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Occurrence, Uptake, Accumulation and Physiological Responses of Nickel in Plants and its Effects on Environment

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Abstract: Literature pertaining to the uptake and accumulation of Nickel (Ni) in plants and its effects on physiology and biochemistry in plants has been reviewed. The review shows that Ni has significance as a nutritive as well as a potentially toxic element in the plants and environment.

Key words: Nickel, enviroument, micronutrient, physiological response, toxic elements, enzymes, accumulation, uptake

INTRODUCTION

Nickel is a heavy metal, present in soil, water and air, usually in trace amounts. However, rapid industrialization and urbanization during the recent past have caused accumulation of Ni and many others trace elements in varied habitats where from the acquisition by the plants and their further transfer to human and animal population may affect the life forms seriously. There are a number of reports of stimulation of growth in higher plants by low concentrations of Ni in the nutrient medium (Mishra and Kar, 1974; Stedman, 1968; Welch, 1981) based on the studies with nutritional requirement of some plants (Welch, 1981; Reinbothe and Mother Urea, 1980) several investigators had suggested that Ni was an essential element for plants growth although no conclusive evidences were provided. However, Browen *et al.* (1987) have demonstrated that Ni is an essential micronutrient for barley which failed to complete its life cycle in the absence of Ni and addition of Ni to the growth medium completely alleviated its deficiency symptoms. Nickel has been demonstrated to be associated as the metallic co-factor of urease from Jack bean (*Canavalia ensiformis*) seeds (Dixon *et al.*, 1980b). This enzyme catalyses the hydrolytic cleavage of urea to ammonia and carbon dioxide and is widely distributed in higher plants (Welch, 1981).

On the other hand, physiological role of Nickel and its toxic effects on higher plants (Seregin and Kozhevnikova, 2006) and phytotoxic effects of the metal have also been observed (Agarwal *et al.*, 1976; Crooke, 1956; Hunter, 1954). Growth of most plants species is adversely affected by tissue concentration above 50 µg g⁻¹ dry weight. These effects are manifested at morphological, physiological and biochemical levels and they may result either because of direct toxicity of the m et al or because of its tendency to compete with other cations such as Ca²⁺, Mg²⁺, Fe²⁺ and Zn²⁺ and thus to cause their artificial deficiencies. Higher levels of Ni in the soil and in the plants tissue of ten induce Zn or Fe deficiency which leads to characteristic symptoms of chlorosis (Anderson *et al.*, 1973). The application of sewage sludge to the crop species results in elevated level of Nickel in plant tissues (Abud and Moneem, 1996; Maranville, 1970). Thus, there is a real concern for the possibility of Ni phytotoxicity in the present industrialized and highly urbanized society. The present review deals with various aspects of Ni in plants and its environment to assess the significance of Ni metal in Plant's life.

Table 1: Physical properties of Nickel (Evenhort, 1971)

Characteristics	Values
Atomic No.	28
Atomic weight	58.71
Boiling point	2913°C
Crystal structure	Face centered cubic
Curic temperature	353°C
Density of the metal (g cm ⁻³)	8.90
General appearance	Soft silvery metal
Melting point	1455°C
Poissons ratio	0.31
Specific heat (25 cal g ⁻¹)	0.106

PROPERTIES OF NICKEL

As with other metals, the biological significance of Ni is related to its physicochemical properties. Some physical properties of Ni are shown in Table 1. The β -emitting radioisotope of Nickel (63 Ni) which has a half life of 92 years and an energy of decay of 0.067 MeV, is very useful for biochemical studies particularly those involving micro-organisms. The preferred oxidation states of Nickel are 0 and +2, but in complexes +3 and +4 states can also occur. Nickel forms stable octahedral complexes with, for example, EDTA, nitrolotriacetate, cysteine and citrate (Bagati and Shorthours, 1991). This property is of physiological importance since these complexing agents have been shown to prevent the uptake of Ni by cultured cells of tobacco, soybean and rice (Polacco, 1976). The complexes are important from storage point of view also in most Ni accumulating species the metal exists as an anionic citratenickelate (II) complex (Los *et al.*, 1966). Complexes with other organic acids such as malic and malonic acids are also found (Evenhort, 1971), such organic acids are in abundance in metabolically active plant tissues.

NICKEL IN THE ENVIRONMENT

Nickel is widely distributed throughout the physical and biological world. In the soil, it is present in the form of its mineral ores; the important ones are liunacite [(Fe. Co. Ni) $_3$ S $_4$)] and spies cobalt [(Co. Fe. Ni) $_4$ S $_2$)]. The soils of Sudbury in Ontario, Canada are rich in another type of Nickel ore, pentlandite, a sulfide of Cu, Fe and Ni, about 20% of the world's Ni supply comes from this area. The metal is extracted from its ore for various industrial, chemical and biological applications. The total world's production of Ni is about 660,000 tours per year, about 40% of which is used in the steel industry. Natural weathering of igneous and metamorphic rocks also releases Ni, which is largely retained in the weathered profile in association with clay minerals and as hydrous ions or as a complex with manganese oxide. Free Ni concentration in the soil is controlled primarily by precipitation reactions with the hydrous oxides of Mn and Fe metals. There are about 42 different oxides of these metals. Further, precipitation of Ni by hydrous oxides of Mn and Fe increases with increase in pH (Jemme, 1968). Liming, which increases the pH of the soil, can reduce Ni toxicity of plants growing on serpentine soils (Crooke, 1956).

Nickel is also occurs in water bodies and in atmosphere, usually in trace amounts. The municipal and industrial effluents contribute significant Ni content to the soil and water but relative contribution depending upon the source of effluent (Table 2). Due to uncontrolled industrial and municipal discharges, some of the rivers in India and other countries are becoming highly polluted with Ni and other toxic metals, which also sediment in riverbed to high level of toxic concentrations. The Ni concentration in river water and in sediments in the upper Ganges (India), for example, has been estimated to be between 35 and 211 ppm and 70,900 and 511,000 ppm, respectively (Israili, 1992).

Table 2: The concentration of Nickel present in different industrial effluents (Srikanth et al., 1992)

Sources	Concentration (range) Ni in ppm
Chemical and electrochemical industry	5-40
Iron and steel industry	10-80
Metallurgical industry	5- 60
Textile industry	3-20
Fertilizer industry	5-80
Oil and vegetable industry	5-30
Leather industry	5-40
Chloral-alkali industry	5-20
Urban sludge	12-35
Rural sludge	18-20

The relatively higher concentration of Ni in sediments indicates that the metal gets deposited by the physico-chemical reactions in water and in riverbed. This is apparently favoured by alkalinity (7.3-7.7 pH) and high concentrations of organic matter and carbonates and oxides of other co-precipitating metals in Ganges water (Israili, 1992).

Atmospheric Ni content is primarily due to pollutant particles laden with the metal and suspended in the air. Fly ash contains appreciable amounts of Ni, which ultimately settles to the soil, water or plants surfaces.

PLANT UPTAKE OF NICKEL

A number of reports (Aschmann and Zasoski, 1987; Crooke *et al.*, 1954; De Kock, 1956; De Kock and Mutehell, 1957) indicate that Ni is easily absorbed by the plants when supplied in the ionic form (Ni²+) and is not as strongly absorbed when chelated. Crooke *et al.* (1954) reported that the level of Nickel in oat plants (*Avena sativa*, Crooke *et al.* (1954) supplied with Ni versenate did not vary significantly from the controls, whereas plants received in ionic form of Ni at the same concentration, the level was more than 10 times as compared to control. The inference is that no absorption of Nickel complex occurred. Turina (1968) reported that in some monocots like rye (*Secale cereale*), wheat (*Triticum vulgare*) and maize (*Zea mays*), the absorption of Ni by roots was through the root caps. Ni uptake appears to be an active process, as it is influenced by temperature and anaerobic condition and by respiratory inhibitors such as dinitrophenol (Aschmann and Zasoski, 1987). However, the study of Ni uptake has been confined to low and medium concentrations of Ni (0.5 to 200 μM) ouly and it is not possible to deduce about the dual or multiphasic nature of the uptake isotherms from these studies (Pinamonti *et al.*, 1997; Cataldo *et al.*, 1978a; Epstein, 1976; Nandi *et al.*, 1987).

Uptake of Ni by plants depends upon various factors, the most important of course, being the ionic, Ni concentration in the medium (Dixon *et al.*, 1980c; Halstead *et al.*, 1969; Miller, 1961; Roth *et al.*, 1991). Soil pH values below 5.6 seem to favour the absorption of Ni and is largely due to the fact that the exchangeable Ni content of the soil increases with the increasing soil acidity (Mizuno, 1968). The Ni absorption is increased by increasing the phosphate content of the soil (Halstead *et al.*, 1969; Polacco, 1976). Foliar spray with Co (NO₃)₂ on tomato and cucumber also increases the Ni content. Inspite of the high Ni content in peat (80 μg g⁻¹) it is unable to supply Ni to barley (*Hordeum vugare*) seedlings (Morrison *et al.*, 1980) addition of peat to Ni-rich soil significantly inhibits Ni uptake. This inhibition may be due to complexing of the soluble Ni to the insoluble forms, competition from other metal ions in the media or both. Organic matter present in the soil inhibits the uptake of Ni by the plants to some extent (Halstead *et al.*, 1969). Fertilizers decrease the total absorption of Ni and other elements like Cu, Zn, Co, Ni (Miller, 1961). The absorption rates of other elements compared with Ni shows that reaction by elements absorbed to the exchangeable element content in the soil decreases in the order Cu > Zn > Co > Ni (Miller, 1961). Tiffin (1971) has

shown that Ni transported in the xylem fluid of crop plants was present as anionic complex. The presence of other divalent cations as in case of soybean and barley in the culture solution also influences the uptake of Ni²⁺ (Klucas *et al.*, 1983; Korner *et al.*, 1987; Lee *et al.*, 1987).

Nickel Accumulation in Plants

Plants can accumulate Ni, which is usually less than 0.1% of the total dry weight; although the New Caledonia species *Sepertia acuminata* can accumulate Ni up to 25% of its dry weight (Lee *et al.*, 1987). The metal is very mobile and can be accumulated in vegetative and reproductive parts (Soon *et al.*, 1980). During vegetative growth, most of the Ni is translocated and accumulated in leaves. However, during senescence of leaves, most of it is transported to seeds, as reported for soybean (Cataldo *et al.*, 1978b) and *Mimulus guttatus* (Tilstone and Mc Niar, 1991).

Studies on the chemical form of Ni in plant tissues have been confined to the leaves of accumulator species (Patel *et al.*, 1976; Timperley *et al.*, 1973). These studies demonstrate that the metal is present in the form of a cationic complex (Krog Nier *et al.*, 1991; Mishra and Kar, 1974; Pelosi *et al.*, 1976). Most of the Ni in *Sepertia acuminate*, *Homalium freancii*, *H. guillgini*, *H. kanaliense*, *Hybanthus austrocaledonicu* and *H. caledonicus* is found as a negatively charged citratenickelate II complex with Ni $(H_2O)^{2+}$ as the major cationic constituent (Lee *et al.*, 1977).

Prasad *et al.* (1997) have shown a high Ni⁺ accumulation in various parts of wheat and Eichhornia plants; Indian mustard is considered as a very significant accumulator of heavy metals including Ni⁺² (Singh *et al.*, 2001). A large number of plants have been identified as Ni⁺² phytoremediator including Indian mastered fragrant geranium sunflower, *Thlaspi* sp. *Alyssum* (Cunningham *et al.*, 1995). *Berkheya coddii* (Kramer *et al.*, 1996) *Sebertia accuminatav* (Ensley *et al.*, 1997), *Eichhornia* etc. (Prasad *et al.*, 1997; Brooks *et al.*, 1998; Sagner *et al.*, 1998; Chaudhry *et al.*, 1998; Persans *et al.*, 1999; Singh *et al.*, 2001).

It has been observed that a 36-fold increase in the concentration of free his in the xylem exudates of the Ni hyper accumulator alyssum canoccur office exposure to Ni, whereas no much significant change was observed in the non-accumulator *Alyssum* sp. Recent research demonstrated recently that histidine is involved in Ni hyper accumulation in Thlaspi goesingense halacsy (Kramer *et al.*, 1996, 1997; Persans *et al.*, 1999).

Its essentiality to higher plants was proposed by Browen et al. (1987), it was generally accepted as a likely essential nutrient element to higher plants (Gerendás et al., 1999; Sirko and Brodzik, 2000; Bloom, 2002; Marschner, 2002) and it was added to the US Department of Agriculture (USDA) list of essential plant nutrient elements (Hull, 2003). Ni was recently recognized as an essential plant nutrient (Association of American Plant Food Control Officials, 2005), thus enabling manufacture and sale of Ni fertilizers in the United States. Several plants species and several environmental factors determine the extent of Ni accumulation in plants. There is a considerable variation among the species, as far as Ni accumulation is concerned (Table 3). Plants growing on serpentine soils are usually assumed to be Nickel (Ni)-tolerant species. It has been reported that Cunonia macrophylla (Virginie Léon et al., 2006) an endemic species common on Ni-rich soils in New Caledonia, is indeed Ni tolerant. In a greenhouse experiment, plants were watered with solutions containing Ni chloride (5 to 1000 mg Ni L⁻¹) for 15 months before their morphological characteristics were measured and the levels of nitrate, protein and nutrients in the plants were determined. The plants grown with 5, 10, 50 and 100 mg Ni L⁻¹ had more number of leaves and there was a tendency toward greater stem thickness and fresh shoot biomass at 50 mg Ni L⁻¹. Manganese (Mn), copper (Cu) and zinc (Zn) concentrations were also higher in the presence of 50 mg Ni L⁻¹. One thousand mg Ni L⁻¹ induced symptoms of Ni toxicity and this effect may account for the lower concentration of potassium (K) in the shoots and roots of plants exposed to this concentration, as well as for inducing higher concentrations of Table 3: Nickel content of various plants and plant parts

	Nickel content (µg g ⁻¹ dry weight		
Plant species	Plant parts	(μg g · ary weight organelle)	References
Alvssum bertolunis	Leaves	2590	Lee et al. (1987)
Alyssum bertolunis	Leaves	5000	Dan et al. (2000)
Alyssum vertotians Alyssum serpyllifulium	Leaves	9330	Lee et al. (1987)
Alyssum trodil	Leaves	8000-10000	Bazzaz et al. (1974)
Avena sativa	Leaves	3 or less	Hunter (1954)
Bean	Leaves	5 or ress 58	Halstead <i>et al.</i> (1969)
Cabbage	Leaves	0.2-186	Halstead et al. (1969)
Casearoa silvanae	Leaves	1490	Dan et al. (2000)
Casearoa savanae Chrysanthemum	Leaves	1.5-139	Patel et al. (1976)
Cru ysaninemiun	Roots	7-900	Patel et al. (1976)
	Leaves	22.26	Hunter (1954)
Clover	Leaves	22.23	Hunter (1954)
	Roots	24.23	
Cynodon dactylon	Roots	24.23 0.4-0.6	Mishra and Kar (1974)
Deucus carota L.			Winker et al. (1983)
Geissois pruinosa	Leaves	13400	Soon et al. (1980)
Glycine max	Seed	0.6	Miller et al. (1970)
	Leaves	58.4	Dixon et al. (1980a)
	Stems	21.0	Dixon et al. (1980b)
	Roots	148.6	Dixon <i>et al.</i> (1980c)
Homalium mathieuanum	Leaves	14.4	Lee et al. (1987)
H. połystachyum	Leaves	46.4	Lee et al. (1987)
H. polystachyum	Leaves	46.4	Lee et al. (1987)
H. intermedium	Leaves	74.6	Lee et al. (1987)
H. leretiorum	Leaves	446	Lee et al. (1987)
H. decurreus	Leaves	505	Lee et al. (1987)
H. austrocaledonicum	Leaves	622	Lee et al. (1987)
H. kanalieuse	Leaves	3730	Lee et al. (1987)
H. francii	Leaves	7210	Lee et al. (1987)
H. guillainii	Leaves	9580	Lee et al. (1987)
Hybanthus caledonicus	Leaves	6820	Lee et al. (1987)
H. austrocaledonicus	Leaves	14900	Lee et al. (1987)
Hybanthus floribundus	Leaves	1300	Dan et al. (2000)
Iponaea prescorpes	Root	1.9-129	Pyatnitskaya (1970)
	Stem	2.1-8.3	Pyatnitskaya (1970)
	Leaves	2.0-7.1	Pyatnitskaya (1970)
Lycopersicon esculentum	Fruit	0.013	Pyatnitskaya (1970)
Lasiochlamys pltata	Leaves	1000	Dan et al. (2000)
Medicago sativa L.	Plant	44	Halstead et al. (1969)
Momordica charantia	Fruit	10	Polacco (1977a)
Nothorfagus fusca	Chloroplast	8.25	Timperley et al. (1973)
	Mitochondria	8.05	Timperley et al. (1973)
	Ribosomes	1.42	Timperley et al. (1973)
	Supernatant	87.48	Timperley et al. (1973)
Panicum maximum	Shoot	0.88	Srikanth et al. (1992)
Panicum miliaceum	Grain	4.33	Los et al. (1966)
Pearsonia metallifera	Leaves	10600	Lee et al. (1987)
Phyllanthus serpentinus	Leaves	38100	Dan et al. (2000)
Psedowinter colorata (heaf, f)	Chloroplast	8.0	Duit 61 (2000)
i sedominer colordia (near, 1)	Mitochondria	14.70	Timperley et al. (1973)
	Ribosomes	1.65	Timperley et al. (1973)
	Supernatant	87. 87	Timperley et al. (1973)
Psychotria douarrei	Leaves	13400	Dan et al. (2000)
Quintinia acntifolia (heaf, f)	Chloroplast	9.93	Dan et al. (2000)
	Mitochondria	6.20	Timperley et al. (1973
	Ribosomes	2.53	Timperley et al. (1973
n 1	Supernatant	87.22	Timperley et al. (1973)
Raphanus sativus L.	Fleshy root	0.11-0.2	Winker et al. (1983)
Rinovea benejalenis	Leaves	5000	Dan et al. (2000)
	Rumex Fruit	0.369	Pyatnitskaya (1970)

Table 3: Continued

Plant species Plant par		Nickel content (μg g ⁻¹ dry weight organelle)	References	
Rye grass	Leaves	48	Smith (1943)	
Sebertia acuminata	Leaves	10200	Lee et al. (1987)	
	Latex	167000	Lee et al. (1987)	
Triticum stivum L.	Grain	1.06-2.69	Mishra and Kar (1974)	
Typha latifolia	Root	300-1200	Prasad et al. (1997)	
	Leaves	500	Prasad et al. (1997)	
Vacciniuon angusti folium	Roots	6.1-62.3	Bagati and Shorthoure (1991)	
	Stems	6.3-49.2	Bagati and Shorthoure (1991)	
	Leaves	3.4-33.3	Bagati and Shorthoure (1991)	
Xylosma vincentii	Bowies	1.3-7.0	Bagati and Shorthoure (1991)	
-	Leaves	3750	Dan et al. (2000)	
Zea mays L.	Grain	1.4	Los et al. (1966)	

soluble protein in the shoots. Calcium (Ca) and iron (Fe) levels were lower in roots exposed to higher concentrations of Ni. Nickel concentrations in the plant as a whole increased with the level of Ni applied and the Ni gradient decreased from roots to stem and from stem to leaves. It was concluded that Cunonia macrophylla (Jaffer et al., 1976) is a Ni-tolerant species that accumulates Ni without any impairment of growth when exposed to levels of up 500 mg Ni L⁻¹. Species endemic to serpentine soils accumulate usually up to 1000 ppm of Ni (Jaffer et al., 1976; Reeves et al., 1980). Levels of over 2.1 ppm have been found in some species e.g., Alyssum bertolonii (Pyatnitskaya, 1970) and of over 3% in Psychotria dovarrei and Peltair emarginata (Borrks and Marfil, 1981). Several of these species which accumulate Ni to high levels also accumulate cobalt but usually to a lesser extent e.g., Hybanthus floribundus, Alyssum species etc. (Brooks et al., 1977; Bollard and Bulter, 1966). A Few genera e.g., Rinorea and Nyssa accumulate more cobalt than Ni (Severne, 1974). Apparently, these species have developed some multiple metal tolerance mechanisms. It would be rather interesting to investigate complexes of Ni in the species, which accumulate very high levels of the metal. Bollard and butler (Prince, 1957) reported that in tolerant species a mechanism has evolved so that Ni is largely excluded from the sites of activity of enzymes. This is accomplished by biosynthesis of usual compounds of organometallic complexes or by spatial segregation of the enzymes and Ni within the cells. In Hybanthus floribundus the Ni is concentrated at epidermal cell walls, which might confer xenomorphic adaptation to the plants by reducing cuticles transpiration (Soane et al., 1956).

Nickel accumulation is also determined by the growth stage and the organ of the plants. In oats, during a 70 days experimental period from germination to maturity, Ni content increased rapidly during the first 30 days and then decreased slowly (Jemme, 1968). In other studies, younger oat seedlings have been shown to accumulate more Ni than the older ones (Hunter, 1954). This might be related to the activity of the root, the Ni absorbing system and/or the metabolic activity of the tissue accumulating the metal. In maize plants, younger leaves contain more Ni than the older ones (Mac Lean and Dekker, 1978). In *Primula obconica, Trifolium repens, Elodea Canadensis* and *Aspidistra elatiar*, Ni is predominantly located in the leaves and flowers (Roth *et al.*, 1991). In tobacco, the leaves accumulate Ni. However, the cereals from the Eastern, Central and Barabas zones of the Novosibirsk region of erstwhile USSR. contain higher quantities of Ni and trace elements in the straw than in the grain. This may be a mechanism to avoid toxic accumulation of trace elements in embryonic and metabolically active tissues adopted by these cereals.

The Ni content of the soil in which the plant is growing has a significant effect on plant's Ni content (Mitchell, 1945). The foliage of crop plants growing in agricultural soils usually contains less than 10 ppm Ni and often below 1 ppm Ni (Lyon *et al.*, 1970; Mitchel *et al.*, 1978). In plants on serpentine soils or on soils to which sewage sludge has been applied the level of Ni is often 100-1000 times higher (Mertin and Meybark, 1997; Singh, 1984; Das *et al.*, 1978).

A seasonal variation in Ni accumulation by plants has also been recorded. For example, in *Ipomoea pescarpes*. Plants growing on a island near Calcutta, Ni accumulation is maximum during the monsoon (July-Oct) season (Pyatnitskaya, 1970). During this season salinity and pH of the medium are also at the lowest, it may be suggested that these factors affect the uptake of Ni from the medium.

PHYSIOLOGICAL EFFECTS

Seed Germination and Seedling Growth

Seed germination and early seedling growth are initial events in the life of a plant projecting the extent of future physiological and biochemical processes. It has been observed that 2.0 ppm solution of Ni (NO₃)₂ and NiSO₄ accelerated the germination of wheat grains. Low level of Ni (NO₃)₂ stimulated the germination of dormant timothy (*Phleum pratense*) seeds but there was a distinct toxicity of the Ni ion at 58.71 ppm (Veer, 1988). When used as a presowing treatment, NiSO₄ solution in the concentration range of 2.68 to 26.3 ppm had a marked stimulating effect on the germination of pea (*Pisum sativum*), bean (*Phaseolus vulgaris*), wheat and castor (*Ricinus communis* (Underwood, 1971)) seeds. Concentrations of the order of 26 ppm and higher were detrimental to the germination of these seeds. NiSO₄ solutions at low concentration have beneficial effects on the germination of white lupine (*Lupinus albus* (Bertrand and Wolf, 1973).

Singh (1984) has suggested that the lower concentration of Ni in Cicer cultivars promotes seed germination as well as seedling growth. On the basis of germination percentage, cultivar CV-C 130 is relatively more resistant than CV-H 208 to Ni pretreatments. It clearly shows that the effect of Ni on seed germination depends upon the species and the Ni concentration in the medium. Attempts have been made to study the physiological basis of the effect of Ni on seed germination. The germination of rice seed and the activities of some oxidative enzymes in the seedlings were stimulated by 3 ppm Ni (Bushnell, 1966). According to Welch (1981), the stimulation of germination by Ni (Pelosi *et al.*, 1976) may be based on the function of Ni as the metal components of urease.

Veer (1988) studied the effect of 1.0 nM concentration of Ni on seedling growth and activities of certain hydrolytic enzymes of seeds of *Phaseolus aureus*. The metal usually inhibited the root and shoot growth and also the activities of α -amylase and protease. The author has suggested that Ni inhibits seedling growth by the suppression of the activities of hydrolytic enzymes (Walker *et al.*, 1985). It has been reported that (Lan Zhang *et al.*, 2007) leaves having high amount of Ni shed by the Nickel hyperaccumulator Alyssummurale inhibit seed germination of competing plant.

Nickel and Nitrogen Assimilation

Requirement of Ni has been demonstrated for both nitrate/ammonia assimilation as well as for symbiotic Nitrogen fixation. The effects of Ni deficiency on soluble anions, amino acid and Nitrogen levels in barley were studied. It was concluded that Ni deficiency in barley plants resulted in the disruption of nitrogen metabolism and affected the concentration of malate and various inorganic anions in roots, shoots and grain of barley (Eskew *et al.*, 1984).

Nickel has been shown to stimulate growth when urea is the sole nitrogen source but to have slight or no effect with other nitrogen sources. Recently, it was demonstrated that soybeans grown in nutrient solution containing $\mathrm{NO_3}^-$ and $\mathrm{NH_4}^+$ sources of Nitrogen developed necrotic lesions on the leaf tips. These necrotic tips contained 2.5% urea (dry weight) and were more frequent and more extensive on plants dependent on $\mathrm{N_2}$ fixation for their Nitrogen. On the basis of these experiments, it was suggested that urea is produced during normal nitrogen metabolism in higher plants and that Ni as a component of urease is required to prevent urea accumulation to toxic levels. Apparently Ni is essential for plants supplied with urea and for plants in which ureides (e.g., allantoin) play a significant role in Nitrogen metabolism. This holds true, in particular, for several legume species in which ureides

Table 4: Examples of plants families that accumulate large amounts of ureides or arginine in various tissues (Smith, 1943)

Family	Examples
Boraginaceae	Borage herbs
Betulaceae	Betula lenta (Birch tree)
Compositae	Sunflower
Hipocostmacae	Tilia sp. (Buckeye tree)
Leguminosae	Legumes
Gleacease	(Ash tree) glea sp.
Oxalidaceae	Oxalis sp.

are important forms of soluble Nitrogen during transport of nitrogen from root nodules to the shoots. The stimulation by Ni of the nodule weight and seed yield of soybean further supports the role of Ni in legumes (Browen *et al.*, 1980). Bertrand and De Wolf (1973) reported that the yield of soybean seeds from plants grown in a soil low in Ni was increased by 21.0% when Ni was added to the soil at a level up to 40 mg ha⁻¹. The plants were dependent on symbiotically fixed nitrogen for most of their Nitrogen requirement. Nickel treatment (up to 40 mg ha⁻¹) also increased the total dry weight of nodules formed on the soybean roots. At 80 mg ha⁻¹, Ni chloride inhibited nodule formation and reduced soybean seed yield. Apparently, low levels of Ni are required either for the growth of Rhizobia or for the utilization of fixed Nitrogen by the plants, or both.

Since Dixon *et al.* (1980a) reported that Ni is an integral part of the enzyme urease (isolated from Jack bean seeds) several investigators have shown a Ni requirement for higher plants grown solely on urea Nitrogen sources. Polacco (1976) reported that soybean cells grown in suspension cultures and supplied only urea- nitrogen required exceedingly low levels of Ni (i.e., less than 0.1 μ M Nil). There was an almost absolute requirement of Ni for soybean cell growth in nutrient media containing 10 mg potassium citrate. Apparently, citrate complexed in Ni made it unavailable for urease production. Free Nickel (Ni²+) is essential for urease synthesis in soybean cells.

Table 4 represents examples of plant families that accumulate large amount of ureides or arginine in various tissues. In these plants urease and, therefore, Ni is required to utilize fully the Nitrogen in these compounds in anabolic reactions.

The legumes of tropical origin including cowpea (Vigna unguiculata (Walker et al., 1985) and soybean (Glycine max (Singh et al., 2001) form ureides in root nodules during nitrogen fixation. Ureides are then transported via the xylem to leaves. They also transfer ureides from old senescing leaves to developing seeds and younger leaves via phloem. Utilization of nitrogen in such ureides by cowpeas and soybeans apparently involves breakdown to urea and then hydrolysis of urea, because without Ni, toxic amount of urea are built up in leaf tips, when plants begin to flower (Singh et al., 2001). When Ni is carefully removed from nutrient solution, plants accumulate much urea in their leaf tips that subsequently cause necrotic spots.

PLANT GROWTH AND PRODUCTIVITY

Numerous reports demonstrate that Ni supply increases the yield of crop plants. The first indications of importance of Ni in increasing the crop yield was given by Roach and Barcloy (1946). In culture experiments with pine seedling, Ni has proved to be an essential element for growth (Polacco, 1977a). Slight increase in the yield of *Eragaria* and *Gossypium* was reported by the addition of Ni to the soil (Dixon *et al.*, 1975). Presowing treatment of wheat seeds with Ni at a concentration of 100 ppm resulted in maximal growth of shoots and roots. Nickel treatment increased the mass of root as well as of the aerial parts of cotton plants. Growth and development of paprica and tomato plants are stimulated by the application of Ni at a concentration less than 1 µg L⁻¹. Applications of Ni increased the yield of soybean (Browen *et al.*, 1980). Patel *et al.* (1976) reported that yield of *Ephedra nevadenssi* was increased by Ni at 250 and 500 mg kg⁻¹ applied to desert soil. *Ephedra*

nevadensis was more tolerant to high concentration of Ni than were the Ambrosis dumosa andersonii and Larrea tridenta desert shrubs.

Spraying cotton plants with 235 µg L⁻¹ Ni in the form of NiSO₄ solution increases the number of buds, blossoms and the rate of boll formation (Gordon *et al.*, 1978). Photo insensitive growth of etiolated pea stem sections is also promoted by Ni (Browen *et al.*, 1980), Buozek and Konarzewski (Bazzaz *et al.*, 1974) reported that Ni significantly increased the elongation and fresh weight of the hypocotyl sections of sunflower. Nickel treatment promoted bean hypocotyl hook opening. Gordon *et al.* (1978) grew *Lemna paucicostata* plants on urea as the sole source of nitrogen. At levels above 2 µM Ni sulfate additions significantly promoted growth. At 50 µM Ni sulfate, the rate of vegetative growth was doubled. They also reported that in a preliminary experiment, other species of duckweed, including *Spirodela polyrhiza* and *Wolffia globosa* (Browen *et al.*, 1987). Responded to Ni and urea in qualitatively the same way as did *Lemna paucicostata* (Gordon *et al.*, 1978). Nickel may also have essential functions in grain maturation and plant senescence (Browen *et al.*, 1987). These reports show that Ni has significant effects on the normal growth and development of higher plants.

PHOTOSYNTHESIS

The decreased plant productivity in some cases may be traced to decreased photosynthetic rate by Ni in these plants. The metal is known to inhibit photosynthesis and over all gas exchange in some plants such as maize and sunflower (Lo and Chen, 1994; Mishra *et al.*, 1973). However, presowing treatment of seeds with Ni salts increases photosynthetic rate in maize, oat, tomato, pea, broad bean and spring wheat this is apparently due to increased levels of chlorophylls and other accessory pigments (Mishra and Kar, 1974). Sheoran and Singh (1993) have suggested that the metal inhibit Photo System (PS) II more effectively possibly at the oxidizing site. Photophosphorylation, activities of the photosynthetic enzymes and PS-I are the other possibilities of action of Ni^{+2z} z. Long-term exposure results in reduced leaf growth decreased photosynthetic pigments, changed chloroplast structure and decreased enzyme activities for CO₂ assimilation (Dan *et al.*, 2000).

Pigment Content

Changes in natural coloration are often a visible sign of a Ni effect, many investigators have tried to determine the effect of Ni on pigment content, especially the chlorophyll and carotenoid levels. In pea plants, the formation of carotene is promoted by treatment with Ni (Mishra and Samal, 1971). An increase in total chlorophyll content with 100-200 $\mu g g^{-1}$ Ni has been observed in presowing treatment of maize and oat seeds. Presowing treatment of potato tubers with Ni solutions of low concentrations increased the synthesis of chloryophyll, carotene and xanthophyll (Mishra and Kar, 1974). The breakdown of chlorophyll in tobacco leaf discs and detached rice leaves is usually accelerated by far red light and is retarded by red light (Mishra *et al.*, 1973; Medvedava, 1972). But surprisingly when detached rice leaves are floated on 237.7 $\mu g L^{-1}$ NiCl₂ solutions under red and far-red light, the breakdown of chlorophyll is accelerated in red light and is retarded in far red light (Nensen and Daly, 1960).

There are a number of reports that Ni ion (Ni²⁺) prevents the destruction of chlorophyll during senescence of detached wheat and rice leaves in the dark (Roach and Barcloy, 1946; Singh *et al.*, 2001; Miller *et al.*, 1970; Lo and Chen, 1945). But in attached wheat leaves Ni fails to maintain the greenness, it has been reported (Lo and Chen, 1945) that Ni protected the chlorophyll from destruction more effectively in the dark than in the light in detached wheat leaves. Medvedeva (1972) reported that a higher level of anthocyanin is maintained in *Petunia* flowers incubated for 96 h in NiSO₄ solution compared to the flowers incubated in water for the same period. This observation has significance in horticulture and may be exploited for prolonging the life of cut flowers.

RESPIRATION

The rate of respiration in the healthy tissues of wheat leaves increases on treatment with Ni salts (Aschmann and Zasoski, 1987; Ensley $et\ al.$, 1997) while the treatment suppresses the increased respiration accompanying rust infection (Singh $et\ al.$, 2001). Miller $et\ al.$ (1970) demonstrated that NiCl₂ at lower concentration increased the respiratory rate of maize mitochondria but at high concentrations the respiratory reaction was blocked. The concentration of Ni producing maximal respiratory response is 4.7 ppm NiSO₄ and the percentage of stimulation is 400. The report of Miller $et\ al.$ (1970) further indicated that Ni at 5.87 ppm NiSO₄ increases the NADH-oxidation in the absence of phosphate by about 5%.

METABOLIC EFFECTS

The physiological abnormalities observed during Ni deficiency suggest that Ni might be involved in several physiological processes. Some of these linked with Ni metabolic effects have been described earlier (Browen *et al.*, 1987; Sagner *et al.*, 1998). One of the most obvious effects of Ni supply has been on the protein metabolism. Nickel increased the total protein content and total Nitrogen content of maize and oat plants (Mishra and Kar, 1974; Welch, 1981). Spraying of infected plants with NiSO₄ solutions at the stage of 5-6 leaves increased the free amino acid content of the leaves (Borrks and Marfil, 1981). In senescing detached rice leaves, Mishra and Samal (1971) have reported that NiCl₂ in combination with benzimidazole was more effective in arresting the breakdown of protein than NiCl₂ alone.

Lo and Chen (1945) have reported that $NiSO_4$ alone fails to increase ascorbic acid content significantly the in tomatoes but this salt in combination with complete Fertilizers increases the ascorbic acid content of *Phaseolus lactuca* and tomatoes (Alagna *et al.*, 1984). Nickel salts increased the ascorbic acid content of *Phaseolus* (Soon *et al.*, 1980), tomatoes and maize (Polacco, 1976; Andrae and Van Ysseltein, 1960). Mishra and Kar (1974) studied the stabilizing effects of salts of Ni and other elements on the stability of ascorbic acid in the medium with water, HCl, H_2SO_4 and NaOH. In aqueous solution and in HCl, Ni amongst elements tested, had the maximum stabilizing effect on ascorbic acid.

Nickel favours the oxidation of phenols in infected wheat plants (Bradfield, 1976). In pea plants, the formation of citrin is promoted by ${\rm NiSO_4}$ treatment (Alagna *et al.*, 1984). Tips of intact roots of pea incubated with indole-acetic acid solution rapidly accumulated indole acety1-aspartic acid but the excised roots had completely lost this ability (Andrae and Van Ysseltein, 1960). However, the presence of sucrose, glucose, fructose, mannitol or Ni in the solution restored this ability in excised roots.

ENZYME ACTIVITY

Several investigators have measured the activities of enzymes in response to Ni. The micronutrient plays a significant role in the enzyme catalysed metabolic processes, often functioning as a cofactor, as is evident from the Table 5. Most of the enzymes studied are increased by Ni treatment. Several possibilities regarding enzyme inhibition by Ni have been suggested. However, studies with purified enzyme preparation are required to define the molecular mechanism of Ni action.

Some doubt as to the specificity of Ni for urease has been casted by work with the duckweed (*Lemna paucicostata*) growing in sterile culture (Gordon *et al.*, 1978). However, the growth of Lemna with urea as a source of Nitrogen was considerably stimulated by the addition of Ni ions but the role of Ni in urease function is unquestionable. Dixon *et al.* (1975) suggested that Ni might have a specific

Table 5: Some examples of effects of anion enzyme activities in plants

	amples of effects of affion enzyme	Ni conc. (ppm)		
Enzyme	Plant material	and salt supplied	Effect	Reference
Acidinorganc				
pyrophosphtes	Rice leaves	237.7 NiCl ₂	Increased	Miller et al. (1970)
Acid phosphates	Germinating maize seeds	5-10 NiCl ₂	Increased	Crooke (1956)
Aldolase (FDP)	Radish and leaves	0.1 NiCl ₂	Increased	Persans et al. (1999)
Germinating	Maize and mung bean seeds	5-10 NiCl ₂	Increased	Mishra and Kar (1974)
Amylase				
Agrinase	Jack bean	293.55 NiSO ₄	Increased	Mishra and Kar (1974)
Catalase	Mature plant seeds		Increased	Delton et al. (1985)
	Germinating barley seeds	293.55 NiSO ₄	Increased	Delton et al. (1985)
	Cucumis milo L.	1467.75 NiSO ₄	Increased	Agarwal et al. (1976)
	Detached buds of median	Low conc. (variable),	
		NiSO ₄	Increased	Agarwal et al. (1976)
	Pink (Dianthus deltoids)	262.5, NiSO ₄	Increased	Agarwal et al. (1976)
	Phaseolus radiates	Variable, NiCl ₂	Increased	Dixon et al. (1980a)
Hydrogenase	Soybean plants and			
	root nodules	$100\mathrm{NiCl_2}$	Increased	
	Soybean plants and root nodules	100 NiCl ₂	Increased	Kersten et al. (1980)
Nitrateredutase	Young grains and of Sorghum	234.84 NiSO ₄	Increased	Nensen and Daly (1960)
	Sudangrass (Sorghum			
	sudanese (P) step.f.)	234.84 NiSO ₄	Increased	Mac Lean and Dakker (1978)
Peroxidesactivity	Mustard leaves	Low con. NiSO ₄	Increased	De Kock and Mitchell (1957)
Ribonuclease	Seescing detached rice leaves	237.7 NiCl ₂	Increased	Roger et al. (1975)
Urease	Soybean Plants	13 NiSO ₄	Increased	Dixon et al. (1980c)

function in higher plants as a component of urease. Nickel is not required for the synthesis of the enzyme protein but as metal component it is essential for the structure and functioning of enzyme (Klucas *et al.*, 1983; Roach and Barcloy, 1946). The urease enzyme consists of six subunits with two Ni atom's in each subunit (Dan *et al.*, 2000; Winker *et al.*, 1983; Polacco, 1977b). The function of Ni is supported by results of Pollacco (1976), Crooke *et al.* (1954), Walker *et al.* (1985), Bazzaz *et al.* (1974) and Persans *et al.* (1999) with cell culture of soybean. In the absence of added Ni, urease activity was low and growth was poor when urea was the source of nitrogen. Browen *et al.* (1987) reported that breakdown of purine bases (adenine and guanine) occurs via ureides in all plants. Thus, it seemed likely that all plants probably required urease and Ni. Urease contains two Ni ions at the active site (Ciurli, 2001). Ni can also replace Zn or Fe and other metal ions, in certain other metalloenzymes of lower plants (Mulrooney and Hausinger, 2003). Circumstantial evidence indicates that ureide-transporting species, such as pecan, possess a higher Ni requirement than amidetransporting species (Wood, 2006), thus raising the possibility that ureide transporters might possess enzymes, other than urease, that require Ni for activation or for enhanced activity. Likely candidates are one or more enzymes affecting ureide catabolism.

In some experiments, it has been demonstrated that citrate inhibits the growth of soybean callus tissue culture when urea is the sole source of nitrogen but not when other Nitrogen sources are used. This inhibition is relieved off by the addition of 0.6 ppm NiCl₂(Maranville, 1970; Bazzaz *et al.*, 1974). It appears that citrate preferentially chelates Ni present in the culture solution, thereby preventing the development of functional urease unless excess Ni ions are added. Analogous studies with tissue culture of two other species of tobacco and rice have shown that the urease of these organisms also requires Ni (Persans *et al.*, 1999).

The question of a more extensive role of urease (and hence of Ni) has also been again raised. The presence of ureides and related guanidine compounds in plants tissue is well established (Browen *et al.*, 1980). For the plant to make full use of nitrogen of such compounds, the presence of urease (and therefore Ni) would be required (Saguer *et al.*, 1998). Delton *et al.* (1985) reported that Ni fertilizers increased leaf urease and nodule hydrogenase activity of soybean grown in low-Nickel soil,

although the yield was not improved. Krog Nier *et al.* (1991) examined the effects of Ni deficiency in soybean and concluded that Ni deficient plants may have a lower urease activity than the plants that are not deficient in Ni and may, therefore, be more susceptible to leaf burn when foliar fertilized with urea.

CONCLUSION

Adequate literature does not exist to understand fully the mechanism of action of Ni in plants, although it is an important environmental contaminant. Several important elements such as N(nitrogen), S(sulphur), P(phosphorus) and metabolism of macro-molecules (Proteins, nucleic acid etc.) are untouched. The literature survey, however demonstrates that the growth of plants certainly responds to Ni, which of course varies according to the species, concentration of Ni and also according to the soil nutrient composition. Plant can not complete their life cycle without adequate Ni. Nickel can not be replaced by Al, Cd for the growth of soybeans. This is, in conjunction with the findings that Ni is essential for cowpeas and produces beneficial growth responses in several plants species, suggests that Ni should be classified as a micronutrient element essential for higher plants.

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