17 Cobalt

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17.1 INTRODUCTION

Cobalt has long been known to be a micronutrient for animals, including human beings, where it is a constituent of vitamin B_{12} (1). However, its presence and function has not been recorded to the same extent in higher plants as in animals, leading to the suggestion that vegetarians and herbivorous animals need to ingest extra cobalt or vitamin B_{12} in diets to prevent deficiency. Vitamin B_{12} is synthesized in some bacteria, but not in animals and plants (1). Intestinal absorption and subsequent plasma transport of vitamin B_{12} are mediated by specific vitamin B_{12} proteins and their receptors in mammals. Vitamin B_{12}, taken up by the cells, is converted enzymatically into methyl and adenosyl vitamin B_{12}, which function as coenzymes. Feeding trials of cattle (*Bos taurus* L.), which also suffer from vitamin B_{12} deficiency, show that the normal diet is deficient in cobalt to the extent that supplemental provision of the element can improve their performance, something that could also be achieved by feeding them feedstuffs grown in cobalt-rich soil (2).

The only physiological role so far definitely attributed to cobalt in higher plants has been in nitrogen fixation by leguminous plants (3).

17.2 DISTRIBUTION

17.2.1 Microorganisms and Lower Plants

17.2.1.1 Algae

Cobalt is essential for many microorganisms including cyanobacteria (blue–green algae). It forms part of cobalamin, a component of several enzymes in nitrogen-fixing microorganisms, whether free-living or in symbiosis. It is required for symbiotic nitrogen fixation by the root nodule bacteria of legumes (3). Soybeans grown with 0.1 $\mu g\ L^{-1}$ cobalt with atmospheric nitrogen and no mineral nitrogen showed rapid nitrogen fixation and growth (4). Cobalt is distributed widely in algae, including microalgae, *Chlorella*, *Spirulina*, *Cytseira barbera*, and *Ascophyllum nodosum*. Alginates, such as fucoiden, in the cell wall play an important role in binding cobalt in the cell-wall structure (5,6).

Bioaccumulation of heavy metals in aquatic macrophytes growing in streams and ponds around slag dumps has led to high levels of cobalt (7). Certain marine species such as diatoms (*Septifer virgatus* Wiegman) and brown algae *Sargassum horneri* (Turner) and *S. thunbergii* (Kuntze) from the Japanese coast act as bioindicators of cobalt (8). Accumulation has been shown to be controlled by salinity of the medium with bladder wrack (brown alga, *Fucus vesiculosus* L.) (9).

The cell walls of plants, including those of algae, have the capacity to bind metals at negatively charged sites. The wild type of *Chlamydomonas reinhardtii* Dangeard, owing to the presence of its cell wall, was more tolerant to metals such as cobalt, copper, cadmium, and nickel, than the wall-less variant (10). When exposed to metals, singly in solutions for 24 h, cells of both strains accumulated the metals. Absorbed metals not removed by chelation with EDTA–$CaC1_2$ wash were considered strongly bound. Cobalt and nickel were present in significantly higher amounts loosely bound to the walled organism than in the wall-less ones. It was concluded that metal ions were affected by the chelating molecules in walled algae, which limited the capacity of the metal to penetrate the cell. Thus, algae appear to contain a complex mechanism involving internal and external detoxification of metal ions (10).

In a flow-through wetland treatment system to treat coal combustion leachates from an electrical power system using cattails (*Typha latifolia* L.), cobalt and nickel in water decreased by an average of 39 and 47% in the first year and 98 and 63% in the second year, respectively. Plants took up 0.19% of the cobalt salts per year. Submerged *Chara* (a freshwater microalga), however, took up 2.75% of the salts, and considerably higher concentrations of metals were associated with cattail roots than shoots (11).

17.2.1.2 Fungi

In fungi, cobalt accumulates by two processes. The essential process is a metabolically independent one presumably involving the cell surface. Accumulation may reach 400 mg g^{-1} of yeast and is rapid in *Neurospora crassa* Shear & BO Dodge (12,13).

In the next step, which is metabolism dependent, progressive uptake of large amounts of cations takes place. Two potassium ions are released for each Co^{2+} ion taken up in freshly prepared yeast-cell suspensions. The Co^{2+} appears to accumulate via a cation-uptake system. Its uptake is specifically related to the ionic radius of the cation (14). Accumulated cobalt is transported (at the rate of 40 µg h^{-1} 100 mg^{-1} dry weight of *N. crassa*) mainly into the intercellular space and vacuoles (13,15). Acidity and temperature of media are factors involved in Co^{2+} uptake and transport. In *N. crassa,* Mg^{2+} inhibits Co^{2+} uptake and transport, suggesting that the processes of the two cations are interrelated. In yeast cells exposed to elevated concentrations of cobalt, uptake is suppressed, and intercellular distribution is altered (15).

Yeast mitochondria passively accumulate Co^{2+} in levels linearly proportional to its concentration in the medium. The density of mitochondria is slightly increased and their appearance is altered, based on observations with electron microscopy (16). The more dense mitochondria are exchanged by hyphal fusion in the fully compatible common A and common AB matings of tetrapolar basidiomycetes *Schizophyllum commune* Fries, but not in the common B matings (17). Toxicity and the barrier effect of the cell wall inhibit surface binding of Co^{2+}. As a result, isolated protoplasts from yeast-like cells of hyphae and chlamydospores of *Aureobasidium pollulans* were more sensitive to intracellular cobalt uptake than intact cells and chlamydospores (18).

17.2.1.3 Moss

The absorption and retention of heavy metals in the woodland moss *Hylocomium splendens* Hedw followed the order of Cu, Pb>Ni>Co>Zn, and Mn within a wide range of concentrations and was independent of the addition of the ions (19).

17.2.2 Higher Plants

Cobalt is not known to be definitely essential for higher plants. Vitamin B_{12} is neither produced nor absorbed by higher plants. It is synthesized by soil bacteria, intestinal microbes, and algae. In naturally cobalt-rich areas, cobalt accumulates in plants in a species-specific manner. Plants such as astragalus (*Astragalus* spp. L.) may accumulate from 2 or 3 to 100 mg kg^{-1} dried plant mass. Cobalt occurs in a high concentration in the style and stigma of *Lilium longifolium* Thunb. It was not detected in the flowers of green beans (*Phaseolus sativus* L.) and radishes (*Raphanus sativus* L.) though the leaves of the latter contain it. It was shown to occur in high amounts in leafy plants such as lettuce (*Lactuca sativa* L.), cabbage (*Brassica oleracea* var. *capitata* L.), and spinach (*Spinacea oleracea* L.) (above 0.6 ppm) by Kloke (20). Forage plants contain 0.6 to 3.5 mg Co kg^{-1} and cereals 2.2 mg kg^{-1} (21). Rice (*Oryza sativa* L.) contains 0.02 to 0.150 mg kg^{-1} plant mass (22).

Cobalt chloride markedly increases elongation of etiolated pea stems when supplied with indole acetic acid (IAA) and sucrose, but elongation is inhibited by cobalt acetate. Cobalt in the form of vitamin B_{12} is necessary for the growth of excised tumor tissue from spruce (*Picea glauca* Voss.) cultured *in vitro*. It increases the apparent rate of synthesis of peroxides and prevents the peroxidative

destruction of IAA. It counteracts the inhibition by dinitrophenol (DNP) in oxidative phosphorylation and reduces activity of ATPase and is known to be an activator of plant enzymes such as carboxylases and peptidases (4). The Co^{2+} ion is also an inhibitor of the ethylene biosynthesis pathway, blocking the conversion of 1-amino-cyclopropane-l-carboxylic acid (ACC) (23).

17.3 ABSORPTION

Kinetic studies of cobalt absorption by excised roots of barley (*Hordeum vulgare* L.) exhibited a Q_{10} of 2.2 in a concentration range of 1 to100 μM $CoCl_2$. It has been suggested that a number of carrier sites are available, which are concentration dependent (24). Entry of divalent cations in the roots of maize is accompanied by a decrease in the pH of the incubation media and of the cell sap and also a decrease in the malate content (25). The uptake by different species probably depends on the various physiological and biological needs of the species (26,27).

Accumulation of cobalt by forage plants has been studied in wetlands, grasslands, and forests close to landfills and mines (11,28,29). Irrigation with cobalt-rich water in meadows has shown high intake of cobalt, which was also demonstrated in the blood serum and plasma of bulls fed on the hay grown in the field (29). African buffalos *(Syncerus caffer* Sparrman*)* in the Kruger National Park (KNP) downwind of mining and refining of cobalt, copper, and manganese showed the presence of the metals in liver in amounts related significantly to age and gender differences (30).

17.4 UPTAKE AND TRANSPORT

17.4.1 Absorption as Related to Properties of Plants

The molecular basis of metal transport through membranes has been studied by several workers. Korshunova et al. (31) reported that IRT 1, an *Arabidopsis thaliana* Heynh (mouse-ear cress) metal-ion transporter, could facilitate manganese absorption by a yeast mutant *Saccharomyces cerevisiae* Meyen ex E.C. Hansen strain defective in manganese uptake (smfl delta). The IRT 1 protein has been identified as a transporter for iron and manganese and is inhibited by cadmium and zinc. The IRT 1 cDNA also complements a Zn-uptake-deficient yeast mutant. It is therefore suggested that IRT 1 protein is a broad-range metal-ion transporter in plants (31).

Macfie and Welbourn (10) reviewed the function of cell wall as a barrier to the uptake of several metal ions in unicellular green algae. The cell walls of plants, including those of algae, have the capacity to bind metal ions in negatively charged sites. As mentioned above, the wild-type (walled) strain of the unicellular green alga *Chlamydomonas reinhardtii* Dangeard was more tolerant to cobalt than a wall-less mutant of the same species. In a study to determine if tolerance to metals was associated with an increased absorption, absorbed metal was defined as that fraction that could be removed with a solution of Na-EDTA and $CaCl_2$. The fraction that remained after the EDTA–$CaCl_2$ wash was considered strongly bound in the cell. When exposed to metals, singly, in solution for 24 h, cells of both strains accumulated the metals. Significantly higher concentrations of cobalt were in the loosely bound fraction of the walled strain than in the wall-less strain.

Passive diffusion and active transport are involved in the passage of Co^{2+} through cortical cells. A comparison of concentration of Co^{2+} in the cytoplasm and vacuoles indicates that active transport occurs outward from the cytoplasm at the plasmalemma and also into the vacuoles at the tonoplast. Light–dark cycles play an important role in transport through the cortical cells of wheat (*Triticum aestivum* L.) (32). A small amount of absorption at a linear rate takes place in the water-free space, Donnan-free space, and cytoplasm in continuous light, whereas a complete inhibition of absorption occurs during the dark periods (32). In ryegrass (*Lolium perenne* L.), 15% of the Co^{2+} absorbed was transported to the shoot after 72 h. Absorption and transport of Co^{2+} markedly increased with increasing pH of the solution, but were not affected by water flux through the plants. With 0.1 μM Co^{2+} treatment, concentration of cobalt in the cytoplasm was regulated by an efflux

pump at the plasmalemma and by an influx pump at the tonoplast. Stored cobalt in the vacuole was not available for transport (33).

Cobalt tends to accumulate in roots, but free Co^{2+} inhibited hydrolysis of Mg-ATP and protein transport in corn-root tonoplast vesicles (34). ATP complexes of Co^{2+} inhibited proton pumping, and the effect was modulated by free Co^{2+}. Free cations affected the structure of the lipid phase in the tonoplast membrane, possibly by interaction with a protogenic domain of the membrane through an indirect link mechanism (34).

Upward transport of cobalt is principally by the transpirational flow in the xylem (35). Usually, the shoot receives about 10% of the cobalt absorbed by the roots, most of which is stored in the cortical cell vacuoles and removed from the transport pathway (32). Distribution along the axis of the shoot decreases acropetally (36). Cobalt is bound to an organic compound of negative overall charge and molecular weight in the range of 1000 to 5000 and is transported through the sieve tubes of castor bean (*Ricinus communis* L.) (37). Excess cobalt leads to thick callose deposits on sieve plates of the phloem in white bean (*Phaseolus vulgaris* L.) seedlings, possibly reducing the transport of ^{14}C assimilates significantly (38).

The distribution of cobalt in specific organs indicates a decreasing concentration gradient from the root to the stem and from the leaf to the fruit. This gradient decreases from the root to the stem and leaves in bush beans (*Phaseolus vulgaris* L.) and *Chrysanthemum* (39,40). No strong gradient occurs from the stem to the leaves because of the low mobility of cobalt in plants, leading to its transport to leaves in only small amounts (41,42). In seeds of lupin (*Lupinus angustifolius* L.), concentrations of cobalt are higher in cotyledons and embryo than in seed coats (43). The distribution depends on the phase of development of the plant. At the early phase of growth of potatoes (*Solanum tuberosum* L.) on lixiviated (washed) black earth, large quantities of cobalt are accumulated in the leaves and stalks (44), whereas before flowering and during the ripening of beans (*Phaseolus vulgaris* L.), the largest amount is in the nodules. Plant organs contain cobalt in the following increasing order: root, leaves, seed, and stems (44). During flowering, a large amount shifts to the tuber of potato and, in the case of beans, to flowers, followed by nodules, roots, leaves, and stems. Movement is more rapid in a descending direction than in an ascending one (36). The cobalt content was observed to be higher in pickled cucumber (*Cucumis sativus* L.) than in young fresh fruit (45). In grains of lupins (*Lupinus* spp. L.) and wheat, the concentration varied with the amount of rainfall and soil types (46).

17.4.2 Absorption as Related to Properties of Soil

Soil pH has a major effect on the uptake of cobalt, manganese, and nickel, which become more available to plants as the pH decreases. Increase in soil pH reduces the cobalt content of ryegrass (*Lolium* spp.) (47). Reducing conditions in poorly drained soils enhance the rate of weathering of ferromagnesian minerals, releasing cobalt, nickel, and vanadium (48). Liming decreased cobalt mobility in soil (49). The presence of humus facilitates cobalt accumulation in soil, but lowers its absorption by plants. Five percent humus has been shown to decrease cobalt content by one-half or two-thirds in cultures (50).

High manganese levels in soil inhibit accumulation of cobalt by plants (51). Manganese dioxides in soil have a high sorption capacity and accumulate a large amount of cobalt from the soil solution. Much of the cobalt in the soil is fixed in this way and is thus not available to plants (52). Water logging of the soil increases cobalt uptake in French bean (*Phaseolus vulgaris* L.) and maize (*Zea mays* L.) (53).

17.4.3 Accumulation as Related to the Rhizosphere

Cobalt may be absorbed through the leaf in coniferous forests, but the majority is through the soil, especially in wetlands. The physicochemical status of transition metals such as cobalt in the rhizosphere is entirely different from that in the bulk soil. A microenvironment is created around the root system

(e.g., wheat and maize), characterized by an accumulation of root-derived organic material with a gradual shift from ionic metal to higher-molecular weight forms such as cobalt, manganese, and zinc. These three metals are increasingly complexed throughout the growth period. Fallow soil has been shown to complex lower amounts (6.4%) of tracers (^{57}Co) than cropped soil, 61% for maize and 31% for wheat (54). Cobalt has a stimulatory effect on the microflora of tobacco (*Nicotiana tabacum* L.) rhizosphere, shown by an intensification of the immobilization of nitrogen and mineralization of phosphorus (55). Cobalt status in moist soil from the root zone of field-grown barley shows seasonal variation, being low in late winter and higher in spring and early summer. Discrete maxima are achieved frequently between May and early July, depending on the extent of the development of the growing crop and on seasonal influences. Increased concentration may result from the mobilization of the micronutrient from insoluble forms by biologically produced chelating ligands.

17.5 COBALT METABOLISM IN PLANTS

Interactions between cobalt and several essential enzymes have been demonstrated in plants and animals. Two metal-bound intermediates formed by Co^{2+} activate ribulose-1,5 bisphosphate carboxylase/oxygenase (EC 4.1.1.39). Studies by electron paramagnetic resonance (EPR) spectroscopy have shown the activity to be dependent on the concentration of ribulose 1,5 bisphosphate (23). This finding suggested that the enzyme–metal coordinated ribulose 1,5 bisphosphate and an enzyme–metal coordinated enediolate anion of it, where bound ribulose 1,5 bisphosphate appears first, constitute the two EPR detectable intermediates, respectively.

Ganson and Jensen (56) showed that the prime molecular target of glyphosate (*N*-[phosphonomethyl]glycine), a potent herbicide and antimicrobial agent, is known to be the shikimate-pathway enzyme 5-enol-pyruvylshikimate-3-phosphate synthetase. Inhibition by glyphosate of an earlier pathway enzyme that is located in the cytosol of higher plants, 3-deoxy-D-arabino-heptulosonate-7 phosphate synthase (DS-Co), has raised the possibility of dual enzyme targets *in vivo*. Since the observation that magnesium or manganese can replace cobalt as the divalent-metal activator of DS-Co, it has now been possible to show that the sensitivity of DS-Co to inhibition by glyphosate is obligately dependent on the presence of cobalt. Evidence for a cobalt(II):glyphosate complex with octahedral coordination was obtained through examination of the effect of glyphosate on the visible electronic spectrum of aqueous solutions of $CoCl_2$.

Two inhibition targets of cobalt and nickel were studied on oxidation–reduction enzymes of spinach (*Spinacia oleracea* L.) thylakoids. Compounds of complex ions and coordination compounds of cobalt and chromium were synthesized and characterized (57). Their chemical structures and the oxidation states of their metal centers remained unchanged in solution. Neither chromium(III) chloride ($CrC1_3$) nor hexamminecobalt(III) chloride [$Co(NH_3)_6C1_3$] inhibited photosynthesis. Some other coordination compounds inhibited ATP synthesis and electron flow (basal phosphorylating, and uncoupled) behaving as Hill-reaction inhibitors, with the compounds targeting electron transport from photosystem II (P680 to plastoquinones, QA and QB, and cytochrome).

The final step in hydrocarbon biosynthesis involves the loss of cobalt from a fatty aldehyde (58). This decarbonylation is catalyzed by microsomes from *Botyrococcus braunii*. The purified enzyme releases nearly one mole of cobalt for each mole of hydrocarbon. Electron microprobe analysis revealed that the enzyme contains cobalt. Purification of the decarbonylase from *B. braunii* grown in $^{57}CoCl_2$ showed that ^{57}Co co-eluted with the decarbonylase. These results indicate that the enzyme contains cobalt that might be part of a Co-porphyrin, although a corrin structure (as in vitamin B_{12}) cannot be ruled out. These results strongly suggest that biosynthesis of hydrocarbons is effected by a microsomal Co-porphyrin-containing enzyme that catalyzes decarbonylation of aldehydes and, thus, reveals a biological function for cobalt in plants (58).

The role of hydrogen bonding in soybean (*Glycine max* Merr.) leghemoglobin was studied (59,60). Two spectroscopically distinct forms of oxycobaltous soybean leghemoglobin (oxyCoLb), acid and neutral, were identified by electron spin echo envelope modulation. In the

acid form, a coupling to 2H was noted, indicating the presence of a hydrogen bond to bound oxygen. No coupled 2H occurred in the neutral form (60). The oxidation–reduction enzymes of spinach thylakoids are also affected by chromium and cobalt (23,57).

The copper chaperone for the superoxide dismutase (CCS) gene encodes a protein that is believed to deliver copper to Cu–Zn superoxide dismutase (CuZnSOD). The CCS proteins from different organisms share high sequence homology and consist of three distinct domains, a CuZnSOD-like central domain flanked by two domains, which contain putative metal-binding motifs. The Co^{2+}-binding properties of proteins from arabidopsis and tomato (*Lycopersicon esculentum* Mill.) were characterized by UV–visible and circular dichroism spectroscopies and were shown to bind one or two cobalt ions depending on the type of protein. The cobalt-binding site that was common in both proteins displayed spectroscopic characteristics of Co^{2+} bound to cysteine ligands (61).

The inhibition of photoreduction reactions by exogenous manganese chloride ($MnCl_2$) in Tris-treated photosystem II (PSII) membrane fragments has been used to probe for amino acids on the PSII reaction-center proteins, including the ones that provide ligands for binding manganese (62,63). Inhibition of photooxidation may involve two different types of high-affinity, manganese-binding components: (a) one that is specific for manganese, and (b) others that bind manganese, but may also bind additional divalent cations such as zinc and cobalt that are not photooxidized by PSII. Roles for cobalt or zinc in PSII have not been proposed, however.

17.6 EFFECT OF COBALT IN PLANTS ON ANIMALS

Cobalt uptake by plants allows its access to animals. Kosla (29) demonstrated the effect of irrigation of meadows with the water of the river Ner in Poland on the levels of iron, manganese, and cobalt in the soil and vegetation. Experiments were also carried out on young bulls (*Bos taurus* L.) fed with the hay grown on these meadows. The levels of iron and cobalt were determined in the blood plasma, and manganese level in the hair of the bulls. The irrigation caused an increase of the cobalt content in the soil, but had no effect on cobalt content in the plants or in the blood plasma of the bulls. Webb et al. (30) stated that animals may act as bioindicators for the pollution of soil, air, and water. To monitor changes over time, a baseline status should be established for a particular species in a particular area. The concentration of minerals in soil is a poor indicator of mineral accumulation by plants and availability to animals.

The chemical composition of the body tissue, particularly the liver, is a better reflection of the dietary status of domestic and wild animals. Normal values for copper, manganese, and cobalt in the liver have been established for cattle, but not for African buffalo. As part of the bovine-tuberculosis (BTB) monitoring program in the KNP in South Africa, 660 buffalo were culled. Livers were randomly sampled in buffered formalin for mineral analysis. The highest concentrations of copper in livers were measured in the northern and central parts of the KNP, which is downwind of mining and refining activities. Manganese, cobalt, and selenium levels in the liver samples indicated neither excess nor deficiency although there were some significant area, age, and gender differences. It was felt that these data could serve as a baseline reference for monitoring variations in the level and extent of mineral pollution on natural pastures close to mines and refineries. Cobalt is routinely added to cattle feed, and deficiency diseases are known. Of interest also are the possible effects of minor and trace elements in Indian herbal and medicinal preparations (64).

17.7 INTERACTION OF COBALT WITH METALS AND OTHER CHEMICALS IN MINERAL METABOLISM

The interaction of cobalt with other metals depends to a major extent on the concentration of the metals used. The cytotoxic and phytotoxic responses of a single metal or combinations are considered in terms of common periodic relations and physicochemical properties, including electronic structure,

ion parameters (charge–size relations), and coordination. But, the relationships among toxicity, positions, and properties of these elements are very specific and complex (65). The mineral elements in plants as ions or as constituents or organic molecules are of importance in plant metabolism. Iron, copper, and zinc are prosthetic groups in certain plant enzymes. Magnesium, manganese, and cobalt may act as inhibitors or as activators. Cobalt may compete with ions in the biochemical reactions of several plants (66,67).

17.7.1 Iron

Many trace elements in high doses induce iron deficiency in plants (68). Combinations of increased cobalt and zinc in bush beans have led to iron deficiency (69). Excess metals accumulated in shoots, and especially in roots, reduce ion absorption and distribution in these organs, followed by the induction of chlorosis, decrease in catalase activity, and increase in nonreducing sugar concentration in barley (70,71). Supplying chelated iron ethylenediamine di(*o*-hydroxyphenyl) acetic acid [Fe-(EDDHA),] could not overcome these toxic effects in *Phaseolus* spp. L. (72). Simultaneous addition of cobalt and zinc to iron-stressed sugar beet (*Beta vulgaris* L.) resulted in preferential transport of cobalt into leaves followed by ready transport of both metals into the leaf symplasts within 48 h (73). A binuclear binding site for iron, zinc, and cobalt has been observed (74).

17.7.2 Zinc

Competitive absorption and mutual activation between zinc and cobalt during transport of one or the other element toward the part above the ground were recorded in pea (*Pisum sativum* L.) and wheat seedlings (75). Enrichment of fodder beet (*Beta vulgaris* L.) seeds before sowing with one of these cations lowers the content of the other in certain organs and tissues. It is apparently not the result of a simple antagonism of the given cations in the process of redistribution in certain organs and tissue, but is explained by a similar effect of cobalt and zinc as seen when the aldolase and carbonic anhydrase activities and intensity of the assimilators' transport are determined (76).

Cobalt tends to interact with zinc, especially in high doses, to affect nutrient accumulation (77). The antagonism is sometimes related to induced nutrient deficiency (69). In bush beans, however, cobalt suppressed to some extent the ability of high concentration of zinc to depress accumulation of potassium, calcium, and magnesium. The protective effect was stated to be the result of zinc depressing the leaf concentration of cobalt rather than the other nutrients (69). Substitution of Zn^{2+} by Co^{2+} reduces specificity of Zn^{2+} metalloenzyme acylamino-acid-amido hydrolase in *Aspergillus oryzae* Cohn (78).

17.7.3 Cadmium

Combinations of elements may be toxic in plants when the individual ones are not (72). Trace elements usually give protective effects at low concentrations because some trace elements antagonize the uptake of others at relatively low levels. For example, trace elements in various combinations (Cu–Ni–Zn, Ni–Co–Zn–Cd, Cu–Ni–Co–Cd, Cu–Co–Zn–Cd, Cu–Ni–Zn–Cd, and Cu–Ni–Co–Zn–Cd) on growth of bush beans protected against the toxicity of cadmium. It was suggested that part of the protection could be due to cobalt suppressing the uptake of cadmium by roots. Other trace elements in turn suppressed the uptake of cobalt by roots (69). These five trace elements illustrated differential partitioning between roots and shoots (40). The binding of toxic concentration of cobalt in the cell wall of the filamentous fungus (*Cunninghamella blackesleeana* Lender) was totally inhibited and suppressed by trace elements (79).

17.7.4 Copper

The biphasic mechanism involved in the uptake of copper by barley roots after 2 h was increased with 16 μM Co^{2+}, but after 24 h, a monophasic pattern developed with lower values of copper absorption, indicating an influence of Co^{2+} on the uptake site (80).

17.7.5 Manganese

Cobalt and zinc increased the accumulation of manganese in the shoots of bush beans grown for 3 weeks in a stimulated calcareous soil containing Yolo loam and 2% $CaCO_3$ (40).

17.7.6 Chromium and Tin

The inhibitory effects of chromium and tin on growth, uptake of NO_3^- and NH_4^+, nitrate reductase, and glutamine synthetase activity of the cyanobacterium (*Anabaena doliolum* Bharadwaja) was enhanced when nickel, cobalt, and zinc were used in combination with test metals in the growth medium in the following degree: Ni>Co>Zn (81).

17.7.7 Magnesium

The activating effect of cobalt on Mg^{2+}-dependent activity of glutamine synthetase by the blue–green alga *Spirulina platensis* Geitler may be considered as an important effect. Its effect in maintaining the activity of the enzyme *in vivo* is independent of ATP (82).

17.7.8 Sulfur

The mold *Cunninghamella blackesleeana* Lendner, grown in the presence of toxic concentration of cobalt, showed elevated content of sulfur in the mycelia. Its cell wall contained higher concentrations of phosphate and chitosan, citrulline, and cystothionine as the main cell wall proteins (79).

17.7.9 Nickel

In moss (*Timmiella anomala* Limpricht), nickel overcomes the inhibitory effect of cobalt on protonemal growth whereas cobalt reduces the same effect of nickel on bud number (83).

17.7.10 Cyanide

Cyanide in soil was toxic to bush beans and also resulted in the increased uptake of the toxic elements such as copper, cobalt, nickel, aluminum, titanium, and, to a slight extent, iron. The phytotoxicity from cyanide or the metals led to increased transfer of sodium to the leaves and roots (40).

17.8 BENEFICIAL EFFECTS OF COBALT ON PLANTS

17.8.1 Senescence

Senescence in lettuce leaf in the dark is retarded by cobalt, which acts by arresting the decline of chlorophyll, protein, RNA and, to a lesser extent, DNA. The activities of RNAase and protease, and tissue permeability were decreased, while the activity of catalase increased (84). Cobalt delays ageing and is used for keeping leaves fresh in vetch (*Vicia* spp.) (85). It is also used in keeping fruits such as apple fresh (86).

17.8.2 Drought Resistance

Presowing treatment of seeds with cobalt nitrate increased drought resistance of horse chestnut (*Aesculus hippocastanum* L.) from the Donets Basin in southeastern Europe (87).

17.8.3 Alkaloid Accumulation

Alkaloid accumulation in medicinal plants such as downy thorn apple *Datura innoxia* Mill. (88), *Atropa caucasica* (89), belladonna *A. belladonna* L. (90), and horned poppy *Glaucium flavum* Crantz (91) is regulated by cobalt. It also increased rutin (11.6%) and cyanide (67%) levels in different species of buckwheat (*Fagopyrum sagittatum* Gilib., *F. tataricum* Gaertn., and *F. emargitatum*) (89,92).

17.8.4 Vase Life

Shelf and vase life of marigold (*Tagetes patula* L.), chrysanthemum (*Chrysanthemum* spp.), rose (*Rosa* spp.), and maidenhair fern (*Adiantum* spp.) is increased by cobalt. Cobalt also has a long-lasting effect in preserving apple (*Malus domestica* Borkh.). The fruits are kept fresh by cobalt application after picking (86,93–96).

17.8.5 Biocidal and Antifungal Activity

Cobalt acts as a chelator of salicylidine-*o*-aminothiophenol (SATP) and salicylidine-*o*-aminopyridine (SAP) and exerts biocidal activity against the molds *Aspergillus nidulans* Winter and *A. niger* Tiegh and the yeast *Candida albicans* (97). Antifungal activities of Co^{2+} with acetone salicyloyl hydrazone (ASH) and ethyl methyl ketone salicyloyl hydrazone (ESH) against *A. niger* and *A. flavus* have been established by Johari et al. (98).

17.8.6 Ethylene Biosynthesis

Cobalt inhibits IAA-induced ethylene production in gametophores of the ferns *Pteridium aquilinum* Kuhn and sporophytes of ferns *Matteneuccia struthiopteris* Tod. and *Polystichum munitum* K. Presl (99); in pollen embryo culture of horse nettle (*Solanum carolinense* L.) (100); in discs of apple peel (101); in winter wheat and beans (102); in kiwifruit (*Actinidia chinensis* Planch) (103); and in wheat seedlings under water stress (104). Cobalt also inhibits ethylene production and increases the apparent rate of synthesis of peroxides and prevents the peroxidative destruction of IAA. Other effects include counteraction of the uncoupling of oxidative phosphorylation by dinitrophenol (4).

Cobalt acts mainly through arresting the conversion of methionine to ethylene (105) and thus inhibits ethylene-induced physiological processes. It also causes prevention of cotyledonary prickling-induced inhibition of hypocotyls in beggar tick (*Bidens pilosa* L.) (106), promotion of hypocotyl elongation (107), opening of the hypocotyl hook (bean seedlings) either in darkness or in red light, and the petiolar hook (*Dentaria diphylla* Michx.) (108,109). Cobalt has also been noted to cause reduction of RNAase activity in the storage tissues of potato (110), repression of developmental distortion such as leaf malformation and accumulation of low-molecular-weight polypeptides in velvet plant (*Gynura aurantiaca* DC) (111), delayed gravitropic response in cocklebur (*Xanthium* spp.), tomato and castor bean stems (112), and prevention of 3,6-dichloro-*o*-anisic acid-induced chlorophyll degradation in tobacco leaves (73). Prevention of auxin-induced stomatal opening in detached leaf epidermis has been observed (85). The effects of ethylene on the kinetics of curvature and auxin redistribution in the gravistimulated roots of maize are known (113). ^{60}Co γ-rays and EMS influence antioxidase activity and ODAP content of grass pea (*Lathyrus sativus* L.) (114).

17.8.7 Nitrogen Fixation

Cobalt is essential for nitrogen-fixing microorganisms, including the cyanobacteria. Its importance in nitrogen fixation by symbiosis in Leguminosae (Fabaceae) has been established (115–119). For example, soybeans grown with only atmospheric nitrogen and no mineral nitrogen have rapid nitrogen fixation and growth with 1.0 or 0.1 μg Co ml^{-1}, but have minimal growth without cobalt additions (4).

17.9 COBALT TOLERANCE BY PLANTS

17.9.1 Algae

Stonewort (*Chara vulgaris* L.) resistant to metal pollution, when cultivated in a natural medium containing $CoCl_2$ showed high level of cobalt in dry matter as insoluble compounds (120). On the

other hand, a copper-tolerant population of a marine brown alga (*Ectocarpus siliculosus* Lyng.) had an increased tolerance to cobalt. The copper-tolerance mechanism of other physiological processes may be the basis of this cotolerance (121).

17.9.2 Fungi

A genetically stable cobalt-resistant strain, Co^R, of *Neurospora crassa* Shear & Dodge, exhibited an approximately ten-fold higher resistance to Co^{2+} than the parent strain. The Co^{2+} toxicity was reversed by Mg^{2+}, but not by Fe^{3+}, indicating that the Co^{2+}did not affect iron metabolism. Alternatively, the mechanism of resistance probably involves an alteration in the pattern of iron metabolism so that the toxic concentration of cobalt could not affect the process (122). Magnesium (Mg^{2+}) may reverse the toxicity of Co^{2+}, either by increasing the tolerance to high intracellular concentration of heavy metal ions or by controlling the process of uptake and accumulation of ions (123). In several mutants of *Aspergillus niger* growing in toxic concentrations of Zn^{2+}, Co^{2+}, Ba^{2+}, Ni^{2+}, Fe^{3+}, Sn^{2+}, and Mn^{2+}, the resistance is due to an intracellular detoxification rather than defective transport. Each mutation was due to a single gene located in its corresponding linkage group. Toxicity of metals is reversed in the wild-type strain by definite amounts of K^+, NH_4^+, Mg^{2+}, and Ca^{2+}. These competitions between pairs of cations indicate a general system responsible for the transport of cations (124). In *Aspergillus fumigatus*, cobalt increased thermophily at 45°C and fungal tolerance at 55°C (125).

17.9.3 Higher Plants

In higher plants, cobalt tolerance has been mainly reported in members of 'advanced' families such as the Labiatae and Scrophulariaceae growing in the copper-field belt of Shaba (Zaire) (126). Among these plants, *Haumaniastrum robertii*, a copper-tolerant species, is also a cobalt-accumulating plant. The plant contains abnormally high cobalt (about 4304 μg g^{-1} dry weight), far exceeding the concentration of copper. This species has the highest cobalt content of any phanerogam (127). *Haumaniastrum katangense* and *H. robertii* grow on substrates containing 0 to 10,000 μg Co g^{-1}. Although they can accumulate high concentrations of cobalt, an exclusion mechanism operates in these species at lower concentrations of the element in the soil. Uptake of cobalt was not linked to a physiological requirement of the element. The plant–soil relationship for Co was significantly high enough for these species to be useful in the biogeochemical prospecting for cobalt (128).

Tolerance and accumulation of copper and cobalt were investigated in three members of phylogenetic series of taxa within the genus *Silene* (Caryophyllaceae) from Zaire, which were regarded as representing a progression of increasing adaptation to metalliferous soils. Effects of both metals (singly and in combination) on seed germination, seedling and plant performances, yield, and metal uptake from soil culture confirmed the ecotypic status of *S. burchelli*, which is a more tolerant variant of the nontolerant *S. burchelli* var. *angustifolia*. But both the ecotype and metallophyte variants of *S. cobalticola* are relatively more tolerant to copper than to cobalt.

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18 Selenium

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18.1 THE ELEMENT SELENIUM

18.1.1 Introduction

Selenium (Se), a beneficial element, is one of the most widely distributed elements on Earth, having an average soil abundance of 0.09 mg kg^{-1} (1). It is classified as a Group VI A metalloid, having

metallic and nonmetallic properties. Selenium was identified in 1818 by the Swedish chemist Jöns Jacob Berzelius as an elemental residue during the oxidation of sulfur dioxide from copper pyrites in the production of sulfuric acid (2). The name selenium originates through its chemical similarities to tellurium (Te), discovered 35 years earlier. Tellurium had been named after the Earth (*tellus* in Latin), so selenium was named for the moon (*selene* in Greek) (3). Although selenium is not considered as an essential plant micronutrient (4), it is essential for maintaining mammalian health (5). Selenium deficiency or toxicity in humans and livestock is rare, but can occur in localized areas (5,6) owing to low selenium contents in soils and locally produced crops (7). Recently, much attention has been given to the role of selenium in reducing certain types of cancers and diseases. Efforts in plant improvement have begun to enhance the selenium content of dietary food sources.

18.1.2 Selenium Chemistry

Selenium has an atomic number of 34 and an atomic mass of 78.96. The atomic radius of Se is 1.40 Å, the covalent radius is 1.16 Å, and the ionic radius is 1.98 Å. The ionization potential is 9.74 eV, the electron affinity is −4.21 eV, and the electronegativity is 2.55 on the Pauling Scale (8). The chemical and physical properties of selenium are very similar to those of sulfur (S). Both have similar atomic size, outer valence-shell electronic configurations, bond energies, ionization potentials, electron affinities, electronegativities, and polarizabilities (8). Selenium can exist as elemental selenium (Se^0), selenide (Se^{2-}), selenite (SeO_3^{2-}), and selenate (SeO_4^{2-}). There are six stable isotopes of selenium in nature: ^{74}Se (0.87%), ^{76}Se (9.02%), ^{77}Se (7.58%), ^{78}Se (23.52%), ^{80}Se (49.82%), and ^{82}Se (9.19%) (8). Some of the commercially available forms of selenium are H_2Se, metallic selenides, SeO_2, H_2SeO_3, SeF_4, $SeCl_2$, selenic acid (H_2SeO_4), Na_2SeO_3, Na_2SeO_4, and various organic Se compounds (9).

In the elemental form, selenium exists in either an amorphous state or in one of three crystalline states. The amorphous form of selenium is a hard, brittle glass at 31°C, vitreous at 31 to 230°C, and liquid at temperatures above 230°C (10). The first of three crystalline states takes the form of flat hexagonal and polygonal crystals called α-monoclinic or red selenium. The second form is the prismatic or needle-like crystal called β-monoclinic or dark-red selenium. The third crystalline state is made up of spiral polyatomic chains of Se_n, often referred to as hexagonal or black selenium. The black forms of crystalline Se are the most stable. At temperatures above 110°C, the monoclinic amorphous forms convert into this stable black form. Conversion of the amorphous form into the black form occurs readily at temperatures of 70 to 210°C. When Se^0 is heated above 400°C in air, it becomes the very pungent and highly toxic gas H_2Se. This gas decomposes in air back to Se^0 and water (10).

Reduction or oxidation of elemental selenium can be to the −2-oxidation state (Se^{2-}), the +4-oxidation state (SeO_3^{2-}), or the +6-oxidation state (SeO_4^{2-}). The Se^{2-} ion is water-soluble (270 ml per 100 ml H_2O at 22.5°C) and will react with most metals to form sparingly soluble metal selenides. Selenium in the +4-oxidation state can occur as selenium dioxide (SeO_2), SeO_3^{2-}, or selenious acid (H_2SeO_3). Selenium dioxide is water-soluble (38.4 g per 100 ml H_2O at 14°C) and is produced when Se^0 is burned or reacts with nitric acid. Reduction back to Se^0 can be carried out in the presence of ammonium, hydroxylamine, or sulfur dioxide. In hot water, SeO_2 will dissolve to H_2SeO_3, which is weakly dibasic. Organic selenides, which are electron donors, will oxidize readily to the higher oxidation states of selenium. Selenites are electron acceptors. At low pH, SeO_3^{2-} is reduced to Se^0 by ascorbic acid or sulfur dioxide. In the soil, SeO_3^{2-} is bound strongly by hydrous oxides of iron and is sparingly soluble at pH 4 to 8.5 (10).

In the +6-oxidation state, selenium is in the form of selenic acid (H_2SeO_4) or SeO_4^{2-} salts. Selenic acid is formed by the oxidation of H_2SeO_3 and is a strong, highly soluble acid. Selenate salts are soluble, whereas SeO_3^{2-} salts and metal Se^{2-} salts are sparingly soluble. Their solubilities and stabilities are the greatest in alkaline environments. Conversion of SeO_4^{2-} to the less-stable SeO_3^{2-} and to Se^0 occurs very slowly (10).

18.2 SELENIUM IN PLANTS

18.2.1 Introduction

The question of whether or not selenium is a micronutrient for plants is still considered unresolved (3). Selenium has not been classified as an essential element for plants, but its role as a beneficial element in plants that are able to accumulate large amounts of it has been considered (11). Uptake and accumulation of selenium by plants is determined by the form and concentration of selenium, the presence and identity of competing ions, and affinity of a plant species to absorb and metabolize selenium (10). Variation in selenium contents of plants seems to exceed that of nearly every other element (12). Nonconcentrator or nonaccumulator plant species will accumulate >25 mg Se kg^{-1} dry weight. Most crops species such as grains, grasses, fruits, vegetables, and many weed species are considered nonconcentrators (8,13). Secondary absorbers normally grow in areas with low to medium soil-selenium concentrations and can accumulate from 25 to 100 mg Se kg^{-1} dry weight. They belong to a number of different genera, including *Aster*, *Atriplex*, *Castelleja*, *Grindelia*, *Gutierrezia*, *Machaeranthera*, and *Mentzelia*. The primary indicator or selenium-accumulator species can accumulate from 100 to 10,000 mg Se kg^{-1} dry weight. This group includes species of *Astragalus*, *Machaeranthera*, *Haplopappus*, and *Stanleya* (14). These plant species are suspects for causing acute selenosis, or selenium toxicity, of range animals that consume the plants as forages (10,15). Selenium-accumulator plants can contain 100 times more selenium than nonaccumulator plants when grown on the same soil (16). Surveys of selenium concentrations in crops reveal that areas producing low-selenium crops (<0.1 mg Se kg^{-1}) are more common than those producing crops with toxic selenium levels (>2 mg Se kg^{-1}) (16).

18.2.2 Uptake

Selenium can be absorbed by plants as inorganic SeO_4^{2-} or SeO_3^{2-} or as organic selenium compounds such as the selenoamino acid, selenomethionine (Se-Met) (10). Selenate and organic selenium forms are taken up actively by plant roots, but there is no evidence that SeO_3^{2-} uptake is mediated by the same process (3). Because of the close chemical and physical similarities between selenium and sulfur, their uptake by plants is very similar. Sulfur is absorbed actively by plants, mainly as SO_4^{2-}. The controlling enzymes for sulfur uptake are sulfur catabolic enzymes such as aryl sulfatase, choline sulfatase, and various S permeases (3,17,18). Uptake of SO_4^{2-} and SeO_4^{2-} was shown to be controlled by the same carrier with a similar affinity for both ions (19). This action demonstrated competition between SO_4^{2-} and SeO_4^{2-} for the same binding sites on these permeases (20,21).

Many studies have demonstrated an antagonistic relationship for uptake between SeO_4^{2-} and SO_4^{2-} (10,19,22–25). When SeO_4^{2-} is present in high concentrations, it can competitively inhibit SO_4^{2-} uptake. Adding SeO_4^{2-} lowered SO_4^{2-} absorption and transport in excised barley (*Hordeum vulgare* L.) roots. Conversely, adding SO_4^{2-} lowered SeO_4^{2-} absorption and transport (19,26). These studies involved an SeO_4^{2-}/SO_4^{2-} ratio of 1:1. In a preliminary solution culture experiment, an SeO_4^{2-}/SO_4^{2-} ratio of 1:3 resulted in the death of onion (*Allium cepa* L.) plant within 6 weeks (D.A. Kopsell and W.M. Randle, University of Georgia, unpublished results, 1994). When the SeO_4^{2-}/SO_4^{2-} ratio was lowered to 1:500 or 1:125 in solution culture, Kopsell and Randle (27) reported significant increases in SO_4^{2-} uptake by whole onion plants. Increasing SO_4^{-2} levels from 0.25 to 10 mM in solution culture inhibited SeO_4^{2-} uptake of broccoli (*Brassica oleracea* var. *botrytis* L.), Indian mustard (*Brassica juncea* Czern.), sugarbeet (*Beta vulgaris* L.), and rice (*Oryza sativa* L.) by 90% (22). Applications of gypsum ($CaSO_4 \cdot 2H_2O$) at the rates of 5.6 to 16.8 t ha^{-1} reduced selenium uptake in alfalfa (*Medicago sativa* L.) and oats (*Avena sativa* L.) grown on fly-ash landfill soils (28).

Although phosphate ($H_2PO_4^-$) is not expected to affect SeO_4^{2-} uptake because of the chemical dissimilarities of the two radicals, the relationship between phosphate additions and selenium

levels in plants has been inconsistent (9,10,29). Hopper and Parker (29) reported that a 10-fold increase (up to 200 µM) in phosphate solution culture decreased the selenium content of ryegrass (*Lolium perenne* L.) shoots and roots by 30 to 50% if selenium was supplied as SeO_3. In contrast, Carter et al. (30) reported that applying up to 160 kg P ha^{-1} either as H_3PO_4 or concentrated superphosphate to Gooding sandy loam increased selenium concentrations in alfalfa.

Selenate can accumulate in plants to concentrations much greater than that of selenium in the surrounding medium. In contrast, SeO_3^{2-} did not accumulate to levels surpassing the selenium levels of the external environment (31). When broccoli, Indian mustard, and rice were grown in the presence of SeO_4^{2-}, SeO_3^{2-}, or selenomethionine (Se-Met), plants accumulated the greatest amount of shoot selenium when selenium was supplied as SeO_4^{2-}, followed by those provided with Se-Met (22). In the same study, sugarbeet (*Beta vulgaris* L.) accumulated the most shoot-Se when treated with Se-Met (22). Broccoli, swiss chard (*Beta vulgaris* var. *cicla* L.), collards (*Brassica oleracea* var. *acephala* D.C.), and cabbage (*Brassica oleracea* var. *capitata* L.) grown in soil treated with 4.5 mg SeO_3^{2-} kg^{-1} or 4.5 mg SeO_4^{2-} kg^{-1} had a tissue concentration of Se in the range from 0.013 to 1.382 g Se kg^{-1} dry weight and absorbed 10 times the amount of selenium if treated with SeO_4^{2-} than with SeO_3^{2-} (32). When roots of bean (*Phaseolus vulgaris* L.) were incubated in 5 mmol m^{-3} Na_2SeO_3 or 5 mmol m^{-3} Na_2SeO_4 for 3 h, there was no significant difference in selenium accumulation, but distribution within the plant was different (33). In contrast, time-dependent kinetic studies showed that Indian mustard absorbed SeO_4^{2-} up to 2-fold faster than SeO_3^{2-} (34).

Increasing levels of selenium in plants may act to suppress the tissue concentrations of nitrogen, phosphorus, and sulfur. It can also inhibit the absorption of several heavy metals, especially manganese, zinc, copper, iron, and cadmium (35). This detoxifying effect of selenium has been demonstrated as reducing cadmium effects on garlic (*Allium sativum* L.) cell division (36). In contrast, the application of nitrogen, phosphorus, or sulfur is known to detoxify selenium. This effect may be due to either lowering of selenium uptake by the roots or to establishment of a safe ratio of selenium to other nutrient elements (35).

Selenomethionine was readily taken up by wheat (*Triticum aestivum* L.) seedlings, and the uptake followed a linear pattern in response to increasing selenium solution concentrations up to 1.0 µM (37). Western wheatgrass (*Pascopyrum smithii* Löve) also showed linear selenium uptake with Se-Met solution concentrations up to 0.6 mg Se L^{-1} (38). Results from Bañuelos et al. (39) showed that alfalfa accumulated selenium in plant tissues when selenium-laden mustard plant tissue was added to the soil. These studies provide evidence that organic selenium compounds in the soils may become available sources of selenium (40).

Genetic differences for selenium uptake and accumulation within species have also been reported. In 1939, Trelease and Trelease reported that cream milkvetch (cream locoweed, *Astragalus racemosus* Pursh.), a selenium-accumulator, produced 3.81 g dry weight in solution culture with 9 mg Na_2SeO_3 L^{-1}, whereas ground plum (*A. crassicarpus* Nutt.), a nonaccumulator, produced only 0.20 g dry weight (41). Shoots of different land races of Indian mustard grown hydroponically in the presence of 2.0 mg Na_2SeO_4 L^{-1} ranged from 501 to 1092 mg Se kg^{-1} dry matter, whereas shoots grown in soil culture at 2.0 mg Na_2SeO_4 kg^{-1} concentration ranged from 407 to 769 mg Se kg^{-1} dry matter (42). Total accumulation of selenium in onion bulb tissue ranged from 60 to 113 µg Se g^{-1} dry weight among 16 different cultivars responding to 2.0 mg Na_2SeO_4 L^{-1} nutrient solution (43).

18.2.3 Metabolism

The incorporation of SeO_4^{2-} into organic compounds in plants occurs in the leaves (44). In a similar manner, SO_4^{2-} is reduced to sulfide (S^{2-}) in the leaves before being assimilated into the S-containing amino acid, cysteine (45). After SO_4^{2-} enters the cell it can be bound covalently in different secondary metabolites or immediately reduced and assimilated (46). Selenate is assimilated in the same metabolic pathways as SO_4^{2-}. Discrimination between SO_4^{2-} and SeO_4^{2-} was

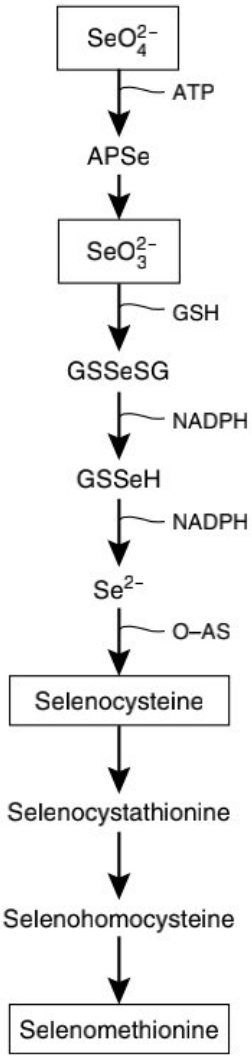

FIGURE 18.1 Proposed pathway for formation of the two Se-amino acids, Se-cysteine and Se-methionine in plants. (Abbreviations: APSe, adenosine 5′-selenophosphate; GSH, reduced glutathione; GSSeSG, selenotrisulphide; GSSeH, selenoglutathione; O-AS, acetylserine.) From A. Läuchli. *Bot. Acta* 106:455–468, 1993.

noted to occur at the level of amino acid incorporation into proteins. Uptake ratios between SO_4^{2-} and SeO_4^{2-} remained constant over a 60-h period for excised barley roots, but the ratio of S/Se decreased for free amino acid content and increased for proteins during assimilation (24).

In a series of solution-culture experiments with corn (*Zea mays* L.), Gissel-Neilsen (47) reported immediate selenium uptake and translocation to the leaves. Xylem sap contained 80 to 90% of ^{75}Se supplied as SeO_3 in amino-acid form, whereas 90% of ^{75}Se supplied as SeO_4 was recovered unchanged (47). In the leaves, selenate is converted into adenosine phosphoselenate (APSe) by ATP sulfurylase (Figure 18.1). In a similar fashion, SO_4^{2-} is first activated by ATP sulfurylase to form adenosine phosphosulfate (48). It has been suggested that ATP sulfurylase is not only the rate-limiting enzyme controlling the reduction of SO_4^{2-} (46), but it also appears to be the rate-limiting step in reduction of SeO_4^{2-} to SeO_3^{2-} (34,49). Overexpression of ATP sulfurylase in Indian mustard increased reduction of supplied SeO_4^{2-} (49). Following reduction of SeO_4^{2-}, APSe is converted into SeO_3^{2-}. Selenite is coupled to reduced glutathione (GSH), a sulfur-containing tripeptide to form a selenotrisulfide. Selenotrisulfide is reduced first to selenoglutathione and then to Se^{2-}. Selenide reacts with O-acetylserine to form selenocysteine (Se-Cys), which is further converted into Se-Met via selenocystathionine and selenohomocysteine (40). Ng and Anderson (50) reported that cysteine synthase enzymes extracted from selenium accumulator and nonaccumulator

plants utilize Se^{2-} as an alternative substrate to S^{2-} to form Se-Cys in lieu of cysteine and that the affinity for Se^{2-} was substantially greater than for S^{2-}.

18.2.4 Volatilization

Biological methylation of selenium to produce volatile compounds occurs in plants, animals, fungi, bacteria, and microorganisms (9). The predominant volatile selenium species is dimethylselenide, which is less toxic (1/500 to 1/700) than the inorganic selenium species (51). Plant species differ in their rates of selenium volatilization, and these rates are correlated with tissue selenium concentrations (52). The ability of plants to accumulate selenium is a good indicator of their potential volatilization rate. It was reported that selenium was more readily transported to the shoots of an accumulator plant (*Astragalus bisulcatus* A. Gray), whereas a barrier to selenium movement to the shoots was seen in the nonaccumulator plant, western wheatgrass (*Pascopyrum smithii* A. Löve) (38). However, in broccoli, the roots were shown to be the primary site for selenium volatilization (53). In an earlier experiment with broccoli, Zayed and Terry (54) revealed that a decrease in selenium volatilization was observed with increased application of SO_4^{2-} fertilizer.

Volatilization of selenium is also influenced by the chemical form of selenium in the growing medium. The rate of selenium volatilization of a hybrid poplar (*Populus tremula* × *alba*) was 230-fold higher in sand culture if 20 µM Se was supplied as Se-Met than as SeO_3^{2-}, and volatilization from SeO_3^{2-} was 1.5-fold that from SeO_4^{2-} (49). Selenium volatilization by shoots of broccoli, Indian mustard, sugarbeet, or rice supplied with Se-Met was also many folds higher than that from plants supplied with SeO_3^{2-} (22). In Indian mustard, Se-volatilization rates were doubled or tripled in sand culture amended with 20 µM SeO_3^{2-} relative to rates with 20 µM SeO_4^{2-} (34). These data indicate that selenium volatilization from SeO_4^{2-} is limited by the rate of SeO_4^{2-} reduction as well as by the form of selenium available (22,34).

18.2.5 Phytoremediation

An increasing problem with irrigation agriculture in arid and semi-arid regions is the appearance of selenium in soils, ground water, and drainage effluents (12,55,56). The greatest concerns for selenium contamination come in areas where water systems drain seleniferous soils. One area of the United States that has come under close investigation because of elevated levels of selenium in the water is the San Joaquin Valley in California (57,58). Selenium enters the groundwater as soluble selenites and selenates and as suspended particles of sparingly soluble and organic forms of the element (8). The mobility of selenium in groundwater is related to its speciation in the aqueous solution, sorption properties of the substrate, and solubility of the solid phases (59). The ability of certain plants to take up, accumulate, and volatilize selenium has an important application in phytoremediation of selenium from the environment (3). Phytoremediation of selenium from contaminated soils is more practical and economical than its physical removal (60). Bioaccumulation of selenium in wetland habitats is also a problem and results in selenium toxicity to wildlife (61). There is a danger of selenium re-entering the local ecosystem if plant tissues that have accumulated selenium are consumed by wildlife or allowed to degrade (62).

The search for germplasm with the potential for effective phytoremediation has begun (63). The most ideal plant species for selenium phytoremediation should have the ability for rapid establishment and growth, ability to accumulate or volatilize large amounts of selenium, tolerate salinity and elevated soil boron, and develop large amounts of biomass on high-selenium soils (3,62–64). Indian mustard was more efficient at accumulating selenium than milkvetch (*Astragalus incanus* L.), Australian saltbush (*Atriplex semibaccata* R. Br.), old man saltbush (*Atriplex nummularia* Lindl.), or tall fescue (*Festuca arundinacea* Schreb.) when grown in potting soil amended with 3.5 mg Se^{6+} kg^{-1} or 3.5 mg Se^{4+} kg^{-1} as selenate or selenite (60).

Two of the options available once selenium is phytoextracted from contaminated soils are volatilization of methylated Se forms or harvest and removal of selenium-enriched plant biomass.

Plant species with a high affinity for phytovolatilization could remove selenium from the environment by releasing it into the atmosphere, where it is dispersed and diluted by air currents (3,11,62). Most of the selenium in the air comes from windblown dusts, volcanic activity, and discharges from human activities such as the combustion of fossil fuels, smelting and refining of nonferrous metals, and the manufacturing of glass and ceramics (8). The large particulate and aerosol forms of selenium generally are not readily available for intake by plants or animals. When 15 crop species were grown in solution culture with 20 µM SeO_4^{2-}, rice, broccoli, or cabbage volatized 200 to 350 µg Se m^{-2} leaf area day^{-1}, whereas sugar beet, bean, lettuce (*Lactuca sativa* L.), or onion volatized less than 15 µg Se m^{-2} leaf area day^{-1} (52). One of the proposed disposal schemes for selenized plants from phytoremediation is as a source of forage for selenium-deficient livestock (3,60) Accurate determination of selenium levels as well as other trace elements in plant tissues and the use of other forages in a blended mixture would be needed to ensure proper dietary selenium levels in animal feeds (60,62).

18.3 SELENIUM TOXICITY TO PLANTS

Selenium toxicity is influenced by plant type, form of selenium in the growth medium, and presence of competing ions such as sulfate and phosphate (9). Interestingly, there are no written reports of selenium toxicity under cultivated conditions (9,12). This result may be because most crop plants show no injury or yield suppression until they accumulate at least 300 mg Se kg^{-1}, which is usually more than they contain even on seleniferous soils (9,14). In nonaccumulator plants, the threshold selenium concentration in shoot tissue that resulted in a 10% restriction in yield ranged from 2 mg Se kg^{-1} in rice to 330 mg Se kg^{-1} in white clover (*Trifolium repens* L.) (10). Wild-plant species growing in areas of elevated soil selenium tend to be adapted to those regions. Indicator plants can hyperaccumulate selenium to levels above 10,000 mg Se kg^{-1}, but possess biochemical means to avoid toxicity.

Descriptions for toxicity symptoms come only from solution-culture experiments. Stunting of growth, slight chlorosis, decreases in protein synthesis and dry matter production, and withering and drying of leaves are most often associated with selenium toxicity (4). Toxicity of selenium appears as chlorotic spots on older leaves that also exhibit bleaching symptoms. A pinkish, translucent color appearing on roots can also occur (65). Onions grown under extremely toxic Se concentrations showed sulfur-deficiency symptoms just before plant death (D.A. Kopsell and W.M. Randle, unpublished data, 1994).

The toxic effect of selenium to plants results mainly from interferences of selenium with sulfur metabolism (10). In most plant species, selenoamino acids replace the corresponding S-amino acids and are incorporated into proteins. Nuehierl and Böck (66) reported on a proposed mechanism of selenium tolerance in plants. In nonaccumulator plant species, Se-cys would either be incorporated into proteins or function as a substrate for downstream-sulfur pathways, which would allow selenium to interfere with sulfur metabolism. Replacing cysteine (Cys) with Se-Cys in S-proteins will alter the tertiary structure and negatively affect their catalytic activity (31). In contrast, accumulator plant species would instantly and specifically methylate Se-cys using Se-Cys methyltransferase, thereby avoiding Se-induced phytotoxicity (31). This action would remove selenium from the pool of substrates for cysteine metabolism. Thus, Se-Cys methyltransferase may be a critical enzyme conferring selenium tolerance in selenium-accumulating plants. Alternatively, tolerance may be achieved by sequestering selenium as selenate or other nonprotein Se-amino acids in the vacuole in accumulator plant cells (3).

18.4 SELENIUM IN THE SOIL

18.4.1 INTRODUCTION

The two forms of selenium that predominate in cultivated soils are SeO_4^{2-} and SeO_3^{2-} (8). Soils also contain organic selenium compounds such as Se-Met (67). Selenium occurs in the highest concentration in the surface layers of soils, where there is an abundance of organic matter (9).

Selenium in soils is generally considered to be controlled by an adsorption mechanism rather than by precipitation–dissolution reactions (68). In acid soils, sesquioxides control the sorption of selenium. Absorption controls the co-precipitation of SeO_3^{2-} by $Fe(OH)_3$. In mineral soils, SeO_4^{2-} was absorbed by soil solids. Adsorption is also believed to control the distribution of selenium in the soil under oxidizing conditions (68).

Transformation of SeO_3^{2-} to SeO_4^{2-} and vice versa occurs very slowly. The transformation of SeO_3^{2-} to Se^0 was found to be even slower (9). After Se^0 is added to soil, it oxidizes rapidly to SeO_3^{2-}. But, after the initial oxidation, the remaining selenium in the soil becomes inert, and any further oxidation proceeds very slowly. The rate of oxidation will vary in different soil types (68).

18.4.2 Geological Distribution

Selenium attracts interest because the amount in which it is present in soils is not evenly distributed geographically. Seleniferous soils and vegetation in North America extend from Alberta, Saskatchewan, and Manitoba south along the west coast into Mexico (12). The mean total selenium in soils of the United States is reported to be 0.26 mg kg^{-1} (69). Considerable variability exists from one location to another, and high Se concentrations occur in a few localized regions. In the United States, seleniferous soils occur in the northern Great Plains states of North Dakota, South Dakota, Wyoming, Montana, Nebraska, Kansas, and Colorado and in the Southwest states of Utah, Arizona, and New Mexico. These soils average 4 to 5 mg Se kg^{-1} and can reach levels as high as 80 mg kg^{-1} in some areas (8). The primary selenium sources are the western shales of the Cretaceous Age and the carbonic debris of sandstone ores of the Colorado Plateau (9).

In the other parts of the world, selenium occurs in high amounts only in the semi-arid and arid regions derived from cretaceous soils (14). Seleniferous soils occur in Mexico, Columbia, Hawaii, and China. Toxic soil selenium levels (>300 mg kg^{-1}) in Europe are limited to a few locations in Wales and Ireland (16). High-selenium soils also occur in Iceland, probably because of the volcanic activity on the island (16). In contrast, soils in Denmark, the Netherlands, Switzerland, Australia, and New Zealand, and Finland are naturally low in selenium (16). In humid climates, or in irrigated areas, most of the selenium is leached from soils (9). The most severe selenium-deficient area in the world is the Keshan region in southeastern China (16), where many children have died owing to insufficient dietary selenium. Variations in soil selenium can give rise to differences of selenium in the food chain (70).

Selenium can enter the soil through weathering of selenium-containing rocks, volcanic activity, phosphate fertilizers, and water movement. The selenium content in the soil reflects the concentration in the parent material, secondary deposition or redistribution of selenium in the soil profile, accumulation and deposition by selenium-accumulating plant materials, and erosion from selenium-containing rocks (71). The highest amounts of selenium are in igneous rock formations, existing as Se^{2-} or sulfoselenides with copper, silver, lead, mercury, and nickel (8). Selenium also occurs under sedimentary rock formations. The weathering of selenium-containing rocks under alkaline and well-aerated conditions releases selenium into the soil, which oxidizes it into the SeO_4^{2-} form. Selenium released from rocks under acidic, poorly aerated conditions will form insoluble Se^{2-} and SeO_3^{2-}. These forms of selenium develop stable adsorption complexes with ferric hydroxide and are less available to plants (8). The level of selenium in a phosphate fertilizer is governed by the concentration of selenium in the phosphatic rock (9). Fifteen different rock-phosphate fertilizers from sources in Canada and the United States ranged in selenium concentration from 0.07 to 178 mg kg^{-1} (72). Ordinary and concentrated super phosphate can be expected to contain between 40 and 60% more selenium than the phosphate rock from which it was made (72).

The distribution of selenium in the soil profile is determined by factors such as soil type, amount of organic matter, soil pH, and to some extent, leaching caused by rainfall. Organic matter helps to retain selenium in the surface horizon and has a greater SeO_3-fixation capacity than clay minerals do (9,16). Soil pH, aeration, water levels, and oxidation–reduction conditions have an effect on the

form of selenium in the soil and its availability to plants. Selenates are highly soluble in water and do not have stable adsorption complexes, thereby making them highly leachable (8).

Metal selenides occur in metal sulfide ores of iron, copper, and lead. Selenium occurs in small quantities in pyrite and in the minerals clausthalite (PbSe), naumannite ((Ag,Pb)Se), and tiemannite (HgSe). The similarity of the ionic radii of Se^{2-} (0.191 nm) and S^{2-} (0.184 nm) results in substitution of Se^{2-} for S^{2-}. Soil pH will affect the capacity of clays and ferric oxides to adsorb selenium (10). Selenite has a strong affinity for sorption, especially by iron oxides like geothite, amorphous iron hydroxide, and aluminum sesquioxides. Adsorption of SeO_3^{2-} is also a function of soil-particle concentration and composition, SeO_3^{2-} concentration, and the concentration of competing anions such as phosphate (10). Being stable in reducing environments, Se^0 can be oxidized to SeO_3^{2-} and to trace amounts of SeO_4^{2-} by some microorganisms.

18.4.3 Selenium Availability in Soils

Soil texture can affect selenium availability and uptake by plants. Because of the adsorption of SeO_3^{2-} to clay fractions in the soil, plants grown on sandy soils take up twice as much selenium as those grown on loamy soils (10). Organic matter has the ability to draw selenium from the soil solution (10). In general, selenium concentrations in plants will increase as the level of soil selenium increases, but will decrease with the addition of SO_4^{2-} (10). Extraction of selenium from soils is increased when SO_4^{2-} is used in the leaching process (9). The presence of low-molecular-weight organic acids in the soil–root interface resulted in the loss of SeO_3^{2-} sorption sites on aluminum hydroxides (73). A decrease in total selenium accumulation from soils supplied with sodium selenate (Na_2SeO_4) resulted under conditions of increasing levels of sodium (NaCl) and calcium (CaCl) salinity for canola (*Brassica napus* L.), kenaf (*Hibiscus cannibinus* L.), and tall fescue (74).

The chemical form of selenium in the soil is determined mainly by soil pH and redox potential (Figure 18.2). In alkaline soils, selenium is in the available SeO_4^{2-} form. When soil conditions become neutral to acidic, sparingly soluble ferric oxide–selenite complexes develop. Since sparingly soluble forms dominate at low pH, liming of the soil to raise the pH also has an effect by increasing the availability of selenium to plants (9). This response to addition of lime is probably

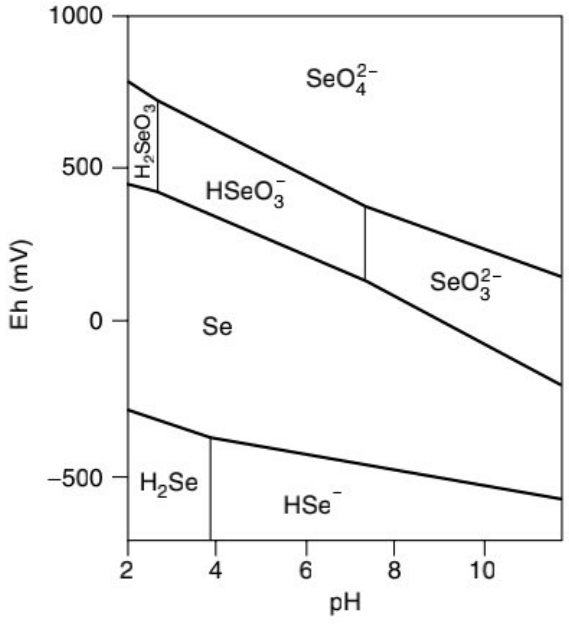

FIGURE 18.2 Selenium speciation in an aqueous system: effect of pH and oxidation–reduction potential E_h. From R.L. Mikkelsen, et al., *Selenium in Agriculture and the Environment*. Madison, WI: American Society of Agronomy, Soil Science Society of America, 1989, pp. 65–94.

caused by the reduced absorption to clays and iron oxides, resulting from increases in the soil pH (75). In the soil solution, the pH can change the speciation of selenium present. Below pH 4.5, soluble selenium speciation was 71% SeO_4^{2-} and 8% SeO_3^{2-}. When the pH was 7.0, the percentages were 51% for SeO_4^{2-} and 23% for SeO_3^{2-}. After 105 days, SeO_4^{2-} accounted for 22% and SeO_3^{2-} for 20% at pH 4.5, and were 12 and 22%, respectively, at pH 7.0 (76).

Selenium can be supplied to plants by application to soil, by foliar sprays, and by seed treatments (16). Slow-release selenium fertilizers were effective over a 4-year period in maintaining selenium levels in subterranean clover (*Trifolium subterraneum* L.) to prevent selenium deficiency in sheep in Australia (77). Use of selenium-enriched $Ca(NO_3)_2$ significantly increased selenium in wheat (*Triticum aestivum* L.) (78). Coal fly ash has been used as a source of soil-applied selenium as well as many heavy metals (9). One should be careful when using phosphate fertilizers as soil amendments, since they may contain substantial amounts of selenium (10). Selenium incorporation into fertilizers is becoming common in some countries with low soil-Se levels. Spraying SeO_4^{2-} onto pumice has been used for the production of selenium prills in New Zealand (16,77).

18.5 SELENIUM IN HUMAN AND ANIMAL NUTRITION

18.5.1 Introduction

After its discovery, selenium was most noted for its harmful effects. Selenium was the first element identified to occur in native vegetation at levels toxic to animals. Poisoning of animals can occur through consumption of plants containing toxic levels of selenium (79). Livestock consuming excessive amounts of selenized forages are afflicted with 'alkali disease' and 'blind staggers.' Typical symptoms of these diseases include loss of hair, deformed hooves, blindness, colic, diarrhea, lethargy, increased heart and respiration rates, and eventually death. On the other hand, selenium deficiency in animal feeds can cause 'white muscle disease,' a degenerative disease of the cardiac and skeletal muscles (9). Perceptions of selenium changed when Schwarz and Foltz (80) reported that additions of selenium prevented liver necrosis in rats (*Rattus* spp.) deficient in vitamin E. Its role in human health was established in 1973 when selenium, the last of 40 nutrients proven to be essential, was shown to be a component of glutathione peroxidase (GSHx), an enzyme that protects against oxidative cell damage (81). The United States' recommended daily allowance for selenium is 50 to 70 µg in human diets (5). Currently, all of the known functions of selenium as an essential nutrient in humans and other animals have been associated with selenoproteins (82).

18.5.2 Dietary Forms

Organic forms of selenium appear to be more bioavailable than the inorganic ones because the organic forms are more easily absorbed, have the ability to be stored in seleno- and other nonspecific proteins, and have lower renal clearance (83). The organic-selenium compounds identified in plants include Se-Cys, Se-methylselenocysteine, selenohomocystine, Se-Met, Se-methyl-selenomethionine, selenomethionine selenoxide, selenocystathionine, and di-methyl diselenide, selenoethionine, and Se-allyl selenocysteine (41,84,85). The majority of selenium in seleniferous wheat was shown to be Se-Met (86). The effect of consumption of seleniferous wheat on urinary excretion and retention in the body was similar to that of Se-Met supplementation (87). The form of selenium in nuts is selenocystathionine (88). The high-selenium-accumulating species of milkvetch (*Astragalus* spp. L.) contain Se-methylselenocysteine and selenocystathionine (89). Most fruits and vegetables contain >0.1 mg Se kg^{-1}, (13) but some have the potential to be enriched. Marine fish such as tuna are high in selenium, but bioactivity is much lower than selenium from other foods (90). Inorganic SeO_3^{2-}, SeO_4^{2-}, and Se^{2-} have been identified in plants at low levels (91). Selenate and SeO_3^{2-} are not regarded as naturally occurring forms of selenium in foods, but they have high biological activity, and animals can metabolize them into more active forms such

as Se-Cys (90). Selenocysteine is a component of glutathione peroxidase and constitutes the majority of selenium in animal proteins.

18.5.3 Metabolism and Form of Selenium

The bioavailability and metabolism of selenium and its distribution in an organism depend on the form of selenium ingested (83). The chemical form of selenium in foods and supplements determines absorption, speciation, and metabolism within the body, bioavailability for selenoproteins, and toxicity (87). Inorganic forms of selenium are absorbed rapidly, but are equally rapidly excreted in the urine, in contrast to Se-Met, which is retained in the body. Total recovery of inorganic forms of selenium in urine and feces of human subjects was 82 to 95% of the total dose, whereas only 26% of the total Se-Met was recovered after being ingested (87). Prolonged consumption of any one single form of selenium can produce side effects such as exaggerated accumulation in body tissues (Se-Met) and changes in cellular glutathione homeostasis (selenite) (92). When high levels of inorganic SeO_3^{2-} or organic Se-Met were fed to rats, higher selenium concentrations in body tissues were found for Se-Met than for SeO_3^{2-}. Selenium levels in erythrocytes, testes, kidney, and lungs were not significantly different between rats fed with 0.2 mg kg^{-1} Se as SeO_3^{2-} and those fed with Se as Se-Met, but higher levels of selenium were found in liver, muscle, and brain tissues for rats fed with Se-Met (93). There was an increase of up to 26-fold in the concentration of selenium localized in muscle tissues for rats fed with high levels of selenium as Se-Met when compared with those fed with SeO_3^{2-}. Selenium from Se-Met and seleno yeast showed higher accumulation in liver and muscle tissues than that from SeO_3^{2-} for channel catfish (94).

18.6 SELENIUM AND HUMAN HEALTH

18.6.1 Introduction

Immune system enhancement, cancer suppression, and cardiovascular disease reduction are all associated with increased dietary selenium (95–97). The chief biological function of selenium is as an essential cofactor to the enzyme GSHx (81). The antioxidant enzyme GSHx protects against oxidative stress by removing DNA-damaging hydrogen peroxide and lipid hydroperoxides. The chemopreventive action of selenium may come from its role in GSHx (98). Other protective qualities attributed to selenium, independent of GSHx activity, include repair of damaged DNA (99), reduction in DNA binding of carcinogens (100), and suppressing genetic mutations (101).

18.6.2 Selenium Deficiency and Toxicity in Humans

The average selenium intake by humans in most countries is sufficient to meet the United States' recommended daily allowances, and selenium deficiency in healthy humans is relatively rare (5,6). Selenium status in a population correlates highly with the selenium content of locally produced crops (7). In areas of the world with low soil selenium, addition of selenium in normal fertility regimes is practiced to avoid selenium deficiencies in humans and livestock (16). A significant inverse relationship between low-selenium status and increased risk of cancer mortality has been established for some rural counties of the United States (102).

The link between selenium deficiency and disease is associated with more than 40 different health conditions (103). The first reports of diseases linked to selenium status came from regions of China having extremely low soil selenium. Keshan disease, an endemic cardiomyopathy, and Kashin-Beck disease, a chronic and deforming arthritis, have been linked to selenium deficiency (104). Selenium deficiency also depresses the effectiveness of immune cells. Selenium deficiency was found to be an independent predictor of survival rates among patients infected with HIV (human immunodeficiency virus) (105). Increasing selenium intake in animals and human beings

increases antitumorigenic activities (106), and selenium-dietary supplementation decreases severity of several viral diseases (107).

The United States National Academy of Sciences has identified selenium intake of up to 200 μg day^{-1} as safe (108). However, sustained consumption of selenium levels exceeding 750 μg day^{-1} can cause selenium poisoning or selenosis (109). Signs of human selenosis include morphological changes in fingernails and hair loss, with an accompanied garlicky breath odor. Human selenosis reports have come from regions in China, where extremely high levels of soil selenium caused human-dietary intake to be >900 μg day^{-1} (110).

18.6.3 Anticarcinogenic Effects of Selenium

There is perhaps no more extensive body of evidence for the cancer preventive potential of a normal dietary component than there is for selenium (106). Evidence for inverse associations between nutritional selenium status and cancer risk exist from epidemiological studies (111,112), experimental animal models (92,113), and most recently, clinical trials (5). Selenium supplementation resulted in a 63% reduction in the incidence of prostate cancer over a 10-year period in an at-risk group of men given 200 μg Se day^{-1} (5). Experimental antitumorigenic effects of selenium are associated with supranutritional levels of at least 10 times those required to prevent clinical signs of selenium deficiency (106). These levels are higher than those experienced by most people, an amount which tends to be <150 to 200 μg Se day^{-1}. Anticarcinogenic activity of selenium may not involve its usual role as a nutrient because selenium-dependent enzyme activities are already at a maximum at levels of selenium below effective anticarcinogenic level and the forms of selenium that lack nutritional activity (not synthesized by Se-dependent enzymes) show good cancer-preventing activity (82). Therefore, for anticarcinogenic effects to be seen, supplementation of selenium in the diet is usually needed. Inorganic SeO_3^{2-} and yeast-derived Se-Met are the most common selenium supplements for human consumption.

18.6.4 Importance of Selenium Methylation in Chemopreventive Activity

Methylation is the best-known fate of selenium, and fully methylated metabolites are regarded as detoxified forms of selenium. Selenium methylselenocysteine has very high chemopreventive activity. This form of selenium is naturally occurring in plants enriched with selenium and does not get incorporated into proteins, thus minimizing excessive accumulation in body tissues. The metabolism of Se-methylselenocysteine produced monomethylated forms of selenium as excretory products (82). The potential activity of selenium can be enhanced in the course of being metabolized in plants, especially in those having specialized alkyl-group capabilities. Some plants such as alliums can transfer allyl groups to sulfur, or possibly, selenium. These allyl groups can undergo methylation to form highly chemopreventive alkylated derivatives (82). Selenium-enriched garlic (*Allium sativum* L.) had higher chemopreventive activity than regular garlic alone in animal models (113). Natural selenium products formed in plants are very active chemopreventive metabolites. They show higher activity in animals than the selenium compounds metabolized from inorganic selenium sources (82).

18.7 SELENIUM ENRICHMENT OF PLANTS

Substantial genetic variation in plants has been reported for mineral (43,114,115), vitamin (116), and phytochemical content (117). Breeding plants that are enriched with mineral nutrients and vitamins could substantially reduce the recurrent costs associated with fortification (118,119). Successful programs are now in place for improving zinc (120) and iron (119) contents of wheat. Selenium fertilizer has been used in Finland on vegetable crops to increase the uptake levels of dietary Se in both humans and other animals (121). However, there is very little information on the

TABLE 18.1
Selenium Tissue Analysis Values of Various Plant Species

Plant		Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			Reference
Common and Scientific Name	Variety					Low	Medium	High	
Alfalfa (*Medicago sativa* L.)		Sand	Shoot	Three cuttings	No Se treatment; pH 4.5	0.10	—	0.20	
					0.25 mg L^{-1} Na_2SeO_3; pH 4.5	14.1	—	28.7	
					0.50 mg L^{-1} Na_2SeO_3; pH 4.5	27.6	—	28.9	
					1.0 mg L^{-1} Na_2SeO_3; pH 4.5	32.7	—	49.9	
					0.25 mg L^{-1} Na_2SeO_3; pH 4.5	21.6	—	24.3	
					0.50 mg L^{-1} Na_2SeO_3; pH 4.5	38.3	—	52.6	
					1.0 mg L^{-1} Na_2SeO_3; pH 4.5	73.8	—	165.4	
					3.0 mg L^{-1} Na_2SeO_3; pH 4.5	478.2	—	912.7	
					No Se treatment; pH 7.0	0.10	—	0.50	133
					0.25 mg L^{-1} Na_2SeO_3; pH 7.0	19.2	—	60.1	
					0.50 mg L^{-1} Na_2SeO_3; pH 7.0	52.7	—	63.5	
					1.0 mg L^{-1} Na_2SeO_3; pH 7.0	92.4	—	131.4	
					3.0 mg L^{-1} Na_2SeO_3; pH 7.0	183.3	—	382.4	

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
					0.25 mg L^{-1} Na_2SeO_3; pH 7.0	28.4	—	65.1	
					0.50 mg L^{-1} Na_2SeO_3; pH 7.0	61.5	—	169.0	
					1.0 mg L^{-1} Na_2SeO_3; pH 7.0	174.4	—	503.30	
					3.0 mg L^{-1} Na_2SeO_3; pH 7.0	722.3	—	1581.60	
	'Germain WL 512'	Sand	Shoot	First harvest	No Se treatment	—	<0.05 mg kg^{-1}	—	134
				Second harvest	No Se treatment	—	<0.05 mg kg^{-1}	—	
				First harvest	0.25 mg L^{-1} Na_2SeO_4	—	44.3 mg kg^{-1}	—	
				Second harvest	0.25 mg L^{-1} Na_2SeO_4	—	30.1 mg kg^{-1}	—	
				First harvest	0.5 mg L^{-1} Na_2SeO_4	—	133.3 mg kg^{-1}	—	
				Second harvest	0.5 mg L^{-1} Na_2SeO_4	—	45.5 mg kg^{-1}	—	
				First harvest	1.0 mg L^{-1} Na_2SeO_4	—	620 mg kg^{-1}	—	
				Second harvest	1.0 mg L^{-1} Na_2SeO_4	—	98.6 mg kg^{-1}	—	
	'Honey-oye'	Soil	Shoot		50 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.13 mg kg^{-1}	—	135

Astragalus, (Two-grooved milkvetch, *Astragalus bisulcatus* A. Gray) See entry under milkvetch.	Solution		No Se treatment	—	44 µg kg^{-1}	—	136	
			0.25 µg L^{-1} SeO_3	—	272 µg kg^{-1}	—		
			5 µg L^{-1} SeO_3	—	6200 µg kg^{-1}	—		
			10 µg L^{-1} SeO_3		10,700 µg kg^{-1}	—		
		Roots	No Se treatment	—	27 µg kg^{-1}	—		
			0.25 µg L^{-1} SeO_3	—	252 µg kg^{-1}	—		
			5 µg L^{-1} SeO_3	—	3480 µg kg^{-1}	—		
			10 µg L^{-1} SeO_3	—	6650 µg kg^{-1}	—		
Astragalus crotalariae A. Gray	Solution	Tops	No Se treatment	—	0.97 µg kg^{-1}	—		
			0.25 µg L^{-1} SeO_3	—	238 µg kg^{-1}	—		
			1 µg L^{-1} SeO_3	—	452 µg kg^{-1}	—		
			2.5 µg L^{-1} SeO_3	—	1530 µg kg^{-1}	—		
			10 µg L^{-1} SeO_3	—	4960 µg kg^{-1}	—		
			50 µg L^{-1} SeO_3	—	26,900 µg kg^{-1}	—		
			100 µg L^{-1} SeO_3	—	30,300 µg kg^{-1}	—		
		Roots	No Se treatment	—	22 µg kg^{-1}	—		
			0.25 µg L^{-1} SeO_3	—	151 µg kg^{-1}	—		
			1 µg L^{-1} SeO_3	—	363 µg kg^{-1}	—		
			2.5 µg L^{-1} SeO_3	—	750 µg kg^{-1}	—		

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
					10 µg L^{-1} SeO_3	—	2400 µg kg^{-1}	—	
					50 µg L^{-1} SeO_3	—	10,200 µg kg^{-1}	—	
					100 µg L^{-1} SeO_3	—	20,800 µg kg^{-1}	—	
Barley (*Hordeum vulgare* L.)		Native soil[a]	Grain		No Se treatment	—	0.09	—	
					1.12 kg ha^{-1} Na_2SeO_3; pH 6.6	—	1.24	—	137
					2.24 kg ha^{-1} Na_2SeO_3 pH 6.6	—	—	—	
	'Iona'	Foliar application	Grain		10 g ha^{-1} Na_2SeO_4	—	2.00	—	
					20 g ha^{-1} Na_2SeO_4	—	0.51	—	138
			Straw		10 g ha^{-1} Na_2SeO_4	—	1.13	—	
					20 g ha^{-1} Na_2SeO_4	—	0.50	—	
						—	0.79	—	
Bean (*Phaseolus vulgaris* L.)	'Tender-crop'	Soil	Pods		100 ton A^{-1} Se as fly ash (16.8 ppm Se)		0.47		139
					50 ton A^{-1} Se as fly ash (16.8ppm Se)	—	0.07	—	135

Rapid-growing brassica (*Brassica oleracea* L.)	RCBP	Solution	Leaves	No Se treatment	—	ND	—	140
				3.0 mg L^{-1} Na_2SeO_4	—	522	—	
				6.0 mg L^{-1} Na_2SeO_4	—	1275	—	
				9.0 mg L^{-1} Na_2SeO_4	—	1916	—	
			Stem	No Se treatment	—	ND	—	
				3.0 mg L^{-1} Na_2SeO_4	—	267	—	
				6.0 mg L^{-1} Na_2SeO_4	—	721	—	
				9.0 mg L^{-1} Na_2SeO_4	—	1165	—	
			Root	No Se treatment	—	ND	—	
				3.0 mg L^{-1} Na_2SeO_4	—	338	—	
				6.0 mg L^{-1} Na_2SeO_4	—	857	—	
				9.0 mg L^{-1} Na_2SeO_4	—	1636	—	
Broccoli (*Brassica oleracea* var. *botrytis* L.)		Soil	Floret	5 mg kg^{-1} Na_2SeO_3	—	155	—	32
				5 mg kg^{-1} Na_2SeO_4	—	1382	—	
			Composite leaves	5 mg kg^{-1} Na_2SeO_3	—	49	—	
				5 mg kg^{-1} Na_2SeO_4	—	377	—	
Cabbage (*Brassica oleracea* var. *capitata* L.)		Soil	Young leaves	5 mg kg^{-1} Na_2SeO_3	—	52	—	32
				5 mg kg^{-1} Na_2SeO_4	—	479	—	

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
			Old leaves		5 mg kg^{-1} Na_2SeO_3	—	38	—	
					5 mg kg^{-1} Na_2SeO_4	—	275	—	
			Composite leaves		5 mg kg^{-1} Na_2SeO_3	—	41	—	
					5 mg kg^{-1} Na_2SeO_4	—	316	—	
	'Scandic'	Native soil	Leaves		No Se treatment	11.00 µg kg^{-1} fresh weight	45.00 µg kg^{-1} fresh weight	100.00 µg kg^{-1} fresh weight	141
	'Golden Acre'	Soil	Leaves		100 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.95	—	139
					50 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.20	—	135
Canola (*Brassica napus* L.)	'Wester'	Soil	Leaves	First harvest	1.5 mg kg^{-1} as SeO_4^{2-} or Se organic materials	1.60	—	283	142
				Second harvest	1.5 mg kg^{-1} as SeO_4^{2-} or Se organic materials	0.80	—	7.70	
			Stems	First harvest	1.5 mg kg^{-1} as SeO_4^{2-} or Se organic materials	0.50	—	57.00	

				Second harvest	1.5 mg kg^{-1} as SeO_4^{2-} or Se organic materials	0.30	—	5.60	
			Roots	First harvest	1.5 mg kg^{-1} as SeO_4^{2-} or Se organic materials	0.60	—	87.50	
				Second harvest	1.5 mg kg^{-1} as SeO_4^{2-} or Se organic materials	0.80	—	5.80	
		Native soil	Shoots	—	40 mg kg^{-1} Se in soil	280	—	470.0	32
				—	0.1 mg kg^{-1} Se in soil	0.20	—	0.60	
			Roots	—	40 mg kg^{-1} Se in soil	25	—	44	
				—	0.1 mg kg^{-1} Se in soil	0.10	—	0.20	
Carrot (*Daucus carota* L.)	'Scarlet Nantes'	Soil	Root		100 ton A^{-1} Se as fly ash (16.8 ppm Se)		0.19		139
					50 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.06	—	135
Celery (*Apium graveolens* L.)	'Seoul'	Solution	Leaves, petioles		6 mg L^{-1} Na_2SeO_4	—	57.3	—	143
Collards (*Brassica oleracea* var *acephala DC.*)		Soil	Leaf		5 mg kg^{-1} Na_2SeO_3	—	36	—	32
					5 mg kg^{-1} Na_2SeO_4	—	398	—	
			Mid-rib/ petiole		5 mg kg^{-1} Na_2SeO_3	—	23	—	
					5 mg kg^{-1} Na_2SeO_4	—	240	—	

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
			Composite leaves		5 mg kg^{-1} Na_2SeO_3	—	33	—	
					5 mg kg^{-1} Na_2SeO_4	—	455	—	
			Seeds		5 mg kg^{-1} Na_2SeO_3	—	18	—	
					5 mg kg^{-1} Na_2SeO_4	—	491	—	
Tall fescue (*Festuca arundinacea* L.)	'Fawn'	Soil	Shoots	First harvest	1.5 mgkg^{-1} as SeO_4^{2-} or Se organic materials	0.40	—	75.2	142
				Second harvest	1.5 mg kg^{-1} as SeO_4^{2-} or Se organic materials	0.80	—	74.6	142
		Native soil	Shoots	First clipping (60 days)	0.46 mg kg^{-1} Se in soil	—	310	—	55
				Second clipping (115 days)	0.46 mg kg^{-1} Se in soil	—	630	—	
				First clipping (60 days)	0.65 mg kg^{-1} Se in soil	—	170	—	
				Second clipping (85 days)	0.65 mg kg^{-1} Se in soil	—	200	—	
				Third clipping (115 days)	0.65 mg kg^{-1} Se in soil	—	270	—	
	'Alta'	Native soil	Shoots		40 mg kg^{-1} Se in soil	10	—	50	62

				0.1 mg kg^{-1} Se in soil	0.01	—	0.14	
Fourwing Saltbush [*Atriplex canescens* Nutt.]		Native soil	Shoots	1.8 mg Se kg^{-1} in soil	—	2.10	—	144
		Soil	Shoots	4.8 mg Se kg^{-1} (3.0 mg Na_2SeO_4 kg^{-1})	—	172	—	
Grape (*Vitis vinifera* L.)	'Cabernet Sauvignon'	Soil	Leaves	0 to 1.5 kg Se ha^{-1} as Na_2SeO_4	0.02 to 0.12 µg g^{-1}	—	10.41	145
		Native soil	Fruit	0.15 ± 0.02 µg Se g^{-1}	—	0.02	—	146
				0.31 ± 0.06 µg Se g^{-1} in soil	—	0.04	—	
				0.49 ± 0.03 µg Se g^{-1} in soil	—	0.06	—	
Kanef (*Hibiscus cannabinus* L.)	'Indian'	Native soil	Shoots	40 mg kg^{-1} Se in soil	36	—	45	42
				0.1 mg kg^{-1} Se in soil	0.75	—	1.10	
			Roots	40 mg kg^{-1} Se in soil	36	—	62	
				0.1 mg kg^{-1} Se in soil	0.86	—	1.10	
		Native soil	Shoots	0.75 mg kg^{-1} Se in soil		520		55
			Roots	0.75 mg kg^{-1} Se in soil		420		
Lettuce (*Lactuca sativa* L.)		Soil	Leaves	No Se treatment	—	0.05	—	147
				0.1 mg kg^{-1} H_2SeO_4	—	6.40	—	
				1.0 mg kg^{-1} H_2SeO_4	—	270.0	—	

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
Milkvetch, two-grooved (*Astragalus bisulcatus* A. Gray)		Solution	Tops		1.0 mg L^{-1} Na_2SeO_3	—	243	—	38
					2.0 mg L^{-1} Na_2SeO_3	—	510	—	
See entry under *Astragalus*.					0.3 mg L^{-1} Se-Met	—	283	—	
					0.6 mg L^{-1} Se-Met	—	274	—	
					0.3 mg L^{-1} Se-Cys	—	46.8	—	
					0.6 mg L^{--1} Se-Cys	—	95.2	—	
			Roots		1.0 mg L^{-1} Na_2SeO_3	—	202	—	
					2.0 mg L^{-1} Na_2SeO_3	—	407	—	
					0.3 mg L^{-1} Se-Met	—	350	—	
					0.6 mg L^{-1} Se-Met	—	428	—	
					0.3 mg L^{-1} Se-Cys	—	124	—	
					0.6 mg L^{-1} Se-Cys	—	222	—	
Millet, Japanese; barnyardgrass (*Echinochloa crusgalli* var. *frumentacea* Wight)		Soil	Grain		100 ton A^{-1} Se as fly ash (16.8 ppm Se)		0.90		139
					50 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.16	—	135
Indian mustard (*Brassica juncea* L.)	Land races	Solution	Shoots		2.0 mg L^{-1} Na_2SeO_4	501.00 ± 26.00 mg kg^{-1}	—	1092	62
			Roots		2.0 mg kg^{-1} Na_2SeO_4	197.00 ± 16.00 mg kg^{-1}	—	470	

		Soil	Shoots	2.0 mg L^{-1} Na_2SeO_4	407.00 ± 26.00 mg kg^{-1}	—	769	
			Roots	2.0 mg kg^{-1} Na_2SeO_4	152.00 ± 38.00 mg kg^{-1}	—	332	
		Native soil	Shoots	0.50 mg kg^{-1} Se in soil	—	950	—	55
				0.86 mg kg^{-1} Se in soil	—	1050	—	
Onion (*Allium cepa* L.)	'Granex 33'	Solution	Leaves	No Se treatment	—	ND	—	27
				0.5 mg L^{-1} Na_2SeO_4	—	47.3	—	
				1.0 mg L^{-1} Na_2SeO_4	—	109.3	—	
				1.5 mg L^{-1} Na_2SeO_4	—	140	—	
				2.0 mg L^{-1} Na_2SeO_4	—	208	—	
			Bulb	No Se treatment	—	ND	—	
				0.5 mg L^{-1} Na_2SeO_4	—	18.9	—	
				1.0 mg L^{-1} Na_2SeO_4	—	41.4	—	
				1.5 mg L^{-1} Na_2SeO_4	—	56.5	—	
				2.0 mg L^{-1} Na_2SeO_4	—	70.9	—	
			Root	No Se treatment	—	[a]ND	—	
				0.5 mg L^{-1} Na_2SeO_4	—	37.7	—	
				1.0 mg L^{-1} Na_2SeO_4	—	78.9	—	
				1.5 mg L^{-1} Na_2SeO_4	—	104.3	—	
				2.0 mg L^{-1} Na_2SeO_4	—	148.5	—	

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
		Solution	Bulb		2.0 mg L^{-1} Na_2SeO_4	65.7	—	156.2	148
	'Downing Yellow Sweet Spanish'	Soil	Bulb		100 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.30	—	139
	'1620 Pedro'	Soil	Bulb		50 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.21	—	135
	'Stuttgart'	Soilless media	Bulb		7.59% Se as coal fly ash (13.3 ppm Se)	—	0.25	—	149
					10% Se as coal fly ash (10.1 ppm Se)	—	0.22	—	
Orach (*Atriplex patula* L.)		Native soil	Shoots		45.20 ± 19.79 mg kg^{-1} Se in soil	—	20.79	—	150
					75.78 ± 28.78 mg kg^{-1} Se in soil	—	79.96	—	
Potato (*Solanum tuberosum* L.)	'Katahdin'	Soil	Tuber		100 ton A^{-1} Se as fly ash (16.8 ppm Se)		0.49		139
					50 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.03	—	135
Raspberry (*Rubus idaeus* L.)		Soil	Roots		0 to 1.5 kg Se ha^{-1} as Na_2SeO_4	0.02	—	0.21	151

			Floricanes		0 to 1.5 kg Se ha^{-1} as Na_2SeO_4	—	—	0.32	
			Primicanes		0 to 1.5 kg Se ha^{-1} as Na_2SeO_4	—	—	1.10	
			Leaves		0 to 1.5 kg Se ha^{-1} as Na_2SeO_4	—	—	1.81	
			Brambles		0 to 1.5 kg Se ha^{-1} as Na_2SeO_4	—	—	0.65	
Rice (*Oryza sativa* L.)		Native soil	Grain	First year	2.4 mg Se kg^{-1} in soil	—	9.9	—	152
				Second year	2.4 mg Se kg^{-1} in soil	—	8.9	—	
			Straw	First year	2.4 mg Se kg^{-1} in soil	—	18.0	—	
				Second year	2.4 mg Se kg^{-1} in soil	—	16.6	—	
	'M101'	Native soil	Grain		No Se treatment (0 to 5 g kg^{-1} OM)	0.10	—	0.40	153
					1.5 mg kg^{-1} Na_2SeO_4 (0 to 5g kg^{-1} OM)	6.0	—	213	
					3.0 mg kg^{-1} Na_2SeO_4 (0 to 5 g kg^{-1} OM)	6.8	—	215	
					6.0 mg kg^{-1} Na_2SeO_4 (0 to 5 g kg^{-1} OM)	13.9	—	455	
			Shoots		No Se treatment (0 to 5 g kg^{-1} OM)	0.10	—	2.00	
					1.5 mg kg^{-1} Na_2SeO_4 (0 to 5 g kg^{-1} OM)	5.60	—	360	
					3.0 mg kg^{-1} Na_2SeO_4 (0 to 5 g kg^{-1} OM)	10.2	—	668	

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
					6.0 mg kg^{-1} Na_2SeO_4 (0 to 5 g kg^{-1} OM)	20.9	—	1233	
Ryegrass (*Lolium perenne* L.)		Soil	Shoots		No Se treatment	—	0.05	—	147
					0.1 mg kg^{-1} H_2SeO_4	—	5.70	—	
					1.0 mg kg^{-1} H_2SeO_4	—	72.0	—	
Sprouts, Brussels (*Brassica oleracea* var. *gemmifera* Zenker)	'Explorer'	Native soil	Leaves		No Se treatment	38.00 µg kg^{-1} fresh weight	66.00 µg kg^{-1} fresh weight	220.00 µg kg^{-1} fresh weight	141
Sweet clover, Yellow [*Melilotus officinalis* Pallas]		Native soil	Shoots		1.8 mg Se kg^{-1} in soil	—	2.75	—	144
		Soil			4.8 mg Se kg^{-1} (3.0 mg Na_2SeO_4 kg^{-1})	—	216	—	
Sweet clover (*Melilotus indica* L.)		Native soil	Shoots		75.78 ± 29.78 mg Se kg^{-1} in soil		183.01		150
Swiss chard (*Beta vulgaris* L.)		Soil	Leaf		5 mg kg^{-1} Na_2SeO_3	—	29	—	32
					5 mg kg^{-1} Na_2SeO_4	—	735	—	
			Mid-rib/ petiole		5 mg kg^{-1} Na_2SeO_3	—	13	—	

					5 mg kg^{-1} Na_2SeO_4	—	120	—	
			Composite leaves		5 mg kg^{-1} Na_2SeO_3	—	30	—	
					5 mg kg^{-1} Na_2SeO_4	—	449	—	
Timothy (*Phleum pratense* L.)		Natural soil	Shoots	First cutting	No Se treatment	—	0.10	—	137
					1.12 kg ha^{-1} Na_2SeO_3; pH 6.6	—	0.68	—	
					2.24 kg ha^{-1} Na_2SeO_3 pH 6.6	—	1.18	—	
Tomato (*Lycopersicon esculentum* Mill.)	'Vendor'	Soil	Fruit		100 ton A^{-1} Se as fly ash (16.8 ppm Se)		0.33		139
	'Super-sonic'	Soil	Fruit		50 ton A^{-1} Se as fly ash (16.8 ppm Se)	—	0.02	—	135
Trefoil, birdsfoot (*Lotus corniculatus* L.)		Native soil	Shoots	First clipping (60 days)	0.39 mg kg^{-1} Se in soil	—	440 µg kg^{-1}	—	63
				Second clipping (115 days)	0.39 mg kg^{-1} Se in soil	—	870 µg kg^{-1}	—	
				First clipping (60 days)	0.82 mg kg^{-1} Se in soil	—	360 µg kg^{-1}	—	
				Second clipping (85 days)	0.82 mg kg^{-1} Se in soil	—	290 µg kg^{-1}	—	
				Third clipping (115 days)	0.82 mg kg^{-1} Se in soil	—	220 µg kg^{-1}	—	
Wheat (*Triticum aestivum* L.)		Native soil	Grain	First year	2.4 mg Se kg^{-1} in soil	—	19.6	—	152
				Second year	2.4 mg Se kg^{-1} in soil	—	12.4	—	

Continued

TABLE 18.1 (*Continued*)

Plant						Selenium Concentration in Dry Matter (mg kg^{-1} unless otherwise noted)			
Common and Scientific Name	Variety	Type of Culture[a]	Type of Tissue Sampled	Age, Stage, Condition, or Date of Sample	Selenium Treatment	Low	Medium	High	Reference
			Straw	First year	2.4 mg Se kg^{-1} in soil	—	16.6	—	
				Second year	2.4 mg Se kg^{-1} in soil	—	11.1	—	
Western wheatgrass (*Pascopyrum smithii* Löve)		Solution	Tops		1.0 mg L^{-1} Na_2SeO_3	—	20.2	—	38
					2.0 mg L^{-1} Na_2SeO_3	—	55.1	—	
					0.3 mg L^{-1} Se-Met	—	31.5	—	
					0.6 mg L^{-1} Se-Met	—	92.8	—	
					0.3 mg L^{-1} Se-Cys	—	17.4	—	
					0.6 mg L^{-1} Se-Cys	—	28.6	—	
			Roots		1.0 mg L^{-1} Na_2SeO_3	—	187	—	
					2.0 mg L^{-1} Na_2SeO_3	—	647	—	
					0.3 mg L^{-1} Se-Met	—	81	—	
					0.6 mg L^{-1} Se-Met	—	161	—	
					0.3 mg L^{-1} Se-Cys	—	158	—	
					0.6 mg L^{-1} Se-Cys	—	220	—	

Note: ND = not determined.

[a]Native soil denotes experiments or studies where crops were harvested from untreated soil and the selenium level was determined from a soil sample to estimate selenium fertility.

inheritance of Se uptake and accumulation in plants. Investigation into the genetic variation for Se content in tall fescue revealed that progress from selection for selenium content is possible and that the trait was heritable (122). Narrow-sense heritability estimates for selenium accumulation in a rapid-cycling *Brassica oleracea* L. population were moderate (0.55), and gains from selection were 4.8 and 4.0% per selection cycle for high and low selenium accumulation, respectively (114). Knowledge of the genetic variances for selenium accumulation will be useful in selecting efficient strategies designed to enhance food crops. Further research is needed to identify the form and dosage of selenium delivered by selenium-enriched plants (92).

18.8 SELENIUM TISSUE ANALYSIS VALUES OF VARIOUS PLANT SPECIES

Selenium is unevenly distributed within plant tissues. Actively growing tissues usually contain the highest amounts of Se (35), and many plant species accumulate higher amounts of selenium in shoot or leaf tissues than in root tissues. Plant species differ greatly in their ability to accumulate seed selenium. Nelson and Johnson (123) reported seed selenium levels up to 3750 μg Se g^{-1} dry weight in native milkvetch (*Astragalus* L.) species. Selenium accumulation in a rapid-cycling *Brassica oleracea* L. population increased linearly with increasing Na_2SeO_4 treatment concentrations in nutrient solution culture, ranging from nondetectable at 0 mg Na_2SeO_4 L^{-1} to 753 μg Se g^{-1} dry weight at 7.0 mg Na_2SeO_4 L^{-1} (124). Selenium is also unevenly distributed within seeds. In dried grains of barley, the husk and pericarp accumulated selenium up to 0.6 μg Se g^{-1}, the scutellum 0.4 μg Se g^{-1}, the embryo 0.3 μg Se g^{-1}, and the aleurone layer, embryonic leaves, and root initials 0.2 μg Se g^{-1} (125).

Selenium treatment and selenium-enriched media will affect seed germination in a number of species. Soybeans (*Glycine max* Merr.) pretreated with 10 to 100 g Se ha^{-1} as either seed or foliar treatments were grown on a nonseleniferous sandy loam soil and subsequently produced seeds accumulating 0.78 to 38.5 mg Se kg^{-1}. When these seeds were planted without application of selenium fertilizer, the concentration of harvested seeds decreased to 0.11 to 1.02 mg Se kg^{-1} (126). Seed germination was reduced if wheat (*Triticum aestivum* L.) was grown in soils with >16.0 mg Se kg^{-1} (127). Weight of fresh Alfalfa seedling was suppressed in response to >10.0 mg Se L^{-1} in solution culture (128). Turnip (*Brassica campestris* L.) seed germination was >98% when seeds were incubated in <484 mg $NaSeO_3$ L^{-1}, but decreased to 51% if the concentration of $NaSeO_4$ was increased to 4.84 g $NaSeO_3$ L^{-1}. In response to $NaSeO_3$, turnip seed germination was 97% at Se levels <95 mg $NaSeO_3$ L^{-1}, 53% at 484 mg $NaSeO_3$ L^{-1}, 17% at 951 mg $NaSeO_3$ L^{-1}, and 0% at 4.84 g $NaSeO_3$ L^{-1} (129). Interestingly, several studies report that seed germination was enhanced in response to <1.0 mg Se L^{-1} in nutrient solutions (127,130,131). Activity of β-galactosidase, an enzyme important in the hydrolysis of complex carbohydrates during seed germination, in fenugreek (*Trigonella foenum-graecum* L.) was enhanced by 40% when exposed to 0.5 mg L^{-1} Na_2SeO_3-seed treatment, but decreased by 60 to 65% if Na_2SeO_3-seed treatment was increased to 1 mg L^{-1} (132). Seed germination was >96% after 72 h in a rapid-cycling *Brassica oleracea* population when the content of selenium in the seed was <700 μg Se g^{-1} dry weight (124).

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