

Review

Morphological, physiological and biochemical responses of plants to nickel stress: A review

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Nickel (Ni), is the 22nd abundant element in the earth crust, being an essential mineral nutrient found in natural soils in trace concentrations. The elevated levels of Ni pollution in the environment are due to industrial and agricultural activities. It is vitally important to understand both, the functional characteristics and toxic effects of Ni in plants. The quantity of Ni required for normal growth and development of plants is very low. However, Ni has been identified as a component of various enzymes in plants and has decisive metabolism for certain enzyme activities, like maintaining proper cellular redox state and various other biochemical, physiological and growth responses. The higher concentration of Ni is associated with serpentine soils, manifestation in plant chlorosis and inhibits root and shoot growth. Excess of Ni inhibits a large number of enzymes and interferes with several aspects of plant biochemistry, including photosynthesis, pigment synthesis, and membrane integrity. This article is based on the overview of available data of past two decades that in core, it encompasses the ill morphological, physiological and biochemical effects of Ni stress on plants.

Key words: Nickel stress, growth, yield, antioxidative system, gas exchange, photosynthetic pigments.

INTRODUCTION

Heavy metals are the elements with specific gravity more than 5.0 and atomic weight ranging from 63 to 200 (NCSU Water Quality, 2006). The plant can use metals in mineral form only as there is no direct metal availability to plants (Appenroth, 2009). The micronutrients are indispensable for biogenesis, proper functioning of nucleic acid, chlorophyll, and hydro carbonates as well as for stress resistance. Some heavy metals play an important role in plants cells as micronutrients (Rengel, 2004), while others have stimulating and provoking

effects on plants even in trace concentrations (Kovacs et al., 2009; Nyitrai et al., 2007). There are two important facts about the heavy metals. Firstly, heavy metals are generally non-toxic but when their quantity exceeds the limit they become toxic. Secondly, some of the heavy metals are essential for development and growth of plants like cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn) (Rengel, 2004).

On the basis of plant growth parameters, the

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phytotoxicity of heavy metals are $\text{As}^{5+} < \text{As}^{3+} < \text{Cr}^{6+} < \text{Co}^{2+} < \text{Zn}^{2+} < \text{Ni}^{2+} < \text{Cu}^{2+} < \text{Ti}^{+} < \text{Hg}^{2+} < \text{Cd}^{2+} < \text{Ag}^{+}$ (Assche and Clijsters, 1990). Ni was discovered by a Swedish chemist Ronstadt in 1751. In the earth crust, Ni hold 22nd position among abundant elements and have igneous origin (Sunderman and Oskarsson, 1991). The natural concentration of Ni in soil and surface waters is lower than 100 and 0.005 ppm, respectively (McGrath, 1995). The most common form of Ni present in soil solution is the hydrated form as $\text{Ni}(\text{H}_2\text{O})_2^{6+}$ (Yusuf et al., 2011).

Ni received very little attention due to its dual character and complicated electronic chemistry which act as barrier to reveal the toxicity mechanism in plants (Yusuf et al., 2011). Ni is considered as a major pollutant all over the world due to the elevated quantities of Ni in soil (Echevarria et al., 1998; Faryal et al., 2007; Atiq-ur-Rehman and Iqbal, 2008). Ni has both natural and manmade sources of emission. The natural source include weathering of rocks, while many compounds of Ni like oxides, hydroxides and acetate released into the environment as a result of devastating human activities (Cempel and Nikel, 2006). Ni concentrations in soil are the result of smelting, burning of fossil fuels, industrial and municipal waste, vehicular emissions, mining of metals and application of Ni fertilizers (Allo-way, 1995; Salt et al., 2000) but the metallurgical and electroplating industries, chemicals used in food industry and electric batteries are major sources of Ni released into the environment (Easton, 1992).

The soil is ultimate sink of Ni compounds where they are deposited and taken up by plants which cause very perilous effects on animals and humans as it moves throughout the food webs (Nieboer and Nriagu, 1992; Cempel and Nikel, 2006).

In 1975, it was discovered that Ni is a part of plant urea (Dixon et al., 1975). Many researchers reported that the plant growth significantly decreased when nitrogen was provided to plants in the form of urea under the deficiency of Ni (Eskew et al., 1983, 1984; Walker et al., 1985; Gerendas and Sattelmacher, 1997, 1999). Brown et al. (1987) stated that the life cycle of barley (*Hordeum vulgare* L. cv. 'Onda') is incomplete without Ni even if the nitrogen was supplied to plants via non-urea source (Brown et al., 1987). In addition, it was exposed that the growth of oats (*Avena sativa* L. cv. 'Astro') and wheat (*Triticum aestivum* L. cv. 'Era') was decreased under Ni deficiency (Brown et al., 1987). Ni played an important role as key enzyme in nitrogen-fixing symbiotic relationship (Maroney et al., 1999).

In 1945, the responses of field crops to Ni fertilizers was observed for potato (*Solanum tuberosum* L.), wheat (*T. aestivum* L.), and bean (*Phaseolus vulgaris* L.) (Roach and Barclay, 1946). The plants showed transparent responses to Ni when nitrogen is provided as urea or by nitrogen fixation (Eskew and Welch, 1982.), Ni plays a significant role in transportation of nitrogen to

seeds (Brown et al., 1987). However, it is evident that the high concentration of Ni induced phytotoxicity at multiple levels. The toxicity of Ni becomes serious problem for the whole world. In sensitive species, the captious level of Ni is higher than $10 \text{ mg kg}^{-1} \text{ DM}$ (Kozlow, 2005), while in normal tolerant species the level of Ni is found to be $>50 \text{ mg kg}^{-1} \text{ DM}$ (Bollard, 1983; Asher, 1991) and in hyperaccumulator species, the level is $1000 \text{ mg kg}^{-1} \text{ DM}$ (Kupper et al., 2001; Pollard et al., 2002). Many factors influenced the toxicity of Ni in plants including concentration of Ni in soil, resident time in soil, cultivation conditions, growth stages and type of plant species (Krupa et al., 1993; Xylander and Braune, 1994; Marschner, 1995; Kabata-Pendias and Pendias, 2001; Assuncao et al., 2003).

MORPHOLOGICAL RESPONSES

Plants show variety of responses to environmental stresses. The growth and yield of plants is affected directly or indirectly in response to internal and external environmental stresses.

Growth

The plant growth is a very essential process to maintain the life on earth. There are many factors that influence the internal and external growth of plants such as mineral resources present in soil, air and genotype of plant species. The access amount of Ni in ecosystem severely affected the growth and development of plants (Yusuf et al., 2010, 2011).

The toxic effect of Ni in plants includes decreased shoot and root growth and reduction in leaf area (Shaw et al., 2004). The growth of *Zea mays* seedling was decreased with the elevated concentration of Ni (Bhardwaj et al., 2007). The germination of pigeon pea was decreased by 20% in a 1.5 mM solution of Ni and the germination percentage was decreased in proportion to the concentration of Ni (Rao and Sresty, 2000). The mass of wheat shoot was decreased by 20 and 26% with Ni application of 100 and 200 μM , respectively (Gajewska and Sklodowska, 2008). The high concentration of Ni is associated with stunted growth of soybean seedlings. This was caused by hyperaccumulation of Ni in soybean leaf (Prasad et al., 2005). Similarly, it was observed in mungbeen spermatophyte exposed to $\text{Ni}_{0.10}$ and $\text{Ni}_{1.00}$ (Gopal et al., 2002). The root growth of onion was inhibited under the 100 mM Ni concentrations (Liu et al., 1994).

In a histochemical study, it was observed that nuclei of onion root expended under the 1 to 10 mM Ni stress (Liu et al., 1994) and the shape of nuclei distorted under the $>10 \text{ mM}$ Ni stress (Liu et al., 1994). The root biomass of barley seedling severely affected under the 200 mM Ni

stress and the barley seedling not survived when they are treated with 400 mM Ni concentration (Brune and Dietz, 1995). Ni alone or with other toxic metals caused reduction in the dry matter production in root system of maize (Brune and Dietz, 1995). The Ni stress >50 mM reduced the dry and fresh mass production of roots and shoots in soybean seedling (El-Shintinawy and El-Ansary, 2000). The decreased fresh weight of wheat's root was observed under 100 μM Ni stress (Gajewska et al., 2009). Burd et al. (1998) discovered that the seeds of canola germinated normally in the presence of up to 1 mmol L^{-1} Ni chloride, but the higher concentration of Ni initiated the plant's roots and shoots elongation. The excessive concentration (0.5 mg^{-1}) of Ni in nutrient solution inhibited the growth of *L. gibba* (Khellaf and Zerdaoui, 2010). Copper and Ni decreased the compound leaves (Khellaf and Zerdaoui, 2010; Mishra and Dubey, 2011).

It is stated that the toxic concentration of Ni and aluminium (Al) collectively reduced the enzymes [nitrate reductase (NR) and glutamine synthetase (GS)] activity in rice seedling and the enzyme GS play very essential role in the assimilation of NH_4^+ in plants. The root system of sun flower had been founded less developed as compared to controlled plants under the Ni stress (Ahmad et al., 2010). The growth of plant stem significantly decreased due the Ni toxicity. Ni toxicity created disorders in metabolic system of plants and quickly inhibited the cell division (Yusuf et al., 2011). It was reported that excess amount of Ni caused chlorosis of plants and leaf necrosis (Assche and Clijsters, 1990; McIlveen and Negusanti, 1994; Marschner, 1995; Seregin and Kozhevnikova, 2006; Kovacevic et al., 1999).

Kovacevic et al. (1999) reported that the Ni stress (100 μM NiSO_4) reduced the size of vascular bundle, width of epidermal cells and mesophyll thickness in *T. aestivum*. The shoot length of wheat seedling decreased by 44% under the Ni stress (Gajewska et al., 2006). It was revealed that the Ni concentration (100 μM) caused chlorosis and necrosis in plants of *T. aestivum* and also reduced the shoot growth (Gajewska and Sklodowska, 2007). It was demonstrated by Guo et al. (2010) that the toxicity of Ni cause stunted growth, reduction in dry weight of grains and straws, chlorosis in maize.

Yield

The toxicity of heavy metals is directly associated with crop yield. The high concentration of Ni has devastating effects on plants which ultimately caused reduction in crop yield (Balaguer et al., 1998). The fresh weight of shoots of sun flower constantly decreased with increasing concentration of Ni from 10 to 40 mgL^{-1} in root medium (accepted manuscript results). All yield attributed to sunflower significantly decreased under the Ni stress.

50% reduction in all yield parameters was observed

under the Ni stress (40 mgL^{-1}) as compared to control. Reduction in the yield of different crops like; cucumber (Gonçalves et al., 2007), tomato (Balaguer et al., 1998), and mungbean (Ahmad et al., 2007) was observed in previous studies. The reduced yield of sunflower is mostly associated with Ni's quantity that accumulates in plant's leaf (Ahmad et al., 2010). The total dry matter deposited in upper and lower part of plants and total biomass reduction was attributed to Ni stress (Rao and Sresty, 2000; Pandey and Sharma, 2002). The yield of *Vigna radiata* clearly reduced at the concentration of 50 mg Ni kg^{-1} soil (Sinha et al., 2011).

Chlorophyll contents

Ni was positively associated with proteins inhibition germination and chlorophyll production (Zhou et al., 2009). The high concentration of Ni significantly decreased the chlorophyll content, stomatal conductance and a potential inhibitor of photosynthesis (Meharg, 1993; Lin and Kao, 2007; Maksimović et al., 2007). The number of leaves and chlorophyll contents decreased with 24 and 47%, respectively under the Ni concentration of 0.025 mM (Wheeler et al., 2001). In the fresh leaves of maize, the concentration of chlorophyll content decreased with increased concentration of Ni from 20 to 100 μM . it was observed that chlorophyll-a decreased with 70% and chlorophyll-b decreased 50% under the Ni stress of 100 μM in maize as compared to control plants. But there was no significant effect on 250 and 500 μM Ni concentration on the chlorophyll content in maize (Baccouch et al., 1998). Accumulation of Ni in lower and upper parts of mungbean's plants significantly decreased the chlorophyll content in the upper parts of plant (Ahmad et al., 2010). The Ni stress in black gram (*Vigna mungo*) created a significant reduction in photosynthetic pigments (Dubey and Pandey, 2011).

Photosynthesis

In the past two decades, number of studies reported that the Ni toxicity is correlated with reduction or inhibition of photosynthesis in plants (Assche and Clijsters, 1985; Seregin and Kozhevnikova, 2006; Ahmed and Häder, 2010). The Ni stress in sunflower reduced the stomatal conductance (g_s) and photosynthetic activity (Bazzaz et al., 1974). Later, the study of Bazzaz et al. (1974) was confirmed by Ouzounidou et al. (2006) in a study on wheat. The Ni stress of 200 μM to Poplar (*Populus nigra*) plants significantly decreased the stomatal conductance (g_s) especially in emerging leaves where the g_s reduced from 0.40 to 0.03 $\text{mol m}^{-2}\text{s}^{-1}$. This decline in g_s resulted in direct decrease in photosynthesis (Velikova et al., 2010). Ni caused destruction of photosynthetic organs including the epidermal tissues and mesophyll cells (Bethkey and

Drew, 1992). Rauser and Dumbroff (1981) stated that the Ni toxicity (200 mM Ni for 24 h) increased the stomatal resistance in *P. vulgaris*. In a study on *Brassica juncea* by Alam et al. (2007), the Ni stress (100 μ M) decreased net photosynthetic rate and chlorophyll content. Photosynthetic rate in five test cultivars of *T. aestivum* significantly decreased under the Ni stress (Yusuf et al., 2010). The Ni has toxic impact on both entire plant and on the chloroplast (Tripathy et al., 1981; Singh et al., 1989; Molas, 2002; Boisvert et al., 2007).

Water relation

It was reported that heavy metals can cause severe dehydration in plants by restricting the movement of water from roots to upper parts of plants (Haag-Kerwer et al., 1999; Chen et al., 2004). Generally, heavy metals can alter the water relation in plants (Barcelo and Poschenrieder, 1990; Prasad, 1997). The toxic effects of heavy metals were observed on multiple levels like stomatal functioning, movement of water through apoplast and symplast and water uptake etc. (Barcelo and Poschenrieder, 2004). Water stability in plants depends on the balance between transpiration and water uptake. Many researchers stated that the Ni toxicity decreased the water content and transpiration rate in plants (Sheoran et al., 1990b; Schickler and Caspi, 1999; Bishnoi et al., 1993; Molas, 1997). When 4-day old plants of *T. aestivum* in sand culture treated with 10 mM Ni added in nutrient solution transpiration rate, stomatal conductance, leaf water potential, and total moisture content were decreased (Bishnoi et al., 1993).

The toxicity of Ni^{2+} reduced the area of transpiring surface (leaf blades) of plants (Chen et al., 2009). The 40% reduction in leaf area of *Cajanus cajan* plant was observed under the Ni stress of 1 mM in nutrient solution (Sheoran et al., 1990b). Similarly, this type of reduction was also observed by Molas (1997) in *Brassica oleracea* grown in agar medium under $5.20 \text{ gm}^{-3} \text{ NiSO}_4 \cdot 7\text{H}_2\text{O}$. The primary toxic impact of heavy metals is the reduced transpiration rate and shutting of stomatal aperture (Molas, 1997; Seregin and Ivanov, 2001). The elevation of abscisic acid (ABA) levels that was responsible for stomata closing, in the leaf tissues of *P. vulgaris* was observed under the Ni stress (Molas, 1997).

Reactive oxygen species (ROS)

ROS continually produced as off-spins of different metabolic reactions that take place in different cellular parts of plants like mitochondria and chloroplast (DelRio et al., 2006; Navrot et al., 2007). In plants, the mitochondria (energy factories) are the major responsible site for the production of ROS (Rasmusson et al., 2004). Many abiotic and biotic stresses disturb the equilibrium

between the cleaning and production of ROS like heavy metals, salinity, droughts, ultraviolet (UV)-radiation, air pollution, extremes of temperature, pathogens and herbicides (Gill et al., 2010). The ROS are comparatively more reactive than O_2 and thus they have severe toxic impacts on living system. The toxicity of ROS can destroy the DNA structure; it can also stimulate the oxidation of lipids and proteins and degradation of chlorophyll pigments (Schutzendubel and Polle, 2002).

Heavy metals as well as Ni have capacity to create the OH by Haber/Fenton-Weiss reaction (Kehrer, 2000) but due to high reduction/oxidation capacity, Ni was not observed as a catalyst in this reaction (Leonard et al., 2004). It is known that the excessive amount of transition metals increased the production of ROS in plants. In normal condition, the ROS expeditiously cleaned by antioxidant system (Dubey and Pandey, 2011). Hydroxyl radical (OH^\cdot), superoxide radical ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^\cdot) are the main mediators for the peroxidative damage (Dubey and Pandey, 2011). The cytotoxic protein damage and DNA disruption in plant tissues may be due to Ni (Gajewska et al., 2006; Gajewska and Skłodowska, 2007). Rao and Sresty (2000) stated that the Ni toxicity increased the production of ROS and causing the peroxidative damage in membrane lipids. It was well documented that over accumulation of lipids peroxidation resulted in toxicity of heavy metals and oxidative damage (Pandolfini et al., 1992; Luna et al., 1994; Chaoui et al., 1997; Mazhoudi et al., 1997). The H_2O_2 quantity significantly increased in leaf of wheat under the Ni stress (Gajewska and Skłodowska, 2007). In the roots of wheat, the ROS content increased under the Ni stress (Hao et al., 2006) and same type of result was observed by Boominathan and Doran (2002) in the hairy roots of *Nicotiana tabacum* and *Alyssum bertolonii*.

Antioxidant enzymes

Many evidences indicated that the toxicity of Ni is associated with oxidative stress in plants (Rao and Sresty, 2000; Gajewska et al., 2006; Boominathan and Doran, 2002; Gonnelli et al., 2001). The H_2O_2 content in plant tissues is cleaned and controlled by different enzymatic and non-enzymatic antioxidants. Ni stress caused significant decline of superoxide dismutase (SOD) in wheat and ascorbate peroxidase (APX) activities increased in the leaves under the Ni stress. APX may play significant role in the cleaning of H_2O_2 from the leaves of Ni-stressed plants in wheat because the highest value of APX coincides with decline in H_2O_2 content (Gajewska and Skłodowska, 2007).

The ROS may not be generated by Ni because it is not a redox-active metal; however, it can cause interference with the number of antioxidant enzymes (Pandey et al., 2002; Hao et al., 2006; Pandolfini et al., 1992; Baccouch

et al., 1998; Baccouch et al., 2001; Gajewska et al., 2005). Ni in very low concentration (0.05 mM) increased the activities of peroxidase (POD), SOD, and guaiacol peroxidase (GOPX) (Freeman et al., 2004, 2001; Gajewska et al., 2005; Schickler et al., 1999; Gomes-Juniora et al., 2006) but the high concentration of Ni reduced the activities of many cellular antioxidant enzymes both *in vitro* and *vivo* plants so for the capability of plants to remove the ROS and finally lead to oxidative stress (Freeman et al., 2004, 2001; Gajewska et al., 2005; Schickler et al., 1999; Gomes-Juniora et al., 2006). The 2 weeks old plants of pea under the Ni stress (10, 100, and 200 μ M for 1, 3, 6 and 9 days) significantly reduced the activities of SOD in both roots and leaves and activity of APX in roots however, the activities of catalase (CAT) remain unaltered (Gajewska et al., 2005). The activities of POD, SOD and glutathione reductase (GR) were increased, while the activity of CAT reduced in the seedling (6 days) of pigeonpea (*C. cajan* L. Millspaugh) under the Ni stress (0.5 mM) (Rao, and Sresty, 2000). It was demonstrated that the activities of POD and CAT significantly decreased under the Ni stress (0.5 mM for 8 days) (Pandey and Sharma, 2002). The same trend was observed for other enzymes like POD, SOD and CAT in leaves of *Hydrocharis dubia* in response to Ni stress (0.5, 1, 2, 3, and 4 mM Ni treatments of 3 days) (Papadopoulos et al., 2007). Lipid peroxidation product malondialdehyde (MDA) content in roots and shoots was increased in the plant of pigeonpea under the Ni stress (0.5 to 1.5 mM) (Rao, and Sresty, 2000). Similar results were observed in wheat, *Alyssum* species and corn (Boominathan et al., 2002; Baccouch et al., 2001; Schickler et al., 1999; Dietz et al., 1999). Ni caused the depletion of low molecular weight proteins like glutathione (GSH); this may caused oxidative stress in plants (Rao and Sresty, 2000; Kukkola et al., 2000).

CONCLUSION

Although Ni is an essential metal and plays an important role in plant metabolism, but its toxicity become a particular apprehension, due to growing industrial use. Many common responses appear in plants under Ni stress conditions. These responses include growth inhibition of plants, wilting and leaf chlorosis, and reduction of total plant yields. Ni toxicity also cause disruption of photosynthetic and enzyme activities. However, the mechanisms operating at both protein and molecular levels results in toxicity symptoms remain largely unknown and require further study. Growing concerns about Ni pollution in the environment have led to research on phytoremediation, that is, the use of hyper accumulator or wetland plants to remove and/or sequester Ni from soil and water. However, many such plants have limited utility for phytoremediation, because of their slow growth, difficult propagation, seasonal growth, and low biomass. These problems require viable

solutions and further research.

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