESTICIDES

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SUBMITTED BY

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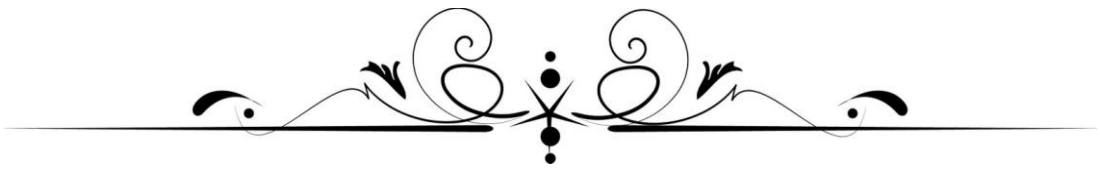
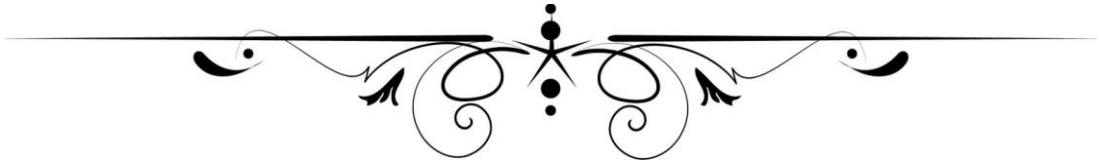


Dedicated to

My Beloved Parents

&

Grand Parents



DECLARATION

I hereby declare that the thesis entitled "**Phytoremediation of soil co-contaminated with copper, cadmium and organochlorine pesticides**" is my own work conducted under the supervision of **Dr. Narendra Kumar** in the Department of Environmental Science at Babasaheb Bhimrao Ambedkar University, Vidya Vihar, Raebareli Road, Lucknow and also approved by Departmental Research Committee (DRC).

I further declare to my best Knowledge, that the thesis does not contain any part of work, which has been earlier submitted for the award of any other degree either in this University or in any other University/Deemed University.

Dhananjay Kumar

Department of Environmental Science

BBA University, Lucknow-226025

CERTIFICATE

This is to certify that the thesis titled "**Phytoremediation of soil co-contaminated with copper, cadmium and organochlorine pesticides**" submitted by **Mr. Dhananjay Kumar** is an original work and has not been previously submitted in part(s) or full for the award of any other degree to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University, Lucknow satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulations-1999, as amended in 2008/2010 and it is fit for submission and evaluation for the award of the Doctor of Philosophy of the University

Supervisor

Head of the Department

PREFACE

Escalated industrialization and unorganized anthropogenic activities has exerted tremendous pressure on all components of the environment by releasing enormous quantities of organic as well inorganic contaminants including metal and pesticides. Soils are important target sink for several contaminants that vary in their composition, concentration and toxicity. Degradation of soil quality due to metals and pesticides has caused global concerns and emerged as a major environmental challenge. Soil contamination with metal and pesticides not only adversely affects the food quality, production and environmental safety but also pose a massive threat to human health and natural ecosystem. Due to ubiquitous degradation of soil and its associated concerns, need of an innovative and environmental friendly technique to restore the soil health has now become a priority in field of pollution treatment techniques. Phytoremediation has been recognized as an economical and eco-friendly technique that exploits the potential of plants to accumulate, degrade, immobilize or detoxify the contaminants. Further, biosorption has proven as an effective, innovative, eco-friendly and cost effective alternative for the removal of contaminants from water.

In this study, *A. paniculata* and *V. zizanioides* plants are used for phytoremediation of Cu, Cd and organochlorine pesticides co-contaminated soil. Further, to optimizes the associated sustainable and cost benefit aspect, the plant biomass generated after the phytoremediation was used as a biosorbent for removal of fluoride. Results revealed that the *V. zizanioides* have high tolerance and potential for the remediation of Cu, Cd and organochlorine pesticides and can be considered as a suitable candidate for phytoextraction. Likewise, *A. paniculata* can be considered as a moderate phytoremediator of Cu and Cd. However, its high adsorption capacity for fluoride makes it a good biosorbent for removal of fluoride from water.

Dhananjay Kumar
(M. Sc., UGC-JRF)

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Dhananjay Kumar

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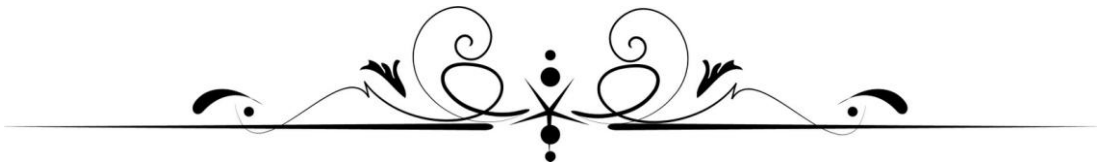
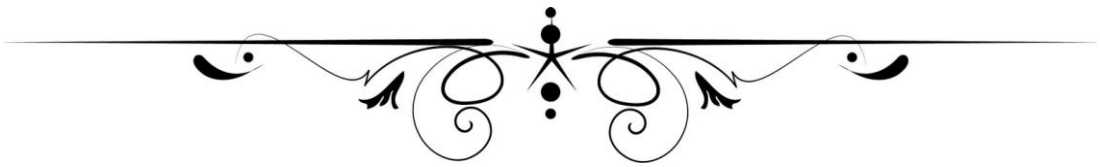
LIST OF ABBREVIATIONS

ROS	Reactive oxygen species
OCPs	Organochlorine pesticides
cm	Centimeter
ºC	Degree Centigrade
FW	Fresh Weight
DW	Dry Weight
%	Percentage
mg g^{-1}	Milligram per gram
µg g^{-1}	Microgram per gram
mgkg^{-1}	Milligram per kilogram
µ mol/dm^3	Micromole per meter cube
Cu	Copper
Cd	Cadmium
Zn	Zinc
Hg	Mercury
Pb	Lead
GI	Germination index
TF	Translocation factor
ECr	Enrichment coefficient (root)
ECs	Enrichment coefficient (Shoot)
ANOVA	Analysis of variance
Fig.	Figure
OC	Organic Content



Chapter 1
Introduction



Over the past few decades, boost in global economy due to industrial revolution and intense agricultural practices created various luxurious facilities and commodities, however, such industrial revolution combined with other anthropogenic happenings like faulty waste management practices, improper landfill operations, mining and application of sewage sludge have caused contamination of soil, air and water (Fig 1.1) (Kumar et al., 2013). Changes in environmental quality is a natural process, but the anthropogenic activities like improper waste management practices, application of wide range of chemical in day to day life and in agriculture have accelerated the level and rate of contamination. These improper anthropogenic deeds release number of organic (e.g. pesticides) and inorganic (e.g. Heavy metals and metalloids) contaminants in biosphere and consequently degraded the air, water and soil quality (Kumar et al., 2012). Now a day, soil co-contamination with heavy metals and pesticides is a global concern leads to situation where many life forms including human being have to face serious threats to their life supporting resources (Ghavri et al., 2013; Kumar et al., 2013; Kumar et al., 2018a). Co-contamination of soil poses technical challenges associated with each class of pollutants (e.g. hydrophobic organochlorine and heavy metals). The other problems and challenges arise due to the differences in the physico-chemical behavior of contaminants. Since, each contaminants present in co-contaminated soil have different physicochemical properties and they respond in different way to the remediation technologies. Additionally, the interaction among the different classes of contaminants may generate new and unforeseen problems that could limit the efficiency of remediation technologies (Reddy, 2011; Chirakkara et al., 2016).

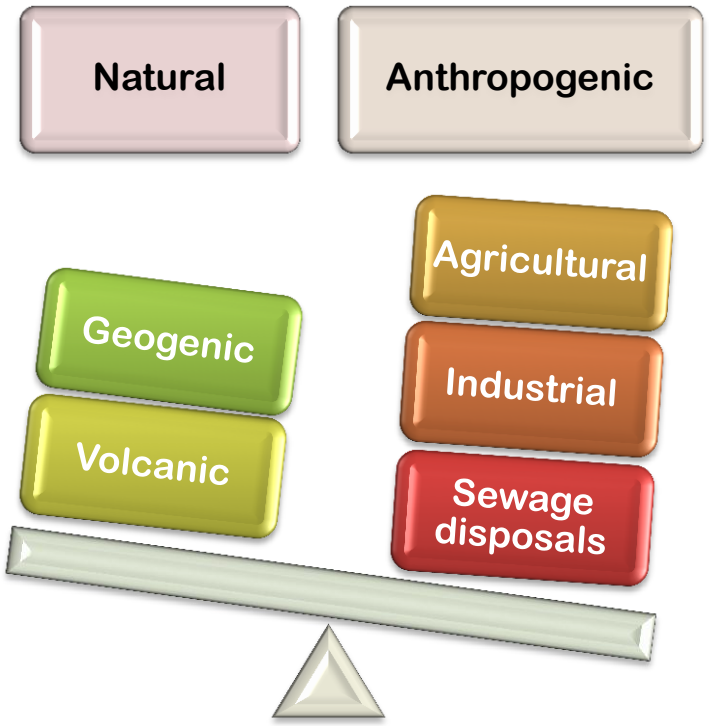


Fig 1.1. Sources of soil contamination

Agricultural land contamination with heavy metals and pesticides not only degrade the quality of soil but also affects the food quality, production and environmental safety (Singh et al., 2010; Reddy, 2011). Heavy metals are transmission metals having atomic mass over 20 and a specific gravity greater than 5 g/cm^3. Some of these heavy metals such as As, Cd, Hg and Pb are non-essential, as they do not have any essential role in the normal physiological function of plant. Besides, non-essential heavy metals some heavy metals like Fe, Zn, Cu are essential because they play very important role in the normal physiological function. Though, at high concentrations, these heavy metals have strong toxic effects and considered as environmental toxicants (Kumar et al., 2016a; Kumar et al., 2018b). When the soil contaminated with heavy metals are used for agriculture, metals effortlessly get transferred from soil to food chain and leads to biomagnification; a serious concern (Jarup, 2003; Kumar et al., 2017a). Certain heavy metals such as Cu being

micronutrient at optimum level essential metal for plant metabolism, however, at higher concentration it is potentially harmful (Wu et al., 2012) whereas, cadmium (Cd) is a non-essential heavy metal and very toxic to plants and animals including human beings (Mejare and Bulow, 2001).

1.1. Cadmium and its physico-chemical properties

Cadmium is a d-block element with atomic number 48, atomic weight 112.4, density 8.65 g/cm^3 and symbol Cd (Table 1.1). Cd is a soft (enough to be cut with a knife), lustrous, silver-white (bluish tinge surface), ductile, very malleable metal. In periodic table, Cd is placed just below Zinc (Zn) and above Mercury (Hg) has much similar chemical properties to that of Zn (exhibits oxidation state +2) and Hg (lower melting point than the transition metals in groups 3 through 11). Chemically similar nature of Cd to the Zn is the key reason of Cd toxicity in plant and animals as Zn is an essential micronutrient and its substitution by Cd causes malfunctioning of metabolic processes (Kumar et al., 2016a; Kumar et al., 2017a). It is easily soluble in acids but not in alkalis. It is similar in many respects to zinc but it forms more complex compounds. About 3/4 of total cadmium uses is re-chargeable Ni-Cd batteries. Remaining 1/4 is used mostly for pigments, coatings and plating, and as stabilizers for plastics. Cd has been used particularly to electroplate steel where a film of cadmium only 0.05 mm thick will provide complete protection against the sea. Cadmium has the ability to absorb neutrons, so it is used as a barrier to control nuclear fission. Naturally, Cd always occurs in combination with Zn as a minor component in most Zn ores. Anthropogenically, it is found as an inevitable by-product of Zn, Pb and Cu production. After being applied it enters the environment mainly through the ground, due to its presence in manures and pesticides. After 20th century, Cd contamination in environment has intensified dramatically, because of recycling of Cd Compound were

rare and often dumped together with domestic waste. About 25,000 tons in a year Cd released into the environment naturally and about half of this are released into water by weathering of rocks and some is released into air by forest fires and volcanic eruptions.

Table 1.1. Some basic properties of Cd

Properties	**Cadmium**
Atomic number	48
Atomic mass	112.4 $gmol^{-1}$
Electro negativity according to Pauling	1.7
Density	8.7 $g.cm^{-3}$at 20°C
Melting point	321 °C
Boiling point	676 °C
Vanderwaals radius	0.154 nm
Ionic radius	0.099 nm (+2)
Isotopes	15
Electronic shell	[Kr] $4d^{10}$ $5s^2$
Energy of first ionisation	866 $kJ.mol^{-1}$
Energy of second ionisation	1622 $kJ.mol^{-1}$
Standard potential	- 0.402 V
Discovered by	Fredrich Stromeyer in 1817

(Source: http://www.lenntech.com/periodic/elements/cd.htm#ixzz2LuFK7yBq)

1.2. Copper and its physico-chemical properties

Copper is a d-block element with atomic number 29, Atomic weight 63.546, density 8.9 g cm^{-3} and symbol Cu (Table 1.2). Copper is a soft (softer than Zn and can be polished to a bright finish) and reddish metal with a face-centered crystalline cubic structure. It is malleable, ductile and a very good conductor of both heat (second highest) and electricity (59.6×10^6 S/m). It occurs in nature in directly usable metallic form which led it to very early human use. Cu is used for electrical equipment (60%), Construction (mainly roofing and plumbing) (20%), industrial machinery (as a heat exchangers) (15%) and alloys (brass and bronze) (5%). Being biostatic and antimicrobial in nature it used in manufacturing of antimicrobial copper products such as bedrails, handrails, sinks, door knobs, toilet hardware, computer keyboards and health club equipment. Sources of Cu in environment are both natural (wind-blown dust, decaying vegetation, forest fires and sea spray) and anthropogenic (electrical industries, mining, metal production, phosphate fertilizer production and waste disposals). The exploitable reserves for Cu are around 300 million tons and about 12 million tons are produced annually. Out of total annual production about 2 million tons are recovered by recycling. Due to wide range of application its production has raised over the last decades and still rising. Cu enters in air, mainly during combustion of fossil fuels and remains there till the rain settled it to ground and ultimately ends up in soil where it strongly attaches to organic matter and materials.

Table 1.2.Some basic properties of Cu.

Properties	**Copper**
Atomic number	29
Atomic mass	63.546 $gmol^{-1}$
Electronegativity according to Pauling	1.9
Density	8.9 $g.cm^{-3}$at 20°C
Melting point	1083 °C
Boiling point	2595 °C
Vander waals radius	0.128 nm
Ionic radius	0.096 nm (+1); 0.069 nm (+3)
Isotopes	6
Electronic shell	[Ar] $3d^{10}4s^{1}$
Energy of first ionisation	743.5 $kJ.mol^{-1}$
Energy of second ionisation	1946$kJ.mol^{-1}$
Standard potential	+ 0.522 V (Cu^{+}/Cu); +0.345 V (Cu^{2+}/Cu)
Discovered	Middle East (9000 BC)

(Source: https://www.lenntech.com/periodic/elements/cu.htm#ixzz5H8dKG5S6)

1.3. Pesticides

Pesticides are chemical or biological substances that are used to kill or control the growth of pests (insects, rodents, and fungi) and unwanted weeds. These are used to kill vectors of diseases, such as mosquitoes, termites, rodents. (WHO, 2012). Pesticides includes herbicides, insecticides, molluscicide, rodenticide, bactericide, insect and animal repellent, antimicrobial, fungicide, disinfectant (antimicrobial) and sanitizers. Pesticides can also be categorized on the basis of target organism (Table 1.3).

Table 1.3. Types of pesticides

Pesticides	**Target group**
Algaecides	Algea
Avicides	Birds
Bactericides	Bacteria
Fungicides	Fungi
Herbicides	Plants
Insecticides	Insects
Larvicides	Larvae
Molluscicide	Snails
Miticides or acaricides	Mites
Nematicides	Nematodes
Rodenticides	Rodents
Virucides	Viruses

(Source: https://en.wikipedia.org/wiki/Pesticide)

Chemically, they are categorized as organophosphate, organochlorine, carbamate, pyrethroid, Triazines. *Organochlorines* are chlorinated organic pesticides e.g. DDT (Dichlorodiphenyltrichloroethane) and BHC (Benzene hexachloride). These are lipophilic and very slowly degradable/decomposing in nature. *Organophosphate* are organic ester of phosphoric, thiophosphoric acids e.g. malathion, parathion, fenitrothion. *Carbamete* are organic derivative of carbamic acid (NH_2COOH) e.g. Aldicarb, Carbofuran, Carbaryl, Ethienocarb, Fenobucarb, Oxamyl and Methomyl. *Pyrethoids* are derived from pyrethrin. Pyrethoidsare of two types: (1) pyrethrin obtained from *chrysanthemum cineraria folium* and (2) rotinoids from roots of rain-forest legumes. These are natural, biodegradable, effective and least harmful to higher

animals such as birds and mammals. *Triazines* are derivatives of urea such as simazine, atrazine. These are a group of herbicides used to control weeds in tea, tobacco and cotton.

Undoubtedly, pesticides improve and increase the crops yield and quality by controlling pest, plant disease vectors and invasive species but their prolong use causes hazard to environment and human being through bioaccumulation/ magnification in nature and in animal and plant tissue. Nearly all the pesticides are non-specific and kill many other life forms that may be useful or harmless (Gill and Garg, 2014). More than 98% of applied insecticides and 95% of herbicides miss their target and ultimately reach to non-targeted destination i.e. air, water and soil. Application of pesticides causes pollinator decline, reduced biodiversity habitat loss and threatens endangered species (Miller, 2004; Gill and Garg, 2014).

1.4. Remediation approaches for contaminated soil

Over past some epochs, the accelerated industrialization and unplanned urbanization throughout the globe had also released a considerable amount of pollutants including heavy metals and pesticides in environments (Adriano, 1992; McIntyre, 2003; Singh et al., 2004; Kumar et al., 2013). Globally around 40% of human deaths are the consequence of environmental degradation (Science Daily, 2007). Soil contamination is a natural process but the anthropogenic happenings have continuously amplified the level and rate. The untreated cumulative industrial discharges into soil increases the problem of soil pollution. When the untreated industrial effluents discharged directly to soil the problem of contamination turns to more complex because of the difference in quality and quantity of contaminants (Srivastava et al., 1994; Kara, 2005; Mapand et al., 2005; Singh et al., 2010). Heavy metals, (e.g. Cu, Cd, As, Ni, Pb, Hg, Zn) are an important class of environmental

contaminants and many of these are highly toxic in both elemental and soluble forms. In the past decades, the problem of co-contamination poses serious concern for human health as the production of synthetic organic chemical and metal processing has increased intensely. The U.S. environmental protection Agency (USEPA), National Priority list (NPL) reported that 40% of the hazardous wastes are co-contaminating the soil/land with organic and heavy metals. Being non-biodegradable/slowly degradable, heavy metals and pesticides are resided in soil for a longer period and gets bioaccumulated/biomagnified in living being via food chain (Sakakibara et al., 2011). Therefore, removal of these pollutants is a major policy priority in many countries including India. The applicability of remediation practices depends on the contaminant type, site-specific conditions such as soil type, associated cost and end use of the land (Chirakkara et al., 2016). Several remediation approaches like stabilization, solidification, soil washing have been well reported for the remediation of contaminated soil. Some commonly used soil remediation methods are discussed below.

1.4.1. Soil washing

Soil washing or soil scrubbing is an *ex situ* remediation approach for removal of inorganic, organic, and radioactive contaminants from soil through chemical or physical treatment methods in aqueous suspension. It involves the washing of contaminated soil (sand and gravel), using liquid solvents (usually water) and mechanical scrubbing. Aqueous solvent used for washing solvents are selected on the basis of their ability to dissolve the contaminants (Khan et al., 2004).

1.4.2. Soil vapor extraction (SVE)

Soil vapor extraction or soil venting or vaccum extraction is an *in-situ* technique, applicable to the unsaturated (vadose) zone of soil. SVE technique is

simple, cheap and applicable for removal of volatile organic compounds (VOCs) and semi-volatile organic compounds (SVOCs) (Fischer et al., 1996). During SVE extraction, a vacuum is applied through extraction wells near the contaminated areas to create pressure and a concentration gradient in the contaminated soil to induce a controlled air-flow for the removal of VOCs and SVOCs (U.S.EPA, 2006).

1.4.3. Solidification and Stabilization (S/S)

Solidification and stabilization is a technique that encapsulates and converts the potentially hazardous waste into a more chemically stable solid form. This technique reduces the potential risk of hazardous wastes by transforming them into a more chemically stable, immobile, and less toxic forms. The solidification technique comprise of micro-encapsulation (fine waste particles) and macro-encapsulation (use of large blocks or containers) of hazardous wastes in a high structural integrated monolithic solid (U.S. EPA, 2006). Further, mobility of contaminants is limited by reducing the surface area exposed for leaching and by isolation within an impermeable capsule (U.S. EPA, 2006).

1.4.4. Encapsulation

Encapsulation involves the mixing of contaminated soil with other materials, such as cement, lime, concrete, or asphalt leading to prevention of spread of the contaminants to surrounding clean soil strata (Camenzuli and Gore, 2013). Lime or concrete encapsulation is used for metals whereas; asphalt encapsulation is used for hydrocarbon contaminated soils. Silica encapsulation is effective and applicable for heavy metals as well as hydrocarbons contaminated soil (Camenzuli and Gore, 2013; U.S.EPA 2004, Dawadi et al., 2004; EPA 2006).

1.4.5. Electro-kinetic

Electro-kinetics is an *in situ* remediation technique, applied for the remediation of heavy metals, radionuclides, and selected organic pollutants from low permeable contaminated soil, mud, sludge and marine dredging spoils. Principally, this is a separation and removal technique which involves electrochemical and electrokinetic (low-intensity, direct current passed through the soil) processes for desorption and removal of contaminants. Additionally, the application of low-intensity, direct current induces an electro-osmotic hydraulic flow that offers a driving force for movement of neutrally charged soluble contaminants (Saichek and Reddy, 2005; U.S. EPA, 2006; Gomes et al., 2012).

Among the above explained remediation techniques, some requires the application of chemical (e.g. solidification, soil washing, stabilization); other are so concentrated that they alters the texture, pH and organic content of soil (e.g. stabilization and solidification, vitrification and electro-kinetic). Additional most of the above mentioned remediation techniques require long treatment time and high energy, so their application results high expense. In this perspective, phytoremediation arise as an economical, eco-friendly and most aesthetically acceptable technique for remediation of mildly and co-contaminated soil (Cameselle et al., 2013; Stingu et al., 2012; Kumar et al., 2017a; Kumar et al., 2017b; Kumar et al., 2018a).

1.4.6. Phytoremediation

The term "phytoremediation" derived from the Greek word "*φυτο* (*phyto*)" means plant and a Latin "*remedium*" means restoration or remediation. Some plants have unique potential to accumulate, degrade, eliminate or immobilize the contaminants from contaminated growing medium. This technique exploit the potential of naturally occurring or genetically engineered plants to mitigate the level

of contaminants like heavy metals, pesticides and its derivatives with the aim of restoration. Phytoremediation is also known as solar driven, botanical remediation (Flathman and Lanza, 1998; Prasad and Freitas, 2003; Kumar et al., 2013; Kumar et al., 2017). Several researchers have encouraged the approach of combining phytoremediation with oil and medicinal plant cultivation to optimize the cost involved in soil restoration during the phytoremediation (Shi and Cai, 2009; Bauddh and Singh, 2012a; Kumar et al., 2018a; Kumar et al., 2013; Kumar et al., 2012; Stingu et al., 2012). Several benefits and limitation associated with the phytoremediation are listed below (Table 1.4).

Table.1.4. Benefits and limitation associated with the phytoremediation

Advantages	**Precincts**
Economical, low-cost, and eco-friendly in *in situ* and *ex situ* condition as compared to other remediation process.	Effective only with respect to surface area covered and limited up to the depth extended by the root system.
Exploit natural tendency of plant and associated microbes for remediation and conserve the natural state of environment.	Tendency of plant and microbes differ under different growth conditions i.e. temperature, climate, light intensity, altitude.
Applicable for the sites contaminated with more than one contaminant.	Its success depends on the tolerance potential of applied plant for phytoremediation.
At the end of process, the hyperaccumulating plants can be used for retrieval of the valuable metals as bio-ores.	Possibility of re-entering of contaminants in the environmental because of the biodegradable nature of plant.

Over last few years, this solar driven, botanical remediation has proven its effectiveness for remediation of co-contaminated soil. Several plant species have proven themself for a better candidate for the remediation of soil contaminated with mixed/multiple contaminants (Chirkkara and Reddy, 2014; Ramamurthy and Memarian, 2014; Chirkkara and Reddy, 2015). Further, it has been suggested that the association of diverse microbial population and activity within the rhizospheric zone of soil promotes endophytic-plant symbioses which support the effective phytoremediation of co-contaminated soil probably because the microbial degradation of organic contaminant may serve as bolster for plant health against toxicity (Mastretta et al., 2006; Batty and Dolan, 2013; Germaine et al., 2013).

1.5. Factors affecting phytoremediation process

Table 1.5: Factors affecting the phytoremediation potential (Sood et al., 2012; De Fillippis, 1979; Rai et al., 1981; Trevor et al., 1990; Wallen et al., 1990; Kelly et al., 1989; Whitton and Burrows, 1989; Mallick et al., 1990; Reed and Gadd, 1990; Wong and Chau, 1990; Mukherjee et al., 2004)

S. No.	Parameter	Effects
1	Temperature	Metal uptake/toxicity decreases at lower temperature
2	Light	Sometime depends upon light
3	pH	Generally metal uptake/accumulation potential decreases at higher pH.
4	Salinity	High salinity decreases the content/toxicity
5	Monovalent Cation	Enhances metal uptake in presence of lower concentration of monovalent cation in growing medium
6	Divalent Cation	Enhances metal uptake in presence of lower

		concentration of divalent cation in growing medium
7	Nitrate	Decreases metal toxicity significantly
8	Sulphate	Decreases metal uptake and toxicity
9	Polysaccharides	Chelate metals, reduces uptake/toxicity
10	Organic acid/Selenite (Se)	Reduces metal uptake and toxicity
11	Heavy Metals	Lessens metal uptake/toxicity by binding metals complexes, reduces uptake/toxicity Zn, Cd, Cu, Ni combination are antagonistic while, Fe can encourage Cu uptake.

1.6. *Vetiveria zizanioides*

Vetiveria zizanioides (L.) Nash, syn. *Chrysopogon zizanioides* (L.) Roberty, is a well-known fast growing, perennial bunchgrass, belongs to the family Poaceae and subfamily Panicoideae, (Bertea and Camusso, 2002). Vetiver grass is native to the Indian sub-continent and commonly found in floodplains and stream. It grows up to 150-180 cm height and can reach to 3 meter under favourable condition. It is erect and form clumps as wide. Stems are tall, erect, and stiff and can survive even in deep water flow. Leaves are thin, long and slightly rigid. Roots are well developed, finely structured and strong, horizontally spread like a matwhich can penetrate to the deeper layer of soil (2-4m). Vetiver is a very versatile plant, has many important applications in area of bioengineering, soil reclamation, water conservation, and environmental protection in non-native environment (Khan, 2006; Dahn et al., 2009). The plant has high adaptability for extreme environmental conditions. It also has unique morphological, physiological and ecological characteristics such as high biomass with

remarkable photosynthetic efficiency, massive and deep root system that makes it tolerant against various adverse climatic and edaphic conditions, including elevated heavy metal contamination. These properties facilitate vetiver to be an ideal candidate for phytoremediation. It can tolerate high levels of heavy metals such as As, Cd, Cu, Cr, Pb, Hg, Ni, Se, Zn contamination in growing medium (Truong and Baker, 1998; Truong and Hart, 2001). Vetiver is also capable of stabilizing Pb, Ar, Cd in their root tissue from fly-ash amended soil (Ghosh and Singh, 2005). Vetiver had been used successfully to stabilize highly saline, alkaline (pH 9.5) coal mined land, and highly acidic (pH 2.7) gold mined land (Pang et al., 2003).

1.7. *Andrographis paniculata*

Andrographis paniculata (Kalmegh) is an annual herb belonging to the family Acanthaceae. Kalmegh is native to the India and Sri Lanka and extensively cultivated in Asia. *A. paniculata* is an erect plant with height of 30–110 cm grows in moist and shady places. Stem is slender, dark green, squared in cross-section with longitudinal furrows and wings along the angles. Leaves are lance-shaped having hairless blades up to 8cm.*A. paniculata* has been widely used in traditional medicine in several Asian countries, Southern parts of India and Sri Lanka. It is cultivated mostly for its various medicinal values. In India, it is used for the treatment of infections and health disorders. In the recent era of phytoremediation, medicinal plants has been also tested and successfully reported as an alternative high-value crop in metal polluted agricultural soils (Zheljazkov et al., 2008). Toxic metal accumulation potential of medicinal herbs has been reported by several investigators (Chaiyarat et al., 2011; Siddiqui et al., 2012; Rania, 2015).

Keeping the above perspective and concepts in consideration the present study is planned with the following specific objectives

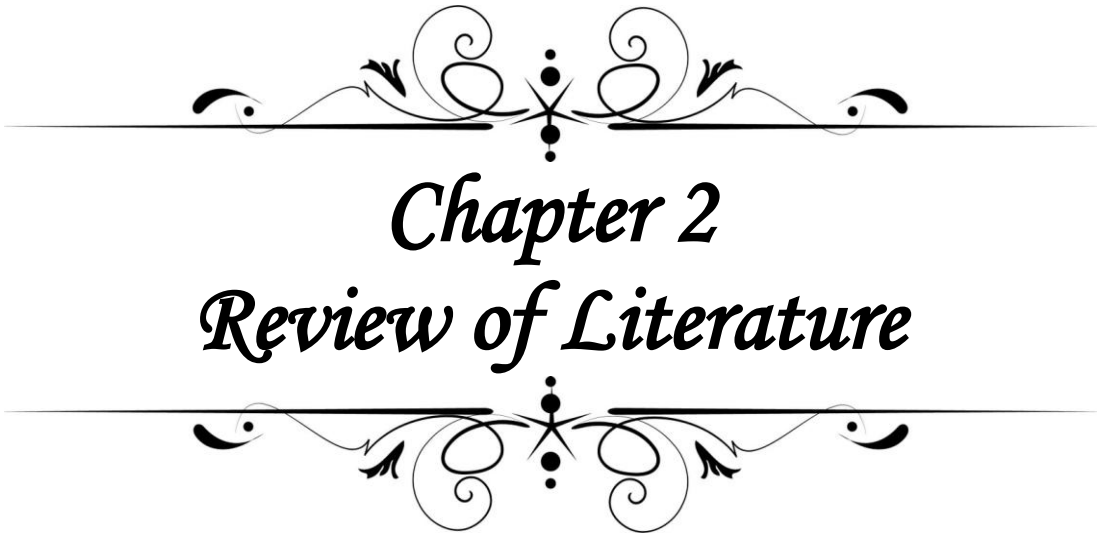
1. To study the individual and collective phytoremediation potential of selected plant species to remediate Cu, Cd and organochlorine pesticides.

2. To study the physiological and biochemical response in selected plants species exposed to different concentration of Cu, Cd and organochlorine pesticides.

3. To study the uptake, accumulation and partitioning of Cu, Cd and organochlorine pesticides in selected plant species.

4. To study the microbial activities and fertility of rhizospheric soil treated with different concentration of Cu, Cd, and organochlorine pesticides in presence of selected plant species.

5. Application of copper phytoremediated plant biomass for the defluoridation of water



Chapter 2
Review of Literature

Over the last few decades, accelerated population growth, urbanization and industrialization have generated a huge amount of undesirable waste and recognized as the main reason for major environmental alterations. Environmental alterations are natural progression but the anthropogenic activities like inadequate waste management practices, faulty landfill operations, application of chemical fertilizers and sewage sludge etc. have accelerated the rate, level and variety of contamination in soil. Each industry generates its own specific waste that pollute ecosystem. Contamination in water being an obvious health hazard, has been acceptably well looked into, but contamination in agricultural soil still requires indispensable attention. Day by day, tons of industrial effluents containing various amounts of toxic contaminants such as metals and organic chemicals are released in to soil in various direct/indirect ways (Kumar et al., 2018; Kumar et al., 2013; Nagajyoti et al., 2010; Rascio and Navari-Izzo, 2011; Ali et al., 2013). When these contaminated soils are used for agricultural purpose, toxic chemicals particularly heavy metals and pesticides become a part of the vegetation. Some of these metals and plants are highly toxic to the plants and animals even at low level (Kumar et al., 2016; Kumar et al., 2017; Shukla et al., 2005; Hassan et al., 2017). When the toxic effect of such contaminants is lethal, some flora and fauna may completely die out, while the sub-lethal effects are more risky because some of the surviving flora and fauna accumulate these pollutants in their body giving rise to biomagnifications, and if they enter into the food chain, directly or indirectly, human beings and other animals occupying the higher tropic levels are endangered (Kumar et al., 2013; Arslam, 2015).

2.1. Sources of Heavy metals

2.1.1. Natural sources

Earth crust is the principal natural source of heavy metals. Heavy metals naturally enter in the environment either by weathering of rocks or volcanic eruptions. Basaltic ingenious rocks contains large amount of Co, Cd, Zn, Cu and Ni which exist in their salts of carbonates, sulfides, and oxides. Although, soil derived from sediment rocks contains high level of Zn, Cu, Mn, Cd, As and Pb, however, due to very low weathering rate these are not considered as significant source of heavy metals. Igneous rocks such as olivine, augite and hornblende are significant source of Mn, Co, Ni, Cu and Zn while, shale rocks are the source of Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Hg, Pb and contribute considerable amount of metal to the environment (Thornton, 1981; Kabata-Pendias and Pendias, 2001; Sarwar et al., 2016). Volcanic eruptions are also considered as an important source of heavy metals because it contains a high level of Al, Zn, Mn, Pb, Ni, Cu and Hg (Kumar et al., 2017b)

2.1.2. Anthropogenic Sources

Advancement in industrial and agricultural sector contributed a large amount of heavy metals and pesticides to soil. Mining and smelting activities, agricultural run-off, industrial and sewage discharge are important anthropogenic sources for metal contamination to soil. During the high temperature metal processing some parts of metals are (Hg, As, Cu, Cd, Sn, Zn and Pb) released into atmosphere in vapor form, where they combine with water droplet to form aerosols. These metallic aerosols may disperse either by wind or precipitate in rainfall and causes many health hazards to nearby flora and fauna including human. In agriculture sector some essential metals especially Zn is used with phosphate fertilizers for better crop production which

finally comes to soil (Chen et al., 2004). Various chemical pesticides (insecticide, fungicides and herbicides) are also used in agricultutre at large scale for protection of plant from insect, pest and diseases. These chemical pesticides are reported to certain heavy metals such as As, Cu, Zn and Fe. (Fageria et al., 2002; Sarwar et al., 2016; Kumar et al., 2018). Further, other organic compound like organic manures, composts and biosolids applied in field to improve soil fertility are also reported for their high metal content than the soil. Water used for irrigation may also be a source of metal and pesticide contamination. Generally, fresh water used for irrigation contributes low level of contamination while sewage and industrial waste waters used for irrigation may be significant source of metal and pesticide contamination. Dry and wet deposits of emissions from cement industry, metal smelters and steel industry are well reported to be great contributor of metal in soil (Freedman and Hutchinson, 1981). Furthermore, deposits of emission release from automobile or vehicles using lead containing fuel are known for Pb enrichment in soil of nearby highways/roads. Sources and toxic effects of metals and pesticides on plant and animals are given in Table 2.1.

Table 2.1. Sources and effects of heavy metals**.**

S. No.	**Metals**	**Sources**	**Effect on plant**
1.	Arsenic (As)	Geogenic, Smelting operation, thermal power plants	Cellular biochemical dysfunction, protein and lipids damage
2.	Chromium (Cr)	Leather tanning, mining, industrial coolants, chromium salt manufacturing,	Chlorosis, membrane and root damage, decrease enzyme activity and plant growth
3.	Mercury (Hg)	Fluorescent lamps, electrical appliances, Thermal power plants, Chlor-alkali plants	Decreases photosynthetic activity, water uptake, antioxidant enzymes. phenol and proline accumulation

4.	Nickel (Ni)	Battery industry, thermal power plant, smelting operations	Decreases dry mass accumulation, protein production, chlorophylls and seed germination, increases free amino acids
5.	Zinc (Zn)	Electroplating, smelting	Reduces seed germination, enhances growth and ATP/chlorophyll
6.	Lead (Pb)	Lead acid batteries, paint, e-waste, smelting operations, coal based thermal power plants, ceramics, bangle industry	Chlorophyll and plant growth reduction, increases superoxide dismutase
7.	Manganese (Mn)	Sewage sludge, mining and mineral processing (particularly Ni), emission from steel and iron industries	Intervienal chlorosis and necrosis, brown spot on leaves, deformation of young leaves and growth retardation
8.	Cadmium (Cd)	Waste batteries, e-waste, paint sludge, incineration and fuel combustion, zinc smelting	Induces phytochelatins production, reduces lipid content, plant growth and seed germination
9.	Copper (Cu)	Electroplating, mining, smelting operations, sulphuric acid plant, vanadium Spent catalyst	Inhibits photosynthesis, plant growth and reproductive processes, reduces thylakoid surface area

2.2. Copper and its toxicity

Copper (Cu) is an essential micronutrient for plants and algae especially because of its important role in photosynthesis (primary electron donor in photosystem I), ATP synthesis and other metabolic processes as a cofactor (Thomas et al., 1998; Mahmood and Islam, 2006; Chatterjee et al., 2006). It is an important constituent of several proteins like plastocyanin of photosystem and cytochrome oxidase of respiratory electron transport chain (Demirevska-kepova et al., 2004). It works as a cofactor in the process of elimination of superoxide (superoxide dismutase

and ascorbate oxidase) and oxidase, mono- and di-oxyegenase (amine oxidases, ammonia monoxidase) radicals. Optimum level of bioavailabity of Cu in soil is well documented for its essentiality for plant growth and development however, its bioavailability even at slightly higher than the optimal levelcausesphytotoxicity (Michaud et al., 2008). Cu toxicity has been reported by several researchers (Moreno-Caselles et al., 2000; Singh and Tewari, 2003). Presence of high level of Cu in soil causes retardation in growth, leaf chlorosis and cytotoxicity, induces stress and causes damages to plants which lead to several deformities (Moreno-Caselles et al., 2000; Lewis et al., 2001; Singh and Tewari, 2003). Exposure of excess Cu is also responsible for oxidative stress and generation of Reactive Oxygen Species (ROS) which causes disturbance in metabolic processes and damage to macromolecules (Hegedus et al., 2001). Higher concentration of Cu in soil inhibits the availability of nutrients by altering their ionic form (Adrees et al., 2015; Habiba et al., 2015; Mei et al., 2015). Further, in combination with Cd, Cu has adverse effects on seed germination, seedling length and lateral roots formation (Neelima and Reddy, 2002; Kumar et al., 2018).

2.3. Cadmium and its toxicity

Cd is a non-essential element for plants and considered to be a potentially hazardous trace metal. Due to its great solubility in water and its high toxicity it has been reported as an extremely significant pollutant among the class of heavy metal pollutants and ranked 7th among the top 20 toxins (Das et al., 1997; Yang et al., 2004). Cadmium toxicity is easily recognizable in the form of stunted growth, chlorosis, browning root tips and finally plant death (Wojciek and Tukiendorf, 2004; Mohanpuria et al., 2007; Guo et al., 2008). Cadmium induces the inhibition of root Fe(III) reductase which led to deficiency of iron, and severely affects the plants

photosynthesis (Alcantara et al., 1994). Cd-induced chlorosis may be attributed to the suppression in uptake of iron and alterations in Fe:Zn ratio (Haghiri, 1973). Further, Cd restricts the uptake, transport and use of essential elements like Ca, Mg, P, K etc. and water(Das et al., 1997). It is also reported that Cd also suppresses the activity of enzyme nitrate reductase causing reduction in absorption of nitrate and its transportation from root to other plant parts (Hernandez et al., 1996). Cytotoxic effects of Cd in plants has also been reported in the form of swelling, vacuolization, degeneration of mitochondria, inhibition in cell proliferation and a low mitotic index (Silverberg, 1976; Rosezs et al., 1984). Chromosomal aberrations were also observed in onions, beans, peas and barley due to the presence of high level of Cd in growing soil (Oehlekers, 1953; Von Rosen, 1954; Degraeve, 1981). Roses et al. (1984) reported that the exposure of 1.5 to 10 mgL^{-1} Cd for 24 hrs had caused physiological and genetical damages in plants by inhibition of cell division and chromosomal alteration. Further, they also stated that the inhibition of cell proliferation, shown by low mitotic index was proportional to the concentration and time of exposure (Roses et al., 1984). Moreover, exposure to Cd causes decrease in nitrogen fixation and primary ammonia assimilation in the root nodules (Balestrasse et al., 2003).

2.4. Persistent Organic Pollutants (POPs) and its toxicity

POPs are group of synthetic lipophilic organic chemicals resistant to chemical, biological and photolytic/photochemical degradation with long-ranged transboundary dispersal potential via wind and ocean current. These chemicals are also capable of getting biomagnified and bioaccumulate in ecosystem including all living biota. The stable, semivolatile and insoluble nature of POPs allows then to travel long distances, even where they were never used i.e. Antarctica and Arctic Circle. Although, POPs are effective in their desirable uses, however, due to their above mentioned

characteristic sand high toxicity to natural settings these are gaining serious concern. Being lipophilic in nature, they get accumulated in fatty tissues of living creatures including human beings, even upto concentration of 70000 times greater than the background levels. Further, POPs bio-magnifies in food chain and tends to have highest concentration at the top trophic level of food chain. Since, human being and tertiary carnivores are at the top of food chain, therefore, are at high risk of toxic exposure (Lee et al., 2014; Wang et al., 2014; Arslan et al., 2015). POPs are absorbed by adipose tissues (fatty tissues) and sooner or later become part of adipose cell and liver (Lee et al., 2014). Human exposure to POPs started prenatally as many of them have potential to cross the placenta. After birth, breast feeding, ingestion, inhalation and dermal contact are the different mode of exposure (Dewailly et al., 1999; Arslan et al., 2015). The first report on the toxicity of POPs was published in 1962 by Carson and Darling in their book "Silent Spring" (Carson and Darling, 1962). In their publication they reported about the accumulation of DDT and its impact on living creature; birds are unable to hatch their egg because of brittleness of the egg shells. As a consequence of this report, production and use of DDT was banned in 1973. Considering the impact of POPs on environment and human health deterioration, the Stockholm Convention was organized in 2001 by the United Nations Environment Program (UNEP). The Stockholm Convention was signed by legislatures from 92 countries to regulate and ban the application of 12 listed chemicals (aldrin, dieldrin, DDT, chlordane, mirex, endrin, heptachlor, toxaphene, hexachlorobenzene (HCB), polychlorinated biphenyls (PCBs), dibenzodioxins and dibenzofurans); collectively known as 'dirty dozen' or 'legacy POPs' which were highly persistence and toxic in nature (UNEP, 2001; Arslam et al., 2017). Human, can be exposed to POPs either through diet and/or environmental exposure. Exposure to POPs can causes allergies,

cancer, hypersensitivity, developmental changes, damage to nervous system and disruption of reproductive, endocrine and immune systems. It is reported that the exposure to POPs causes increased rate of diseases and/or abnormalities in wildlife (birds and mammals) and aquatic species (USEPA, 2007). On the basis of their origins and use these chemicals are classified into three major categories *viz.* Organochlorine pesticides (OCPs), industrial chemicals (ICs) and unintended byproducts (UIBPs) (USEPA, 2006; Arslan et al., 2015). Organochlorine pesticides (OCPs) are a group of synthetic chlorinated organic compound mostly used as pesticides such as DDT, methoxychlor, dieldrin, chlordane, toxaphene, lindane, benzene hexachloride etc. It is reported that the 40% of pesticides belong to the class of Organochlorine. Earlier, they were successfully used as insecticides to control malaria and typhus, but now these chemicals are banned in most of the developed countries (Gupta et al., 2004; FAO, 2005; Aktar et al., 2009).

2.5. Phytoremediation: An Eco-Friendly Cost effective technique

Phytoremediation, involves the application of naturally occurring or genetically engineered plants to remove, degrade or immobilize various soil contaminants such as heavy metals and pesticides from growing medium (Prasad and Freitas, 2003; Rajkumar et al., 2012). The concept of use of plants for the soil remediation is not new. With interdisciplinary approach related with a chain of enthralling scientific research has endorsed the phytoremediation to develop into a most promising, cost effective, eco-friendly and aesthetically acceptable technique (Padmavathiamma et al., 2007; Kumar et al., 2013; Sainger et al., 2014; Arslam et al., 2015). The concept of phytoremediation was reintroduced and developed by Utsunamyia (1980) and Chaney (1983), whereas, the first field trial was piloted by Baker et al., (1991). Phytoremediation alsoconsist of the application of plants and

their associated rhizospheric microbes for the remediation of heavy metals, radionuclides, and organic xenobiotics (Malik et al., 2010). It is a solar-driven biological remediation practice (Chaney et al., 1997; Wang et al., 2016).

Some plants species have a special genetic setup to survive in contaminated growing medium along with potential to extract and accumulate significant amount of metals in their tissues. Identification and application of such plants that have this kind of adaptive mechanism can help to a great extent in remediating a metal-contaminated matrix (Lasat, 2002). Further, plant-based remediation systems take advantage of the roots for unique and selective uptake potential along with translocation, bioaccumulation, and stabilization of potentially toxic elements. Various forms and mechanism of phytoremediation are given in Fig 2.1.

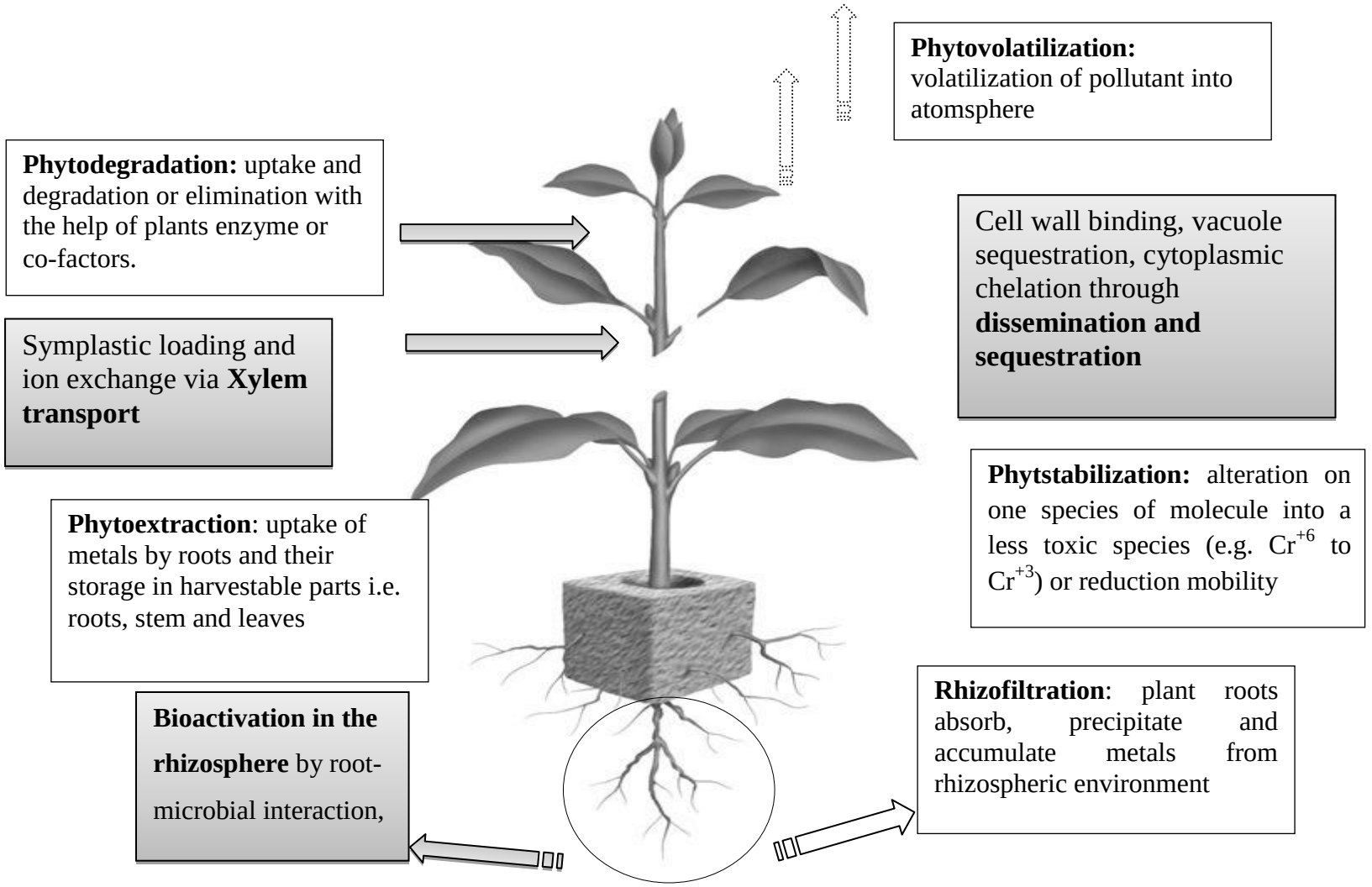


Fig.2.1 Mechanism involves in different form of phytoremediation

On the basis of their potential towards accumulation, extraction, and tolerance, plants can be categorized as indicators, excluders, accumulators, and hyperaccumulators (Prasad, 2004). *Indicators* are those plants in which metal uptake and translocation imitate metal concentrations in the soil strata, and display phytotoxicity symptoms. Plant growth and development reduce along with the increase of metal concentration(s) in the growing medium. *Excluders* obstruct the metal uptake and accumulation into the shoot accumulate high amount of metals in their roots thereby restricting their translocation. These plants are used for the stabilization of soil contamination. *Accumulators* uptake and accumulates metals from soil and translocate them to shoot without showing noticeable phytotoxic symptoms. These plants accumulate a high level of metals in their shoots; high translocation. *Hyperaccumulators* plants have potential to accumulate metals at concentration up to 100 times greater than metal concentration in growing medium; very high translocation. Moreover, the metal accumulation potential depends on the uptake ability and movement of intracellular binding sites (Baker et al., 1989; Yuan et al., 2016).

2.6. Hyperaccumulator of heavy metals

Plant species having ability to accumulate 100 mg Cd, 1000 mg Ni, Cu, Co, Cr and Pb and 10,000 mg kg^{-1} Zn and Mn dry weight are called as hyperaccumulator (Baker et al., 1989; Kumar et al., 2013; Brooks, 1998; Yuan et al., 2016). Further, translocation of metals from roots to above ground plant parts is also an essential feature of phytoremediator plants and is deduced by calculating the translocation factor (TF) (Kumar et al., 2013). TF higher and lower than unity is a key feature of metal accumulator and excluder plant species, respectively (Singh et al., 2010).

Identification and use of hyperaccumulator plants for soil remediation can help upto a great extent in the remediation of metal contaminated matrix (Lasat et al., 2002). Till date more than 500 hyperaccumulator plants have been reported belonging to the family Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunouniceae, Fabaceae, Flaconrtiaceae, Laminaceae, Poaceae, Violaceae and Euphorbiaceae. Some of the hyperaccumulator plants having high phytoremediation potential and TF of > 1 for selected metals are mentioned in Table 2.2. And the key important factors affecting the phyoremediation potential are mentioned in Table1.5.

Table 2.2. Hyperaccumulators of various metals

Plant Name	**Family**	**Contaminants**	**References**
Ricinus Comunis	Euphorbiaceae	Cd	Bauddha and Singh, 2012b
Brassica juncia	Brassicaceae	Cd, Se	Qadir et al., 2004; Banuelos et al., 2005
Vetiveria zizanioides	Poaceae	Cd, Pb	Danh et al., 2009; Kumar et al., 2018
Cyperus rotundus	Cyperaceae	Cr, Cu, Ni, Pb, Cd, As, Sn	Asharaf et al., 2011; Kumar et al., 2013; Jaison and Muthukumar, 2016; Tuan et al., 2016; Yuan et al., 2016
Parthenium hysterophorus	Asteraceae	Mg Fe, Pb, Zn,Cd,	Mazumdar and Das 2015; Sanghamitra et al., 2011; Ahmad and Al-Othman, 2014
Amaranthus cruentus	Amaranthaceae	Mg, Fe, Pb, Zn, Cr	Mazumdar and Das, 2015; Liu et al., 2008

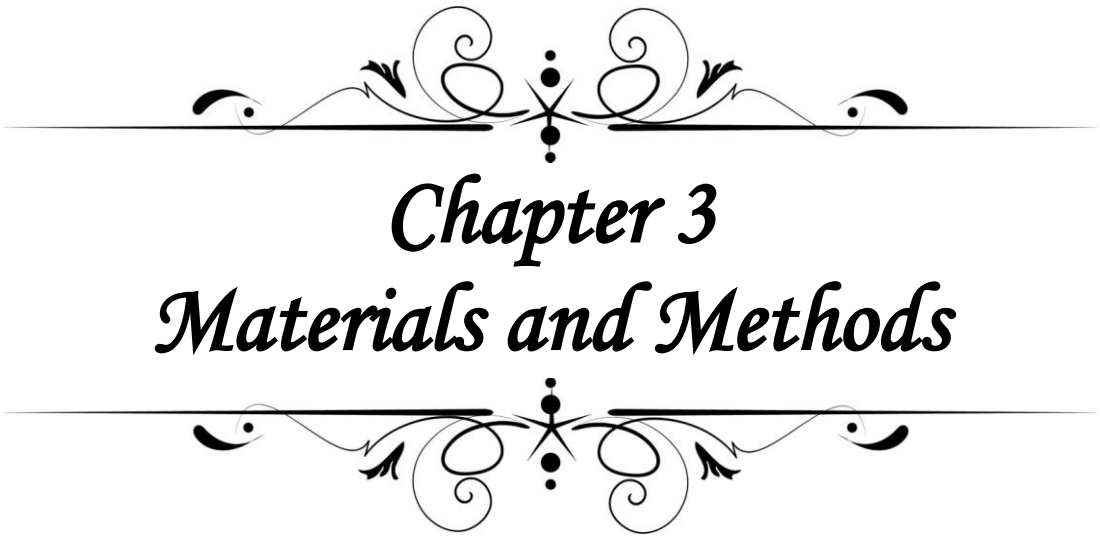
Populus tremula	Salicaceae	Zn, Cd, Cu	Ruiz et al., 2011; Pierre et al.,2011
Solanum americanum	Solanaceae	Mg, Fe, Pb, Zn	Mazumdar and Das, 2015
Croton bonplandianum	Euphorbiaceae	Cr, Cu, Ni, Pb, Cd	Kumar et al., 2013
Arabidopsis thaliana	Brassicaceae	Cd, As	Guo et al., 2012; Kiyono et al., 2012
Solanum nigrum	Solanaceae	Zn, Mn, Cu, Cr, Ni, Co, Cd, Pb	Malik et al., 2010; Varun et al., 2012
Cannabis sativa	Cannabaceae	Pb, Cu, Zn, Ni, Co, Cr	Malik et al., 2010

Contamination of land and water by toxic pollutants such as heavy metals and pesticides has occurred in developing and developed countries. In recent years, phytoremediation is developed as a leading technology for removal of toxic metals from contaminated land and water. Metal transfer from the roots to the shoots is an important feature of phytoremediator plant species, and the extent of transfer can be calculated based on the translocation factor (TF) (Kumar et al., 2013).

2.7. Role of microbes in phytoremediation

In contemporary world, contamination of soil by heavy metals and pesticides caused degradation of soil. Remediation of soil by plant based remediation approach i.e. phytoremediation not only revitalizes the degraded or contaminated soil but also simultaneously helps to meet the food and energy supply. Since, the effectiveness of phytoremediation is a consequence of function of a complete ecosystem and thus depends on the functional stability and interaction of plants with associated

rhizospheric microbes. It is reported that several plants involved in the interaction with root associated microbes to cope up with the contaminant toxicity and nutrients limitations (Weyens et al. 2009; Abhilash et al., 2012). Plant growing in contaminated soil harbor a diverse group of metal tolerant microbes capable of enhancing the efficiency of phytoremediation by improving the bioavailability of a metal due to alteration in soil pH, and chelators (siderophores and organic acid) (Khan et al., 2009; Uroz et al., 2009; Ma et al., 2011) Bioavailability/mobilization of contaminant in soil is an important aspect for a successful phytoremediation. In recent years, chemical amendments such as EDTA and limestone has been applied in the soil to increase the contaminants mobilization/bioavailability may be toxic to the beneficial soil microbes. However, use of microbe-associated processes could be better alternative to chemical amendments in which microbial processes and metabolites alters the contaminants mobility and bioavailability (Rajkumar et al., 2009; Ma et al., 2010; Aafi et al., 2012; Rajkumar et al., 2012). In addition, Plant roots-associated microbes produces several growth promoting substances such as 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, siderophores and plant growth hormones which improve the plant growth growing even in metal or pesticide contaminated soil (Wu et al., 2006; Rajkumar et al., 2010; Glick, 2010; Wang et al., 2011; Miransari, 2011, Luo et al., 2012). Microbial association with plant enhances the efficiency of remediation process by two complementary means: (i) direct enhancement in phytoremediation by altering metal mobilization/bioavailability (ii) indirect promotion by improving biomass production in order to accumulate/remove the contaminants and/or confer the plant tolerance (Rajkumar et al., 2012).



Chapter 3
Materials and Methods

3.1. Study Area

The pot experiment was carried out naturally in the net house of experimental research field station of Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India (N:26°46'05.4"; E: 80°55'38.8"). The minimum, maximum and average temperature during the whole study was ranged between 6.7-23.8 ºC, 19.4-37.2 ºC and 13.5-30.2 ºC with relative humidity between 84-95% at 6:30 am and 36-59% at 2:30 pm.

3.2. Plants

Vetiveria zizanioides and ***Andrographis paniculata*** were selected for the study.

3.2.1. Vetiveria zizanioides: *Vetiveria zanioides*(L.) Nash, syn. *Chrysopogon zizanioide s*(L.) Roberty, a fast growing, perennial bunchgrass, belongs to the family Poaceae (Bertea and Camusso, 2002). It is a multipurpose plant has many important applications in area of bioengineering, soil reclamation, water conservation, environmental protection particularly in non-native environment (Khan, 2006; Dahn et al., 2009). Its morphological, physiological and ecological characteristics such as high biomass with noteworthy photosynthetic efficiency, massive and deep root system makes it tolerant against various adverse environmental conditions.

3.2.2. Andrographis paniculata: Kalmegh (*Andrographis paniculata*), is an annual herb belonging to family Acanthaceae. It is cultivated mostly for its various medicinal values and extensively used in traditional medicine. In India, it is well known for the treatment of infections and health disorders. In the recent era of phytoremediation, medicinal plants has been also tested and successfully reported as an alternative high-value crop in metal polluted

agricultural soils (Zheljazkov et al., 2008). Toxic Metal accumulation potential of medicinal herbs has been reported by several investigators (Chaiyarat et al., 2011; Siddiqui et al., 2012; Rania, 2015). However, application of medicinal plant like *A.paniculata* for remediation purposes is still scanty.

(a) *Vetiveria zizanioides*

(b) *Andrographis paniculata*

Fig: 3.1. Selected plant for the experiment

3.3. Experimental Design

T_0	Control	T_8	$Cu_{100}+Cd_{50}$
	Control		$Cu_{100}+Cd_{50}$
	Control		$Cu_{100}+Cd_{50}$
	Control		$Cu_{100}+Cd_{50}$
	Control		$Cu_{100}+Cd_{50}$
T_1	Cu_{50}	T_9	$Cu_{150}+Cd_{75}$
	Cu_{50}		$Cu_{150}+Cd_{75}$
	Cu_{50}		$Cu_{150}+Cd_{75}$
	Cu_{50}		$Cu_{150}+Cd_{75}$
	Cu_{50}		$Cu_{150}+Cd_{75}$
T_2	Cu_{100}	T_{10}	$Pest_1$
	Cu_{100}		$Pest_1$
	Cu_{100}		$Pest_1$
	Cu_{100}		$Pest_1$
	Cu_{100}		$Pest_1$

T_3	Cu_{150}	T_{11}	$Pest_2$
	Cu_{150}		$Pest_2$
	Cu_{150}		$Pest_2$
	Cu_{150}		$Pest_2$
	Cu_{150}		$Pest_2$
T_4	Cd_{25}	T_{12}	$Pest_3$
	Cd_{25}		$Pest_3$
	Cd_{25}		$Pest_3$
	Cd_{25}		$Pest_3$
	Cd_{25}		$Pest_3$
T_5	Cd_{50}	T_{13}	$Cu_{50}+Cd_{25}+Pest_1$
	Cd_{50}		$Cu_{50}+Cd_{25}+Pest_1$
	Cd_{50}		$Cu_{50}+Cd_{25}+Pest_1$
	Cd_{50}		$Cu_{50}+Cd_{25}+Pest_1$
	Cd_{50}		$Cu_{50}+Cd_{25}+Pest_1$
T_6	Cd_{75}	T_{14}	$Cu_{100}+Cd_{50}+Pest_2$
	Cd_{75}		$Cu_{100}+Cd_{50}+Pest_2$
	Cd_{75}		$Cu_{100}+Cd_{50}+Pest_2$
	Cd_{75}		$Cu_{100}+Cd_{50}+Pest_2$
	Cd_{75}		$Cu_{100}+Cd_{50}+Pest_2$
T_7	$Cu_{50}+Cd_{25}$	T_{15}	$Cu_{150}+Cd_{75}+Pest_3$
	$Cu_{50}+Cd_{25}$		$Cu_{150}+Cd_{75}+Pest_3$
	$Cu_{50}+Cd_{25}$		$Cu_{150}+Cd_{75}+Pest_3$
	$Cu_{50}+Cd_{25}$		$Cu_{150}+Cd_{75}+Pest_3$
	$Cu_{50}+Cd_{25}$		$Cu_{150}+Cd_{75}+Pest_3$

T= Treatment

Cu_{50}= Copper at conc. of 50ppm; $Cu_{100=}$ Copper at conc. of 100ppm; $Cu_{150=}$ Copper at conc. of 150ppm.

Cd_{25}= Cadmium at conc. of 25ppm; $Cd_{50=}$ Cadmium at conc. of 50ppm; $Cd_{75=}$ Cadmium at conc. of 75ppm.

$Pest_{1=}$ Organochlorine Pesticide efluent100%; $Pest_{2=}$ Organochlorine Pesticide effluent 75%; $Pest_{3=}$ Organochlorine Pesticide effluent 50%

3.4. Detail of experimental soil and its physicochemical analysis

3.4.1. Soil

Soil was collected from the research field station of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India and air dried for 4 weeks before passing through 1.5 mm sieve. Physico-chemical parameter of experimental soil viz. pH, organic carbon, electrical conductivity and essential minerals like Nitrogen, Phosphorus, Potassium, Calcium, Iron, Mangnese, Sodium and Zinc were examined before initiating the pot experiment following Maiti,

2003. Ground water used for the irrigation was also analyzed for various physico-chemical characteristics including trace metals using APHA, (2005).

3.4.1.1. pH

Each soil sample was dissolved in Double Distilled Water in ratio of 1:5 and pH was measured by electronic pH meter (Direct Readout Ion Meter) (Piper, 1966).

3.4.1.2. Electrical conductivity (EC)

The same suspension used for pH analysis was used for measurement of EC. EC was recorded by conductivity meter (Toshniwal TCM 15) in dSm^{-1} (Anderson-Cook, 2002).

3.4.1.3. Organic Carbon

Organic carbon content of soil was measured by following Walkleyand Black (1934)

Reagents

- Standard 1N Potassium dichromate: 49.04 g of Analytical Reagent grade $K_2Cr_2O_7$ was dissolved in doubled distilled water and the volume was made up to 1 litre.
- 0.5N ferrous ammonium sulphate: 196 g of the hydrate crystalline salt was dissolved in 1 litre double distilled water containing 20 ml of concentrated H_2SO_4.
- O-phenanthroline (Ferroin) was used as indicator
- Concentrated H_2SO_4 containing 1.25% of silver sulphate
- Ortho-phosphoric acid (85%) or NaF

Procedure

- 1g of soil was taken in a dry 500 ml conical flask.
- 10 ml of 1N $K_2Cr_2O_7$ was pipetted in and swirled.
- The flask was kept on an asbestos sheet, and 20 ml of conc. H_2SO_4 containing 1.25% of silver sulphate was added. Swirled the solution 2-3 times again.
- Allowed the flask to stand for 30 minutes and then 200 ml of double distilled water added to it.
- Added 10 ml of phosphoric acid, 0.5g of sodium fluoride and 1 ml of diphenyl indicator in the solution.
- Titrated above solution with 0.5N ferrous ammonium sulphate solution till the color changes from blue-violet to green at end point.
- Simultaneously a blank was run without soil.

Calculation

Organic Carbon (%)= N × (B-C) × 0.03 × 100/Wt. of Soil (g)

Where,

N= Normality of ferrous ammonium sulphate

B= Vol. of 0.5N ferrous ammonium sulphate required to neutralize 10 ml of 1N $K_2Cr_2O_7$ (blank reading)

C= Vol. of 0.5N ferrous ammonium sulphate required for titration of soil sample.

There is incomplete oxidation of the organic matter in this procedure. Therefore, the organic carbon obtained by the above method is multiplied by a factor 1.3 based on the assumption that there is 77% recovery.

Therefore,

Organic Carbon = Organic carbon estimated × 1.3

3.4.1.4. Organic Matter

Organic matter content of soil was calculated by multiplying the value of organic carbon by Van Bemmelen factor of 1.724 because organic content contains 58% of organic carbon.

Organic Matter= Organic Carbon × Van Bemmelen factor of 1.724

3.4.1.5. Total Nitrogen

Total nitrogen present in soil was calculated by Kjeldahl methods (EPA, 1983). The nitrogen in the sample was converted into ammonium sulphate nitrogen when treated with sulphuric acid using potassium selenite and copper sulphates catalyst. As excess of alkali is then added and the ammonia distilled into as excess of boric acid solution was determined by titration with standard sulphuric acid. 1 to 5g of dried sample was taken into a 500 ml Kjeldahl flask, 20 ml of concentrated H_2SO_4 and one Kjeltab $CuSO_4$ and K_2CuSO_4 were added (containing $CuSO_4$ and K_2CuSO_4 to the flask). After thorough mixing, slow digestion was done until frothing ceased and after 30 min the liquid became clear, the sample was estimated for N by AutoKjeltec 1030 analyzer and direct reading in percent was obtained.

3.4.1.6. Available Nitrogen

Available nitrogen in soil was determined by the alkaline potassium permanganate method of Subbaih and Asija, (1956).

Reagent used

- Potassium permanganate solution (0.32%)

- Sodium hydroxide solution (2.5%)
- Sulphuric acid (0.02N)
- Boric acid solution
- Methyl red indicator

Soil sample (2 g) was taken in distillation flask and mixed with 20 ml distilled water. Then 100 ml of potassium permanganate solution and 100 ml sodium hydroxide solution were added to it and immediately fitted up in the distillation apparatus. 20 ml of sulphuric acid was pipetted out in a conical flask and the end of the delivery tube was dipped in it. The ammonia gas was distilled from the distillation flask and collected in about 30 ml of filtrate. Then five drops of methyl red indicator were added and titrated against 0.02N sulphuric acid solutions. The available nitrogen was calculated by the titrant used.

3.4.1.7. Sodium and potassium

Sodium and potassium were determined by fast sequentional atomic absorption spectrophotometer in flame mode on a model VARIAN AA240FS. 1g of oven dried soil sample was taken in conical flask and kept overnight in HNO_3: $HClO_4$ (5:1) digested at 70 to 80 ºC on hotplate. The solution was allowed to evaporate to dryness until all the tissues had been digested and raised the temperature to 105 ºC to reduce the volume to 0.5-1.0 ml. The solution was filtered through Whatman filter paper No. 40 in a volumetric flask. The residue was re-dissolved and diluted to 15 ml with distilled water. The analysis was carried out in three replicates.

Sodium and Potassium content in ($\mu g\ g^{-1}$dwt)= $\frac{XV}{W}$

Where,

X=Reading in ppm on AAS,

V= Final volume of digested samples (ml)

W= Dry weight of the sample (g)

3.4.1.8. Total Phosphorous

Inorganic phosphorous was measured by complexing it as molybdophosphoric acid and reducing it with stannous chloride. 50 ml of sample taken in a conical flask was made free from and color and colloidal impurities with activated charcoal and filtering. To this clean filtrate, addition of 2 ml of ammonium molybdate was followed by 5 drops of stannous chloride solution. The blue color of the complex was read in a spectrophotometer (AA 240 FS, Varian) at 690 nm after 5 min (but before 12 minute of addition of the last reagent) and inorganic phosphorous was determined by referring to a standard calibration curve. Inorganic phosphorous has been expressed as µg/g dry wt.

3.4.1.9. Available phosphorous (P_2O_5)

Available Phosphorous in soil was determined by the method of Olsen et al., (1954).

Reagent

- Sodium bicarbonate (1/2 N) pH 8.5
- Activated carbon; Darco G-60
- Ammonium molybedate

Working Solution

Stannous chloride ($SnCl_2.2H_2O$) **(**10 g) was dissolved in 25 ml of concentrated hydrochloric acid (HCl) and the volume was made upto 100 ml with distilled water.

Procedure

- Black Darco G-60 (2-3 g) and 50 ml of sodium bicarbonate (1/2 N) solution were added to 2 g dried powdered soil.
- It was shaken thoroughly for 30 minute and then filtered through Wattman No. 40 filter paper.
- To 5 ml of filtered soil extract 5 ml of ammonium molybedate was added and this was diluted to about 20 ml with distilled water.
- Stannous chloride solution (working solution) (1 ml) was then added to it and the final volume was made upto 25 ml with distilled water and shaken thoroughly.
- The color intensity which was red measured using the colorimeter at 660 nm after 10 minutes.

Preparation of Standard Curve

To prepare standard curve of P, 1, 2, 3, 4, and 5 ml of 5 ppm solution was taken in 50 ml volumetric flasks. 5 ml of extracting solution i.e., sodium bicarbonate was added to these flasks. 10 ml of distilled water and one drop of p-nitrophenol indicator was added to this solution. Then 2.5M H_2SO_4 was added to the solution drop wise till the solution becomes transparent. At the point where indicator's yellow colour disappears, the correct pH(5) for the colour development has been attained. If the end point was exceeded through addition of excessive acid, the pH may be raised by adding NaOH. 8 ml of Murphy-Riley solution was added to each flask. Volume was made to 50 ml with distilled water and mixed thoroughly. Now standards have P concentration 0.1, 0.2, 0.3, 0.4 and 0.5 μg g^{-1}. A blank was prepared with $NaHCO_3$ solution, distilled water and Murphy-Riley reagent. After waiting for 15 minutes the intensity of the blue colour was read on spectrophotometer at 730nm. Absorbance

values for the standard having 0.1, 0.2, 0.3, 0.4, and 0.5 µg P/ml were used to construct a standard curve between absorbance values and concentration of P in standards.

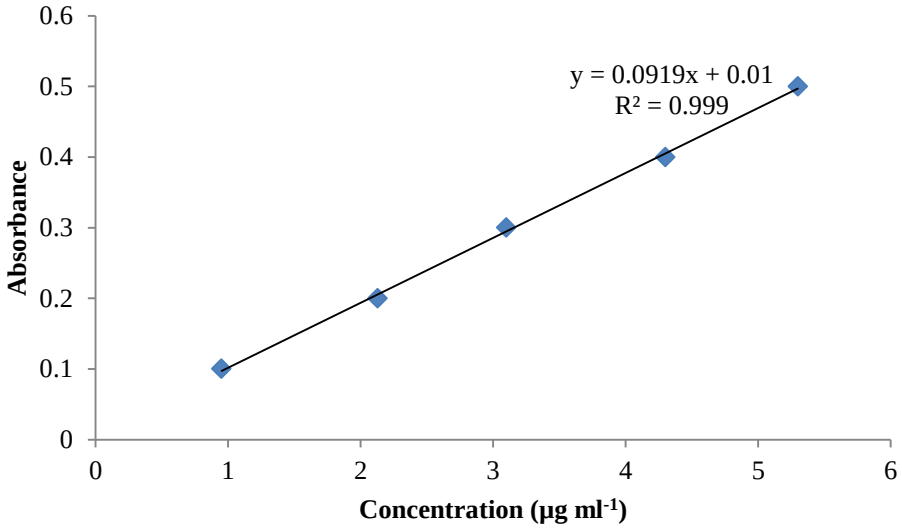


Fig 3.2 Standard curve for phosphorous

Method

- 5.0 g of air dried soil was placed in a 125 ml Erlenmeyer flask.
- Small amount of phosphorus free Darco-G-60 was added.
- 50 ml of $NaHCO_3$ was added to each flask at 25 ºC.
- Shaken on a reciprocating shaker at 120 strokes per minute for 30 min
- Simultaneously a blank was run without soil
- Solution was filtered through Whatman No. 40/42 filter paper
- 10 ml aliquot of the extract was pipette in a 50 ml volumetric flask.
- 10 ml of distilled water and one drop of p-nitrophenol indicator was added to this solution. Then content were acidified to pH 5 by adding 2.5M H_2SO_4 drop wise till color disappeared.

- 8 ml of Murphy-Riley solution was added to each flask. Volume was made to 50 ml with distilled water and mixed thoroughly. After waiting for 15 minutes the intensity of the blue color was read on spectrophotometer at 730nm.

The calculations were made as follows:

If concentration of P in the aliquot as read from the standard curve against x= C μg g^{-1} which is the concentration present in 5 ml aliquot.

Therefore, the concentration of P in 50 ml aliquot=C/5 × 50 μg g^{-1}

Hence,

Avail. P in μg g^{-1}= C × 50/5 × wt. of soil (g)

3.4.1.10. Available Potassium (K)

Potassium was estimated by flame photometer (Perkin-Elmer model 52, flame photometer with acetylene of propane burner) following Jackson (1967).

Reagent

A. **Ammonium acetate 1N:** To 800 ml of water, 57 ml of concentrated acetic acid and then 68 ml of concentrated ammonium hydroxide was added. It was diluted to a volume of 1 liter and was adjusted to pH 7 by the addition of more ammonium hydroxide or acetic acid.

B. **Potassium chloride 0.02N:** Dry potassium chloride (1.49 g) in water was dissolved in distilled water and was diluted to a volume of 1 liter.

C. **Potassium chloride:** 0.**02N KCl in 1N ammonium acetate:** Dry potassium chloride (KCl) (1.49 g) was dissolved in reagent A and diluted to a volume of exactly 1.0 liter with additional A solution.

D. **Lithium chloride, 0.05N:** Dry lithium chloride (2.12 g) was dissolved in distilled water and diluted to 1 liter.

Procedure

- Reagent B and D were used to prepare a series of standard potassium chloride solution, each containing the same concentration of lithium chloride.
- A similar series of standard potassium solutions using reagents C and D which was diluted by reagent A were prepared.
- The concentrations of potassium chloride were 0.1, 0.2, 0.3, 0.4, 0.5, 1, 1.5 and 2 moles equivalent/Liter (meq/l). The optimum concentration of lithium chloride varies with individual flame photometers but was usually 5 to 10 meq/l.
- Standard solutions were made in water employed for the analysis of water and water extracts of soil; whereas, those made up in ammonium acetate solution were used for the analysis of ammonium acetate extracts of soils.
- The flame photometer was calibrated for operation over the concentration range 0 to 0.5 meq/l of potassium, using the first 6 standard solutions of the appropriate series. The first and the last 4 solution of the appropriate series were used to calibrate the instrument for operation over the concentration range 0 to 2 meq/l of potassium.
- An aliquot of the solution was pipetted out to analysis containing less than 0.1 meq of potassium into a 50 ml volumetric flask.
- An amount of reagent D was added, which when diluted to a volume of 50 ml, gave a concentration of lithium chloride exactly equal to that in the standard potassium chloride solutions.
- It was diluted to volume with A, and determined the potassium concentration by use of the flame photometer.

3.5. Plant Growth Analysis

3.5.1. Fresh Weight

The fresh weight of plant parts was recorded at 30, 60 and 90 DAS using single pan electric balance. Root and Shoot were washed with distilled water and excessive water was removed by placing within two layers of filter paper before weighing.

3.5.2. Dry weight

The dry weight of the same root and shoot were recorded after drying in hot air oven at 60 ºC for 48 hr. when the dry weight became constant.

3.5.3. Chlorophyll

The chlorophyll, green pigments of plants, is the most important pigments active in the photosynthetic process.

Reagent

- **80% Phenol:** 80 ml Phenol was taken in to a 100 ml volumetric flask and the volume was made up to 100 ml by adding distilled water.

Procedure

About 0.5 g of fresh leaf was crushed in 10 ml of 80% (v/v acetone/water) chilled acetone with the help of pestle mortar in dark. Centrifuged it at 5000 rpm at 10 ºC for 15 min. The supernatant was taken and absorbance was measured at wavelength 663 nm and 645 nm by spectrophotometer (AA 240 FS, Varian). 80% acetone was used as blank.

The chlorophyll (in mg/g fresh weight) was determined by following formula Machalachlan and Zalik (1963).

Chlorphyll (a)(mg/g)= $\frac{12.7 \times OD\ 663nm\ - 2.63 \times OD\ 645}{1000 \times W} \times xV$

Chlorphyll (b)(mg/g)= $\frac{22.9 \times OD\ 645nm\ - 4.68 \times OD\ 663}{1000 \times W} \times xV$

Total Chlorphyll (mg/g)= $\frac{20.2 \times OD\ 645nm\ + 4.68 \times OD\ 663}{1000 \times W} \times xV$

Where,

V= volume of acetone= 10 ml

W = weight of sample = 0.5 g

O.D. = Asorbance at wavelength 663 nm and 645 nm

3.5.4. Protein Content

Protein content was estimated by Lowry et al., (1951).

- Fresh leaves (100 mg) of the control and treated plants were homogenized separately in 3 ml of 10% chilled trichloroacetic acid (TCA) in pestle and mortar and centrifuged at 10,000 rpm for 10 min.
- After transferring the supernatants, the pellets were washed and heated for 7 min with 3 ml of 1N NaOH (sodium hydroxide), cooled and centrifuged again at 10,000 rpm for 10 min.
- 0.5 ml of extracted sample was taken in 2.5 ml of 0.5% $CuSO_4$ (copper sulfate in 1% potassium sodium tartarate), 48 ml of 5% sodium carbonate was added. 0.5 ml (1N) of folin-phenol reagent was added after 10 min.
- 30 min incubation developed a blue color complex in the mixture. Absorbance was taken at 700 nm against a blank without sample. Protein content was calculated by a standard curve made by bovine serum albumin (BSA).

3.5.5. Malondialdehyde (MDA)

The level of lipid peroxidation products in leaf samples was expressed as MDA content and was determined by Heath and Packer (1968).

Procedure

- About 200 mg fresh leaves were ground in 0.25% 2-thiobarbituric acid (TBA) in 10% trichloroacetic acid (TCA) using a mortar and pestle.
- After heating at 95 ºC for 30 min, the mixture was quickly cooled in an ice bath and centrifuged at 10,000 rpm for 10 min.
- The absorbance of the supernatant was read at 532 nm and corrected for unspecific turbidity by subtracting the absorbance of the same at 600 nm.
- The blank was 0.25% TBA in 10% TCA.
- The concentration of lipid peroxides together with oxidatively modified proteins of plants were thus quantified in terms of MDA level using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as $mmolg^{-1}$ fresh weight (FW).

3.5.6. Assay of Antioxidant Enzymes

Fresh leaves (500 mg) were homogenized in 100 mM chilled potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA and 1% polyvinylpyrrolidone (w/v) at 4 ºC. Homogenate was squeezed through four layer of cheese cloth, and extract thus obtained was centrifuged at 20000 rpm for 20 min at 4 ºC. Supernatant was used to measure the activities of various antioxidants. The protein content in the supernatant was measured according to Bradford (1976). Activity of all the enzymes is expressed in unit mg^{-1} protein.

3.5.7. Catalase (CAT)

Catalase (CAT) activity was determined by monitoring the decrease in absorbance at 240 nm as consequence of H_2O_2 consumption. For measurement of the catalase activity, extraction was done in the buffer containing 50 mMTris-HCl (pH 7.0), 0.1 mM EDTA, 1 mM PMSF and 0.3 g g^{-1}fw PVP. Activity was measured by the method of Aeby (1974). The 3 ml reaction mixture comprised of 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and a suitable aliquot of enzyme. Decrease in the absorbance was taken at 240 nm (molar extinction coefficient of $H_2O_2$was 0.04 cm^2 $mmol^{-1}$). Enzyme activity was expressed as unit mg^{-1} protein.

3.5.8. Peroxidase (POD)

Peroxidase activity was assayed following Hemeda and Klein (1990). POD activity was examined by the variation in absorbance at 470 nm due to guaiacol oxidation in a reaction solution (3ml final volume) composed of 50 mmol/L phosphate buffer (pH 7.0) 20 mmol/L guaiacol, 10 mmol/L H_2O_2 and 0.5 mL of crude extract.

3.6. Heavy metal estimation in soil and plant

One gram of oven dried soil/plant sample was taken in conical flask and kept overnight in HNO_3: $HClO_4$ (5:1) digested at 70 to 80 °C on hotplate. The solution was allowed to evaporate to dryness until all the tissues were digested and raised the temperature was raised to 105 °C to reduce the volume to 0.5-1.0 ml. The solution was filtered through Whatman filter paper No. 40 in a volumetric flask. The residue was re-dissolved and diluted to 15 ml with distilled water. Metal content was estimated using Atomic Absorption Spectrophotometer (Varian Model Spectra AA-250 plus). The analysis was carried out in three replicates.

Metal concentration (µg g^{-1}dwt) = $\frac{XV}{W}$

Where,

X = AAS Reading (in ppm)

V = Final volume of digested sample (ml)

W = Dry weight of the sample (g)

3.6.1. Calculation of Translocation factor (TF), Enrichment coefficient,

3.6.1.1. ***Translocation factor*** or mobilization ratio of each metal was calculated to determine the translocation of metals from the root to shoot of the plant species (Barman et al., 2000; Gupta et al., 2008).

$$TF = \frac{\text{Concentration of metal in plant shoots}}{\text{Concentration of metal in plant roots}}$$

3.6.1.1. ***Enrichment coefficient*** has been determined to derive the degree of heavy metal accumulation in plants growing on contaminated environment (Kisku et al., 2000).

$$EC = \frac{\text{Concentration of metal in roots or shoots}}{\text{Concentration of metal at contaminated site}}$$

3.7. Pesticides Estimation

1 g of soil or plant sample was suspended in 80 ml of petroleum ether-acetone mixture (1/1 v/v) and shaken on Excella E24 incubator shaker series for 3 hr. The extract was filtered and concentrated to exactly 1 ml by using rotatory evaporator IKARV 10 and nitrogen stream respectively. The samples were dissolved in mixture of acetone and n-hexane (1:1; v/v). Column cleanup was done with anhydrous sodium sulphate and Florisil (activated magnesium silicate). 50 ml of eluting solvent (n-

hexane: ethyl acetate: dichloromethane in the ratio of 70:15:15) was used for each sample. The samples were run on GC (Agilent 7890 A) equipped with ECD detector.

3.8. Microbial Analysis

3.8.1. Isolation and Enumeration of Bacteria

For microbiological analysis soil samples was collected from rhizospheric region of both the plants i.e. *A. paniculata* and *V. zizanioides* from all treatments including control. For microbiological examination soil were collected from the surface layer (at depth of 15 cm) in polyethylene plastic bags and collected samples were stored in an ice cooler box and delivered immediately to the laboratory for analysis. The samples were stored in 4ºC for further analysis. Isolation was done by following serial dilution method. This method is a very simple and inexpensive technology for the isolation of soil microbes and CFU (Colony forming unit) count was done by serial dilution method. The main value of colony counts lies in comparing the results of repeated samples from the same source. The colonies that develop are counted. A single measurement is not very reliable, so the procedure is repeated at least three times and the results are averaged. Protocol for isolation and enumeration of bacteria are as:

- Firstly 10 g of soil sample was added in 90 ml autoclaved distilled water and suspension was made through shaking.
- 1 ml of soil suspension was and added 9 ml of blank. This process continued up to 5 tubes and resulted in serial dilutions of 10^{-1} to 10^{-5} of soil samples.
- From last three dilutions 0.1 aliquot spread on nutrient agar plates for bacterial colonies.
- After spreading plates were incubated at 28±2 ºC for 48 hr. All the plates in inverted position to prevent moisture.

- Further all the plates were observed for bacterial colonies and count the no of colonies were counted.
- Colony forming unit was calculated by using no of colonies by applying the following formula

$$\text{CFU/g soil} = \frac{1}{Dilution\ factor} \times \text{number of colonies}$$

3.8.2. Dehydrogenase activity Test

Dehydrogenase 2-3-5-Triphenyl tetrazolium chloride (TTC) reduction technique (Casida, 1977) was used for the estimation of dehydrogenase activity in soil. Protocol used for dehydrogenase activity was as follows:

- One-gram fresh soil taken in test tube and 0.1 gm of calcium carbonate ($CaCO_3$) and 1 ml of 1 % TTC solution was added in tube
- Tubes were incubated at 30° C for 24 hours in an incubator, after shanking.
- Produced soil slurry was transferred on Whatman filter paper No.1 and extraction was done by adding successive aliquots of concentrated methanol. The volume of the filtrate was made to 50 ml by adding methanol.
- The optical density of the obtained filtrate was read at 485 nm by spectrophotometer, using methanol extract as a reference sample.
- Dehydrogenase activity was represented by concentration of Formazan, which was calculated by a standard curve of triphenylformazan in methanol and expressed in terms of milligram formazan per gram dry soil per hour.

3.9. Defluoridation of water

100 mg/l stock solution of F was prepared by dissolving 221mg of anhydrous sodium fluoride (NaF) with 99.5% purity in one liter of distilled deionized water from Millipore. Test concentration of 5 mg/l was prepared from stock solution following serial dilution technique. Test concentration of 5 mg/l was selected for adsorption experiment since it is considered to be normal fluoride concentration in groundwater.

Procedure

- Fresh shoot of *A. paniculata*and *V. zizanioides* of 90 days age were collected from the experimental pots used for phytoremediation of Cu from the soil and marked as T0 (control), T1 (Cu50), T2 (Cu100) and T3 (Cu150).
- Plant parts were washed carefully with double distilled deionized water and sun dried for 3 days.
- Dried plant biomass samples were ground manually with the help of mortar pestle and sieved to obtain powder below 1.5 mm diameter.
- Before applying as biosorbent; the obtained powdered sample was subjected to acid and alkali treatment.
- Powdered sample of biosorbent (50 g) was mixed with 500 ml of 1 M HNO_3 and heated gently for 20 minutes on open flame burner then filtered out and washed with double distilled water till the elimination of color.
- Acid treated sample was subsequently subjected to the alkali treatment with 500 ml of 0.5 M NaOH.
- The treated plant material was washed repetitively with double distilled water till clear solution having pH 7 was obtained.
- The obtained powdered biomass was oven dried for 3 hours at 110 ^{0}C and subsequently cooled in air to room temperature for use.

3.10. Statistical analysis

Data (n=5) were analyzed statistically by one way analysis of variance (SPSS, 20 Statistical package and MS Excel) using Duncan's Multiple Range Tests (DMRT) and t test to determine the significance of differences among treatments at probability (p) 0.05 and 0.01.

Chapter 4

Studies on physiological and biochemical responses of A. paniculata and V. zizanioides exposed to different concentration of Cu, Cd and organochlorine pesticides

4.1. Physico-chemical examination of experimental soil and groundwater used for irrigation of pot

Physico-chemical parameters of experimental soil were examined before sowing. Soil was found slightly alkaline with pH 7.45, EC 0.46 dsm^{-1} and organic carbon 1.27%. Soil was found to be rich in Ca: 3.07; Fe: 112.38; Mn: 7.42; Na: 3.22; S: 15.43; and Zn: 2.78 ppm. N, P and K content of the soil was found to be 1.38, 0.91 and 3.48 g kg^{-1} respectively. Ni, Pb, Cu and Cr were present in traces (Table 4.1).

Table 4.1. Physico-chemical characteristics of soil

Parameters	**Mean Value ± SD**
pH	7.45 ± 0.3
EC ($dsm^{-1)}$	0.46 ± 0.02
Organic C (%)	1.27 ± 0.07
N(g kg^{-1})	1.38 ± 0.04
P(g kg^{-1})	0.91 ± 0.006
K(g kg^{-1})	3.48 ± 0.08
Na (ppm)	3.22 ± 0.05
Ca (ppm)	3.07 ± 0.04
Fe (ppm)	112.38 ± 2.8
Mn (ppm)	7.24 ± 0.06
Zn (ppm)	2.78 ± 0.08
Cd (ppm)	0.01 ± 0.003
Cu (ppm)	2.29 ± 0.13
Pb(ppm)	0.72 ± 0.03
Ni (ppm)	0.016 ± 0.007
Cr (ppm)	0.002 ± 0.0001

All results have been expressed as mean of five replicates i.e. n=5 ± SD.

Groundwater used for irrigating the pots was examined for various physico-chemical characteristics *viz.* Total dissolved solids (300 mgl^{-1}), nitrate (6.29 mgl^{-1}), chloride (28.23 mgl^{-1}), sulphate (13.09 mgl^{-1}), alkalinity (28.7 mgl^{-1}) hardness (150

mgl^{-1}), Na (13.2 mgl^{-1}), Ca (6.2 mgl^{-1}), K (3.54 mgl^{-1}) and were found to be within the limits for drinking water as prescribed by WHO (2012), whereas, trace metals; As, Cd and Cu were not detected. Organochlorine pesticides (OCPs) were examined in the effluent collected from the outlet of pesticide industry (Table 4.2.).

Table 4.2. Analysis of organochlorine pesticides in pesticide industry effluent GC-2

Compound	**Concentration (µg/l)**
α-HCH	105.882±4.7
β-HCH	10.139±0.84
γ-HCH	115.017±7.3
δ-HCH	91.917±4.4
Aldrin	9.074±0.61
α-Endosulfan	0.107±0.012
pp-DDD	0.022±0.004
op-DDT	0.437±0.0072
pp-DDT	0.009±0.0001

Results are expressed as means of five replicates i.e. n= 5± SD.

4.2. Effect of Cd and Cu contamination on seed germination of *A. paniculata*

Several researchers reported that germination assay is an elementary procedure to assess the toxic effects of metal on plant species (Adrees et al., 2015; Gang et al., 2013). Since *V. zizanioides* was planted after the propagation of numerous slips (containing roots and small stem) in pots. Hence, seed germination test was applied only for *A. paniculata.* The results of seed germination study conducted on *A. paniculata* exposed to varying concentration of Cu and Cd are presented in Table 4.3. Results clearly reveal that the seed germination percentage was decreased with increase in metal concentration. Similar results were reported by Gang et al., 2013;

Muccifora and Bellani, 2013. The maximum seed germination percent was recorded at 50 µg/g concentration of Cu i.e. 67% while minimum was observed at concentration of Cu 150 and Cd 75 µg/g i.e. 47.4 and 42.1% respectively. It was also observed that at similar concentration of Cd and Cu the decline in seed germination in presence of Cu (67.1-47.4%) were low as compare to Cd (65.5-42.1%) in germinating medium indicating Cd is more toxic than Cu. However, in case of Cu and Cd combination, the germination percent was found minimum i.e. 39.45% indicating that both the metal produces synergistic effect.

Table 4.3. Effect of Cd and Cu on seed germination of *A. paniculata*

Treatments	**Germination %**
Control	70 ± 4.2^{a}
Cu50	67 ± 3.5^{b}
Cu100	56.5 ± 3.2^{d}
Cu150	47.4 ± 2.2^{de}
Cd25	65.5 ± 4.5^{c}
Cd50	49.3 ± 3.7^{d}
Cd75	42.1 ± 2.6
Cu50+Cd25	48.3 ± 2.9^{d}
Cu100+Cd50	44.3 ± 4.1^{f}
Cu150+Cd75	39.45 ± 3.6^{g}

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p < 0.05$).

4.3 Influence on Biomass production of *A. paniculata* exposed to Cu and Cd

Effect of Cd and Cu on *A. paniculata* growth was assessed by observing the biomass of root and shoot after 30, 60 and 90 day of sowing (DAS) (Table 4.4.). Results indicate that as the concentration of Cd and Cu increases in soil the rate of biomass production of roots and shoots decreases. In case of Cu exposure, the maximum rate of biomass production was found at Cu 50 µg/g (2.83-10.24 g) and

minimum at Cu 150 µg/g (2.38-9.20 g). In case of Cd exposure, the maximum rate of biomass production was found at concentration of 25 µg/g (2.69-10.08 g) and minimum at concentration of 75 µg/g (2.47-8.24 g). In case of combination i.e. Cd + Cu, the maximum rate of biomass production was found at Cu50+Cd25 µg/g (2.62-9.59 g) and minimum at Cu150+Cd75 µg/g (2.12-6.95 g). Further, on comparing all the treatments, the rate of decline in biomass production of root and shoot of plant grown at of 50 µg/g Cu were found minimum. Further, it was observed that, in case of metal combination (Cu+Cd) treatment the rate of biomass production of root and shoot were minimum as compared to individual Cd and Cu treatments which shows the level of phytoxicity in combination of Cu and Cd is higher than individual Cu and Cd exposure.

Table 4.4. Influence on Biomass production of *A. paniculata* exposed to Cu and Cd

	Root (g)			**Shoot (g)**		
Treatment	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
Control	0.38 ± 0.02^{a}	0.68 ± 0.03^{a}	1.06 ± 0.04^{a}	2.47 ± 0.06^{a}	5.74 ± 0.07^{a}	9.46 ± 0.07^{a}
Cu 50	0.35 ± 0.01^{a}	0.65 ± 0.04^{ab}	0.93 ± 0.04^{b}	2.48 ± 0.03^{a}	5.71 ± 0.08^{a}	9.31 ± 0.08^{a}
Cu 100	0.33 ± 0.02^{b}	0.56 ± 0.02^{c}	0.86 ± 0.03^{c}	2.15 ± 0.06^{c}	5.43 ± 0.04^{d}	8.72 ± 0.06^{d}
Cu 150	0.31 ± 0.02^{ab}	0.52 ± 0.03^{e}	0.76 ± 0.03^{cd}	2.07 ± 0.05^{d}	4.87 ± 0.05^{e}	8.44 ± 0.07^{d}
Cd25	0.33 ± 0.04^{d}	0.62 ± 0.05^{b}	0.82 ± 0.05^{d}	2.36 ± 0.07^{a}	5.54 ± 0.07^{ab}	9.26 ± 0.08^{b}
Cd50	0.30 ± 0.05^{e}	0.54 ± 0.04^{c}	0.75 ± 0.02^{e}	2.26 ± 0.07^{b}	5.13 ± 0.05^{c}	8.78 ± 0.04^{cd}
Cd75	0.26 ± 0.02^{b}	0.48 ± 0.02^{d}	0.68 ± 0.03^{e}	2.21 ± 0.05^{bc}	4.70 ± 0.08^{f}	7.56 ± 0.04^{f}
Cu50+Cd25	0.28 ± 0.04^{a}	0.58±0.03c	0.78 ± 0.05^{d}	2.34 ± 0.04^{b}	5.10 ± 0.06^{c}	8.81 ± 0.07^{c}
Cu100+Cd50	0.25 ± 0.03^{d}	0.45 ± 0.02^{e}	0.57 ± 0.04^{f}	2.28 ± 0.06^{b}	4.58 ± 0.04^{f}	8.05 ± 0.06^{e}
Cu150+Cd75	0.21 ± 0.01^{e}	0.38 ± 0.02^{f}	0.48 ± 0.03^{g}	1.91 ± 0.05^{e}	3.52 ± 0.07^{g}	6.95 ± 0.05^{g}

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p< 0.05$).

4.4. Influence on biomass production of *V. zizanioides* exposed to Cu, Cd and organochlorine pesticide

The effect of exposure of Cd, Cu and organochlorine pesticides on biomass production of root and shoot of *V. zizanioides* after 30, 60 and 90 days after sowing are presented in Table 4.5. Effect of metal and pesticide exposure on biomass production varied with concentration and with various combinations. As the concentration of Cd, Cu and organochlorine pesticide increases in soil the rate of biomass production of roots and shoots were found to decrease simultaneously. In case of exposure of Cu, the maximum rate of biomass production during 30-90 DAS was found at Cu 50 µg/g (2.33-5.22 g) and minimum at Cu 150 µg/g (2.02-4.32 g). In case of Cd exposure, the maximum rate of biomass production was found at concentration of 25 µg/g (2.23-4.95g) and minimum at 75 µg/g (1.96-3.97 g). Similarly, In case of combination, metals and pesticide, the maximum rate of biomass production was found at lowest given exposure and while minimum at highest concentration. Further, on comparing all the treatments (within groups), the variation among the rate of decline in biomass production on organochlorine pesticide exposure was comparatively lower than the other treatments. Further, comparing with control, the total biomass production during 30-90 DAS was found lowest at Cu150+Cd75+P3, while maximum at Cu50. Results obtained indicates that the combination of organochlorine pesticides with metal were more toxic than that of individual exposure. Further the trend of toxicity (among group) were found as Cd+Cu+P > Cd+Cu > OCPs > Cd > Cu.

Table 4.5. Influence on biomass production of *V. zizanioides* exposed to Cu, Cd and Organochlorine pesticide

	Root (g)			Shoot (g)		
Treatment	**30 DAS**	**60 DAS**	**90 DAS**	**30 DAS**	**60 DAS**	**90 DAS**
Control	0.68±0.02[a]	1.38±0.06[a]	1.76±0.21[a]	1.65±0.13[a]	2.51±0.17[a]	3.46±0.42[a]
Cu 50	0.65±0.04[a]	1.27±0.07[b]	1.73±0.20[a]	1.63±0.06[a]	2.38±0.11[c]	3.35±0.25[b]
Cu 100	0.62±0.02[b]	1.16±0.05[c]	1.64±0.09[b]	1.50±0.07c	2.13±0.12[e]	3.12±0.43[d]
Cu 150	0.57±0.04[c]	1.12±0.04[d]	1.58±0.09[c]	1.45±0.05[d]	2.02±0.16[f]	2.74±0.19[e]
Cd25	0.61±0.03[b]	1.22±0.08[b]	1.69±0.06[b]	1.62±0.08[a]	2.47±0.41[b]	3.26±0.21[c]
Cd50	0.56±0.03[c]	1.17±0.07[c]	1.52±0.2[d]	1.46±0.10[d]	2.23±0.24[d]	2.78±0.17[e]
Cd75	0.51±0.04[d]	1.15±0.05[d]	1.41±0.05[f]	1.44±0.13[d]	2.17±0.53[e]	2.56±0.18[g]
Cu50+Cd25	0.48±0.05[d]	1.08±0.06[e]	1.48±0.10[e]	1.54±0.21[b]	2.21±0.43[d]	2.81±0.21[e]
Cu100+Cd50	0.45±0.02[e]	0.85±0.04[f]	1.27±0.06[g]	1.38±0.04[e]	1.78±0.14[g]	2.45±0.15[g]
Cu150+Cd75	0.41±0.03[f]	0.78±0.06[g]	1.08±0.07[i]	1.21±0.08[g]	1.52±0.12[i]	1.95±0.13[i]
P1	0.46±0.01[e]	0.83±0.04[f]	1.25±0.07[g]	1.27±0.05[f]	1.84±0.06[f]	2.23±0.14[h]
P2	0.42±0.01[f]	0.81±0.03[f]	1.16±0.08[h]	1.23±0.07[g]	1.75±0.07[f]	2.05±0.12[i]
P3	0.41±0.03[f]	0.74±0.05[g]	1.08±0.05[i]	1.21±0.06[g]	1.65±0.05[h]	1.89±0.08[i]
Cu50+Cd25+P1	0.43±0.03[e]	0.82±0.05[f]	1.12±0.20[i]	1.23±0.60[g]	1.48±0.21[i]	1.63±0.09[j]
Cu100+Cd50+P2	0.41±0.01[f]	0.80±0.03[f]	1.11±0.30[i]	1.17±0.71[h]	1.32±0.08[j]	1.62±0.18[j]
Cu150+Cd75+P3	0.41±0.03[f]	0.77±0.06[g]	1.04±0.08[i]	1.13±0.49[h]	1.23±0.28[j]	1.57±0.09[j]

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p < 0.05$).

4.5. Effect on Chlorophyll content in *A. paniculata* exposed of Cu and Cd

Chlorophyll is important phosynthetic pigments mainly compose of chlorophyll *a* and *b.* Addition of Cu and Cd in soil significantly affected the chlorophyll content in *A. paniculata*. As the concentration of Cu and Cd in soil increased the level of chlorophyll content in leaves decreased as compared to control (Table 4.6). In comparison to control, the decrease in total chlorophyll content was about 8.11, 23.42, 46% at 30 DAS; 8.66, 22.83, 47.24% at 60 DAS and 7.64, 27.78, 48.61 % at 90 DAS at 50, 100, 150 mg Cu kg^{-1} of soil respectively. Similarly, with Cd treatment, the decrease in total chlorophyll content was found to be 37.39, 47.75, 59.14% at 30 DAS; 43.31, 53.54, 58.66% at 60 DAS; 48.61, 58.68, 63.96 % at 90 DAS in 25, 50, 75 mg Cd kg^{-1} of soil respectively. While in case of treatment of combination of Cd and Cu, the decrease in total chlorophyll content was observed as 47.75, 56.76, 69.37 % at 30 DAS; 48.03, 59.84, 66.54 at 60 DAS; 53.13, 60.76, 69.27 at 90 DAS in Cu50 + Cd25, Cu100+Cd50, Cu150 + Cd75 $mgkg^{-1}$of soil respectively.

Table 4.6. Effect on Chlorophyll content (mg/g) in *A. paniculata* exposed of Cu and Cd

	30 DAS			60 DAS			90 DAS		
Treatments	**Chl a**	**Chl b**	**Total Chl**	**Chl a**	**Chl b**	**Total Chl**	**Chl a**	**Chl b**	**Total Chl**
Control	1.87 ± 0.14^{a}	0.35 ± 0.06^{a}	2.22 ± 0.24^{a}	2.17 ± 0.14^{a}	0.37 ± 0.02^{a}	2.54 ± 0.03^{a}	2.43 ± 0.32^{a}	0.45 ± 0.02^{a}	2.88 ± 0.42^{a}
Cu50	1.77 ± 0.16^{b}	0.27 ± 0.04^{b}	2.04 ± 0.18^{b}	2.01 ± 0.13^{b}	0.31 ± 0.03^{b}	2.32 ± 0.03^{b}	2.32 ± 0.04^{b}	0.34 ± 0.04^{b}	2.66 ± 0.32^{b}
Cu100	1.54 ± 0.12^{c}	0.16 ± 0.01^{c}	1.70 ± 0.11^{a}	1.74 ± 0.06^{c}	0.22 ± 0.012^{c}	1.96 ± 0.07^{c}	1.85 ± 0.21^{c}	0.23 ± 0.05^{c}	2.08 ± 0.49^{c}
Cu150	1.08 ± 0.15^{e}	0.11 ± 0.02^{d}	1.19 ± 0.06^{d}	1.18 ± 0.07^{de}	0.16 ± 0.01^{e}	1.34 ± 0.04^{e}	1.31 ± 0.08^{d}	0.17 ± 0.04^{d}	1.48 ± 0.52^{e}
Cd25	1.26 ± 0.06^{d}	0.13 ± 0.01^{cd}	1.39 ± 0.12^{c}	1.26 ± 0.10^{d}	0.18 ± 0.01^{d}	1.44 ± 0.07^{d}	1.28 ± 0.04^{d}	0.25 ± 0.02^{c}	1.54 ± 0.37^{d}
Cd50	1.06 ± 0.07^{e}	0.10 ± 0.01^{d}	1.16 ± 0.07^{d}	1.03 ± 0.07^{f}	0.15 ± 0.01^{ef}	1.18 ± 0.02^{f}	1.03 ± 0.03^{e}	0.16 ± 0.05^{d}	1.19 ± 0.22^{g}
Cd75	0.82 ± 0.04^{f}	0.09 ± 0.08^{d}	0.91 ± 0.06^{e}	0.94 ± 0.06^{g}	0.11 ± 0.01^{g}	1.05 ± 0.02^{g}	0.95 ± 0.02^{ef}	0.09 ± 0.003^{f}	1.04 ± 0.08^{h}
Cu50+Cd25	1.02 ± 0.06^{e}	0.14 ± 0.02^{c}	1.16 ± 0.07^{d}	1.15 ± 0.08^{e}	0.17 ± 0.01^{e}	1.32 ± 0.03^{e}	1.20 ± 0.05^{d}	0.15 ± 0.06^{d}	1.35 ± 0.21^{f}
Cu100+Cd50	0.86 ± 0.05^{f}	0.10 ± 0.04^{d}	0.96 ± 0.04^{e}	0.89 ± 0.04^{g}	0.13 ± 0.01^{g}	1.02 ± 0.04^{g}	1.01 ± 0.03^{e}	0.12 ± 0.02^{e}	1.13 ± 0.23^{gh}
Cu150+Cd75	0.63 ± 0.02^{g}	0.05 ± 0.001^{e}	0.68 ± 0.02^{f}	0.78 ± 0.03^{h}	0.07 ± 0.003^{h}	0.85 ± 0.03^{h}	0.81 ± 0.04^{f}	0.08 ± 0.003^{f}	0.89 ± 0.06^{h}

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p< 0.05$).

4.6. Effect on Chlorophyll (mg/g) content in *V. zizanioides* in response to application of Cd, Cu and organochlorine pesticide.

Photosynthesis is a vital process for plant growth and development. The interventral chlorosis of leaves is the first observable sign of phytotoxicity caused by heavy metals which is associated with chlorophyll content (Stiborova et al., 1986; Aibibu et al., 2010). At lower concentration, both chlorophyll a and b contents were found to be higher (Table 4.7). With increase in concentration of Cd, Cu and organochlorine pesticide increases the total chlorophyll content was found to be declined. In case of Cu stress on 30 DAS, at lowest concentration (50 $mgkg^{-1}$) chlorophyll content was found to be almost equal (1.80 mg/g FW) to the control (1.83 mg/g FW) while at highest centration i.e. 150 $mgkg^{-1}$ total chlorophyll content was recorded as 1.49 mg/g FW. The concentration dependent reduction percentage in total chlorophyll was recorded as 1.83 to 18.58, 9.26 to 26 and 13.78 to 28.44% of control plants at 30, 60 and 90 DAS respectively. However, the level of total chlorophyll content was slightly increased at 60 and 90 DAS. In case of Cd stress, the total chlorophyll content was decreased on increasing the level of Cd, the reduction percentage was found to be 13.11 to 28.58, 14.35 to 36.11 and 21.33 to 42.53% with respect to control at 30, 60 and 90 DAS. On increasing the level of pesticides, the reduction percentage was recorded as 28.42 to 46.45, 41.20 to 51.67 and 52.44 to 60% of control level on 30, 60 and 90 DAS respectively. Furthermore, the plant growing in the soil contaminated with combination of metal (Cu + Cd) and metal along with pesticides also show the decline in the level of chlorophyll content on increase in the level of treatments. The maximum level of concentration dependent reduction was found to be 47 to 62% in plant growing in soil treated with highest level of Cu, Cd and organochlorine pesticide. The reduction in chlorophyll clearly reveals that the co-

contamination of Cd, Cu and organochlorine pesticides is more toxic than the any individual agent. However, on comparison of individual treatment with Cu, Cd and organochlorine pesticide, the pesticide treated plants showed the highest reduction in chlorophyll indicating its higher toxic nature than Cu and Cd to the exposed plant.

Table 4.7. Effect on Chlorophyll content (mg/g) in *V. zizanioides* exposed to Cu, Cd and organochlorine pesticides

	30 DAS			60 DAS			90 DAS		
Treatments	**Chl a**	**Chl b**	**Total Chl**	**Chl a**	**Chl b**	**Total Chl**	**Chl a**	**Chl b**	**Total Chl**
Control	1.52±0.13^{a}	0.31±0.41^{a}	1.83±0.24^{a}	1.83±0.21^{a}	0.33±0.05^{a}	2.16±0.23^{a}	1.93±0.17^{a}	0.32±0.015^{a}	2.25±0.43^{a}
Cu50	1.51±0.11^{a}	0.29±0.17^{a}	1.80±0.21^{a}	1.66±0.23^{b}	0.30±0.06^{a}	1.96±0.25^{b}	1.72±0.21^{b}	0.22±0.014^{b}	1.94±0.21^{b}
Cu100	1.44±0.13^{b}	0.24±0.03^{c}	1.68±0.23^{b}	1.44±0.13^{d}	0.26±0.07^{c}	1.70±0.31^{d}	1.67±0.13^{c}	0.18±0.031^{c}	1.85±0.42^{c}
Cu150	1.30±0.16^{c}	0.19±0.04^{d}	1.49±0.18^{d}	1.38±0.21^{e}	0.21±0.04^{d}	1.59±0.12^{e}	1.49±0.12^{e}	0.15±0.023^{d}	1.61±0.32^{e}
Cd25	1.32±0.11^{c}	0.27±0.013^{ab}	1.59±0.21^{c}	1.57±0.14^{c}	0.28±0.04^{b}	1.85±0.12^{c}	1.60±0.11^{d}	0.17±0.014^{c}	1.77±0.24^{d}
Cd50	1.26±0.10^{d}	0.23±0.023^{d}	1.49±0.17^{d}	1.33±0.12^{f}	0.26±0.03^{c}	1.59±0.08^{f}	1.37±0.10^{f}	0.13±0.04^{d}	1.50±0.53
Cd75	1.12±0.12^{e}	0.19±0.011^{d}	1.31±0.10^{e}	1.18±0.09^{g}	0.20±0.01^{d}	1.38±0.05^{g}	1.21±0.08^{g}	0.08±0.004^{e}	1.29±0.21^{f}
Cu50+Cd25	1.02±0.08^{f}	0.24±0.012^{c}	1.26±0.11^{e}	1.14±0.10^{h}	0.17±0.04^{e}	1.31±0.23^{h}	1.09±0.06^{h}	0.15±0.051^{d}	1.24±0.11^{g}
Cu100+Cd50	0.96±0.04^{f}	0.17±0.021^{d}	1.13±0.09^{f}	1.09±0.05^{i}	0.13±0.05^{e}	1.22±0.04^{i}	1.02±0.07^{i}	0.12±0.021^{d}	1.14±0.08^{i}
Cu150+Cd75	0.83±0.02^{i}	0.11±0.052^{e}	0.94±0.05^{f}	0.95±0.04^{i}	0.07±0.004^{f}	1.02±0.03^{j}	0.86±0.04^{j}	0.08±0.003^{e}	0.94±0.05^{jk}
P1	0.92±0.04^{f}	0.06±0.004^{f}	0.98±0.02^{f}	0.91±0.07^{j}	0.05±0.006^{f}	0.96±0.06^{j}	0.98±0.04^{i}	0.09±0.006^{e}	0.97±0.06^{j}
P2	0.90±0.05^{f}	0.02±0.007^{f}	0.92±0.04^{g}	0.91±0.04^{j}	0.02±0.003^{g}	0.93±0.07^{k}	0.91±0.05^{ig}	0.06±0.005^{de}	0.97±0.04^{j}
P3	0.87±0.02^{g}	0.04±0.002^{f}	0.91±0.03^{g}	0.85±0.05^{j}	0.04±0.006^{f}	0.89±0.06^{k}	0.85±0.04^{j}	0.05±0.004^{ef}	0.90±0.03^{k}
Cu50+Cd25+P1	0.95±0.057^{f}	0.04±0.006^{f}	0.99±0.04^{f}	1.11±0.08^{h}	0.06±0.003^{f}	1.17±0.13^{i}	1.12±0.21^{h}	0.06±0.003^{e}	1.18±0.50^{h}
Cu100+Cd50+P2	0.91±0.64^{f}	0.03±0.002^{f}	0.94±0.05^{g}	1.02±0.08^{j}	0.04±0.002^{f}	1.06±0.09^{j}	0.98±0.72^{i}	0.05±0.004^{ef}	1.03±0.70^{i}
Cu150+Cd75+P3	0.86±0.42^{g}	0.03±0.001^{f}	0.89±0.03^{g}	0.94±0.06^{j}	0.02±0.003^{g}	0.96±0.06^{k}	0.83±0.54^{j}	0.02±0.005^{f}	0.85±0.05k

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p< 0.05$).

4.7. Effect on the level of Protein and Lipid peroxidation

4.7.1. Influence on protein content

Change in protein content of *A. paniculata* leave under Cd and Cu exposed conditions are show in Fig 4.1.1. The result showed that Cu and Cd had inhibitory effect on protein content. Exposure to high level of Cu and Cd caused significant decrease in protein content. Presence of high concentration of Cu and Cd in soil resulted in reduction of protein content. The protein content of leave were reduced by 9.29, 24.06, 40.40% in 30 DAS; 5.21, 19.41, 33.55% at 60 DAS and 5.12, 13.65, 29.50% at 90 DAS on exposure to 50, 100, 150 mg Cu kg^{-1} of soil respectively, as compared to control. In soil contaminated with 25, 50 and 75 ppm Cd, the observed reduction in protein content was 26.84, 39.84, 53.57% at 30 DAS; 27.37, 38.81, 51.97% at 60 DAS and 18.95, 34.13, 46.64% at 90 DAS respectively, as compared to control. Similarly in case of combination of Cd and Cu exposure the reduction was found to be 42.80, 50.47, 61.51% at 30 DAS; 34.89, 51.64, 61.20 % at 60 DAS and 26.79, 45.37, 52.45 % at 90 DAS in plant exposed to Cu50+Cd25, Cu100+Cd50 and Cu150+Cd75 mg Kg^{-1} of soil respectively, as compared to control. The above result indicates that the exposure of high level of Cd and Cu had toxic effect on *A. paniculata.*

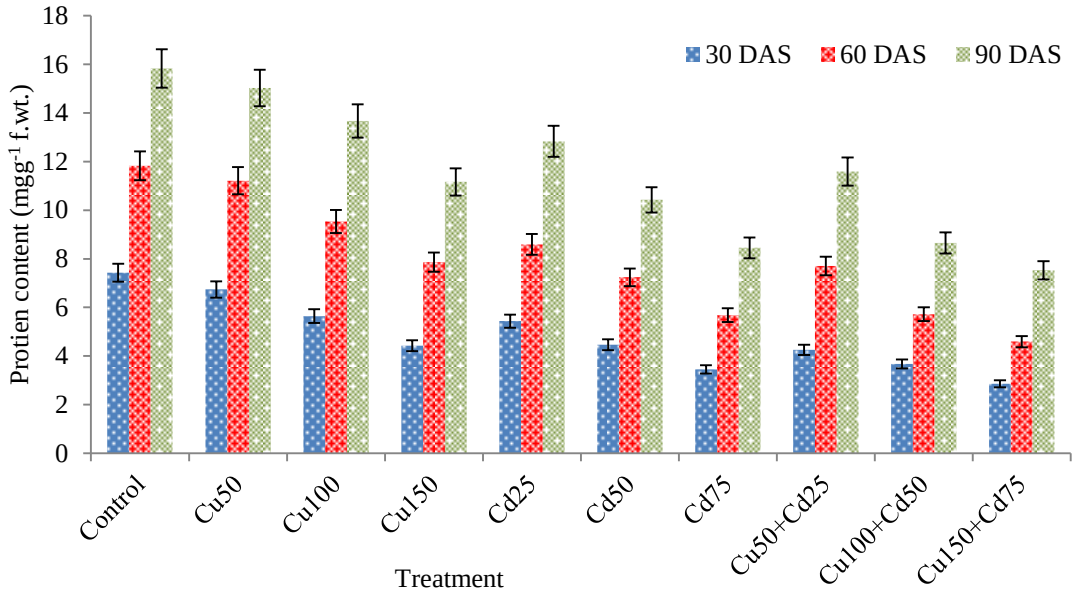


Fig. 4.1.1. Effect of Cd and Cu on protein content in leaves of *A. paniculata*

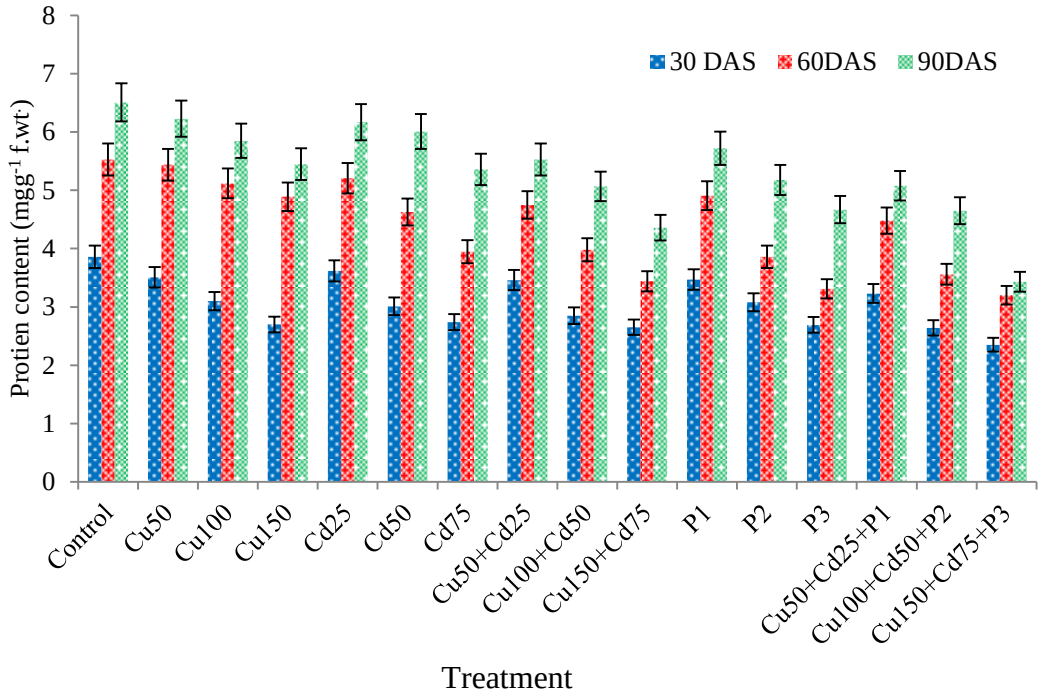


Fig. 4.1.2. Effect of Cd, Cu and organochlorine pesticide on protein content in leaves of *V. zizanioides*

It is reported that the protein content is a significant indicator for reversible and irreversible alterations in the metabolism. It is also recognized indicator to a wide variety of environmental stresses like metal and organochlorine pesticides (Singh et al., 2003; Liu et al., 2005; Sulaiman and Mohammad, 2013). Effect of exposure of Cd, Cu and organochlorine pesticide on protein content are shown in Fig 4.1.2. Results clearly indicates that the presence of Cd, Cu and pesticide in soil had negative effect; protein content decreased on increasing the level of Cd, Cu and organochlorine pesticides. The protein content in leaves of exposed plants was reduced by 9.07, 19.69, 30.05% at 30 DAS; 1.63, 7.41, 11.57% at 60 DAS and 4.30, 10.14, 16.28% at 90 DAS on exposure to be 50, 100, 150 mg Cu kg^{-1} of soil respectively, as compared to control. In plant exposed to Cd at 25, 50 and 75 $mgkg^{-1}$ of soil, the reduction % was observed as 6.22, 22.02, 29.02% at 30 DAS; 5.79, 16.27, 28.57% at 60 DAS and 5.22, 7.68, 46.64% at 90 DAS respectively, as compared to control. Further, the similar trend in reduction of protein content was also observed in the plant leave growing in presence of pesticide industry effluent at 25, 50 and 100%. Similarly in case of combined effect of Cd and Cu stress, the reduction was observed as 10.36, 26.17, 31.35% at 30 DAS; 14.10, 28.03, 37.79 % at 60 DAS and 15.05, 22.12, 33.03 % at 90 DAS in plant growing at Cu50+Cd25, Cu100+Cd50 and Cu150+Cd75 mg kg^{-1} of soil respectively, as compared to control. The maximum reduction was observed in plant exposed with the Cd, Cu and organochlorine pesticide in combination which was as follows: 16.32, 31.61, 39.12 % at 30 DAS; 18.99, 35.62, 42.13% at 60 DAS and 21.97, 28.57, 47.31% at 90 DAS under treatment of Cu50+Cd25+P1, Cu100+Cd50+P2 and Cu150+Cd75+P3 respectively as compared to control. The results also showed that the combination of Cd, Cu and organochlorine pesticide had strongest inhibitory effect on protein content than the other treatments. Furthermore,

the above result clearly reveals that the reduction of protein content in *V. zizanioides* is comparatively lower than the *A. paniculata* indicating its high tolerance.

4.7.2. Effect on lipid peroxidation

It is reported that the main consequence of metal and pesticides contamination in growing medium is the enhanced production of reactive oxygen species (ROS) which consecutively causes oxidative stress. The outcomes of this oxidative stress often come in the form of radical chain reaction that damages membrane lipids by peroxidation. The lipid peroxidation is considered as a sensitive measureof oxidative damage and therefore appropriate for the biomarker of oxidative stress (Polle et al., Aibibu et al., 2010). It is reported that the malondialdehyde (MDA), a primary lipid peroxidation by-product can be used for the estimation of level of lipid peroxidation. Accumulation of high level of MDA often indicates severe lipid peroxidation (Aibibu et al., 2010). The level and production of MDA due to lipid peroxidation under Cd, Cu and organochlorine pesticide stress condition are shown in the Fig. 4.2.1 and 4.2.2. Result showed that the accumulation of MDA increases significantly on increasing the concentration of Cd, Cu and organochlorine pesticide. Several researches showed that the presence of metal and pesticide in soil caused increased level of MDA in plant tissue (Aibibu et al., 2010; Zong et al., 2017; Chakraborty et al., 2017).Results also revealed that the combination of metal and pesticide leads to generation high level of MDA in comparison to other individual treatments indicating the greater level of production of superoxide radicals resulting increased oxidative stress and lipid peroxidation.

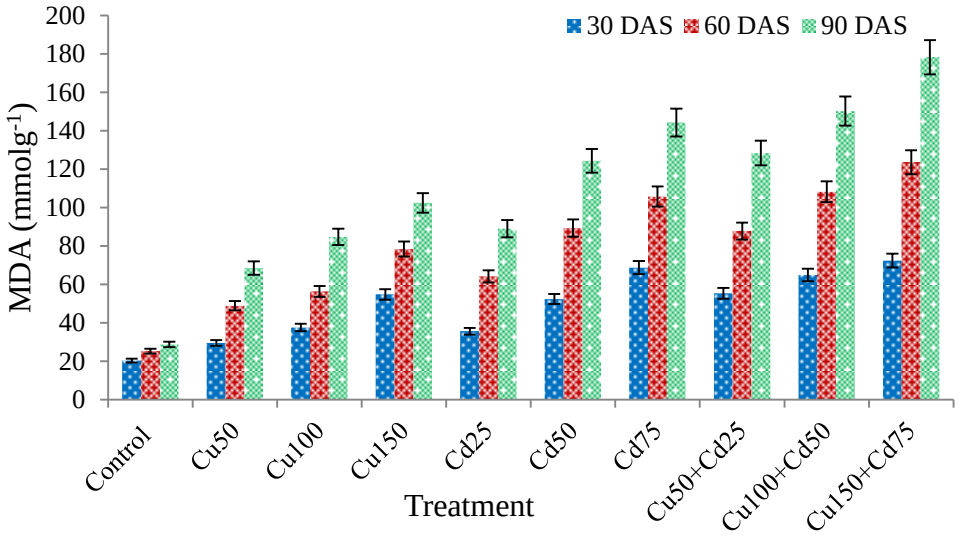


Fig 4.21. Effect on level of MDA production in *A. paniculata* under Cu and Cd stress

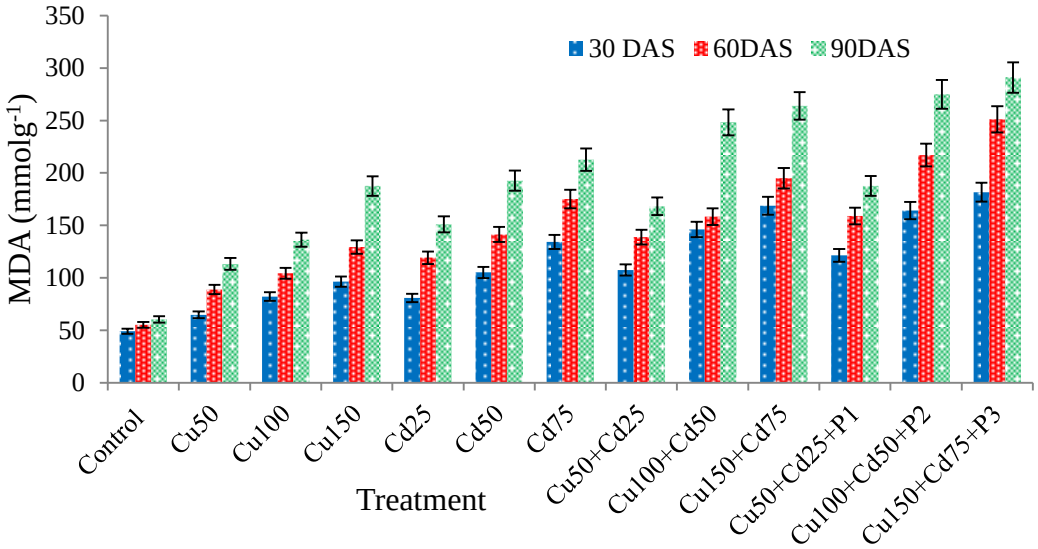


Fig 4.2.2. Effect on level of MDA production in *V. zizanioides* under Cu, Cd and organochlorine pesticide stresses

4.9. Effect on level of antioxidative enzymes (peroxidase and Catalase) on exposure to Cu, Cd and organochlorine pesticide

It is reported that the plant exposure to metal and pesticides can cause oxidative stress by generating superoxide radicals that leads to increased lipid peroxidation (MDA production) and oxidative stress (ROS). ROS generated during

oxidative stress, rapidly react with all types of biomolecules such as protein and nucleic acids, leading to irreversible metabolic dysfunction and finally cell death (Singh et al., 2006). Therefore, the stimulation of antioxidant enzymes like peroxidase (POD) and catalase (CAT)is a vital protective mechanism to alleviate the oxidative damage in contaminated environment.

POD is a principle enzymes involved in the eradication of active oxygen species (AOS).Change in the generation of POD under Cd, Cu and organochlorine pesticide contaminated environment are shown in Fig 4.3.1 and Fig 4.3.2 The level of POD activity was found to be higher at higher concentration of Cd, Cu and organochlorine pesticides. However, in Cd treated plant, the POD activity was found to be higher as compare to the Cu and pesticide. It shows that the Cd is strongest producer of ROS amongst all treatments. However, in case of metal combination with pesticide, the plants showed highest POD activity at highest concentration (Cu150+Cd75+P3).Further, the POD activity was found to be high in *V. zizanioides* as compared to *A. paniculata* indicating that the *V. zizanioides* was much able to maintain the high POD activity at higher level of stresses (Fig. 4.3.1 and Fig 4.3.2).It is reported that the POD also involved in lignin biosynthesis to build up a physical barrier against toxic metals (Scaler et al., 1995). It also indicates that *V. zizanioides* can efficiently minimize the damage caused by metal toxicity.

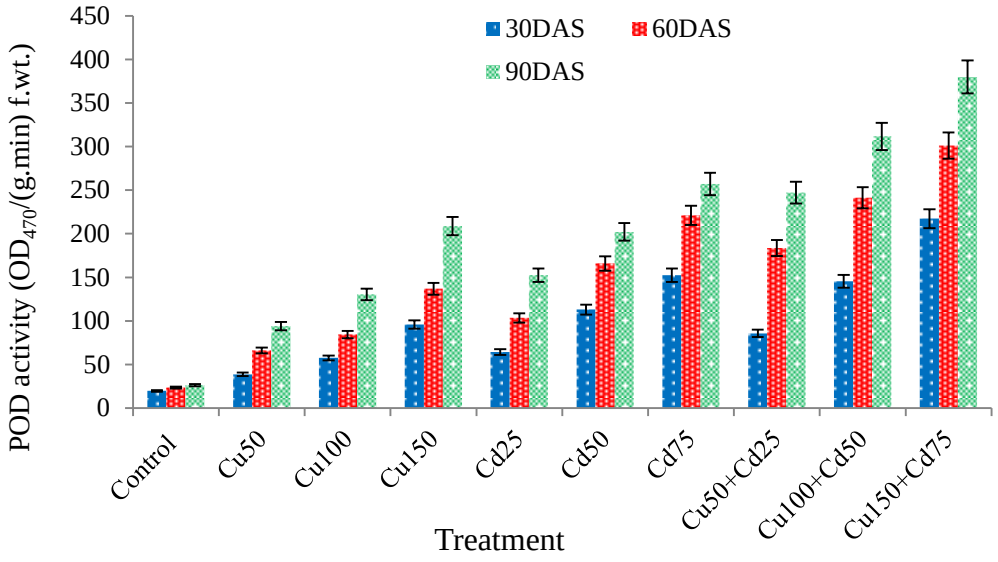
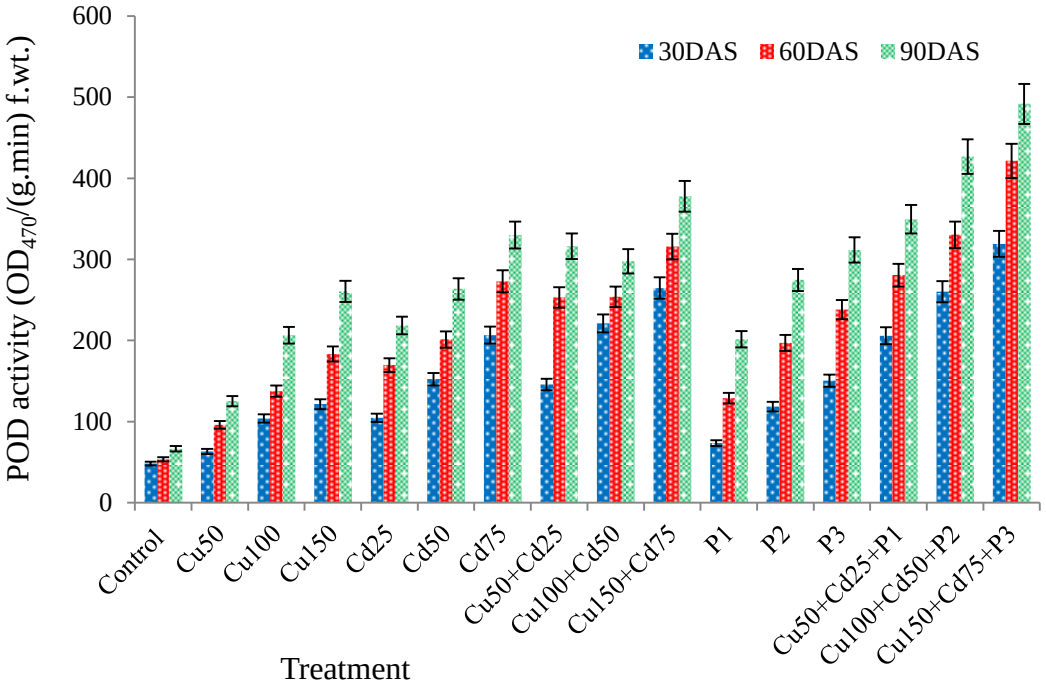


Fig. 4.3.1. Effect on level of peroxidase in *A.paniculata*on exposure to Cu and Cd



(A) *V. zizanioides*

Fig. 4.3.2. Effect on level of peroxidase in *V. zizanioides* on exposure to Cu, Cd and organochlorine pesticide

Catalase (CAT) is an important enzyme which participated in elimination of ROSs in plant cells. It is reported that the CAT involves in main defense system against accumulation and toxicity of H_2O_2. It catalyzes H_2O_2 and converts into water and oxygen. The CAT activity against the Cd, Cu and organochlorine pesticides are shows in Fig 4.4.1 and Fig 4.4.2. The result show that the CAT activity increased on increased level of Cd, Cu and organochlorine pesticides. CAT activity was observed higher in case of treatments in combination (Cd+Cu/Cd+Cu+Pesticide) than individual (Cd/Cu/pesticide) contaminants indicating that the multiple contaminations in soil cause generation of higher ROS and toxicity than individual contaminants. The CAT activity in presence of metals and pesticide combination (Cd+Cu+Pesticide) was found higher than the exposure of only metals combination (Cd+Cu). This indicates that as the numbers of contaminants increased the toxicity/generation of ROS also increased hence the CAT activity increased simultaneously. Further, *V. zizaniodes* showed high catalase activity than the*A. paniculata,*at same concentration of metal. Thus, we can conclude that the *V. zizaniodes* has more potential than *A. Paniculata* to protect against the Cd and Cu involving stimulation of several enzymes serve as key factor of antioxidant defense mechanism.

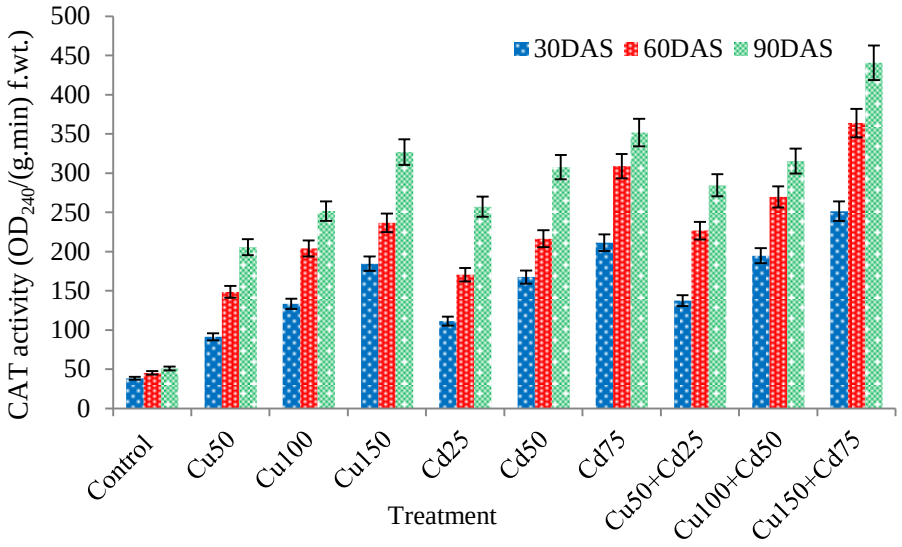
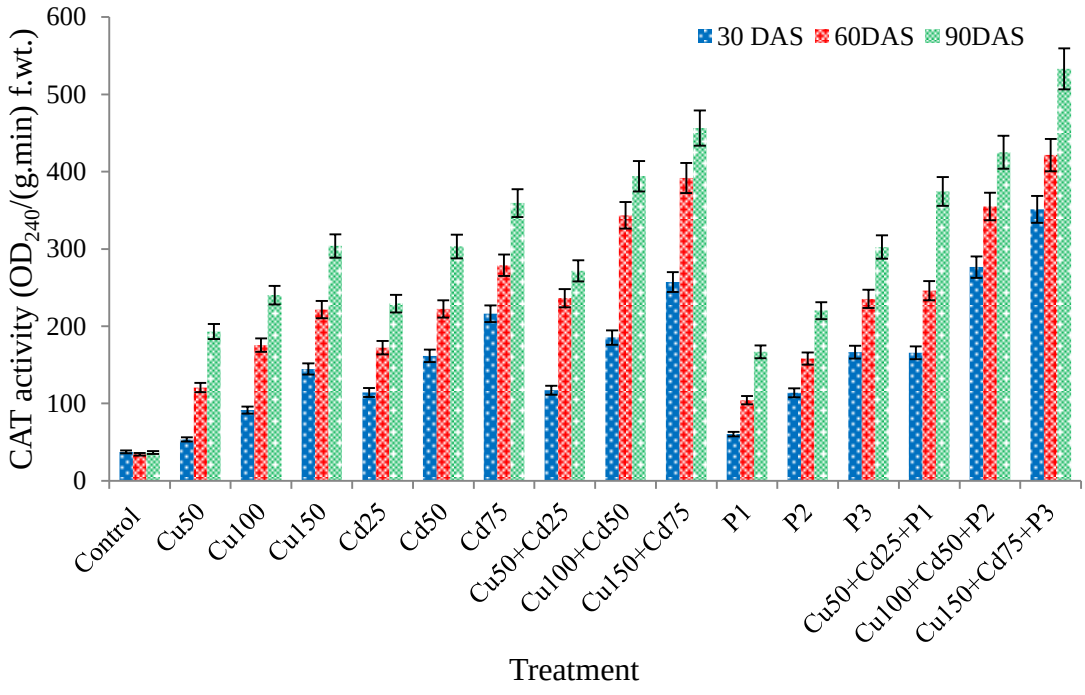


Fig. 4.4.1. Effect on level of catalase in *A.paniculata* on exposure to Cu and Cd



(B)

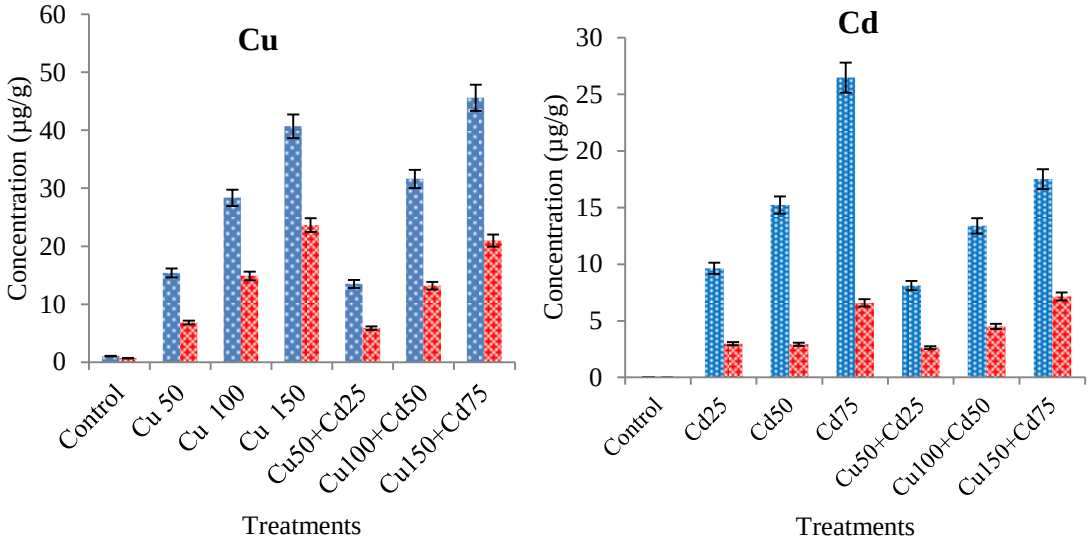
Fig. 4.4.2. Effect on level of catalase in *V. zizanioides* on exposure to Cu, Cd and organochlorine pesticide

Chapter 5

Studies on individual and collective phytoremediation potential of A. paniculata and V. zizanioides to remediate Cu, Cd and organochlorine pesticides

5.1. Accumulation of Cu and Cd in *A. paniculata*

It was observed that the accumulation of Cu and Cd increased significantly (p> 0.005) on increasing their concentration in the soil and exposure time (p> 0.005). *A. paniculata* plant have accumulated significant amount of Cu and Cd in their roots and shoots (Fig 5.1). However, accumulation of Cu and Cd in roots was higher than the shoots in all treatments including control. Accumulation of Cu in roots and shoots at 30, 60 and 90 DAS was ranged between 13.49-45.60, 27.64-67.82 & 46.73-145.64 and 5.86-23.63, 17.75-31.39 & 19.76-67.32 $\mu g g^{-1}$dwt respectively. Similarly, Cd accumulation in roots and shoots at 30, 60 and 90 DAS was ranged between 8.11-17.51, 12.99- 27.61 & 24.30-54.76 and 2.63-7.14, 4.31-9.59 & 6.67-16.31 $\mu g g^{-1}$dwt respectively. In case of soil treated with Cd and Cu combination i.e. Cd+Cu, the level of Cd or Cu accumulation was lower than their individual treatment (non-combination). Further, it was also observed that the amount of Cu accumulated in shoots was comparatively higher than Cd. Probably being essential as a micronutrient it is prerequisite for plant growth.



(A)

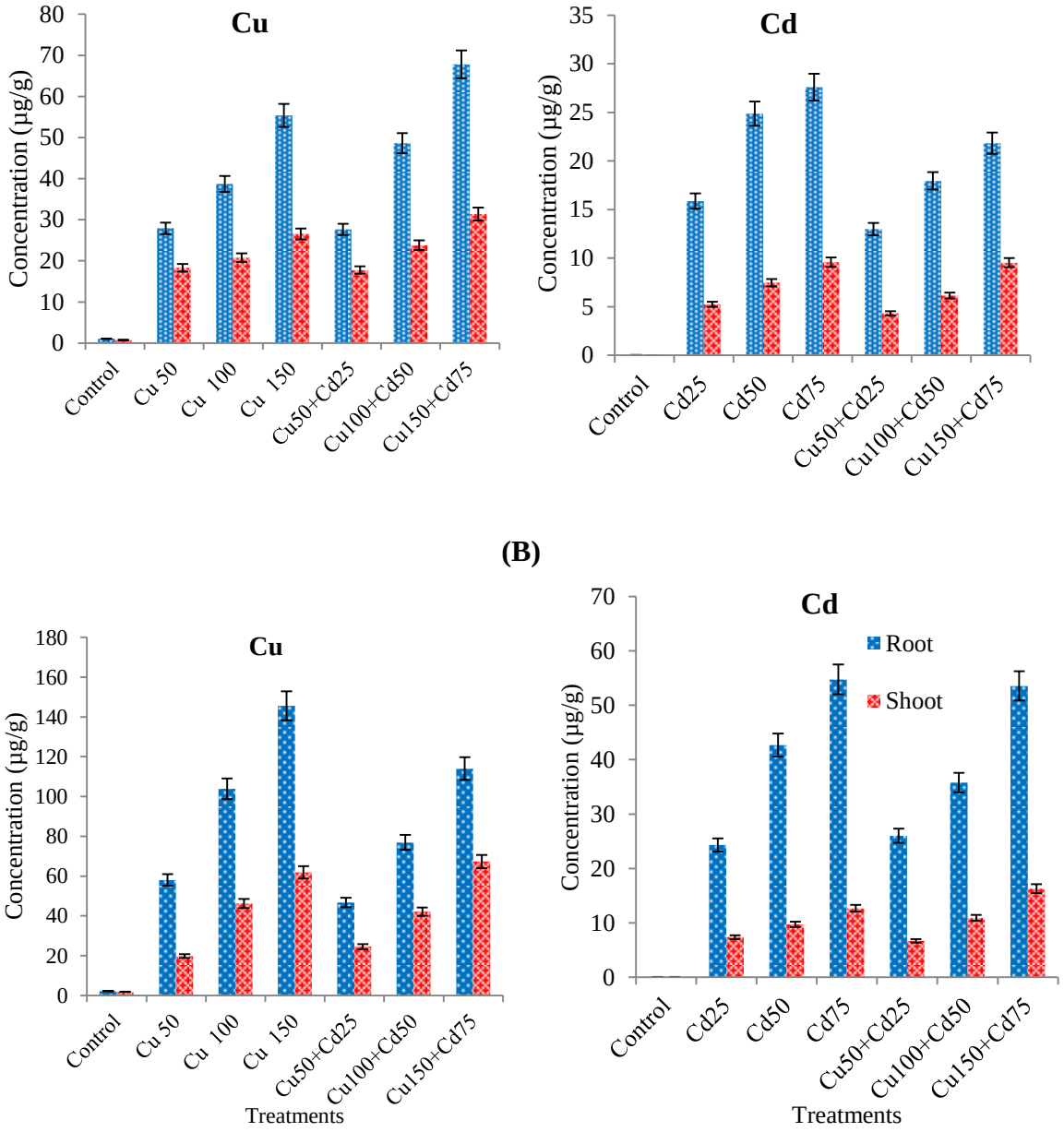


Fig 5.1. Accumulation (µgg^{-1}dwt) of Cu and Cd in root and shoot of *A. paniculata* at 30 DAS (A) 60 DAS (B) and (C) 90 DAS.

5.2. Accumulation of Cu, Cd and Organochlorine pesticides in *V. zizanioides*

The uptake and accumulation of Cu, Cd and organochlorine pesticides (OCPs) in roots and shoots of *V. zizanioides* varied significantly at different level of contamination (p> 0.005) (Fig 5.2 - 5.4). It is reported that *V. zizanioides* is tolerant to Cu and can survive in soil contaminated with upto 1762 mgkg^{-1} of Cu (Danh, 2009).

In present study, accumulation of Cu in roots ranged from 72.99 to 178.28, 115.9–396.94 and 276.24–513.66 $\mu g g^{-1}$dwt while in shoot it ranged between 5.19–12.50, 20.48–45.54 and 33.38-58.33 $\mu g g^{-1}$dwt at 30, 60 and 90 DAS, respectively. The maximum accumulation of Cu in *V. zizanioides* was found to be 513.66 $\mu g g^{-1}$in soil treated with 150 $\mu g g^{-1}$. Cd is well known for its toxicity to plant and its threshold limit in soil is about 1.5 $mg kg^{-1}$ (Baker and Eldershaw, 1993). In the present study it was observed that the Cd accumulation in roots ranges from 197.85 to 569.07, 326.91–669.84 and 498.07–1069.62 $\mu g g^{-1}$dwt while the corresponding values in shoots were 8.74–16.62, 13.92–15.19 and 16.7–23.12 $\mu g g^{-1}$dwt at 30, 60 and 90 DAS respectively; results manifest the tolerant nature of *V. zizanioides* against Cd. Similarly, accumulation of organochlorine pesticides in roots ranged from 1.62 to 8.01, 3.65–11.63 and 5.71–17.04 $ng g^{-1}$dwt while the corresponding values in shoots were 0.28-3.53, 3.65–11.63 and 3.93-11.11 $ng g^{-1}$dwt at 30, 60 and 90 DAS respectively. It has been suggested that the threshold limit of Cd and Cd in shoots of *V. zizanioides* ranged between 13–15 $\mu g g^{-1}$and 45–48 $\mu g g^{-1}$dwt. Results showed that the accumulation of Cd, Cu and organochlorine pesticides were higher in roots than shoots. Similar results were also reported by several authors (Das and Maiti, 2009; Dahn et al., 2009; Danh et al., 2010; Aibibu et al., 2010).

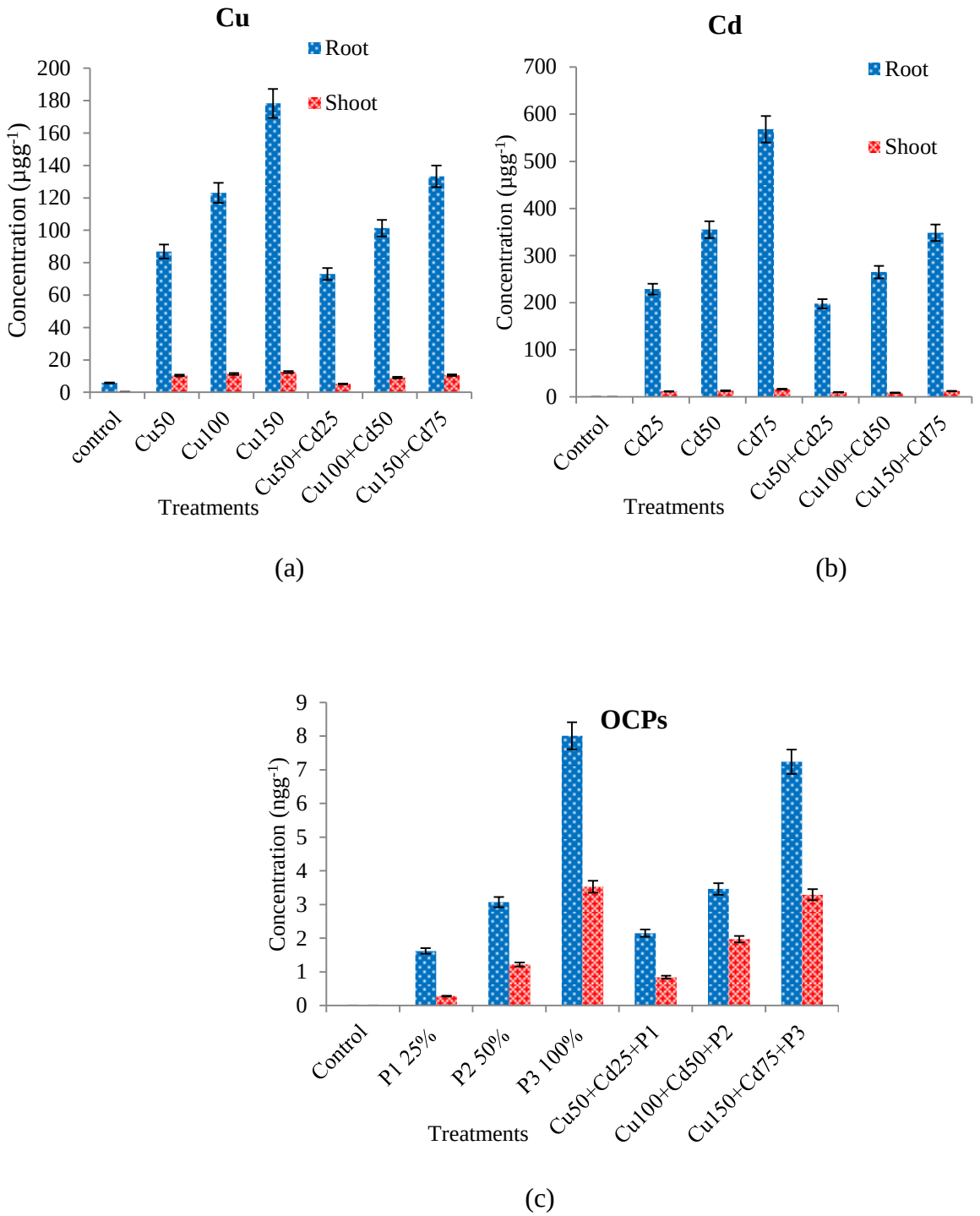
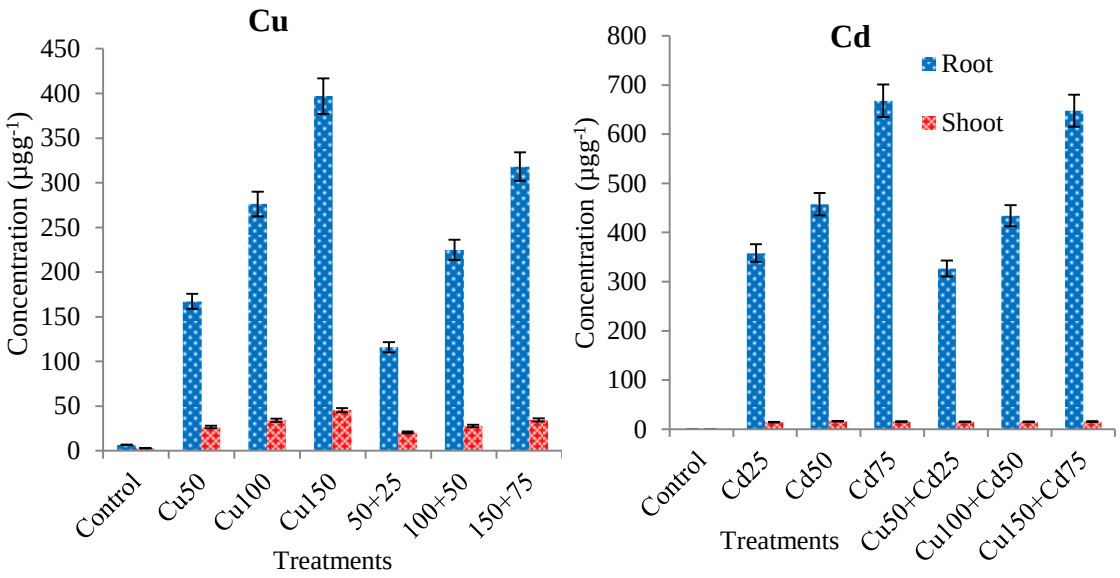
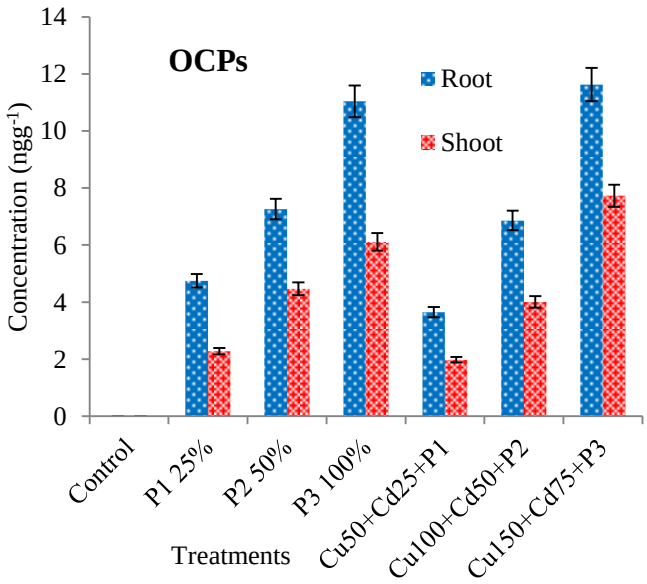


Fig 5.2. Accumulation of Cu, Cd (µgg^{-1}dwt) and organochlorine pesticides (OCPs) (ngg^{-1}dwt) in root and shoot of *V. zizanioides* at 30 DAS



(a)

(b)



(c)

Fig 5.3. Accumulation of Cu, Cd (µgg^{-1}dwt) and organochlorine pesticides (OCPs) (ngg^{-1}dwt) in root and shoot of *V. zizanioides* at 60 DAS

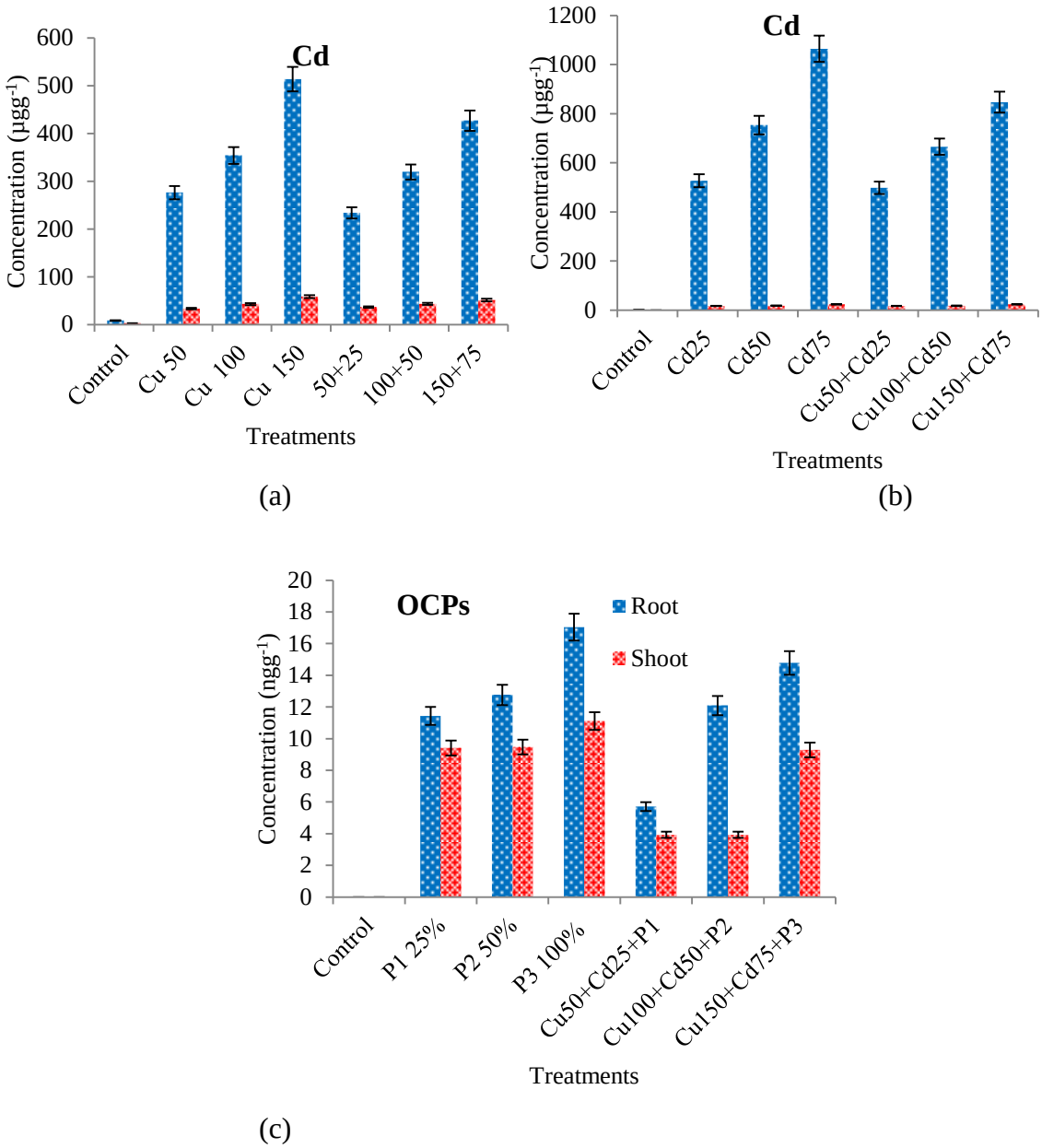


Fig 5.4. Accumulation of Cu, Cd (µgg^{-1}dwt) and organochlorine pesticides (OCPs) (ngg^{-1}dwt) in root and shoot of *V. zizanioides* at 90 DAS

5.3. Accumulation of Cu, Cd and OCPs in *A. paniculata* and *V. zizanioides* grown in soil co-contaminated with Cu, Cd and organochlorine pesticides

The uptake and accumulation of Cu, Cd and Organochlorine pesticides (OCPs) in roots and shoots of *A. paniculata* and *V. zizanioides* grown in soil co-contaminated with Cu, Cd and organochlorine pesticides varied significantly at

different level of contamination (Table 5.1). In case of plant growing in the combination, the level of their individual metal accumulation was observed lower than the plant grown in non-combination. However, the total removal of metal and OCPs from soil was higher than the soil in which plant were grown separately. The maximum accumulation of Cu was observed as 120.64 and 454.66 $\mu g g^{-1}$dwt in *A. Paniculata* and *V. zizanioides,* respectively in soil treated with 150 $\mu g g^{-1}$.The maximum accumulation of Cd was observed as 93.76 and 985.62 $\mu g g^{-1}$dwt in *A. paniculata* and *V. zizanioides* respectively in soil treated with 75 $\mu g g^{-1}$. Further, *A. paniculata* could not survive in all treatments of OCPs except P1 (25%). Hence the study of collective phytoremediation potential of selected plant species for organochlorine pesticides could not be further carried out.

Table 5.1. Accumulation of Cu, Cd and OCPs in *A. paniculata* and *V. zizanioides* growing in soil co-contaminated with Cu, Cd and Organochlorine pesticides.

	Root		**Shoot**			**Root**		**Shoot**			**Root**		**Shoot**	
	Cu (μgg^{-1})					**Cd (μgg^{-1})**					**OCPs (ngg^{-1})**			
						30 DAS								
Treatment	AP	VZ	AP	VZ	Treatment	AP	VZ	AP	VZ	Treatment	AP	VZ	AP	VZ
Control	0.8±0.01^{g}	1.1±0.01^{g}	0.4±0.002^{g}	0.02±0.002^{g}	Control	0.00^{g}	0.01±0.001^{f}	BDL	BDL	Control	BDL	BDL	BDL	BDL
Cu50	2.4±0.01^{f}	40.8±1.3^{e}	1.8±0.2^{f}	3.36±0.21^{d}	Cd25	6.6±0.2^{e}	172.5±14.2^{d}	0.99±0.03^{e}	4.51±0.2^{d}	P1 25%	BDL	0.09±0.002^{f}	BDL	0.16±0.01^{f}
Cu100	18.3±1.7^{d}	77.1±3.7^{c}	9.9±0.3^{c}	4.33±0.4^{b}	Cd50	12.2±1.1^{c}	298.9±21.7^{b}	1.9±0.07^{c}	6.1±0.6^{b}	P2 50%	0.001	1.54±0.02^{d}	BDL	1.1±0.05^{d}
Cu150	30.7±2.6^{b}	132.3±9.3^{a}	18.6±0.8^{a}	5.5±0.3^{a}	Cd75	23.5±1.6^{a}	512.1±26.9^{a}	3.58±0.1^{a}	9.62±0.7^{a}	P3 100%	ND	6.48±0.40^{a}	ND	3.4±0.2^{a}
Cu50+Cd25	3.5±0.2^{e}	27±1.5^{f}	4.9±0.3^{e}	2.19±0.1^{e}	Cu50+Cd25	5.11±0.7^{f}	141.9±12.7^{e}	0.63±0.03^{f}	2.79±0.1^{e}	Cu50+Cd25+P1	ND	0.62±0.02^{e}	ND	0.72±0.04^{e}
Cu100+Cd50	21.6±1.6^{c}	55.3±3.1^{d}	8.2±0.5^{d}	2.07±0.6^{f}	Cu100+Cd50	10.39±1.2^{d}	209.1±15.4^{c}	1.52±0.7^{d}	1.74±0.1^{f}	Cu100+Cd50+P2	ND	1.93±0.43^{c}	ND	1.85±0.2^{c}
Cu150+Cd	35.6±2.9^{a}	87.3±3.5^{b}	16.0±1.2	3.62±0.8^{c}	Cu150+Cd	14.51±1.2	292.3±17.	2.14±0.08	5.31±0.3^{c}	Cu150+Cd75+	ND	5.71±0.43	ND	3.17±0.2^{b}

75			b	75	b	9^{bc}	b		P3		b		
					60 DAS								
Control	1.0±0.06^{g}	1.2±0.31^{g}	0.6±0.02 g	0.03±0.001^{f}	Control	0.01±0.00 2^{g}	0.01±0.00 1^{g}	0.03±0.00 1^{f}	0.01±0.00 1^{f}	Control	ND	ND	ND	BDL
Cu50	12.9±1.1^{f}	101.2±7.2^{e}	13.3±0.4 e	12.63±0.6^{e}	Cd25	10.9±0.35 e	249.2±18. 1^{e}	3.26±0.10 d	4.92±0.02 e	P1 25%	ND	3.72±0.2^{e}	ND	1.9±0.1^{d}
Cu100	23.7±1.4^{d}	210.2±17.5 c	15.8±1.3 d	17.12±0.8^{d}	Cd50	19.8±0.81 b	348.6±24. 3^{c}	5.47±0.32 b	7.20±0.54 d	P2 50%	ND	6.23±0.3^{c}	ND	3.2±0.2^{c}
Cu150	40.4±3.9^{b}	330.9±23.5 a	21.6±1.5 b	26.54±1.4^{a}	Cd75	22.61±0.9 a	558.8±34. 7^{a}	7.59±0.43 a	9.91±0.72 a	P3 100%	ND	10.1±0.1^{a} b	ND	5.8±0.2^{b}
Cu50+Cd2 5	12.6±1.3^{e}	49.9±3.8^{f}	12.8±0.4^{f}	18.47±1.1^{c}	Cu50+Cd2 5	7.99±0.32^{f}	217.9±60. 7^{f}	2.31±0.21 e	8.75±0.34 c	Cu50+Cd25+P 1	ND	2.62±0.2^{f}	ND	1.7±0.1^{e}
Cu100+Cd 50	33.6±3.5^{c}	158.8±9.2^{d}	18.8±0.8 c	23.80±1.9^{b}	Cu100+Cd 50	12.9±0.92 d	325.2±21. 4^{d}	4.14±0.27 c	8.77±0.38 c	Cu100+Cd50+ P2	ND	5.83±0.7^{d}	ND	3.6±0.2^{c}
Cu150+Cd 75	52.8±6.7^{a}	252.0±17.5 b	26.4±1.7 a	18.57±0.7^{c}	Cu150+Cd 75	16.8±0.82 c	538.7±33. 2^{b}	7.53±0.43 a	9.34±0.54 b	Cu150+Cd75+ P3	ND	10.6±0.2^{a}	ND	7.4±0.3^{a}
					90 DAS								
Control	1.1±0.01^{g}	1.5±0.11^{g}	0.8±0.00 3^{g}	0.04±0.002^{f}	Control	0.05±0.00 2^{g}	0.01±0.00 1^{g}	0.06±0.00 3^{g}	0.02±0.00 1^{f}	Control	ND	ND	ND	BDL

Cu 50	46.1±2.1[e]	207±16.3[e]	14.8±1.2[f]	19.38±0.7[e]	Cd25	39.30±1.4[d]	348.6±27.5[e]	5.36±0.4[e]	10.7±0.6[e]	P1 25%	ND	5.9±0.3[e]	ND	3.3±0.3[e]
Cu 100	78.9±3.2[c]	285±19.2[c]	31.3±2.2[c]	32.77±2.4[b]	Cd50	67.69±2.4[b]	674.2±43.6[b]	7.72±0.3[d]	12.1±0.8[c]	P2 50%	ND	7.23±0.5[c]	ND	6.4±0.5[b]
Cu 150	120.6±8.4[a]	447±32.7[a]	46.8±2.8[b]	44.35±3.6[a]	Cd75	93.76±3.2[a]	985.6±67.8[a]	10.67±0.7b	17.1±1.1[a]	P3 100%	ND	11.51±1.1[a]	ND	8.9±0.7[a]
Cu50+Cd25	21.7±1.5[f]	164.7±10.4[f]	19.6±1.4[e]	32.48±2.4[b]	Cu50+Cd25	21.02±1.8[f]	319.1±28.1[f]	4.67±0.3[f]	10.8±0.7[e]	Cu50+Cd25+P1	ND	0.18±0.01[f]	ND	2.8±0.3[f]
Cu100+Cd50	51.9±3.4[d]	250.4±21.3[d]	27.1±1.6[d]	29.20±2.1[d]	Cu100+Cd50	30.80±2.3[e]	486.4±36.2[d]	8.89±0.5[c]	11.6±0.8[d]	Cu100+Cd50+P2	ND	6.56±0.42[d]	ND	3.81±0.2d
Cu150+Cd75	89.0±6.7[b]	357.9±27.8[b]	52.3±3.2[a]	31.29±2.3[bc]	Cu150+Cd75	48.56±3.6[c]	668.1±42.5[c]	14.31±0.7[a]	14.1±0.6[b]	Cu150+Cd75+P3	ND	9.25±0.63[b]	ND	6.16±0.04[c]

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p< 0.05$).

Chapter 6
Studies on uptake, accumulation and partitioning of Cu, Cd and organochlorine pesticides in selected plant species

6.1. Enrichment potential of *A. paniculata*

Enrichment coefficient (EC) also known as bioconcentration factor was used to determine the amount of Cu and Cd accumulation in plant tissues with respect to the metal concentration in growing soil. ECroot (ECr) is used to calculate the degree of metal transferred from soil to plant root, while ECshoot (ECs) determines the degree of transfer of metal from soil to shoot. EC also represents the potential of the plant to uptake the concern metals from growing medium, and subsequently transport them into above ground parts (Zhao et al., 2003; Chen et al., 2005). ECr and ECs for Cu at 30, 60, 90 DAS ranged between 0.27-0.32, 0.39-0.56, 0.76-1.16 and 0.12-0.16, 0.18-0.37, 0.29-0.49, respectively. ECr and ECs for Cd at 30, 60, 90 DAS ranged between 0.23-0.39, 0.29-0.63, 0.71-1.04 and 0.06-0.12, 0.12-0.21, 0.17-0.29, respectively (Table 6.1). A higher value of ECr as compared to ECs indicates that the metal was largely retained in root tissues. It has been reported that EC >1 indicates the potential of plants to extract and transport metals from the substrate to different plant parts (Wei et al., 2002). Such plant species are considered as hyperaccumulator and can be applied for phytoextraction of metals (Barman et al., 2000; Kumar et al., 2013).

Table 6.1. Metal enrichment coefficient with respect root and shoots of *A. paniculata* after 30, 60 and 90 days of sowing (DAS)

Cu			Cd		
30 DAS					
Treatments	**ECr**	**ECs**	**Treatments**	**ECr**	**ECs**
Control	0.16 ± 0.03^{f}	0.11 ± 0.03^{f}	Control	1.30 ± 0.08^{a}	0.72 ± 0.05^{a}
Cu50	0.31 ± 0.04^{ab}	0.14 ± 0.01^{c}	Cd25	0.39 ± 0.04^{b}	0.12 ± 0.02^{c}
Cu100	0.28 ± 0.03^{d}	0.15 ± 0.02^{b}	Cd50	0.30 ± 0.02^{e}	0.06 ± 0.003^{f}
Cu150	0.27 ± 0.05^{e}	0.16 ± 0.02^{a}	Cd75	0.35 ± 0.04^{c}	0.09 ± 0.003^{e}
Cu50+Cd25	0.27 ± 0.04^{e}	0.12 ± 0.03^{e}	Cu50+Cd25	0.32±0.02d	0.11 ± 0.04^{b}
Cu100+Cd50	0.32 ± 0.06^{a}	0.13 ± 0.02^{d}	Cu100+Cd50	0.27 ± 0.01^{f}	0.09 ± 0.003^{e}
Cu150+Cd75	0.30 ± 0.04^{bc}	0.14 ± 0.02^{c}	Cu150+Cd75	0.23 ± 0.01^{g}	0.10 ± 0.03^{d}
60 DAS					
Control	0.17 ± 0.03^{g}	0.11 ± 0.03^{f}	Control	1.50 ± 0.12^{a}	1.14 ± 0.26^{a}
Cu50	0.56 ± 0.07^{a}	0.37 ± 0.05^{a}	Cd25	0.63 ± 0.04^{b}	0.21 ± 0.03^{b}
Cu100	0.39 ± 0.05^{e}	0.21 ± 0.05^{d}	Cd50	0.50 ± 0.04^{d}	0.15 ± 0.02^{d}
Cu150	0.37 ± 0.03^{f}	0.18 ± 0.02^{e}	Cd75	0.37 ± 0.02^{e}	0.13 ± 0.01^{e}
Cu50+Cd25	0.55 ± 0.06^{b}	0.36 ± 0.04^{b}	Cu50+Cd25	0.52 ± 0.04^{c}	0.17 ± 0.03^{c}
Cu100+Cd50	0.49 ± 0.03^{c}	0.24 ± 0.02^{c}	Cu100+Cd50	0.36 ± 0.02^{f}	0.12 ± 0.01^{f}
Cu150+Cd75	0.45 ± 0.03^{d}	0.21 ± 0.01^{d}	Cu150+Cd75	0.29 ± 0.021^{g}	0.13 ± 0.02^{e}

			90 DAS		
Control	0.35±0.02[f]	0.29±0.02[g]	Control	2.80±0.5[a]	4.80±0.34[a]
Cu50	1.16±0.07[a]	0.40±0.03[f]	Cd25	0.97±0.06[c]	0.29±0.04[b]
Cu100	1.04±0.06[b]	0.46±0.04[b]	Cd50	0.85±0.04[d]	0.19±0.02[e]
Cu150	0.97±0.04[c]	0.41±0.03[e]	Cd75	0.73±0.05[e]	0.17±0.02[f]
Cu50+Cd25	0.93±0.03[d]	0.49±0.06[a]	Cu50+Cd25	1.04±0.06[b]	0.27±0.03[c]
Cu100+Cd50	0.77±0.04[e]	0.42±0.03[d]	Cu100+Cd50	0.72±0.05[f]	0.22±0.02[d]
Cu150+Cd75	0.76±0.05[e]	0.45±0.03[c]	Cu150+Cd75	0.71±0.06[g]	0.22±0.01[d]

Results are expressed as means of five replicates i.e. n= 5± SD. Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p < 0.01$).

6.2. Translocation of Cu and Cd from root to shoot in *A. paniculata.*

Translocation factor (TF) or mobilization ratio used to determine the translocation of metals from soil to root and then shoot can be used to assess the potential of a plant for phytoremediation. TF for Cu, at 30, 60 and 90 days after treatment ranged between 0.42-0.58, 0.46-0.66 and 0.42-0.59, respectively (Table 6.2). While TF for Cd, at 30, 60 and 90 days after treatment ranged between 0.19-0.41, 0.30-0.44 and 0.23-0.30, respectively. The results showed that the translocation of Cu from root to above ground part was comparatively higher than the Cd. Cd was largely retained in the roots. Further, it was also observed that the translocation of metal was not affected by exposure time and application rate of Cu and Cd in growing medium.

Table 6.2. Translocation factor of Cu and Cd in *A. paniculata*

Cu		**Cd**	
30 DAS			
Treatments	**TF**	**Treatments**	**TF**
Control	0.65 ± 0.03^{a}	Control	0.28 ± 0.03^{e}
Cu50	0.44 ± 0.03^{e}	Cd25	0.31 ± 0.02^{d}
Cu100	0.52 ± 0.05^{c}	Cd50	0.19 ± 0.01^{g}
Cu150	0.58 ± 0.04^{b}	Cd75	0.25 ± 0.02^{f}
Cu50+Cd25	0.43 ± 0.03^{f}	Cu50+Cd25	0.32 ± 0.03^{c}
Cu100+Cd50	0.42±0.03g	Cu100+Cd50	0.34 ± 0.04^{b}
Cu150+Cd75	0.46 ± 0.04^{d}	Cu150+Cd75	0.41 ± 0.03^{a}
60 DAS			
Control	0.68 ± 0.05^{a}	Control	0.38 ± 0.03^{b}
Cu50	0.66 ± 0.06^{b}	Cd25	0.33 ± 0.02^{de}
Cu100	0.54 ± 0.04^{d}	Cd50	0.30 ± 0.02^{f}
Cu150	0.48 ± 0.04^{f}	Cd75	0.35 ± 0.03^{cd}
Cu50+Cd25	0.64 ± 0.05^{c}	Cu50+Cd25	0.33 ± 0.02^{e}
Cu100+Cd50	0.49 ± 0.04^{e}	Cu100+Cd50	0.34 ± 0.03^{d}

Cu150+Cd75	0.46±0.03[g]	Cu150+Cd75	0.44±0.05[a]
90 DAS			
Control	0.84±0.05[a]	Control	0.86±0.06[a]
Cu50	0.34±0.04[g]	Cd25	0.30±0.03[b]
Cu100	0.45±0.05[e]	Cd50	0.23±0.02[d]
Cu150	0.42±0.03[f]	Cd75	0.23±0.01[d]
Cu50+Cd25	0.53±0.04[d]	Cu50+Cd25	0.26±0.02[c]
Cu100+Cd50	0.55±0.05[c]	Cu100+Cd50	0.30±0.03[b]
Cu150+Cd75	0.59±0.05[b]	Cu150+Cd75	0.30±0.02[b]

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p< 0.01$).

6.3. Enrichment potential of *V. zizanioides*

ECr and ECs for Cu at 30, 60, 90 DAS ranged between 0.89-1.74, 1.12-3.35, 1.33-5.52 and 0.07-0.21, 0.23-0.53, 0.34-0.73, respectively. ECr and ECs for Cd at 30, 60, 90 DAS ranged between 4.64-9.14, 8.64-14.33, 11.30-19.92 and 0.16-0.46, 0.20-0.59, 0.31-0.67, respectively. Similarly, ECr and ECs for OCPs at 30, 60, 90 DAS ranged between 0.041-0.102, 0.074-0.132, 0.10-0.319 and 0.008-0.026, 0.041-0.064, 0.05-0.262, respectively (Table 6.3). Results showed that the higher value of ECr than ECs manifest that greater amount of metal was largely retained in root tissues than shoot tissues. Noticeably, it was also observed that the EC for Cd was much higher than for Cu indicating the high accumulation potential of *V. zizanioides* for Cd than Cu. ECr for OCPs was observed higher than ECs indicating the OCPs were largely retained in roots. Further, EC for Cu and Cd were found to be greater than 1 indicating the concentration of Cu and Cd is higher than in growing soil. Further, It was suggested that a good phytoremediator plant possess EC value greater than 1 (Zhang et al., 2002; Barman et al., 2000; Kumar et al., 2013). Similar trend of metal accumulation and translocation was observed by several authors (Dahn et al., 2010; Vargas et al., 2016; Banerjee et al., 2016; Meyer et al., 2017).

Table 6.3. Cu, Cd and organochlorine pesticides (OCPs) enrichment in different parts of *V. zizanioides* at 30, 60 and 90 DAS

	Cu		**Cd**			**OCPs**	
	ECr	**ECs**	**ECr**	**ECs**		**ECr**	**ECs**
Treatments			**30 DAS**				
Control	0.93±0.05^{f}	0.07±0.003^{d}	Control	4.30±0.32^{g}	0.40±0.02^{b}	Control	BDL	BDL
Cu50	1.74±0.4^{b}	0.21±0.02^{a}	Cd25	9.14±0.51^{a}	0.46±0.03^{a}	P1 25%	0.045±0.002^{c}	0.008±0.0004^{e}
Cu100	1.23±0.5^{c}	0.11±0.01^{b}	Cd50	7.10±0.42^{d}	0.26±0.02^{c}	P2 50%	0.041±0.002^{d}	0.016±0.002^{d}
Cu150	1.19±0.2^{d}	0.08±0.003^{cd}	Cd75	7.57±0.45^{c}	0.22±0.01^{cd}	P3 100%	0.054±0.003^{a}	0.024±0.002^{b}
Cu50+Cd25	1.46±0.3^{a}	0.10±0.02^{b}	Cu50+Cd25	7.91±0.65^{b}	0.39±0.02^{b}	Cu50+Cd25+P1	0.102±0.01^{a}	0.023±0.001^{b}
Cu100+Cd50	1.01±0.1^{e}	0.09±0.004^{c}	Cu100+Cd50	5.30±0.32e	0.17±0.01^{e}	Cu100+Cd50+P2	0.046±0.002^{bc}	0.026±0.002^{a}
Cu150+Cd75	0.89±0.04^{fg}	0.07±0.003^{cd}	Cu150+Cd75	4.64±0.28^{f}	0.16±0.01^{f}	Cu150+Cd75+P3	0.049±0.002^{b}	0.022±0.001^{c}
			60 DAS					
Control	1.07±0.03^{f}	0.43±0.02^{b}	Control	5.60±0.42^{f}	2.30±0.12^{a}	Control	BDL	BDL
Cu50	3.35±0.32^{c}	0.53±0.04^{a}	Cd25	14.33±1.17^{a}	0.56±0.03^{c}	P1 25%	0.133±0.01^{a}	0.064±0.003^{a}
Cu100	2.76±0.12^{a}	0.34±0.01^{d}	Cd50	9.15±0.65^{c}	0.32±0.04^{d}	P2 50%	0.097±0.004^{bc}	0.060±0.004^{b}
Cu150	2.65±0.17^{b}	0.30±0.02^{e}	Cd75	8.90±0.53^{d}	0.20±0.01^{f}	P3 100%	0.074±0.003	0.0412±0.003^{e}
Cu50+Cd25	2.32±0.15^{c}	0.41±0.03^{bc}	Cu50+Cd25	13.08±0.78^{b}	0.59±0.03^{b}	Cu50+Cd25+P1	0.102±0.021^{b}	0.055±0.003^{c}
Cu100+Cd50	2.25±0.021^{d}	0.28±0.02^{f}	Cu100+Cd50	8.68±0.54^{e}	0.30±0.02^{e}	Cu100+Cd50+P2	0.092±0.004^{e}	0.054±0.004^{c}

Cu150+Cd75	2.12±0.17^{e}	0.23±0.01^{g}	Cu150+Cd75	8.64±0.43^{e}	0.20±0.01^{f}	Cu150+Cd75+P3	0.079±0.003^{d}	0.052±0.002^{d}
				90 DAS				
Control	1.33±0.21^{g}	0.45±0.021^{c}	Control	7.00±0.37^{g}	4.50±0.65^{a}	Control	BDL	BDL
Cu50	5.52±0.32^{a}	0.67±0.04^{b}	Cd25	21.09±3.48^{b}	0.67±0.03^{b}	P1 25%	0.319±0.02^{a}	0.262±0.03^{a}
Cu100	3.54±0.24^{c}	0.43±0.03^{d}	Cd50	15.07±1.27^{c}	0.36±0.02^{b}	P2 50%	0.171±0.04^{b}	0.127±0.01^{b}
Cu150	3.42±0.21^{d}	0.39±0.02^{e}	Cd75	14.19±1.37^{d}	0.31±0.01^{d}	P3 100%	0.115±0.02^{e}	0.075±0.003^{c}
Cu50+Cd25	4.67±0.34^{b}	0.73±0.04^{a}	Cu50+Cd25	19.92±1.63^{a}	0.67±0.03^{b}	Cu50+Cd25+P1	0.159±0.02^{d}	0.110±0.02^{b}
Cu100+Cd50	3.19±0.25^{e}	0.43±0.02^{d}	Cu100+Cd50	13.31±0.78^{de}	0.35±0.02^{c}	Cu100+Cd50+P2	0.162±0.03^{c}	0.053±0.003^{e}
Cu150+Cd75	2.85±0.21^{f}	0.34±0.02^{f}	Cu150+Cd75	11.30±0.68^{f}	0.31±0.01^{d}	Cu150+Cd75+P3	0.101±0.02^{f}	0.063±0.004^{d}

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p< 0.01$).

6.4. Translocation of metals and Organochlorine pesticides (OCPs) from root to shoot in *V. zizanioides*

TF for Cu, at 30, 60 and 90 days after treatment ranged between 0.07-0.12, 0.11-0.18 and 0.11-0.16, respectively. While TF for Cd ranged between 0.029-0.050, 0.023-0.045 and 0.022-0.034 at 30, 60 and 90 days after treatment, respectively. Results showed that the translocation of metal was not affected by the time of exposure to contaminants and availability of different concentration of Cu and Cd in growing medium. Further, the translocation of OCPs from root to above ground part was recorded relatively higher than the Cu and Cd. It was reported in earlier studies that the translocation of Cu is generally less than 10% (Yang et al., 2003; Dunn et al., 2005 Wong et al., 2006; Dahn et al., 2009). In case of Cd contamination, the accumulation of Cd was observed much higher in roots than shoot and the translocation was very less in shoots hence, its consecutive TF was much lower. Similar results were also observed by several authors (Truong, 1999; Yang et al., 2003). However, the TF values for OCPs were much higher than metals indicating the better translocation rate of OCPs.

Table 6.4. Translocation factor for Cu, Cd and organochlorine pesticides (OCPs0 in *V. zizanioides*

Cu		Cd		OCPs	
30 DAS					
Treatment	**TF**	**Treatment**	**TF**	**Treatment**	**TF**
Control	0.08±0.003^{c}	Control	0.09±0.005^{a}	Control	ND
Cu50	0.12±0.011^{a}	Cd25	0.05±0.003^{b}	P1 (25%)	0.17±0.012^{f}
Cu100	0.09±0.003^{b}	Cd50	0.04±0.003^{c}	P2 (50%)	0.40±0.015^{d}
Cu150	0.07±0.002^{d}	Cd75	0.03±0.001^{d}	P3 (100%)	0.44±0.021^{c}
Cu50+Cd25	0.07±0.001^{d}	Cu50+Cd25	0.05±0.002^{b}	Cu50+Cd25+P1	0.32±0.016^{e}
Cu100+Cd50	0.09±0.003^{b}	Cu100+Cd50	0.03±0.001^{d}	Cu100+Cd50+P2	0.57±0.023^{a}
Cu150+Cd75	0.08±0.004^{c}	Cu150+Cd75	0.04±0.002^{c}	Cu150+Cd75+P3	0.45±0.021^{b}
60 DAS					
Control	0.40±0.01^{a}	Control	0.41±0.02^{a}	Control	ND
Cu50	0.16±0.01^{c}	Cd25	0.04±0.002^{c}	P1 (25%)	0.48±0.01^{e}
Cu100	0.12±0.006^{d}	Cd50	0.04±0.002^{c}	P2 (50%)	0.62±0.02b
Cu150	0.11±0.005^{e}	Cd75	0.02±0.001^{e}	P3 (100%)	0.55±0.009^{d}
Cu50+Cd25	0.18±0.007^{b}	Cu50+Cd25	0.05±0.003^{b}	Cu50+Cd25+P1	0.54±0.02^{d}
Cu100+Cd50	0.12±0.006^{d}	Cu100+Cd50	0.03±0.002^{d}	Cu100+Cd50+P2	0.58±0.008^{c}
Cu150+Cd75	0.11±0.008^{e}	Cu150+Cd75	0.02±0.001^{e}	Cu150+Cd75+P3	0.66±0.02^{a}
90 DAS					
Control	0.34±0.01^{a}	Control	0.64±0.02^{a}	Control	ND
Cu50	0.12±0.004^{d}	Cd25	0.03±0.001^{b}	P1 (25%)	0.82±0.005^{a}
Cu100	0.12±0.008^{d}	Cd50	0.02±0.001^{c}	P2 (50%)	0.74±0.03^{b}
Cu150	0.11±0.004^{e}	Cd75	0.02±0.001^{c}	P3 (100%)	0.65±0.008^{d}
Cu50+Cd25	0.16±0.002^{b}	Cu50+Cd25	0.03±0.002^{b}	Cu50+Cd25+P1	0.69±0.009^{c}
Cu100+Cd50	0.14±0.006^{c}	Cu100+Cd50	0.03±0.007^{b}	Cu100+Cd50+P2	0.33±0.011^{f}
Cu150+Cd75	0.12±0.004^{d}	Cu150+Cd75	0.03±0.006^{b}	Cu150+Cd75+P3	0.63±0.008^{e}

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($p< 0.01$).

Chapter 7

Studies on microbial activities and fertility of rhizospheric soil treated with different concentration of Cu, Cd, and organochlorine pesticides in presence of selected plant species

7.1. Microbiological and dehydrogenase activity in rhizospheric soil

7.1.1. Microbial load

Findings of microbiological analysis of soil samples showed that microbial load in all treatments was decreased significantly by increasing concentration of metal and organochlorine pesticides in soil. However, at highest level of contamination the colony forming unit (cfu) were almost same because probably due to growth of resistant bacteria only. In present study microbial load was ranged from 40-301×10^4, 35-263×10^4 and 19-197×10^4 cfu at 30 days, 60 days and 90 DAS respectively in rhizospheric soil of *V. zizanioides* and the corresponding values in *A. paniculata* were ranged from 40-284×10^4, 35-183×10^4 and 19-166×10^4 cfu at 30, 60 and 90 DAS respectively. After 30 days of treatment, maximum microbial load was observed as 297 ×10^4 cfu and 284 ×10^4 cfu in rhizospheric soil of *V. zizanioides* and *A. paniculata* respectively at 25 µgg^{-1} of Cd kg^{-1} in soil. Compare to control, difference in cfu count in rhizospheric soil having concentration of 25 µgg^{-1} of Cd kg^{-1} in soil was very less. However, with the progress of time, numbers of colonies were decreased in case of all treatments (Table 7.1 and Table 7.2). It was found that the concentration of contaminants affects the microbial population. Minimum cfu count i.e. 40×10^4 was reported at treatment Cu150+Cd150 in case of both plants after 30 days of treatment. However, after 90 days of treatment the lowest corresponding value were 19 ×$10^4$and 21×10^4 cfu for *A. paniculata* and *V. zizanioides* respectively at same treatment i.e. Cu150+Cd150.

Microbial load was higher at concentration of 25 µgg^{-1} Cd kg^{-1} of soil than control. Similar results were reported by Gikas, 2007. They reported that less concentration of heavy metals in soil often stimulates the growth of microorganisms. On the basis of findings, it can be assumed that an enhanced of concentration of metals and organochlorine pesticides caused reduction of microbial growth. It is reported that higher concentration of metals result in reduction of microbial activity

and that affects the microbial population and diversity as well (Gikas et al., 2009). Similar finding were also found at 60 days and 90 days of treatment in case of both the plants.

7.1.2. Dehydrogenase (DHA) activity

It was observed that dehydrogenase activities in rhizospheric soil of both the plants were decreased with increasing the exposure time and concentration of heavy metal and OCPs (Table.7.1 and 7.2). Dehydrogenase (DHA) activity was ranged from 0.75-2.31 and 0.81-2.11 TPF µg/g/h after 30 days of treatment, however, after 90 days of treatment it was reduced to 0.51-2.09 and 0.66-2.05 TPF µg/g/h in rhizospheric soil of *V. zizanioides* and *A. paniculata* respectively. Maximum dehydrogenase activity was observed in control condition and the values were 2.39 TPF µg/g/h and 2.45 TPF µg/g/h in rhizospheric soil of *V. zizanioides* and *A. paniculata* respectively. Minimum dehydrogenase activity was observed as 0.60, 0.51 and 0.45 TPF µg/g/h in rhizospheric soil of *A. paniculata* treated with Cu150+Cd25+P3 after 30, 60 and 90 days for treatment respectively. Similarily, minimum dehydrogenase activity was observed as 0.74 µg/g/h, 0.74 µg/g/h and 0.51 µg/g/h in rhizospheric soil of *V. zizanioides* treated withCu150+Cd25+P3after 30, 60 and 90 days for treatment respectively.

Microbiological population and dehydrogenase activity of soil of both the plants was correlated with each other and reduced simultaneously with the treatment after 30, 60 and 90 days. Dehydrogenase activity of soil is considered as an indication of microbial load in soil. For microbial oxidative activity, analysis of soil dehydrogenase is a noble tool (Ross, 1971). Dehydrogenase is an extra-cellular enzyme and considered an indicator of any disturbance in soil caused by heavy metals and pesticides (Reddy and Faza, 1989). It can be measured as microbial biomass and soil respiration (Ladd, 1978).

Table 7.1. Microbiological load and dehydrogenase activity in rhizospheric soil of *A. paniculata*

Treatment	Microbial load* (×10⁴)			Dehydrogenase activity** (TPF µg/g/h)- TPF – Triphenylformazan		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Control	270±15^{b}	285±16^{a}	280±19^{a}	2.42±0.2^{a}	2.58±0.16^{a}	2.45±0.14^{a}
Cu50	120±9.1^{e}	101±8.1^{e}	93±5.8^{d}	1.45±0.1^{e}	1.37±0.06^{e}	1.15±0.09^{e}
Cu100	71±4.5^{f}	58±4.7^{f}	49±3.2^{e}	1.20±0.1^{e}	1.09±0.06^{f}	0.86±0.03^{f}
Cu150	49±3.2^{g}	42±3.6^{g}	37±2.8^{f}	1.01±0.1^{f}	0.96±0.04^{g}	0.81±0.04^{f}
Cd25	284±24^{a}	183±16^{b}	166±15^{b}	2.11±0.2^{b}	2.04±0.1^{b}	2.03±0.2^{b}
Cd50	199±17^{c}	136±11^{c}	128±14^{c}	2.01±0.1^{c}	1.89±0.12^{c}	1.94±0.3^{c}
Cd150	148±11^{d}	130±12^{d}	126±11^{c}	1.93±0.5^{cd}	1.75±0.07^{d}	1.53±0.3^{d}
Cu25+Cd50	46±2.6^{g}	39±3.5^{h}	31±2.5^{g}	0.97±0.05^{g}	0.84±0.04^{h}	0.75±0.03^{g}
Cu100+Cd50	45±3.2^{g}	37±2.1^{h}	21±2.3^{h}	0.84±0.04^{h}	0.71±0.04^{i}	0.76±0.01^{g}
Cu150+Cd150	40±3.4^{gh}	35±2.3^{h}	19±1.5^{h}	0.81±0.04^{h}	0.67±0.03^{i}	0.66±0.02^{h}

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($^{*}p< 0.05$ and $^{**}p<0.01$).

Table.7.2: Microbiological load and dehydrogenase activity in rhizospheric soil of *V. zizanioides*

Treatment	Microbial load* (×10⁴)			Dehydrogenase activity** (TPF µg/g/h)- TPF – Triphenylformazan		
	30 Days	60 Days	90 Days	30 Days	60 Days	90 Days
Control	285±23^{b}	259±21^{a}	280±26^{a}	2.21±0.09^{b}	2.02±0.09^{b}	2.39±0.9^{a}
Cu50	117±10^{f}	101±8.7^{f}	96±6.5^{f}	1.36±0.08^{e}	1.27±0.07^{g}	1.15±0.1^{g}
Cu100	67±4.3	53±3.2^{g}	43±3.3^{h}	1.10±0.07^{f}	1.03±0.05^{h}	0.86±0.06^{h}
Cu150	50±3.2^{h}	44±2.7^{h}	39±2.2^{h}	0.98±0.05^{g}	0.90±0.07^{i}	0.81±0.04^{h}
Cd25	297±25^{a}	197±17^{b}	176±14^{c}	2.31±0.3^{a}	2.17±0.1^{a}	2.03±0.3^{b}
Cd50	201±17^{c}	146±13^{d}	138±10^{d}	2.19±0.09^{ab}	2.03±0.1^{b}	1.94±0.5^{c}
Cd150	150±12^{e}	130±11^{e}	126±9.5^{e}	1.70±0.3^{d}	1.69±0.1^{e}	1.53±0.3^{f}
Cu25+Cd50	45±3.4^{i}	42±3.1^{h}	36±2.6^{hi}	0.90±0.06^{g}	0.83±0.07^{i}	0.75±0.04^{j}
Cu100+Cd50	43±4.1^{ij}	36±2.7^{i}	27±1.5^{j}	0.86±0.05^{h}	0.79±0.06^{j}	0.76±0.03^{j}
Cu150+Cd150	40±3.5^{j}	35±2.5^{i}	22±1.6^{j}	0.84±0.04^{h}	0.75±0.06^{j}	0.66±0.02
P1	301±19^{a}	263±19^{a}	197±16^{b}	2.04±0.1^{c}	1.96±0.5^{c}	1.90±0.1^{d}
P2	195±17^{cd}	167±15^{c}	174±14^{c}	1.96±0.4^{c}	1.90±0.4^{cd}	1.83±0.1^{e}
P3	190±15^{d}	167±12^{c}	136±11^{d}	1.50±.01^{e}	1.39±0.3^{f}	1.25±0.1^{g}

Cu50+Cd25+P1	94±6^{g}	86±6.2^{g}	73±5.4^{g}	0.84±.005^{h}	0.78±0.05^{j}	0.69±0.03^{i}
Cu100+Cd50+P2	50±3^{i}	39±3^{i}	30±2j	0.79±0.03^{i}	0.62±0.02^{i}	0.58±0.02^{j}
Cu150+Cd25+P3	59±5^{h}	47±4^{h}	36±3^{h}	0.74±0.04^{i}	0.74±0.03^{j}	0.51±0.02

Results are expressed as means of five replicates i.e. n= 5± SD. Data was analyses by one way analysis of variance (DMRT). Different letters indicates that means are significantly different from each other ($^{*}p< 0.05$ and $^{**}p<0.01$).

7.2. Effect of soil Cu, Cd and organochlorine pesticides (OCPs) on NPK content in soil.

The balance level and ratio of NPK in soil is considered as beneficial for plant growth and development (Chu et al., 2007). However, contamination of metal and pesticide disturbs the level and ration of NPK which ultimately affects the soil fertility. Effect of Cd, Cu and OCPs contamination on organic carbon, N, P and K content of soil in which *A. paniculata* and *V. zizanioides* were grown are presented in Table 7.3 and 7.4. It was observed that the level of organic carbon (%) and available NPK in soil were decreased significantly on increasing the concentration of Cd, Cu and OCPs (Table 7.3 and Table 7.4). Result also revealed that the decline of NPK and OC was observed higher in case of Cd contamination than Cu. However, the combination of metals and OCPs has caused rapid decline than the combination of metals only. The trend of decline of NPK and OC content in contaminated soil was as follows: Cu+Cd+P>Cu+Cd>Cd>Cu. Further, the *V. zizanioides* planted soil have higher NPK and OC content than the *A. paniculata* planted soil. It was reported that the *V. zizanioides* have well developed massive roots system and higher root activity which help to survive in adverse condition. Further, the microbial activity in rhizospheric soil was reported high and presence of high microbial activity favor the building up of NPK and organic carbon content in the soil. However, at higher level of contamination, it was observed that the microbial activity was declined and simultaneously the level of NPK and OC was also reduced. Hence, it may be inferred that the presence of microbial activity could be a possible reason for the soil fertility.

Table 7.3. Change in NPK and organic content of *A. paniculata* planted soil contaminated with Cu, Cd and OCPs

	30 DAS				60 DAS				90 DAS			
Treatments	**OC (%)**	**Available N**	**Available P**	**Soluble K**	**OC (%)**	**Available N**	**Available P**	**Soluble K**	**OC (%)**	**Available N**	**Available P**	**Soluble K**
Control	1.37±0.3	79.1± 3.4	67.2± 2.7	118±5.4	1.44±0.3	85.3± 4.2	74.6±2.3	123±0.7	1.5±0.22	90.2±3.6	78.6±3.5	133.5±7
Cu 50	1.03±0.2	65.3±2.7	50.5±1.7	93±4.3	0.83±0.04	51.3±0.3	47.6±3.5	78.1±3.8	0.71±0.05	42.5±2.4	40.4±1.5	65.9±3.2
Cu 100	0.89±0.04	54.1±2.3	41.9±1.6	80.5± 4	0.71±0.04	40.3±2.4	34.3±1.2	67.2±3.2	0.58±0.02	33.8±2.1	32.2±2.4	53.5±2.8
Cu 150	0.71±0.03	43.8±2.5	35.8±1.9	64.2±3.1	0.55±0.03	32.5±1.5	27.4±1.6	55.6±2.8	0.46±0.02	25.6±1.3	23.8±1.5	42.1±1.7
Cd25	0.94±0.05	59.1±3.6	54.8±3.2	98.1±4.2	0.81±0.05	51.6±1.8	44.1±1.9	76.5±4.1	0.72±0.04	40.1±2.2	38.5±3.2	63.6±3.2
Cd50	0.79±0.04	47.3±3.1	44.1±2.4	83.7±3.4	0.65±0.04	39.8±1.6	32.2±1.2	57.1±2.4	0.58±0.03	31.4±1.7	27.6±2.1	46.4±2.3
Cd75	0.56±0.03	36.5±2.2	32.3±2.1	61.1±2.3	0.46±0.03	27.6±1.3	25.6±1.1	45.3±2.3	0.43±0.02	20.7±1.3	20.3±1.3	36.7±2.1
Cu50+Cd25	0.87±0.06	51±2.8	47.1±2.6	79.4±3	0.73±0.04	44.5±1.9	47.1±2.5	56.3±3.4	0.64±0.03	35.8±2.4	34.1±1.9	43.8±2.5
Cu100+Cd50	0.68±0.03	40.1±1.7	36.9±2.1	58.2±2.5	0.56±0.03	34.2±1.4	29.9±2.4	43.3±2.2	0.47±0.02	27.6±1.4	25.7±1.6	32.7±2.1
Cu150+Cd75	0.45±0.02	28.7±1.6	29.5±1.7	45.2± 3	0.35±0.02	23.2±1.1	21.5±1.2	35.3±1.6	0.32±0.02	18.5±0.7	18.4±1.2	29.02±3

Results are expressed as means of five replicates i.e. n= 5± SD.

Table 7.4. Change in NPK and organic content of *V. zizanioides* planted soil contaminated with Cu, Cd and OCPs

	30 DAS				60 DAS				90 DAS			
Treatments	**OC**	**Available N**	**Available P**	**Soluble K**	**OC**	**Available N**	**Available P**	**Soluble K**	**OC**	**Available N**	**Available P**	**Soluble K**
Control	1.37±0.05	79.1±5.8	67.2±2.9	118±7.8	1.44±0.07	85.3±4.5	74.6±4.2	123.0±8.6	1.5±0.06	90.2±6.1	78.6±4.7	133.5±9.2
Cu 50	1.18±0.04	73.4±5.4	62.0±2.6	97.4±6.5	1.02±0.06	67.3±3.4	56.3±3.2	91.5±7.3	0.87±005	60.1±3.5	48.3±3.1	83.5±6.5
Cu 100	0.96±0.05	60.1±4.6	48.6±2.1	86.6±6.2	0.84±0.04	51.3±2.7	43.6±2.1	77.5±5.6	0.73±0.04	43.8±3.2	37.7±2.1	68.6±4.3
Cu 150	0.78±0.05	47.4±3.2	37.4±1.5	68.7±4.4	0.68±0.03	42.0±1.8	32.8±1.4	63.7±4.3	0.62±0.04	35.2±2.6	28.0±1.6	56.4±3.5
Cd25	1.05±0.07	74.2±6.1	63.2±2.7	101.6±7.8	0.95±0.07	65.6±3.7	55.9±3.2	90.4±7.4	0.89±0.07	54.1±3.5	43.3±2.4	80.3±6.1
Cd50	0.89±0.04	59.6±3.2	49.5±2.4	88.3±6.4	0.82±0.06	53.0±3.5	40.2±1.7	76.4±4.5	0.81±0.04	46.1±3.1	35.6±2.1	70.0±5.2
Cd75	0.77±0.05	43.7±2.5	38.7±1.5	70.2±5.6	0.71±0.03	40.1±1.7	32.4±1.5	65.8±3.6	0.63±0.04	30.0±2.5	26.8±1.8	54.2±3.1
Cu50+Cd25	0.97±0.06	56.8±3.1	55.4±2.2	83.6±7.3	0.82±0.06	46.4±2.8	47.5±2.3	76.1±5.6	0.86±0.06	41.4±1.8	38.5±2.7	70.5±4.6
Cu100+Cd50	0.72±0.05	44.2±2.1	42.6±1.8	67.8± 4.6	0.67±0.05	37.5±2.6	38.6±1.7	55.7±4.7	0.72±0.05	30.2±1.3	27.4±1.8	47.1±3.2
Cu150+Cd75	0.56±0.03	29.8±1.4	35.0±1,6	53.6±3.7	0.48±0.02	26.0±1.7	30.5±1.4	41.4±3.2	0.56±0.03	20.1±1.1	23.7±1.1	38.0±2.7
P1	0.88±0.06	83.5±4.7	62.4±2.8	94.4±7.2	0.81±0.07	71.2±6.2	54.7±3.8	86.6±6.4	0.88±0.05	63.5±4.3	47.3±2.4	79.3±4.5
P2	0.72±0.04	66.3±3.2	48.4±2.2	76.1±5.3	0.66±0.03	58.5±3.1	43.5±3.5	70.4±5.3	0.65±0.04	50.6±2.8	35.2±2.6	65.4±3.8
P3	0.56±0.04	50.6±2.2	36.8±1.5	62.5±2.8	0.57±0.04	46.0±3.1	30.9±2.6	57.5±4.2	0.48±0.03	43.0±3.2	27.1±2.1	50.5±4.2
Cu50+Cd25+P1	0.79±0.06	57.2±2.7	54.4±1.8	83.7±5.9	0.72±0.04	46.8±2.2	42.7±3.2	75.7±6.3	0.66±0.04	35.1±2.3	34.1±2.4	72.4±6.2
Cu100+Cd50+P2	0.61±0.04	41.3±1.8	42.6±1.5	63.5±3.2	0.55±0.03	38.6±1.8	31.1±2.1	58.4±3.4	0.51±0.02	29.4±1.6	26.6±1.7	49.5±3.5
Cu150+Cd75+P3	0.53±0.05	29.4±1.1	33.8±1.4	48.1±2.1	0.44±0.03	24.1±1.1	26.4±1.7	44.1±3.1	0.35±0.02	19.8±1.1	20.2±1.4	43.5±2.6

Results are expressed as means of five replicates i.e. n= 5± SD.

Chapter 8

Application of copper phytoremediated plant biomass for the defluoridation of water

Several batch experiments were executed to study the adsorption of fluoride using *A. paniculata* and *V. zizanoides* leaves as biosorbent after phytoremediation under different conditions. The adsorption capacities of both biosorbent found to be influenced by contact time, pH, initial fluoride concentration and adsorbent dose. Detailed results are described below.

8.1 Influence of contact time on adsorption

Influence of contact time on the adsorption efficiency of Fluoride (F) was studied by varying the contact time from 20 to 120 minute at the adsorbent dose of 5 g per 25 ml i.e. 1:5 (w/v), pH 3 and temperature 28±2 ºC. It was observed that initially, the fluoride removal efficiency increased with increase in contact time, however, after 100 min it has become almost stagnant denoting the attainment of adsorption saturation (Fig 8.1.(a) and 8.2. (a)). The trend of influence of contact time on the adsorption efficiency was observed similar in case of both the biosorbent though, the adsorption efficiency of *A. paniculata* was recorded higher than *V. zizanoides*. Rapid rate of adsorption at the initial stage may be due to availability of enough active sites for fluoride sorption, however, with the progress of experiment the active sites became saturated and ultimately the adsorbent might have been exhausted at the final stages. Similar findings were reported with various other bisorbents; *Pleurotus ostreatus* 1804 (Ramanaiah et al., 2007), protonated chitosan beads (Viswanathan et al., 2009), Citrus limonum leaf (Tomar et al., 2014), wheat straw, sawdust and activated bagasse (Yadav et al., 2013).

8.2 Influence of pH on adsorption

It is a documented fact that the process of biosorption is reliant on the pH, functional groups of the biosorbent and their ionic state (Liu et al., 2014; Yadav et al.,

2013). It was observed that defluoridation efficiency found to be decrease with increase pH in case of both the biosorbent (Fig 8.1.(b) and 8.2. (b)). Highest defluoridation efficiency of both biosorbents was recorded at pH 3. Biosorbent contains a high amount of polysaccharides and some of them are associated with proteins and other biomolecules (Williams and Edyvean, 1997; Panumati et al., 2008). These biomolecules have several chemically active functional groups such as amine, carboxyl, thiol, sulfhydryl, alcohol, phenol and phosphate. Further, the process of biosorption generally depends on the protonation or deprotonation of these functional groups (Ilhami et al., 2005). The ionic form of fluoride in aquous solution and the electric charge of the functional groups of biomolecules (the surface biosorbent) depend on the pH of the solution. At higher pH the defluoridation efficiency was found to be decline probably due to the adsorbent surface becomes negatively charged at higher pH which leads to electrostatic repulsion between fluoride and adsorbent surface resulting in low adsorption capacity (Panumati et al., 2008; Viswanathan et al., 2009; Tomar et al., 2014).

8.3. Influence of adsorbent dose

The influence of adsorbent dose on the defluoridation was examined at pH 6 and contact time of 90 minute. The amount of dosage were varied between 0.5 g to 3 g per 25 ml. Results revealed that up to a certain level, an increase in adsorbent dose resulted in a simultaneous increase in defluoridation efficiency, probably due to high availability surface area and pore volume. Defluoridation efficiency was found to be increased from 49.4, 50.8, 49.8 and 51.4 to 79.2, 82.14, 82.8 and 83.6% respectively at 0.5-3 g dose in differently treated biosorbent of *A. paniculata* biosorbent (Fig 8.1 (c) and Fig 8.2 (c)). Similarly, the fluoride removal efficiency of differently treated biosorbent of *V. zizanioides* found to be increased from 29.4, 30.8, 33.8 and 35.4 to

65.6, 66.8, 66.6 and 68% respectively. All the treatments of both type of biosorbent followed the similar trend of Fluoride removal, however, over 2.5 g adsorbent doses, there were no significant changes in the fluoride removal was observed, probably being the overlapping of active sites at a higher dosage, thus decrease in the net available surface area for adsorption (Killender and Bhargava, 1993). Similar observations for influence of adsorbent dose on the defluoridation were reported by several researchers using other biosorbents (Viswanathan et al., 2009; Chakrabarthy and Sharma, 2012; Yadav et al., 2013; Tomar et al., 2014).

8.4. Influence ofinitial fluoride concentration

Influence of initial fluoride concentration on the defluoridation efficiency was examined by adding the 2 g of adsorbent dose into water fluoridated with different concentrations of fluoride (2.5, 5, 7.5, 10, 12.5 and 15 mgL^{-1}) contact time of 100 minute. Results revealed that F removal efficiency decreased with increase in the initial fluoride concentration (Fig 8.1 (d) and Fig 8.2 (d)). Decrease in removal efficiency indicates that the capacity of adsorbent gets exhausted abruptly on increasing the initial fluoride concentration probably due to instant saturation of active adsorbent sites at higher F concentration. Similar trends have been reported by several authors for fluoride removal from water using protonated chitosan beads (Viswanathan et al., 2009), algal biomass (Mohan et al., 2007), wheat straw, sawdust, activated bagasse of sugarcane (Yadav et al., 2013), *Azadirachta indica* (Chakrabarty and Sarma, 2012) and Lemon leaf (Tomar et al., 2014).

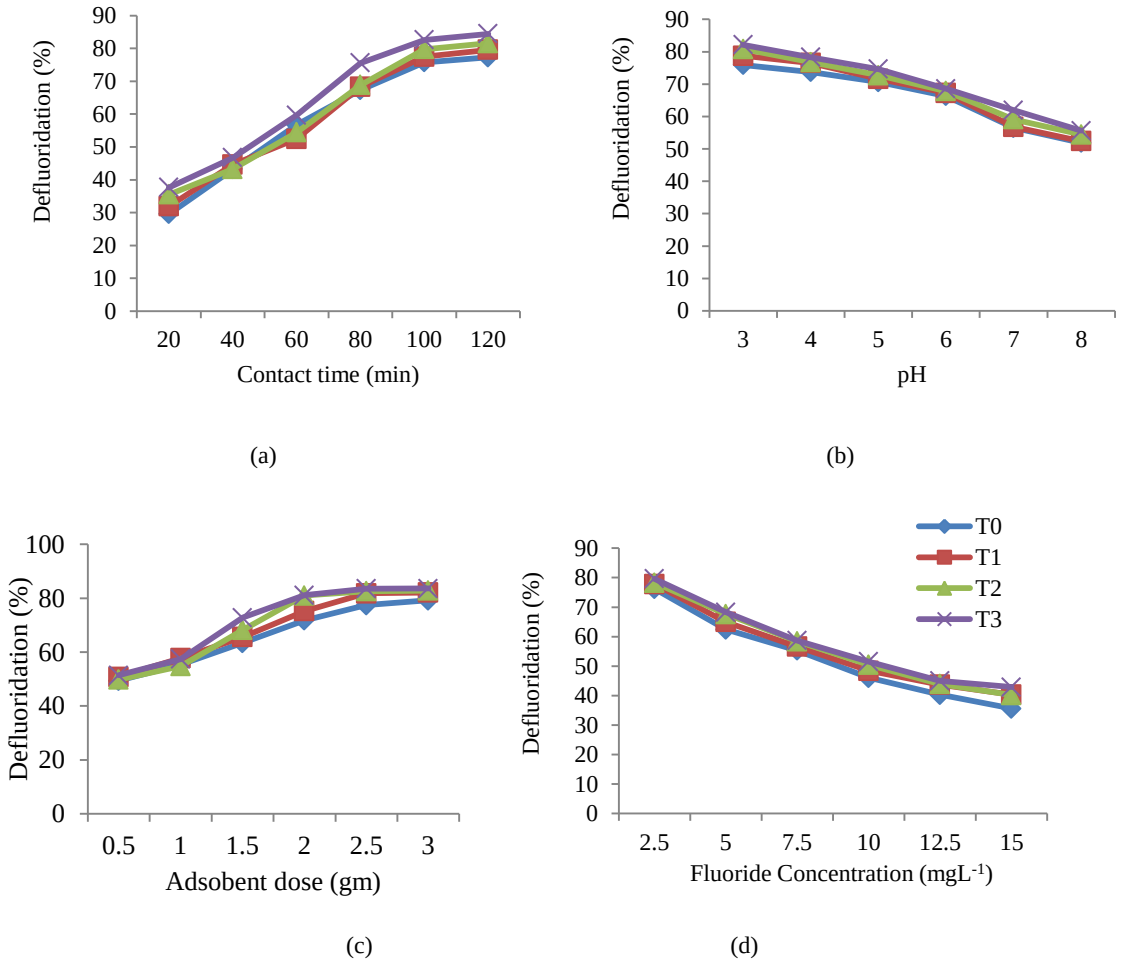
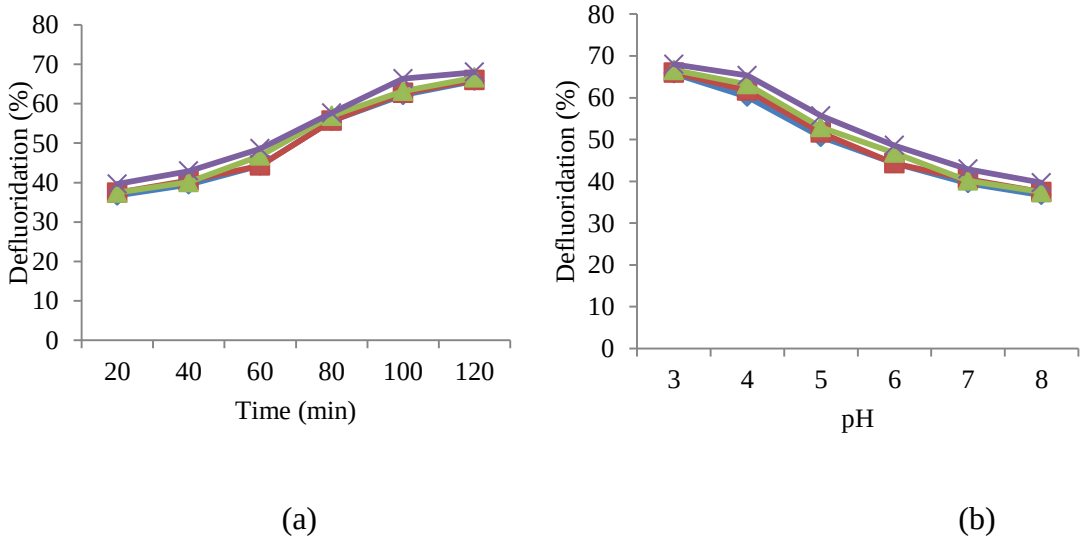
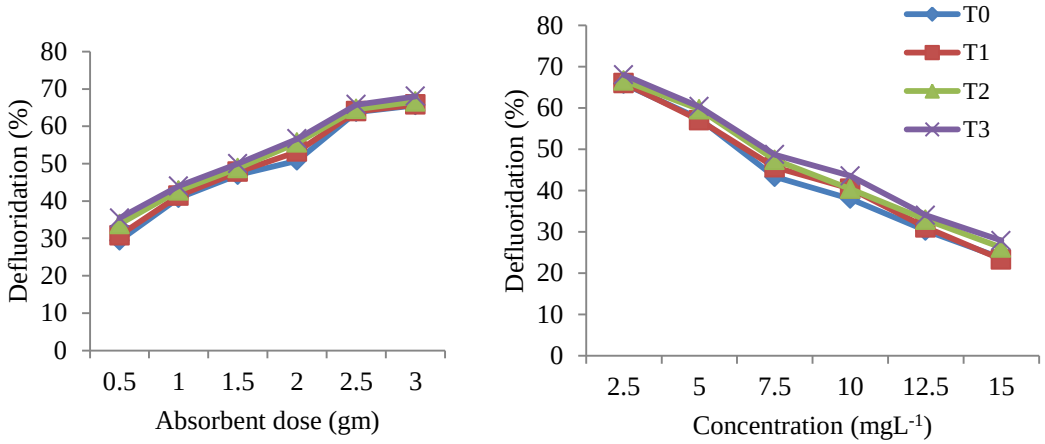


Fig. 8.1. Effect of (a) contact time, (b) pH, (c) adsorbent dose and (d) initial fluoride concentration on the fluoride removal from water by Cu phytoremediated *A. paniculata* plant biomass.





(c) (d)

Fig. 8.2. Effect of (a) contact time, (b) pH, (c) adsorbent dose and (d) initial fluoride concentration on the fluoride removal from water by Cu phytoremediated *V. zizanoides* plant biomass.

8.5. Adsorption isotherms

Langmuir and Freundlich isotherms are considered as essential models for understanding the adsorption process. The Langmuir isotherms is based on saturated molecular layer (monolayer) adsorption on the active sites of the adsorbate while Freundlich isotherms is based on adsorption on a heterogeneous surface and a multilayer adsorption with an energetic nonuniform distribution (Freundlich, 1906; Langmuir, 1918).

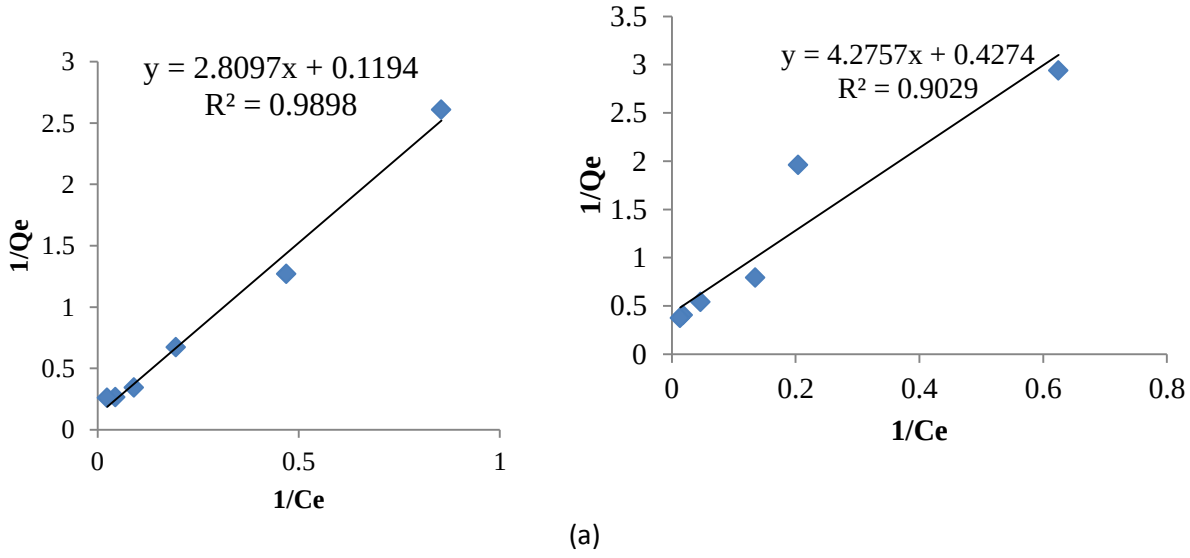
Langmuir isotherm can be expessed as:

$$Q_e = Q_{max}\, bC_e / 1 + b\, C_e \qquad (1)$$

Where Q_e (mg/g) is the equilibrium adsorption capacity, C_e (mg/L) is the equilibrium constant, Q_{max} is the maximum adsorption capacity per gram of adsorbent and b is the langmuir constant.

Langmuir isotherm can be also expressed as

$$1/Q_e = 1/Q_{max} + 1/ b + Q_{max} C_e \quad (2)$$



(a)

(b)

Fig. 8.3. Linear model of Langmuir isotherm for adsorption of fluoride at 1g/10ml Volume. pH 3, temp 30 ºC, contact time of 120 min and different initial concentration of biosorbent (a) *A. paniculata* (b) *V. zizanoides*.

A plot of 1/ Q_e Vs 1/C_e has been shown in fig.8.3 (a) and (b), where the straight line has a slope of slope of 1/b Q_{max} which indicates that the adsorption follow Langmuir isotherm. Further, seperation factor (R_L) for equlibrium parameter can be calculated as:

$$R_L = 1/ 1 + bC_0 \quad (3)$$

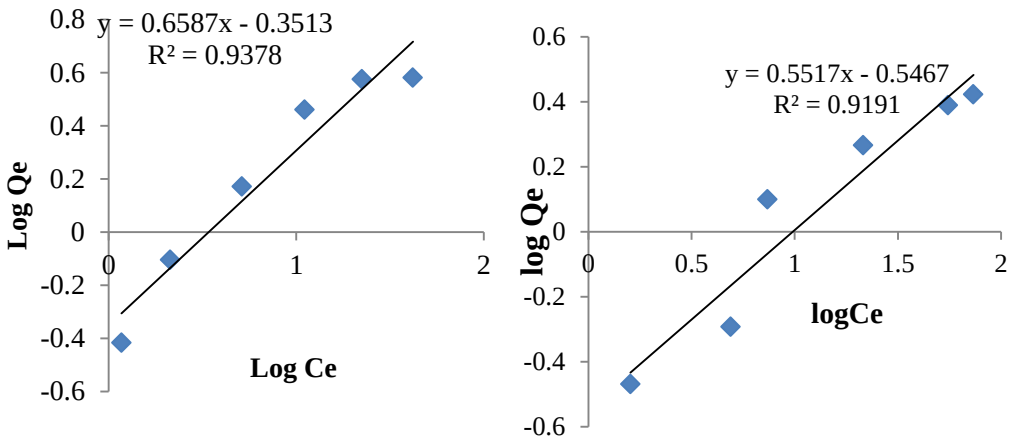
Where C_0 (mg/L), is the initial concentration of the fluoride and b (L/mg) is the Langmuir constant. R_L value indicates the types of isotherms i.e. favourable (0 >R_L> 1), unfavourable (R_L> 1), Linear (R_L= 1) and irreversible (R_L = 0).

Freundlich isotherm can be expressed as:

$$Q_e = K_f C_e^{1/n} \quad (4)$$

Where, Q_e (mg/g) is the equilibrium adsorption capacity, $C_e$is the equilibrium concentration. Taking logrithum of equation (4) shows the linear form of Freundlich isotherm which can be written as:

$$\text{Log } Q_e = 1/n \text{ Log } C_e + \text{Log } K_f$$



(a) (b)

Fig.8.4. Linear model of Freundlich isotherm for adsorption of fluoride at 1g/10 ml Volume. pH 3, temp 30 ºC, contact time of 120 min and different initial concentration ofbiosorbent (a) *A. paniculata* and (b) *V. zizanioides*.

Where K_f and n are the Freundlich constant. K_f characterises the adsorption capacity of adsorbent and 1/n reflects the adsorption intensity of adsorbent. A plot of Log Q_e against Log C_e is shown in Fig. 8.4 (a) and (b), a linearity of Freundlich isotherm plot confirms the applicability of Freundlich model for defluoridation at different initial concentrations. The calculated Lengmuir and Freundlich isotherms parameters are listed in Table 8.1 and Table 8.2

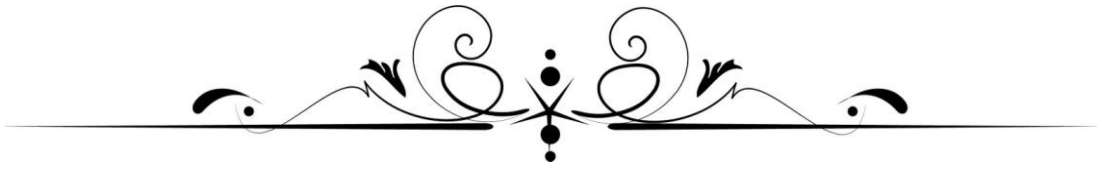
Table 8.1. Value of Lengmuir and Freundlich parameters for *A. paniculata* biosorbent

Lengmuir isotherm			**Freundlich isotherm**		
Q_{max}	b	R^2	K_f	1/n	R^2
3.8	0.094	0.98	0.445	0.66	0.93
R_L = 0.681					

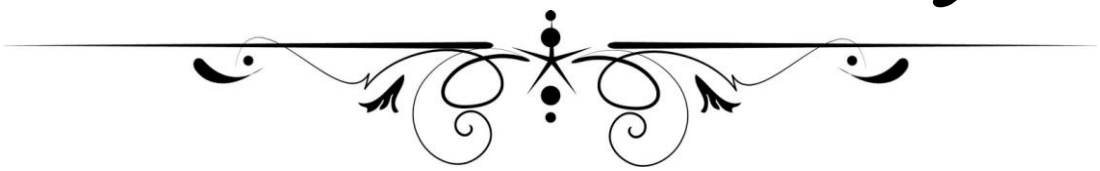
Table 8.2. Value of Lengmuir and Freundlich parameters for *V. zizanioides* biosorbent

Lengmuir isotherm			**Freundlich isotherm**		
Q_{max}	b	R^2	K_f	1/n	R^2
2.32	1.84	0.90	3.548	0.55	0.91
R_L = 0.202					

Correlation coefficient (R^2) of Lengmuir and Freundlich isotherms for both biosorbents (*A. paniculata* and *V. zizanioides*) was greater than 0.9 indicating that both of the model supports the adsorption of fluoride on biosorbents. Maximum adsorption capacity (Q_{max}) from langmuir isotherm model for *A. paniculata* and *V. zizanioides* was 3.8 and 2.32 mg/g which is higher than wheat straw, sawdust and activated bagasse; 1.93, 1.73 and 1.15 mg/g respectively as reported by Yadav et al., (2013). Results confirmed that adsorption capacity of *A. paniculata* was higher than *V. zizanioides.* R_L values were in the range of 0.118-0.681 indicating the adsorption process is favourable. Furthermore, Freundlich constant n is greater than 1 which also confirms the favourable adsorption process.



Chapter 9
Conclusion and Summary



Today, contamination of soil with heavy metal and pesticides is becoming most serious concern. Soils/lands are important target sink for number of contaminants that vary in their composition, concentration and toxicity. Contaminants including metals and pesticide entering the soil system as consequences of various anthropogenic deeds such as faulty waste management practices, improper landfill operations, mining and application of sewage sludge etc. pose a massive threat to human health and natural ecosystem. Contamination of agricultural land not only worsens the soil health but also affects the food quality, production and environmental safety. Due to ubiquitous soil contamination and its associated concerns, finding an innovative and ecofriendly way to clean metal and pesticide pollutant has now become a priority in remediation field.

Phytoremediation has been recognized as an economical technique that explores the potential of plant to accumulate, degrade, immobilize, metabolize or detoxify inorganic and organic contaminants. Till date, there are several promising results indicating that this solar driven botanical remediation might become sustainable alternative to other mechanical and chemical approaches for remediation of mildly contaminated soil. Several phytoremediation studies have been conducted by using edible crops such as wheat (*Triticum aestivum* L), tomato (*Solanum lycopersicum*), spinach (*Spinacia oleracea* L), radish (*Raphanus sativus*), potato (*Solanum tuberosum* L) Brassica (*Brassica juncea*) etc. for the removal of various organic and inorganic contaminant, particularly heavy metals. However, the use of edible crops is not safe, because they are consumed by animals and human beings and may cause toxicity on single or chronic exposure. A good phytoremediator plant should be perennial, un-palatable, have high biomass productivity, and tolerance to biotic and abiotic stresses.

The present work was undertaken to conduct a comparative study of phytoremediation potential of *A. paniculata* with a relatively studied plant *V. zizanioides.* The study was conducted to investigate the physiological and biochemical responses of *A. paniculata* and *V. zizanioides* growing under different concentration of Cu, Cd and organochlorine pesticides. The study was made to investigate the individual and collective phytoremediation potential of *A. paniculata* and *V. zizanioides,* Further, uptake, accumulation and partitioning of Cu, Cd and OC pesticide in *A. paniculata* and *V. zizanioides* have also been examined. Simultaneously, an effort was made to examine the microbial activity and fertility of rhizospheric soil treated with different concentration of Cu, Cd, and organochlorine pesticides in presence of selected plant species. Moreover, to optimize the associated sustainable and cost-benefit aspect, many researchers have advocated the strategy of combining phytoremediation with energy crops. However, intensive research is still required in this area for better and economically sustainable application. In harmony to this approach, the present study also explored the use of biomass generated after phytoremediation of Cu for the removal of fluoride from contaminated water; a chronic problem in developing countries. Although, low level of fluoride in water is considered as a nutrient because it is essential for bone and teeth development. However, when its concentration exceeds above the permissible level it becomes toxic and leads to fluorosis.

Studies on physiological and biochemical responses of *A. paniculata* and *V. zizanioides* exposed to different concentration of Cu, Cd and organochlorine pesticides

The objective of this study was to study of physiological and biochemical response of *A. paniculata* and *V. zizanioides* exposed to different concentration of Cu,

Cd, and organochlorine pesticides. During the study, seed germination, biomass production, chlorophyll content, change in level of protein and lipid peroxidation and anti-oxidative enzymes (catalase and peroxidase) has been examined. Seed germination of *A. paniculata* was found to be decreased on increasing the concentration of Cd and Cu. However, at lower concentration of Cd and Cu, the value of seed germination percentage was nearly similar to control. The rate of biomass production of roots and shoots of *A. paniculata* and *V. zizanioides* was decreased significantly on increasing the concentration of contaminants in growing medium. Further, decline in seed germination and biomass production was observed maximum in case of co-contaminated (i.e. Cd+Cu and Cd+Cu+P). It was observed that the chlorophyll content in the leaves of *A. paniculata* and *V. zizanioides* was declined simultaneously on increasing the degree of contamination. However, compared to Cd and Cu, the reduction percentage was high in case of organochlorine pesticides contamination indicating that pesticides are relatively more phytotoxic.

The effect of Cu, Cd and organochlorine pesticides on protein and malondialdehyde (MDA) content in fresh leaves were investigated and it was found that the protein content decreased with increase in Cu, Cd and organochlorine pesticide concentration in soil, whereas, MDA was found to be increased in both the species. The reduction in protein content was observed on increasing the concentration of contaminants which indicates the exposure of high level of Cu, Cd and organochlorine contamination had toxic effect on *A. paniculata* and *V. zizanioides.* However, reduction in protein content in *V. zizanioides* is comparatively lower than the *A. paniculata* indicating its higher tolerance as compare to *A. paniculata*. The degree of negative effect was directly correlated with contamination level and exposure. The level of MDA content was found higher in *V. zizanioides* than

A. paniculata which indicates more lipid peroxidation in the leaves of *V. zizanioides* as compared to *A. paniculata*. As the degree of contamination increased the generation of superoxide radical also increased that has caused increased lipid peroxidation (MDA production) and oxidative damages. In order to diminish the oxidative stress the level of anti-oxidative enzymes (peroxidase and catalase) content increased simultaneously. The peroxidase (POD) and catalase (CAT) activity was found to be increased on increasing the level of Cu, Cd and organochlorine pesticides.

Studies on individual and collective phytoremediation potential of *A. paniculata* and *V. zizanioides* to remediate Cu, Cd and organochlorine pesticides

It was observed that the accumulation of Cu and Cd increased significantly on increasing the level of contamination and exposure time ($p > 0.005$). Both the plants have accumulated significant amount of metal in their root and shoots. While accumulation of organochlorine was observed only in root and shoot of *V. zizanioides* as *A. paniculata* plants were not survived in organochlorine pesticide contaminated soil (except in combination with *V. zizanioides* at lowest concentration i.e. P1). Further, the accumulation of Cd and Cu was higher in roots of both the plants than shoots. The level of accumulation of Cu in shoot of *A. paniculata* was higher than the shoot of *V. zizanioides.* However, the accumulation of Cd and Cu in root of *V. zizanioides* was many folds higher than that of *A. paniculata* roots. In case of collective phytoremediation i.e. when both the plants were grown in same pot, the accumulation of metal in their individual roots and shoots were lower than the plants grown individually. However, if we collectively sum-up the metal accumulation by *A. paniculata* and *V. zizanioides* growing in same pot was found to be higher than the individual accumulation in *A. paniculata* or *V. zizanioides* grown in separate pots.

Studies on uptake, accumulation and partitioning of Cu, Cd and organochlorine pesticides in selected plant species

Uptake and accumulation of Cu, Cd in both the plants and Organochlorine pesticides (OCPs) in *V. zizanioides* varied significantly at different level of treatments and exposure time. As the level of contaminants in growing medium increases the level of accumulation also increased significantly. Although, *A. paniculata* had accumulated significant amount of Cd and Cu in their tissues however, uptake of Cd and Cu in *V. zizanioides* was many fold higher than *A. paniculata.* Translocation factors (TF) of metal for both the plants were lower than 1 indicating the accumulation of metal in shoots (per gram of dry weight) was lower than root. TF for metal in *V. zizanioides* was very low, which indicates that the metals were mainly concentrated in root tissue. The total metal accumulation in shoot and root may vary because of the higher biomass of shoots compared to roots. TF for Cu in *A. paniculata* and *V. zizanioides* was higher than Cd indicating that the translocation of Cu in shoot was higher than Cd. However, TF for OCPs in *V. zizanioides* was observed higher than metals indicating that the translocation of OCPs in above ground parts was higher than the Cd and Cu.

Studies on microbial activities and fertility of rhizospheric soil treated with different concentration of Cu, Cd and pesticides in presence of selected plant species

Microbiological load/Colony forming unit (cfu) and dehydrogenase activity were found to be decreased significantly on increasing the concentration of metal and OCPs. However, at high level of contamination the values are approximately same because only resistant bacteria can survive at high level of contamination. High concentration of metals in soil caused reduction of microbial activitythat affects the

microbial population and diversity as well. Microbiological population and dehydrogenase activity of soil of both the plants are correlated with each other and reduced simultaneously with the treatment after 30, 60 and 90 days.

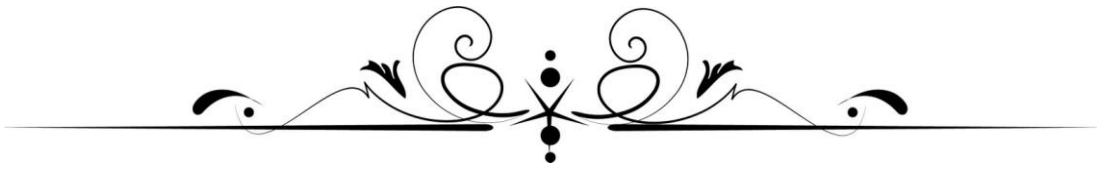
Fertility of soil treated with different concentration of Cd, Cu and OCPs were analyzed by measuring the NPK and organic carbon content. The balance level and ratio of NPK in soil is considered as beneficial for plant growth and development. It was observed that the level of organic carbon (%) and available NPK in soil were decreased significantly on increasing the concentration of Cd, Cu and OCPs. However, the decline of NPK and OC content was higher in case of Cd contamination than Cu. Further, the *V. zizanioides* planted soil have higher NPK and OC content than the *A. paniculata* planted soil probably because *V. zizanioides* have well developed massive root system and higher microbial root activity which helps to maintain the NPK and OC level in soil. At high concentration of contaminant, the microbial activity was declined and simultaneously the level of NPK and OC was also reduced. Thus the presence of microbial activity could be a possible reason for the soil fertility.

Application of copper phytoremediated plant biomass for the defluoridation of water

Several batch experiments were executed to study the feasibility of defluoridation of water using *A. paniculata* and *V. zizanoides* leaves as biosorbent under different conditions and found that the adsorption capacities of both biosorbent found to be influenced by contact time, pH, initial fluoride concentration and adsorbent dose. It was found that the removal efficiency of fluoride was increased on increasing the contact time, initial F concentration, adsorbent dose and decreasing pH. The fluoride removal efficiency of *A. paniculata* was found to be higher than the *V.*

zizanoides.Further the Q_{max}(maximum adsorption capacity) obtained from langmuir isotherm model for *A. paniculata* (Q_{max}=3.8mg/g) was higher than *V. zizanoides* (Q_{max}= 2.32 mg/g) incating that the adsorption capacity of *A. paniculata* was higher than *V. zizanioides.* Furthermore, the R_L value and Freundlich constant (n) is greater than 1 which confirms the favorable adsorption process.

From the above study, we can conclude that the *V. zizanioides* have high tolerance and potential for the remediation of Cd, Cu and OCPs and can be considered as a suitable candidate for phytoextraction. Likewise, *A. paniculata* can be considered as a moderate phytoremediator of Cu and Cd. However, its high adsorption capacity for fluoride makes it a good biosorbent for deflouridation of water.



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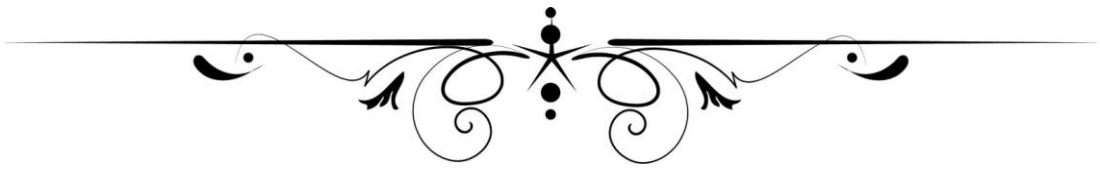

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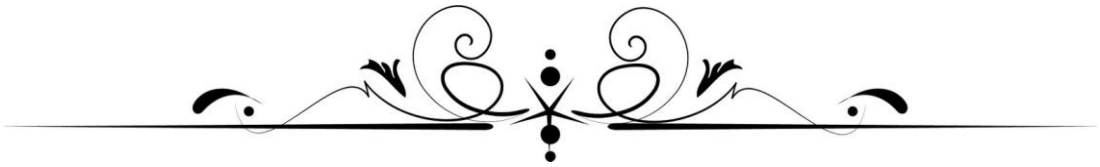
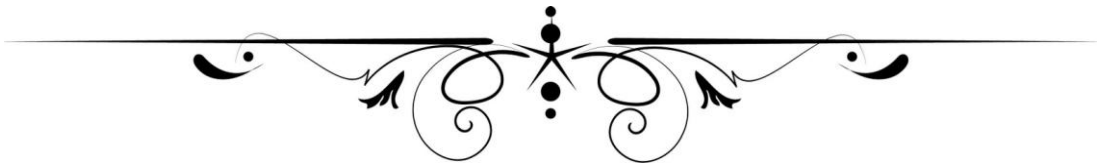


Publications



Research Papers:

1. Dhananjay Kumar, Sushil Kumar Bharti, Sangeeta Anand and Narendra Kumar (2018) Bioaccumulation and biochemical responses of *Vetiveria zizanioides* grown under Cadmium and Copper stresses. *Environmental Sustainability*. https://doi.org/10.1007/s42398-018-0009-z.

2. Dhananjay Kumar, Sushil Kumar Bharti,Sangeeta Anand and Narendra Kumar (2018) Defluoridation of water with the help of copper phytoremediated *Andrographis paniculata* plant biomass. *Journal of Environmental Biology*. 39 (Accepted).

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2. Dhananjay Kumar, Sanjeev Kumar, Vertika Shukla, Narendra Kumar (2017) Adaptation Strategies of Plants Against Common Inorganic Pollutants and Metals. In: Shukla et al. (eds) *Plant Adaptation Strategies in Changing Environment*, Springer Nature Singapore 315-328.

3. Dhananjay Kumar, Poonam, Kuldeep Bauddh, Jaya Tiwari, D. P. Singh and Narendra Kumar (2017), *Ricinus Communis*: An ecological engineer and a biofuel resource. In: Bauddh et al. (eds) *Phytoremediation Potential of Bioenergy Plants*. Springer Singapore, 139-167

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ORIGINAL ARTICLE

Bioaccumulation and biochemical responses of *Vetiveria zizanioides* grown under Cadmium and Copper stresses

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Abstract
Pot experiments were carried out to investigate the effects of Copper (Cu) and Cadmium (Cd) on *Vetiveria zizanioides*. Results revealed that increasing metal concentration in growing medium caused reduction in dry biomass, water content, leaf chlorophyll and soluble protein on exposure of Cu and Cd for 30 days. Cu and Cd accumulation in root and shoot enhanced on increasing Cu and Cd concentration in soil and reached a maximum of 514 and 1069 mg kg^{-1} dry weight (DW) in root and 58.03 and 17.87 mg kg^{-1} DW in shoot at exposure of 150 and 75 mg kg^{-1} of Cu and Cd respectively. Results also showed that the exposure of Cu and Cd in combination was more phytotoxic than the individual treatments. However, Bioconcentraion Factor (BCF) was found to be greater than one indicating suitability of *V. zizanioides* for phytoremdiation of Cu and Cd from soil. Metal accumulation in roots was much higher than shoots (TF $\ll$ 1) indicating that the mechanism involved was of phytoextraction.

Keywords Bioconcentraion · Cadmium · Metal translocation · Phytoextraction · Vetiver

Introduction

Heavy metals are important class of environmental contaminants and are potentially toxic in both elemental and soluble forms. Various anthropogenic activities such as unplanned industrialization, injudicious waste management, faulty landfill operations, mining and manufacturing have led to the increased metal contamination in soil (Zhang et al. 2010; Nagajyoti et al. 2010). Heavy metals are taken up into the plant system along with the other nutrients through nutrient absorption process (Liu et al. 2007). Cu is considered as an essential micronutrient for plants because of its key role in photosynthesis and other metabolic processes as a cofactor for several enzymes (Thomas et al. 1998; Mahmood and Islam 2006; Chatterjee et al. 2006). However, presence of excess Cu in growing medium causes phytotoxicity such as leaf chlorosis and retardation in plant growth (Moreno-Caselles et al. 2000; Lewis et al. 2001; Singh and Tewari 2003). Excess of Cu also generates oxidative stress and Reactive Oxygen Species (ROS) which cause disturbance in metabolic pathways and damage to macromolecules (Hegedüs et al. 2001). Contrary to Cu, Cd is a non-essential element for plants and considered to be a potentially hazardous trace metal. On exposure of excess Cd, plants display toxicity in the form of stunted growth, chlorosis, browning of root tips etc. (Das et al. 1997; Wojcik and Tukiendorf 2004; Mohanpuria et al. 2007; Guo et al. 2008). Noticeably, in combination with Cd, Cu has been reported for its adverse effects on seed germination, length of seedling, number of lateral roots and generation of ROS (Neelima and Reddy 2002; Zhang et al. 2015; Sidhu et al. 2017). The ROS include hydrogen peroxide, superoxide ($O_2^{\bullet -}$) and hydroxyl ($OH^{\bullet -}$) radicals. ROS are byproducts generated during electron transport activities and metabolic pathways and cause damage to the biomolecules like proteins, lipids, chlorophyll, enzymes, etc. (Wang et al. 2008; Sidhu et al. 2016).

Being persistent in nature; Cu and Cd remain in soil for long time and have a tendency to get bio-accumulate in ecological food chain which poses the threats of metal toxicity to the living beings occupying the higher tropic levels (Sakakibara et al. 2011; Kumar et al. 2012, 2013). Hence, remediation of metals from soil is of utmost importance, i.e. in order to protect humans and animals from metal toxicity. Heavy metals are naturally found in their discrete

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form in rocks. However, uncontrolled industrialization and unplanned urbanization have caused amplification of heavy metals in biosphere. During the last few decades, attentiveness towards the plant based remediation, i.e. phytoremediation, particularly in mildly contaminated soils has increased not because of their effectiveness but also due to associated additional benefits like carbon sequestration and improvement of soil health. Further, phytoremediation is also gaining attraction being ecofriendly, economical and aesthetically acceptable in nature.

In the race of exploration of plants for remediation purpose, vetiver can be a suitable player because of its extensive and deep root system, rapid and high biomass production, ability to survive in diverse stress conditions and high metal accumulation potential. Vetiver has been found to be successful in stabilization of alkaline, acidic and coal mined land (Aibibu et al. 2010). Present study deals with the bioaccumulation and biochemical responses of *Vetiver zizanioides* on exposure to Cd and Cu at different levels. Further, the grass has been studied for its metal accumulation behavior and applicability for the remediation of land mildly contaminated/co-contaminated with Cu and Cd.

Materials and methods

Soil

Soil was collected from the research field station of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow (N:26°46′05.4″; E: 80°55′38.8″), India and air dried for 5 weeks before passing through 1.5 mm sieve. Physico-chemical parameters of experimental soil viz. pH, organic carbon, electrical conductivity and essential minerals like nitrogen, phosphorus, potassium, calcium, iron, mangnese, sodium and zinc were examined before initiating the pot experiment according to Maiti (2003). Ground water used for the irrigation was also analyzed for various physico-chemical characteristics including trace metals as per APHA (2005).

Plant

Seedlings of *V. zizanioides* were procured from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, Uttar Pradesh, India. All the seedlings were grown in 30-cm-diameter earthen pots filled with 9 kg soil. Each pot was watered regularly with ground water to keep the soil moist. Soil treatments of Cu and Cd were prepared by mixing the appropriate amount of $CuSO_4$ and $Cd(NO_3)_2{\cdot}4H_2O$ to achieve 50, 100 and 150 mg Cu kg^{-1} soil and 25, 50 and 75 mg Cd kg^{-1} soil. Garden soil without any metal treatment served as control. The pots sown with the seedling of *V. zizanioides* were kept in the net house of research field station of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India. To determine the metal bioaccumulation potential of *V. zizanioides*, one plant from each pot was uprooted at the interval of 30, 60 and 90 days after sowing (DAS).

Biomass, Water Content (WC), Chlorophyll and Protein content

Biomass per plant was measured gravimetrically. Water content of plant was calculated by applying the following equation:

$$\mathrm{WC}\ (\%) = (\mathrm{FW} - \mathrm{DW})/\mathrm{DW} \times 100,$$

where WC (%) is the water content, FW (g) fresh weight and DW (g) dry weight of plant. Before the FW measurement, freshly uprooted plants were washed twice with deionized water, subsequently blotted with filter paper and weighed.

Chlorophyll and protein content was determined in fresh leaves of *V. zizanioides* after 45 days exposure using spectrophotometer as described by Lichtenthaler (1987), whereas, total protein content was calculated following Bradford (1976) using bovine serum albumin (BSA) as standard.

Metal content, bioconcentration factor (BCF), translocation factor (TF)

For determination of metal content, 1 g of dried plant sample was digested in a solution of HNO_3:$HClO_4$ (3:1) at 70–80 °C. The solution was allowed to evaporate by raising the temperature to 105 °C until the solution became transparent. Final volume was diluted to 25 ml with 0.1 N HNO_3, filtered through Whatman (no. 42) filter paper and subsequentially analyzed by atomic absorption spectrophotometer (AA 240 FS, Varian) (Piper 1942).

BCF was determined to derive the degree of heavy metal accumulation in plants following Zayed et al. (1998).

$$\mathrm{BCF} = \frac{\text{Concentration of metal in roots/shoots}}{\text{Concentration of metal at contaminated site}}.$$

TF or mobilization ratio of each metal was calculated to determine the translocation of metals from root to shoot of the plant species following Barman et al. (2000).

$$\mathrm{TF} = \frac{\text{Concentration of metal in plant shoots}}{\text{Concentration of metal in plant roots}}.$$

Results and discussion

Physico-chemical parameters of experimental soil were examined before sowing. Soil was found slightly alkaline with pH 7.45, EC 0.46 ds m^{-1} and organic carbon 1.39%. Soil was found to be rich in Ca: 3.07; Fe:112.38; Mn: 7.42; Na: 3.22; S: 15.43; and Zn: 2.78 ppm. N, P and K content of the soil was found to be 1.38, 0.91 and 3.48 g kg^{-1} respectively. Ni, Pb, Cu and Cr were present in traces (Table 1).

Groundwater used for irrigating the pots was examined for various physico-chemical characteristics viz. Total dissolved solids (300 mg l^{-1}), nitrate (6.29 mg l^{-1}), chloride (28.23 mg l^{-1}), sulphate (13.09 mg l^{-1}), alkalinity (28.7 mg l^{-1}), hardness (150 mg l^{-1}), Na (13.2 mg l^{-1}), Ca (6.2 mg l^{-1}), K (3.54 mg l^{-1}) and were found to be within the limits prescribed by WHO (2012), whereas, trace metals; As, Cd and Cu were not detected.

Effect on biomass production and water content

The effect of exposure of Cd and Cu on growth of plant was assessed by observing the total biomass production after 45 days growth (Table 2.). Different concentrations and combination of Cu and Cd exerted variable effects on the biomass of *V. zizanioides*. At moderate exposure to Cu (50 mg kg^{-1}) plant showed slight increase in biomass. However, further increase in Cu concentration caused gradual decrease in biomass. In case of Cd exposure, the plant biomass decreased as the level of exposure increased. Compared to control, the total plant biomass at Cd 75 mg kg^{-1} declined up to 33.3%. In case of combination of Cu and Cd exposure, gradual decrease in the plant biomass was observed. However, in combination of Cu and Cd @ 50 and 25 mg kg^{-1} the plant biomass was more than that of Cd @ 50 and 75 mg kg^{-1}. Cu being an essential element for plant growth at low level and Cd tolerance ability of vetiver may be probable reason for the higher biomass. On increase in metal concentration in the soil, the water content of plant decreased gradually along with the biomass production. On exposure of Cu, Cd and Cu + Cd @ 150, 75 and 150 + 75 mg kg^{-1} the water content declined to 81.57, 77.76 and 76.31% of control, respectively. The substantial reduction in biomass and water content with increased level of exposure showed that high level of exposure leads to phytotoxicity and decline in biomass production. Similar results for reduction in biomass of *V. zizanioides* exposed to Cd have been reported by several authors (Liu et al. 2009; Aibibu et al. 2010; Zhang et al. 2014).

Table 1 Physicochemical characteristics of soil

Parameters	Mean value ± SE
pH	7.45 ± 0.3
EC ($dsm^{-1)}$	0.46 ± 0.02
Organic C (%)	1.35 ± 0.07
N (g kg^{-1})	1.38 ± 0.04
P (g kg^{-1})	0.91 ± 0.006
K (g kg^{-1})	3.48 ± 0.08
Na (ppm)	3.22 ± 0.05
Ca (ppm)	3.07 ± 0.04
S (ppm)	15.43 ± 0.32
Fe (ppm)	112.38 ± 2.8
Mn (ppm)	7.24 ± 0.06
Zn (ppm)	2.78 ± 0.08
Cd (ppm)	0.01 ± 0.003
Cu (ppm)	2.29 ± 0.13
Pb (ppm)	0.72 ± 0.03
Ni (ppm)	0.016 ± 0.007
Cr (ppm)	0.002 ± 0.0001

All results have been expressed as mean of five replicates, i.e. n = 5 ± SE

Metal accumulation, bioconcentration and translocation factor

The uptake and accumulation of Cu and Cd in roots and shoots of *V. zizanioides* varied significantly at different concentrations (Table 3). Cu accumulation in roots of treated plants ranged from 73.29 to 133.31, 116.04–397, 234–514 mg kg^{-1} while in shoot it ranged between 9.01–10.95, 26.71–37, 43.07–58.03 mg kg^{-1} at 30, 60 and 90 DAS, respectively. Cd accumulation in roots of treated plants ranged from 197 to 569, 327–669, 497–1069 mg kg^{-1} while the corresponding values in roots were 9.87–16.27, 13.43–17.27 and 17.87–24.21 mg kg^{-1} at 30, 60 and 90 DAS respectively. Truong (1999) had reported the threshold limit of 13–15 mg kg^{-1} and 45–48 mg kg^{-1} for Cu and Cd in shoots of vetiver. Similar results were also reported by Das and Maiti (2009) and Danh et al. (2010). Most of the Cu and Cd was found to be accumulated in the roots of *V. zizanioides* whereas, comparatively very low amount was translocated to above ground part, i.e. shoots [Fig. 1(c) and (d)]. Availability of Cu and Cd at different concentrations in soil did not significantly affect the translocation of metal from root to shoot. BCF for Cu and Cd was found to be greater than 1 indicating that the metal concentration in tissue is higher than in the growing medium [Fig. 1(a) and (b)]. A good phytoremediator species possesses BCF value of greater than 1 (Zhang et al. 2002). Noticeably the BCF for Cd was found to be higher compared to Cu exhibiting that the Cd accumulation potential *V. zizanioides* was higher than the Cu. Similar trend of metal accumulation and translocation was observed by several authors (Danh et al. 2010; Banerjee et al. 2016; Vargas et al. 2016; Meyer et al. 2017).

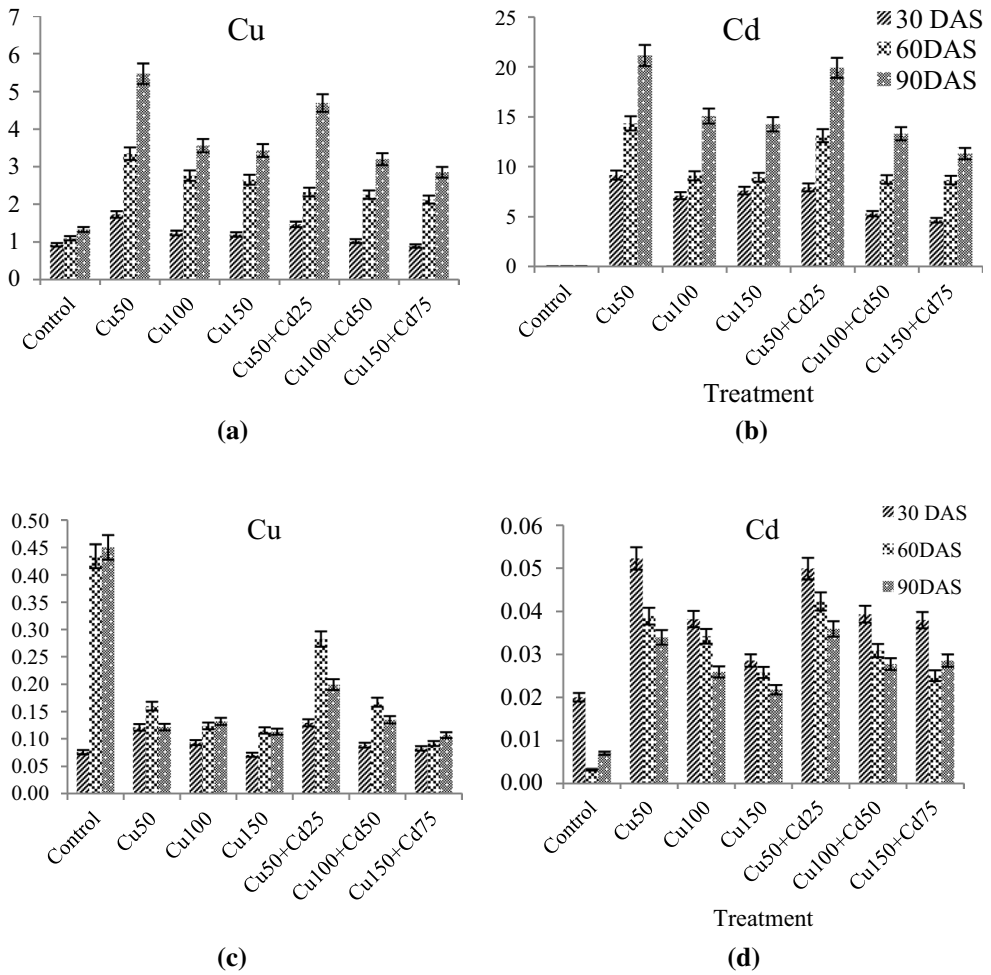

Fig. 1 Bioconcentration factor (**a**, **b**) and tanslocation factor (**c**, **d**) of Cu and Cd in *V. zizanioides*. Values represent mean ± SE based on five independent observations and bar indicate 5% standard error

Table 2 Influence of Cu and Cd on dry biomass of *V. zizanioides* after 45 days of treatment

Treatments ($mgkg^{-1}$)	Root biomass (g/plant)	Shoot biomass (g/plant)	Water content (%)
Control	1.68 ± 0.041	2.01 ± 0.065	75.20 ± 0.91
Cu50	1.77 ± 0.034*	1.98 ± 0.043*	70.70 ± 2.12
Cu100	1.56 ± 0.034*	1.83 ± 0.057	67.69 ± 1.43*
Cu150	1.15 ± 0.051*	1.34 ± 0.023	61.40 ± 2.21**
Cd5	1.22 ± 0.064**	1.27 ± 0.033*	72.99 ± 2.76*
Cd10	0.86 ± 0.012	1.03 ± 0.054*	68.05 ± 3.21**
Cd25	0.56 ± 0.021*	0.67 ± 0.022*	58.53 ± 0.89
Cu50 + Cd25	1.08 ± 0.042	1.21 ± 0.021*	69.41 ± 0.97
Cu100 + Cd50	0.85 ± 0.053*	0.76 ± 0.012	54.18 ± 1.72*
Cu150 + Cd75	0.68 ± 0.032*	0.72 ± 0.006*	57.44 ± 0.45*

The significant differences are represented by $*p < 0.05$, $**p < 0.01$. Values represent mean of five replicates ± SE

Influence of Cu and Cd stress on Chlorophyll content

Photosynthesis is a vital process for plant growth and significantly reduces on increasing the metal concentration in growing medium. It is reported that the interventral chlorosis of leaves is the first visible symptom of metal

Table 3 Accumulation of Cu and Cd (mg kg^{-1}DW) in the roots and shoots of *V. zizanioides* after 30, 60 and 90 days of sowing

Treatments (mg kg^{-1})	Cu (mg kg^{-1})			Cd (mg kg^{-1})	
	Roots	Shoots		Roots	Shoots
30 days after showing					
Control	5.81 ± 0.53*	0.47 ± 0.02	Control	0.05 ± 0.002*	0.01 ± 0.001
Cu50	86.19 ± 2.17**	10.41 ± 0.42	Cd25	228.70 ± 27.21*	11.95 ± 0.62
Cu100	123.75 ± 13.26	11.48 ± 0.86	Cd50	354.09 ± 17.39	13.52 ± 0.43
Cu150	179.70 ± 19.11	12.72 ± 0.06	Cd75	569.560 ± 28.66	16.27 ± 1.8
Cu50 + Cd25	73.29 ± 3.62**	9.47 ± 0.71*	Cu50 + Cd25	197.82 ± 12.53	9.87 ± 0.32
Cu100 + Cd50	102.22 ± 16.87*	9.01 ± 0.44	Cu100 + Cd50	265.19 ± 22.31*	10.43 ± 0.52
Cu150 + Cd75	133.31 ± 17.82	10.95 ± .51	Cu150 + Cd75	348.19 ± 25.23	13.21 ± 0.81
60 days after showing					
Control	6.91 ± 0.07	2.73 ± 0.08	Control	0.07 ± 0.002	0.02 ± 0.001
Cu50	167.22 ± 11.92*	26.71 ± 3.17	Cd25	358.70 ± 27.94**	13.95 ± 0.89
Cu100	276.05 ± 20.05	34.07 ± 3.75	Cd50	454.09 ± 33.98*	15.52 ± 2.12
Cu150	397.37 ± 27.11	45.75 ± 4.07*	Cd75	669.56 ± 52.17	17.27 ± 2.68
50 + 25	116.04 ± 9.25*	32.75 ± 2.78	50 + 25	327.82 ± 30.06*	13.87 ± 1.01
100 + 50	225.23 ± 18.64	37.60 ± 3.82*	100 + 50	435.19 ± 38.21	13.43 ± 1.92*
150 + 75	318.22 ± 27.18	28.99 ± 2.97	150 + 75	648.15 ± 47.33*	16.21 ± 1.23
90 days after showing					
Control	8.37 ± 0.76	2.83 ± 0.009	Control	0.08 ± 0.003*	0.04 ± 0.001
Cu 50	273.47 ± 22.81*	33.16 ± 2.68*	Cd25	528.70 ± 45.39**	17.95 ± 3.56*
Cu 100	355.67 ± 29.67*	46.87 ± 3.42*	Cd50	754.09 ± 63.82*	19.52 ± 1.40
Cu 150	514.24 ± 46.21	58.03 ± 3.88*	Cd75	1069.56 ± 69.34	23.27 ± 1.78
50 + 25	234.73 ± 18.73*	46.75 ± 3.64*	50 + 25	497.82 ± 36.21*	17.87 ± 1.01
100 + 50	319.74 ± 27.56	43.07 ± 3.41	100 + 50	665.19 ± 46.32	18.43 ± 1.69*
150 + 75	427.25 ± 37.83**	45.52 ± 4.05	150 + 75	848.16 ± 58.34	24.21 ± 1.92

The significant differences are represented by $*p < 0.05$, $**p < 0.01$. Values represent mean of five replicates ± SE

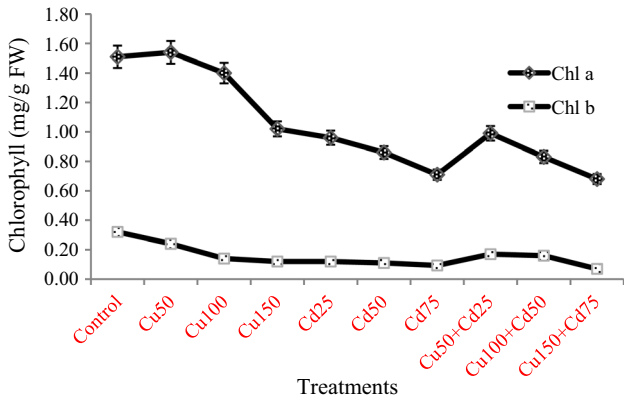

Fig. 2 Chlorophyll contents in leaves of *V. zizanioides* after 45 days of treatment periods. Values represent mean ± SE based on five independent observations and bar indicate 5% standard error

phytotoxicity and closely related to chlorophyll content (Stiborova et al. 1986; Aibibu et al. 2010). At lower concentration of Cu and Cd, both chlorophyll a and b contents of leaves were found to be higher (Fig. 2.). However, an increase in concentration of Cd and Cu in soil caused decline in chlorophyll content. In case of Cu, at concentration of 50 mg kg^{-1}, chlorophyll content was found to be slightly higher (1.54 mg/g FW) than the control (1.51 mg/g FW). At highest exposed concentration of Cu (150 mg kg^{-1}) the chl *a* and chl *b* content was recorded as 1.02 and 0.12 mg/g FW. While, at highest Cd concentration (75 mg kg^{-1}) the chl *a* and chl *b* content was found as 0.71 and 0.09 mg/g FW which was comparatively lower than the highest Cu stress condition indicating that Cd is comparatively more toxic to *V. zizanioides* than Cu. In case of highest concentration of combined treatment of Cu and Cd, i.e. 150 and 75 mg kg^{-1} respectively the chl *a* and chl *b* content was recorded as 0.68 and 0.07 mg/g FW which was found to be lowest establishing that the combined effect of Cu and Cd were more toxic.

Influence of Cu and Cd stress on protein content

Effect of Cu and Cd on protein content at different concentrations and combinations are presented in Fig. 3. Soluble protein content in roots and shoots of *V. zizanioides* was found to decrease gradually on increasing Cu and Cd

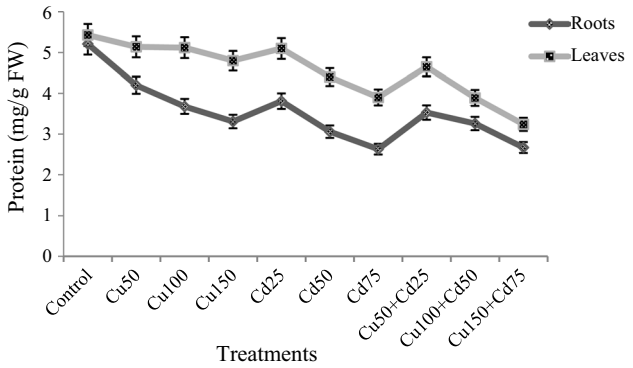

Fig. 3 Soluble protein contents in roots and leaves of *V. zizanioides* after 45 days of treatment periods. Values represent mean ± SE based on five independent observations and bar indicate 5% standard deviation

concentration. Decline in soluble protein of roots was found to be more than shoots. Maximum value of soluble protein was recorded in control plants, i.e. 5.21 and 5.43 mg/g FW in root and shoot respectively while, minimum level of protein content was recorded as 2.67 and 3.24 mg/g FW in plant exposed to metal combination (Cu 150 and Cd 75 mg kg^{-1}). At high concentration of Cu (150 mg kg^{-1}), the reduction in soluble protein was 63.53 and 88.39% compared to control in root and shoot, respectively. While in case of high level of Cd exposure (75 mg kg^{-1}), the reduction was found to be 51.36 and 71.82% of control in root and shoot, respectively, showing that the exposure of Cd is more toxic for the plant in comparison to Cu. Influence on soluble protein content is a key indicator of instabilities in metabolic process. Soluble protein is well known to respond to variety of stresses including metals (Singh and Tewari 2003) and its functionality can be exaggerated by presence of ROS either by oxidation of side chains of amino acids or secondary reaction of ROS with aldehydic products of lipid peroxidation (Reinheckel et al. 1998; Singh et al. 2017). Therefore, decrease in soluble protein content on increasing the metal concentration showed metal induced oxidative stress.

Conclusion

Exposure of Cu and Cd at high concentrations leads to several toxic effects. Although, at some instances exposure of Cu at comparatively low level did not cause significant toxic effects. Significant variation ($p < 0.001$) in biochemical parameters on exposure to Cu and Cd reveals their phytotoxic potential. Accumulation of high amount of Cd in comparison to Cu in vetiver reveals its high tolerance towards Cd and can be considered as a hyperaccumulator of Cd. In view of its ability to survive in diverse stress conditions, rapid growth, high biomass production, and high accumulation potential, *V. zizanioides* can be considered as a suitable candidate for the remediation of soils mildly contaminated with Cd and Cu. Furthermore, accumulation of metal in roots was much higher than shoots indicating that it could be a better option for the phytoextraction or phytostabilization of Cu and Cd with least chance of their entry into the food chain.

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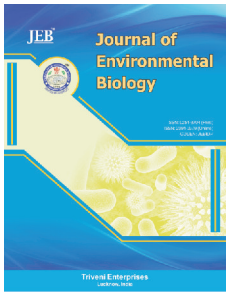

Defluoridation of water with the help of copper phytoremediated *Andrographis paniculata* plant biomass

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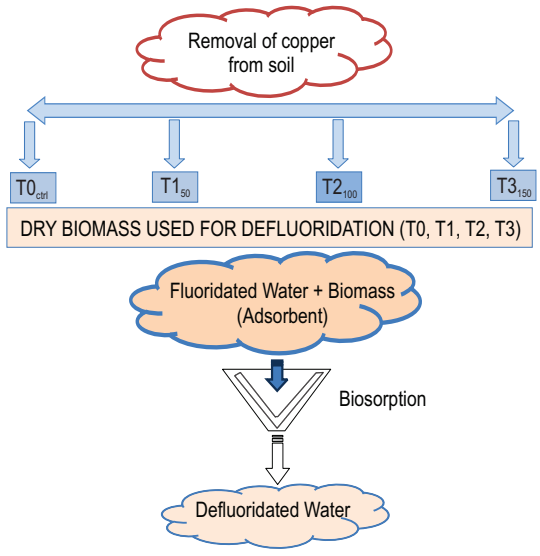
Abstract

Aim : The present study aimed to defluoridate water with the help of copper phytoremediated *A. paniculata* plant biomass.

Methodology : One gram of dry root and shoot of *A. paniculata* (30, 60 and 90 days) grown in Cu treated soil (50, 100, 150 mg kg^{-1}) was digested separately in a solution of HNO_3:$HClO_4$ (3:1) at 70–80°C and was subsequently analyzed on atomic absorption spectrophotometer (AA 240 FS, Varian). Biomass of fresh shoot of *A. paniculata* 90 days after sowing (DAS) were collected from the experimental pots (T0, T1, T2 and T3) and washed carefully with double distilled deionized water and sun dried till constant weight. Dried plant biomass samples were ground manually and sieved to obtain powder of below 1.5 mm diameter and used as a biosorbent. Before applying as a biosorbent, the obtained powdered sample was subjected to acid/alkali treatment. The treated plant material was washed repetitively with double distilled water till clear solution having pH 7 was obtained.

Plant (*A. paniculata*)



Results : Cu accumulation in roots and shoots after 30, 60 and 90 DAS ranged between 38.19-70.70, 57.23-97.38 and 73.47-184.24 and 25.41-51.23, 26.71-55.75 and 43.16-118.03 µg g^{-1} d.wt. respectively. Enrichment coefficient of Cu in root (EC_{root}) and shoots (EC_{shoot}) at 30, 60 and 90 days after treatment ranged between 0.47-0.92, 0.65-1.14, 1.23-2.60 and 0.34-0.51, 0.37-0.53, 0.67-1.48 respectively. The dried and ground biomass of *A. paniculata* had successfully reduced fluoride concentration in water from 5 to 0.784 (mg l^{-1}) accounting for 84% removal at an adsorbent dose of 1.5 mg, contact time 100 min. and pH 3, respectively.

Interpretation : The correlation coefficient (R^2) confirmed the suitability of Langmuir and Freundlich isotherms. The R_L values indicated favorable adsorption process. Furthermore, Freundlich constant (n) was found to be greater than 1 which also confirmed the favorable adsorption process.

Introduction

Rampant industrialization and unorganized anthropogenic activities throughout the world has laid increasing pressure on all components of the environment by releasing enormous quantities of organic as well inorganic contaminants including metal and metalloids. Soil contamination with metals due to several anthropogenic deeds like improper waste management practices, landfill operations, mining, manufacturing and application of sewage sludge etc. has emerged as a major environmental challenge. Agricultural field contamination with metals is not only degrading the quality of soil and foods,but also poses a threat to human health and ecosystem (Mapanda *et al.,* 2005; Singh *et al.,* 2010). Bioavailability of copper (Cu) in soil at optimum concentration is considered as essential micronutrient because it play an important role in plant growth and development (Yruela, 2009), however, its bioavailability even at slightly higher than the optimal level can cause phytotoxicity (Michaud *et al.,* 2008). Anthropogenic deeds e.g. use of pesticide, fungicides and Cu-rich slurries during agricultural practices elevates the level of Cu in soil (Legros *et al.,* 2010; Nagajyoti *et al.,* 2010). Higher concentration of Cu in soil interferes with the availability of various nutrients by converting ionic species to non-ionic form. At elevated concentration, Cu can hamper the plant growth by enhancing the generation of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) (Adrees *et al.,* 2015; Habiba *et al.,* 2015; Mei *et al.,* 2015). Various *in situ* and *ex situ* remediation approaches like stabilization, solidification, soil washing, vitrification, electrokinetic treatment and phytoremediation are being used for the restoration of metal contaminated land. Among these techniques phytoremediation is a technique that utilizes living plants to reduce, remove, degrade or immobilize the contaminants. This technique emerges as an economical, eco-friendly and most aesthetically acceptable technique (Kumar *et al.,* 2012; Stingu *et al.,* 2012; Kumar *et al.,* 2013; Chayapan *et al.,* 2015). However, proper utilization of biomass generated after phytoremediation is an important concern.

Globally, more than 200 million people are affected with fluorosis in more than twenty nine countries including India (Ayoob *et al.,* 2008). Several techniques are available for the removal of fluoride from water *viz.,* precipitation (Reardon and Wang, 2000), ion exchange, reverse osmosis (Ndiayea *et al.,* 2005), electrodialysis (Kabay *et al.,* 2008) and adsorption (Karthikeyan *et al.,* 2009; Mohan *et al.,* 2012a). Among the available techniques, adsorption had been proven to be effective and economical. Various adsorbents like aluminum based material (Ayoob *et al.,* 2008), algal biosorbent (Mohan et al., 2007), chitosan beads (Liu *et al.,* 2014; Miretzky and Cirelli, 2011), clay soil, carbon based material (Eric *et al.,* 2010; Mohan et al., 2012b), powdered *Tinospora cordifolia* (Pandey *et al.,* 2012), *Echhornia crassipes* (Sinha *et al.,* 2003), lime (Islam and Patel, 2006; Gogoi *et al.,* 2015), wheat straw dust, saw dust raw and activated biogass carbon (Yadav *et al.,* 2013) etc. have been applied for the defluoridation. In view of the above, the present study was carried out to assess defluoridation of water with the help of copper phytoremediated *Andrographis paniculata* plant biomass.

Materials and Methods

Phytoremediation of Cu; plant materials and treatments : Certified seeds of *Andrographis paniculata* were procured from the National Seed Disposal Centre, Lucknow, Uttar Pradesh, India. Seed were sown in 30 cm-diameter earthen pots filled with 8.5 Kg (approx) soil. All the pots were watered regularly with ground water to keep the soil moist. Nitrogen (N), phosphorus (P) and potassium (K) @120, 30 and 80 mg kg^{-1} soil respectively were applied at the time of sowing in the form of urea, single super phosphate and potash. Soil treatments of Cu were prepared by mixing the appropriate amount of $CuSO_4$ to achieve 50, 100 and 150 mg Cu kg^{-1} soil along with control *i.e.,* garden soil. The pots sowed with the seed of *A. paniculata* were kept in the net house of research field station of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, India. Seed germination was recorded after six days of sowing and thereafter, plants were thinned to three plants per each pot for further studies. To determine the metal accumulation potential one plant from each pot was uprooted at the interval of 30, 60 and 90 days after sowing (DAS). Physico-chemical parameters of experimental soil were examined before initiating the pot experiment.

Estimation of metal content, Enrichment coefficient and Translocation factor : One gram of dried plant sample was digested in a solution of HNO_3: $HClO_4$ (3:1) at 70–80°C for metal estimation. The solution was allowed to evaporate by raising the temperature to 105°C until the solution becomes transparent. The final volume was diluted to 25 ml with 0.1 N HNO_3, filtered through Whatman no. 42 filter paper and analyzed on atomic absorption spectrophotometer (AA 240 FS, Varian) (Piper, 1942).

Enrichment coefficient (EC) has been determined to derive the degree of heavy metal accumulation in plants following Kisku *et al.* (2000). Translocation factor (TF) or mobilization ratio of each metal was calculated to determine the translocation of metals from root to shoot of the plant species following Barman *et al.* (2000).

Defluoridation of water : A 100 mg l^{-1} stock solution of fluoride was prepared by dissolving 221 mg of anhydrous sodium fluoride of 99.5% purity in one liter of distilled deionized water from Millipore. Test concentration of 5 mg l^{-1} was prepared from stock solution following serial dilution technique. Test concentration of 5 mg l^{-1} was selected for adsorption experiment since it is considered to be normal fluoride concentration in groundwater. Fresh shoot of *A. paniculata* of 90 days age were collected from the experimental pots (T0, T1, T2 and T3) used for phytoremediation of Cu from the soil. Plant parts were washed carefully with double distilled deionized water and sun dried for 3 days. Dried plant biomass samples were ground manually with the help of mortar pestle and sieved to obtain powder of below 1.5 mm diameter. Before applying as biosorbent; the obtained powdered sample was subjected to acid/alkali treatment. Powdered sample of *A. paniculata* (50 gm) was mixed with 500 ml of 1 M HNO_3 and heated gently for 20 min. on open flame burner

then filtered out and washed with double distilled water till the elimination of color. Acid treated sample was subsequently subjected to alkali treatment with 500 ml of 0.5 M NaOH. The treated plant material was washed repetitively with double distilled water till a clear solution of pH 7 was obtained. The obtained powdered biomass was oven dried for 3 hrs at 110°C and subsequently cooled in air to room temperature for use.

Results and Discussion

Physico-chemical properties of experimental soil were investigated prior to seed sowing. Soil was found slightly alkaline with pH 7.41, EC 0.46 ds m^{-1} and organic carbon 1.38%. Soil was found to be rich in Ca : 3.02 ppm; Fe : 114.32 ppm; Mn: 7.2 ppm; Na: 3.17 ppm; S: 16.58 ppm; and Zn: 2.73ppm. N, P and K content of the soil was found to be 1.39, 0.94 and 3.52 g kg^{-1}, respectively. Ni, Pb, Cu and Cr were present in traces.

Accumulation of Cu in roots of *A. paniculata* was higher than the shoots in all treatments. Cu accumulation in roots and shoots after 30, 60 and 90 DAS ranged between 38.19-70.70, 57.23-97.38 & 73.47-184.24; 25.41-51.23, 26.71-55.75 and 43.16-118.03 µg g^{-1} d.wt., respectively (Table 1). Enrichment coefficient (EC) was used to determine the degree of metal accumulation in roots and shoots with respect to metal concentration in growing medium. EC_{root} is used as an index for the transfer of metal from soil to plant root, while EC_{shoot} determines the degree of transfer of metal from soil to shoot. EC also represents a special feature of the plant to absorb the metals from soil, and subsequently transport them to aerial parts (Zhao *et al.,* 2003; Chen *et al.,* 2005). EC_{root} and EC_{shoot} for Cu at 30, 60, 90 days after treatment ranged between 0.47-0.92, 0.65-1.14, 1.23-2.60 and 0.34-0.51, 0.37-0.53, 0.67-1.48, respectively (Fig. 1). A higher value of EC_{root} as compared to EC_{shoot} indicates that largely the metal was retained in roots.

It has been reported that EC>1 indicates the potential of plants to extract and transport metals from the substrate to different plant parts (Wei *et al.,* 2002). Such plant species are considered as hyperaccumulator and can be applied for phytoextraction of metals (Barman *et al.,* 2000; Kumar *et al.,* 2013). Translocation factor (TF) or mobilization ratio determines the translocation of metals from root to shoot and can be used to assess the potential of a plant for phytoremediation. TF for Cu, at 30, 60 and 90 days after treatment ranged between 0.42-0.72, 0.44-0.58 and 0.53-0.64, respectively. The results revealed that the metal was moderately translocated from root to shoot in *A. paniculata* (Fig. 1). Several batch experiment were performed to study the adsorption of flouride using *A. paniculata* leaves as biosorbent under different conditions. It was found that the adsorption capacity of biosorbent (powdered leaf sample) was influenced by contact time, pH, initial F concentration and adsorbent dose.

The influence of contact time on the adsorption efficiency of F was studied by varrying the contact time from 20 to 120 min. at the adsorbent dose of 5 g per 25 ml, pH 3 and temparature 28±2°C. It was found that initially, the fluoride removal efficieny increased with increase in contact time, however, after 100 min. it became almost stagnant denoting the attainment of adsorption saturation (Fig. 2a). Rapid adsorption at the initial stage may be due to availability of enough active sites for fluoride adsorption, however, with the progress of experiment the active sites became saturated and ultimately, the adsorbent might have been exhausted at the final stages (Killedar and Bhargava, 1993; Chen *et al.* 2015; Kumari and Khan, 2017; Kofa *et al.* 2017). Similar findings were reported with various other bisorbents; *Pleurotus ostreatus* 1804 (Ramanaiah *et al.,* 2007), protonated chitosan beads (Viswanathan *et al.,* 2009), *Citrus limonum* leaf (Tomar *et al.,* 2014), wheat straw, sawdust and activated bagasse (Yadav *et al.,* 2013).

It is an established fact that the process of biosorption is reliant on the pH, functional groups of the biosorbent and their ionic state (Liu *et al.,* 2014; Yadav *et al.,* 2013). Fluoride removal efficiency was found to decrease with increased pH (Fig. 2b). Biosorbent contains a high amount of polysachharides and some of them are associated with protiens and other biomolecules (Williams and Edyvean, 1997; Panumati *et al.,* 2008). These biomolecules have several functional groups e.g., amine, carboxyl, thiol, sulfhydryl, alcohol, phenol and phosphate. The phenomenon of biosorption depends on the protonation or deprotonation of these functional groups (Ilhami *et al.,* 2005). The ionic form of fluoride in aquous solution and the electric charge of the functional groups of biomolecules (the surface biosorbent) depends on the pH of the solution. Further, at higher pH, the adsorbent surface becomes negatively charged which leads to electrostatic repulsion between fluoride and

Table 1 : Accumulation of Cu (µg g^{-1} d.wt.) in different plant parts of *A. paniculata* after 30, 60 and 90 days of sowing (DAS)

	30 DAS		60 DAS		90 DAS	
Sample	Root	Shoot	Root	Shoot	Root	Shoot
T0	5.81±0.023[a]	2.47±0.005[a]	6.91±0.32[a]	2.83±0.06[a]	16.37±0.56[a]	9.31±0.56[a]
T1	38.19±1.05[b]	25.41±0.93[b]	57.23±1.09[b]	26.71±1.02[b]	73.47±6.2[b]	43.16±2.92[b]
T2	53.75±1.12[c]	38.48±1.43[c]	76.06±2.41[c]	44.07±1.13[c]	125.51±7.01[c]	66.87±3.12[c]
T3	70.70±2.82[d]	51.23±2.03[d]	97.38±4.08[d]	55.75±2.17[d]	184.24±7.42[d]	118.03±6.64[d]

Values represent mean of five replicates ± SE; Different letters indicates that means are significantly different from each other (p<0.05)

adsorbent surface resulting in low adsorption capacity (Gajbhiye *et al.,* 2017; Kumari and Khan, 2017).

The effect of adsorbent dose on the fluoride removal from water was examined at pH 6 and contact time of 80 min. The amount of dosage were varied between 0.5 gm to 3 gm per 25 ml. The results revealed that upto a certain level, an increase in adsorbent dose resulted in a simultaneous increase in F removal, probably due to high availibity of surface area and pore volume. Flouride removal efficiency increased from 45.4, 50.8, 49.8 and 51.4 to 79.2, 82.14, 82.8 and 83.6%, respectively at 0.5-3 gm dose in different treatments (Fig. 2 c). All the treatments have followed the similar fluoride removal trend, however, over 1.5 gm adsorbent doses, there were no significant changes in the fluoride removal was noticed; the reason being the overlapping of active sites at a higher dosage, thus decrease in the net available surface area for adsorption (Killender and Bhargava, 1993; Mondal *et al.,* 2016). Similar results were reported with various other bisorbents *viz.* protonated chitosan beads (Viswanathan *et al.,* 2009), *Citrus limonum* leaf (Tomar *et al.,* 2014), *Azadirachta indica* (Chakrabarthy and Sharma, 2012), wheat straw, sawdust and activated bagasse (Yadav *et al.,* 2013).

The effect of initial fluoride concentration on the removal efficiency was investigated by adding the fixed amount of adsorbent dose (2 gm) into different concentrations of fluoride solution (2.5, 5, 7.5, 10, 12.5 and 15 mg l^{-1}) for 100 min. The results demonstrated that fluoride removal efficiency decreased with increase in the initial fluoride concentration (Fig. 2d). The deacrease in fluoride removal efficiency indicates that the capacity of adsorbent gets exhausted abruptly on increasing the initial fluoride concentration probably due to instant saturation of active adsorbent sites at higher fluoride concentration (Mondal *et al.,* 2016). Similar trends have been reported by several authors

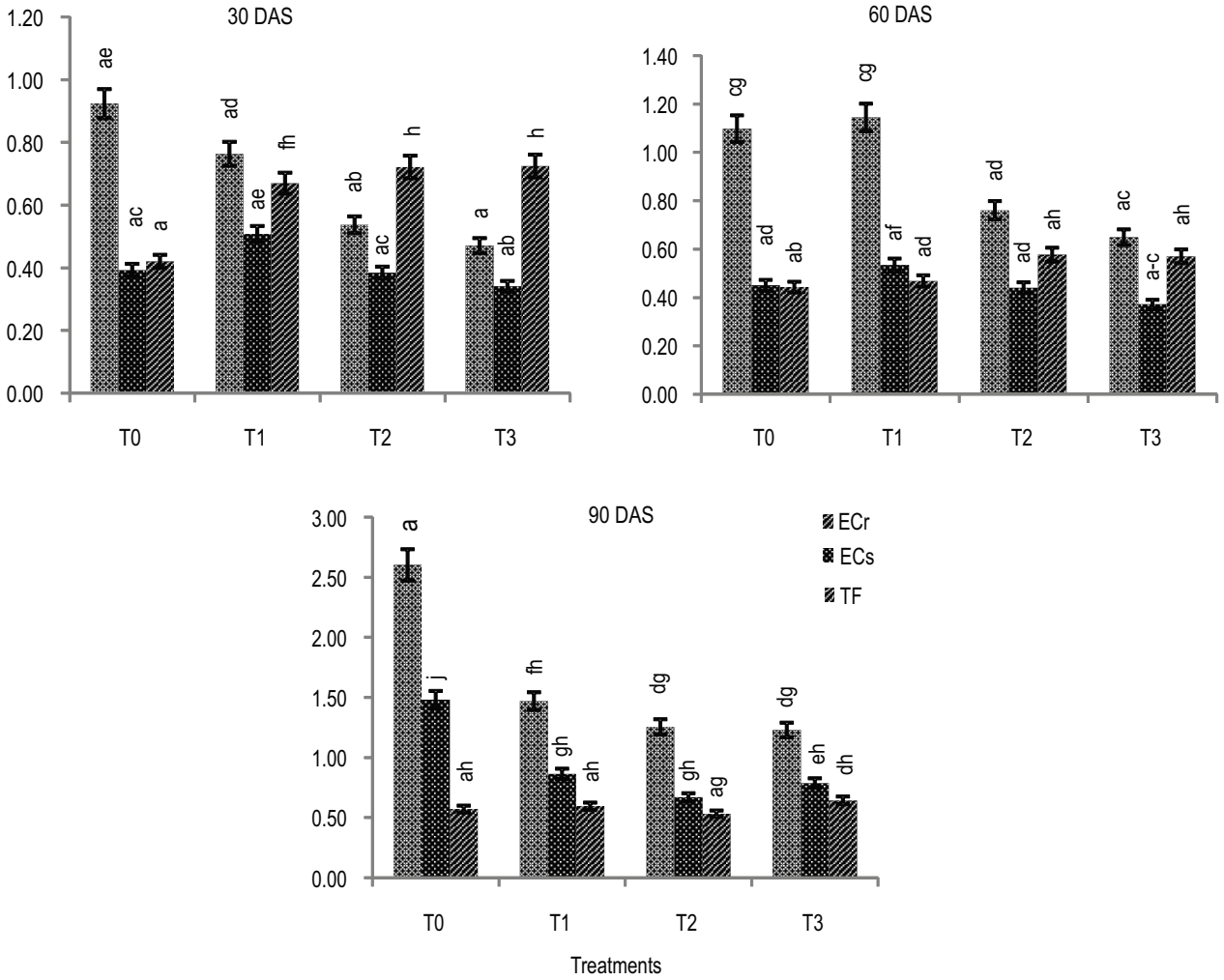


Fig. 1 : Accumulation of Cu (µg g^{-1} d.wt.) in different plant parts of *A. paniculata* after 30, 60 and 90 days of sowing (DAS). ECr- Enrichment Co efficient of root, ECs- Enrichment Coefficient of shoot, TF- Translocation Factor

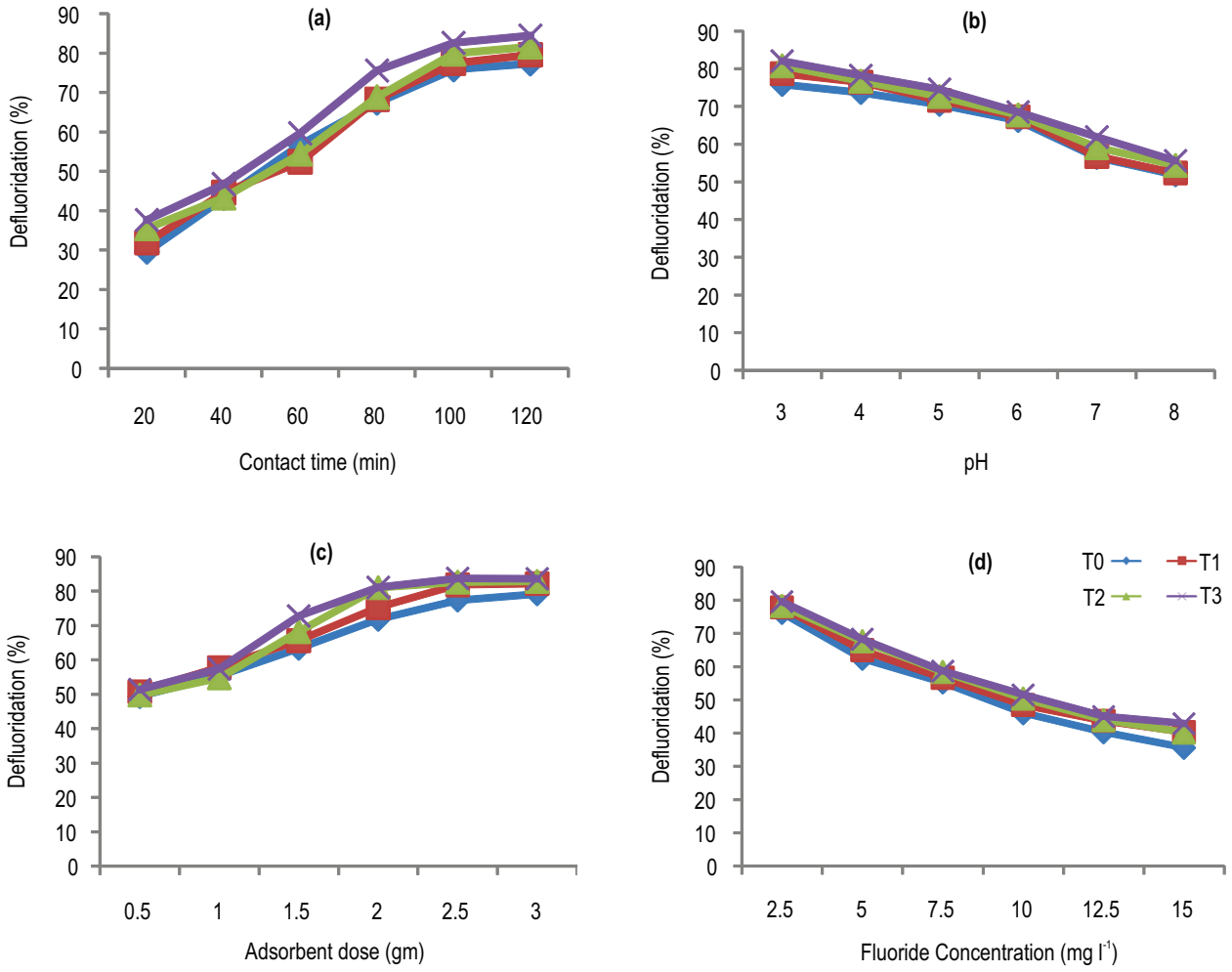


Fig. 2 : Effect of (a) contact time, (b) pH, (c) adsorbent dose and (d) initial fluoride concentration on the pecent fluoride removal from water by Cu phytoremediated *A. paniculata* plant biomass

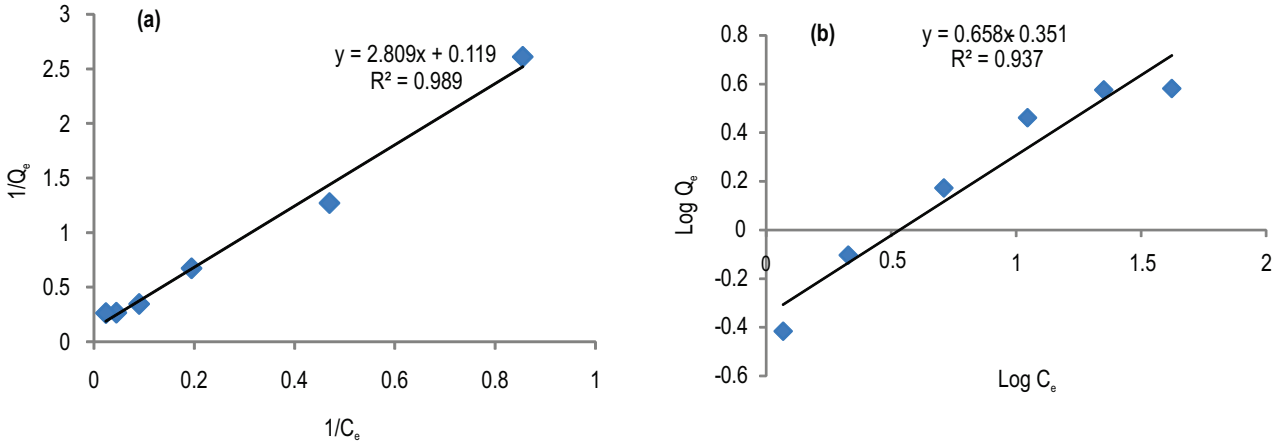


Fig. 3 : Linear model of Langmuir isotherm (a) and Freundlich isotherm (b) for adsorption of fluoride at 1g 10 ml^{-1} volume, pH 3, temp 30°C, contact time of 120 min and different initial concentration of adsorbent

for fluoride removal from water using protonated chitosan beads (Viswanathan *et al.,* 2009), algal biomass (Mohan *et al.,* 2007), wheat straw, sawdust, activated bagasse of sugarcane (Yadav *et al.,* 2013), *Azadirachta indica* (Chakrabarty and Sarma, 2012) and lemon leaf (Tomar *et al.,* 2014).

Langmuir and Freundlich isotherms are considered as essential models for understanding the adsorption process. The Langmuir isotherm is based on saturated molecular layer (monolayer) adsorption on the active sites of the adsorbate, while Freundlich isotherm is based on adsorption on a heterogeneous surface and a multilayer adsorption with an energetic non uniform distribution (Liu *et al.,* 2011; Cai *et al.,* 2015). A plot of $1/Q_e$ against $1/C_e$ shows a straight line which indicates that the adsorption process followed the Langmuir isotherm (Fig. 3a). Further, a plot of Log Q_e against Log C_e showed a linearity of Freundlich isotherm plot which confirms the applicability of Freundlich model for defluoridation at different initial concentrations (Fig. 3b).

The correlation coefficient (R^2) of Langmuir and Freundlich isotherms was greater than 0.93 indicating that both the models could explain the adsorption of fluoride. Maximum adsorption capacity (Q_{max}) from Langmuir isotherm model was 3.8 mg g^{-1} which was higher than wheat straw, sawdust, and activated bagasse (1.93, 1.73 and 1.15 mg g^{-1}) as reported by Yadav *et al.,* (2013). The values of RL ranged between of 0.118-0.681, indicating favorable adsorption process. Furthermore, Freundlich constant 'n' (n=1.51) was found to be greater than 1 which also confirms the favorable adsorption process (Zhang and Jia, 2016; Ghada *et al.,* 2018). The present study concludes that *A. paniculata* plant can be used as a moderate phytoremediator of Cu, however, use of *A. paniculata* biomass for removal of fluoride from contaminated water is an added advantage to the remediation process.

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Chapter 12

Phytoremediation of Heavy Metal Pollutants from Wastewater Environment using Aquatic Macrophytes

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SUMMARY

Various toxic metals like Cr, Cd, Pb, Ni, As, Mn, etc. are released into the environment as a consequence of natural (geogenic) and anthropogenic (mining, urban sewage, industries, agriculture, construction etc.) activities and finally contaminate the ground water and surface water and also create some serious toxic effects in the living beings. These metals enter into the aquatic environment through either point source, large area non-point source or combination of these. Being persistent in nature and when present in significant amount in water; these toxic metals pose serious health hazards to human, animal and aquatic life including both flora and fauna. The removal of these pollutants from contaminated water or wastewater is achieved by several techniques like reverse osmosis, ion-exchange, electrodialysis, adsorption etc. But, these techniques are very expensive and cause waste disposal problem. Phytoremediation is

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Ricinus communis: An Ecological Engineer and a Biofuel Resource

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Dhananjay Kumar, Poonam, Kuldeep Bauddh, Jaya Tiwari, D.P. Singh, and Narendra Kumar

Abstract

Degradation of soil quality due to industrial and agricultural activities has caused worldwide concerns. In order to remediate the soil contamination, several physicochemical techniques like soil washing, soil vapor extraction, solidification, stabilization, vitrification, electrokinetic, etc. had been applied. These modes of remediation may irretrievably affect the soil quality, destroy biodiversity, and render the soil useless for the plant growth. Hence, there is need of suitable, cost-effective, and eco-friendly techniques for remediation of soil without interfering with soil fertility. Phytoremediation being interdisciplinary in nature validated with a series of enthralling scientific research has emerged as a most promising, cost-effective, eco-friendly, and aesthetically acceptable technique for soil restoration. During phytoremediation, whole life span of a plant produces profound effects on the chemical, physical, and biological processes that occur in its instant vicinity. Processes like water and mineral acquisition, senescence, and biomass decay can greatly influence the rhizospheric and subsequently result in land restoration. Further, in the search of a suitable phytoremediator, *Ricinus communis* has proven its potential as a good phytoremediator for several organic and inorganic toxic chemicals particularly heavy metals and polyaromatic hydrocarbons along with other associated benefits being a medicinal and oil-producing plant. Unlike

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Heavy Metal and Their Regulation in Plant System: An Overview

2

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Abstract

Unplanned industrialization and improper waste disposal have resulted in the release of enormous quantities of inorganic toxicants like metal, metalloids, and radionuclides in the biosphere. Since, metals are non-biodegradable and tend to bioaccumulate via food chain, they pose threat to human health. Indiscriminate disposal of industrial waste to the environment causes adverse impact on ecosystem. Plants growing on metal-contaminated sites display several disturbances related to physiology and biochemical process like gaseous exchange, CO_2 fixation, respiration, nutrient absorption, etc. These disturbances subsequently cause reduction in plant growth and lower biomass production. Although being an essential micronutrient, some heavy metals at lower concentrations are vital for plant growth; however, at higher concentrations they become very toxic. To cope up with the metal toxicity, plants have developed various mechanisms like immobilization, exclusion, chelation, and compartmentization. Plants have distinct cellular mechanism such as chelation and vacuolar compartmentization of metals to withstand the metal toxicity. Phytochelatins, the thiol peptides, potentially chelate metals and form complexes in cytoplasm; subsequently these metal-thiol complexes are sequestrated into vacuole via ATP-binding cassette transporters (ABC transporters). In the last couple of

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