Discussion

The present study investigated the phytotoxic effects of Ni\(^{2+}\) in terms of seedling growth, induction of oxidative stress, alterations in carbohydrate and nitrogen metabolism in *Triticum aestivum* and *Zea mays* seedlings. In addition, Ni-caused anatomical and ultrastructural changes were also analyzed in the target tissue. The significant observations and results obtained are discussed hereunder:

**Excess Ni is a potent root inhibitor**

Ni (5–50 \(\mu\)M) treatment strongly inhibited the radicle emergence and seedling growth of test plant in a dose-dependent manner. The inhibitory effect was more pronounced on roots than on shoots suggesting that Ni is a potent root inhibitor. The reduction in seedling growth is in agreement with many reports investigated in past. For example, Ni has been reported to inhibit root growth in cabbage (Pandey and Sharma 2002), pigeon pea (Rao and Sresty 2000) watercress (Duman and Ozturk 2010), and cereal crops such as barley (Kumar *et al.* 2012), rice (Maheshwari and Dubey 2009), wheat (Gajewska and Sklodowska 2007), and oat (Poulík 1997). However, it is in contrast to an earlier study reporting non-inhibitory effect of 10 \(\mu\)M Ni on shoot growth of wheat (Gajewska *et al.* 2006). According to Demchenko *et al.* (2005), a reduction in root growth in plants exposed to excess Ni might be associated with decline in mitotic activity and restriction of cell elongation. Ni interferes with the uptake and translocation of nutrients and metal ions, and thus negatively affects germination and initial growth of seedlings (Kovacevi *et al.* 1999). Additionally, excess Ni has been reported to cause poor branching system (Reeves *et al.* 1996), distortion of various plant parts (Wright and Welbourn 2002), irregular flower shape (Mellvæen and Negusanti 1994), leaf spotting (Gajewska *et al.* 2006), mitotic root tip disturbances (Mellvæen and Negusanti 1994), Fe deficiency that induces chlorosis (Kirkby and Romheld 2004), and foliar necrosis (Seregin *et al.* 2006; Kumar *et al.* 2012).
**Ni exposure reduces plant biomass**

Ni exposure negatively affected biomass accumulation in plants. Earlier, many workers have reported a reduction of plant biomass under metal stress (Rao and Sresty 2000; Gajewska and Sklodowska 2007; Mishra et al. 2010). In fact, accumulation of Ni seriously affects the yield of plants, decreases the number of seeds per pod, seed weight and reduces seed yield (Tripathy et al. 1981). Excess Ni altered the yield of crop plants, 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). A reduction in yield under Ni stress is mainly due to the fall in nutrient absorption by roots (Kochian 1991; Hasinur et al. 2005), impaired plant metabolism (Pandey and Sharma 2002), and inhibition of photosynthesis and reduction in transpiration rates (Sheoran et al. 1990; Shi and Cai 2008). According to Gajewska et al. (2006), reduction in fresh weight may be partly due to the metal induced decline in tissue water content. The reduced biomass of plants subjected to excess Ni may be attributed to decreased photosynthetic activity due to inhibition of enzymes of Calvin cycle and loss of photosynthetic pigments (Gabbrielli et al. 1990).

**Ni accumulates in roots**

Present study revealed that Ni accumulates greatly in the roots of treated plant, which paralleled the more inhibitory effect of Ni on roots than on shoots. It is in agreement with past studies that Ni accumulates mostly in roots than shoots (Cardoso et al. 2005; Maheshwari and Dubey 2009; Duman and Ozturk 2010). Deng et al. (2004) suggested that the rate and extent of translocation of metals within plants depends upon concentration of metal and type of plant species. The accumulation of Ni increased consistently with increasing Ni concentration applied to the roots (Parida et al. 2003).

**Ni interferes with photosynthetic efficiency of plant**

Under Ni stress chlorophyll content and photosynthetic pigments (chl a, chl b, and carotenoids) were reduced in *T. aestivum* and *Z. mays*. It is in agreement with earlier reports that excess Ni altered photosynthetic pigments including chl a, b and carotenoids in plants, thereby affecting photosynthetic activity (McIvceen and Negusanti 1994; Balaguer et al. 1998; Seregin and Kozhevnikova 2006). The reduction in both chl a, and chl b, could be consequence of (Fe and Mg deficiency in plants under excess Ni
(Ouzounidou et al. 2006), or excessive ROS production (Hao et al. 2006), or stimulation of chlorophyllase activity by Ni (Abdel-Basset et al. 1995). Excess Ni may directly damage the photosynthetic apparatus of leaves by destroying mesophyll cells, epidermal cells, and thylakoid membranes of chloroplast (Bethkey and Drew 1992; Szalontai et al. 1999; Molas 2002).

The reduced photosynthetic rate could be due to the inhibition of components of light and dark reactions. The toxic effects of heavy metals on metabolic processes during light and dark reactions result in direct inhibition of photosynthesis. Heavy metals can also hold back the dark reactions of photosynthesis by inhibiting the key enzymes of Calvin cycle, such as Rubisco, 3-phosphoglycerate kinase, fructose 1,6-bisphosphatase, etc. Ni disrupts the electron transport system during light reaction (Krupa and Baszynski 1995; Malkin and Niyogi 2000). Such effects have been demonstrated in Cajanus cajan leaves following several days of incubation on 1 mM NiCl₂ (Sheoran et al. 1990).

Ni alters the structure of carrier proteins, which resulted in electron transport inhibition from pheophytin via plastoquinone Qₐ and Fe to plastoquinone Qₙ (Mohanty et al. 1989). Moreover, the reduction in cytochromes, ferredoxin and plastocyanin content in thylakoid region, results in inhibition of electron transport chain (Veeranjaneyulu and Das 1982).

**Ni alters the protein metabolism**

In the present study, Ni-caused reduction in radicle emergence was accompanied by alteration in protein content and activity of protein-hydrolyzing enzyme–proteases. Decline in protein content obtained in present experiment is in sharp contrast to the earlier observations in maize leaves (Gajewska and Sklodowska 2007), but is in agreement with the results obtained in radish in the presence of Ni (Latif 2010), beet root in the presence of Ni and Cd (Kevresan et al. 1998), and in barley exposed to Cu (Demirevska-Kepova et al. 2003). Duman and Ozturk (2010) suggested that this decrease could be due to the degradation of a number of proteins. Maheshwari and Dubey (2007) also reported an inhibition in the activity of proteases in rice seedlings under Ni-toxicity. Previously, heavy metals, including Ni, have been reported to cause ROS-mediated oxidation of proteins and other biomolecules (Schutzendubel and Polle 2002; Gajewska et al. 2006).
**Ni alters the activity of enzyme proteases**

Parallel to results obtained in the present work, reduced activity of proteases was also observed by many authors in the past under heavy metal stress. For example, decline in protease activity was noticed in rice seedlings subjected to Ni and Pb stress (Maheshwari and Dubey 2008; Shah and Dubey 1997). Likewise, a decline in protease activity in the presence of Cd was also reported in soybean root nodules (Balestrasee et al. 2003) and in mung bean roots under As-toxicity (Kaur et al. 2011). Proteases are linked with protein hydrolysis, which mediates protein mobilization to amino acids and thus their decreased activity in the Ni-exposed roots may be due to severe cell damage (Schlereth et al. 2001). Hence, it is speculated that reduction in protease activity in the presence of metal stress declines the protein synthesis, and thus a reduction in the protein content in plants. Moreover, Ni has been shown to exhibit high affinity for protein in comparison to DNA and prevent proteins from binding to DNA (Costa et al. 1994).

**Ni alters the activity of oxidoreductase enzymes**

Under Ni stress, activity of oxidoreductase enzymes—peroxidases (POD) and polyphenol oxidases (PPO)—increased significantly. PODs play a role in building up physical barriers to prevent the entry of toxic metals into the cell (Díaz et al. 2001). In support to present results, significant rise in POD and PPO was also reported in R. sativus (Latif 2010), T. aestivum (Gajewska et al. 2006; Pandolfini et al. 1992), Capsicum annum (Diaz et al. 2001), Hordeum vulgare (Simonovicova et al. 2004) and Brassica juncea (Alam et al. 2007) in response to Ni. Further, their activity increases in response to abiotic stresses and they protect plant cells from ROS as well as provide inducible plant defense (Batish et al. 2008). In the present study, induction of peroxidases activity was correlated with decrease in seedling length and it is assumed that involvement of POD in lignification process reduces cell wall plasticity and consequently leads to inhibition of growth which is in agreement with the results obtained by Diaz et al. (2001).

**Ni exposure causes lipid peroxidation**

Lipid peroxidation (LPO) is the most common indicator of oxidative stress resulting in the disturbance in the membrane integrity and consequently in its enhanced permeability. MDA (malondialdehyde) is a major thiobarbituric acid reactive substance (TBARS)
formed by peroxidation of unsaturated fatty acids releasing free radicals, and consequently damaging biological membranes as well as associated physiological activities (Dhindsa et al. 1981). The cyclic reactions initiated by lipid peroxidation lead to the formation of short-chain alkane and aldehydes completely destroying lipid structure, which further causes dimerization and polymerization of proteins, thus damaging the membranes (Logani and Davies 1980). In the present study, MDA content increased with increase in Ni concentrations and duration of exposure. In support to present results, stimulation of lipid peroxidation was observed in Z. mays roots only 6 h after Ni application (Baccouch et al. 2001). Earlier, accumulation of MDA content was reported in Triticum aestivum (Pandolfini et al. 1992) and Silene cucubalus (De Vos et al. 1992) due to Ni and Cu treatments, respectively. This fast rise of membrane lipid peroxidation might be due to direct contact of roots with the metal. Baccouch et al. (2001) suggested that lipid peroxidation could be one of the primary phytotoxic effects of Ni on membrane integrity resulting in growth inhibition.

Ni induced lipid peroxidation was further confirmed by enhanced conjugated dienes content, another byproduct of lipid peroxidation. In the present study, conjugated dienes content increased initially with increase in metal concentration but decreased after 24 h of Ni treatment. Reduced content of conjugated dienes was also observed in mung bean roots in response to arsenic (As) toxicity (Singh et al. 2007). According to Singh et al. (2007), As-induced membrane damage in mung bean roots was reported as an enhanced electrolyte leakage, which paralleled the results obtained in present study. Relative electrolyte leakage (REL) is an indicator of membrane damage and it occurs due to membrane peroxidation resulting from an oxidative burst (Bajji et al. 2002). Increased REL in the present study thus suggests Ni toxicity causes membrane damage in Z. mays roots.

**Ni exposure leads to accumulation of H$_2$O$_2$ content**

H$_2$O$_2$, one of the ROS, acts as signaling molecule and serves a dual role in plant defense mechanism. At lower concentrations it helps in stress acclimation, whereas at higher concentrations, it induces cellular damage leading to cell death (Stone and Yang 2006). H$_2$O$_2$ is also suggested to play an important role in the regulation of root growth and it acts as a substrate for peroxidases, hence participates in lignification, which consequences in the restriction of cell growth (Gaspar et al. 1991).

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Significant accumulation of H$_2$O$_2$ content in Z. mays roots in present study suggests the induction of oxidative stress. Ni, being a non-redox metal, cannot generate active oxygen species (AOS) directly by Fenton-type reaction. Previously, accumulation of H$_2$O$_2$ in response to Ni was reported in rice (Lin and Kao 2005), and wheat (Gajewska et al. 2006; Gajewska and Sklodowska 2007). Gajewska et al. (2006) opined that the accumulation of H$_2$O$_2$ content might be generated from increased diamine oxidase activity or from non-enzymatic disproportionation of superoxide ion. Dumas et al. (1995) also reported that oxalate oxidase, another source for H$_2$O$_2$ generation, degrades oxalate to CO$_2$ and H$_2$O$_2$ (Dumas et al. 1995). This was later supported by the findings of Delisle et al. (2001) in wheat roots under Al stress.

**Ni induces the generation of hydroxyl radical (‘OH)**

In our studies, increased level of ‘OH, in concentration- and time-dependent manner, was observed in Z. mays roots. Continuous generation of ROS, including ‘OH, was earlier reported in many plants under heavy metal stress (Foyer and Noctor 2005; Kaur et al. 2012). Earlier, Fridovich (1978) reported that interaction between O$_2$‘ and H$_2$O$_2$ results in the formation of ‘OH and O$_2$, which are more destructive end product and further consequences in greater peroxidation of the unsaturated lipids of cell membrane.

**Ni alters activities of antioxidant enzymes**

Plants have well evolved a well-defined machinery to scavenge the ROS generated during normal physiological processes. However, overproduction of ROS occurs in response to different stress conditions (Apel and Hirt 2004). The balance between generation and degradation of ROS is essential to maintain normal metabolic functions and to avoid oxidative injuries under stress conditions (Apel and Hirt 2004). According to Gratão et al. (2005), antioxidant machinery plays an important role in regulating cellular metabolism in response to heavy metal induced stress in plant tissue. In the present study, increased activity of superoxide dismutase (SOD) was observed initially (4–24 h) with increase in concentration as well as duration of exposure. However, with further increase in duration, a decline in SOD activity in a concentration-dependent manner was observed. Such a trend of changes in SOD under Ni stress paralleled earlier findings in maize leaves under Ni stress (Kumar et al. 2007). The stimulation of antioxidant system by heavy metal stress is well-known. SOD is key enzyme which plays first line of defense mechanism
against oxidative stress (Gratao et al. 2005). It catalyzes disproportionation of superoxide anion to hydrogen peroxide and oxygen (Foyer et al. 1997). SOD belongs to family of metallo-enzymes containing Cu-Zn, Mn, and Fe in their prosthetic groups, depending on the enzyme type. Maheshwari and Dubey (2009) suggested that among all, Cu-Zn SOD plays key role in scavenging O$_2^{**}$ and H$_2$O$_2$. Though we did not assess the activities of their individual isoforms by native PAGE, yet any change due to deficiency or excess of metal ions is likely to affect their specific activities (Gajewska et al. 2006). The significant increase in SOD activity observed in the present study clearly depicts efficient breakdown of O$_2^{**}$. Parallel to results obtained in present study, enhanced SOD activity was exhibited in pigeon pea (Rao and Sresty 2000), rice (Maheshwati and Dubey 2009), and maize (Mishra et al. 2010) in response to Ni. Reduction in SOD activity, with increase in duration, was also observed in present experiment, which is in agreement with earlier reports given by Bhoominathan and Doran (2002). Decline is SOD activity was accompanied by increase in O$_2^{**}$ (Gajewska and Sklodowska 2007). Since excess amount of Ni have been shown to decrease Fe content (Pandey and Sharma 2002), Cu and Zn content (Parida et al. 2003) in plant tissues, so, it is suggested that reduction in SOD activity in response to excess Ni could be consequence from deficiency of essential metals required for catalytic action of enzyme. According to Hodgson and Fridovich (1975), reduction in enzyme activity could be due to its inactivation by ROS.

Catalase (CAT), another antioxidant enzyme plays an important role in the removal of toxic peroxides in peroxisomes and mitochondria by dismutating H$_2$O$_2$ into O$_2$ and H$_2$O (Zhang et al. 2007). Decline in CAT activity in the present study in response to Ni, is in accordance with the findings for cabbage (Pandey and Sharma 2002), pigeon pea (Rao and Sresty 2000), and wheat (Gajewska and Sklodowska 2007). Contrary to this, induction of CAT activity has been reported in many plants exposed to Ni (Baccouch et al. 1998). According to Boominathan and Doran (2002), Ni did not affect CAT activity in roots of Alyssum bertolonii and Nicotiana tabacum. A decline in CAT activity noticed in the present study suggested that catalase enzyme is not able to cope up with increasing H$_2$O$_2$ levels in plants in response to Ni. Das et al. (1978) also suggested that decrease in CAT activity can be attributed to inhibition of the synthesis of enzyme and other oxidase proteins.
Besides CAT, peroxidases (APX and GPX) also participate in H$_2$O$_2$ scavenging mechanism. However, the substrate affinity of APX is higher than that of CAT (Siedlecka and Krupa 2002). Significant increase in APX and GPX activity in Ni-treated roots can be correlated with the level of H$_2$O$_2$ measured, as these enzymes consume H$_2$O$_2$ and reduce it to water (Nakano and Asada 1981; Singh et al. 2006). Earlier, increased activity of peroxidases in response to Ni was reported in Cajanus cajan (Rao and Sresty 2000), Silene italica (Gabbrielli et al. 1987), and Phaseolus vulgaris (Van Assche et al. 1986). APX is one of the most important enzymes, having various physiological roles in plant cells and also participates in biochemical reactions. Activation of this enzyme can cause alteration in the cell wall, and this in turn may decrease the growth rate (Batish et al. 2006).

GR, another enzyme, along with APX is also involved in quenching of H$_2$O$_2$ from plant cell, and has been well established in ascorbate-glutathione pathway (Aono et al. 1995; Polle 2001). GR converts oxidized glutathione (GSSG) to reduced glutathione (GSH), a compound able to scavenge ROS (Smirnoff 1996) and it is also known to maintain the sulphhydryl group of thylakoid protein in the reduced form and thus protect them from dehydration stress (Navari-Izzo et al. 1997). The participation of GR in the conversion of oxidized to reduced glutathione was observed by many workers (Foyer et al. 1994; Smith et al. 1989). In agreement to results obtained in present study, stimulation of GR activity was also reported in pigeon pea in response to Ni stress (Rao and Sresty 2000). Christie and Costa (1984) attributed that reduced glutathione forms very stable complexes with metal ions (Zn$^{2+}$, Ni$^{2+}$, Pb$^{2+}$ etc.), thus disturbs the inter-conversion of oxidized and reduced glutathione, which consequences in lowering of available antioxidants level in cells. Since all the antioxidant enzymes belong to various cellular compartments such as mitochondria, cytosol, plastids or peroxisomes, the changes in their activities are correlated with the differential sensitivity of different organelles to diverse type of stresses (Bailly et al. 2001).

Besides enzymatic, the non-enzymatic radical scavengers, namely ascorbates and glutathione, also play a crucial role in sequestration of free radicals (Gupta et al. 1999; Schutzenbudel and Polle 2001). In the present study, increase in non-enzymatic antioxidants such as ascorbates (total and reduced) as well as non-protein thiols was observed with increasing concentrations of Ni$^{2+}$. Ascorbates are known as key substance in the network of antioxidants and it plays various functions in plant growth, such as cell
division, cell wall expansion, and acts as a co-factor for many other enzymes (Smirnoff 1996; Pignocchi and Foyer 2003). Ascorbate decontaminates H$_2$O$_2$, which is resulted by the dismutation of O$_2$•• (Noctor and Foyer 1998). Increased level of thiol compounds in the present study represents another defensive mechanism against oxidative stress Yadav (2010).

The accumulation of all these enzymatic and non-enzymatic antioxidants in the present study could, therefore, be justified as a defense measure adapted against Ni-induced stress.

**Ni interferes with carbohydrate metabolism**

The increase in carbohydrate content in response to Ni is supported by earlier observations of Rabie et al. (1992), who reported an accumulation of carbohydrate content in corn seeds when soaked in Ni solution. Likewise, Agarwala et al. (1977) reported an accumulation of carbohydrate content in barley plant exposed to Ni. Increase in total carbohydrate content was also observed in *P. vulgaris* by 8.0, 5.7, and 5.6 fold upon exposure to Ni, Zn and Co, respectively (Samarakoon and Rauser 1979). Increased carbohydrate content indicated either failure of the plant to hydrolyze carbohydrates or *de-novo* synthesis of enhanced carbohydrates under Ni-stress. Jha and Dubey (2005) opined that most of the essential and non-essential metal ions when present in excess amount in soil cause a marked perturbation in the carbohydrates metabolism.

The observed decline in reducing sugars under Ni-treatment are paralleled by observations made in maize roots under Cr(VI) stress (Mahajan et al. 2013). In contrast to present result, anomalous accumulation of reducing sugars in unifoliate leaves of white beans upon exposure to higher concentration of Ni, Zn and Co (Samarakoon and Rauser 1979) and in maize leaves under Cr(VI) stress (Mahajan et al. 2013) has been reported. It is speculated that this may be due to disturbance in phloem translocation in the presence of heavy metal-stress, which leads to accumulation of reducing sugars in leaves (Samarakoon and Rauser 1979).

In the present study, reduced activity of starch hydrolyzing enzymes such as α-amylases, β-amylases and starch phosphorylase was noticed in *Z. mays* roots exposed to Ni. Similar observations have been reported in chick pea seedlings growing under water deficit stress (Kaur et al. 2000), rice seedlings growing under salinity stress (Dubey and
Singh 1999), as well as under As (Jha and Dubey 2004) and Cd treatments (Verma and Dubey 2001). Decrease in starch hydrolyzing enzymes, α- and β-amylases and starch phosphorylase with As-treatment was also observed in endosperms of rice seeds (Jha and Dubey 2005). According to Yang et al. (2001), α-amylase, β-amylase and starch phosphorylase are the major starch hydrolyzing enzymes present in plants, which hydrolyze the stored endospermic starch into metabolizable sugars, and provide energy for germination of seeds and growth of roots and shoots (Kaneko et al. 2002). Thus, it is speculated that decline in starch hydrolyzing enzyme activity in presence of Ni lessens starch degradation, which further leads to reduction in seed germination and inhibits radicle and plumule growth of the plant (Negi et al. 2014).

In agreement to results obtained in present work, declined activity of invertases has been reported in Ni-stressed seedlings of rice (Mishra and Dubey 2012) and mustard exposed to Pb (Singh et al. 2011). According to Roitsch and Gonzalez (2004), a direct correlation exists between invertases and hexoses. Weakening activity of acid invertases in the presence of Ni toxicity reduces hexose formation (Negi et al. 2014). Hexoses play an important role in maintaining osmotic pressure, cell wall extension, and cell elongation (Sturm 1999) and a reduction in hexose formation results in poor plant growth.

Acid phosphatases are the key enzymes involved in acquisition, transport and recycling of phosphorus (Yan et al. 2001). The reduced activity of phosphorolytic enzymes in Z. mays roots in response to Ni is supported by earlier reports in rice seedlings under Ni stress (Maheshwari and Dubey 2011), in roots of mustard in response to Pb-exposure (Singh et al. 2011) and in maize roots under Cr(VI)-toxicity (Mahajan et al. 2013). Contrary to our results, an increase in acid phosphatase activity was reported in Ni-tolerant plant of Alyssum species (Gabrielli et al. 1989). Inhibition in phosphohydrolytic enzymes resulted in a decline in the level of the phosphate pool, which consequently leads to poor plant growth and yield (Maheshwari and Dubey 2011).

**Ni interferes with Nitrogen metabolism**

Nitrogen is considered to be a vital nutrient for determining the growth and the productivity of plants. In the present study, a significant reduction in the activity of nitrate assimilating enzymes (Nitrate reductase, NR and Nitrite reductase, NiR) was observed with increased Ni concentration and duration of exposure. This is in agreement with the
past observations of Singh (2002) in response to Cu and Ni stress, Richharia et al. (1997) in response to salt stress, and El-Shora and Ali (2011) in response to Cd stress. Adverse environmental conditions lead to reduction in NO\textsubscript{3}\textsuperscript{−} uptake and its assimilation, which consequences in decreased level of NR in many plant species (Dubey and Pessarakli 1995). An inhibition of NO\textsubscript{3}\textsuperscript{−} uptake can result due to the presence of ~SH groups in the proteins of NO\textsubscript{3}\textsuperscript{−} uptake system, which are sensitive to heavy metals including Ni (Singh et al. 1989; Tan et al. 2000). According to Richharia et al. (1997), reduction in NR activity might be due to decreased synthesis of NR protein or due to the direct inhibitory effect of salt ions on NR activity. As NR is a substrate-inducible enzyme, its decreased activity has also been attributed to a reduced NO\textsubscript{3}\textsuperscript{−} uptake by the plants under metal stress (Lacuesta et al. 1990).

Parallel to observations made by El-Shora and Ali (2011), substantial reduction in ammonium assimilating enzymes (Glutamine synthetase, GS, and Glutamate synthase, GOGAT) was observed in the present study in response to Ni. A decrease in these enzyme activities was also observed under heavy metal stress in other species, such as maize (Boussama et al. 1999), pea (Chugh et al. 1992), bean (Gouia et al. 2000) and rice (Kumar and Dubey 1999; Chien et al. 2002), and reflects a general inhibition of primary nitrogen assimilation. Ireland and Lea (1999) suggested that assimilation of NH\textsubscript{4}\textsuperscript{+} in higher plants is mediated by GS/GOGAT cycle and converts NH\textsubscript{4}\textsuperscript{+} to glutamine and glutamate. A decline in GS/GOGAT activity observed in the present study suggested that ammonium assimilating enzyme are not able to cope up with increasing NH\textsubscript{4}\textsuperscript{+} levels in plants in response to Ni.

In the present study, Ni enhanced GDH activity in both roots and shoots of Z. mays seedlings was observed. Stimulation of GDH activity was also reported in response to Cd in marrow (Cucurbita pepo) seedlings (El-Shora and Ali 2011) and bean plant grown with N source (Boussama et al. 1999; Papazoglou et al. 2005). Chaffie et al. (2006) suggested that the stimulation of GDH activity under Cd-stress resulted in increase of GDH protein content and in induction of the transcription of GDH gene accompanied by an increase of mRNA content. Some studies support the involvement of GDH in the assimilation of ammonia induced in response to metal stress (Chaffie et al. 2003; Masclaux-Daubresse et al. 2006).
Significant accumulation of NH$_4^+$ content and total nitrogen content, in concentration- as well as duration-dependent manner, was observed in present study in Z. mays seedlings in response to Ni-toxicity. It is assumed that accumulation of NH$_4^+$ content might be due to decline in ammonium assimilating enzyme activity or it might be due to the presence of heavy dosage of N (Sanchez et al. 2004).

**Ni disrupts ultrastructure of root cells**

Ni exposure induces various irregularities in the plant roots. Studies through transmission electron microscopy and hand cut sections revealed some interesting anatomical changes in the roots of Z. mays grown under Ni treatment. Analysis of hand cut sections revealed that number of root hair decreased in Z. mays roots exposed to Ni in comparison to the control. Decline in root hair in present study in response to Ni, is in accordance with the findings for Z. mays exposed to metal stress (İ.Huillier et al. 1996). These results are also supported by Fernandez and Henriques (1991) who revealed that Cu-induced toxicity leads to reduction in the root hair. Root hairs play an important role of water conduction from soil into roots; ultimately it affects the conduction of water into xylem tissue cells. According to Askari et al. (2007), absence or presence of distorted root structure also inhibits nutrient uptake by plant.

Besides this, the epidermal tissue of the root treated with Ni seemed to be completely disintegrated or contracted. Increase in size of areenchyma and destruction of parenchymatous cells present in cortical region was also reported in the present study. This is in agreement with the observations of Sridhar et al. (2007), who reported breakdown of cortical cells in response to Cd toxicity. Moreover, irregular arrangement of epidermis and cortical cells observed in present study is parallel to the observations made by Ishikawa et al. (2003) in response to Al toxicity. Kovacevi et al. (1999) reported a decrease in parenchymatous cell area of stem, leaf midrib tissues, and pith and cortex of root under high Ni application. Ni stress decreased cell wall thickness in epidermal and hypodermal tissues of stem (Setia and Bala 1994). It is suggested that destruction of cortical cells might be responsible for the disrupted diffusion of materials into the central cylinder of root and interrupted storage of food in the form of starch. Previously, Molas (2002) reported a series of alterations in chloroplast in Ni-treated cabbage. These include: alterations in shape of chloroplast, reduction in size and number of grana, swelling of thylakoids and increase in number of plastoglobuli.
In addition to above results, abnormalities in cell membrane, nucleus and mitochondria were also observed in Ni-treated Z. mays roots. Cell wall act as an excretory organ for heavy metals and it prevents their movement in protoplasm. One of the most noticeable changes in Ni-exposed Z. mays roots was the thinning of cell wall and formation of intercellular spaces. Ni-induced toxicity resulting in formation of folds was also reported in present study, which is in agreement with the findings of Kurkova et al. (2002) in response to salt stress, and of Kaur et al. (2012) in response to Pb. It is suggested that such changes in cell wall consequences in enhanced permeability, which further results in improved transport of ions and water (Kurkova et al. 2002). This may also affect the regulation and function of the membrane bound enzymes that alter the synthesis of many cell wall polysaccharide components (i.e. glutan, chitin and mannan), cell growth and morphogenesis (Sanchez et al. 2004).

The observed increase in the number of mitochondria under Ni stress is corroborated by earlier findings made in response to Al (Konarska 2008), Cd (Gzyl et al. 2009), Pb (Kaur et al. 2012) and abiotic stresses (Stoynova et al. 1997). It is assumed that increased mitochondria possibly enhanced ATP generation, which is required by plant to cope up with Ni-induced stress. Earlier, degradation of mitochondria due to mineral deficiency in cytoplasm was also reported by Vázquez et al. (1992) and Koyro (1997).