INTRODUCTION

A rapid, unprecedented change in environmental conditions is likely to supersede the adaptive potential of plants. These environmental changes occur naturally as well as from anthropogenic activities, and result in air, water and soil pollution and degradation. The adding up of different kinds of pollutants in the plant environment, particularly the addition of heavy metals is of great concern. Contamination of the biosphere with heavy metal has accelerated severely during the last century due to smelting, mining and amendment of agricultural soils with municipal sewage sludge, and waste disposal practices (Diwan et al., 2010). The excess amount of heavy metals in the environment is reported to be very dangerous to human health (Olowoyo et al., 2012).

Widespread contamination of soils with metals has posed huge threat to the human health as the majority of them have been verified to be carcinogenic (Krishna and Govil, 2004). Heavy metals enter the environment through natural as well as anthropogenic activities. In nature, these metals are weathered from natural rock formations, occur in particulate or dissolved form in soils, lakes, rivers, sea water and sea floor sediments. Heavy metals are also released into the atmosphere through volcanic eruptions (Babula et al., 2008). Anthropogenic sources of heavy metal contaminants include mining and smelting, metallurgical industries, sewage sludge treatment, military training and warfare, waste disposal sites, agricultural fertilizers and electronic industries (Alloway, 1995).

Metals exist in a number of forms, soluble as well as particulate forms, which influence their mobility and bioavailability (Ge et al., 2005). In soils, a high content of heavy metals may increase the potential uptake of these metals by plants (Tomáš et al., 2012). Metals like copper (Cu), nickel (Ni), zinc (Zn), cobalt (Co), iron (Fe) and molybdenum (Mo) are essential for algae and plants as they are the important constituents of pigments and enzymes. However, metals like cadmium (Cd), lead (Pb), mercury (Hg), aluminum (Al), arsenic (As) etc. are the non essential metals and cause toxicity when present in excess. In plants, metal toxicity causes oxidative stress and leads to alterations of physiological processes at cellular and molecular level by inactivating
enzymes, blocking functional groups of metabolically important molecules, displacing or substituting for essential elements and disrupting membrane integrity (Rascio and Navari Izzo, 2011).

Oxidative stress is induced by heavy metals as they generate free radicals and toxic reactive oxygen species (ROS) (Sanita di toppi et al., 2002; Arvind and Prasad, 2003). ROS include variety of radicals like superoxide radicals (O$_2^-$), singlet oxygen (O$_2$°), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radicals (OH$^-$). These ROS are the partly reduced forms of atmospheric oxygen and under normal growth conditions, their production in cells is low and strongly controlled (Dat et al., 2000). These species react with lipids, proteins, pigments and nucleic acids and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting the cell viability. Unlike other organic pollutants, these metals do not get degraded and converted into harmless compounds through biological processes. Thus, these can persist for long durations in the environment and enter into food chain (Ramasubbu and Chandra Prabha, 2012).

Among different essential metals needed as vital micronutrients for growth and development, Ni is one of them. It is a transition metal and found in natural soils in trace concentrations except in ultramafic or serpentinitic soils (Yadav, 2010). The requirement of Ni in the plants is generally low [1.7μmol Kg$^{-1}$(Ni) or even lesser] (Dalton et al., 1988). Symptoms of Ni toxicity can be observed between 0.19 to 0.85 mmol Kg$^{-1}$(Ni) in plant dry biomass. These symptoms include the inhibition in root elongation, photosynthesis, and respiration, and interveinal chlorosis (Marschner, 1986). Moreover, the toxic concentration of Ni also inhibits enzyme activities and protein metabolism (Kevrešan et al., 1998). However, Ni also accelerates the activities of anti-oxidative enzymes (Schickler and Caspi, 1999; Prasad et al., 2005). An elevated level of Ni$^{2+}$ in soil causes diverse physiological alterations and toxicity symptoms like chlorosis and necrosis in different plant species (Zornoza et al., 1999; Pandey and Sharma, 2002; Rahman et al., 2005).

Similarly, Cr is a heavy metal that causes severe contamination in soil, sediments, and underground water table. Lethal effects of Cr on plant growth and development
include alterations in the germination process and the growth of roots, stems and leaves. Hence, exposure to elevated concentration of Cr affects total dry matter production and yield of plants. Cr is also reported to cause harmful effects on physiological processes like photosynthesis, water relations and mineral nutrition in plants. Likewise, Cr exposure in plants either has a direct effect in the form of alteration of enzymes and metabolites or indirect effect as it generates reactive oxygen species (ROS) (Shanker et al., 2005).

A variety of As compounds are found in plant tissues e.g inorganic arsenite, arsenate, methylated As species, arsleno-betaine and arsleno-sugars. Arsenate is an analog of phosphate (P) and competes for its uptake in the root plasmalemma of plants (Meharg and Macnair, 1992). Plant species, which are not resistant to As, experience significant stress upon exposure, with diverse symptoms ranging from inhibition of root growth to death (Meharg and Hartley-Whitaker, 2002).

Plants respond to heavy metal toxicity in different ways. The responses of plants include immobilization, exclusion, chelation and compartmentalization of metal ions and the expression of stress response mechanisms like ethylene and stress protective proteins. Another defensive mechanism is an antioxidant system, composing of both enzymatic and non-enzymatic antioxidants (Shanker et al., 2005). The antioxidative defence system scavenges toxic free radicals to protect the plant from oxidant stress caused by heavy metals (Cao et al., 2005). The resistance of plants to oxidative stress depends on the equilibrium between the generation of reactive oxygen species (ROS) and antioxidant capacity of the cell. The antioxidative enzymes includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APOX), guaiacol peroxidase (POD), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) (Zhou et al., 2005). Several plant growth regulators (PGRs) like ABA, Ethylene, Auxin, Jasmonic acid and Brassinosteroids (BRs) have been reported to modulate this antioxidant defense system and thus scavenging the free radicals and helping the plant to be protected from oxidative stress (Verma et al., 2012).

BRs, the new class of plant steroidal hormones, show quicker response at very low concentrations. These compounds are present in the plant kingdom with higher levels
in young growing tissues. Pollen and immature seeds are the richest sources of these steroidal compounds (Bajguz and Tretyn, 2003). The era of BRs began in 1970, when a new class of putative plant growth factors was detected in pollen extracts of *Brassica napus* and named “Brassins” (Mitchell *et al*., 1970). Brassinolide (BL), a novel steroid of unique structure, was shown to be responsible for the growth-promoting activity of "bras-sins" (Grove *et al*., 1979). Since the discovery of BL, till date 70 BRs have been isolated from 60 plant species including 51 angiosperms, 6 gymnosperms, 1 pteridophyte, 1 bryophyte and 1 chlorophyte (Haubrick and Assmann, 2006). Among all these BRs, 65 were unconjugated and 5 were conjugated ones. Depending upon the length of side chain, these compounds are classified as C$_{27}$, C$_{28}$ or C$_{29}$ types (Bajguz and Tretyn, 2003).

Along with the isolation, the focus of attention during the past few decades is on the physiological properties of BRs (Krishna, 2003). These have multiple involvement in the regulation of plant physiological activities such as cell expansion, cell division, alteration of membrane properties, vegetative growth, reproductive biology, senescence, seed germination and stress management (Khripach *et al*., 2000; Tanaka *et al*., 2003; Bhardwaj *et al*., 2006). Further, BRs have the ability to protect the plants from various environmental stress conditions such as drought, extreme temperatures, heavy metals, herbicidal injuries and salinity (Cui *et al*., 2011). Due to their pleiotropic involvement in the various physiological processes, they are now well accepted as a sixth group of plant growth hormones besides the five classic plant hormones.

Another aspect of study of BRs is their ability to regulate the uptake of metal ions into the plants and control their absorption and accumulation (Khripach *et al*., 2000). Use of industrial effluents and sewage sludge on agricultural lands has become a common practice as a result of which, these toxic metals can be transferred and concentrated into plant tissues from soil (Athar and Ahmad, 2002). Since most of the crops are utilized by animals including humans, the heavy metals being non-degradable in nature keep on cycling from plants to animals resulting into accumulation. BRs influence accumulation of heavy metals and radioactive elements in plants grown in polluted areas (Bajguz 2000b; Janeczko *et al*., 2005). The accumulation of metals (Zn, Cd, Pb and Cu) under the
influence 24-epibrassinolide had been studied in different agricultural plants such as barley, tomatoes, radish, sugar beet and winter rape (Volynets et al., 1997 a,b; Janeczko et al., 2005). It was observed that the application of BRs in appropriate doses in a certain stages of development reduces the metal absorption significantly.

In agriculture, the potential of BRs to increase crop yields is being exploited (Khripach et al., 2000). In the field of medicines, they have been studied for antiviral properties against many viruses like Measles, Herp and Arena virus (Wachsman et al., 2000, 2002, 2004a).

*Brassica juncea* L., an amphidiploid species is commonly known as Indian mustard or mustard green. It is an important genus of the *Cruciferae* family. *B. juncea* L. is grown as oilseed crop in India with striking medicinal value. It is used as a folk remedy for arthritis, foot ache, lumbago, and rheumatism (Duke and Wain, 1981). Mustard greens are rich source of vitamin A, vitamin C, iron, calcium and contains novel phytochemicals which are protective against carcinogenesis (Steinmetz and Potter, 1991). Brassica is grown over 15% arable land in India but its productivity is considerably hindered by various abiotic and biotic stresses (Shah, 2002).

The data on stress protective properties of BRs indicate that exogenous application of BRs can act as immuno-modulators when applied at appropriate doses at the appropriate stage of plant development. However no information is available till date on the expression of specific group of brassinosteroids which are overexpressed/underexpressed under heavy metals stress in such a valuable plant. Keeping these lacunae in mind, the present proposal has been framed to meet the following objectives:

i) To isolate and characterize various BRs, produced under the stress of different heavy metals (Ni, Cr, As) in *B. juncea* L. plants at various stages of growth.

ii) To look for a novel class of BRs in *B. juncea* L. which may pinpoint a biosynthetic pathway of BRs production.

iii) To study the metal uptake and antioxidative defence system of *B. juncea* L. plants subjected to stress of heavy metals (Cr, As, Ni) by assessment of protein content,
lipid peroxidation, osmolytes and activities of antioxidant enzymes like catalase (CAT), guaiacol peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and ascorbate peroxidase (APOX)

iv) To study the bioactivities of BRs isolated from *B. juncea* L. plants grown under the stress of heavy metals (Cr, As, Ni) by assessment of antiproliferative activity and antioxidant potential.