14 Nickel

Patrick H. Brown
University of California, Davis, California

CONTENTS

14.1 INTRODUCTION

Nickel (Ni), the most recently discovered essential element (1), is unique among plant nutrients in that its metabolic function was determined well before it was determined that its deficiency could disrupt plant growth. Subsequent to the discovery of its essentiality in the laboratory, Ni deficiency has now been observed in field situations in several perennial species (2). The interest of plant scientists in the role of nickel was initiated following the discovery in 1975 (3) that it was a critical constituent of the plant enzyme, urease. The ultimate determination that nickel was essential for plant growth (1) depended heavily on the development of new techniques to purify growth media and to measure extremely low concentrations of nickel in plants. The establishment of nickel as an essential element, however, highlights the limitations of the current definition of essentiality of nutrients as applied to plants (4). It has been argued, for example, that even though nickel is clearly a normal and functional constituent of plants, it does not fulfill the definition of essentiality, since urease is not essential for plant growth and nickel deficiency apparently does not prevent the completion of the life cycle of all species, even though that criterion has not been explicitly satisfied for any element (5). Several authors (5,6) now suggest that the criteria for essentiality should be modified to include elements that are normal functional components of plants.

As our ability to determine the molecular structure, function, and regulation of biological systems improves, it is quite likely that additional elements will be shown to have irreplaceable functions in discrete biochemical processes that are important for plant life. This determination will be supplemented by advances in molecular and structural biology that will help predict the occurrence of similar processes across all organisms, allowing the relevance of discoveries made in bacterial systems to be immediately tested in plant and animal systems. The discovery of the essentiality of nickel is a good illustration of this principle and is likely to be repeated in the coming years. Nickel represents the first of several likely new essential elements that will be shown to be critical for certain metabolic processes normally active in plants, but not necessarily essential for the completion of the species' life cycle under all conditions.

The current definition of essentiality is clearly inadequate and its acceptance likely stifles the search for new essential elements. It is proposed, therefore, that the definition of essentiality be modified to more closely resemble that utilized in animal biology (7).

> An element shall be considered essential for plant life if a reduction in tissue concentrations of the element below a certain limit results consistently and reproducibly in an impairment of physiologically important functions and if restitution of the substance under otherwise identical conditions prevents the impairment; and, the severity of the signs of deficiency increases in proportion to the reduction of exposure to the substance. (Nielson (7))

By this criterion, nickel is an essential element as are silicon and cobalt, which are essential elements for nitrogen-fixing plants.

14.2 DISCOVERY OF THE ESSENTIALITY OF NICKEL

The discovery in 1975 that nickel is a component of plant urease (3) prompted the first detailed studies on the essentiality of nickel for plant life. In 1977, Polacco (8) determined that tissue-cultured soybean (*Glycine max* Merr.) cells could not grow in the absence of nickel when provided with urea as the sole nitrogen source. Subsequently, many researchers demonstrated that plant growth is severely impacted by nickel deficiency when urea is the sole nitrogen source (9–14).

These results, though compelling, demonstrated a role for nickel only in certain species when grown with urea as the sole nitrogen source and as such did not satisfy the established criteria for essentiality, which state that an element is essential if without the element, the plant cannot complete its life cycle and the element is a constituent of an essential plant metabolite or molecule (4). Essentiality of nickel was subsequently established in 1987, when Brown et al. (1) demonstrated that barley (*Hordeum vulgare* L. cv. 'Onda') could not complete its life cycle in the absence of added nickel, even when plants were supplied with a nonurea source of nitrogen. In addition, it was shown that growth of oats (*Avena sativa* L. cv. 'Astro') and wheat (*Triticum aestivum* L. cv. 'Era') were significantly depressed under nickel-deficient conditions (15). The laboratory-based observations that Ni deficiency impacts a diversity of plant species has recently been verified in a diverse number of perennial species (*Carya*, *Betula*, *Pyracantha*) growing in the acidic low-nutrient soils of southeastern United States (2).

Nickel is now generally accepted as an essential ultra-micronutrient (16); however, the only defined role of nickel is in the metabolism of urea, a process that is not thought to be essential for plants supplied with a nitrogen source other than urea. The possibility that additional roles for nickel in plants exist was suggested by the results of Brown et al. (1,15), who demonstrated an effect of nickel deprivation in plants grown in the absence of urea and is implied in the work of Wood et al. (2), who demonstrated field responses to Ni supplementation in many ureide-transporting hydrophiles. A broader biological significance of nickel is also implied in the demonstration that nickel is essential for animal life and for a range of bacterial enzymes, including key enzymes in the nitrogen-fixing symbiont, *Bradyrhizobium japonicum* (17).

Our knowledge of the complete biological significance of nickel for plant productivity is still quite limited; however, with the demonstration of the essentiality of nickel in diverse species (1,2)

and the increased use of urea as a nitrogen source, the importance of understanding the chemistry and biology of nickel and its potential impact on agricultural production has never been greater. Evidence that nickel plays an important function in animal and bacterial systems also suggests that nickel plays a larger role in plant productivity than is currently recognized. To obtain a full understanding of the potential role and management of nickel in agricultural systems, it is necessary to review the roles of nickel in other biological systems and to understand the plant and soil conditions under which nickel deficiency is likely to occur.

14.3 PHYSICAL AND CHEMICAL PROPERTIES OF NICKEL AND ITS ROLE IN ANIMAL AND BACTERIAL SYSTEMS

Nickel is a first-row transition metal with chemical and physical characteristics ideally suited to biological activity (18). Divalent nickel is the only oxidation state of nickel that is likely to be of any importance to higher plants. Nevertheless, Ni^{2+} forms a bewildering array of complexes with a variety of coordination numbers and geometries (19). Nickel readily binds, complexes, and chelates a number of substances of biological interest and is ubiquitous in all biological systems. Nickel is now known to be a functional constituent of seven enzymes, six of which occur in bacterial and animal systems, but not known to be active in plants, but the seventh enzyme, urease, is widely distributed in biology. The sensitivity of known biological nickel–complex equilibriums to temperature, concentration, and pH also make nickel an ideal element for the fine control of enzyme reactions (18).

14.3.1 Nickel-Containing Enzymes and Proteins

The field of nickel metallobiochemistry has seen tremendous growth over the preceding 10 years, and nickel is clearly a biologically important element in a diverse range of organisms. Indeed, it is highly likely that with the advent of molecular techniques to search for genetic and functional homology rapidly, the diversity of known functions of nickel in biology will increase substantially in the coming years. Advances in the field of bacterial and animal biology will rapidly flow to the plant sciences.

To date, seven nickel-dependent enzymes have been identified. Two of these enzymes have nonredox function (urease and glyoxylase), and the remaining five involve oxidation–reduction reactions (Ni-superoxide dismutase, methyl coenzyme M reductase, carbon monoxide dehydrogenase, acetyl coenzyme A synthase, and hydrogenase).

In all microorganisms that produce nickel-dependent metalloenzymes, there exist a number of proteins involved in nickel uptake, transport storage, and incorporation into the metalloenzyme. In bacteria, the transport of nickel into the cell involves two high-affinity transport systems, an ATP-dependent Nik family (Nik a–e) in *Escherichia coli* and a variety of nickel permeases (NixA, HoxN, etc.) in diverse species (17). Incorporation of nickel into the metalloenzyme involves a number of accessory proteins including metallo-chaperones (UreE, HypB, and CooJ) involved in nickel storage and in protein assembly (17).

Of the established nickel enzymes and proteins, urease is the sole nickel-specific enzyme known to function in plants; however, nickel-dependent hydrogenase also indirectly influences plant productivity through its role in nitrogen-fixing symbionts (20) and in leaf commensal bacteria (21). Currently, none of the bacterial proteins involved in nickel uptake and assimilation (NikA, NixA, UreE, etc.) is known to be present in plants. Interestingly, the hydrogenase and urease activities of leaf-surface symbionts are clearly inhibited when they colonize urease-deficient soybean mutants (21). The mechanism by which this inhibition occurs is unknown but may suggest that the urease-deficient mutants lack key nickel assimilatory proteins, thus preventing the transfer of nickel to the leaf-surface bacterial enzymes. This possibility would suggest that plants might contain nickel-dependent assimilatory proteins.

Nielsen reported the first description of a dietary deficiency of nickel in animals in 1970 for chickens and later for rats (*Rattus* spp.), goats (*Capra hircus*), sheep (*Ovis aries*), cows (*Bos taurus*), and mini pigs (*Sus scrofa*) (7). Nickel deficiency in these animals results in growth depression, physiological and anatomical disruption of liver function, and disruption of iron, copper, and zinc metabolism resulting in reduced levels of these enzymes in blood and various organs (22). Nickel deficiency also markedly reduces the activity of a number of hepatic enzymes, including several hydrogenases, urease, and glyoxylase, though a specific functional role for nickel in these enzymes in animals has not been determined.

One of the important and consistent findings from animal studies is that nickel deficiency induces iron deficiency, an observation that is also made in plants (15). In rats (22), and in sheep (23), nickel deprivation resulted in decreased iron uptake and reduced tissue-iron concentrations. Nielsen et al. (24) have suggested several possible roles for nickel in iron metabolism and oxidation–reduction (redox) shifts that draw upon the observation that nickel and iron are associated in a number of bacterial redox-based enzymes (17).

The suggestion that additional nickel-dependent enzymes and proteins are present in higher plants is supported by the observation that several of the known bacterial nickel-containing enzymes have analogs in plants and animals (including superoxide dismutase, glyoxylase, acetyl coenzyme A synthase, and hydrogenase). Our current failure to identify additional nickel-dependent enzymes in plants is likely a result of the relatively primitive state of plant enzymology, in contrast to bacterial enzymology, and the difficulty involved in research on complex organisms involving ultra-trace elements. The similarity between the effects of nickel deficiency in animals and plants also provides evidence of a common biological role for nickel in all organisms.

14.3.2 Essentiality and Function of Nickel in Plants

The first evidence of a response of a field crop to application of a nickel fertilizer was demonstrated in 1945 for potato (*Solanum tuberosum* L.), wheat (*Triticum aestivum* L.), and bean (*Phaseolus vulgaris* L.) crops (25). In these crops, the application of a dilute nickel spray resulted in a significant increase in yield. These experiments were conducted on the 'Romney Marshes' of England, a region that is well known for its trace mineral deficiencies, particularly of manganese and zinc. These experiments were conducted very carefully and excluded the possibility that the nickel applied was merely substituting for manganese, zinc, iron, copper, or boron, suggesting that the growth response was indeed due to the application of nickel. Interestingly, the soils of this region may be low in nickel since the conditions that limit manganese and zinc availability in these soils (acid sands of low mineral content) would also limit nickel availability to crops, and the concentrations of nickel provided were appropriate based on the current knowledge of nickel demand. These same soil types also dominate the region of southeast United States where Ni deficiency is now known to occur.

Mishra and Kar (26) and Welch (27) reviewed the evidence of the role of nickel in biological systems and cited many examples of yield increases in field-grown crops in response to the application of nickel to the crop or to the soil. The significance of these purported benefits of field applications of nickel is difficult to interpret since the majority of the reported experiments used very high nickel application rates. None of these reports considered the possibility that nickel influenced plant yield through its effect on disease suppression, nor was the nickel concentration in the crops determined. Indeed, prior to the availability of graphite-furnace atomic absorption spectrophotometers and inductively coupled plasma mass spectrometers (in the mid-1970s), it was exceedingly difficult to measure nickel at the concentrations (<0.1 mg Ni kg^{-1} dry weight) later shown to be critical for normal plant growth. In the absence of information on tissue-nickel concentrations, it is impossible to conclude that the observed yield increases were the result of a correction of a nickel deficiency in the plant.

Clear evidence that nickel application benefited the growth of nitrogen-fixing species of plant was demonstrated by Bertrand and DeWolf (28), who reported that soil-nickel application to field-grown

soybean (*Glycine max* Merr.) resulted in a significant increase in nodule weight and seed yield. The authors suggested that the yield increase was the result of a nickel requirement of the nitrogen-fixing rhizobia. A specific role for nickel in nitrogen-fixing bacteria is now well established with the determination that a nickel-dependent hydrogenase is active in many rhizobial bacteria (20) and is thus essential for maximal nitrogen fixation (29). Nickel is also known to be essential for nitrogen fixation of the free-living cyanobacterium, *Nostoc muscorum* C.A. Adargh, though the specific mechanism has not been determined (30).

A role for nickel in plant disease resistance has long been observed and has been variously attributed to a direct phyto-sanitary effect of nickel on pathogens, or to a role of nickel on plant disease-resistance mechanisms. Mishra and Kar (26) concluded that nickel likely acted to reduce plant disease by direct toxicity to the pathogen. Nickel, however, is not particularly toxic when applied directly to microorganisms, and Graham et al. (31) demonstrated that nickel supplied to the roots of cowpea (*Vigna unguiculata* Walp.) that contained only 0.03 mg Ni kg^{-1} dry weight effectively reduced leaf-fungal infection by 50%. Whether this effect was directly due to a role of nickel in plant defense reactions (possibly involving superoxide dismutase-mediated processes) or a consequence of the alleviation of deficiency-induced changes in nitrogen metabolites (urea, amino acids, etc.) is uncertain. Regardless of the mechanism, a positive effect of nickel supplementation on disease tolerance was clearly documented.

The discovery that nickel is a component of the plant urease in 1975 (3) prompted a renewed interest in the role of nickel in plant life. In 1977, Polacco (32) determined that tissue-cultured soybean cells could not grow in the absence of nickel when provided with urea as the sole nitrogen source. Subsequently, an absolute nickel requirement was demonstrated for tissue-cultured rice (*Oryza sativa* L.) and tobacco (*Nicotiana tabacum* L.) (26,27). This finding was followed in 1981 by a review of nickel in biology that suggested that leguminous plants might have a unique requirement for nickel (28).

Using a novel chelation chromatography technique to remove nickel as a contaminant from the nutrient media, Eskew et al. (9,33,34) and Walker et al. (11) demonstrated that, under nickel-deficient conditions, urea accumulated to toxic levels in the leaves of soybean and cowpea. Leaflet tips of nickel-deficient plants contained concentrations of urea as high as 2.4% dry weight. The accumulation of urea occurred irrespective of the nitrogen source used and was assumed to have occurred as a result of urease-dependent disruption of the arginine-recycling pathway. Eskew et al. (9) concluded that nickel was an essential element for leguminous plants though they did not demonstrate a failure of nickel-deficient plants to complete their life cycles. Recently, Gerendas et al. (12–14), in a series of elegant studies demonstrated a profound effect of nickel deficiency on the growth of urea-fed tobacco, zucchini (*Cucurbita pepo* L.), rice, and canola (*Brassica napus* L.), but observed no growth inhibition when nitrogen sources other than urea were used.

Confirmation that nickel was essential for higher plants was provided by Brown et al. (1), who demonstrated that barley seeds from nickel-deprived plants were incapable of germination even when grown on a nitrogen source other than urea. Significant restrictions in shoot growth of barley, oats, and wheat (*Triticum aestivum* L.) were subsequently demonstrated under nickel-deficient conditions when the plants were supplied with mineral nitrogen sources (15). Brown et al. (15) also observed a marked suppression in tissue-iron concentrations in nickel-deficient plants, a response that is also observed in nickel-deficient animals (7). Reductions in tissue-malate concentrations have also been observed in nickel-deficient animals and plants (15,24,35). Confirmation of the essentiality of Ni under field conditions was provided in 2004 by Wood et al. (2), who observed a marked and specific positive response to application of Ni fertilizer to pecan (*Carya illinoinensis* K. Koch) and other species (2) that could not be corrected with any other known essential element.

The demonstration of a role for nickel in diverse plant species, the presence of nickel in a discrete metabolic process, and the failure of plants to complete their life cycles in the absence of nickel, satisfies the requirement for the establishment of essentiality (4).

Although nickel has been accepted generally as an essential element, there is reason to be cautious about this conclusion, and some authors suggest that nickel may not fully satisfy the most stringent interpretation of the laws of essentiality primarily since its role in a specific essential metabolic function has not been identified. Furthermore, even though nickel has a clear role in metabolism, it is now clear that urease is not, by itself, essential for plant life as evidenced by the observation that urease-null soybean mutants can complete their life cycles (37). There has also been no independent replication of the effect of nickel on barley grain viability though Horak (36) did observe a marked increase in seed viability with the addition of nickel to pea (*Pisum sativum* L.) seeds grown in nickel-deficient soils.

Regardless of these apparent contradictions, nickel is still clearly required for normal plant metabolism. As a component of urease, nickel is required for urea and arginine metabolism, and both of these metabolites are normal constituents of plants (5). Nickel is also an essential component of hydrogenases involved in nitrogen fixation and other associative bacterial processes, and nickel clearly influences plant response to disease. Nickel is clearly a normal constituent of plant life.

Many of the reported effects of nickel on plant growth cannot be attributed solely to the role of nickel in urease, and many symptoms of nickel deficiency (disrupted iron and malate metabolism) are also observed in animals (7). It is likely, therefore, that additional nickel-dependent enzymes and proteins await discovery and will help resolve the remaining questions on the function of nickel in plants.

14.3.3 Influence of Nickel on Crop Growth

Many early reports of the role of nickel in agricultural productivity have been questioned since they did not adequately exclude the possibility that nickel was acting directly as a fungicidal element (27). Regardless of the many questionable reports, a compelling body of literature exists in which appropriate concentrations of nickel were applied or where the plant response is consistent with current knowledge of nickel functions including effects on nitrogen fixation, seed germination, and disease suppression (26,27,31,34,38,39).

The clearest agronomic responses to nickel have been observed when nitrogen is supplied as urea or by nitrogen fixation. The most illustrative example of the relationship between nickel and urea metabolism is provided from studies with foliar urea application and tissue-culture growth of plants. Plants without a supply of nickel have low urease activity in the leaves, and foliar application of urea leads to a large accumulation of urea and severe necrosis of the leaf tips (34). Nicoulaud and Bloom (40) observed that nickel, provided in the nutrient solution of tomato (*Lycopersicon esculentum* Mill.) seedlings growing with foliar urea as the only nitrogen source, significantly enhanced growth. The authors speculated that the effect of nickel was more consistent with its role in urea translocation than that on urease activity directly (40). This result is in agreement with the findings of Brown et al. (15), who suggest that nickel has a role in the transport of nitrogen to the seed thereby influencing plant senescence and seed viability.

The first demonstration of an agricultural Ni deficiency did not occur until 2004 (Wood et al., 2004), when it was observed in pecan (*Carya illinoinensis*). Nickel deficiency in pecan is associated with a physiological disorder 'mouse-ear' which occurs sporadically, but with increasing frequency, throughout the southeastern United States (portions of South Atlantic region) where it represents a substantial economic impact. In agreement with the results of Brown et al. (1), Ni deficiency in pecan results in a disruption of nitrogen metabolism and altered amino acid profiles (72).

The value of addition of nickel to Murashige and Skoog plant tissue-culture medium was shown by Witte et al. (41). These authors suggested that the lack of nickel and urease activity may represent a stress factor in tissue culture and recommended that the addition of 100 nM Ni be adopted as a standard practice. The benefits of adding nickel to solution cultures was also demonstrated by Khan et al. (42), who determined that a mixture of 0.05 mg Ni L^{-1} and 20% nitrogen as urea resulted in optimal growth of spinach (*Spinacia oleracea* L.) under hydroponic conditions.

14.4 DIAGNOSIS OF NICKEL STATUS

14.4.1 Symptoms of Deficiency and Toxicity

In legumes and other dicotyledonous plants, nickel deficiency results in decreased activity of urease and subsequently in urea toxicity, exhibited as leaflet tip necrosis (9–11). With nitrogen-fixing plants or with plants grown on nitrate and ammonium, nickel deficiency results in a general suppression in plant growth with development of leaf tip necrosis on typically pale green leaves (9,10) (Figure 14.1 and Figure 14.2). These symptoms were attributed to the accumulation of toxic levels of urea in the leaf tissues.

In graminaceous species (Figure 14.3), deficiency symptoms include chlorosis similar to that induced by iron deficiency (1), including interveinal chlorosis and patchy necrosis in the youngest leaves. Nickel deficiency also results in a marked enhancement in plant senescence and a reduction in tissue-iron concentrations. In monocotyledons and in dicotyledons, the accumulation of urea in leaf tips is diagnostic of nickel deficiency. In early or incipient stages of nickel toxicity, no clearly visible symptoms develop, though shoot and root growth may be suppressed. Acute nickel toxicity results in symptoms that have variously been likened to iron deficiency (interveinal chlorosis in

FIGURE 14.1 Nitrogen-fixing cowpea seedlings (*Vigna unguiculata* Walp.) were grown for 40 days in nutrient solutions containing either 1 (left) or 0 $\mu g\ L^{-1}$ (right) and supplied with no inorganic nitrogen source. In the absence of nickel, plants developed pronounced leaf tip necrosis and marked yellowing and growth stunting. The observed symptoms closely resemble those of nitrogen deficiency. (Photograph by David Eskew.) (For a color presentation of this figure, see the accompanying compact disc.)

FIGURE 14.2 Leaf tip necrosis in soybean plants (*Glycine max* Merr.) grown in nutrient solution provided with equimolar concentrations of nitrate and ammonium. Solutions were made free from nickel by first passing solutions through a nickel-specific chelation resin. Leaf tip necrosis was observed coincident with the commencement of flowering. (Photograph by David Eskew.) (For a color presentation of this figure, see the accompanying compact disc.)

monocotyledons, mottling in dicotyledons) or zinc deficiency (chlorosis and restricted leaf expansion) (1,2,43). Severe toxicity results in complete foliar chlorosis with necrosis advancing in from the leaf margins, followed by plant death.

In pecan growing in the southeastern United States, the long-described but poorly understood symptoms of 'mouse-ear' or 'little-leaf disorder' (Figure 14.4) have recently been shown to be due

FIGURE 14.3 Nickel deficiency symptoms in barley (*Hordeum vulgare* L. cv. Onda) following 50 days growth in nutrient solution containing equimolar concentrations of nitrate and ammonium. Symptoms include leaf-tip chlorosis and necrosis, development of thin 'rat-tail' leaves, and interveinal chlorosis of young leaves. (Photograph by Patrick Brown.) (For a color presentation of this figure, see the accompanying compact disc.)

FIGURE 14.4 Branches of nickel-sufficient (left) and nickel-deficient (right) pecan (*Carya illinoinensis* K. Koch). Symptoms include delayed and decreased leaf expansion, poor bud break, leaf bronzing and chlorosis, rosetting, and leaf tip necrosis. (Photo courtesy of Bruce Wood.) (For a color presentation of this figure, see the accompanying compact disc.)

to nickel deficiency that can be cured by application of nickel (at 100 mg L^{-1}) (2). Nickel deficiency in pecan and in certain other woody perennial crops (e.g., plum, peach and pyracantha, and citrus) is characterized by

> early-season leaf chlorosis, dwarfing of foliage, blunting of leaf or leaflet tips, necrosis of leaf or leaflet tips, curled leaf or leaflet margins, dwarfed internodes, distorted bud shape, brittle shoots, cold-injury-like death of over-wintering shoots, diminished root system with dead fibrous roots, failure of foliar lamina to develop, rosetting and loss of apical dominance, dwarfed trees, and tree death (Wood et al. (2))

Nickel deficiency was long unrecognized in this region because of its similarity to zinc deficiency and as a consequence of a complex set of factors that influences its occurrence. Nickel deficiency is induced by: (a) excessively high soil zinc, copper, manganese, iron, calcium, or magnesium; (b) root damage by root-knot nematodes; or (c) dry or cool soils at the time of bud break (2). The conditions under which Ni deficiency occurs also commonly result in a deficiency of zinc or copper, and this fact has resulted in the extensive use of copper and zinc fertilizers over many years further exacerbating the nickel deficiency. In many horticultural tree species, heavy application of fertilizers with zinc, copper, or both nutrients is common for their nutritional values and benefits for leaf removal and disease protection. In many orchard crops recalcitrant physiological disorders and poorly understood replant 'diseases' are frequent suggesting that induced nickel deficiency may be much more widespread than was previously recognized.

14.5 CONCENTRATION OF NICKEL IN PLANTS

The nickel concentration (Table 14.1) in leaves of plants grown on uncontaminated soil ranges from 0.05 to 5.0 mg Ni kg^{-1} dry weight (27,44,45). The adequate range for nickel appears to fall between 0.01 and 10 mg Ni kg^{-1} dry weight, which is an extremely wide range compared to that for the other elements (5). The critical nickel concentration required for seed germination in barley, shoot growth in oat, barley, and wheat, and shoot growth of urea-fed tomato, rice, and zucchini (*Cucumus pepo* var. *melopepo* Alef.) has been estimated independently by two groups to be approximately 100 mg Ni kg^{-1} (1,5), which is similar to the recently determined Ni requirement for pecan (2).

TABLE 14.1
Concentration Ranges of Nickel in Crop Species

		Concentrations of Nickel in Plants (mg Ni kg^{-1})				
Plant Species	**Scientific Name**	***Deficient***	***Critical (deficiency)***	***Adequate***	***Critical (toxicity)***	***Reference***
Barley	*Hordeum vulgare* L., *H. distichon* L.	—	0.1	—	—	1,15
Wheat	*Triticum aestivum* L., *T. durum* Desf	0.037		0.084	63–113	15,53
Cowpea	*Vigna unguiculata* Walp	<0.01–0.142		0.22–10.3		11
Beans	*Phaseolus vulgaris* L.				10–83	54
Oats	*Avena sativa* L.	0.017		0.10		15
Soybean	*Glycine max* Merr.		0.02–0.04			10
Italian ryegrass	*Lolium multiflorum* Lam.			0–8	>80	55
Pecan	*Carya illinoinensis* K. Koch		0.1			2

Nickel concentrations above the toxicity levels of >10 mg kg^{-1} dry weight in sensitive species, and >50 mg kg^{-1} dry weight in moderately tolerant ones (44,45,46) result in impaired root and shoot growth without any remarkable defining characteristics (47).

The nickel content of a plant is determined by the nickel availability in the soil, plant species, plant part, and season. Plants growing on serpentine soils (derived from ultramific rocks) or contaminated soils can accumulate high levels of nickel and other heavy metals (48,49). In naturally occurring high-nickel soils (serpentine soils) highly specialized plant species have evolved including several species that hyperaccumulate nickel, sometimes up to 1 to 5% of tissue dry weight (50,51). Species growing on the same soil can also vary dramatically in nickel content and within plant distribution. In general, nickel is transported preferentially to the grain, particularly under conditions of marginal nickel supply (52).

14.6 UPTAKE AND TRANSPORT

In bacterial systems, several families of nickel permeases and ATP-dependent nickel carriers have been characterized. No equivalent mechanism has yet been identified in animals or plants (17). In plant systems, most studies have been conducted at unrealistically high soil-nickel concentrations and as such may be relevant for nickel toxicity, but are not relevant for nickel uptake under normal conditions. Cataldo et al. (56) using ^{63}Ni indicated that a high-affinity Ni^{2+} carrier functioned at 0.075 or 0.25 μM Ni^{2+} with a K_m of 0.5 μM which approaches the nickel concentration in uncontaminated soils (48). Either Cu^{2+} or Zn^{2+} competitively inhibits Ni^{2+} uptake suggesting that all the three elements share a common uptake system (57). Uptake at higher nickel-supply levels (0.5 to 30 μM) was energy dependent and had a K_m of 12 μM indicative of an active, low-affinity transport system.

No evidence suggests that associations with arbuscular mycorrhizal fungus increase nickel accumulation by plants (58,59).

Nickel, unlike many other divalent cations, is readily re-translocated within the plant likely as a complex with organic acids and amino acids (60). Nickel rapidly re-translocates from leaves to young tissues in the phloem, particularly during reproductive growth. Indeed, up to 70% of nickel in the shoots was transported to the seed of soybean (61). Nickel is associated primarily with organic acids and amino acids in the phloem. Above pH 6.5, histidine is the most significant chelator, whereas at pH <5, citrate is the most significant one (5).

14.7 NICKEL IN SOILS

14.7.1 Nickel Concentration in Soils

Nickel is abundant in the crust of the Earth, comprising about 3% of the composition of the earth. Nickel averages 50 mg Ni kg^{-1} in soils and commonly varies from 5 to 500 mg Ni kg^{-1} but ranges up to 24,000 to 53,000 mg Ni kg^{-1} in soil near metal refineries or in dried sewage sludge, respectively. Agricultural soils typically contain 3 to 1000 mg Ni kg^{-1}, whereas soils derived from basic igneous rocks may contain from 2000 to 6000 mg Ni kg^{-1} (62).

Total nickel content is, however, not a good measure of nickel availability. At pH>6.7, most of the nickel exists as sparingly soluble hydroxides, whereas at pH<6.5, most nickel compounds are relatively soluble (48). Depending on the soil type and pH, nickel may also be highly mobile in soil and is further mobilized by acid rain. The role of pH in nickel availability was illustrated by Van de Graaff et al. (63), who observed that long-term irrigation with sewage effluent increased heavy metal loading in soil, but that plant metal contents did not increase, apparently owing to the increased soil pH, iron complexation and coprecipitation, and precipitation of phosphorus–metal complexes.

Truly nickel-deficient soils have not been identified to date; however, Ni deficiency can occur as a result of excessive use of competing ions (Zn, Cu, and MgO and unfavorable growth conditions (2)).

Nickel is the 24th-most abundant element in the crust of the earth, and plant nickel requirement ($<0.05\,mg\,kg^{-1}$ dry weight) is the lowest of any essential element. Although a large number of analyses have been conducted for nickel in plant tissues, no recorded levels have been below $0.2\,mg\,kg^{-1}$ dry weight in field-grown plants. Nickel can be supplied by atmospheric deposition, at rates that easily exceed the removal from the crops in the field (64). The ubiquitous nature of nickel is illustrated by the experiments that established the essentiality of nickel (1). In these experiments, the authors went to extraordinary lengths to purify or re-purify all chemical reagents, equipment, and water and to maintain contaminant-free growing conditions. Even under these conditions, it required three generations of crop growth to deplete the nickel carried over from the grain before the first evidence of nickel deficiency was observed.

The possibility that nickel-deficient soils exist, however, cannot be discounted particularly as purity of fertilizers is improved, the use of urea is increased, and atmospheric deposition of pollutant nickel is decreased. Plants grown under specialized conditions (greenhouses and tissue culture), particularly with urea as a nitrogen source, may be especially susceptible to nickel deficiency (40).

Nickel toxicity, which is usually associated with serpentine soils, sewage-sludge application, or industrial pollution, is a well-described constraint on crop production in many parts of the world. In serpentine soils (derived from basic igneous rocks), nickel concentrations may range from 1000 to $6000\,mg\,kg^{-1}$ dry weight and are frequently associated with high concentrations of iron, zinc, and chromium and an unfavorable ratio of magnesium to calcium. Values for ammonium acetate-extractable nickel in these soils varies from 3 to $70\,mg\,kg^{-1}$; however, it is not always clear that poor plant growth can be ascribed to any single factor concerning nickel.

Similarly, in sewage-amended soils or in contaminated soils, it is often difficult to relate total nickel load with plant productivity as factors such as the chemical properties of the contaminant and base soil, pH, and oxidation–reduction state affect results (48,65). Indeed, the importance of considering soil pH is well illustrated by Kukier and Chaney (65 and references therein), who demonstrated that addition of limestone to raise soil pH is highly effective in immobilizing nickel *in situ* and in reducing phytotoxicity. Plant species also differ in their ability to obtain nickel from soils and hence any measurement of soil nickel must be interpreted with consideration of the plant species of interest.

14.7.2 Nickel Analysis in Soils

A large number of approaches, including diethyltriaminepentaacetic acid (DTPA), $BaCl_2$, $Sr(NO_3)_2$, and ammonium acetate among others (48,65) are used to extract metals from soils in an attempt to predict nickel availability to plants. The DTPA method, however, is probably the most commonly used (48,66,67) and has been shown to be quite effective for a variety of soils to define Ni excess. The DTPA method is improved significantly if factors such as soil pH and soil bulk density are incorporated into the resulting regression equation (65). Many authors (48,65), however, observe that plant species and soil environment (water, oxygen content, and temperature) can markedly affect the relationship between soil-extractable and plant-nickel concentrations (2). These results suggest that the condition under which the soil is collected and tested can significantly influence the interpretation of results. Nickel deficiency is also known to be exacerbated by environmental conditions that limit uptake (cold, wet weather) and by the oversupply of apparently competing elements such as Cu, Mn, Mg, Fe, Ca, and Zn (2). Nickel bioavailability can also be determined by the ion-exchange resin (IER) method, which has been used quite successfully in a limited number of soil types and facilitates the *in situ* assessment of exchangeable nickel (68).

14.8 NICKEL FERTILIZERS

Essentially under all normal field conditions, it is unlikely that application of nickel fertilizer will be required. Exceptions to this concept occur when urea is the primary source of nitrogen supply, in species in which ureides play an important physiological role (2), when excessive applications of

Zn, Cu, Mn, Fe, Ca, or Mg have been made over many years (2) and perhaps also in nitrogen-fixing crops grown on mineral-poor or highly nickel-fixing (high pH, high lime) soils. In experiments utilizing highly purified nutrient solutions or tissue-culture media, supplemental nickel may also be beneficial. In all of these cases, the nickel demand is quite low and can be satisfied easily with $NiSO_4$ or other soluble nickel sources including Ni–organic complexes (Bruce Wood, personal communication). In solution-grown plants and as a supplement to foliar urea applications, a nickel supply of 0.5 to 1 μM is sufficient.

Nickel is currently being applied to many fields in sewage sludge (48,69). In general, this usage does not represent a threat to human health, as its availability to crop plants is typically low. The total extractable nickel in these amended soils can also be controlled by selection of plant species and management of soil pH, moisture, and organic matter (65).

In recent years, a great deal of attention is being focused on nickel-accumulating plants that can tolerate otherwise nickel-toxic soils and accumulate substantial concentrations of nickel, up to 5% on a dry weight basis (70). Three nickel hyperaccumulators showed significantly increased shoot biomass with the addition of 500 mg Ni kg^{-1} to a nutrient-rich growth medium, suggesting that the nickel hyperaccumulators have a higher requirement for nickel than other plants (71). Considerable attention is also being focused on utilizing hyperaccumulating species for phytoremediation and phytomining, where they can be grown in a nickel-contaminated soil and then harvested and exported from the field. To date, however, this approach has not been successful owing to the small size and slow growth rate of many of the hyperaccumulating species. With a better understanding of the genetic basis of metal hyperaccumulation, it may be possible to transfer this trait into a fast-growing agronomic species and hence develop an effective phyoremediation strategy.

14.9 CONCLUSION

Nickel is the latest element to be classified as essential for plant growth in both laboratory and field conditions and an absolute requirement for nickel fertilizer under field conditions in perennial species growing in the southeast of the United States has now been established. Nickel clearly has a significant effect on the productivity of field-grown, nitrogen-fixing plants, those in which ureides are a significant form of nitrogen and those utilizing urea as a primary nitrogen source. The symptoms of nickel deficiency in barley, wheat, and oats observed by Brown et al. (1) and Wood et al. (2) are consistent with the observations made in nickel-deficient animals and are indicative of a role of nickel in nitrogen metabolism that cannot be easily explained through an exclusive role of nickel in urease. This finding in combination with the diverse known functions of nickel in bacteria suggests that nickel may indeed play a role in many, yet undiscovered processes in plants.

REFERENCES

1. P.H. Brown, R.M. Welch, E.E. Cary. Nickel: A micronutrient essential for higher plants. *Plant Physiol.* 85:801–803, 1987.
2. B.W. Wood, C.C. Reilly, A.P. Nyczepir. Mouse-ear of Pecan: A nickel deficiency. *HortScience* 39 (6):1238–1242, 2004.
3. N.E. Dixon, C. Gazzola, R.L. Blakeley, B. Zerner. Jack bean urease (EC 3.5.1.5). A metalloenzyme. A simple biological role for nickel. *J. Amer. Chem. Soc.* 97:4131–4133, 1975.
4. D.I. Arnon, P.R. Stout. The essentiality of certain elements in minute quantity for plants with special reference to copper. *Plant Physiol.* 14:371–375, 1939.
5. J. Gerendas, J.C. Polacco, S.K. Freyermuth, B. Sattelmacher. Significance of nickel for plant growth and metabolism. *J. Plant Nutr. Soil Sci.* 162:241–256, 1999.
6. E. Epstein, A.J. Bloom. Mineral Nutrition of Plants: Principles and Perspectives. 2nd edition. Sinauer Associates, Sunderland, MA, 2004, pp. 45.

7. F.H. Nielson. Nickel. In: E. Frieden, ed. *Biochemistry of the Essential Ultratrace Elements*. New York: Plenum Press, 1984, pp. 293–308.
8. J.C. Polacco. Nitrogen metabolism in soybean tissue culture: II Urea utilization and urease synthesis requires Ni^{2+}. *Plant Physiol*. 59:827–830, 1977.
9. D.L. Eskew, R.M. Welch, W.A. Norvell. Nickel: an essential micronutrient for legumes and possibly all higher plants. *Science* 222:621–623, 1983.
10. D.L. Eskew, R.M. Welch, W.A. Norvell. Nickel in higher plants. Further evidence for an essential role. *Plant Physiol.* 76:691–693, 1984.
11. C.D. Walker, R.D. Graham, J.T. Madison, E.E. Cary, R.M. Welch. Effects of nickel deficiency on some nitrogen metabolites in cow peas, *Vigna unguiculata*. *Plant Physiol*. 79:474–479, 1985.
12. J. Gerendas, S.B. Sattelmacher. Significance of Ni supply for growth, urease activity and the contents of urea, amino acids and mineral nutrients of urea-grown plants. *Plant Soil* 190:153–162, 1997.
13. J. Gerendas, S.B. Sattelmacher. Significance of N source (urea vs. NH_4NO_3) and Ni supply for growth, urease activity and nitrogen metabolism of zucchini (*Cucurbita pepo* convar. *giromontiina*). *Plant Soil* 196:217–222, 1997.
14. J. Gerendas, S.B. Sattelmacher. Influence of Ni supply on growth, urease activity and nitrogen metabolites of *Brassica napus* grown with NH_4NO_3 or urea as nitrogen source. *Ann. Bot*. 83:65–71, 1999.
15. P.H. Brown, R.M. Welch, E.E. Cary, R.T. Checkai. Beneficial effects of nickel on plant growth. *J. Plant Nutr*. 10:2125–2135, 1987.
16. H. Marschner. *Mineral Nutrition of Higher Plants,* 2nd edition. London: Academic Press, 1995, pp. 364–369.
17. M.J. Maroney. Structure/function relationships in nickel metallobiochemistry. *Curr. Opin. Chem. Biol*. 3:188–199, 1999.
18. D.A. Phipps. *Metals and Metabolism*. Oxford: Clarendon Press, 1976, pp. 28–56.
19. E. Frieden. *Biochemistry of the Essential Ultratrace Elements*. New York: Plenum Press, 1984, pp. 59–62.
20. R. Cammack. Splitting molecular hydrogen. *Nature* 373:556–557, 1995.
21. M.A. Holland, J.C. Pollacco. Urease-null and hydrogenase-null phenotypes of a phylloplane bacterium reveal altered nickel metabolism in two soybean mutants. *Plant Physiol*. 98:942–948, 1992.
22. Von A. Schnegg, M. Kirchgessner. Aktivitatsanderungen von enzymem der leber und niere im nickel- bzw. Eisen-Mangel. *Zh. Tierphysiol.*; *Tierernahrg u Futtermittelkde* 38:200–205, 1977.
23. M. Anke, P. Gropel, H. Kronemann, M. Gunn. Nickel—An essential element. In: F.W. Sunderman, Jr., A. Aito, eds. *Nickel in the Human Environment*. Lyon: IARC Scientific Publishers, 1984, pp. 339–365.
24. F.H. Nielson, T.J. Zimmerman, M.E. Collings, D.R. Myron. Nickel deprivation in rats: nickel-iron interactions. *J. Nutr*. 109:1623–1632, 1979.
25. W.A. Roach, C. Barclay. Nickel and multiple trace deficiencies in agricultural crops. *Nature* 157:696, 1946.
26. D. Mishra, M. Kar. Nickel in plant growth and metabolism. *Bot. Rev*. 40:395–452, 1974.
27. R.M. Welch. The biological significance of nickel. *J. Plant Nutr*. 31:345–356, 1981.
28. D. Bertrand, A. de Wolf. Nickel, a dynamic trace element for higher plants. *C R Academic Sci*. 265:1053–1055, 1967.
29. S.L. Albrecht, R.J. Maier, F.J. Hanus, S.A. Russel, D.W. Emerich, H.J. Evans. Hydrogenase in *Rhizobium japonicum* increases nitrogen fixation by nodulated soybeans. *Science* 203:1255–1257, 1979.
30. L.C. Rai, M. Raizada. Nickel-induced stimulation of growth heterocyst differentiation carbon-14 dioxide uptake and nitrogenase activity in *Nostoc muscorum*. *New Phytol*. 104:111–114, 1986.
31. R.D. Graham, R.M. Welch, C.D. Walker. A role of nickel in the resistance of plants to rust. *Proceedings of the Third Australian Agronomic Conference*, Hobart Tasmania, Australia, 1985.
32. J.C. Polacco. Is nickel a universal component of plant ureases? *Plant Sci. Lett*. 10:249–255, 1977.
33. D.L. Eskew, R.M. Welch, E.E. Cary. A simple plant nutrient solution purification method for effective removal of trace metals using controlled pore glass 8-hydroxyquinoline chelation column chromatography. *Plant Physiol*. 76:103–105, 1982.
34. D.L. Eskew, R.M. Welch. Nickel supplementation 1 µg L^{-1} prevents leaflet tip necrosis in soybeans grown in nutrient solutions purified using 8 hydroxy quinoline-controlled pore glass chromatography. *Plant Physiol*. (Supp.) 69:134, 1982.
35. M. Kirchgessner, Von A. Schnegg. Malate dehydrogenase and glucose-6-phosphate dehydrogenase activity in livers of Ni-deficient rats. *Bioinorg. Chem*. 6:155–161, 1976.

36. O. Horak. The importance of nickel in Fabaceae II. Uptake and requirement of nickel by *Pisum sativum*. *Phyton (Horn)* 25:301–307, 1985.
37. J.C. Polacco. A soybean seed urease-null produces urease in cell culture *Glycine max*. *Plant Physiol*. 66: 1233–1240, 1981.
38. O. Horak. The importance of nickel in Fabaceae I. Comparative studies on the content of nickel and certain other elements in vegetative parts and seeds. *Phyton (Horn)* 25:135–146, 1985.
39. P.K. Das, M. Kar, D. Mishra. Nickel nutrition of plants: I. Effect of nickel and some oxidase activities during rice (*Oryza sativa* L.) seed germination. *Zh. Pflanzenphysiol*. 90:225–233, 1978.
40. B.A.L. Nicoulaud, A.J. Bloom. Nickel supplements improve growth when foliar urea is the sole nitrogen source for tomato. *J. Amer. Soc. Hortic. Sci*. 123:556–559, 1998.
41. C.-P. Witte, S.A. Tiller, M.A. Taylor, H.V. Davies. Addition of nickel to Murashige and Skoog medium in plant tissue culture activates urease and may reduce metabolic stress. *Plant Cell Tissue Organ Culture* 68:103–104, 2002.
42. N.K. Khan, M. Wantanabe, Y. Wantanabe. Effect of different concentrations of urea with or without nickel on spinach (*Spinacia* oleraceae L.) under hydroponic culture. In: T. Ando, K. Fujita, T. Mae, S. Matsumoto, S. Mori, J. Sekiya, eds. *Plant Nutrition for Sustainable Food Production and Environment*. Dordrecht: Kluwer Academic Publishers, 1997, pp. 85–86.
43. H.D. Chapman. *Diagnostic Criteria for Plants and Soils*. Riverside: Division of Agricultural Science, University of California, 1966.
44. R.R. Brooks. Accumulation of nickel by terrestrial plants. In: J.O. Nriagu, ed. *Nickel in the Environment*. New York: Wiley, 1980, pp. 407–430.
45. E.G. Bollard. Involvement of unusual elements in plant growth and nutrition. In: A. Lauchli, R.L. Bielski, eds. *Encyclopedia of Plant Physiology*, New Series, Vol. 15B, 1983, pp. 695–755.
46. CJ Asher. Beneficial elements, functional nutrients, and possible new essential elements. In: JJ Mortvedt, ed. *Micronutrients in Agriculture*, 2nd edition. SSSA book series #4. Madison, WI: Soil Science Society of America, 1991, pp. 703–723.
47. R. Gabrielli, T. Pandolfini, O. Vergnano, M.R. Palandri. Comparison of two serpentine species with different nickel tolerance strategies. *Plant Soil* 122:271–277, 1990.
48. P.H. Brown, L. Dunemann, R. Schultz, H. Marschner. Influence of redox potential and plant species on the uptake of nickel and cadmium from soils. *Zh. Pflanzenernahr. Bodenkd*. 152:85–91, 1989.
49. D.E. Salt, M. Blaylock, N.P.B.A. Kumar, V. Dushenkov, B.D. Ensley, I. Chet, I. Raskin. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Biotech*. 13:468–474, 1995.
50. F.H. Nielson, H.T. Reno, L.O. Tiffin, R.M. Welch. Nickel. In: *Geochemistry and the Environmen,. Vol. II, The Relation of Other Trace Elements to Health and Disease*. Washington, DC: National Academy of Science, 1977, pp. 40–53.
51. R.D. Reeves, A.J.M. Baker, A. Borhidi, R. Berazain. Nickel hyperaccumulation in the serpentine flora of Cuba. *Ann. Bot*. 83:29–38, 1999.
52. M.M. Guha, R.L. Mitchell. Trace and major element composition of the leaves of some deciduous trees. II. Seasonal changes. *Plant Soil* 24:90–112, 1966.
53. B. Singh, Y.P. Dang, S.C. Mehta. Influence of nitrogen on the behavior of nickel in wheat. *Plant Soil* 127:213–218, 1990.
54. R.D. Macnicol, P.H.T. Beckett. Critical tissue concentrations of potentially toxic elements. *Plant Soil* 85:107–129, 1985.
55. A. Cottenie, A. Dhaese, R. Camerlynck. Plant quality response to uptake of polluting elements. *Qual. Plant*. 26:293–319, 1976.
56. D.A. Cataldo, T.R. Garland, R.E. Wildung. Nickel in plants. I. Uptake kinetics using intact soybean seedlings. *Plant Physiol*. 62:5636–5665, 1978.
57. L.V. Kochian. Mechanisms of micronutrient uptake and translocation in plants. In: J.J. Mortvedt, ed. *Micronutrients in Agriculture,* 2nd edition. SSSA book series #4. Madison, WI: Soil Science Society of America, 1991, pp. 229–296.
58. Y. Guo, E. George, H. Marschner. Contribution of an arbuscular mycorrhizal fungus to the uptake of cadmium and nickel in bean and maize plants. *Plant Soil* 184:195–205, 1996.
59. U. Ahonen-Jonnarth, R.D. Finlay. Effects of elevated nickel and cadmium concentrations on growth and nutrient uptake of mycorrhizal and nonmycorrhizal *Pinus sylvestris* seedlings. *Plant Soil* 236:129–138, 2001.

60. D.A. Cataldo, K.M. McFadden, T.R. Garland, R.E. Wildung. Organic constituents and complexation of nickel(II), iron(III), cadmium(II) and plutonium(IV) in soybean xylem exudates. *Plant Physiol.* 86:734–739, 1988.
61. L.O. Tiffin. Translocation of nickel in xylem exudates of plants. *Plant Physiol.* 48:273–277, 1971.
62. D.G. Barceloux. Nickel. *J. Toxicol.: Clin. Toxicol.* 37:239–242, 1999.
63. R.H.M. van de Graaff, H.C. Suter, S.J. Lawes. Long-term effects of municipal sewage on soils and pastures. *J. Environ. Sci. Health A* 37:745–757, 2002.
64. M.F. Hovmand. Cycling of Pb, Cd, Cu, Zn and Ni in Danish agriculture. In: S. Berglund, R.D. Davis, P.L. Hermite, eds. *Utilisation of Sewage Sludge on Land: Rates of Application and Long-Term Effects of Metals*. Dordrecht: D. Reidel, 1984, pp. 166–185.
65. U. Kukier, R.L. Chaney. Amelioration of nickel phytotoxicity in muck and mineral soils. *J. Environ. Qual.* 30:1949–1960, 2001.
66. D.R. Sauerbeck, A. Hein. The nickel uptake from different soils and its prediction by chemical extractions. *Water Air Soil Pollut.* 57–59:861–871, 1991.
67. A.U. Haq, M.H. Miller. Prediction of available soil Zn, Cu, Mn using chemical extractants. *Agron. J.* 64:779–782, 1972.
68. T. Becquer, F. Rigault, T. Jaffre. Nickel bioavailability assessed by ion exchange resin in the field. *Commun. Soil Sci. Plant Anal.* 33:439–450, 2002.
69. M. Khan, J. Scullion. Effects of metal (Cd, Cu, Ni, Pb or Zn) enrichment of sewage-sludge on soil microorganisms and their activities. *Appl. Soil Ecol.* 20:145–155, 2002.
70. R.S. Boyd, M.A. Davis, M.A. Wall, K. Balkwill. Nickel defends the South African hyperaccumulator *Senecio coronatus* (Asteraceae) against *Helix aspersa* (Mollusca: Pulmonidae). *Chemoecology* 12:91–97, 2002.
71. H. Kupper, E. Lombi, F.-J. Zhao, G. Wieshammer, S.P. McGrath. Cellular compartmentation of nickel in the hyperaccumulators *Alyssum lesbiacum*, *Alyssum bertolonii* and *Thlaspi goesingense*. *J. Exp. Bot.* 52:2291–2300, 2001.
72. C. Bai, C.C. Reilly, B.W. Wood. Nickel deficiency disrupts metabolism of ureides, amino acides, and organic acids of young pecan foliage. *Plant Physiol.* 140:433–443, 2006.