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Physiological Effects Of Nickel Contamination On Plant Growth

[1*]Sammia Ghazanfar , [2]Ayesha Komal , [3]Aqsa Waseem , [4]Waseem Hassan , [5]Rana Javaid Iqbal , [6]Saiqah Toor , [7]Muhammad Asif , [8]Irfan Ahmad Saleem , [9]Saftain Ullah Khan , [8]Zahid Hassan Tarar , [10]Saima Nazar , [11]Hafeez-u-Rehman , [12*]Muhammad Ibrar Ahmed , [13*]Ansa Rebi

[1*]Department of Soil Science, The Islamia University Bhawalpur-63100, Pakistan.

[2]Department of Bioengineering, Cyprus International University-99010, Cyprus.

[3]Department of Environmental Science, Cyprus International University-99010, Cyprus.

[4]Pesticide Residue Lab Kala Shah Kaku-39350, Sheikhupura, Punjab, Pakistan.

[5]Soil Fertility Research Institute, Lahore-54000, Punjab, Pakistan.

[6]Soil and water Testing Lab, Gujrat-50700, Punjab, Pakistan.

[7]Soil and Water Testing Laboratory Bahawalnagar-62300, Punjab, Pakistan.

[8]Soil and Water Testing Laboratory, Mandi Bahauddin-50400, Punjab, Pakistan.

[9]Soil and water Testing Laboratory Mianwali-42201, Punjab, Pakistan.

[10]Soil Fertility Section, Gujranwala-52230, Punjab, Pakistan.

[11]Soil and Water Testing Laboratory, Sialkot-51480, Punjab, Pakistan.

[12]Soil and Water Testing Laboratory, Sargodha 40100, Punjab, Pakistan.

[13]Jianshui Research Station, School of Soil and water conservation, Beijing Forestry University, Beijing 100083, China.

Abstract

The mobility of nickel in the environment, soil and water is of considerable concern. In this review we want to determine characteristics of nickel and its significance effect on growth, photosynthesis, mineral nutrition and enzyme activity of plants. Nickel effects the plant physiological processes such as photosynthesis, water relation and mineral nutrition. Toxic effects of nickel on plant includes alteration in germination process, total dry matter production and yield which cause detrimental effects on plant. plant metabolic reactions are also strongly influenced by nickel because it has the ability to generate reactive oxygen species that may cause oxidative stress. Small amount of nickel is desirable for optimum plant growth and development however unwarranted amount of nickel cause detrimental effect on plant growth. In non-tolerant plants elevated concentration of Ni can inhibit cell division at root meristem and cause decrease in plant growth. Studies have revealed that Ni has a cynical effect on photosynthesis and respiration. High uptake of Ni causes a decline in water content of dicot and monocot plant species which act as an indicator for Ni toxicity in plants. Due to its complicated electronic chemistry Nickel has received very little attention which act as barrier to reveal the toxicity mechanism in plants.

1.Introduction:

Nickel (Ni) is the 24th profuse element in the earth crust. Among the heavy metals it holds a particular place (Esfandiari et al.2022). Ni is an essential micronutrient for plant growth. Due to progress of industrialization nickel and nickel compounds have many industrial and commercial uses and increase the emission of pollutant in environment (Begum et al.2022). Nickel is widely distributed in nature and is found in plants, animals and soil. The Ni contents to soil vary in a wide range from 3 to 1000 mg Kg^{-1} (Acquah et al.2022). Large amount of nickel is released in atmosphere due to natural as well as anthropogenic activities including energy consumption, industrial processes (mining, smelting and refining), use and dumping of Ni compounds, alloys and waste incineration (Kamura et al.2022). Nickel is a metal that contaminating soils in the vicinity of industrial sites (Priyadarshanee et al.202). It including smelters and mining sites where concentrations can be as high as 40–4600 μg [g soil]$^{-1}$ (Ji et al.2022). Nickel has venomous effects on human, plants, soil micro flora and fauna. it is regarded as belonging to the far-reaching group of heavy metals (Tasin et al.2022). In addition, Ni2+ and other metal cations can exchange nutrient cations (Ca2+, Mg2+ and Mn2+) from the cation exchange places of soils and causes a decrease in amount of existing nutrients (Goytain et al.2005). Still a few tools are established to assess the environmental consequence of Ni contamination in the soil. Since the Ni concentration in soil is not directly associated with its potential biological effects. Although Nickel (Ni+2) in general is known as an essential micronutrient because it is a component of some plant enzymes (Lall,2022). But its excessive concentration is highly phytotoxic for plant species. Physiological processes such as growth inhibition, changes in chloroplast and chlorophyll concentrations, especially photosynthesis have been related to Ni+2 toxicity (Riyazuddin et al.2021).

In soil solution nickel is frequently absorbed in ionic form (Ni++) by the plants and is not as strongly absorbed when chelated 5, 6. Turina explored that the absorption of nickel by roots is through the root caps in some monocots like rye (Secale cereale L.) and corn (Zea mays L.). Absorption of nickel is mostly favored below Soil pH 5.6 while values above 5.6 do not. With Increase in soil acidity the exchangeable nickel content of the soil increases.

Ni is considered as an essential element for growth of plants and its deficiency has been observed in some perennial species (Wang, et al.2022). Ni deficient soils showed encouraging results of field crops with application of Ni, but on other hand soils amended with industrial and municipal wastes results addition of excessive amount of Ni, which has toxic effect on plants and soil microorganisms due to its mobility and availability in soil (Turan et al.2022). Although essentiality of Ni is well established, however its higher concentration becomes toxic in most plant species. Alterations in plant metabolisms, inhibition of growth and other common symptoms of heavy metal toxicity like chlorosis, wilting and necrosis are induced by Ni (Giannakoula et al.2021). In plants Ni inhibits photosynthesis and decrease root growth by inhibiting cellular division in the root apex (Gao et al.2022). However, an excess of Ni is more commonly found in plants as compared to the deficiency. The excess of Ni is associated with many adverse effects in plants like reduction in germination. plant growth cell division, biomass production nutrient absorption photosynthesis transpiration leaf chlorosis, and necrosis (Shanker et al.2005) Therefore, all the toxic effects due to Ni stress reduce crop yield and quality. In view of the above-cited evidence, Ni enters the environment through various sources, although is beneficial at low concentration and becomes toxic at high concentration with alteration in plant processes and final yield. In this review, we discuss the importance of Ni for plants and its entrance into the environment, its toxic effects, and possible remediation strategies in order to mitigate Ni toxicity.

2. Sources of Nickel:

Ni is a permeating trace metal released into the environment from both natural and anthropogenic sources which are found within biosphere in soil, water and air samples (Rajendran et al. 2022). In industrial processes various nickel compounds are used such as nickel acetate, nickel carbonate, nickel hydroxide and nickel oxides (Narender et al. 2022). Ultimately fate of these compounds is they accumulate in soil and environment and taken up by plants, they can enter the food chain and cause venomous effects to animals and human (Ashar et al.2022). In ambient air the level of Ni is generally small (about 6–20 ng m-3) while in air contaminated by anthropogenic sources levels up to 150 ng Ni m-3 could be present (Huremović, et al.2020). Ni derives from biological cycles and solubilization of nickel compounds from soils, as well as from the sedimentation of Ni from the atmosphere in water. Water which is uncontaminated usually contains about 300 ng dm-3 Ni while the Farm soils contain approximately 3–1,000 mg kg-1 Ni (Langasco et al.2022). In soil near metal refines Ni concentration can reach up to 24,000 mg kg-1 Ni. Ni compounds exist in soil in various soluble or insoluble hydroxide form which are relatively soluble at pH\6.5 whereas at pH -6.7 mostly Ni exists in form of insoluble hydroxides. Wind-blown dust, volcanic emissions, forest fire and vegetation are natural sources of atmospheric nickel derived from weathering of soil and rocks. Ni find its way as a result of the combustion of coal, diesel oil and fuel oil in ambient air.

2.1 Air :

Concentration of Ni in ambient air vary significantly. From industrialized areas highest values of Ni have been reported. In remote areas average levels of airborne nickel is 0.00001-0.003 µg/m3 while in urban areas 0.0030.03 µg/m3 having no metallurgical industry; 0.07-0.77 µg/m3 in nickel processing areas. In atmospheric air the recommended nickel concentration is set as 0.025 µg/m3 (Choi et al.2022). Ni forms to which workers are exposed differs in the various industries in which nickel is used. It occurs usually in areas where there are poor industrial hygiene practices through inhalation or dermal contact with ingestion (Afata et al.2022). It occurs mostly due to t the inhalation of insoluble nickel compounds, aerosols derived from nickel solutions (soluble nickel) and gaseous forms containing nickel (usually nickel carbonyl). Several studies conducted revealed that Ni industrial concentrations may vary in a wide range from micrograms to milligrams of nickel per m3 of air. In industries where nickel is produced graft potency of 0.2% may be exposed to ample amounts of airborne nickel that clue to the maintenance of 100 µg of nickel per day.

2.2 Water:

Water is also a source of nickel but very low concentration of nickel is present in drinking water less than 10 µg/l (Paulelli et al.2022). Presumptuous if one intake 1.5 l of water daily and a level of 5-10 µgNi/l than mean daily intake of nickel from water for adults would be between 7.5 and 15.0 µg which is very toxic. In USA Several research conducted and publicized that 97% of the drinking water samples tested had nickel concentrations below 20 µg/l and 80% of the samples had less than 10 µg/l. while highest values up to 75 µg/l or 200 µg/l Ni were documented only in the Ni ore mining areas. Ni concentration in cold and hot areas mostly depends on the quality of pipes. In case of metal pipes level of Ni in hot water is lower than in cold water However, concentration are opposite when PVC pipes are properly used (Hasan et al.2022). Acidic beverages soft drinking water may dissolve nickel from pipes and containers (Klein et al.2022). plant water regime stability depends on the balance between water uptake and transpiration. However, decline in plant respiration and water content due to nickel toxicity has been reported by several authors. 10mMNi added to the nutrient solution by following four days of growth of Triticum aestivum plants in the sand culture. In areas where metal accumulation was more pronounced uppermost leaf water potential, stomatal conductance, the transpiration rate, and total moisture content will decrease. As a concern of several metal-induced changes transpiration rate may drop that are also produced by other heavy metals and area of leaf blade first will decrease due to toxic effects of Ni^{2+} on plant growth. Such leaf area decreases by 40% was observed in Cajanus Cajan plants grown in sand culture when 1 mM $NiCl_2$ was added to the nutrient solution. Second because of lower stomatal number per unit of leaf area transpiration may decrease. Due to reduction of leaf area and the size of epidermal cell in some cases, stomata density may even increase. The decline in moisture content and stomatal conductance prompted by Ni is also one of the important mechanisms of its toxicity towards photosynthesis. Nickel is easily accumulated in aquatic plants, which are sensitive bio indicators of water pollution. Ni can be deposit in the sediment by processes such as precipitation, complexation and adsorption on clay particles. Fresh water catchment level of precipitation of Ni of 0.9 mg/m2/year over long periods were found to be dangerous for biological system. Ni is transported mainly as a precipitated coating on particles in rivers. Ni is generally transported via rivers into the Baltic Sea in poland in this way the average value of anthropogenic Ni input is 57% while in sea water nickel is present at concentrations of 0.1- 0.5 µg/l (Achary et al.2016).

2.3 Soil:

Nickel is generally distributed uniformly through the soil profile it accumulates from deposition by industrial and agricultural activities at the surface. In industrial or agriculture land receiving wastes such as sewage sludge nickel may serve as a major problem. Ni content in soil varies in range from 3 to 1000 mg/kg (Riaz et al.2020). In soil solution Ni can exist in several forms: inorganic crystalline minerals or precipitate, water soluble, free-ion or chelated metal complexes (Campel et al.2006). In urban areas this metal become a major problem due to decrease in soil PH caused by reduced use of soil liming in agriculture and mobilization as a distress of increased acid rain but actually it does not appear to be a major disquiet in outside urban areas at this time (De Burgh et al.2011). Effect of anthropogenic metals on the geochemical properties of urban soils studied by Mielke et al (Wang et al.2022). The content of median Ni for fresh alluvium samples was 3.9 µg/g and for urban alluvial soil 9.8 µg/g (New Orleans and stratified by census tracts (Banning et al. 2012). Overall, lower values of Ni occur in outlying areas and significantly higher metal values occur in the inner city. The level of nickel in 60 samples of the soil collected from the stalowa wola areas were higher (average 17.20 mg/kg) which is affected by industrial emissions than that in the reference samples (average 9.72 mg/kg) but all the values, however, were below the highest allowable concentration. In post-flooded industrialized areas of the Dolnośląski Region during (2000-01) in allotment garden Ni content also did not exceed the highest allowable concentration. Permitting to the contemporary elegance parameter the allowable limit for nickel in the soil depends on many factors, and for industrialized areas is set as 50 mg/kg d.w.

3.Essentiality of Nickel:

Ni is a essential micronutrient in plants because it hydrolyze urea in plant tissues and is a part of the active site of the enzyme ureas (Awasthi et al.2022). Two major forms of urease known as glyoxalases and glutathione are present in plants. They are present in seeds and in vegetative tissues (ubiquitous). Highly active form of urease is seed form while the activity of the ubiquitous form is low in vegetative tissues. Despite it playing an important role in N recycling in plants (Rebi et al.2021). By action of enzyme urease the toxic amounts of urea are metabolized into ammonia and the N from the ammonia may be recycled in other metabolic pathways, e.g., synthesis of amino acids, polyamines, and other nitrogen compounds (Nawaz et al.2021).

Ni also participate in GSH homeostasis in plant cells .single and double amount of GSH1 and GSH2 produce lethal phenotypes. Ni play an important role in plants as its metabolism is very crucial for enzyme activities, cellular redox state and for biochemical and physiological growth responses (Hänsch et al.2009). In hydroponic studies beneficial effects of Ni on urease activity and improving N use efficiency by plants were investigated by several authors. However, it is also investigated deficiency of Ni in soil is very rare which affects plant growth, plant senescence, nitrogen (N) metabolism and Fe uptake and may play role in disease resistance. Ni concentration in the majority of plant species is very low (0.05-10 mg/kg dry weight) which effects the plant growth and metabolism.

4. Toxicity of Nickel:

Ni is essential microelement for plants, animals, and humans. Higher concentration of Ni is toxic and surpassing optimum intake values. Ni excess concentration in plants cause chlorosis and necrosis as with other heavy metals and cause disruption of Fe uptake and metabolism (Manzoor et al 2022). In non-tolerant plants elevated concentrations of Ni inhibit cell division at root meristem and decrease plant growth (Ivanov et al.2021). Ni is a major component of plant enzyme as it is an important metal for metabolism. For illustration, small quantities of Ni (0.01–5 lg/g dry wt) are essential for some plant species as it is a component of urease. Concentration of Ni that present in natural soils are (10-1,000 mg/kg) 20-30 fold lower than concentration present in polluted soil (200-26,000 mg/kg. In a variety of plant species preeminent levels of Ni in soil may cause numerous physiological modifications and diverse toxicity symptoms such as chlorosis and nerosis (Hassan et al.2019). Plants grown in soil containing high Ni2 concentration resulted complaint of cell membrane function and loss of nutrient balance. As it is reported in Oryza sativa shoots nickel effects the lipid composition and H-ATPase activity of the plasma membrane. MDA concentration also enhanced in wheat plants if they exposed to higher level of Ni. Moreover, other symptoms observed in Ni treated plants were related with decline in water contents of monocot and dicot plant species which cause changes in water balance. Important indicator of Ni toxicity in plant species is decrease in water content.

4.1 Effect on Morphology:

Heavy metals may persuade changes in plant morphology in addition to toxic effects on growth. Thus, exposure of Triticum aestivum leaves to 1 mM $NiSO_4$ solution decreased the mesophyll thickness, the size of vascular bundles and the vessel diameter in the main and lateral vascular bundles (Bhalerao et al.2015). Whereas in Brassica oleracea plant leaves if grown in agar in the presence of $NiSO_4 7H_2O$ (10–20 g/m3) than the volumes of intercellular spaces and sponge mesophyll decreased relative to control plants (Molas 1997). Heavy metals are known to decrease the malleability of cell walls, perhaps by direct binding to pectin's and by stimulating peroxidise activity in the cell walls and intercellular spaces. However these peroxidases are essential for lignification and association between extension and polysaccharides containing ferulic acid (Pandolfini et al. 1992).

Ni can also inhibit growth by impeding cell division (Amosova et al. 2003). Decreased mitotic index in Vicia faba roots at the incubation on 1.5–5 mM $NiCl_2$ solution and at a concentration of 60 mM, in Z. mays roots was perceived. In the embryonic root of T.aestivumi cell division were clogged in rhizoderm, exoderm and middle cortex except in distal cell of these tissues and peripheral cells of caliptrogen at concentration of 0.1 mM, $NiSO_4$ (Seregin and Kozhevnikova 2006). Ni may cause mitosis disorder and produce various chromosome aberrations: C-metaphases, sticky chromosome, and Chromosome Bridge while interphase cells contained micronucli. At higher concentration of Ni (1–10 mM), the nuclear material was originate in the cytoplasm, whereas the nuclei confined nucleoli of irregular form (oval, oblong, and dumbbell like). Inhibition of plant growth by Ni and other toxic heavy metals results from general metabolic disorder and gradual inhibition of cell divisions.

4.2 Effect on Mineral Nutrition:

Reduction of cation and anion absorption by plant roots is one of the non-specific mechanisms of heavy metal toxicity. The effects of Ni on plant mineral nutrition is slightly inconsistent. Mineral nutrients in plant organs may increase, decrease, or be unaffected in presence of Ni. Due to comparable ionic radii of Ni and other cation competition for common binding sites occur which cause decrease the uptake of macro-and micro nutrients. Such mechanisms may have activated when the uptake of Mg2 (78 pm), Fe2 (82 pm), and Zn2 (83 pm) decreased in the presence of Ni (78 pm) where the ionic radii in digressions (Emsley, 1991). One of the prime causes of chlorosis induced by excess of environmental Ni is reduced uptake of Mg and Fe (Khalid and Tinsley 1980). Ni induced metabolic disorder cause decline in nutrient uptake which effects the structure and enzyme activities of cell membranes (Seregin and Ivanov 2001). In O.sativa shoots Ni affects the sterol and phospholipid composition of the plasma membrane and induce changes in the ATPase activity (Ros et al. 1990). In this way ionic balance in cytoplasm changes due to change in membrane permeability. Ni effects on nutrient uptake mostly depends on the Ni concentration that present in the environment. Trials with ryegrass revealed that at low nickel concentration Fe content in the shoots increased while at higher concentration decreased (Khalid and Tinsley 1980). An increase in soil nickel content decreased the contents of Cu and Mg in the caryopses and Mg and Ca in the shoots of T. aestivum . It is concluded that due to disordered absorption and transport at high Ni concentrations (about 0.1–1 mM), the contents of macro and micro-nutrients in plant tissues decreases (Pandolfini et al. 1992). At the identical interval, contents of nutrients did not change even in some cases increases at low Ni concentrations in the environment (Barsukova and Gamzikova 1999). Such a spectacle was designated as a ‘‘concentrating effect’’, because they were observed as a result of growth inhibition (dry biomass decreases) in the plants While the rate of metal absorption stayed the same in the control plants. Subsequently, the contents of heavy metals increased per unit of dry matter.

4.3 Inhibition of Photosynthesis:

There are several direct and indirect means by which heavy metals are known to cause non-specific inhibition of photosynthesis. The reduced rate of photosynthesis is related to disrupted chloroplast structure, blocked chlorophyll synthesis and disordered electron transport. The activities of the Calvin cycle enzymes inhibited and CO2 deficiency caused by stomatal cessation (Seregin and Ivanov 2001). In B. oleracea plants grown in agar in the presence of NiSO47H2O at 10–20 g/m3 decrease in chloroplast size and numbers and the disorganization of chloroplast ultra-structure were described. In Brasicca plants other changes such as reduced numbers of grana and thylakoids, their deformation, the formation of plastoglobuli were also observed. Such changes arises from an oxidative stress resulting in peroxidation of membrane lipids (Molas 1997). Several journalists conveyed reduced chlorophyll content in the leaves of Ni-treated plants, where Fe or Mg deficiency causes such chlorosis and inhibition of chlorophyll synthesis. Other mechanism of decreased photosynthesis is disruption of electron transport. Ni as well as other heavy metals, primarily affects PSII (Seregin and Ivanov 2001) and inhibit electron transport from pheophytin via plastoquinone QA and Fe to plastoquinone QB by changing the structure of carriers, such as plastoquinone QB, or the reaction center protein. Effeciency of electron transport in the thylakoids decreases which diminished the contents of cytochromes b6f and b559, as well as ferredoxin and plastocyanin. Activity of calvin cycle enzymes decreases that inhibit photosynthesis.

4.6 Effects on plant growth:

Ni is heavy metal and important micronutrient for plant growth. When high concentration of Ni is given to the plant, it becomes toxic. Access amount of nickel destroys cell membrane functions (Naz et al.2022).It also inhibits the seed germination. Ultimately plant growth and development decreases. It has been demonstrated that the excess amount of nickel and cadmium the metal leads to increase the content of hydrogen per oxide (H_2O_2). Plants have different abilities to take minerals from their environment. In the recent study, when wheat seeding were grown in hydro phonic culture in presence of Ni concentrations.

The result shows that Ni was toxic to wheat seeding. The greater the amount of Ni affects the cellular mechanism of roots. However the optimum level of Ni is significant for reduction in dry matter yield. Ni is essential micronutrient of plants. In this form it is helpful as a nutrient for roots and leaves but our results shoes high amount of Ni prevents the seeding.

4.7 Chlorophyll content:

Ni is important element for activation of ureas in higher plants. The effect of Ni on chlorophyll content of different plants like wheat plant; grow in nutrient supplies either with ammonium nitrate or urea as two different kinds of nitrogen sources was investigated as experiment (Gheibi, et al.2009). Plants was allowed to grow for near about six weeks then leaf chlorophyll content ,roots, shoots fresh and dry weights and the concentration of Ni in shoots and roots are determined. Its concentration of ammonium nitrate and urea both in shoots and roots increased significantly with increase in Ni concentration. Chlorophyll content in the leaves fed plants has seen increasing when Ni percentage was high in solution as 0.05mg/L and decreased at 0.1mg Ni/L which is growing in media containing ammonium nitrate with similar Ni levels. When Ni and urea both were supplied to the plants, the chlorophyll content was also higher. Wheat plants required Ni and it is dependent on the kind of nickel which is used with supply of ammonium nitrate and urea. Amount of Ni was needed about 0.01mg/l and 0.05 mg/l of nutrient solution respectively.
Chlorophyll content in the leaves of plants is decreased when Ni is treated to plants. Ni also affects lipids per oxidations in plants but it depends on the element doses.
At the end we concluded that Ni is growth stimulant as well as growth retardant for a wide variety of plants such as wheat. Ni has a negative effect on photosynthesis and respiration.

4.8 Effect on yield:

When different varieties of plants like wheat, sorghum etc on loamy soil in greenhouse was treated with different amount of foliar nickel sulphate (Moosavi et al. 2014). Nickel levels of 15, 30, 45 and 60 ppm shows the accumulation of nickel in plant and results shows a little increase in the dry matter production so yield is slightly increased. When plants regard to the heavy metal absorption the cat ions were accumulated in this order Fe > Mn >Ni. Faba beans absorb higher number of elements then wheat and sorghum (Emamverdian et al.2015). Significant amounts of nickel were present in wheat grains. In addition to heavy metals which may present in sewage material or in industrial wastes i.e. 2m cr, cu, nickel was the element which in responsible to damage crops. There is an adverse effect of nickel elements in very high and low concentration in species to species. Nickel is also responsible in clerisies and necrosis process when applied with excess amount. Nickel concentration in shoot of most plants was increased with increase of the rate of Ni. The working ability of nickel varies with other heavy metals like Mn, Fe, Mg, 2n etc. sometimes it causes beneficial effects on plant growth especially in dry yielded production i-e seeds. It has also been seen that in the presence of some heavy metals Ni has adverse effects on crop production i-e shoot production nickel concentration of 30pm were significantly developed in grains.

4.9 Effect of nickel on enzyme activity:

Nickel affects various physiological processes in plants, including enzyme activities (Van Assche and Glijsters 1990) however, direct and indirect effects of heavy metals on enzyme activities is not always easy to discriminate. Indirect effects are due to competitive inhibiton of absorption and transport of nutrient such as Cu, Fe and Zn arise from ion-induced imbalances. Direct effects of heavy metals inhibit enzymes and there by causing inactivation of enzymes by interacting with the SH groups of proteins. About a hundred enzymes are known presently to be inhibited by binding of SH-group ensuing in concomitant metabolic disorders (Muhammad Sajid and Muhammad 2012). In O.sativa shoots Ni promote in vivo Mg+2 dependent ATPases in plasmalemma. However due to decreased enzyme content total decline in enzyme activity take place.

The toxic effects of metals on enzyme activity in vitro do not always agree with the in vivo effects at the same salt concentration. Such disagreement may stem from the presence of efficient cellular mechanisms for detoxification and the physiological barriers that curb metal translocation into the cytoplasm. To illustrate, Ni2? was shown to promote in vivo Mg2. dependent ATPases in the plasmalemma of O. sativa shoots (Ros et al. 1990). Total decline of enzyme activities is sometimes observed due to decreased enzyme contents. Activity of glutamine synthetase and alanine aminotransferase also dropped which depends on the cytoplasmic levels of nitrate and their substrates (El-Shintinawy and El-Ansary 2000). Other heavy metals also have indirect influence on nitrate reductase activity. Ni ions can both stimulate and inhibit enzyme activities in plant tissues depending upon its concentration. Thus, in O.sativa seedlings activities of IAA oxidase, ascorbate oxidase, catalase, and peroxidase at their highest when Ni concentration is lower but the enzyme activities considerably declined at a higher Ni concentration . Ni also inhibit the activity of calvin cycle enzymes while, the inhibitory effect was insignificant when Ni salt was added at advanced progressive phases (70 days after sowing) (Sheoran et al. 1990). However, the appliance of this phenomenon is unknown.

Conclusion

Aim of this review is to correlate the essentiality of nickel in proper growth and development. Ni has vital roles in a varied range of morphological and physiological functions, starting from germination to the productivity. Moreover, Ni is an important metal without it plants cannot complete their life cycle. Excess Ni toxicity is illustrated by the inhibition of lateral root development, photosynthesis, mineral nutrition and enzymatic activity which differs from that of other heavy metals such as Ag, Cd, Pb, Zn, Cu, Co, and Hg.

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