Review of Literature

Environmental Pollution by Heavy Metals

Heavy metals are conventionally defined as the elements that have high density and high atomic number (>$20$) (Adriano 2001). In the current era of industrial development, heavy metals have gained considerable attention as potential environmental pollutants and the contamination of biosphere with these toxic metals has accelerated dramatically (Swaminathan 2003). Common examples of heavy metals in our environment include Chromium (Cr), Manganese (Mn), Iron (Fe), Cobalt (Co), Nickel (Ni), Copper (Cu), Zinc (Zn), Lead (Pb), Cadmium (Cd), Aluminum (Al), and Mercury (Hg). However, all metals are not toxic as some of them function as essential micro-nutrients at low concentration (Timbrell 2005). Some of the metals function as a cofactor of a number of metabolic reactions in plants. Among the heavy metals, Fe, Zn, Cu, Ni and Mo have known biological functions in plants and some of them are even integral components of a number of metallo-enzymes (Taiz and Zeiger 2006). But increase in their concentration exerts toxic effects on various metabolic processes (Parida et al. 2003; Negi et al. 2014). Other heavy metals such as Cr, Cd, Pb, As and Hg seems to be more and less toxic to plants and microorganisms, since they do not have any biological function in plants and microorganisms (Lamb et al. 2010). Heavy metals are ubiquitous in nature and are an integral component of the biosphere. Beside the natural activities, they are emitted from a wide spectrum of man-made sources such as pesticides, electronic waste, vehicular exhausts, and industrial effluents in the environment, and it may damage or alter both natural and man-made ecosystems (Tyler et al. 1989). According to Wang (1987), Hg, Cd, As, Cu, Zn, Ni, Cr and Pb are serious pollutants of the environment and are highly toxic to plants and animals. Migration of these contaminants into non-contaminated areas as dust or leachates through the soil and heavy metals containing sewage sludge leads to contamination of various ecosystems (Gaur and Adholeya 2004). Heavy metal pollution has harmful effect on biological systems and these do not undergo biodegradation.
Contamination of soil by heavy metals has become a critical environmental concern due to their acute and chronic toxic effect on plants grown on such soils (Yadav 2010). Heavy metal toxicity to plants is depicted by impaired seed germination, reduced seedling growth and biomass reduction (Ewais 1997). Apart from the morphological changes, toxic levels of heavy metal affect a variety of metabolic processes in plants (Maksymiec 1997; Siedlecka et al. 2001). One of the major consequences is the enhanced production of reactive oxygen species (ROS), which damage cell membranes, chloroplast pigments, and all types of biomolecules such as proteins, nucleic acids, lipids, and amino acids, leading to irreparable metabolic dysfunction and cell death (Apel and Hirt 2004; Mittler et al. 2004). Accumulation of ROS may be the result of the imbalance between their generation and the scavenging activity of antioxidative enzymes (Apel and Hirt 2004). Moreover, the toxicity of heavy metals can be transferred to higher trophic levels through the food chain and the danger of their bioaccumulation poses a major threat to environment (Peralta-Videa et al. 2009).

Nickel (Ni)
Nickel (Ni) (Atomic number: 28; Atomic mass: 58.69) was first isolated by the Swedish chemist, Axel Fredrik Cronstedt in 1751. It is the 22nd most abundant element in the earth's crust (Nielsen 1987; Sunderman et al. 1991). Ni is a member of the transition series and belongs to group VIII B of the periodic table along with iron, cobalt, palladium, platinum and five other elements. Ni is a nutritionally essential element for plants and micro-organisms and is required in very low concentrations but when taken up in excess, it can cause toxicity symptoms as well. With increasing levels of Ni pollution, plants readily accumulate Ni in high concentrations. Moreover, the toxic effects excess Ni have been reported in plants, such as alterations in mitotic index (Rao and Sresty 2000), plant growth and biomass reduction (Molas 2002) and negative effects on fruit quality and yield (Gajewska et al. 2006). Extreme high concentrations of Ni in soil have left some farmland unsuitable for growing crops, vegetables and fruits (Duarte et al. 2007). Therefore, it is vital to know the useful roles and its toxicity in plants.

Occurrence and sources of Ni
Ni either occurs as a free metal in igneous rocks or in combination with iron. Natural Ni is a mixture of five stable isotopes. Although there are various valence states of Ni (from
Ni is widely distributed in the environment. Volcanic emissions, forest fires and vegetation are some of the natural sources that add Ni to the atmosphere. Apart from the natural sources, it is also released into the environment from anthropogenic activities such as mining processes, fossil fuel burning, smelting, vehicular exhausts, domestic disposals, wastes released by industries, fertilizers and manures, etc. (Alloway 1995). Ni is mostly used as a raw material in metallurgical and electroplating industries. It is used as a catalyst in the chemical and food industry, and as a component in the electrical batteries (Easton et al. 1992). Ni finds its way into the environment by tobacco smoke, dental and orthopedic implants, stainless steel, kitchen utensils and inexpensive jewelry. According to Cempel and Nikel (2005), 1.1 to 3.1 μg Ni is present in each cigarette and it is in the form of nickel carbonyl. In current years, Ni pollution has been reported globally, including Asia (Zarcinas et al. 2004; Zhao et al. 2008), Europe (Kozlow 2005; Papadopoulous et al. 2007) and North America (Kukier et al. 2004).

**Nickel (Ni) as an essential micronutrient**

Ni is an essential micronutrient for plants and in small amounts is known to enhance the growth and yield of plants (Ahmad and Ashraf 2011; López and Magnitski 2011). Dixon et al. (1975) explained Ni as an integral component of urease enzyme in plants. Polacco (1977) later demonstrated that Ni is an essential requirement for soybean cells when grown up with urea, as the only source of N. In the absence of Ni, plants are not able to complete their life cycle. Moreover, any other nutrient is not potent enough to replace it (Eskew et al. 1983; Andreeva et al. 2001). Ni helps in the stimulation of urease enzyme, so most of the studies related to Ni essentiality have concentrated on legumes, because activity of urease enzyme and transportation of ureids within the plant is very high in the seeds of legumes (Bollard 1983; Walker et al. 1985).
Urease enzyme helps in the hydrolysis of urea, derived from different pathways and leads to generation of ammonia, which further takes part in the various processes (anabolic), mainly in glutamine synthesis. In the past, many studies were carried out in the presence of Ni with rye, rapeseed, zucchini, and sunflower, and a subsequent increase in the glutamine content was observed in all the plants (Gerendas and Sattelmacher 1997, 1999). At low concentrations, Ni has been reported to improve the nutritional status such as that of iron and other minerals and thus, has been found to improve the germinating ability of seeds of many plants (Brown et al. 1987a,b; Gerendas et al. 1999), including rice (Das et al. 1978), wheat, timothy grass, pea, bean, soybean, and lupine (Welch 1981).

In some legumes, a slight concentration of Ni is considered to be important for nodule growth, and activation of hydrogenase enzyme, which is responsible for recycling of hydrogen produced during side reaction of nitrogenase in \( \text{N}_2 \)-fixation process (Albrecht et al. 1979). The proficiency of nitrogen fixation is highly regulated by the activity of hydrogenase enzyme as the oxidation of hydrogen delivers ATP required for N reduction to ammonia. Past studies have shown that the hydrogenase activity of \textit{Rhizobium japonicum} in the nodules of soybean plant was enhanced by 45% over the control, when fed up with NiCl\(_2\) (Dalton et al. 1985). Andreeva et al. (2001) reported that cotton plants sprayed with Ni solution raised the number of flowers and buds, followed by enhanced seed oil content and rate of ball formation.

Moreover, a shortage of Ni indicates a decline in the activity of urease (Gad et al. 2007) and some other enzymes, leading to accumulation of urea, amino acids and nitrate (Shimada and Watanabe 2004), and further results in yellowing of leaves in many plants including soybeans (Eskew et al. 1984) and cucumber (Shimada and Watanabe 2004). Ni deficiency also results in growth inhibition, early senescence and poor grain development. It also reduces grain viability and alters the iron content in plant tissues. Thus, it is confirmed that higher plants need Ni, and therefore, it is categorized amongst the essential ultra-micronutrients (López and Magnitski 2011).

**Ni availability and its uptake**

Availability of Ni for plants depends upon its solubility, and the solubility of Ni depends upon the soil pH. As per Temp (1991), extreme pH values decline the availability of Ni\(^{2+}\) because of the generation of low-soluble complexes. An increase in Ni uptake by
*Lathyrus sativus* has been reported with rising pH up to 5.0 (Panda et al. 2007). Upon absorption by roots, Ni is taken up by plants through passive diffusion as well as active transport (Seregin and Kozhevnikova 2006). Though the uptake of Ni through passive and active transport mechanism varies with plant species, oxidative state, soil pH, presence of other metals and Ni application in the soil or nutrient solution (McIlveen and Negusanti 1994; Dan et al. 2002; Podar et al. 2004; Vogel-Mikus et al. 2005; Antoniadis et al. 2008), the absorption of soluble Ni through cation transport system has also been reported (Harter 1983; Smith 1994; Weng et al. 2004). The chelated Ni compounds can be taken up through secondary active transport system because some proteins can specifically bind with Ni (Wolfram et al. 1995). On the other hand, the insoluble compounds of Ni enter into the cells mainly through endocytosis (Costa et al. 1994; Morgan et al. 2002). In addition, the soluble Ni compounds could also be absorbed through the Mg\(^{2+}\) transport system because of the like size and charge of the two metal ions (Oller et al. 1997). Since Ni\(^{2+}\) uptake is inhibited by Cu\(^{2+}\) and Zn\(^{2+}\) competitively, all of these metal ions appear to be absorbed by the same passage system (Cataldo et al. 1978; Kochian 1991). In some cases, however, the high mobility of Ni has also been observed even under neutral or alkaline conditions (Willaert and Verloo 1988; Alloway 1995; Kabata-Pendias and Pendias 2001).

**Transport and Distribution of Ni in Plants**

Parallel to others, Ni\(^{2+}\) is primarily translocated from roots to shoots (Krupa et al. 1993; Peralta-Videa et al. 2002) via transpiration pathway (Neumann and Chamel 1986). Owing to its high mobility in plants, it can be readily re-translocated from older to younger leaves (Zhao et al. 1999; Gray and McLaren 2006). Moreover, being a vital micronutrient, Ni\(^{2+}\) is able to move to seeds, buds and fruits via phloem stream (Fismes et al. 2005; Page et al. 2006) and this process is intensely controlled by metal ligand complexes such as histidine and organic acids (Rauser 1999; Kim et al. 2005; Pianelli et al. 2005; Haydon and Cobbett 2007) and some proteins that specifically bind and transport Ni (Colpas and Hausinger 2000).

Nearly 50% of the Ni taken up by plants is retained in the plant root system (Cataldo et al. 1978). This may be due to its sequestration in the cation exchange sites of the vessel walls of xylem parenchyma cells and immobilization in the vacuoles of roots (Seregin and Kozhevnikova 2006). Moreover, root vascular cylinders contain a high percentage of
Ni, while less than 20% is present in the corticular region. This may explain its good mobility in xylem and phloem tissue (Marschner 1995; Riesen and Feller 2005). However, its distribution is different in stems and leaves, where it is distributed preferentially in the epidermal cells, more likely in the vacuoles rather than in the cell wall (Kupper et al. 2001). On the other hand, the distribution of Ni in leaf organelles and cytoplasm are different. Ni content has been found to be accumulated maximally in the supernatant of cytoplasm and vacuoles followed by chloroplast, mitochondria and ribosomes (Brooks et al. 1981).

Phloem is considered as the principal pathway for translocation of Ni\(^{2+}\) to the fruits and seeds (McIlveen and Negusanti 1994; Page et al. 2006), and its distribution within the various parts of seeds depends upon numerous factors such as species type and presence of pathogens and insects, etc. (Boyd et al. 2006). Bhatia et al. (2003) reported that lesser absorption of Ni within endospermic and cotyledonary tissues as compared to the pericarp of Ni hyperaccumulating species, *Stackhousia tyronii*, is responsible for its better survival even in the presence of high Ni concentrations, as Ni present within the fruit walls had no inhibitory effect on its seed germination. Therefore, it can be inferred that metal exclusion from embryonic tissue ensures normal growth, development and reproductive fitness of hyperaccumulator plants when growing on metal enriched soil.

**Ni Phytotoxicity**

Though Ni plays a significant role in plant metabolism and therefore, it is categorized as an essential metal (Hänsch and Mendel 2009; Ahmad and Ashraf 2011; López and Magnitski 2011), the unwanted concentrations of Ni in soil and nutrient solution have been reported to be phytotoxic (Ahmad and Ashraf 2011). Under Ni stress, plants respond in various ways such as formation and accumulation of Ni\(^{2+}\)-organic acid and Ni\(^{2+}\)-NA (nicotinamine) complexes (Krämer et al. 2000; Küpper et al. 2001; Persans et al. 2001), over production of NA and it's synthase (Vacchina et al. 2003; Weber et al. 2004), and high levels of free histidine (Wycisk et al. 2004), including the stimulation of MTs (metallothionenes) and thiol glutathione (Courbot 2004; Bellion 2007), and high concentrations of glutathione, cys and o-acetyl-L-serine (Freeman et al. 2004). It is further followed by enhancement of some enzyme activities, such as serine acetyltransferase (SAT) and glutathione reductase (Ali et al. 2008).
**Effect on growth and yield**

Under elevated concentrations of Ni, toxicity symptoms become visible in plants. The general signs linked with Ni toxicity in plants include inhibition of germination (Nedhi *et al.* 1990), root and shoot growth (Rahman *et al.* 2005), poor branching system (Reeves *et al.* 1996), distortion of various plant parts (Wright and Welbourn 2002), irregular flower shape (McIlveen and Negusanti 1994), reduced biomass (Pandey and Sharma 2002; Rahman *et al.* 2005), leaf spotting (Gajewska *et al.* 2006), mitotic root tip disturbances (McIlveen and Negusanti 1994), Fe deficiency that induces chlorosis (Ewais 1997; Kirkby and Römheld 2004) and foliar necrosis (Kukkola *et al.* 2000; Seregin and Kozhevnikova 2006; Kumar *et al.* 2012). Ni stress causes partial browning of roots and shoots of pigeon pea, and at high dose the roots were dark brown in color (Rao and Sresty 2002). Excess Ni also affects nutrient absorption by roots (Kochian 1991; Hasinur *et al.* 2005), impair plant metabolism (Pandey and Sharma 2002), and inhibit photosynthesis and transpiration (Sheoran *et al.* 1990; Shi and Cai 2008). In due course, all these processes lead to reduction in crop yield (Ahmad *et al.* 2007). Accumulation of Ni severely affects the yield of plants, decreases the number of seeds per pod, seed weight and reduces seed yield per plant (Tripathy *et al.* 1981). Excess Ni alters the yield of number of crops including mung bean, tomato, cucumber, and sunflower (Balaguer *et al.* 1998; Matraszek 2002; Lavado 2006; Ahmad *et al.* 2007; Aziz *et al.* 2007; Tabatabaei 2009).

**Leaf chlorosis, necrosis and wilting**

Necrosis and chlorosis has been reported in leaves of water spinach (Sun and Wu 1998), maize (Mishra *et al.* 2010), barley (Rahman *et al.* 2005) and wheat (Gajewska and Sklodowska 2007) in response to Ni toxicity. The visible effects were also observed on young leaves of sponge gourd as an interveinal chlorosis along with marked depression in growth within 3 days of Ni exposure (Awasthi and Sinha 2013). Ni is also known to cause reduction in relative water content of wheat shoots (Gajewska *et al.* 2006) and rice seedlings (Llamas *et al.* 2008). Deficiency of other essential metals (Fe, Cu, Zn and Mn) could also be the reason of occurrence of these visual symptoms under Ni toxicity (Khalid and Tinsley 1980; Hasinur *et al.* 2005). Moreover, the reduction in water potential and transpiration rate observed in plants exposed to Ni result in leaf necrosis and wilting (Pandey and Sharma 2002).
**Effect on photosynthesis**

Most noticeable effects of Ni stress on plants are reduced rate of photosynthesis and alteration in various gas exchange aspects—stomatal conductance and transpiration rate (Bishnoi *et al.* 1993; Krupa and Baszynski 1995; Pandey and Sharma 2002). On exposure to heavy metals, photosynthesis is inhibited non-specifically by various direct and indirect ways. With this, the rate of photosynthesis is reduced as a consequence of disrupted chloroplast structure, blocked chlorophyll synthesis, disordered electron transport, inhibited activities of the Calvin cycle enzyme, and CO₂ scarcity caused by stomatal closure (Seregin and Ivanov 2001). Excess Ni altered photosynthetic pigments including chlorophyll *a, b,* and carotenoids in plant species (Seregin and Kozhevnikova 2006). Another mechanism that could be involved in reduced photosynthetic rate could be inhibition of components of light and dark reactions. The toxic effects of heavy metals on metabolic processes during light and dark reactions would result in direct inhibition of photosynthesis. Heavy metals can also hold back the dark reactions of photosynthesis by inhibiting the activities of key enzyme of Calvin cycle, such as Rubisco, 3-phosphoglycerate kinase, fructose 1,6-bisphosphatase, etc. Ni also disrupts the electron transport system during light reaction (Krupa and Baszynski 1995; Malkin and Niyogi 2000) and inhibit oxygen-evolving complex of photosystem-II (Boisvert *et al.* 2007).

**Interference with other metal ions**

In the presence of phytotoxic concentrations of Ni in growing medium, nutrient translocation and their uptake becomes difficult for seed germination and initial growth of seedlings (Cataldo *et al.* 1978; Kovacevi *et al.* 1999). Excess Ni not only leads to alterations in the uptake pattern of water, but mineral nutrients in germinating seeds were also altered (Seregin and Kozhevnikova 2005). Ni competes with essential macro- and micro-nutrients (Ca, Na, K, Mg, Fe, Cu, Zn, and Mn) in absorption and transpiration processes, which are the common signs of heavy metal stress (Heale and Ormrod 1982; Körner *et al.* 1987; Kochian 1991; Marschner 1995; Küpper *et al.* 1996; Nieminen and Helmisäari 1996). At high concentrations, Ni inhibits their absorption, decreases their concentration and leads to its deficiency in plants (Van Assche *et al.* 1990; Gabbielli *et al.* 1990; Rubio *et al.* 1994; Ahmad *et al.* 2007), and thus results in impairment of various physiological processes (Gajewska *et al.* 2006; Goncalves *et al.* 2007).
Oxidative stress and antioxidant systems

Different metabolic processes in plants generate ROS, such as superoxide anion radical ($O_2^-$), hydrogen peroxide ($H_2O_2$) and singlet oxygen ($^1O_2$), as byproducts (Dat et al. 2000). During respiration, electrons depart from their normal route and reach to $O_2$. This leakage induces reduction of $O_2$ to $O_2^*$, where the main site of leakage is complex I (NADH-coenzyme Q Reductase (Møller 2001). Anyhow, ROS are comparatively more reactive as compared to $O_2$ and consequently, they are potentially toxic to the living system. These toxic ROS can cause DNA damage, oxidation of proteins and lipids, and degradation of chlorophyll pigments (Apel and Hirt 2004). The toxicity of ROS leads to the evolution of non-enzymatic and enzymatic detoxification mechanisms in plants capable of quenching ROS without undergoing conversion to a destructive radical itself (Gratão et al. 2005; Pitzschke et al. 2006). The ROS scavenging enzymatic mechanisms of plants include superoxide dismutase (SOD) acting against $O_2^*$, by dismutating $O_2^*$ to $H_2O_2$. Subsequently, ascorbate peroxidase (APX) or glutathione peroxidase (GPX) detoxify $H_2O_2$ to $H_2O$ (Gratão et al. 2005), mainly in apoplast (Zoller et al. 2003), and by catalases (CAT) in peroxisomes (Igamberdiev and Lea 2002). Removal of $H_2O_2$ by APX occurs by the oxidation of ascorbate to monodehydroascorbate, which can be regenerated by MDHAR (monodehydroascorbate reductase) using NAD(P)H as reducing equivalent. Dismutation of monodehydroascorbate into dehydroascorbate occurs spontaneously. Ascorbate regeneration is mediated by DHAR (dehydroascorbate reductase) followed by oxidation of GSH and GSSH. Rapid disproportion to MDHA radical means that some DHA is always produced when ascorbate is oxidized in leaves and other tissues. Finally, GR can regenerate GSH from GSSG by GR (Apel and Hirt 2004).

Usually, transition metals have the ability to produce $OH^*$ via Haber–Weiss reaction (Kehrer 2000). However, Ni does not appear to be an effective catalyst of this reaction due to high oxidation/reduction potential (Leonard et al. 2004). But it has been reported that Ni dependent reduction of $H_2O_2$ leading to $OH^*$ formation may be increased by certain chelating agents. ROS can also be originated from the reactions catalyzed by NADPH oxidases (Sagi and Fluhr 2006).
Ni is toxic only at higher concentrations and its toxicity in plants is associated with oxidative stress (Gonnelli et al. 2001; Gajewska et al. 2006) through the generation of free radicals. Excessive exposure to Ni inhibits the activity of various cellular antioxidant enzymes and reduces the capability of plants to scavenge ROS, thereby, resulting in accumulation of ROS and causing oxidative stress in plants (El-Maraghy 2001; Del Carmen et al. 2002; Zhao et al. 2008; Gajewska and Sklodowska 2007). In response to excess Ni, positive correlation of antioxidant system has been reported in several studies conducted in the past. For example, Ni treatment increased the activity of SOD and peroxidases (POX) in the roots and shoots of pigeon pea (Rao and Sresty 2000), rice (Maheshwari and Dubey 2009), shoots of maize (Baccouch et al. 1998; Mishra et al. 2010) and barley (Kumar et al. 2012). Increased POX activity was also observed in the seedlings of wheat (Pandolfini et al. 1992), pepper (Diaz et al. 2001) and barley (Simonovicova et al. 2004). A decline in CAT activity was observed in sunflower (Pillay et al. 1996), pigeon pea (Rao and Sresty 2000), wheat (Gajewska and Sklodowska 2007), and seedlings of maize (Mishra et al. 2010). Contrary to this, increased activity of CAT was observed in barley leaves exposed to Ni (Kumar et al. 2012). Increase in GR activity has been reported in the shoots of rice (Maheshwari and Dubey 2009) and maize (Baccouch et al. 1998) when grown in phytotoxic concentrations of Ni. Elevated activity of MDHAR and DHAR was observed in roots and shoots of rice grown under 200 μM and 400 μM Ni treatments (Maheshwari and Dubey 2009).

Effect on carbohydrate metabolism

Starch is the major carbohydrate stored in most of the seeds along with other carbohydrates. Upon imbibition, reserves of carbohydrate from endosperm are mobilized thereby, leading to germination of seeds and development of embryo axis (Briggs 1992). The key enzymes involved in the hydrolysis of starch are α-amylase, β-amylase and starch phosphorylase (Wang et al. 2000). Few studies have been carried out in past to investigate the Ni-induced alterations on sugar metabolism and associated enzymes. Abnormal accumulations of sucrose, reducing sugars, and starch (~1.6–3.0 fold over the control) in the unifoliated leaves of white beans were also observed following 1-day exposure of Ni (Samarakoon and Rauser 1979). Increase in total carbohydrate, reducing and non-reducing sugar content has been observed in corn seeds when soaked in Ni solutions (Rabie et al. 1992). Ni treatment led to a significant decrease in activities of...
starch degrading enzymes, α-amylases and β-amylases, in rice seedlings treated with 200 µM and 400 µM NiSO₄ (Mishra and Dubey 2012). The contents of non-reducing, reducing, and total sugars, and the activities of starch phosphorylases and acid invertases increased in the shoots of Ni-treated rice seedlings compared to control (Mishra and Dubey 2012). Ni⁺² impairs sugar metabolism as indicated by decline in the activity of sucrose and starch hydrolyzing enzymes in wheat (Negi et al. 2014).

Similarly, accumulation of carbohydrates due to excess of Ni has been reported in barley (Agarwala et al. 1977). However, Ni stress causes particularly, the accumulation of reducing sugars. Such accumulation of reducing sugars and starch would be alternatively interpreted, by a reduction of their translocation from leaves to the other parts of the seedlings, particularly the root system (Agarwala et al. 1977; Samarakoon and Rauser 1979).

**Effect on nitrogen metabolism**

Nitrogen is considered to be a vital nutrient for determining the growth and the productivity of plants. Although atmosphere contains ~78% N₂, which is most abundant element among all others present in atmosphere. Due to lack of genes required for the nitrogen fixation in plants, they are dependent on the external sources like soil/atmospheric activities. For higher plants, the most available form of element is nitrate and its uptake and transport into the cells depends on the availability of metabolic energy, utilized for cell membrane polarization. The main role in this process is played by H⁺-ATPase proton pump (McClure et al. 1990 a,b).

According to the past results, significant reduction in NR (nitrate reductase, which supplies the organic nitrogen to the plants) activity was observed in Brassica juncea (Alam et al. 2007), wheat seedling (Gajewska and Sklodowska 2009; Yusuf et al. 2011) and in soybean (El-Shintinawy and El-Ansary 2000) under Ni stress. Reduction in NR activity was more significant in leaves than roots at high Ni level (100 µM) (Alam et al. 2007). This might be hindering the mechanism of nitrogen uptake by plant roots. An inhibition of NO₃⁻ uptake can result due to the presence of ~SH groups in the proteins of NO₃⁻ uptake system which are sensitive to heavy metals including Ni (Singh et al. 1989; Tan et al. 2000). Gajewska and Sklodowska (2009) reported that the activities of glutamine synthetase and glutamate synthase decreased, whereas ammonium and proline content increased under Ni stress.
Ni-induced anatomical changes

In addition to toxic effects on germination, growth and photosynthesis, Ni has been reported to alter plant anatomy. Past studies have revealed that Ni exposure decreases the thickness of mesophyll cells and size of vascular bundle in wheat leaves (Kovacevi et al. 1999). Ni exposure led to reduction in size of palisade and spongy mesophyll cells in cabbage leaves and shrinkage in intercellular spaces, palisade and sponge mesophyll cells in *Brassica oleracea* leaves (Molas 1997). Formation of large inclusions, mainly in the mesophyll and bundle sheath cells, observed in the leaves of *Dianthus repens* grown in excess Ni (Kravkina 2000), were assumed to be results of complex formation of Ni with proteins.

Ni treated cabbage exhibits elliptical or oblong-shaped chloroplasts with reduced volume, reduction in size and number of grana, swollen thylakoids (of grana and stroma), decrease in electron density of stroma, and increased number of starch grains and plastoglobuli in chloroplasts (Molas 2002). There was a marked reduction in the total number of stomata per unit area in the leaves of cabbage when grown in high Ni concentrations. In addition, many stomatal deformations have been observed in the presence of Ni stress resulting in increased number of defective stomata in both adaxial and abaxial leaf surfaces (Molas 1997).

Ni has also been observed to cause alterations in stem tissue organization. Disorganization of epidermal cells, distortion and disintegration of root cortical cells were observed in pigeon pea roots exposed to Ni (Sresty and Rao 1999). Moreover, Ni stress reduce stem diameter, number of vascular bundles and cell size of the storage regions. Ni stress decreased cell wall thickness in epidermal and hypodermal tissues of stem (Setia and Bala 1994). Ni has been reported to decrease stem and root diameter as well as area of cortical cells of wheat plants grown in sand culture. Reduction in number of cortical cells, with some elongated aerenchyma like cells instead of the normal parenchymatous tissue and thickening of cell wall were observed in *Colocasia esculentum* roots treated with 100 mg/kg of Ni (Parmar et al. 2012).

According to Demchenko et al. (2005), both centripetally and acropetally inhibition of cell division has been noticed in the root tissues of *Triticum aestivum* exposed to Ni. A further increase in incubation time of roots with Ni stopped mitotic cycle in rhizodermis and the layers of cortex, which further leads to the formation of vacuolated cells. In few
root tissues of *T. aestivum* reduction in length of the zone of cell division has also been noticed. Disturbance in intercellular contacts in the rhizodermis and formation of additional cell layer in the exodermis was also noticed in longitudinal sections of the meristemic areas in the roots of *T. aestivum* grown under NiSO₄ supply (Demchenko *et al.* 2005).

The width and thickness of midrib in leaves and diameter of xylem vessels of root, stem and leaves was decreased in plants subjected to high Ni concentrations. In addition, parenchymatous cell area of stem, leaf midrib tissues, and pith and cortex of root was decreased under high Ni application (Kovacevi *et al.* 1999). Ni stress also altered dimensions of stem vascular bundles, reduced number of xylem vessels in root, and frequency of stomata on abaxial leaf surface. Such variations in anatomical structures might be due to the heavy metal-induced ultrastructural modifications and consequently the function of the parenchymatous, corticular and vascular tissues, and stomatal apparatus. These changes could be a direct effect of metal induced inhibition of cell elongation that could result in reduced cell size of various tissues including stem, root and leaves (Kovacevi *et al.* 1999).

**Ni hyperaccumulation**

Plant species vary in their capacity to accumulate heavy metals. High accumulation of heavy metals and the ratio of metals in various organs of plant species largely depend on plant morpho-physiological characteristics. Among the Ni accumulating plants, there is a discrete group of hyper-accumulators that accumulate metals over 1000 mg/kg dry weight in the shoots (Brooks *et al.* 1977). According to Minguzzi and Vergnano (1948), Ni hyperaccumulation was first time reported in *Alyssum bertolonii*. Till now about 300 such species have been described and they mostly belong to the families of Asteraceae (Merkusheva *et al.* 2001), Brassicaceae (Van Assche *et al.* 1990), Buxaceae (Dalton *et al.* 1985), Euphorbiaceae (Kositsin 1991), Flacouriaceae (Krogmeier *et al.* 1989), Rubiaceae (Gerendas and Sattelmacher 1999), and Violaceae (Welch 1981) and grow on serpentine soils in the tropical and subtropical zones. Hyperaccumulation of metal in plants depends on a mechanism of tolerating high levels of metal in the environment. Hyperaccumulating plants transfer absorbed metal into the compartments of low physiological activity or into plant organs to be shed in future (Boyd and Martens 1998; Brooks 1998). Davis *et al.* (2001) reported that the old leaves of *Psychotria douarrei* stored considerably more Ni.
than the young leaves. The drought resistance hypothesis entitles that Ni accumulation in plant tissues reduces the cuticular transpiration and hence, enhances plant tolerance to moisture deficit (Severne 1974; Brooks 1998; Boyd and Martens 1998).

*Alyssum inflatum* is a serpentine endemic Ni hyperaccumulator, accumulating Ni in trichomes, particularly at the base, and it gets extended to rays and cell walls with increase in the Ni supply (Ghasemi *et al.* 2009). *Alyssum murale*, another hyperaccumulator, has a remarkable ability to accumulate Ni from soils (mostly insoluble). A significant increase in metal concentration has been observed in *Thlaspi goesingense* in the presence of rhizosphere bacteria (Idris *et al.* 2004). Abou-Shanab *et al.* (2006) also reported that the presence of bacterial inoculants in soil increases the phytoavailability of Ni, thus enhancing Ni accumulation by *A. murale*. Another study revealed that *A. murale* is sensitive to cytokinin treatment, and this treatment enhances plant biomass and transpiration rate, whereas no significant variation in Ni accumulation was observed. Treatment of cytokinin increased the phytoextraction capability by increasing biomass (Cassina *et al.* 2011). Cassina *et al.* (2011) also stated that Ni hyperaccumulation mechanism is independent of water flux and transpiration rate.

Ni is predominantly accumulated in the roots of *Typha latifolia* (Taylor and Crowder 1983), *Glycine max* (Dalton *et al.* 1985), *Avena sativa* (Andreeva *et al.* 2000), *Zea mays* (Yang *et al.* 1997; Seregin *et al.* 2003), *T. aestivum* and *Hordeum vulgare* (Andreeva *et al.* 2001), *Alyssum montanum*, and *Thlaspi arvense* (Krämer *et al.* 1996, 1997), *Cyperus difformis*, *Chenopodium ambrosioides*, and *Digitaria sanguinalis* (Ewais 1997). Plants capable of taking up metals in the thousands of ppm possess additional detoxification mechanisms; for example, in *T. goesingense*, a Ni hyper-accumulator, high tolerance is due to the formation of complex by histidine that supports in phytoassimilation but reduced its phytotoxicity (Krämer *et al.* 1996, 1997). Lee *et al.* (1977) revealed that Ni tolerance is elevated by complex formation of Ni with low molecular weight organic compounds. It has also been reported that the Ni complexation may decrease its uptake by plants but may increase the removable metal concentration. However, when Ni accumulation exceeds a characteristic limit, plant mechanisms of detoxification become less efficient. As a result, the control over metal entry into the shoots is lost, and such plants perish (Antosiewicz 1992; Seregin and Ivanov 2001).
Ni-Genotoxicity

Ecotoxicological studies further required valuation up to gene level to understand the extent of toxicity of metals including Ni, as the metal toxicity are not directly visible. Mičieta and Murin (1998) revealed that genotoxicological effect develops shortly even in the presence of very low metal concentrations, which do not able to cause phytotoxic effects. Substantial rise in chromosomal aberration rate was reported in the root tips of Z. mays, Raphanus sativus, and Vicia sativa under Ni stress (Fargašová 2012). Kovalchuk et al. (1998) described that genotoxicity can occur as a result of multipolar anaphase and c-mitosis or damage of protein synthesis in the presence of a DNA toxicant. Ni significantly stimulated micronuclei frequency in comparison to the control (Fargašová 2012). Rossman (1995) found that mutations after Ni applications can result from DNA damage and DNA-protein cross links formation.

Free radicals produced by Ni ions can cause point mutations and strand breakages directly or indirectly interfering with DNA repair system. It is also known that Ni ions change cytosine methylation patterns in the cell causing hypo- or hyper-methylation of DNA (Kovalchuk et al. 2001). Hypomethylation can increase the susceptibility of the chromosomes to breakages. On the other hand, hypermethylation can cause chromosomal instability with its harmful effects in the cell (Kovalchuk et al. 2001). Ni ions show high affinity for proteins in comparison with DNA. Therefore, it can be stated that the genotoxicity caused by Ni ions is dependent on the interactions of Ni ions with chromatin proteins. These interactions may lead to the deleterious effects on heterochromatic chromosome regions that have compact protein-DNA structure. It was shown that Ni compounds form DNA-protein cross-links (Costa et al. 1994). Lee et al. (1995) also indicated that Ni ions have interactions with proteins and other intracellular molecules and likely have a similar effect on gene expression. In addition, Ni ions prevent proteins from binding to DNA. In a micronucleus assay on Tradescantia spp. and Vicia spp., Knasmüller et al. (1998) reported that Ni induces mutagenicity stemming from genotoxicity. Ni-induced mutations are caused due to single strand DNA breaks and DNA-protein cross-links (Sen et al. 1987).