Section IV

Beneficial Elements

16 Aluminum

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16.1 INTRODUCTION

Soils contain an average of 7% total aluminum (Al), and under acidic conditions, aluminum is solubilized (1), increasing availability to plants and aquatic animals. Soil acidification due to application of fertilizers, growing of legumes, or acid rain is an increasing problem in agricultural and natural ecosystems (2–4).

No conclusive evidence suggests that aluminum is an essential nutrient for either plants (5) or animals (6,7), although there are a few instances of beneficial effects. Aluminum is toxic to plants and animals, interfering with cytoskeleton structure and function, disrupting calcium homeostasis, interfering with phosphorus metabolism, and causing oxidative stress (discussed in later sections).

16.2 ALUMINUM-ACCUMULATING PLANTS

Relative to aluminum accumulation, there appears to be two groups of plant species: aluminum excluders and aluminum accumulators (8). Most plant species, particularly crop plants, are aluminum excluders. Aluminum contents in most herbaceous plants averaged 200 mg kg^{-1} in leaves (Hutchinson, cited in [9]). Chenery (10,11) analyzed leaves of various species of monocots and dicots for aluminum content, and defined aluminum accumulators as those plants with 1000 mg Al kg^{-1} or greater in leaves. Aluminum accumulation appears to be a primitive character, found frequently among perennial, woody species in tropical rain forests (9,12).

Masunaga et al. (13) studied 65 tree species and 12 unidentified species considered to be aluminum accumulators in a tropical rain forest in West Sumatra and suggested that aluminum accumulators be divided further into two groups: (a) those with aluminum concentrations lower than 3000 mg kg^{-1}; and (b) those with higher aluminum concentrations. For trees with foliar aluminum concentrations greater than 3000 mg kg^{-1}, positive correlations were noted between aluminum concentrations and phosphorus or silicon concentrations in leaves.

Although Chenery (11) did not consider gymnosperms to be aluminum accumulators, Truman et al. (14) proposed that most *Pinus* species are facultative aluminum accumulators. In Australia, values of foliar aluminum ranged from 321 to 1412 mg kg^{-1} for Monterey pine (*Pinus radiata* D. Don), 51 to 1251 mg kg^{-1} for slash pine (*Pinus elliotii* Engelm.), and 643 to 2173 mg kg^{-1} for loblolly pine (*Pinus taeda* L.) (15). In addition, foliar aluminum concentrations $\geq$ 1000 mg kg^{-1} were reported in Monterey pine and black pine (*Pinus nigra* J.F. Arnold) grown in nutrient solutions containing aluminum (14,16,17).

Tea (*Camellia sinensis* Kuntze) is one crop plant considered to be an aluminum accumulator, with aluminum concentrations of 30,700 mg kg^{-1} in mature leaves, but much lower concentrations of only 600 mg kg^{-1} in young leaves (18). Most of the aluminum was localized in the cell walls of the epidermis of mature leaves (18).

Another well-known aluminum-accumulating plant is hydrangea (*Hydrangea macrophylla* Ser.), which has blue-colored sepals when the plant is grown in acidic soils and red-colored sepals when grown in alkaline soils. The blue color of hydrangea sepals is due to aluminum complexing with the anthocyanin, delphinidin 3-glucoside, and the copigment, 3-caffeoylquinic acid (19).

Two excellent reviews of aluminum accumulators are by Jansen et al. (9) and Watanabe and Osaki (8). Possible mechanisms of aluminum tolerance will be discussed in later sections.

16.3 BENEFICIAL EFFECTS OF ALUMINUM IN PLANTS

16.3.1 Growth Stimulation

Not surprisingly, aluminum addition has a growth stimulatory effect on aluminum accumulators. In tea, addition of aluminum and phosphorus increased phosphorus absorption and translocation as well as root and shoot growth (20,21). Similarly, the aluminum-accumulating shrub, *Melastoma malabathricum* L., exhibited increased growth of leaf, stem, and roots as well as increased phosphorus accumulation when aluminum was added to culture solutions (22).

Low levels of aluminum sometimes stimulate root and shoot growth of nonaccumulators. Turnip (*Brassica rapa* L. subsp. *campestris* A.R. Clapham) root lengths were increased by increasing aluminum levels up to 1.2 μM at pH 4.6 (23). Soybean (*Glycine max* Merr.) root elongation and $^{15}NO_3^-$ uptake increased with increasing aluminum concentrations up to 10 μM, but were reduced when aluminum levels increased further to 44 μM (24). Shoot and root growth of Douglas fir (*Pseudotsuga menziesii* Franco) seedlings were stimulated by increasing aluminum levels up to 150 μM but were reduced at higher aluminum levels (25). Root elongation of an aluminum-tolerant race of silver birch (*Betula pendula* Roth) increased as solution aluminum increased up to 930 μM Al but then decreased at 1300 μM Al (26). Several researchers (23–25,27,28) have hypothesized that low levels of Al^{3+} ameliorated the toxic effects of H^+ on cell walls, membranes, or nutrient transport, but aluminum-toxic effects predominated at higher aluminum levels.

16.3.2 Inhibition of Plant Pathogens

Aluminum can be toxic to pathogenic microorganisms, thus helping plants to avoid disease. Spore germination and vegetative growth of the black root rot pathogen, *Thielaviopsis basicola* Ferraris, were inhibited by 350 μM Al at pH 5 (29). Similarly, mycelial growth and sporangial germination of potato late blight pathogen, *Phytophthora infestans*, were inhibited by 185 μM Al, and Andrivon (30) speculated that amendment of soils with aluminum might be used as a means of disease control.

16.4 ALUMINUM ABSORPTION AND TRANSPORT WITHIN PLANTS

16.4.1 Phytotoxic Species

The most phytotoxic form of aluminum is Al^{3+} (more correctly, $Al(H_2O)_6^{3+}$), which predominates in solutions below pH 4.5 (31–33) (Figure 16.1). Possibly, hydroxyl-aluminum ($AlOH^{2+}$ and $Al(OH)_2^+$) ions are also phytotoxic, particularly to dicotyledonous plants (31,34). However, as pointed out by many researchers (35,36), these aluminum species are interrelated along with the pH variable, so it is difficult to rank their relative toxicity.

In contrast, Al-F, Al-SO_4, and Al-P species are much less toxic or even nontoxic to plants (34,37). Barley (*Hordeum vulgare* L.) roots were unaffected by aluminum when 2.5 to 10 μM F^- was added to nutrient solution containing up to 8 μM total soluble aluminum (37). Also using nutrient solution, Kinraide and Parker (38) positively demonstrated the nontoxic nature of Al-SO_4 complexes ($AlSO_4^+$ and $Al(SO_4)_2^-$) for wheat (*Triticum aestivum* L.) and red clover (*Trifolium pratense* L.). Soybean had longer root growth when increasing amounts of phosphorus were added to nutrient solutions having constant total aluminum concentrations (39).

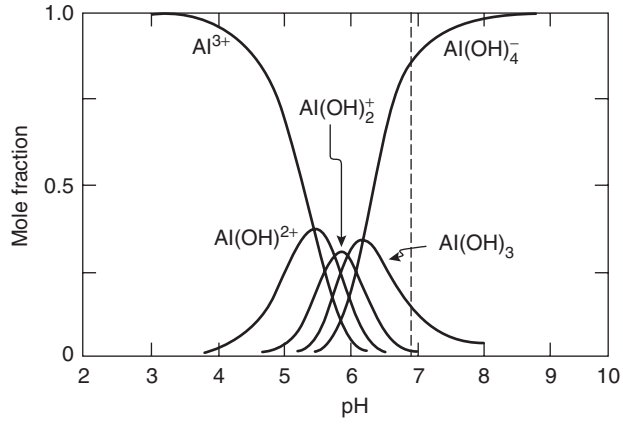


FIGURE 16.1 Speciation of aluminum as affected by solution pH. (46)

16.4.2 Absorption

Since aluminum is a trivalent cation in its phytotoxic form in the external medium, it does not easily cross the plasma membrane. Akeson and Munns (40) calculated that the endocytosis of Al^{3+} could contribute to its absorption. Alternatively, it is possible that Al^{3+} could be absorbed through calcium channels (41) or nonspecific cation channels.

Our understanding of aluminum absorption across plant membranes has been limited by the complex speciation of Al, its binding to cell walls, lack of an affordable and available isotope, and lack of sensitive analytical techniques to measure low levels of aluminum in subcellular compartments (42). Aluminum absorption by excised roots of wheat, cabbage (*Brassica oleracea* L.), lettuce (*Lactuca sativa* L.), and kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov.), and by cell suspensions of snapbean (*Phaseolus vulgaris* L.) followed biphasic kinetics (43–45). A rapid, nonlinear, nonmetabolic phase of uptake occurred during the first 20 to 30 min. This nonsaturable phase was thought to be accumulation in the apoplastic compartment due to polymerization or precipitation of aluminum or binding to exchange sites in cell walls (44). A linear, metabolic phase of uptake was superimposed over the nonlinear phase and thought to be accumulation in the symplasmic compartment (i.e., within the plasma membrane).

Using the rare ^{26}Al isotope and accelerator mass spectrometry on giant algal cells of *Chara corallina* Klein ex Willd., Taylor et al. (42) provided the first unequivocal evidence that aluminum rapidly crosses the plasma membrane into the symplasm. Accumulation of ^{26}Al in the cell wall was nonsaturable during 3 h of aluminum exposure and accounted for most of aluminum uptake. Absorption of aluminum into the protoplasm occurred immediately but accounted for less than 0.05% of the total accumulation (42). Accumulation in the vacuole occurred after a 30-min lag period (42).

16.4.3 Aluminum Speciation in Symplasm

The pH of the cytoplasmic compartment generally ranges from 7.3 to 7.6 (5). Once aluminum enters the symplasm, the aluminate ion, $Al(OH)_4^-$ or insoluble $Al(OH)_3$ could form (Figure 16.1) (46). Alternatively, Al^{3+} could precipitate with phosphate as variscite, $Al(OH)_2H_2PO_4$ (47). Based on higher stability constants, it is likely that Al^{3+} would be complexed by organic ligands, such as adenosine triphosphate (ATP) or citrate (47,48). Martin (47) hypothesized that based on their similar effective ionic radii and affinity for oxygen donor ligands, Al^{3+} would compete with Mg^{2+} rather than Ca^{2+} in metabolic processes.

16.4.4 Radial Transport

The main barrier to radial transport of aluminum across the root into the stele appears to be the endodermis. Rasmussen (49) used electron microprobe x-ray analysis to show little penetration of aluminum past the endodermis of corn (*Zea mays* L.) roots. Similarly, in Norway spruce (*Picea abies* H. Karst.) roots, a large aluminum concentration was detected outside the endodermis, but very low aluminum concentrations on the inner tangential wall (3,50). Using secondary-ion mass spectrometry, Lazof et al. (51) confirmed that the highest aluminum accumulation occurred at the root periphery of soybean root tips, with substantial aluminum in cortical cells, but very low aluminum in stellar tissues. Similar to calcium, aluminum is thought to bypass the endodermis, entering the xylem in maturing tissues where the endodermis is not fully suberized.

16.4.5 Mucilage

Aluminum must cross the root mucilage before it can penetrate to the root apical meristem. Mucilage is produced by the root cap and is a complex mixture of high-molecular-weight polysaccharides, a population of several thousand border cells, and an array of cell wall fragments (52). Archambault et al. (53) showed that aluminum binds tightly to wheat mucilage, with 25 to 35% of total aluminum remaining after citrate desorption.

16.5 ALUMINUM TOXICITY SYMPTOMS IN PLANTS

16.5.1 Short-Term Effects

Owing to the numerous biochemical processes with which aluminum can interfere, researchers have attempted to determine the primary phytotoxic event by searching for the earliest responses to aluminum. Symptoms of aluminum toxicity that occur within a few hours of aluminum exposure are inhibition of root elongation, disruption of root cap processes, callose formation, lignin deposition, and decline in cell division.

16.5.1.1 Inhibition of Root Elongation

The first, easily observable symptom of aluminum toxicity is inhibition of root elongation. Elongation of adventitious onion (*Allium cepa* L.) roots (54), and primary roots of soybean (55,56), corn (57,58), and wheat (59–61) were suppressed within 1 to 3 h of aluminum exposure. The shortest time of aluminum exposure required to inhibit elongation rates was observed in seminal roots of an aluminum-sensitive corn cultivar BR 201F after 30 min (62).

Application of aluminum to the terminal 0 to 3 mm of corn root must occur for inhibition of root elongation to occur; however, the presence of the root cap was not necessary for aluminum-induced growth depression (63). Using further refinement of techniques, Sivaguru and Horst (58) determined that the most aluminum-sensitive site in corn was between 1 and 2 mm from the root apex, or the distal transition zone (DTZ), where cells are switching from cell division to cell elongation.

Lateral root growth of soybean was inhibited by aluminum-containing solutions to a greater extent than that of the taproot (64,65). Interestingly, Rasmussen (49) observed greater aluminum accumulation in lateral roots that emerged from the root surface, breaking through the endodermal layer. Similarly, root hair formation was more sensitive to aluminum toxicity than root elongation in white clover (*Trifolium repens* L.) (66).

16.5.1.2 Disruption of Root Cap Processes

The Golgi apparatus is the site of synthesis of noncellulosic polysaccharides targeted to the cell wall (67). Activity of the Golgi apparatus in the peripheral cap cells of corn was disrupted at 18 μM Al,

a concentration below that necessary to inhibit root growth (68). In wheat, mucilage from the root cap disappeared within 1 h of aluminum exposure, and dictyosome volume and presence of endoplasmic reticulum decreased within 4 h (69). Death of root border cells (a component of root mucilage) occurred within 1 h of exposure to aluminum in snapbean roots (70).

16.5.1.3 Callose Formation

Callose is a polysaccharide consisting of 1,3-β-glucan chains, which are formed naturally by cells at a specific stage of wall development or in response to wounding (67). An early symptom of aluminum toxicity is formation of callose in roots. Using fluorescence spectrometry, callose could be quantified in soybean root tips (0 to 3 cm from root apex) after 2 h of exposure to 50 μM Al (55). In root cells surrounding the meristem of Norway spruce roots, distinct callose deposits were observed after 3 h of exposure to 170 μM Al (71). Zhang et al. (72) showed that callose accumulated in roots of aluminum-sensitive wheat cultivars exposed to 75 μM Al and they proposed using callose synthesis as a rapid, sensitive marker for aluminum-induced injury. However, callose was not accumulated in two aluminum-sensitive arabidopsis (*Arabidopsis thaliana* Heynh.) mutants exposed to aluminum, indicating no obligatory relationship between callose deposition and aluminum-induced inhibition of root growth (73). Sivaguru et al. (74) showed that aluminum-induced callose deposition in plasmodesmata of epidermal and cortical cells of aluminum-sensitive wheat roots reduced movement of micro-injected fluorescent dyes between cells.

16.5.1.4 Lignin Deposition

Lignins are complex networks of aromatic compounds that are the distinguishing feature of secondary walls (67). Deposition of lignin in response to aluminum was found in wheat cortical cells located 1.4 to 4.5 mm from the root tip (elongating zone [EZ]) after 3 h of exposure to 50 μM Al (75). Lignin occurred in cells with damaged plasma membranes as indicated by staining with propidium iodide, and Sasaki et al. (61) proposed that aluminum-induced lignification was a marker of aluminum injury and was closely associated with inhibition of root elongation. Interestingly, Snowden and Gardner (76) showed that a cDNA induced by aluminum treatment in wheat exhibited high homology with the gene for phenylalanine ammonia-lyase, a key enzyme in the pathway for biosynthesis of lignin.

16.5.1.5 Decline in Cell Division

A decrease in abundance of mitotic figures was observed in adventitious roots of onion after 5 h of exposure to 1 mM Al (54). Similarly, a decrease in the mitotic index of barley root tips was found within 1 to 4 hours of exposure to 5 to 20 μM AI (pH 4.2) (77).

16.5.2 Long-Term Effects

Although they may not be indicative of initial, primary phytotoxic events, long-term effects of aluminum are important for plants growing in aluminum-toxic soils or subsoils. Long-term exposure to aluminum over several days or weeks results in suppressed root and shoot biomass, abnormal root morphology, suppressed nutrient uptake and translocation, restricted water uptake and transport, suppressed photosynthesis, and inhibition of symbiosis with rhizobia.

16.5.2.1 Suppressed Root and Shoot Biomass

Increasing aluminum concentrations in solution, sand, or soil decreased fine root biomass of red spruce (*Picea rubens* Sarg.) (78). Typically, aluminum reduces root biomass to a greater degree than

shoot biomass, resulting in a decreased root/shoot ratio (78–80). In contrast, in 3-year-old Scots pine (*Pinus sylvestris* L.), increasing solution of aluminum up to 5.6 mM produced no obvious aluminum toxicity symptoms on roots but decreased needle length and whole shoot length, resulting in increased needle density (81).

16.5.2.2 Abnormal Root Morphology

Often, one symptom of aluminum toxicity is 'coralloid' root morphology with inhibited lateral root formation and thickened primary roots (54). Cells in the elongation zone of primary wheat roots exposed to aluminum had decreased length and increased diameter, resulting in appearance of lateral swelling (61). This abnormal root morphology combined with reduced root length could result in decreased nutrient uptake and multiple deficiencies.

16.5.2.3 Suppressed Nutrient Uptake and Translocation

Increasing aluminum levels in the medium have been reported to decrease uptake and translocation of calcium, magnesium, and potassium (78,82). Forest declines in North America and Europe have been proposed to be due to aluminum-induced reductions in calcium and magnesium concentrations of tree roots and needles (3). Excess aluminum reduced magnesium concentration of Norway spruce needles to a level considered to be critical for magnesium deficiency (3). Also, aluminum toxicity reduced calcium and magnesium leaf concentrations in beech (*Fagus sylvatica* L.) (83). In sorghum (*Sorghum bicolor* Moench), magnesium deficiency was a source of acid-soil stress (84).

In the case of phosphorus, concentrations increased in roots but typically decreased in shoots. In roots of red spruce, ^{32}P accumulation increased but ^{32}P translocation to shoots decreased (85). Clarkson (86) proposed that there were two interactions between aluminum and phosphorus: (a) an adsorption–precipitation reaction in the apoplast; and (b) reaction with various organic phosphorus compounds within the symplasm of the cell. Aluminum and phosphorus were shown to be coprecipitated in the apoplast of corn roots, using x-ray microprobe analysis (49). Excised corn roots exposed to 20 h of 0.1 to 0.5 mM Al had decreased mobile inorganic phosphate (40%), ATP (65%), and uridine diphosphate glucose (UDGP) (65%) as shown by ^{31}P-NMR (nuclear magnetic resonance), indicating aluminum interference with phosphorus metabolism within the symplasm (87,88).

16.5.2.4 Restricted Water Uptake and Transport

Typically, aluminum toxicity decreases water uptake and movement in plants. Stomatal closure of arabidopsis occurred after 9 h of exposure to 100 μM Al at pH 4.0 (89). In wheat, transpiration decreased after 28 days of exposure to 148 μM Al (90). Treatment of 1-year-old black spruce (*Picea mariana* Britton) with 290 μM Al resulted in wilting and reduced water uptake within 7 days (91). Hydraulic conductivity of red oak roots was reduced after 48 to 63 days of exposure to aluminum, although no effect was observed after only 4 days (92). In contrast, transpiration in sorghum increased after 28 days of aluminum treatment (90).

16.5.2.5 Suppressed Photosynthesis

Net photosynthesis is reported to decrease with excess aluminum relative to normal rates. Exposure to 250 μM Al for 6 to 8 weeks reduced the photosynthetic rate of red spruce, and McCanny et al. (79) attributed this effect to an aluminum-induced decrease in root/shoot ratio. Similarly, exposure of beech seedlings to 0.37 mM Al for 2 months significantly decreased net CO_2 assimilation rates (83).

16.5.2.6 Inhibition of Symbiosis with Rhizobia

Biological nitrogen fixation results in release of H^+, acidification of legume pastures, and increased solubilization of aluminum (2). Excess aluminum has an inhibitory effect on rhizobial symbiosis. In an Australian pasture, the percentage of plant nitrogen derived from the atmosphere declined in subterranean clover (*Trifolium subterraneum* L.) as foliar concentration of aluminum increased (93). In four tropical pasture legumes, aluminum at >25 μM for 28 days delayed appearance of nodules, decreased percentage of plants that nodulated, and decreased number and dry weight of nodules (94). In phasey-bean (*Macroptilium lathyroides* Urb.) and centro (*Centrosema pubescens* Benth.), nodulation was more sensitive to aluminum toxicity than host plant growth (94).

Aluminum also inhibited the multiplication and nodulating ability of the symbiotic bacterium, *Rhizobium leguminosarum* bv. *trifolii* Frank (66). Recent research efforts have focused on identifying aluminum-tolerant rhizobial strains. For example, strains of *Bradyrhizobium* spp. that were isolated from acid soils were found to more tolerant of 50 μM Al at pH 4.5 than commercial strains (95).

16.6 MECHANISMS OF ALUMINUM TOXICITY IN PLANTS

Controversy exists over mechanisms of aluminum phytotoxic effects (96–99). Researchers long have debated whether the primary toxic effect of aluminum is on inhibition of cell elongation or inhibition of cell division. Lazof and Holland (28) demonstrated in soybean, pea (*Pisum sativum* L.), and bean (*Phaseolus vulgaris* L.) that both effects occur, with rapid, largely reversible responses to aluminum toxicity due to cell extension effects and irreversible responses due to cell division effects.

Another question puzzling researchers is whether the primary injury due to aluminum in plants is symplasmic or apoplastic. Horst (100) and Horst et al. (101) reviewed the evidence supporting the apoplast as the site of the primary aluminum-toxic event. However, dividing aluminum effects into symplasmic or apoplastic can be arbitrary, because aluminum could enter the symplasm to produce effects in the cell wall or outer face of the plasma membrane.

Since cell walls occur in plants and not animals, aluminum injuries at this site are unique to plants. Possible mechanisms of aluminum injury in cell walls include: (a) aluminum binding to pectin; or (b) modification of synthesis or deposition of polysaccharides. Jones and Kochian (102) proposed that the plasma membrane is the most likely site of aluminum toxicity in plants. Possible mechanisms of toxicity in the plasma membrane are: (a) aluminum binding to phospholipids; (b) interference with proteins involved in transport; or (c) signal transduction. Once aluminum enters the symplasm, there are many possible interactions with molecules containing oxygen donor ligands (47,48). Probable mechanisms of aluminum toxicity within plant cells include: (a) disruption of the cytoskeleton, (b) disturbance of calcium homeostasis, (c) interaction with phytohormones, (d) oxidative stress, (e) binding to internal membranes in chloroplasts, or (f) binding to nuclei.

16.6.1 Cell Wall

Pectins are a mixture of heterogenous polysaccharides rich in D-galacturonic acid; one major function is to provide charged structures for ion exchange in cell walls (67). Under acidic conditions, aluminum binds strongly to negatively charged sites in the root apoplast, sites consisting mostly of free carboxyl groups on pectins. Klimashevskii and Dedov (103) isolated cell walls from pea roots, exposed them to aluminum, and found that aluminum decreased plasticity and elasticity of cell walls. Blamey et al. (104) demonstrated in vitro a rapid sorption of aluminum by calcium pectate and proposed that aluminum phytotoxicity is due to strong binding between aluminum and calcium pectate in cell walls. Reid et al. (105) proposed that aluminum could disrupt normal cell wall growth either by reducing Ca^{2+} concentration below that required for cross-linking of pectic residues or through formation of aluminum cross-linkages that alter normal cell wall structure. Using x-ray microanalysis, Godbold and

Jentschke (106) showed that aluminum displaced calcium and magnesium from root cortical cell walls of Norway spruce. Using a vibrating calcium-selective microelectrode, Ryan and Kochian (107) observed that addition of aluminum commonly resulted in an initial efflux of calcium from wheat roots, probably due to displacement of calcium from cell walls.

Pectin is secreted in a highly esterified form from the symplasm to the apoplast, where demethylation takes place by pectin methylesterase (PME), resulting in free carboxylic groups available to bind aluminum (108). Transgenic potato (*Solanum tuberosum* L.) overexpressing PME is more sensitive to aluminum based on inhibition of root elongation relative to unmodified control plants, indicating that increased binding sites for aluminum in the apoplast are associated with increased aluminum sensitivity (108).

16.6.1.1 Modification of Synthesis or Deposition of Polysaccharides

In addition to external binding to cell wall components, aluminum also could interfere with the internal synthesis or deposition of cell wall polysaccharides. Exposure of wheat seedlings to 10 μM Al for 6 h decreased mechanical extensibility of subsequently isolated cell walls (109). Tabuchi and Matsumoto (109) showed that aluminum treatment modified cell wall components, increasing the molecular mass of hemicellulosic polysaccharides, thus decreasing the viscosity of cell walls, and perhaps restricting cell wall extensibility.

Uridine diphosphate glucose (UDGP) is the substrate for cellulose synthesis. Using ^{31}P-NMR, Pfeffer et al. (87) demonstrated that a 20-h exposure of excised corn roots to 0.1 mM Al decreased UDGP by 65%, and they speculated that such suppression could limit production of cell wall polysaccharides. In barley, one of the most aluminum-sensitive cereals, callose was excreted from the junction between the root cap and the root epidermis after 38 min of exposure to 37 μM Al, and Kaneko et al. (110) proposed that aluminum-induced inhibition of root elongation could be due to reduced cell wall synthesis caused by a shortage of substrate to form polysaccharides.

16.6.2 Plasma Membrane

16.6.2.1 Binding to Phospholipids

Biological membranes are composed of phospholipids that contain a phosphate group (67), and aluminum can bind to this negatively charged group. Using electron paramagnetic resonance spectroscopy, Vierstra and Haug (111) demonstrated that 100 mM Al at pH 4 decreased fluidity in membrane lipids of a thermophilic microorganism (*Thermoplasma acidophilum* Darland, Brock, Samsonoff and Conti). Using physiologically significant concentrations of aluminum, Deleers et al. (112) showed that 25 μM Al increased rigidity of membrane vesicles as indicated by the increased temperature required to maintain a specific polarization value. In addition, aluminum at < 30 μM could induce phase separation of phosphatidylserine (PS; a negatively charged phospholipid) vesicles, as shown by leakage of a fluorescent compound (113).

Phosphatidylcholine (PC) is the most abundant phospholipid in plasma membranes of eukaryotes, and Akeson et al. (114) showed that in vitro, Al^{3+} has a 560-fold greater affinity for the surface of PC than Ca^{2+}. Further, Jones and Kochian (102) found that lipids with net negatively charged head groups such as phosphatidyl inositol (PI) had a much greater affinity for aluminum than PC with its net neutral head group. Interestingly, Delhaize et al. (115) found that expression of a wheat cDNA (TaPSS1) encoding for phosphatidylserine synthase (PSS) increased in response to excess aluminum in roots. Overexpression of this cDNA conferred aluminum resistance in one strain of yeast (*Saccharomyces cerevisiae*) but not in another. In addition, a disruption mutant of the endogenous yeast *CHO1* gene that encodes for PSS was sensitive to aluminum (115).

Aluminum reduced membrane permeability to water as shown by a plasmometric method on root disks of red oak (116). To remove the confounding effect of aluminum binding to cell walls, Lee et al. (117) used protoplasts of red beet (*Beta vulgaris* L.). Within 1 min of exposure to 0.5 mM

Al, volumetric expansion of red beet cells was reduced under hypotonic conditions, and Lee et al. (117) hypothesized that aluminum could bridge neighboring negatively charged sites on the plasma membrane, stabilizing the membrane.

Binding of Al^{3+} to the exterior of phospholipids reduces the surface negative charge of membranes. Kinraide et al. (27) proposed that accumulation of aluminum at the negatively charged cell surface plays a role in rhizotoxicity and that amelioration of aluminum toxicity by cations is due to reduced negativity of the cell-surface electrical potential by charge screening or cation binding. Kinraide et al. (27) found a good correlation between the reduction in relative root length of an aluminum-sensitive wheat cultivar with aluminum activity as calculated at the membrane surface, but not in the bulk external solution. Ahn et al. (118) measured the zeta potential (an estimate of surface potential) of plasma membrane vesicles from squash (*Cucurbita pepo* L.) roots and showed that aluminum exposure resulted in a less negative surface potential. Measuring uptake of radioisotopes by barley roots, Nichol et al. (119) showed that influx of cations (K^+, NH_4^+, and Ca^{2+}) decreased whereas influx of anions (NO_3^-, HPO_4^{2-}) increased in the presence of aluminum. They speculated that binding of Al^{3+} to the exterior of a plasma membrane forms a positively charged layer that retards movement of cations to the membrane surface and increases movement of anions to the surface.

In contrast, Silva et al. (120) demonstrated that Mg^{2+} was 100-fold more effective than Ca^{2+} in alleviating aluminum-induced inhibition of soybean taproot elongation. They (120) suggested that such an effect could not be explained by changes in membrane surface potential and proposed that the protective effects of Mg could be due to alleviation of aluminum binding to G-protein.

16.6.2.2 Interference with Proteins Involved in Transport

In addition to phospholipids, biological membranes are composed of proteins, many of which are involved in transport functions across the membrane (5,67). Aluminum is reported to interfere with the uptake of many nutrients, perhaps through interactions with cross-membrane transporters or channels.

16.6.2.2.1 H^+-ATPases

Transmembrane electric potential (V_m) is the difference in electric potential between the external environment and the symplasm; typically, the interior of the cell is negatively charged with respect to the outside (67). The potential depends on transient fluxes of H^+ through membrane-bound H^+-ATPases, as well as fluxes of K^+ and other cations through membrane transporters. Measurements of net H^+ flux using either a microelectrode or vibrating probe demonstrated that net inward currents of H^+ occurred between 0 to 3 mm from root tips of wheat (60,121). Exposure of roots of an aluminum-sensitive wheat cultivar to 10 μM Al for 1 to 3 h inhibited H^+ influx; however, there was no obligatory association between inhibition of H^+ influx and inhibition of root elongation (60). Ryan et al. (60) speculated that the H^+ influx near the root apex could be due to cotransport of H^+ with unloaded sugars and amino acids into the cytoplasm, or a membrane more permeable to H^+.

Conducting an in vitro enzyme test, Jones and Kochian (102) found little effect of aluminum on H^+-ATPase activity. Similarly, Tu and Brouillette (122) found no effect of aluminum on plasma membrane-bound ATPase activity in the presence of free ATP; however, exposure of Mg^{2+}-ATP to 18 μM Al competitively inhibited hydrolysis of ATP. Based on immunolocalization, H^+-ATPases in epidermal and cortical cells (2 to 3 mm from tip) of squash roots decreased after 3 h of exposure to 50 μM Al (118). Similarly, 2 days of exposure to $\geq$ 75 μM Al decreased activity of plasma membrane-bound ATPases in 1-cm root tips of five wheat cultivars (123). Since H^+-ATPases generate the proton motive force that drives secondary transporters and channels (5,67), a decrease in activity of this membrane-bound enzyme could result in an overall decrease in nutrient uptake.

16.6.2.2.2 Potassium Channels

Uptake of K^+ by pea roots was depressed by aluminum (124). Similarly, exposure of mature root cells ($\geq$ 10 mm from root tip) of an aluminum-sensitive wheat cultivar to 5 μM Al inhibited K^+ influx (121). In addition, Reid et al. (105) showed partial inhibition of Rb^+ (analog for K^+) uptake by $>$ 50 μM Al

in giant algal (*Chara corallina*) cells, and they attributed this effect to partial blocking by aluminum of K^+ channels. Using the patch-clamp technique on isolated plasma membranes or whole cells from an aluminum-tolerant corn cultivar, Pineros and Kochian (125) showed that instantaneous outward K^+ channels were blocked by 12 μM Al, whereas inward K^+ channels were inhibited by 400 μM Al.

A strong dysfunction in K^+ fluxes between guard cells and epidermal cells was observed in beech (*Betula* spp.) seedlings exposed to excess aluminum for 2 months (83). Measuring currents of inside-out membrane patches from fava bean (*Vicia faba* L.) guard cells, Liu and Luan (41) demonstrated that the K^+ inward rectifying channel (KIRC) was inhibited by 50 μM Al when exposed on the inward-facing side of the membrane. They (41) proposed that calcium channels conduct Al^{3+} across the plasma membrane because, verapamil, a Ca^{2+} channel blocker, prevented aluminum-induced inhibition of KIRC in the whole cell configuration. In addition, Liu and Luan (41) expressed the gene, *KAT1*, which encodes for a KIRC, in *Xenopus* oocytes, injected aluminum into the cytoplasm, and observed inhibition of the KAT1 current.

16.6.2.2.3 Calcium Channels

Uptake by roots and translocation of ^{45}Ca to shoots was decreased in wheat by 100 μM Al (126). Similar results occurred with 4-week-old Norway spruce seedlings, in which uptake of ^{45}Ca was reduced by 77 to 92% by 100 to 800 μM Al (3). Net Ca^{2+} influx was highest between 0 and 2 mm from the root apex of wheat, based on a calcium-selective vibrating microelectrode (127). Addition of 20 μM Al to roots of an aluminum-sensitive wheat cultivar resulted in a dramatic decrease in Ca^{2+} influx, and this effect was attributed to blockage by aluminum of a putative calcium channel (128). However, Ryan and Kochian (107) did not find an obligatory relationship between inhibition of calcium uptake and reduction of root growth in wheat. Similarly, in *Chara corallina* cells, aluminum inhibited calcium influx by less than 50% at 100 μM Al, and Reid et al. (105) thought it unlikely that such a small degree of inhibition would be sufficient to inhibit growth so rapidly.

16.6.2.2.4 Magnesium Transporters

Exposure of annual ryegrass (*Lolium multiflorum* Lam.) to 6.6 μM Al competitively inhibited net Mg^{2+} uptake (129). Interestingly, McDiarmid and Gardner (130) isolated two yeast genes, ALR1 and ALR2, that encode proteins homologous to bacterial Mg^{2+} and Co^{2+} transport systems. Overexpression of these genes conferred increased tolerance to Al^{3+}, indicating that aluminum toxicity in yeast is related to reduced Mg^{2+} influx (130).

16.6.2.2.5 Nitrate Uptake

In white clover, 3 weeks of exposure to 50 μM Al inhibited nitrate uptake as measured by nitrogen content in plants (131). In all regions of soybean roots, $^{15}NO_3^-$ influxes were reduced within 30 min of exposure to 80 μM Al (132). In corn, 30 min of exposure to 100 μM Al decreased NO_3^- uptake as measured by NO_3-N depletion in solution, but aluminum-induced inhibition of root elongation was not attributed to inhibition of nitrate uptake (133). Aluminum treatment for 3 days followed by measurement of $^{15}NO_3^-$ uptake in the final hour decreased $^{15}NO_3^-$ uptake in soybean at $\geq$ 44 μM Al but increased $^{15}NO_3^-$ uptake at aluminum levels below 10 μM, probably as a result of Al^{3+} amelioration of H^+ toxicity (24).

16.6.2.2.6 Iron Uptake

Iron acquisition in Strategy II plants (gramineous plants) involves secretion of mugineic acids (MA) and uptake of MA–Fe^{3+} complexes (67). Chang et al. (134) demonstrated that exposure to 100 mM Al for 21 h depressed biosynthesis and secretion of 2′-deoxymugineic acid in wheat.

16.6.2.2.7 Water Channels

Aluminum is reported to reduce permeability of the plasma membrane to water, perhaps through reduced aquaporin (water channel) activity. Milla et al. (135) found that expression of a rye (*Secale cereale* L.) gene encoding for aquaporin (water channel) was decreased by aluminum.

16.6.2.3 Signal Transduction

16.6.2.3.1 Interference with Phosphoinositide Signal Transduction

Under in vitro conditions, aluminum interacted strongly with the phosphoinositide signal transduction element, the plasma-membrane-bound phosphatidylinositol-4,5-bisphosphate (PIP_2) (136). In animals, cleavage of the plasma membrane lipid, PIP_2, by phospholipase C (PLC) releases inositol 1,4,5-triphosphate (IP_3) into the cytoplasm. Then, IP_3 could produce a signaling cascade by binding to a Ca^{2+} channel and releasing Ca^{2+} into the cytosol. In microsomal membranes of wheat roots, aluminum $\geq 20\,\mu M$ dramatically inhibited PLC activity (136). Under in vitro conditions, aluminum was shown to block the PLC-activated cleavage of PIP_2 to IP_3 (136).

16.6.2.3.2 Transduction of Aluminum Signal

Cell wall-associated kinases could serve as a connecting molecule between the cell wall and the cytoplasmic cytoskeleton. These kinases span the plasma membrane, with the extracellular portion covalently bound to pectin in the cell wall and the cytoplasmic portion containing kinase activity. Recently, expression of a cell wall associated kinase (WAK1) in arabidopsis was induced within 3 h of exposure to aluminum (89). Sivaguru et al. (89) hypothesized that WAK1 could be involved in the aluminum signal transduction pathway.

16.6.3 Symplasm

16.6.3.1 Disruption of the Cytoskeleton

The cytoskeleton is a network of filamentous protein polymers that permeates the cytoplasm, providing structural stability and motility for macromolecules and organelles (67). In plants, there are two major families of proteins: actin and tubulin (67). Actin binds and hydrolyzes the nucleotide, ATP, during polymerization to form microfilaments. Proteins α- and β-tubulin bind and hydrolyze guanosine triphosphate (GTP) during polymerization to form microtubules.

Actin filaments are important in cytoplasmic streaming in giant algal cells. With an alga (*Vaucheria longicaulis* Hoppaugh), Alessa and Oliveira (137) demonstrated that cytoplasmic streaming of chloroplasts and mitochondria (mediated by microfilaments) decreased within 30 s of aluminum exposure and completely ceased within 3 min. Using suspension-cultured soybean cells, Grabski and Schindler (138) demonstrated that aluminum rapidly increased rigidity of the transvacuolar actin network, and they proposed that the cytoskeleton is the primary target of aluminum toxicity in plants. Grabski et al. (139) hypothesized that phosphorylated sites on myosin or other actin-binding proteins could bind aluminum, preventing access to phosphatases and resulting in a stabilized actin network. Alternatively, they hypothesized that a calcium-dependent phosphatase could be inhibited directly by aluminum. Interestingly, aluminum toxicity in wheat causes increased expression of a gene encoding for a fimbrin-like (actin-binding) protein involved in maintenance of cytoskeletal function (140). They speculated that the increased tension of cytoskeletal actin by aluminum (138) could involve cross-linking of actin filaments by fimbrins, leading to increased fimbrin gene expression.

Aluminum could disrupt microtubule assembly and disassembly through inhibition of GTP hydrolysis and reduced sensitivity to regulatory signals from Ca^{2+}. When magnesium concentrations were below 1.0 mM, MacDonald et al. (141) demonstrated in vitro that 4×10^{-10} M Al could replace Mg^{2+} in polymerization of tubulin. Disappearance of microtubules was observed sometimes in cells of the EZ of aluminum-treated (3 h, 50 μM Al) wheat roots (61). In outer cortical cells of the DTZ of aluminum-sensitive corn roots, microtubules disappeared within 1 h of exposure to 90 μM Al (142). Treatment of corn roots with 50 μM Al for 3 h resulted in random or obliquely oriented microtubules in inner cortical cells compared to the transverse orientation of those from control roots (57). In addition, a 1 h pretreatment with aluminum prevented auxin-induced reorientation of microtubules in inner cortical cells of corn, and Blancafor et al. (57) proposed that aluminum induced greater stabilization of microtubules. Microfilaments seemed to be less sensitive to aluminum toxicity, with random arrays detectable in the inner cortical cells after 6 h (57).

16.6.3.2 Disturbance of Calcium Homeostasis

Siegel and Haug (143) proposed that the primary biochemical injury due to aluminum was caused by aluminum complexes with calmodulin (a calcium-dependent, regulatory protein). Similarly, Rengel (144) proposed that aluminum is the primary environmental signal, with Ca^{2+} as the secondary messenger that triggers aluminum-toxic events in plant cells. Using a fluorescent calcium-binding dye, Fura 2, Lindberg and Strid (145) showed that exposure of wheat root protoplasts to 50 μM Al caused a transient and oscillating increase in cytoplasmic Ca^{2+} concentration. Similarly, using a cytosolic calcium indicator dye, Fluo-3, in intact wheat apical cells, Zhang and Rengel (146) showed an increase in cytoplasmic Ca^{2+} after 1 h treatment with 50 μM Al. Using Fluo-3 and an indicator of membrane-bound Ca^{2+}, chlorotetracycline (CTC), Nichol and Oliveira (147) found increased calcium concentration in the zone of elongation of an aluminum-sensitive barley cultivar. Since aluminum is known to block calcium channels that allow calcium to move into the cytoplasm, Nichol and Oliveira (147) suggested that Ca^{2+} was released from intracellular storage sites. Interestingly, aluminum-induced callose formation, a rapid marker of aluminum toxicity, is always preceded by elevated cytoplasmic Ca^{2+} (67).

In contrast, Jones et al. (148) used the fluorescent dye, Indo-1, and showed a rapid reduction in cytosolic Ca^{2+} in suspension cultures of tobacco (*Nicotiana tabacum* L.) cells. They (148) attributed this effect to blockage of calcium channels in the plasma membrane by aluminum.

16.6.3.3 Interaction with Phytohormones

The spatial separation between the most aluminum-sensitive site, the DTZ, and the root region that exhibits reduced cell elongation, the EZ, indicates that a signaling pathway is involved. Perhaps, the phytohormones, auxin (IAA) or cytokinin, are involved in the transduction of an aluminum-stress signal.

16.6.3.3.1 Auxin

Corn roots were observed to curve away from unilaterally applied aluminum (149). Similar results were found for snapbean roots that curved away from an agar surface containing aluminum (52). Hasenstein and Evans (150) showed that aluminum inhibited basipetal transport of indoleacetic acid (IAA), perhaps resulting in the tropic root response. Kollmeier et al. (151) confirmed this result, showing that exogenous ^{3}H-IAA application to the meristematic zone of corn roots with aluminum application to the DTZ resulted in decreased basipetal transport of auxin to the EZ. They also showed that exogenous IAA application to the EZ partially ameliorated the aluminum-induced (Al applied to DTZ) inhibition of root elongation. Kollmeier et al. (151) hypothesized that aluminum inhibition of auxin transport mediated the aluminum signal between the DTZ and EZ. Sivaguru et al. (74) speculated that aluminum-induced callose in plasmodesmata could be a primary factor in aluminum inhibition of root growth through disturbance of auxin transport.

16.6.3.3.2 Cytokinin

Bean root elongation was inhibited after 360 min of exposure to 6.5 μM Al (152). Ethylene evolution as well as the level of zeatin (a cytokinin) from root tips increased after 5 min of aluminum exposure. Massot et al. (152) suggested a role for cytokinin and ethylene in transduction of aluminum-induced stress signal.

16.6.3.4 Oxidative Stress

Aluminum is redox inactive and is not able to initiate oxidation of lipids or proteins on its own. Yet, lipid peroxidation has been observed in barley roots after 3 h incubation with aluminum (100 μM $AICI_3$, pH, 4.3) (153). Similarly, in pea roots, increase of lipid peroxidation and inhibition of root elongation occurred after 4 h of exposure to 10 μM aluminium (154). Sakihama and Yamasaki (153) proposal that aluminum stabilizes the oxidized form of phenolics (normally unstable), resulting in phenoxyl radicals that initiate lipid peroxidation. Alternatively, aluminum could increase formation

of reactive oxygen species (ROS). Cell defense against ROS includes the enzymes, superoxide dismutase (SOD) and glutathione peroxidase (PX), which reduce ROS (153). If levels of these enzymes are not sufficient, then ROS could lead to oxidation of lipids, proteins, and DNA, and even cell death. In corn, 24 h of exposure to aluminum increased activities of SOD and PX, and increased protein oxidation in the aluminum-sensitive genotype (155).

Another possibility proposed by Ikegawa et al. (156) is aluminum-enhanced, Fe(II)-medicated peroxidation of lipids as a cause of cell death. Exposure of tobacco suspension cultures to aluminum alone for 24 h resulted in aluminum accumulation but no significant cell death (156). Addition of Fe(II) (a redox active metal) to cells with accumulated aluminum after 12 h resulted in enhanced lipid peroxidation and cell death. Lipid peroxidation does not appear to be the mechanism involved in reduction of root elongation (154). In pea roots, treatment with an antioxidant prevented aluminum-enhanced lipid peroxidation, reduced callose formation, but did not prevent aluminum-induced inhibition of root elongation (154).

Interestingly, three of four cDNA up-regulated by aluminum stress in *Arabidopsis thaliana* encoded genes were induced also by oxidative stress (157). Similarly, the vast majority of isolated cDNAs, whose expression increased in response to aluminum toxicity in sugarcane (*Saccharum officinarum* L.), showed greater expression in response to oxidative stress (158). These results indicate that oxidative stress is an important component of the plant's response to aluminum toxicity. Overexpression of a tobacco gene encoding for glutathione S-transferase (*parB*) in *Arabidopsis thaliana* conferred a degree of aluminum resistance as well as resistance to oxidative stress induced by diamide, providing genetic evidence of a linkage between aluminum stress and oxidative stress in plants (159).

16.6.3.5 Binding to Internal Membranes in Chloroplasts

As discussed earlier, one long-term effect of aluminum toxicity is the suppression of photosynthetic activity (79,90). Photosynthetic $^{14}CO_2$ fixation of isolated spinach (*Spinacia oleracea* L.) chloroplasts was inhibited by 10 μM Al at pH 7 (160). Hampp and Schnabel (160) attributed this effect to damage of the membrane system. Aluminum exposure of wheat for 14 days decreased the maximum photochemical yield F_v/F_m of photosystem II, (ratio of variable fluorescence over maximum fluorescence, as measured by a fluorometer) (161). Moustakas and Ouzounidou (161) attributed this effect to loss of Ca^{2+}, Mg^{2+}, and K^+ from chloroplasts. Seventy days of aluminum exposure decreased F_v/F_0, or the ratio of variable fluorescence over initial fluorescence (162). Pereira et al. (162) speculated that this decrease was an indicator of aluminum-induced structural damage in the thylakoids. In the cyanobacterium, *Anabaena cylindrica* Lemm., aluminum was found to degrade thylakoid membranes (163).

16.6.3.6 Binding to Nuclei

Aluminum entered soybean root cells and was associated with nuclei only after 30 min of exposure to 1.45 μM Al (164). In corn root tips, high chromatin fragmentation and loss of plasma membrane integrity occurred after 48 h exposure to 36 μM Al (155). However, Al^{3+} binding to DNA is very weak and cannot compete with phosphate, ATP, or other organic ligands such as citrate (47,48). Martin (47) stated that the observed association of aluminum with nuclear chromatin must be due to its complexation to other ligands and not to DNA.

16.7 GENOTYPIC DIFFERENCES IN ALUMINUM RESPONSE OF PLANTS

Comparative studies of aluminum effects in 22 species in seven plant families have established that some species or genotypes within species can resist aluminum toxicity (82). Foy (165) proposed 'tailoring the plant to fit the soil; in other words, he suggested that it was more economical to develop mineral-stress-resistant plants than to correct the soil for nutrient deficiencies or toxicities. This statement is particularly true for acid subsoils, where it is not economically feasible to lime at such depths, or for developing countries, where farmers cannot afford the high-input costs of lime.

16.7.1 Screening Tests

Screening for genotypic differences in response to aluminum toxicity can be conducted in pots or in fields with aluminum-toxic soil. A more rapid screening test for differences in aluminum tolerance among species or genotypes within species utilizes the aluminum-induced inhibition of root elongation as a measure of aluminum sensitivity (166). These tests are conducted with varying levels of aluminum in solution at an acid pH (≤ 4.5) to maintain a high activity of Al^{3+}, the phytotoxic ion. Some researchers have found a poor correlation between plant responses in soil with those in nutrient solution (167). Others have found a good correlation (168–171).

Hematoxylin stains extracellular aluminum phosphate compounds that result from aluminum damage to root cells (172). Another quick screening test is to stain roots grown in an aluminum-containing solution with hematoxylin and to assess the intensity of staining (173). With wheat, Scott et al. (174) found a good agreement between root elongation results and those using hematoxylin. However, Bennet (175) warned that many aspects of hematoxylin staining are not well understood and that aluminum-treated roots do not always respond to hematoxylin even when symptoms of aluminum toxicity occurred. Further, sometimes roots will stain in the absence of aluminum (175).

Moore et al. (176) proposed that recovery of root elongation after 48 h of exposure to aluminum is a better measure of irreversible damage to the root apical meristem. Hecht-Buchholz (177) reported that aluminum toxicity in barley caused stunted roots, destruction of root cap cells, swelling, and destruction of both root epidermal and cortical cells. She found large differences between cultivars and proposed that aluminum resistance could be attributed to greater resistance of the root meristem of the aluminum-tolerant genotype to irreversible destruction. Lazof and Holland (28) suggested that root recovery experiments in soybean, pea, and snapbean allowed separation of H^+ toxicity effects from Al^{3+} toxicity effects. Zhang et al. (178) showed that root regrowth after aluminum stress could be used to improve aluminum tolerance in triticale (*Triticosecale* spp.).

16.7.2 Genetics

Aluminum tolerance is a heritable trait in sorghum (179), barley (180), wheat (181,182), rice (*Oryza sativa* L.) (183), soybean (184), and *Arabidopsis thaliana* (185). With sorghum, Magalhaes (cited in 179) has found a pattern of inheritance of aluminum tolerance that is consistent with a single locus. With barley, Tang et al. (180) confirmed that aluminum tolerance segregation in F_2 genotypes was due to a single gene, *Alp*, and they proposed the use of molecular markers in selection of aluminum tolerance in barley genotypes without the need for field trials, soil bioassays, or solution culture tests. In wheat, controversy exists over the number and location of genes that are involved in aluminum tolerance (181,182). In rice, nine different genomic regions on eight chromosomes have been associated with genetic control of plant response to aluminum, indicating that aluminum tolerance is a multigenic trait (183). Similarly, with soybean, aluminum tolerance is likely to be governed by 3 to 5 genes (184). In *Arabidopsis*, two quantitative trait loci occurring on two chromosomes could account for 43% of total variability in aluminum tolerance among a recombinant inbred population (185). A recent review of genetic analysis of aluminum tolerance in plants is found in Kochian et al. (179).

16.8 PLANT MECHANISMS OF ALUMINUM AVOIDANCE OR TOLERANCE

There are two types of mechanisms whereby a plant can avoid or tolerate aluminum toxicity: (a) exclusion of aluminum from the symplasm, or (b) internal tolerance of aluminum in the symplasm. Good reviews on this subject are in Taylor (186,187), Matsumoto (99), Kochian et al. (179, 188), and Barcelo and Poschenrieder (96).

16.8.1 Plant Mechanisms of Aluminum Avoidance

Based on chemical analysis of aluminum in root sections, Horst et al. (189) showed that the root tips of an aluminum-tolerant cultivar of cowpea (*Vigna unguiculata* Walp.) had a lower aluminum

concentration than those of an aluminum-sensitive cultivar, suggesting that reduced aluminum absorption into the root tip was responsible for its higher aluminum tolerance. Using direct measurement of aluminum with atomic absorption spectrophotometry or ion chromatography, Rincon and Gonzales (190) showed that aluminum content was 9 to 13 times greater in the 0-to-2-mm root tips of an aluminum-sensitive wheat cultivar than in an aluminum-tolerant cultivar. Similar results were reported by Delhaize et al. (191), who showed using x-ray microanalysis that aluminum-sensitive wheat root apices accumulated 5 to 10 times greater aluminum than aluminum-tolerant root apices.

These results indicate that aluminum exclusion occurs in several plant species. Possible mechanisms of aluminum avoidance include: (a) root avoidance response, (b) organic acid release, (c) exudation of phosphate, (d) exudation of polypeptides, (e) exudation of phenolics, (f) alkalinization of rhizosphere pH, (g) binding to mucilage, (h) binding to cell walls, (i) binding to external face of membrane, and (j) interactions with mycorrhizal fungi.

16.8.1.1 Avoidance Response of Roots

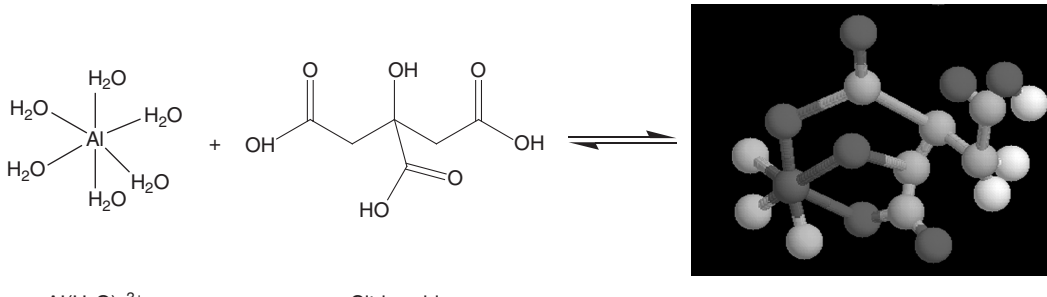
Classic avoidance response of roots to aluminum toxicity was shown by research (149) in which corn roots curved away from aluminum applied to one side of root. Also, aluminum toxicity killed cells in the corn root apical meristem, and Boscolo et al. (155) speculated that this phenomenon would result in loss of apical dominance and greater lateral root growth into environments with lower aluminum levels. Interestingly, taproots of corn cv. SA-6 and soybean cv. Perry did not penetrate much into an aluminum-toxic subsoil layer, although lateral root lengths increased in the nontoxic top soil layer (192). However, although increased lateral root growth in topsoil layers could help to maintain crop yields in areas with acid subsoils, under drought conditions, lack of root growth into deeper layers could limit water uptake.

16.8.1.2 Organic Acid Release

Considerable evidence supports organic acid release as a mechanism of aluminum avoidance in plants (179,188,193,194). Hue et al. (195) used elongation of cotton (*Gossypium hirsutum* L.) taproots as a measure of aluminum toxicity to document the aluminum detoxification effect of several low-molecular-weight organic acids or anions. The relative ameliorative capacity of the organic acids followed closely the stability constants of the aluminum–organic acid complexes in the order:

$$\text{Citric} > \text{Oxalic} > \text{Tartaric} > \text{Malic} > \text{Acetic}$$

The formation of stable rings (5-, 6-, and to a lesser extent 7-membered structures) between aluminum and organic anions or molecules seems to be responsible for the detoxification (195). Structure of an aluminum–citrate complex is shown below.



The first evidence of aluminum-induced root exudation of an organic acid was identified in snapbean, in which an aluminum-tolerant cultivar exuded ten times as much citrate as an aluminum-sensitive cultivar in the presence of aluminum (196). Aluminum-induced root release of malate was characterized thoroughly in wheat by Delhaize and co-workers (197–200). They showed that exposure of an aluminum-tolerant genotype to 10 μM Al induced malate exudation from roots within 15 min. Wheat root apices contained sufficient malate for excretion for over 4 h (198). After 24 h of exposure to 100 μM Al, de novo synthesis of malate was demonstrated by measuring ^{14}C incorporation into malate (199). The efflux of malate from root apices was electroneutral, because it was accompanied by an efflux of K^+ (198). Evaluating 36 wheat cultivars, Ryan et al. (200) showed a significant correlation between relative tolerance of wheat genotypes to aluminum and amount of malate released from root apices. Other researchers have argued against the effectiveness of malate exudation on alleviating aluminum toxicity because of rapid degradation by soil microorganisms (201) and the low concentrations and relatively weak chelating ability of malate for aluminum (202).

Other plant species have been shown to exude organic acids in response to aluminum stress. Aluminum-tolerant corn genotypes exuded higher concentrations of citrate (203). An aluminum-tolerant tree species, *Senna tora* Roxb. (formerly *Cassia tora*), exuded citric acid after 4 h of exposure to 50 μM Al (204). In rye, after 10 h of exposure to 10 μM Al, increased activity of citrate synthase (CS) occurred along with increased citrate secretion (205). In all soybean genotypes, citrate exudation increased within 6 h of aluminum exposure; however, only citrate efflux in aluminum-tolerant genotypes was sustained for an extended time period (206). A positive correlation was found between citrate in root tips of soybean and aluminum tolerance (206). The aluminum-accumulating plant, buckwheat (*Fagopyrum esculentum* Moench), was found to exude oxalate, a strong aluminum chelator (207). Taro (*Colocasia esculenta* Schott), a tropical root crop that is not an aluminum accumulator, also exuded oxalate from roots in response to aluminum (208). Aluminum-resistant mutants of *Arabidopsis thaliana* constitutively released higher concentrations of citrate or malate compared to the wild type (209). A mutant carrot (*Daucus carota* L.) cell line that solubilized phosphate from aluminum phosphate exuded citrate from roots (210). This cell line had a greater activity of mitochondrial CS and a lower activity of a cytoplasmic enzyme, NADP-specific isocitrate dehydrogenase (NADP-ICDH), involved in citrate degradation (211,212).

Anion channels are involved in the aluminum-activated exudation of organic anions. Using electrophysiology to measure current passing across whole apical cells of wheat roots, Ryan et al. (213) showed that 20 to 50 μM Al activated an anion channel. Genotypic differences were found with the aluminum-induced currents across protoplasts from the aluminum-tolerant wheat genotype occurring more frequently and being sustained for a longer period of time than those from the aluminum-sensitive genotype (214). Using subtractive hybridization of cDNAs from near-isogenic lines of aluminum-sensitive and aluminum-tolerant wheat, Sasaki et al. (215) found greater expression of a gene that cosegregated with aluminum tolerance. Heterologous expression of this gene, named ALMT1 (aluminum-activated malate transporter), in *Xenopus* oocytes, rice, and cultured tobacco cells conferred an aluminum-activated malate efflux, and enhanced the ability of tobacco cells to recover from 18 h of exposure to 100 μM AI (215). Transgenic barley cultivars with the ALMT1 transgene showed increased malate effux and increased root grwoth at concentrations up to 12 μM AI (216).

Another means of increasing aluminum tolerance in plants is to increase synthesis as well as exudation of organic acids. De la Fuente et al. (217) overexpressed a CS gene from the bacterium, *Pseudomonas aeruginosa* Migula, in the cytoplasm of transgenic tobacco and found increased citrate levels within roots, increased citrate efflux, and increased root elongation in the presence of $\geq$ 100 μM Al. However, Delhaize et al. (218) were unsuccessful in repeating this work (217), and they suggested that the activity of *P. aeruginosa* cytoplasmic CS in transgenic tobacco is either sensitive to environmental conditions, or that the improved aluminum tolerance observed by de la Fuente et al. (217) was due to other factors. Koyama et al. (219) overexpressed a mitochondrial CS gene, isolated from carrot, in *Arabidopsis thaliana* and found increased CS activity, increased

excretion of citrate, and slightly increased amelioration of aluminum toxicity based on root elongation at pH 5.

Tesfaye et al. (220) overexpressed genes for nodule-enhanced forms of the enzymes that catalyze malate synthesis, phosphoenolpyruvate carboxylase and malate dehydrogenase in alfalfa (*Medicago sativa* L.). They found increased enzyme activities, increased root exudation of organic acids (citrate, oxalate, malate, succinate, and acetate), and increased root elongation in the presence of 50 to 100 μM Al. However, such root exudation represented a drain of plant resources, and transgenic lines had reduced biomass compared to untransformed control plants when grown at soil pH 7.25. In acid soils, however, transgenic alfalfa had 1.6 times greater biomass than untransformed control plants.

Although abundant evidence exists for aluminum-induced organic acid excretion as a mechanism of aluminum tolerance, other mechanisms probably exist. Ishikawa et al. (221) found no correlation between species or within species for organic acid exudation and aluminum tolerance. Similarly, Wenzl et al. (222) reported that the greater aluminum tolerance of signalgrass (*Urochloa decumbens* R.D. Webster, formerly *Brachiaria decumbens*) relative to ruzigrass (*Urochloa ruziziensis* Crins, formerly *Brachiaria ruziziensis*) was not due to greater exudation of organic acids.

16.8.1.3 Exudation of Phosphate

Root apices of an aluminum-tolerant genotype of wheat exuded phosphate as well as citrate in response to aluminum exposure (223). Pellet et al. (223) speculated that phosphate release contributed to aluminum tolerance in wheat. In contrast, no major differences in phosphate release were found among near-isogenic lines of wheat that differed in aluminum tolerance (224).

16.8.1.4 Exudation of Polypeptides

Aluminum-resistant lines of wheat exuded an aluminum-induced 23 kDa polypeptide (225). This polypeptide, synthesized de novo in response to aluminum, binds aluminum, and cosegregates with the aluminum-resistant phenotype in F_2 populations (225,226). The gene encoding this polypeptide still needs to be isolated.

16.8.1.5 Exudation of Phenolics

Phenolics are aromatic secondary metabolites of plants (e.g., quercetin, catechin, morin, or chlorogenic acid) that can bind aluminum (67,227). Silicon ameliorates aluminum toxicity in some plants (228, 229). In an aluminum-resistant corn cultivar, silicon and aluminum triggered the release of phenolic compounds (e.g., catechol, catechin, and quercetin) up to 15 times the release by plants not pretreated with silicon (230). However, the binding capacity of many of these phenolic compounds for aluminum is greater at pH 7 than at pH 4.5 (227).

16.8.1.6 Alkalinization of Rhizosphere

The solubility of aluminum is dependent on pH; as pH rises above 5.0, precipitation of aluminum as $Al(OH)_3$ increases (Figure 16.1). An aluminum-tolerant wheat cultivar grown in a nutrient solution increased the pH, whereas an aluminum-sensitive cultivar lowered the solution pH (231). Foy et al. (231) proposed that aluminum tolerance is associated with plant-induced alkalinization of pH. However, rhizosphere pH associated with apical root tissues did not appear to be a primary mechanism of differential aluminum tolerance in wheat. The root apex of an aluminum-tolerant wheat genotype had only a slightly higher rhizosphere pH in the presence of aluminum than an aluminum-sensitive genotype, resulting in a 6% decrease in free Al^{3+} activity (121). Yet the aluminum-tolerant wheat genotype had 140% greater relative root elongation compared to the aluminum-sensitive

genotype, indicating that rhizosphere pH did not play a major role in differential aluminum tolerance (121). In contrast, Degenhardt et al. (232) reported that aluminum exposure induced a doubling in net H^+ influx at the root tip of an aluminum-resistant *Arabidopsis* mutant relative to the wild-type, increasing pH by 0.15 units. Although the pH difference was small, solution pH maintained at 4.5 was shown to increase *Arabidopsis* root growth relative to that at pH 4.4.

16.8.1.7 Binding to Mucilage

Horst et al. (233) reported that mucilage from root tips of cowpea had a high binding capacity for aluminum and that removal of this mucilage resulted in greater inhibition of root elongation by aluminum. They proposed that mucilage served to protect the apical meristem against aluminum injury. Similarly, Brigham et al. (234) showed that removal of snapbean mucilage (including root border cells) resulted in reduced root elongation and greater aluminum accumulation in root tips as shown by lumogallion staining. Pan et al. (777) demonstrated that the presence of mucilage and border cells in wheat reduced aluminum injury to root meristems, as shown by a greater mitotic index. In contrast, Li et al. (235) found that although mucilage from corn root apices binds strongly to aluminum, the presence or absence of mucilage did not affect aluminum-induced inhibition of root elongation.

16.8.1.8 Binding to Cell Walls

Some researchers observed that root cation exchange capacity (CEC) of Al-tolerant genotypes were lower than that of aluminum-sensitive ones (236); however, other researchers found no such correlation (237,238). Interestingly, a transgenic potato overexpressing PME exhibited greater activity of PME (which should result in more free carboxylic groups in cell walls), greater aluminum accumulation in root tips, and greater sensitivity to aluminum as shown by aluminum-induced callose formation and inhibition of root elongation (108). These results suggest that genotypic differences in number of negatively charged binding sites in the cell wall could result in differential aluminum tolerance.

Interestingly, overexpression of WAK1 in arabidopsis conferred increased aluminum tolerance as shown by increased root elongation in the presence of aluminum (89). Sivaguru et al. (89) speculated that WAKs could interact with cell wall components such as callose or pectins, alleviating aluminum toxicity. Alternatively, they speculated that the cytoplasmic kinase domain could be cleaved off from WAKs and participate in cytoplasmic aluminum response pathways.

16.8.1.9 Binding to External Face of Plasma Membrane

Among five plant species differing in aluminum tolerance, the zeta potential (i.e., an estimate of plasma membrane surface potential) was higher (membrane surface less negative) in aluminum-resistant plant species than in sensitive ones (239). Wagatsuma and Akiba (239) hypothesized that aluminum-sensitive plant species had more negative charges on the plasma membrane, resulting in greater aluminum-binding to its surface. Similarly, Ishikawa and Wagatsuma (240) pretreated protoplasts of four plant species with aluminum for 10 min followed by a hypotonic aluminum-free solution. They found that protoplasts from aluminum-sensitive species exhibited greater leakage of K^+ and proposed that aluminum binding to plasma membrane induced greater rigidity, reduced extensibility, and increased leakage under hypotonic conditions.

In contrast, Yermiyahu et al. (241) found that the surface-charge density of vesicles isolated from an aluminum-sensitive wheat cultivar was 26% more negative than those from an aluminum-tolerant wheat cultivar. However, they (241) argued that this small difference in surface-charge density did not account for the large difference in sensitivity to aluminum (50%).

16.8.1.10 Interactions with Mycorrhizal Fungi

Conflicting reports occur in the literature with a few researchers finding negative or no effect of mycorrhizal colonization on host-response to aluminum toxicity (242–245) and a greater number showing a beneficial effect of colonization with either ectomycorrhizal (ECT) (246,247) or arbuscular mycorrhizal fungi (AMF) (248–250). Host response to aluminum toxicity depended on the species of ECT (242) or AMF (243). Scots pine (*Pinus sylvestris* L.) colonized by an aluminum-sensitive ECT fungus (*Hebeloma* cf. *longicaudum* Kumm. ss. Lange) exhibited decreased shoot and root biomass compared to nonmycorrhizal plants in the presence of 2500 μM Al (242). In contrast, Scots pine colonized by an aluminum-tolerant ECT fungus (*Laccaria bicolor* Orton) had greater shoot and root biomass, greater shoot P, and lower shoot aluminum compared to nonmycorrhizal plants in the presence of 740 μM Al (242). Similarly, only five of eight isolates of AMF increased growth of switchgrass and reduced foliar Al concentrations in an acid soil (243).

Pitch pine (*Pinus rigida* Mill.) colonized with the ECT fungus, *Pisolithus tinctorius* Coker and Couch, had greater shoot and root biomass at 50 to 200 μM Al than noninoculated plants (246). Colonization of white pine (*Pinus strobus* L.) with the ECT fungus, *P. tinctorius*, resulted in greater shoot dry weight, height, and needle length relative to nonmycorrhizal seedlings at aluminum levels $\geq$ 460 μM (247). Schier and McQuattie (247) attributed the beneficial effects of ECT fungi to reduced aluminum concentrations and higher phosphorus concentrations in needles.

Colonization of switchgrass (*Panicum virgatum* L.) with the AMF, *Glomus occultum* Walker, resulted in higher total shoot biomass at 500 μM Al as well as lower tissue aluminum and higher calcium concentrations (248). In an aluminum-sensitive barley cultivar, colonization with the AMF, *Glomus etunicatum* Becker and Gerdemann, resulted in greater shoot biomass and greater P concentrations in shoots and roots at 600 μM Al (249). Colonization of tissue-cultured banana (*Musa acuminata* Colla) with the AMF, *Glomus intraradices* N.C. Schenck & S.S. Sm., increased shoot dry weight, water uptake, and nutrient uptake and decreased aluminum content in roots and shoots (250). Apparently, one of the benefits of either ecto- or endomycorrhizal colonization is to ameliorate the detrimental effects of aluminum toxicity on root growth and nutrient or water uptake.

Aluminum has toxic effects also on mycorrhizal fungi, adversely affecting the quality and quantity of mycorrhizal colonization (243,251). Differences in response to aluminum have been found between ECT fungal species (243). Also, genotypic differences within an ECT fungal species have been found in response to aluminum. For example, isolates of ECT fungus, *P. tinctorius*, from old coal-mining sites (pH 4.3, 12.1 mM Al) exhibited greater aluminum tolerance based on mycelial mass at $\geq$ 440 μM Al than isolates from rehabilitated mine sites (pH 4.9, 800 μM Al) and those from forest sites (pH 4.3, 220 μM Al) (252). Strains of the ECT fungus, *Suillus luteus* Gray, that differed in aluminum sensitivity were inoculated on Scots pine, and the extramatrical mycelia developed by the aluminum-resistant strain were more abundant in the presence of aluminum compared to those of the aluminum-sensitive strain (251). Scots pine seedlings colonized by this aluminum-tolerant ECT strain in the presence of aluminum had greater shoot heights compared to noninoculated seedlings (251).

Cuenca et al. (253) showed that the tropical woody species, *Clusia multiflora* Knuth., inoculated with AMF accumulated less aluminum in roots; instead aluminum was bound to the cell walls of the fungal mycelium and in vesicles. Using ^{27}Al-NMR, aluminum was found to be taken up and accumulated into polyphosphate complexes in the vacuole of the ECT fungus, *Laccaria bicolor* Orton (254). Martin et al. (254) suggested that sequestration of aluminum in polyphosphate complexes could help to protect mycorrhizal plants against aluminum toxicity. An aluminum-adapted strain of an ECT fungus, *Suillus bovines* Kuntze, had a shorter average chain length of mobile polyphosphates and greater terminal phosphate groups (255). Gerlitz (255) proposed that this change increased binding and detoxification of polyphosphates to aluminum. A good review of possible aluminum tolerance mechanisms in ECT is found in Jentschke and Godbold (256).

16.8.2 Plant Mechanisms of Aluminum Tolerance

Mechanisms of internal tolerance of aluminum involve: (a) complexation with organic acids, (b) complexation with phenolics, (c) complexation with silicon, (d) sequestration in the vacuole or other storage organs, and (e) trapping of aluminum in cells.

16.8.2.1 Complexation with Organic Acids

In the leaves of aluminum-accumulating hydrangea, Ma et al. (257) used molecular sieve chromatography to determine that citrate eluted at the same time as aluminum and that the molar ratio of aluminum to citric acid was approximately 1:1. In the aluminum accumulator, buckwheat, aluminum was complexed with citrate in the xylem (258), but with oxalic acid in vacuoles of leaf cells (259,260). In the aluminum accumulator, *Melastoma malabathricum* L., aluminum citrate occurred in the xylem sap and was then transformed into aluminum oxalate for storage in leaves (261,262).

16.8.2.2 Complexation with Phenolics

In aluminum-accumulating tea, Nagata et al. (263) used ^{27}Al-NMR to demonstrate that aluminum was bound to catechin in young leaves and buds; in mature leaves, aluminum–phenolic acid and aluminum–organic acid complexes were found. Interestingly, Ofei-Manu et al. (227) showed that at pH 7 (cytoplasmic pH), aluminum binding capacity is in the order: quercetin > catechin, chlorogenic acid, morin > organic acids. Among ten woody plant species and two marker crop species, a positive linear correlation was found between root phenolic compounds and aluminum tolerance, based on aluminum-inhibited root elongation (227).

16.8.2.3 Complexation with Silicon

Cocker et al. (229) proposed that amelioration of aluminum toxicity by silicon is due to formation of an aluminosilicate compound in the root apoplast. Hodson and Sangster (264) proposed that codeposition of aluminum and silicon in needles of conifers is responsible for aluminum detoxification by silicon. Hodson and Evans (228) reviewed the evidence in support of various mechanisms of silicon amelioration of aluminum toxicity, and they divided plants into four groups: (a) aluminum accumulators in arborescent dicots, (b) silicon accumulators in grasses, (c) gymnosperms and arborescent dicots with moderate amounts of aluminum and silicon, and (d) herbaceous dicots that exclude aluminum and silicon. Obviously, aluminum can codeposit with silicon only in plants that accumulate both elements. Aluminum was deposited in phytoliths (hydrated silica deposits) of conifers, graminaceous plants, and dicots in the Ericaceae family (265,266). Using x-ray microanalysis, Hodson and Sangster (267) found codeposition of aluminum and silicon in the outer tangential wall of the endodermis of sorghum. In *Faramea marginata* Cham., a woody member of the Rubiaceae family that is known to accumulate aluminum and silicon in leaves, colocalization of aluminum and silicon in a molar ratio of 1:2 occurred in the cortex of stem sections and throughout leaves (268). A good review of aluminum and silicon interactions can be found in Hodson and Evans (228), Cocker et al. (229), and Hodson and Sangster (264).

16.8.2.4 Sequestration in the Vacuole or in Other Organelles

Aluminum ions could be sequestered in vacuoles or other storage organelles where they would not affect metabolism in the cytoplasm adversely. The presence of 50 μM Al increased pyrophosphate-dependent and ATP-dependent H^+ pump activity in tonoplast membrane vesicles isolated from barley roots, and Kasai et al. (269) hypothesized that Al^{3+} was sequestered in the vacuole perhaps by an Al/nH^+ exchange reaction. Interestingly, expression of two 51 kDa proteins is strongly induced in an aluminum-tolerant wheat cultivar, and only weakly expressed in an aluminum-sensitive wheat

cultivar (270). Sequence analysis of the purified peptides showed that one is homologous to the B subunit of the vacuolar H^+-ATPase (V-ATPase) (270).

In an aluminum-tolerant unicellular red alga (*Cyanidium caldarium* Geitler), aluminum accumulated in spherical electron-dense bodies in the cytoplasm near the nucleus (271). These bodies contained high levels of iron and phosphorus, and the researchers speculated that they might be iron-storage sites under normal culture conditions. Interestingly, transferrin, an iron carrier, is the main protein that binds Al^{3+} in the blood plasma of animals (47).

16.8.2.5 Trapping of Aluminum in Cells

Fiskesjo (272) proposed that aluminum could be trapped in root border cells, which were then detached and sloughed away from roots. Consistent with this hypothesis, detached root border cells of snap bean were killed by aluminum within 2 h of aluminum exposure (70).

A punctated pattern of cell death was observed in aluminum-tolerant wheat roots after 8 h of exposure to aluminum, with an increase in oxalate oxidase activity and H_2O_2 production after 24 h (273). Delisle et al. (273) speculated that cell death could be a means for root tip cells to trap or exclude aluminum from live tissues. Interestingly, a hypersensitive cell death response is a common means for plants to trap pathogens, not allowing them to spread to other cells. Many genes up-regulated by aluminum in wheat are similar to pathogenesis-related genes (274).

16.9 ALUMINUM IN SOILS

Aluminum in soil forms the structure of primary and secondary minerals, especially aluminosilicates, such as feldspars, micas, kaolins, smectites, and vermiculites (275). As the soils continue to weather (especially under conditions of high rainfall and warm climates), silicon is leached away, usually as $Si(OH)_4$ in solution, leaving aluminum behind in the solid forms of aluminum oxyhydroxides, such as boehmite and gibbsite, as shown below (276):

$$Al_2Si_2O_5(OH)_4\ (\text{kaolinite}) + 5H_2O \rightleftharpoons 2Al(OH)_3\ (\text{gibbsite}) + 2Si(OH)_4$$

The soils themselves become 'older,' more acidic, and more aluminum toxic and would be classified as Oxisols or Ultisols.

16.9.1 Locations of Aluminum-Rich Soils

According to FAO/UNESCO recent maps (277), most Oxisols and Ultisols are located in the Tropics and Subtropics (Figure 16.2 and Figure 16.3). More specifically, about one third of the Tropics (1.5 billion ha) has sufficiently strong soil acidity for soluble aluminum to be toxic to most crops (278). Geographically, Latin America has 821 million ha, Africa 479 million ha, South and Southeast Asia 236 million ha (278). In the United States (Figure 16.4), a major portion of acid Ultisols is in the Southeast (88 million ha), from Alabama, Arkansas to Virginia (279). Other states, such as California, New York, Oregon, Pennsylvania, and Washington, also have acid Ultisols, but to a much smaller extent (280). In contrast, only Hawaii and Puerto Rico have Oxisols (Figure 16.5). A detailed review of global distribution of acid soils was given by Sumner and Noble (281).

16.9.2 Forms of Aluminum in Soils

To be bioavailable, soil aluminum must first be in solution (279). Soluble aluminum, however, is controlled by several processes (Figure 16.6). For example, aluminum-containing minerals, such as gibbsite and kaolinite, can dissolve under acidic conditions, release aluminum into solution, and

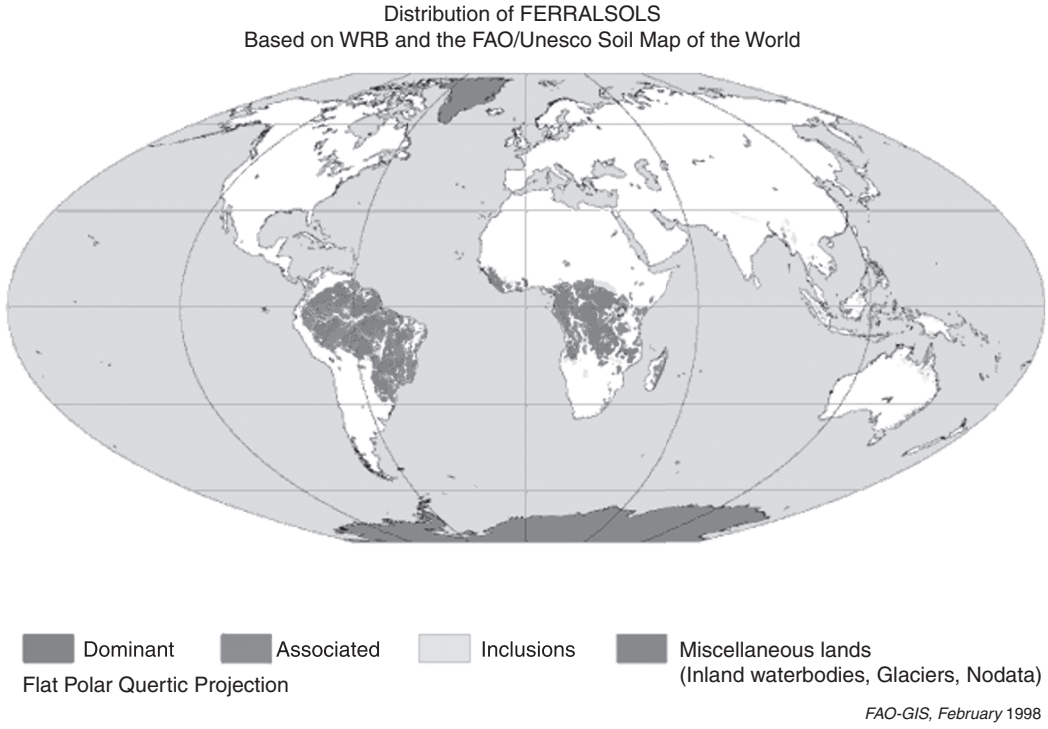


FIGURE 16.2 Oxisols distribution in the world. (From http://www.fao.org/ag/agl/agll/wrb/mapindex.stm) (For a color presentation of this figure, see the accompanying compact disc.)

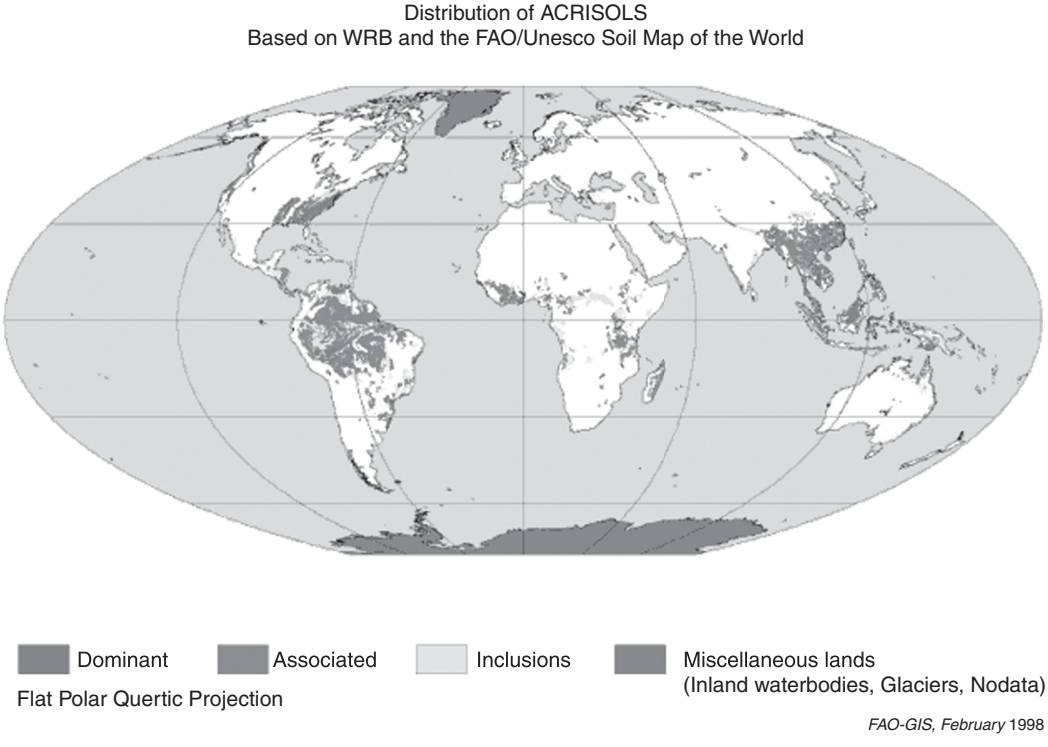


FIGURE 16.3 Ultisols distribution in the world. (From http://www.fao.org/ag/agl/agll/wrb/mapindex.stm) (For a color presentation of this figure, see the accompanying compact disc.)

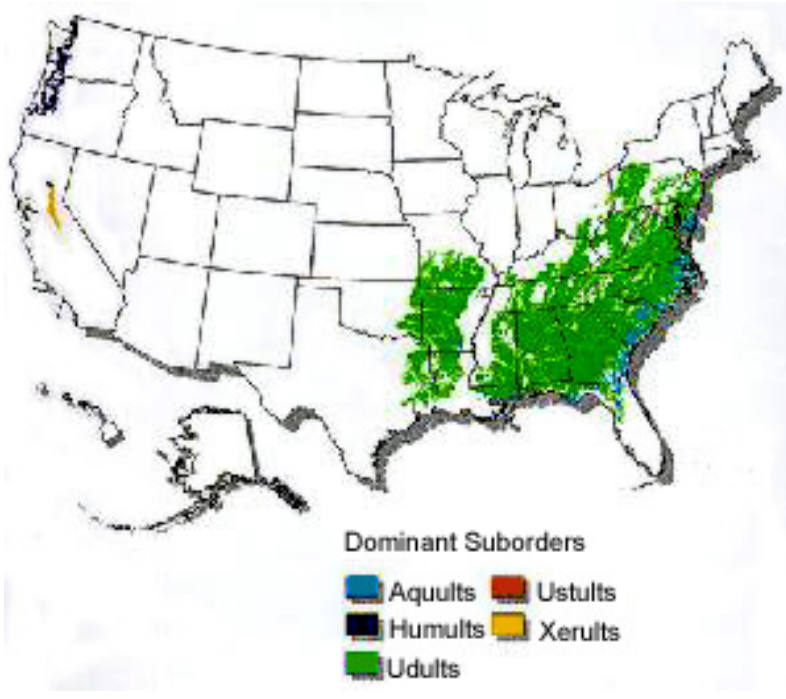


FIGURE 16.4 Ultisols distribution in the United States. (From http://soils.usda.gov/technical/classification/orders/ultisols_map.html) (For a color presentation of this figure, see the accompanying compact disc.)

FIGURE 16.5 Oxisols distribution in the United States. (From http://soils.usda.gov/technical/classification/orders/oxisols_map.html) (For a color presentation of this figure, see the accompanying compact disc.)

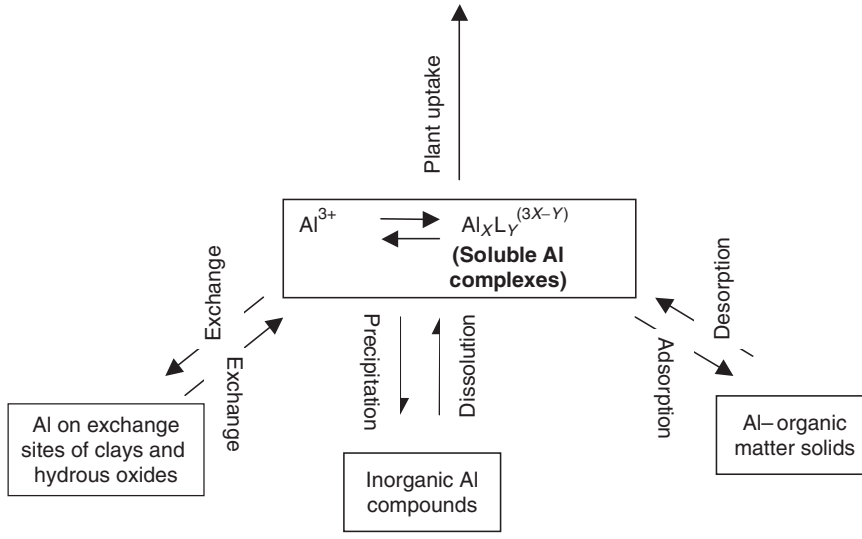


FIGURE 16.6 Processes controlling forms, solubility, and availability of Al in soils. (Adapted from G.S.P. Ritchie, in *Soil Acidity and Plant Growth*, Academic Press Australia, Marrickville, Australia, 1989, pp. 1–60.)

thus, control soluble aluminum concentration and activity (282). The dissolution of gibbsite is expressed by

$$\gamma\text{-}Al(OH)_3(\text{gibbsite}) + 3H^+ \rightleftharpoons Al^{3+}(\text{aqueous}) + 3H_2O$$

On the other hand, clay minerals with negative charges on their surface, resulting from isomorphic substitution (permanent charge) or from hydrolysis of hydroxyl (OH^-) groups at broken edges (variable charge), can take aluminum from solution by electrostatic attraction in cation exchange. Allophane and imogolite, which are amorphous aluminosilicates with large surface areas and high variable charges, can retain large quantities of aluminum (283). So can solid organic matter (OM) with many negative charges from carboxyl ($-COO^-$) functional groups. Solid OM also can retain aluminum strongly by another process called specific adsorption or complexation. Bloom et al. (284) proposed that aluminum–solid OM interactions were central to the exponential decreases of soluble aluminum at $pH < 5$. They reported a 40% reduction in soluble aluminum after adding 2% of a decomposed leafy material to an acid B horizon of an inceptisol.

Aluminous minerals in soils are numerous (275). Besides the aluminosilicates and aluminum oxyhydroxides mentioned previously, aluminum can form sparingly soluble compounds with common soil anions, such as phosphates and sulfates (1). Alunite [$KAl_3(OH)_6(SO_4)_2$], basaluminite [$Al_4(OH)_{10}SO_4$] and jurbanite [$Al(OH)SO_4 \cdot 5H_2O$] have been found in soils where concentration of SO_4^{2-} was high from fertilization with gypsum or by acid sulfate natural occurrence (282,285,286). With prolonged phosphorus fertilization, soluble phosphorus concentration was increased with time, and Al-P minerals, such as variscite, could be formed (287).

The concentration and activity of Al^{3+} in soil solutions not only depend on the processes by which aluminum is distributed between the solid and liquid phases, but also on its many reactions in solution. The extent of these aqueous reactions depends on (a) solution pH, (b) ionic strength, (c) kind and concentration of complexing ligands, and (d) kind and concentration of competing cations (288). Important among these reactions are hydrolysis, polymerization, and complexation with inorganic (e.g., SO_4^{2-}, F^-) and organic anions (e.g., citrate, malate, fulvates) (Table 16.1) (289).

Thus, there are several different species of aluminum in the soil solution, with widely different bioavailability or toxicity (35,37,195). Another implication is that Al^{3+} concentration (activity) makes up only a relatively small fraction of the total soluble aluminum. Wolt (285) found that free

TABLE 16.1
Possible Reactions of Al^{3+} in the Soil Solution

	log K^a (at 25°C)
1. Hydrolysis reactions	
$Al^{3+} + H_2O = Al(OH)^{2+} + H^+$	−5.0
$Al^{3+} + 2H_2O = Al(OH)_2^+ + 2H^+$	−10.1
$Al^{3+} + 3H_2O = Al(OH)_3^0 + 3H^+$	−16.8
$Al^{3+} + 4H_2O = Al(OH)_4^- + 4H^+$	−22.99
2. Polymerization	
$2Al^{3+} + 2OH^- = Al_2(OH)_2^{4+}$	
$13Al^{3+} + 28OH^- = Al_{13}O_4(OH)_{24}^{7+} + 4H^+$	
3. Complexation with inorganic anions	
$Al^{3+} + SO_4^{2-} = Al(SO_4)^+$	3.5
$Al^{3+} + F^- = AlF^{2+}$	7.0
$Al^{3+} + H_2PO_4^- = Al(H_2PO_4)^{2+}$	3.1
4. Complexation with organic anions	
$Al^{3+} + oxalate^{2-} = (Al\text{-}oxalate)^+$	6.0
$Al^{3+} + citrate^{3-} = (Al\text{-}citrate)^0$	8.1
$Al^{3+} + fulvate^{n-} = (Al\text{-}fulvate)^{(n-3)-}$	

[a]From D.K. Nordstrom, H.M. May, in *The Environmental Chemistry of Aluminum*, CRC Press, Boca Raton, FL, 1996, pp. 39–80.

Al^{3+} comprised 2 to 61% of total aluminum in soil solutions of acid Ultisols where SO_4^{2-} was the dominant ligand. Similarly, Hue et al. (195) reported that 76 to 93% of total soil solution aluminum of two acid Ultisols in Alabama was complexed with low-molecular-weight organic acids.

As discussed earlier, it is generally accepted that Al^{3+} and monomeric Al-hydroxy species are more toxic to plants than other forms (35,37,195). Several lines of evidence have shown the nontoxic nature of organically complexed aluminum (195,207,217,290–292). In addition, ionic strength of the soil solution also plays an important role in modifying aluminum toxicity (293). Expressing aluminum species in terms of activity instead of concentration significantly improved the correlation between plant growth and aluminum toxicity across many soils and soil horizons (293,294).

In addition to monomeric aluminum species, polymeric aluminum species have recently been studied intensively perhaps because of their reportedly acute phyto/rhizo-toxicities (31,35,295,296). The 'Al_{13}' polymer $[AlO_4Al_{12}(OH)_{24}(H_2O)_{12}^{7+}]$ was identified using ^{27}Al NMR spectroscopy, where 'clean' solutions containing relatively high aluminum (> 10 mM) were partially neutralized (297). However, this polymeric aluminum species (Al_{13}) could not be detected in soil solutions containing SO_4^{2-} or silicates (298).

16.9.3 Detection or Diagnosis of Excess Aluminum in Soils

As discussed earlier, soil aluminum can exist in many different pools, and its reactions within the soil solution are also quite intricate. It is generally accepted that the activity of monomeric hydroxyaluminum species should be a good predictor of aluminum toxicity for a given plant species if (a) the aluminum absorption by plants is small relative to the quantity of toxic aluminum species in the soil solution such that the solution activity remains virtually constant as the plant grows (steady-state condition) or (b) any decrease in the activity of toxic aluminum species is readily compensated for by solid phase aluminum or nontoxic aluminum in solution (equilibrium condition). In reality, these conditions are hardly met, thus solution activity (intensity factor) and an estimate of the aluminum-buffering capacity (capacity factor) are required to evaluate or predict the toxicity of soil aluminum.

16.9.3.1 Extractable and Exchangeable Aluminum

Different methods have been used to extract solid-phase aluminum, which presumably correlates well with aluminum phytotoxicity (299). An unbuffered solution of 1 M KCl is commonly used to extract the fraction of aluminum (often referred to as 'exchangeable'), which is presumably held by negative charges on the soil surface. When exchangeable aluminum is expressed as a percentage of the effective cation exchange capacity (ECEC), it is referred to as the aluminum saturation percentage. Table 16.2

TABLE 16.2
Selected Chemical Properties of Some Acid Soils from Latin America

Horizon (cm)	pH (H_2O)	Org. C (g kg^{-1})	Exchangeable Al	Ca	Mg	K	ECEC	Al Sat. (%)
			($cmol_c$ kg^{-1})					
Florencia, Colombia. Typic Tropudult								
0–16	4.8	20	3.60	0.95	0.80	0.23	5.58	64
16–85	4.7	5	7.76	0.22	0.43	0.03	8.44	92
Napo, Ecuador. Orthoxic Tropudult								
0–13	4.7	10	0.30	2.06	0.50	2.15	5.01	6
13–25	4.3	6	1.97	0.20	0.09	0.64	2.90	68
25–40	4.0	5	2.07	0.20	0.06	0.18	2.51	82
40–60	4.2	2	2.27	0.22	0.17	0.04	2.70	84
Yurimaguas, Peru. Typic Paleudult								
0–10	4.4	17	1.29	1.13	0.60	0.28	3.30	39
10–30	4.4	5	3.31	0.29	0.14	0.08	3.82	87
30–50	4.5	3	4.26	0.29	0.22	0.07	4.45	87
Iquitos, Peru. Typic Paleudult								
0–16	4.0	24	5.9	1.0	0.2	0.20	7.30	81
16–35	4.5	10	6.7	0.4	0.1	0.08	7.28	92
35–70	4.3	5	9.5	0.2	0.1	0.08	9.88	96
Manaus – AM, Brazil. Typic Acrorthox								
0–8	4.6	30	1.1	1.7	0.3	0.19	3.29	33
8–22	4.4	9	1.1	0.2	—	0.09	1.39	79
22–50	4.3	7	1.2	0.2	—	0.07	1.47	82
Paragominas – PA, Brazil. Typic Acrorthox								
0–6	4.2	28	1.45	2.08	0.88	0.14	4.55	32
6–23	4.1	9	1.86	0.64	0.56	0.07	3.13	59
23–60	4.7	7	1.03	0.48	0.48	0.04	2.03	51
Barrolandia – BA, Brazil. Typic Paleudult								
0–30	4.7	13	0.7	0.8	1.3	0.07	2.87	24
10–23	4.7	10	0.9	0.0	0.6	0.06	1.56	58
23–49	4.8	5	1.0	0.0	0.6	0.04	1.64	61
Porto Velho – RO, Brazil. Orthoxic Palehumult								
0–5	4.5	31	2.2	0.6	—	0.20	3.00	73
5–20	4.2	13	1.4	0.1	—	0.08	1.58	93
20–40	4.4	10	1.1	0.1	—	0.05	1.25	88
40–60	4.7	7	1.0	0.1	—	0.04	1.14	88

Source: From P.A. Sanchez, in *Management o.f Acid Tropical Soils for Sustainable Agriculture*. IBSRAM Proceedings No. 2, 1987, pp. 63–107.

lists values of exchangeable aluminum and aluminum saturation percentage for some acid soils from Latin America (300). The amount of aluminum extracted by neutral salts, such as 1 M KCl or 0.01 M $CaCl_2$, however, varies with extraction time, concentration of the extracting solution (301), and with the number of successive extractions (302).

Other solutions such as 1 M NH_4Cl, 0.01 M $CaCl_2$, or 0.01 M $Ca(NO_3)_2$ have also been used to extract aluminum. There are indications that aluminum extracted with 0.01 M $CaCl_2$, an extractant that mimics the ionic strength (and composition) of highly weathered acid soils, correlates well with the free Al^{3+} activity in soil solution and with aluminum phytotoxicity (303–304).

Also, 0.5 M $CuCl_2$ and 0.33 M $LaCl_3$ have been used to extract organically bound aluminum (284,305). Copper reacts strongly with carboxylate sites that bind aluminum and can readily replace aluminum bound to the solid organic matter. Lanthanum is less effective than copper, but more effective than potassium, in displacing organically bound aluminum (306).

Despite potential difficulties in extracting toxic forms of aluminum with neutral salt solutions, exchangeable aluminum and aluminum saturation percentage have been used extensively as an indicator of aluminum toxicity in acid soils and in estimating the lime requirement (307). Growth of many plants in acid soils was reduced by 50% or more compared to growth in limed soil when the soil aluminum saturation was $> 60\%$ (307). As for lime requirement, it is generally accepted that the amount of $CaCO_3$ required to neutralize toxic aluminum can be estimated as follows:

$$\text{The } CaCO_3 \text{ requirement (t ha}^{-1}) = K \times \text{exchangeable aluminum (cmol}_c \text{ kg}^{-1})$$

where K ranges from 1.5 to 3.0 and averages 2.0 (307). Often K is > 1 to partly account for the fraction of aluminum that is not extracted by KCl. On the other hand, as pointed out by Adams (279), the critical aluminum-saturation percentage, above which relative plant growth would be restricted by 10% or more, varies markedly with soils and crops. For example, the critical aluminum saturation for soybean was about 20 to 25% for Ultisols in Alabama and North Carolina (308–310). It was about 6% for an Ultisol in South Carolina (308), 5% for a Spodosol in Florida (311), and 30% for an Oxisol in Brazilian Amazon (312). As for different crops, the critical aluminum saturation was 4 to 5% for alfalfa, white clover, tall fescue (*Festuca arundinacea* Schreb.), and sericea lespedeza (*Kummerowia striata* Schindl., formerly *Lespedeza striata*) (313,314). It was 40 to 50% for corn grown on three Ultisols in North Carolina (315), 1 to 8% for six Ultisols in Georgia (316) and 30% for an Oxisol in Brazil (312). Similarly, Adams and Moore (317), using the elongation rate of cotton taproot as an indicator of aluminum toxicity, found that the critical aluminum saturation was 2% in the Bt2 horizon of one soil but more than 56% in the Bt1 of another soil in Alabama. For peanut (*Arachis hypogaea* L.), the critical aluminum saturation was 60% (312). Evidently, additional and perhaps better methods for identifying the toxic aluminum forms are needed.

16.9.3.2 Soil-Solution Aluminum

Soil solution can be collected by several techniques, such as zero-tension lysimeters (in situ field sampling), column displacement with a miscible liquid, or high-speed centrifugation with or without a heavy liquid that is immiscible with water (laboratory sampling) (299,318). These techniques, however, are time consuming and often require high skills and care (in terms of pH changes due to CO_2 loss, and contamination) especially when aluminum concentrations are at micromolar levels.

Once in solution, be it soil solution or dilute neutral salt extracts, soluble aluminum can be quantified readily using atomic absorption (preferably flameless) spectroscopy or inductively coupled plasma emission spectroscopy. Alternatively, total soluble aluminum can be measured colorimetrically after forming a colored complex with an organic agent (319).

The separation of total soluble aluminum into different forms (speciation) is more involved, and many techniques have been proposed, which can be grouped into three main categories: (a) analytical separation of various aluminum fractions based on differential reaction kinetics with complexing agents

or the physico-chemical separation of aluminum fractions based on size and charge; (b) computational differentiation of aluminum species from an analytically determined 'total' aluminum fraction, using a thermodynamically based geochemical speciation model with mass balance constraints (320); and (c) combination of one or more analytical techniques with a geochemical speciation model (321).

The most common timed spectrophotometric methods for aluminum determination include 8-hydroxyquinoline (HQ) and pyrocatechol violet (PCV) (322–325). James et al. (322) used a 15 s reaction with HQ buffered at pH 5.2, followed by extraction into butyl acetate, as a method for measuring monomeric aluminum species; a 30-min reaction would measure the total soluble aluminum. The PCV method requires a longer reaction time (approximately 20 min as suggested by Menzies et al. (325)) to complex completely with monomeric aluminum; thus, it is more suitable for an automated procedure.

Aluminum fractionation methods based on size or charge include dialysis, ultrafiltration, size-exclusion chromatography, ion chromatography, capillary zone electrophoresis, and C-18 reverse-phase chromatography (299). Soluble aluminum can also be measured indirectly by reacting it with F^-, then measuring the unreacted free F^- with an ion-elective electrode (326). A quantitative ^{27}Al NMR method is often preferred for the measurement of the 'Al_{13}' polymer (327).

The use of solution Al^{3+} activities to predict or characterize aluminum phytotoxicity are discussed in the later section on soil analysis.

16.9.4 INDICATOR PLANTS

Baker (328) proposed that there are three types of plant responses to increasing heavy metal contents in soil: (a) accumulators, where heavy metals are concentrated in above-ground plant parts; (b) indicators, where internal concentrations reflect external levels; and (c) excluders, where metal concentrations in shoots are low and constant over a wide range of soil concentrations up to a critical soil level above which unrestricted transport occurs. It might be expected that aluminum accumulators would be good indicator plant species; however, this relationship has not been found to be true. Truman et al. (14) reported that only a weak linear relationship was found between foliage aluminum concentration of *Pinus* spp. and exchangeable aluminum in soil. Even in controlled nutrient solution culture, foliar aluminum levels of red spruce varied almost fivefold at a similar solution of aluminum concentration (78).

An alternate method of determining the status of soil aluminum is to grow pairs of aluminum-tolerant and sensitive genotypes of some common crops, such as barley or snapbean, then observe their differential responses. For example, shoots of the aluminum-sensitive 'Romano' snapbean showed a significant response to liming of an acid (pH 5.1) soil from Beltsville, Maryland, but those of the aluminum-tolerant 'Dade' did not; this dry weight difference indicated that aluminum toxicity was the main factor limiting growth (329). Sanchez (300) reported that there was a high degree of tolerance to acid (mostly Al) soil in many varieties of upland rice and cowpea. Such knowledge would be very useful in identifying and managing aluminum-toxic soils.

16.10 ALUMINUM IN HUMAN AND ANIMAL NUTRITION

16.10.1 Aluminum as an Essential Nutrient

Speculation that aluminum is an essential nutrient has persisted for at least 70 years (330); yet to date, there is no conclusive evidence for its essentiality in the diets of animals or humans (6,7). One of the earliest speculations about the essentiality was by E. E. Smith, president of the New York Academy of Sciences in the early 1900s. In his 1928 book on aluminum, he described the effects of adding different elements to milk on the growth and fertility of rats consuming only a milk diet (330,331). Aluminum was one of the added elements that appeared to be necessary for normal fertility and survival of offspring. On this basis, and the fact that aluminum was present in

tissues of the rat, Smith concluded that aluminum 'exercises a true and essential biological function.' This early research with milk diets must be considered equivocal, however, and has never been repeated.

Since this early work, few studies have directly addressed the question of aluminum's essentiality. In 1980, the National Academy of Sciences reviewed the existing research and stated that 'aluminum has not been proven to be essential to animals, but indirect evidence suggests it may be' (332). The indirect evidence included accumulation of aluminum in regenerating bone, stimulation of certain enzyme systems, effective use as an adjuvant, and a report that aluminum stimulated growth in poultry.

Despite this optimism, recent reviews conclude that the evidence for the essentiality of aluminum remains quite limited (6,7). The reports of aluminum accumulation in regenerating bone, stimulation of certain enzymes, and the often-cited ability of aluminum to combine with fluoride and activate the guanine nucleotide (GTP) binding regulatory element of adenylate cyclase (333) are actions of aluminum that have never been proven to be required for normal biological function in any organism. This leaves, then, two isolated studies indicating that a deficiency of aluminum in the diet may modestly inhibit the growth of goats and chickens as the only support for essentiality (6,7). These studies, however, have yet to be validated by others. If aluminum is ever shown to be essential, it appears that the levels required in the diet are so low (less than 200 $\mu g\ kg^{-1}$ diet in the goat study) that dietary deficiency would be very rare.

16.10.2 Beneficial Effects of Aluminum

Although the essentiality of aluminum as a nutrient is questionable, aluminum compounds have been used for many years in animal agriculture, environmental management, and the food and pharmaceutical industries for beneficial purposes. In animals and humans, the beneficial effects usually occur at levels of aluminum intake far above that found in typical diets and, as such, in pharmacological treatments that may carry some risk of aluminum toxicity.

16.10.2.1 Beneficial Effects of Aluminum in Animal Agriculture

Aluminum is generally not added to animal diets because of the lack of any known nutritional function, and no evidence suggests beneficial effects occur in livestock grazing high-aluminum pastures. Rather, aluminum toxicity is of concern as some forages contain over 2000 mg Al kg^{-1} (334). For a variety of useful reasons, however, aluminum compounds have been added to animal diets.

One of the oldest uses of aluminum compounds in agriculture is the use of bentonite clay (Al silicates of sodium, calcium, or other cations) as a binder for pelleted feeds. Studies in the 1950s with poultry indicated no detrimental effects of ingesting bentonite, and some indicated a beneficial effect on growth rate. Benefits were attributed to an increase in feed intake and a delay in the passage of feed through the digestive tract resulting in better absorption of nutrients (335). More recently, bentonite and other aluminosilicates have been investigated for their ability to ameliorate the toxic effects of aflotoxin-contaminated feeds on growth and feed intake in poultry and swine (336,337). Feeding hydrated sodium calcium aluminosilicates has also been shown to reduce the passage of aflatoxins into milk (338). The mechanism of action appears to be adsorption of aflatoxins by the aluminosilicates, reducing aflatoxin bioavailability.

The addition of aluminosilicates to poultry diets has also been reported to enhance eggshell quality (339). Feeding sodium zeolite A, a synthetic aluminosilicate with a 1:1 ratio of aluminum to silicon, increased the levels of silicon and aluminum in the blood. The authors suggested that the increase in blood silicon stimulated calcium use for eggshell formation. Wisser et al. (340), however, were able to show small increases in eggshell quality by adding aluminum sulfate to poultry diets, suggesting that aluminum had an effect independent of silicon. With aluminum sulfate, however, aluminum accumulated in the bones of the hens and reduced fertility. Similar, but less severe

toxic effects were reported with sodium zeolite A, suggesting that zeolites may be a safer way to stimulate eggshell formation (341).

Sodium zeolite A has also been shown to prevent a condition referred to as milk fever (parturient hypocalcemia) in dairy cows, a relatively common problem in the dairy industry (342). Around the time of calving, the metabolic demand for calcium to support gestational growth and milk production is large. This demand for calcium can result in hypocalcemia leading to muscle tremors, weakness, and eventually death if not treated. Sodium zeolite A added to the ration for 3 weeks prior to calving was found to stimulate calcium mobilization from bone and enhance the efficiency of calcium absorption, preventing hypocalcemia (342). The stimulus for these changes in calcium metabolism appeared to come from an aluminum-induced reduction in phosphate availability, since treated cows had significantly lower plasma inorganic phosphate levels.

Similar to the above concept of using aluminum to inhibit phosphate absorption, aluminum has been shown to inhibit fluoride absorption and protect against fluoride toxicity in poultry (343). Aluminum fluoride complexes may be formed in the body, however, and may have detrimental effects of their own (344). Aluminum has also been studied for its beneficial effects on reducing lead toxicity (345).

Some of the beneficial roles of aluminum compounds in animal agriculture are unrelated to aluminum ingestion. Aluminum sulfate has been used to acidify poultry litter to reduce the growth and transmission of bacterial infections caused by *Campylobacter*. *Campylobacter* is a common cause of diarrhea in humans, and undercooked poultry is a potential source. In a recent study, litter contaminated with this bacterium was treated with aluminum sulfate, then, newly hatched chicks were raised on the treated litter (346). No transmission of *Campylobacter* to the chicks was observed. Unfortunately, the treatment was not effective against *Salmonella*. Aluminum compounds have also been used to treat animal manure prior to land applications to reduce environmental impacts. This practice will be discussed in the next section.

16.10.2.2 Beneficial Uses of Aluminum in Environmental Management and Water Treatment

The use of animal manures as fertilizers can increase water pollution problems due to runoff of soluble phosphorus. Several aluminum-containing compounds have been shown to reduce phosphate runoff if applied to manure. Applications of aluminum sulfate or aluminum chloride to swine manure reduced soluble phosphate in runoff by 84%, presumably by forming insoluble phosphate complexes (347). In a large scale, on-farm trial, aluminum sulfate was applied over a 16-month period to litter in 97 poultry houses on the Delmarva Peninsula. Compared to litter from untreated houses, treated litter had decreased soluble phosphates, a lower pH, and higher total nitrogen and sulfur concentrations, thereby increasing its value as a fertilizer (348). Zeolite and aluminum sulfate were evaluated in amending slurries of dairy manure (349). Aluminum sulfate eliminated soluble phosphorus, and zeolite reduced it by over half. Both aluminum compounds reduced ammonia emissions by 50%, presumably by reducing the pH or by adsorbing ammonium cations. Peak et al. (350) used x-ray absorption near edge spectroscopy to determine the chemical species of aluminum and phosphorus in treated manures. No evidence of aluminum phosphate precipitation was found. Therefore, the mechanism of action is not clear and brings up the possibility that soluble forms of aluminum may be present in the treated manures and, hence, in the runoff, especially if excess aluminum is used in the treatment process.

Aluminum sulfate also has been used to treat algal-rich, eutrophied lakes. Welch and Cooke (351) reported the effectiveness and longevity of treatments in 21 lakes across the United States. They concluded that aluminum sulfate effectively reduced total soluble phosphate levels (and the algae that depend on this nutrient) for 8 years on average, especially in lakes without large external inputs of phosphorus. Aluminum is thought to form insoluble aggregates of aluminum phosphate, hydroxide, and organic material that settle to the bottom of the lake and remain in the sediment

unless solubilized by acidic conditions. Acid conditions release soluble forms of aluminum that can be toxic to fish, prompting guidelines that lake pH should remain between 5.5 and 9.0.

Very little evidence suggests that aluminum is beneficial to aquatic species under normal circumstances. Short-term protective effects of aluminum against acid (H^+) toxicity have been shown in some studies (352). Uptake of protons from acidic water can fatally disrupt electrolyte regulation in fish. However, under acidic conditions, monomeric aluminum (Al^{+3}) may bind to gill surfaces blocking the binding and systemic uptake of H^+, thereby improving survival. This protective effect may only last a few hours and has been reported only under laboratory conditions. Aluminum in acidic water (pH 5.2 to 5.9) was also shown to eliminate ectoparasites on Atlantic salmon better than acidic water alone (353).

Municipal water treatment facilities often use aluminum sulfate as a water-clarifying agent in a process similar to that described above for treating eutrophied lakes. The basic process is ancient, originating in China thousands of years ago. When aluminum sulfate is added to turbid water at pH 6.5 to 8, aluminum hydroxide forms as a gel-like precipitate (floc). Suspended particles and oils are trapped in the floc, which is then removed by various methods. Some aluminum, however, can remain in solution. Concentrations of aluminum in treated drinking water have ranged from undetectable to 2.7 mg L^{-1}, with a median of 0.1 mg L^{-1} (354). The Environmental Protection Agency has suggested a maximum contamination level for aluminum in drinking water at a concentration range of 0.05 to 0.2 mg L^{-1}. Recently, other types of aluminum-based clarifying agents such as polyaluminum chloride have been used that may result in less residual aluminum and different chemical species of residual aluminum in treated water compared to current methods (355,356). Clarification of water by aluminum compounds has been investigated for its potential to reduce drinking water fluoride concentrations in regions where fluoride toxicity is a concern (357).

16.10.3 Toxicity of Aluminum to Animals and Humans

The ubiquitous presence of aluminum in soil, water, food, and pharmaceuticals makes exposure to this metal unavoidable for most species. The potential toxicity to humans has been debated since at least the 1920s with the advent of commercially available aluminum-containing baking powders (330). In natural habitats, concern about toxicity increased in the 1970s with the knowledge that acidification of natural waters from acid rain, mine drainage, and deforestation increased the mobilization and bioavailability of soil aluminum (352). The growing awareness of increased exposure to aluminum and the clear demonstration of its potential toxicity to animals and humans (discussed below), combined with its possible association with Alzheimer's disease has given rise to an exponential increase in research related to the metabolism and toxicity of this metal. In the decade from 1970 to 1980, only 140 publications are listed by a bibliographic search using the keywords 'aluminum toxicity,' compared to 1035 publications in the decade from 1990 to 2000. For this reason, a detailed review of aluminum toxicity and metabolism in animals and humans is outside the scope of this chapter and the reader is referred to several recent reviews for this purpose (358–360). The focus of this section will be on the consequences of aluminum exposure from common sources in the food chain with reference, when possible, to potential toxic mechanisms.

16.10.3.1 Toxicity to Wildlife

Much of the concern about aluminum toxicity to wildlife stems from the fact that many lakes and streams have been acidified by natural or industrial causes resulting in increased concentrations of aluminum in their waters. Sparling and Lowe (352) presented a comprehensive review of the environmental toxicity of aluminum and discuss its toxicity in invertebrates, fish, and other wildlife.

Aquatic species, especially freshwater fish, have been studied the most, and it is clear that their survival can be reduced greatly as aluminum concentrations increase in acidic water (361). In fact,

aluminum toxicity is thought to be the most common cause of fish die-offs. Levels of aluminum above 100 to 500 $\mu g\ L^{-1}$ are usually needed to cause death depending on fish species and water conditions such as the amount of dissolved organic matter and pH. Acidity is also toxic and is additive to the effects of aluminum.

The mechanisms of aluminum toxicity fall into two categories based on water pH: asphyxiation in the pH range of 6.5 to 5.5, and loss of electrolytes from the blood in the pH range of 5.5 to 4.5. At the more acidic pH range, soluble cationic species of aluminum are thought to bind to negatively charged sites on the gill surface, displacing bound calcium ions that regulate electrolyte fluxes. This displacement results in the diffusion of sodium and chloride out of the body. In the less acidic pH range of 5.5 to 6.5, the formation of uncharged $Al(OH)_3$ is more likely. These uncharged species form colloids and precipitates that collect on the gill surface, stimulating excess mucus formation. The excess mucus inhibits oxygen and CO_2 diffusion leading to asphyxiation (362). Aluminum appears to be relatively nontoxic to fish at basic pHs where anionic species would predominate.

Dissolved organic matter, such as humic acid, can chelate positively charged aluminum species preventing aluminum from interacting with the gill, thereby reducing aluminum stress (352). Birchall (363) has proposed that silicon can also ameliorate aluminum toxicity by forming colloidal hydroxyaluminosilicates that limit the availability of aluminum for binding to gill surfaces.

Much less is known about aluminum toxicity to other aquatic species such as crustaceans, mollusks, and insect larvae. In general, these invertebrate species are more tolerant to aluminum than fish, but toxic mechanisms appear to be similar in those that have gills, i.e., related to alterations in calcium and electrolyte balance or respiration rates. In contrast to fish, however, invertebrates may accumulate large amounts of aluminum on or within their bodies reaching concentrations as high as 1000 mg kg^{-1} (352,363,364).

There has been some concern about transfer of aluminum up the food chain. Nyholm (365) postulated that elevated levels of aluminum in invertebrates could affect wild birds feeding in or near aluminum-laden waters. In studies with flycatchers, it was reported that female birds had elevated bone aluminum levels and laid deformed eggs with soft shells leading to dehydration and reduced hatchability. Other concerns were with bone growth and body weight gain in growing chicks since aluminum in the diet at a level of 1000 mg kg^{-1} has been shown to inhibit phosphate absorption, reduce feed intake, and accumulate in bone (366). Not all studies, however, have found significant toxic effects on wild birds (352).

Although the ecological impacts of aluminum mobilization into acidified water has been an important concern, recent studies by Palmer and Driscoll (367) indicate, at least in northern hardwood forests in the United States, that stream water aluminum concentrations are declining. They suggested that within 10 years, at the current rate of decline, aluminum toxicity would no longer pose a threat to fish. Remediation of acidic aluminum-laden water also is being accomplished by adding powered limestone ($CaCO_3$) to increase pH and reduce levels of soluble aluminum and, in some cases, total aluminum (352).

16.10.3.2 Toxicity to Agricultural Animals

Generally, aluminum toxicity has not been a serious problem in livestock production (cattle, swine, sheep, and poultry). Levels of aluminum in most common feedstuffs, forages, pastures, and water supplies usually are not high enough to cause problems in animal performance or in the safety of food derived from animals, i.e., they result in diets that contain less than the maximum tolerable levels listed by the National Research Council: 1000 mg kg^{-1} dry feed for cattle and sheep and 200 mg kg^{-1} for swine, poultry, horses, and rabbits (332). These values are for highly soluble forms of aluminum, and higher levels of less soluble forms may be tolerated.

Nevertheless, there has been concern about the toxic levels of intake in cattle and sheep foraging on plants that either accumulate high levels of aluminum or are contaminated with large

amounts of soil, and in poultry consuming diets that contain aluminum from contaminated feed ingredients or from added zeolites. Toxicity symptoms are rather consistent across species. Symptoms include decreased feed intake, reduced efficiency in converting feed to body weight gain, disturbances in mineral metabolism including reduced phosphate absorption, hypercalcemia, reduced bone mineralization, and accumulation of aluminum in body tissues. Large intakes of soluble forms of aluminum (above 3000 to 4000 mg kg^{-1} diet) can be fatal, especially in young animals, or when dietary calcium or phosphorus is low (332).

Storer and Nelson (368) were one of the first to compare the toxicity of different chemical forms of aluminum using young chickens as an animal model. They showed that compounds that were not soluble in dilute acid or water, such as aluminum oxide, did not produce symptoms of toxicity even at dietary levels up to 16,000 mg kg^{-1} diet. Compounds that were soluble such as aluminum chloride, sulfate, acetate and nitrate produced severe toxicity at the 5000 mg kg^{-1} level. Interestingly, aluminum phosphate, which is soluble in dilute acid but not in water, did not produce toxicity apparently due to precipitation in the alkaline environment of the small intestine and its inability to reduce the bioavailability of other forms of dietary phosphate.

16.10.3.2.1 Toxicity to Ruminants (Cattle and Sheep)

Aluminum toxicity to ruminants has not been reported under most livestock production systems. But, some concern has been expressed about the risks of inducing either a phosphorus deficiency or a condition known as grass tetany when ruminants consume large amounts of aluminum from soil or aluminum-rich forages. In general, soil does not appear to be toxic, but the more soluble forms of aluminum in plants may pose some risk.

Ruminants can consume large amounts of soil under some pasture conditions and, therefore, may consume large amounts of aluminum (up to 1.5% of the diet dry matter) (369). Since phosphorus is the mineral most likely to be deficient in the diet of grazing cattle, studies have looked at the effects of soil intake on phosphorus nutrition. Most have shown that soil intake has a minimal effect on phosphorus balance and animals are able to maintain normal serum phosphate levels (370,371). Apparently, the aluminum species in soil are not soluble enough in the intestinal tract of the ruminant to cause significant precipitation of available phosphate.

It is clear, however, that soluble forms of aluminum can induce toxicity. Crowe et al. (369) fed diets that contained soluble aluminum chloride hexahydrate at 2000 mg Al kg^{-1} diet to Holstein dairy calves for 7 weeks. The results are typical of studies in ruminants using soluble forms of aluminum (370). Feed intake decreased by 17%, average daily weight gain decreased by 47%, and the amount of feed needed to produce a kilogram of weight gain increased by 50%. Fecal phosphorus excretion increased by 79% and plasma inorganic phosphate concentrations dropped to levels found in phosphorus-deficient animals. Aluminum accumulated in bone thereby causing demineralization, serum calcium concentrations rose, and urinary and fecal calcium excretion increased. To what extent natural aluminum species in forages can cause these symptoms is not known.

Grass tetany is a serious, often fatal metabolic disorder, characterized by low magnesium levels in the blood. Grass tetany occurs most often in female ruminants in the early stages of lactation while grazing on succulent, immature, magnesium-deficient grasses in springtime. Symptoms include poor coordination, convulsions, and death, presumably related to a metabolic deficiency of magnesium. Several outbreaks of grass tetany have been associated with pastures and forages containing high aluminum concentrations such as wheat and tall fescue containing 1000 to 2000 mg Al kg^{-1} (372). Although most studies looking at soil aluminum intake have not shown significant effects on serum magnesium levels, some studies using soluble aluminum (such as aluminum citrate) have shown small decreases (370,372). It was suggested that the decrease in serum magnesium was not caused by reduced magnesium absorption. Rather, aluminum can cause hypercalcemia, which induces the loss of magnesium in urine. This loss may contribute to the appearance of grass tetany.

16.10.3.2.2 Toxicity to Poultry

Aluminum toxicity has not been reported as a significant problem in poultry production, but concerns have been raised due to the possible intake of soluble aluminum compounds from feed ingredients such as aluminum-flocculated algae, aluminum-contaminated mineral mixes, or the intentional use of zeolites to improve eggshell quality.

Sodium zeolite A ($Na_{12}[(AlO_2)_{12}\ (SiO_2)_{12}]$- $27H_2O$) is a synthetic aluminosilicate with cation exchange properties that has been shown to improve eggshell quality when added to the diet at 0.75 to 1.5%, as mentioned earlier under beneficial effects. When added to the diets of young chicks, however, it caused reductions in feed intake, growth, bone ash, and serum phosphate, and increased serum calcium and bone aluminum content (373–375).

The soluble forms of aluminum are relatively more toxic, but generally show the same biological effects as sodium zeolite A (340,366,376). Interestingly, however, soluble forms tend to inhibit calcium absorption from low calcium diets, whereas, zeolites seem to enhance it. No studies have been done to evaluate the effects of including natural, aluminum-loaded plant or animal products in the diet.

The fact that consuming high levels of aluminum usually decreases food intake makes it difficult to identify toxic effects of aluminum that are independent of reduced nutrient intakes. Wisser et al. (340), however, showed that adding aluminum sulfate to the diet of laying hens decreased egg production and fertility, and increased serum calcium without causing significant decreases in food intake or plasma phosphate. This implies that systemic aluminum can have direct toxic effects on metabolism.

16.10.3.3 Toxicity to Humans

There is no doubt that aluminum intake can be toxic to humans under certain conditions. Regular intake of large doses of aluminum hydroxide can cause bone disease, anemia, and neurological problems in patients with poor renal function that cannot adequately excrete aluminum from the body. Similar effects can occur in healthy individuals if aluminum intake is high enough, over a long enough period. There are questions about the relationship of aluminum to Alzheimer's disease and the health consequences of long-term, low-level exposures that remain unanswered. The reader is referred to several recent reviews for detailed discussions of these topics (358–360).

16.10.3.3.1 Overview of Aluminum Metabolism

The intestine is viewed as a protective barrier against aluminum toxicity as only a small fraction (0 to 0.5%) of ingested aluminum is absorbed from any source. However, of the small amount absorbed, about half is retained in tissues and the other half is excreted, primarily in urine. Elimination from tissues is not rapid so, in the face of constant intake, tissues accumulate aluminum over time.

Drueke (377), and Yokel and McNamara (359) provide recent reviews of the absorption and metabolism of aluminum. A number of factors influence the efficiency of absorption. Most are dietary factors that affect solubility; hence, phosphate reduces absorption as does ingesting insoluble forms of aluminum such as aluminum oxide. Silicon has shown conflicting results, but does not appear to reduce absorption except when given as insoluble, oligomeric forms. The soluble aluminum salts have higher absorption efficiencies, although the hydroxide appears to be less bioavailable than more soluble forms. Citrate, as well as other organic acids including ascorbic, oxalic, lactic, and tartaric acids can greatly enhance absorption possibly by increasing solubility or charge neutralization when complexed species are formed. The mechanism, however, is not yet understood. Aluminum-accumulating plants which store aluminum bound to organic acids would be expected to contain bioavailable aluminum, but this concept has never been tested. Polyphenolic acids have recently been shown to increase tissue uptake of aluminum from food, suggesting increased absorption (378). Fluoride may also enhance absorption.

The mechanism of aluminum absorption is not well understood but appears to involve active transport through the intestinal cells as well as passive diffusion. High iron diets inhibit transport whereas low iron diets enhance it, suggesting that aluminum can follow iron transport pathways.

In the blood, about 80% of aluminum is bound to iron binding sites on transferrin, the major iron transport protein in plasma (47,48). The remainder is bound to low-molecular-weight molecules, possibly citrate. Since most tissues take up transferrin to acquire iron, this process provides a mechanism for aluminum to enter cells, including the brain. Tissue uptake from the citrate-bound form is also possible. In fact, increased dietary citrate appears to enhance tissue accumulation of aluminum as well as urinary excretion. In renal-failure patients, citrate greatly enhances risk of toxicity.

Bone is the major tissue deposition site with aluminum accumulating at areas of active mineralization, possibly as aluminum citrate. Aluminum also enters and is toxic to the bone forming cells (osteoblasts). Other tissues accumulate lesser amounts of aluminum, usually in the order: bone > liver > kidney > spleen > brain. Contrary to other tissues, the brain has not always been found to accumulate aluminum in association with increased dietary intake. Nevertheless, aluminum is routinely found in the brain in measurable amounts. Elimination of aluminum from tissues is relatively slow compared with its rapid uptake, with half-lives estimated in terms of months or years. Elimination from bone is the most rapid, and that from brain is the slowest. Body loads are typically low, 30 to 50 mg in healthy individuals on usual diets.

The intracellular metabolism of aluminum is poorly understood. Presumably, it initially follows the pathways of iron metabolism being incorporated with transferrin-bound iron into endosomes. Its subsequent fate, or the fate of citrate bound aluminum are unknown.

16.10.3.3.2 Overview of the Biochemical Mechanisms of Aluminum Toxicity

The biochemical mechanisms of aluminum toxicity leading to neurodegeneration, bone loss, and anemia are not understood and an explanation for these symptoms cannot be made at this time. At its most fundamental level, the systemic toxicity of aluminum is probably related to its strong binding affinity for three-oxygen-donor ligands, especially negatively charged oxygen donors found in organic phosphates and proteins with carboxylic acid or phosphorylated residues (379). This strong binding can displace magnesium ions, alter the structure and function of substrates, enzymes, regulatory and structural proteins, and in poorly understood ways interfere with iron metabolism. The biochemical aspects of aluminum toxicity in animals and man have recently been reviewed (360). It is likely that the basic biochemical effects of aluminum are similar in plant and animal cells.

Before systemic toxicity is discussed, it should be remembered that dietary aluminum toxicity often induces a phosphate deficiency. Appetite and growth are depressed. Bone mineral is dissolved in an attempt to raise serum phosphate levels and hypercalcemia may result. Skeletal muscle may also lose intracellular phosphorus and magnesium to the blood, resulting in lowered ATP synthesis and a general lack of phosphate for metabolic use within the muscle. Intracellular calcium levels become elevated. Bone pain, muscle weakness, and neurological symptoms including confusion, seizures, and coma can occur (380).

Once aluminum gains entry into the body and enters cells, it is thought to bind to phosphate ligands, particularly ATP. It also binds to proteins. Bound aluminum may alter enzyme activity by displacing cofactors such as Mg^{++}, by affecting the binding of substrates such as ATP, or by inducing conformational changes. For example, aluminum has been shown to inhibit ATP dependent enzymes such as hexokinase. The mechanism is thought to involve formation of Al-ATP that is much more stable and binds much tighter to proteins than Mg-ATP, inhibiting enzyme action. More than 20 other enzymes are reportedly inhibited or stimulated by aluminum (379).

Aluminum may also influence protein–protein interactions (381). For example, aluminum may bind to calmodulin, a calcium-activated regulatory protein that controls the activity of more than 40 different enzymes by binding to them via hydrophobic interactions resulting in the induction or inhibition of activity. Aluminum binding does not affect calcium binding to calmodulin; rather, aluminum induces conformational changes that inhibit the ability of calmodulin to bind target proteins.

Aluminum also can cross-link proteins by forming intermolecular bridges between binding sites on amino acid side chains. The binding of aluminum to proteins may also affect their turnover, either stabilizing them, such as in insoluble aggregates, or enhancing degradation via conformational changes.

Since many signal transduction processes involve phosphate group transfers, this is another likely site for aluminum toxicity (382). The phosphatidylinositol 4,5-bisphosphate (PIP_2) signaling pathway has been inhibited by aluminum. Aluminum apparently binds to phosphate groups of PIP_2 in membrane phospholipids inhibiting PIP_2 hydrolysis by phospholipase C. An alteration in signal transduction pathways may help explain the altered pattern of gene expression seen in tissues exposed to aluminum (383). G-proteins and protein kinases are also reportedly affected by aluminum (383).

Aluminum has been shown to interfere with iron metabolism. It blocks the incorporation of iron into heme resulting in poor hemoglobin production and anemia (384). Aluminum also appears to disrupt the mechanisms that control intracellular iron homeostasis. The result may be altered iron distribution in the cell leading to increased levels of reactive or "free" iron and iron-induced oxidative stress (384–386). Normally, increasing intracellular "free" iron concentrations coordinately stimulate the synthesis of the iron storage protein ferritin, and inhibit the synthesis of transferrin receptors that control iron uptake. Studies suggest that aluminum antagonizes the ability of intracellular iron to regulate the translation of mRNAs for both ferritin and the transferrin receptor. Under these conditions, the amount of "free" iron in the cell becomes elevated relative to the amount of its storage and detoxification by ferritin, thus increasing the risk for iron-induced oxidative stress. Aluminium has also been shown to inhibit the ATP-dependent proton pump on endosomes, resulting in the trapping of transferrin-bound iron inside these vesicles. The trapping of iron would limit its ability to stimulate ferritin synthesis. Aluminium may also inhibit the incorporation of iron into ferritin, further increasing the levels of reactive "free" iron in the cell.

Recent studies have shown that aluminum can induce oxidative stress even though it is not a redox metal, and that antioxidants can attenuate this effect supporting the concept that aluminum toxicity involves oxidative damage (387,388). Oxidative stress could result from altered membrane structure, a reduction in antioxidant defense systems, or the induction of free radical generating systems such as increased levels of reactive "free" iron.

16.11 ALUMINUM CONCENTRATIONS

16.11.1 In Plant Tissues

16.11.1.1 Aluminum in Roots

Increasing aluminum levels in the medium tended to result in increasing aluminum concentrations in roots of aluminum accumulators or aluminum excluders (Table 16.3). Concentrations of aluminum in roots were 2- to 250-fold higher than those in shoots (Table 16.3). In red spruce, root aluminum concentrations associated with a 20% decrease in root biomass ranged from 1700 to 6000 mg Al kg^{-1} (78). Aluminum in roots is present mostly as precipitated hydroxy or phosphate compounds outside the root cells (86). As a result, it is difficult to use aluminum concentrations in roots as a measure of aluminum toxicity unless an effort is made to remove or prevent extracellularly precipitated and adsorbed aluminum. Alternatively, it might be possible to analyze aluminum concentrations in root apices alone as a measure of toxicity (189–191).

16.11.1.2 Aluminum in Shoots

In accumulators, foliar aluminum concentrations of 65 tree species and 12 unidentified trees from an Indonesian rain forest ranged from 1 g kg^{-1} in delta tree (*Aporusa* spp. Blume, Euphorbiaceae) to 37 g kg^{-1} in *Maschalocorymbosus corymbosus* Bremek. (Rubiaceae) (13). Aluminum accumulators (*Melastoma malabathricum* L., *Hydrangea macrophylla* Ser., and *Fagopyrum esculentum* Moench.) exposed to increasing aluminum in solution showed increasing aluminum concentrations in leaves (22) (Figure 16.7). Facultative aluminum accumulators, jack pine (*Pinus banksiana*

TABLE 16.3
Aluminum Concentrations in Roots and Leaves

Species	Al Level (μM)	Effect on Growth[a]	Root Al (mg kg^{-1})	Young Foliar Al (mg kg^{-1})[b]	Reference
Al accumulators					
Jack pine (*Pinus banksiana* Lamb.)	0	0	211	39	390
	185	0	411	85	
	370	0	747	139	
	740	0	849	196	
	1480	−	1227	251	
	2960	−	1744	380	
	5930	−	3654	988	
Black pine	0	0	108[c]	189[d]	16
(*Pinus nigra* Arnold)	100	+	1863	891	
	500	+	1593	999	
	1000	−	5400	999	
Al excluders					
European white birch	0	0	—	—	26
(*Betula pendula*	74	−	1050	70	
Roth race SMM)	185	−	270	160	
	370	+	270	100	
	555	+	260	120	
	930	+	240	40	
	1296	+	310	130	
Tomato (*Lycopersion*	0	0	59	15	397
esculentum Mill.)	10	−	1937	14	
	25	−	5888	51	
	50	−	11,838	48	
Phasey bean	0	0		125	398
(*Macroptilium*	18	+		125	
lathyroides Urb.)	37	+		125	
	74	+		140	
Alfalfa	0	0		70	398
(*Medicago sativa* L.)	18	−		100	
	37	−		150	
	74	−		315	
Red spruce	0	0	243	29	390
(*Picea rubens* Sarg.)	185	−	446	47	
	370	−	739	67	
	740	−	1690	162	
	1480	−	2212	272	
	2960	−	2905	492	
	5930	−	5351	772	
Douglas fir	0	0	304	27[d]	25
[*Pseudotsuga menziesii*	148	+	1350	157	
(Mirb.) Franco]	296	0	1753	369	
	593	0	2375	430	
	1185	0	3591	447	
Northern Red oak	56	0	7560	66	399
(*Quercus rubra* L.)	169	−	6567	168	

Continued

TABLE 16.3 (*Continued*)
Aluminum Concentrations in Roots and Leaves

Species	Al Level (μM)	Effect on Growth[a]	Root Al (mg kg^{-1})	Young Foliar Al (mg kg^{-1})[b]	Reference
	360	−	6422	138	
Stylo	825	−	6982	147	
[*Stylosanthes guianensis*	0	0	180	74	22
(Aubl.) Sw.]	111	−	886	61	
	555	−	890	146	
African marigold	0	0	71	36	396
(*Tagetes erecta* L.)	37	+	650	32	
	148	+	1230	33	
White clover	0	0	1120	<25	131
(*Trifolium repens*[c] L.)	25	−	1621	44	
	50	−	2998	83	
	100	−	4008	66	
Corn	0	0	116	30	400
(*Zea mays* L.)	93	−	2150	38	
	185	−	2470	142	
	370	−	2500	163	
	741	−	2730	282	

[a]Positive (+), negative (−), or no effect (0) on growth relative to control (0 Al).
[b]Foliar concentration in young leaves if young and old leaves were analyzed separately; otherwise, foliar concentration averaged across all leaves.
[c]Al concentrations in coarse roots.
[d]Al concentrations in needles.
[e]Plants supplied with N and no further Al given after pretreatment with Al.

Lamb.) and loblolly pine (*Pinus taeda* L.), also had increasing foliar aluminum concentrations as solution aluminum increased (389) (Table 16.3).

Efforts to establish critical aluminum concentrations for toxicity in plants generally have been unsuccessful (78,82,390). For example, foliar concentrations in red spruce associated with a 20% decrease in foliar biomass ranged from 70 to 250 mg kg^{-1} (78). Similarly, foliar aluminum concentrations in red oak associated with a 20% decrease in leaf biomass ranged from 93 to 188 mg kg^{-1} (391). Within slash pine families, aluminum sensitivity was correlated positively with foliar aluminum concentration; however, no such correlation was found within loblolly pine families (392).

In accumulators, internal complexation of aluminum by organic anions, silicate, or other ligands resulted in poor correlations between foliar aluminum concentrations and restrictions in biomass growth. Raynal et al. (78) reported the absence of any significant correlation between biomass response and foliar aluminum levels in *Pinus* species. In the case of aluminum excluders, aluminum concentrations in shoots do not increase with increasing aluminum levels in the medium until a toxic threshold is exceeded (328), again resulting in poor correlation between foliar aluminum levels and biomass response. For example, in rice and barley, only trace amounts of aluminum were found in leaves at solution aluminum levels up to 111 μM, then foliar aluminum concentrations increased as aluminum levels in solution increased to 555 μM (22) (Figure 16.7). Similarly, increasing solution aluminum levels from 0 to 620 μM had no effect on biomass growth of Western hemlock (*Tsuga heterophylla* Sarg.), then foliar aluminum concentrations decreased from 300 to

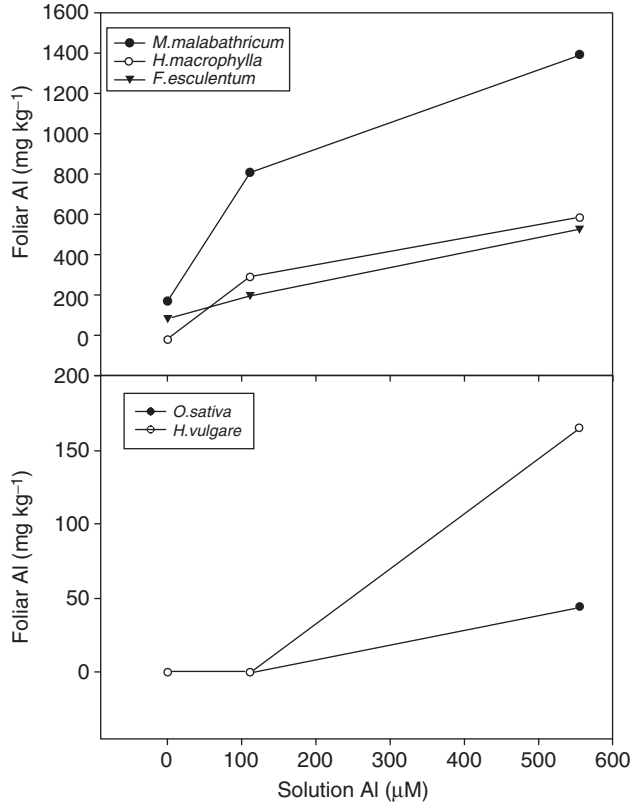


FIGURE 16.7 The pattern of increasing foliar aluminum concentrations with increasing solution aluminum differs in aluminum accumulator species (top) and aluminum excluder species (bottom) (22).

250 mg kg^{-1} when biomass was affected adversely by solution aluminum (393). In sugar maple (*Acer saccharum* Marsh.), aluminum concentrations in leaves increased from 50 to 200 mg kg^{-1} as aluminum levels in solution increased from 0 to 600 μM, but then foliar aluminum concentration dropped to 150 mg kg^{-1} when shoot growth was restricted at 1000 μM aluminum in solution (394). Other examples of a lack of correlation between aluminum-induced growth inhibition and foliar aluminum concentrations can be found in Table 16.3 (395–399).

16.11.2 Soil Analysis

Aluminum bioavailability in soils and toxicity to plants is difficult to quantify because toxic levels vary with species and even with cultivars within a species (82). For example, 1.5 μM Al^{3+} activity was reportedly toxic to cotton roots (294), and 4.0 μM Al^{3+} was toxic for coffee (32). For rice, an aluminum-tolerant crop, the critical Al^{3+} activity was approximately 100 μM (400).

Chemical composition of some soil solutions, including aluminum and its various species, is listed in Table 16.4 (294). Table 16.5 lists critical Al^{3+} activities, as measured by root elongation, for selected plants (401). In general, trees are more tolerant of aluminum than most agronomic crops (Table 16.5). For 2-year-old seedlings of Norway spruce, aluminum toxicity was not evident when Al^{3+} activities in soil solutions ranged from 7.7 to 64.3 μM (402).

Instead of using Al^{3+} activity as the sole indicator of phytotoxicity, Alva et al. (34) used the sum of the activities of monomeric aluminum species ($Al^{3+} + AlOH^{2+} + Al(OH)_2^+ + Al(OH)_3^0 + AlSO_4^+$). They observed 50% reductions in root elongation, relative to roots of plants not receiving

TABLE 16.4
Range of Values, Means, and Standard Deviations (s.d.) for Attributes of Soil Solutions from 48 Surface and 48 Subsoil Samples from Queensland, Australia

		Surface Soil			Subsoil		
Attribute	**Unit**	**Range**	**Mean**	**S.d.**	**Range**	**Mean**	**S.d.**
pH		3.73–7.99	5.4	0.85	3.78–6.77	5.28	0.7
EC	dS m^{-1}	0.13–1.92	0.48	0.35	0.03–1.12	0.24	0.28
I	mM	1.2–22.6	5.3	4.2	< 0.1–13.1	2.4	3.3
Ca	µM	34–1854	38	339	8–1437	79	206
Mg	µM	82–1366	345	240	14–560	138	134
Na	µM	262–8378	1279	1591	106–6960	1333	1730
K	µM	65–3171	386	481	12–2110	143	304
SO_4	µM	63–3858	585	597	14–1369	220	264
Al	µM	2.1–101	23	25	0.05–378	12	54
Al^{3+}	µM	0.05–34	3.4	7	0.05–126	3.6	18
$Al(OH)^{2+}$	µM	0.05–8.3	1.4	1.9	0.05–7.2	0.5	1.1
$Al(OH)_2^+$	µM	0.05–38	0.8	8.9	0.05–11	1.3	1.8
$Al(OH)_3^0$	µM	0.05–22	4.1	4.2	0.05–5.8	0.5	0.9
$Al(SO_4)^+$	µM	0.05–30	2.1	5.3	0.05–7.7	0.4	1.2
Σ(Al)	µM	2.1–67	19	18	0.05–143	6.2	21

Source: From R.C. Bruce et al., *Aus. J. Soil Res*., 27:333–351, 1989.

TABLE 16.5
Threshold of Al Toxicity to Some Plants Where Root Elongation was the Measure of Response and Where Available Al was Expressed as Al^{3+} Activity in Solution

Plant	**Al^{3+} at Phytotoxic Threshold (µM)**	**Rooting Medium**
Gramineae spp.	0.90	Solution, soil
Cotton	1.5	Solution, soil
Barley	1.5	Solution
Coffee	4.0	Solution, soil
Cotton	6.0	Soil
Wheat	20	Soil
Honeylocust	40	Solution
Red spruce	50	Solution
Hybrid poplar	100	Solution
Red spruce, balsam fir	300	Solution
Autumn-olive	400	Solution
Pine, oak, birch	800	Solution

Source: From J.D. Wolt, in *Soil Solution Chemistry*, Wiley, New York, 1994, pp. 220–245.

any aluminum, as this sum ranged from 12 to 17 µM for soybean, < 8 to 16 µM for sunflower (*Helianthus annuus* L.), < 7 to 15 µM for subterranean clover, and < 5 to 10 µM for alfalfa. Alternatively, Cronan and Grigal (390) proposed the use of calcium/aluminum ratios as indicators of aluminum stress in forest ecosystems.

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