Chapter-1

INTRODUCTION

The twentieth century has seen man making significant strides in science and technology. However, it is also for the first time in the entire cultural history, that the humanity is facing a horrible ecological crisis wrought by environmental pollution. The fast pace of industrialization, galloping demand for energy and reckless exploitation of natural resources during the last century have been mainly responsible for the ecological holocaust, which is now set to pose a serious threat to biodiversity and ecosystem processes.

1.1 HEAVY METAL POLLUTION

Concomitant with technological revolution, man is losing precious land and water resources, on which rely the sustainability of our civilization. All the components of the biosphere are getting polluted with a variety of inorganic and organic pollutants originating from anthropogenic activities that alter the normal biochemical cycling. A variety of chemicals (heavy metals, insecticides, pesticides, surfactants, chlorinated solvents etc.) find their way to soil, water and air (Ostroumov, 2001, 2002; Turgut, 2003; Bhardwaj et al., 2006). Soil and water contamination by heavy metals is becoming one of the most serious ecological problems all over the world. Recent urbanization and industrialization have lead to the creation of large number of barren sites impregnated with toxic heavy metals such as Cr, Cd, Cu, Pb, Hg etc. the world over (Lone et al., 2008). Although trace elements are essential in microquantities to sustain the metabolic activities in the organisms, these prove to be lethal beyond certain limits. High concentrations of these metals in the environment results in their incorporation, and subsequent biomagnification in higher trophic levels of nutritional pyramid, which adversely affect the behavioural, structural and functional activities of living organisms (Islam et al., 2007). The sources of metal contamination include smelting of metalliferous ores, gas exhaust, electroplating, energy and fuel production, application of fertilizers and municipal wastes to land, and industrial manufacturing (Blaylock and Huang, 1999; Clemens, 2006). These metals emanate to the environment at the stages of their manufacture, use and discard. Agricultural soils are relatively rich
in heavy metals on account of extensive use of various agrochemicals containing heavy metals as constituents or impurities such as phosphate fertilizers, fungicides or herbicides (Liu et al., 2005) Heavy metals are difficult to remove from the environment, and unlike many other pollutants cannot be chronically or biologically degraded and are ultimately indestructible. This has lead to sharp increase in metal contamination of the biosphere and poses major problems of environment and human health world wide (Einsley, 2000).

All countries have been affected by widespread pollution, though the area and severity of pollution vary enormously (Lone et al., 2008). In Western Europe, 140,000 sites were affected by heavy metals (Mc Grath et al., 2001); the estimated total number of such sites in Europe could be much larger (Gade, 2000). McKeehan (2000) reported 600,000 brown fields contaminated with heavy metals that need immediate reclamation. As per government statistics, more than 100,000 ha of crop land, 55,000 ha of pasture and 50,000 ha of forest have been lost due to heavy metal pollution. Liu (2006), observed that the problem of pollution is also a great challenge in China, where arable land has been polluted by heavy metals. Soil and water pollution are also severe in other developing countries of Asia, like India, Pakistan and Bangladesh, where unplanned small industrial units are pouring their untreated effluents into the surface drains, the water of which is used for irrigation, thus polluting the land and water resources.

Mining, smelting and the associated activities are one of the important sources by which soils, plants and surface waters are contaminated. Thon (1993) reported that soils located in the vicinity of mining and smelting areas of England and Wales have been heavily contaminated by heavy metals. It is estimated that the median value of world wide emissions of Cd, Cu, Pb and Zn into soils were 22,954, 796 and $1.372 \times 10^6$ kg $y^{-1}$ respectively; more than half of these metals were associated with base metal mining and smelting (Nriagu and Pacyna, 1988). There are various documented cases of different metals causing toxicity problem. According to the report released by a US Environment Activation Group (EAS-2006), the world’s most polluted places threaten the health of more than 10 million people in many countries. Jadia and Fulekar (2008) reported that the Chinese city of Linfen, located in the country’s coal region, is facing severe pollution. The residents of Hania, Dominican Republic, the site of automobile
battery recycling smelter, suffer from widespread lead poisoning. In the Indian city Raniapat, 35 million people are affected by tannery waste containing hexavalent Cr and azodyes. Mailuu-Sui, Kyrgyzstan, home to a former Soviet uranium plant is severely contaminated with radioactive mine wastes. In the Russian industrial city of Norilsk, where world’s largest heavy metal smelting complex is located, more than 4 million tons of Cd, Cu, Pb, Ni, As, Se and Zn emissions are released annually. Developing countries like Brazil, China, India etc., contribute large proportion of the world’s mining products. Mining activities cause severe health hazards due to contamination of local water sources, as well as reduce biodiversity (WHO, 2008).

Moreover, a number of industries in the coastal areas have resulted in significant discharge of industrial effluents into the coastal water (Jadia and Fulekar, 2008). Each source of contamination has its own damaging effects on plants, animals and man. Incessant addition of heavy metals to soils and water are of serious concern due to their persistence in the environment, bioaccumulation and carcinogenicity to human beings. Several studies have been conducted throughout the world to determine the toxic effects of heavy metals on edible plants. Intawongse and Dean (2006) studied the uptake of heavy metal by vegetable crops grown on contaminated soil, and their bioavailability in the human gastrointestinal tract. The results indicated that uptake of Cd, Cu, Mn and Zn by plants corresponded to increasing level of soil contamination. The in vitro gastrointestinal study showed that the greatest extent of metal releasing was found in lettuce (Mn 63.7%, Zn 45.2%) and in radish (Cu 62.5%, Cd 54.9%, Mn 45.8%). In two separate studies, Bansal (1998) and Aleem (2003) reported high accumulation of toxic substances in soils, and in the crops grown on sewage. Liu et al. (2005) studied metal contamination in soils and crops affected by the Chenzhou lead and zinc mine spills in Hunan, China, and found exceedingly high levels of As, Cd, Zn and Cu in the soils.

1.2 TECHNOLOGIES FOR RECLAMATION OF POLLUTED SOILS

The 21st century can be characterized as a time of increasing environmental awareness. In the first half of the 20th century, the disposal of industrial waste by many industries was regarded as a productive function to be achieved at the least possible cost. This notion, coupled with insufficient government action and legislation, led to the massive contamination of ground water recourses and soil world wide (Huq et al., 2002;
Koundouri, 2005)). However, outbreak of silent epidemics of itai-itai disease and the infamous Minamata episode in Japan, gained considerable public attention, and brought about monumental changes in the society. Various positive steps were taken by all nations to raise public awareness and to curtail environmental pollution by implementing stringent government regulations. Pressure to meet new standards for environmental quality propelled whole industries to reengineer their fundamental processes and products (Cunningham et al., 1995). The proper disposal of hazardous wastes and the need to clean existing contaminated sites became a productive function for many public and private institutions, government agencies, private industry and researchers all over the world. They began a search for efficient and cost effective technology, which could be used to remediate waste sites, an initiative that remains to the present day.

The reclamation of contaminated soils at a very large scale is not as easy as it appears to be, and needs the implementation of several regulatory steps. Different approaches have been used or developed to mitigate the polluted soils and waters including the landfill/damping sites. These may be broadly classified as physico-chemical and biological approaches. The physicochemical approach includes excavation and burial of soil at hazardous waste site, fixation / inactivation (chemical processing of the soil to immobilize the metals), leaching by using acid solutions, followed by the return of clean residue to the site (Salt et al., 1995, 1998), precipitation, flocculation followed by sedimentation, reverse osmosis and microfiltration. These conventional physicochemical techniques are generally costly and are not ecofriendly (Ghosh and Singh, 2005). Moreover, these techniques are technically limited to small areas and can drastically change the soil structure and fertility (Qiu et al., 2006).

Biological approach of remediation includes: (i) the use of microorganisms to detoxify the metals by valence transformation, extra-cellular chemical precipitation or volatilization, and (ii) use of specific plants to decontaminate soil or water, by inactivating metals in rhizosphere or translocating them into aerial parts. The second approach is called phytoremediation, which is considered as a new and highly
promising technique for the reclamation of polluted sites, and is cheaper than physicochemical techniques (Garbisu and Alkorta, 2001).

1.3 PHOTOREMEDIATION AS GREEN CLEAN TECHNOLOGY

Phytoremediation, also referred to as the “botanical bioremediation”, is becoming an area of current research, which has tremendous prospects towards decontamination of polluted soils and water (Lai and Chen, 2009). The term phytoremediation actually refers to diverse collection of plant based technologies that use either naturally occurring or genetically engineered plants, for cleaning contaminated environment (Cherian and Oliveira, 2005; Dowling and Doty, 2009).

It has long been known that the life cycle of a plant has profound effects on chemical, physical and biological environment that occur in their immediate vicinity. In the processes of shoot and root growth, water and mineral acquisition, senescence and eventual decay, the plants can profoundly alter the surroundings. A living plant can be considered as a “solar driven pump” which can concentrate particular elements from the soil and translocate these to shoots which can be subsequently removed by harvesting. The harvested plant tissue, rich in accumulated contaminant can be easily and safely processed by microbial, physical or chemical means.

The earliest evaluation of the use of plants for heavy metal decontamination was probably done by Chaney (1983, 1997). Phytoremediation is generally viewed as more efficient and cost effective, compared with other methods of decontamination such as engineering and/or microbial approaches (Raskin et al., 1997; Mohan and Hossetti, 2002). Plant based remediation is preferable to microbial remediation, even though the latter is more versatile in degradative potential. Plants can remove contaminants from the environment via harvesting, which is unpractical with microbes. Microbes may sometimes produce intermediate compounds which are more toxic than the original substances. Furthermore, it is often easier to establish an effective plant population than a microbial population. The roots of plants also stabilize the soil, and prevent erosion and spread of contaminants. Roots may also secrete compounds to enhance microbial growth, and improve aeration and microbial activity.

The history of metal hyperaccumulation in plants started at the end of 19th century when it was observed that *Thlaspi caerulescens* and *Viola calaminaria* growing
on soils naturally enriched with Zn, contained extraordinary high levels of Zn. This prompted research on the identification of metal hyperaccumulating plants. Although uncommon, these remarkable plants were reported to be taxonomically widespread in plant kingdom (Baker and Brooks, 1989). The term “hyperaccumulation” was interdicted by Jaffre (1977) describing abnormal levels of Ni accumulation in the plant and this term was later extended to other metals. At present, the criteria used for hyperaccumulation vary with metal, and range from 100 mg kg\(^{-1}\) dw for Cd, to 1000 mg kg\(^{-1}\) dw for Ni, Cu, Co, Cr and Pb, to 10,000 mg kg\(^{-1}\) dw for Zn and Mn. It may be mentioned that these values have to be found in any of the above ground parts of plants growing in their natural habitat, but not under artificial conditions. As usual, these plants exhibit shoot–to–soil metal concentration ratio, the so-called bioaccumulation factor (BCF) higher than 1. Hyperaccumulator plants are usually found on metalliferous soils; calamine soils rich in Zn and Pb; serpentine soils derived from Fe and Mg rich ultramafic rocks, enriched also in Ni, Cr and Co. According to Ernst (2004) natural exposure of plants to a surplus of various metals has driven the evolution of metal hyperaccumulation, as well as plant resistance to heavy metals.

To date, more than 400 metal accumulating taxa belonging to at least 45 plant families have been identified (Reeves and Bakar, 1999). They have been found on all continents, both in temperate and tropical environments. Most of the hyperaccumulator plant species are able to accumulate just one metal, some may be multimetal accumulators. Some families and genera are known as sources of specific metal hyperaccumulator: Ni (Brassicaceae–Thlaspi), and Cu and Co (Lamiaceae, Scrophulariaceae). Ipomoea alpina, T. rodungifolium T. caerulescens etc. have been extensively studied for their metal accumulation potential. The ability to accumulate a heavy metal varies significantly between species and among cultivars within a species, as different mechanisms of ion uptake are operative in each species, based on their genetic, morphological, physiological and anatomical characteristics. There are different techniques of phytoremediation including phytoextraction, phytostabilization, phytofiltration and phytovolatilization depending upon the mechanism of remediation (Salt et al., 1995). Metal hyperaccumulating plants have been deemed for use in phytoextraction of heavy metals from contaminated environments and represent ideal
model systems to study genetic, molecular, and physiological and root-rhizosphere processes responsible for metals uptake, tolerance and accumulation in plants (Prasad and Strazalka, 2002). Thus phytoremediation is a form of ‘ecological engineering’ that takes advantage of plant root system, together with the translocation, bioaccumulation and contaminant storage/degradation abilities of the entire body of the hyperaccumulator plant. The generation of scientific information on heavy metal accumulating plants is so extensive that during the last decade, a commercial industry came up for the application of phytoextraction to restore metal contaminated sites (Glass, 1999).

1.4 *B. juncea* AS A PHYTOREMEDIATOR

Since phytoremediation offers an innovative green clean technology for combating the pollution, it has gained wide popularity and interest in academic studies and practical applications. The existence of plants that acquire high levels of metals in harvestable tissue was thought impossible until the discovery of a variety of wild hyperaccumulating plants (Brooks *et al.*, 1998), often endemic to naturally mineralized soils, which concentrate high amounts of heavy metals in the foliage. Reeves and Baker (1999) compiled an exhaustive list of plant species that hyperaccumulate Cd, Cu, Cr, Ni, Zn, Pb and Se.

With the growing interest in the advancement of this technology, numerous plant species have been identified and tested for their tolerance and accumulation of different metals. Research efforts are being directed to evaluate the metal accumulating capacity of high biomass plants that can be easily cultivated using established agronomic practices, and to make the recycling of metal loaded plants practically feasible. This inherently depends upon several plant characteristics, the two most important being the ability of the plant to rapidly acquire large quantities of biomass rapidly, and its ability to accumulate large quantities of metals in shoots. It is the combination of high metal accumulation and high biomass production that result in the most efficient hyperaccumulator (Cunningham and Ow, 1996).

The selection of phytoremediating species is possibly the single most important factor affecting the extent of metal removal. Although the potential for metal extraction is of primary importance, other criteria, such as ecosystem protection is also of great
concern when selecting remediating plants. As a general rule, native species are preferred to the exotic ones which can be invasive and endanger the harmony of ecosystem. To avoid propagation of weedy species, crops are in general preferred, although some crops may be too palatable and pose a risk to grazing animals (Vassilev et al., 2004). The rate of metal removal depends upon the biomass harvested and metal concentration in harvested biomass. One of the most debated controversies in the field refers to the choice of remediative species; metal hyperaccumulators vs common nonaccumulator species. Hyperaccumulator plants have the potential to bioconcentrate high metal levels. However, their use may be limited if they have small size and slow growth. In common non-accumulator species, low potential for metal bioconcentration is often compensated by the production of significant biomass.

Family Brassicaceae represent a potential and promising group of plants to be used for phytoremediation. It contains a large number of hyperaccumulating species and their ability to accumulate the widest range of heavy metals. Particular emphasis has been placed on Brassica species because of their relationship to wild metal accumulating mustards (Kumar et al., 1995). The members are well adaptive to a range of environmental conditions, and have demonstrated the potential for moderate levels of heavy metal accumulation under experimental conditions (Blaylock, 1998; Brooks et al., 1998). They are of economic value as oilseeds and forages, and can produce extensive biomass. They are also well suited to genetic manipulation and in vitro culture techniques, and are attractive candidates for the introduction of genes aimed at phytoremediation.

Among the Brassica species, B. juncea has been the most extensively studied plant that exhibits superior heavy metal accumulating characteristics (Blaylock et al., 1997; Nanda-Kumar et al., 1995; Ebbs and Kochain, 1997, 1998). The improved lines of B. juncea have already been developed for its metal accumulation ability by conventional selection and plant breeding methods. Further, through genetic manipulations, genes for enhancing metal uptake and accumulation have been introduced into the species to obtain transgenic varieties for more efficient phytoremediation.
Though *T. caerulescens* is known to be the best hyperaccumulator of various metals, it is not very promising and effective to be used commercially for phytoremediation due to its low biomass production. Ebbs *et al.* (1997) reported that *B. juncea*, while having one third of the concentration of Zn in its tissue, is more effective for Zn removal from soil than *T. caerulescens*. This advantage is due primarily to the fact that *B. juncea* produces 10 times more biomass than *T. caerulescens* and has a more rapid growth. According to Renault (2000), *B. juncea* can accumulate metals up to 0.5% to 1% of its dry weight. The results suggest that a greater biomass can more than compensate for a lower shoot metal concentration. Therefore, *B. juncea* is considered as a promising candidate for phytoremediation and also a model system to investigate the physiology and biochemistry of metal accumulation in plants.

1.5 MECHANISM OF HEAVY METAL UPTAKE

Metal hyperaccumulator plants have the genetic potential to clean up contaminated soils, and a great deal of research has been conducted to elucidate the physiology and biochemistry of metal hyperaccumulation. It was observed that hyperaccumulation depends on several factors, including the extent of soil contamination, presence of more than one metal in the soil, metal bioavailability, and plant’s ability to intercept, absorb and accumulate metals in shoots. Recently, it is widely recognised that because of the real problem faced by the plants, it is more representative to examine the effects of complex metal mixtures on plants than single metal studies. It is notable that the habitats are often contaminated by more than one metal in potentially toxic concentrations. During the uptake of heavy metals by plants, positive or negative interactions occur among the metals. According to the classical definition, interactions between nutrients occur when the supply of one nutrient affects absorption, distribution or function of another nutrient. Thus, depending on nutrient supply, interactions between nutrients can induce deficiencies or toxicities, and can modify growth response (Robson and Pitman, 1983). The interactions may be positive or negative, giving rise to synergy and antagonism (Martin-Prevel *et al.*, 1987). The existence and magnitude of a particular interaction is however, generally dependent on the actual concentrations of the individual metals involved in the interaction. There is
almost an endless chain of possible interactions in which heavy metal concentration at one stage affect that at the next, during absorption, translocation, and penetration into cells, in metabolic reactions, remobilization and reallocation. Moreover, these interactions influence the rate of uptake, transfer, accumulation and utilization of metal ions in the plant body. Symeonidis and Karataglis (1992) divided plant responses to different combinations of metals in the growth medium into three groups:

1. **Additive**—When plant growth under multiple metal stress is equal to that observed in the presence of metals supplied separately.

2. **Antagonistic**—When plant growth parameters under multiple metal stress exceed those observed.

3. **Synergistic**—When plant growth parameter under multiple metal stress is observed to be diminished, compared to separate supply of each metal.

The development of an interaction also depends upon the internal physicochemical state of the plant, which is further influenced by genotype, age, growth stage and environmental factors.

### 1.6 PLANT’S DEFENSE AGAINST HEAVY METAL STRESS

Plants survive in a constantly fluctuating environment which has driven to evolution of a highly flexible metabolism and growth and development necessary for the sessile lifestyle. When plants are subjected to different environmental stresses such as high light intensity, temperature extremes, droughts, high salinity, herbicide treatments or metal deficiencies, the balance between various biochemical and physiological systems gets disturbed. Heavy metal stress leads to the accumulation of elevated levels of metal ions in the plant body, which are extremely phytotoxic. Plants have developed two basic strategies - metal exclusion and metal detoxification, to defend against this potential stress by evolving several mechanisms to control the homeostasis of intracellular ions. This includes immobilisation, chelation and complexation of ions, expression of stress proteins and activation of ethylene response to stress (Cobbett, 2000). A common feature of heavy metal stress exposure is
associated with oxidative damage at cellular level (Allen, 1995). Heavy metal hyperaccumulation also shows elevated signs of oxidative stress, even if the plant growth is unaffected (Boominathan and Doran, 2003).

Heavy metals, especially with redox properties, such as Cu, Mn, Fe, or those that are highly reactive such as Cd, are known to increase the amount of reactive oxygen species (ROS) such as $\cdot OH$, $H_2O_2$, and $O_2^-$, leading to the development of oxidative stress (Gallego et al., 1996) in the plant tissue. Transition metals with redox properties (Fe, Cu etc.) produce ROS by autooxidation and Fenton reactions, whereas nonredox reactive metals (Cd, Hg etc.) indicate promoted lipid peroxidation leading to $H_2O_2$ accumulation and oxidative burst. These ROS are also generated in plant tissues during normal metabolic processes. ROS can seriously disrupt the normal metabolism through oxidative damage of lipids, proteins and nucleic acids. Normally plants have evolved specific protective mechanism against oxidative stress by exhibiting an adaptive biochemical response termed as antioxidative defence system. Oxidative stress is essentially a regulated process and the equilibrium between the oxidative stress and antioxidative potential determines the fate of the plant. Under stressful conditions including heavy metal stress, this normal balance between the production of ROS and the quenching activity of the antioxidative defence mechanism gets upset. The stress situation provokes increased production of toxic oxygen derivatives, which in turn also stimulate an increase in the activity of antioxidative defence system. Plants with active and efficient antioxidants, either constitutive or induced, have been reported to provide sufficient resistance against oxidative stress. Stress tolerance in plants is a result of enhancement of antioxidative defense system to combat negative consequences of heavy metals.

Generally, the defence system of plants falls into two classes (Iannelli et al., 2002; Nunez et al., 2003):

(a) Low molecular weight antioxidants, which consist of

(i) Lipid soluble, membrane associated antioxidants which directly quench free radicals of lipid peroxidation ($\alpha$-tocopherol).

(ii) Water soluble antioxidants that detoxify $O_2$ and $H_2O_2$ (glutathione and ascorbate).
(b) Antioxidative enzymes belonging to ascorbate-glutathione cycle, *e.g.* Superoxide dismutase (SOD), catalase (CAT), Glutathione reductase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR).

SOD converts superoxide and hydroxyl radicals into H$_2$O$_2$, which is further degraded into H$_2$O and molecular O$_2$ by CAT and peroxidases (POX). The difference between catalases and peroxidases is in their function. CAT operates alone, has low substrate affinity and needs two molecules of H$_2$O$_2$ per cycle, whereas POX has much higher affinity to H$_2$O$_2$ and requires a reductant. In plant cells, ascorbate is the main reductant, and ascorbate peroxidase is the main POX (Noctor and Foyer, 1998). Glutathione reductase participates not only in H$_2$O$_2$ scavenging, but also favours a high GSH/GSSG ratio, and is a proper cellular redox (Srivastava *et al.*, 2004). All these enzymes are affected by heavy metal stress. As long as the stress is not too strong for the plant defence system, the main response is an increase in the SOD and POX activities, along with decrease in the CAT activity. These results were obtained for a number of heavy metals, including Cd, Cu, Mn, Ni, Pb and Zn and for numerous plant species, although the responses varied depending upon the plants sensitivity, specific organ, selected metal and its concentration, as well as the duration of the stress (Rao and Sresty, 2000; Siedlecka and Krupa, 1996).

In nature, though, the plants are continuously exposed to a wide variety of environmental stresses, and also bear the ill effects of unprecedented increase in pollution; they are also the ultimate savours which can provide solution to revive the lost ecological balance of the nature. The innovative concept of phytoremediation embraces the plant’s unique uptake and accumulation capabilities to decontaminate polluted soils and waters. However, metal pollutants, in their due course of uptake in the plant body, affect numerous biochemical and physiological processes. Therefore, a thorough understanding of the physiology and biochemistry of metal uptake and their possible effects on plants metabolism are necessary to streamline the concept of phytoremediation in multielemental contaminated sites. Moreover, the research on antioxidative defence system of higher plants helps to provide ecophysiological and metabolic strategies to counteract the heavy metal stress during phytoremediation.
1.7 AIMS AND OBJECTIVES

Based on the information regarding the phytoremediation capacity of *B. juncea*, possibility of various metal interactions occurring during their uptake and the antioxidative defence system in metal stressed plants, the present research was undertaken to study metal accumulation in *B. juncea* plants under situations similar to those found in the field when exposed to the different concentrations of various heavy metals present in multielemental contaminated sites.

The present thesis is structured into two parts:

(a) An introductory part, reviewing the literature on the effects of heavy metals on the physiology of plants, and the phytoremediation potential of hyperaccumulators when exposed to multielement contaminated sites.

(b) An experimental part which presents the study carried out on the phytoremediation potential of *B. juncea* under the influence of binary, ternary and six metal combinations of Cr, Mn, Ni, Co, Cu and Zn at various concentrations.

The investigation envisages:

1. The effects of binary, ternary and six metal combinations on germination, growth and heavy metal uptake by *B. juncea* seedlings and mature plants under multiple metal stress.

2. Assessment of interactive effects of binary, ternary and six metal combinations on the growth parameters, and metal uptake with the help of statistical tools like two-way ANOVA, Tukey’s multiple comparison test and multiple regression analysis.

3. To find out the effective concentrations of different heavy metal combinations which can increase the plant growth and efficiency of metal uptake.

4. To study the natural defence system of *B. juncea* under multiple metal stress by estimating the activities of SOD, catalase, GPX, APX and GR.

5. To recommend the remedial measures for decontamination of soils using phytoremediation technology.
The study in its modest will delineate the mechanism underlying heavy metal uptake by *B. juncea* ensuing from metal interactions under multielement heavy metal polluted sites, and identify the combinations of metals for which this species will be most suitable for phytoremediation.