

# 6

## Some Abiotic Plant Stressors – Oxygen, Minerals, and Salt

Plants are subjected to enormous biotic and abiotic stresses during their lifetime (Fig. 6.1). No matter what the source of the stress, the impact is ultimately at the cellular level, eventually manifesting itself at the whole plant level. This manifestation is often seen as reduced performance in the field, reflected in reduced yield or quality.

The basis for many plant responses to stress revolves around a cells' relationship with oxygen. This is a major paradox of biology. While oxygen is required to sustain life, in a slightly altered form, oxygen can wreak havoc on life processes. This chapter explores the many faces of oxygen, the physiology of oxidative stress in plants, and the protective role of antioxidants in plants. In addition, stresses imposed upon plants by minerals and salt are explored.

### Forms of Oxygen

Most of us think of oxygen as an essential component of respiration and a wonderful product of photosynthesis. Oxygen also has a dark side. In order to understand the dark aspect of oxygen, we need to understand its dangerous, mutant forms, reactive oxygen species (ROS) (Table 6.1).

Atmospheric oxygen ( $O_2$ ), also called triplet oxygen, in general does not oxidize (remove electrons from) organic substances. It is the stable form of oxygen we are familiar with. It has two unpaired electrons with parallel spins, and this parallel spin prevents reactivity. In general, in order to participate in chemical reactions with organic molecules oxygen must be activated. Activation can occur through the reversal of the spin of one of the unpaired electrons by absorption of energy to form singlet oxygen ( $O_2^1$ ). Singlet oxygen is very reactive with many organic molecules and can be involved in divalent reduction (transfer of two electrons at once). Activation can also occur by adding electrons to  $O_2$ . If an oxygen molecule ( $O_2$ ) gains one

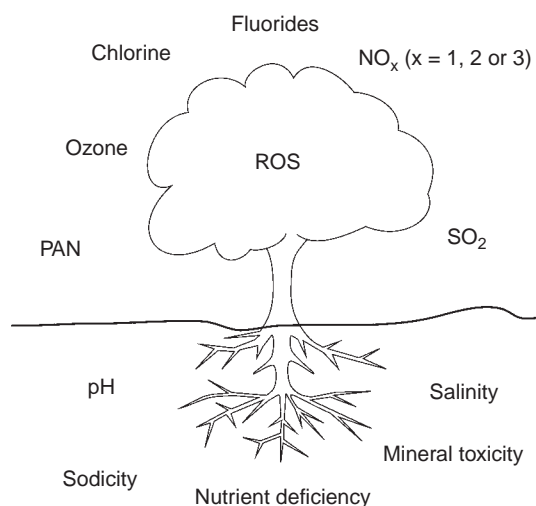
electron, it becomes a superoxide radical ( $O_2^-$ ). This process is endothermic, absorbing heat as it occurs. If ( $O_2^-$ ) gains two electrons, it forms hydrogen peroxide ( $H_2O_2$ ), and if it gains three electrons, it forms a hydroxyl radical ( $HO^\cdot$ ). Both of these reactions are exothermic, releasing heat as they take place (Afanasev, 1985).

These altered forms of oxygen are capable of unrestricted oxidation (removal of electrons from) of cellular components which can ultimately result in cell death. Cell death may occur due to peroxidation of membrane lipids, oxidation of proteins, enzyme inhibition, and nucleic acid damage. ROS also act as signaling molecules in plants, leading to the activation of metabolic pathways for stress resistance.

$H_2O_2$  is a particularly important ROS. It passes through membranes easily and is not compartmentalized within the cell.  $H_2O_2$  is also involved in the formation of many complex organic molecules. Even though  $H_2O_2$  is known to be very reactive, it alone is not that impressive. However, in the presence of a metal reductant (electron donor), it forms the highly reactive hydroxyl radical which is the strongest oxidizing agent known.

### Sources of These Altered Forms

Many ROS are byproducts of photosynthesis and respiration (Gill and Tuteja, 2010). In the light, most ROS come from chloroplasts and peroxisomes (Foyer and Noctor, 2003) while mitochondria produce most of the ROS formed in the dark (Moller, 2001). They are also produced during abiotic stress, pathogen defense or normal senescence. A normal rate of ROS production in cells is  $240 \mu M O_2^-/s$  which includes around  $0.5 \mu M H_2O_2/s$ . Under stressful conditions production can increase to  $700 \mu M O_2^-/s$  with up to  $5-15 \mu M H_2O_2$ . Increased ROS production can be caused by drought, chilling, heat, high light, desiccation,



**Fig. 6.1.** The many abiotic plant stressors. PAN, Peroxyacetyl nitrate; ROS, reactive oxygen species.

**Table 6.1.** Forms of oxygen important in plant physiology.

Form of oxygen	Molecular formula	How it is formed
Atmospheric oxygen (triplet oxygen)	O <sub>2</sub>	
Singlet oxygen	O <sub>2</sub> <sup>1</sup>	Reverse the spin of one of the unpaired electrons in O <sub>2</sub> by adding energy
Superoxide radical	O <sub>2</sub> <sup>-</sup>	Add one electron to O <sub>2</sub>
Hydrogen peroxide	H <sub>2</sub> O <sub>2</sub>	Add two electrons to O <sub>2</sub>
Hydroxyl radical	HO <sup>-</sup>	Add three electrons to O <sub>2</sub>

heavy metals, salt, ultraviolet (UV) radiation, nutrient deficiencies, air pollution (ozone and sulfur dioxide, SO<sub>2</sub>), pathogen attack, and mechanical stress. Since ROS can be both beneficial (in the case of signaling for pathogen defense) and harmful (as in protein or lipid oxidation), ROS levels must be precisely regulated at the cellular level.

## Eliminating ROS

Excessive amounts of ROS must be eliminated from plant cells to avoid damage. This is accomplished

either through direct elimination via specific metabolic pathways or by avoiding or preventing their production in the first place.

The major ROS scavengers in plants are enzymes that include superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) (Gill and Tuteja, 2010). Their balance and activity is important in regulating the levels of superoxide radicals and H<sub>2</sub>O<sub>2</sub>. This balance coupled with heavy metal ion sequestration is important for avoiding the formation of the highly toxic hydroxyl radical.

The most effective enzymatic antioxidant is SOD, which is found in all cells (Beyer *et al.*, 1991). Its activity depends on metal cofactors including copper, zinc, manganese or iron. As the name implies, SOD is responsible for the dismutation of the superoxide molecule. Dismutation is a simultaneous reduction and oxidation of a substance to produce two different products. Superoxide molecules are dismutated into O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>.

APXs are a group of enzymes responsible for reducing H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O by transferring an electron from H<sub>2</sub>O<sub>2</sub> to ascorbate. In the process dehydroascorbate is produced, which is reduced to ascorbate at the expense of creating oxidized glutathione. The oxidized glutathione is reduced using an electron from nicotinamide adenine dinucleotide phosphate (NADPH). The overall process is the transfer of an electron from NADPH to H<sub>2</sub>O<sub>2</sub> through glutathione and ascorbate, reducing the H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and making it harmless. This is called the glutathione-ascorbate cycle and is important for removing H<sub>2</sub>O<sub>2</sub> from plants.

CATs are a group of enzymes with the ability to dismutate H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub>. CAT is a very effective antioxidant enzyme: one molecule of CAT can dismutate millions of molecules of H<sub>2</sub>O<sub>2</sub>/min.

In addition to enzyme-mediated removal of ROS, antioxidants such as ascorbic acid and glutathione are important in a plant's defense against oxidative stress.

Rather than scavenging ROS during stress conditions, avoiding their production in the first place is an effective mechanism for avoiding damage. Some mechanisms that help reduce ROS production during stress include anatomical, physiological and metabolic adaptations. Anatomically, leaf curling in corn (*Zea mays*) to reduce water loss during drought stress may also help reduce ROS production. Physiologically, C4 and CAM metabolism

alter 'normal' photosynthesis by incorporating additional steps into carbon fixation. This helps prevent the transfer of electrons to  $O_2$  instead of  $CO_2$  that often occurs in C3 metabolism. The rearranging of the photosynthetic machinery in response to light quality and quantity represents a molecular mechanism to balance light absorption with  $CO_2$  availability. This prevents the production of excess electrons under high light levels thus reducing the transfer of electrons to  $O_2$ .

The production of ROS in chloroplasts and mitochondria can be reduced by enzymes called alternative oxidases (AOX). These enzymes channel electrons flowing through electron transport chains of photosynthesis and respiration to  $O_2$ , producing water instead of  $O_2^-$ . The levels of ROS are decreased in two ways: (i) by producing water instead of  $O_2^-$ ; and (ii) by reducing the level of  $O_2$  available for further ROS production.

### ROS and Plant Defense

ROS are a key cellular signal to the plant that it is under attack by a pathogen. As a pathogen attacks a plant, ROS are produced in plant cells through enhanced activity of plasma-membrane-bound NADPH oxidases, amine oxidases located in the apoplast, and peroxidases bound to the cell wall.  $H_2O_2$  is produced during this response and seems to diffuse into cells and along with the plant hormone salicylic acid, activates many of the plant defenses against the pathogen.

Salicylic acid at the same time suppresses the activity of ROS-scavenging enzymes, allowing ROS levels to increase indicating the attack. Without this reduction in ROS-scavenging activity, ROS levels could never reach a level needed to signal the need for defense.

The function of ROS is very different during a biotic attack compared with an abiotic attack. During the biotic attack, defenses are signaled via accumulated ROS while during an abiotic stress, increased ROS levels summon ROS scavengers to decrease high levels of ROS produced during the stress. But what happens if a plant comes under a biotic attack while undergoing an abiotic stress such as drought? Plants that are under an abiotic stress before or during a biotic attack generally develop less resistance to the biotic attack than plants not under an abiotic stress before or during the attack.

## Plant Antioxidants

### Ascorbic acid

Vitamin C (L-ascorbic acid, ascorbate) is plentiful in plant tissues (Foyer, 1993). It has received much attention as an important human nutrient. However, little attention has been given to its importance in plant health. Ascorbate, which is directly produced through conversion of D-glucose, is an important plant antioxidant, found mostly in the chloroplast, directly scavenging free radicals to minimize oxidative stress in plants (Smirnoff, 2005). Ascorbate is also capable of indirect ROS scavenging by reducing (adding an electron to) tocopherol, which in its reduced form is a ROS scavenger. Much of a plant's ascorbate is localized within the chloroplast, where it scavenges  $H_2O_2$ . In addition, ascorbate may also be important for cell wall biosynthesis.

### Glutathione

Glutathione, a tripeptide, has antioxidant function due to the sulfhydryl group of cysteine, one of the three amino acids which form the molecule. Most glutathione exists in the reduced form, in the chloroplast and cytosol, and its concentration declines with tissue age (Larson, 1988; Alscher, 1989).

Glutathione can effectively react with singlet oxygen, superoxide and hydroxyl radicals, functioning directly as a ROS scavenger. Glutathione might also help protect membrane integrity by getting rid of products of lipid peroxidation. It is also the reducing agent during recycling of ascorbate from its oxidized to its reduced form.

Glutathione also has other cellular functions besides acting as a ROS scavenger: (i) it appears to help in sulfur transfer from source to sink tissues; (ii) it helps detoxify xenobiotics (substances not normally found in an organism, or found in much higher than normal concentrations); (iii) it is a precursor to heavy metal binding chelates in plants; and (iv) it confers tolerance of corn (*Z. mays*) plants to the herbicide triazine.

### Tocopherol

The tocopherols, and particularly the most active  $\alpha$ -tocopherol (vitamin E), are a well-known family of antioxidants located exclusively in cell membranes (Diplock *et al.*, 1989; Fryer, 1992; Hess, 1993). They are particularly effective in quenching singlet oxygen and peroxides (Kamal-Eldin and

Appelqvist, 1996). Tocopherols are found predominantly in plants in chloroplast thylakoid membranes, thus leaves are a particularly rich source.  $\alpha$ -Tocopherol also stabilizes membranes by sequestering free fatty acids which can act like detergents disrupting membrane integrity.

### Carotenoids

Carotenoids are 40 carbon isoprenoids and tetraterpenes located in plastids of all plant cells. They are accessory pigments in chloroplasts and also detoxify activated oxygen and triplet chlorophyll that are produced during light harvesting in photosynthesis (Peñuelas and Munné-Bosch, 2005).

The two classes of carotenoids include carotenes and xanthophylls. Carotenes are hydrocarbon carotenoids and xanthophylls are derived from carotenes that contain one or more oxygen atoms. Carotenoids exist in the ground state or in one of two excited states during light harvesting.

Carotenoids function as antioxidants in many ways (Young, 1991). They effectively terminate lipid peroxidation chain reactions and thereby reduce damage to membranes by ROS. They scavenge singlet oxygen and dissipate the energy as heat. This is particularly important when light levels are above the saturation level and singlet oxygen is plentiful. Carotenoids, especially  $\beta$ -carotene, react with triplet or excited chlorophyll preventing the formation of singlet oxygen. Finally, cycling of xanthophyll between two forms, violaxanthin and zeaxanthin, dissipates excess energy formed by both photosystems I and II (Demmig-Adams and Adams, 1993).

### Proline

Proline is an effective antioxidant (Chen and Dickman, 2005) as well as an important osmolyte. Though proline is most often associated with osmotic adjustment occurring during drought stress, it is also a potent ROS scavenger (Ashraf and Foolad, 2007; Trovato *et al.*, 2008). Proline is especially important in quenching ROS produced by drought, salt or heavy metal stress.

### Flavonoids

Flavonoids are a group of polyphenolic compounds found especially in leaves and flowers which have

a variety of functions in plant metabolism, including functioning as powerful antioxidants. Their ring structure makes them particularly effective in neutralizing ROS. Flavonoids are also important in signaling resistance to pathogens and may also act as feeding deterrents (Gould and Lister, 2006).

### Herbicides and ROS

Several of the herbicides widely used in agriculture function via the production of ROS. Some plants have developed resistance to these herbicides by increasing their ability to scavenge for ROS.

Paraquat and diquat, both bipyridylium (viologen) herbicides, are non-selective herbicides applied to leaves inducing rapid wilting and leaf desiccation followed by necrosis within 24 h (Calderbank, 1968). Both herbicides require light and chlorophyll to induce injury. The first cellular signs of injury include chloroplast swelling, quickly followed by tonoplast and plasmalemma breakdown.

Paraquat's toxicity relies on its ability to very effectively generate ROS. When an electron is donated to the paraquat<sup>2+</sup> molecule (the original bipyridylium divalent cation), the free radical paraquat<sup>1+</sup> is formed. An electron is transferred from paraquat<sup>1+</sup> to oxygen, regenerating paraquat<sup>2+</sup> and a superoxide radical. Further reactions convert the superoxide radical to a hydroxyl radical.

The electron donated to the paraquat<sup>2+</sup> molecule comes from the primary electron acceptor in PSI, ferredoxin. Since other reducing agents are also effective electron contributors to paraquat<sup>2+</sup>, all other living organisms subject to ROS damage are also injured by paraquat, and this includes humans. Resistance to paraquat has been generated by a number of weed species including perennial ryegrass (*Lolium perenne*). The resistance is the result of increased ROS-scavenging enzymes (Harper and Harvey, 1978; Fuerst and Vaughn, 1990).

Some herbicides, the p-nitrodiphenyl ethers and aminolevulinic acid-based modulators, work by causing the accumulation of intermediates involved in chlorophyll production called tetrapyrroles. Light energy is absorbed by the tetrapyrroles which is then used to create toxic singlet oxygen. Since chlorophyll production is inhibited, the leaves become bleached. Wilting, desiccation, and finally necrosis soon follow.

## Cellular Activity of ROS

The reactions of ROS with cellular components are complex. Consideration must be given to: (i) the cellular component in question; (ii) its location within the cell; (iii) the ROS interacting with it; (iv) electrical charges; (v) membranes; (vi) macromolecular binding; and (vii) compartmentalization of enzymes, substrates, and catalysts. While the effects of ROS at the cellular level are complex, examining their general effects on lipids and proteins emphasizes their potential impact on plants.

### Lipid peroxidation

Lipids are an integral part of cellular membranes and are particularly important for cellular compartmentalization and integrity (Frankel, 1985). Oxidation of lipids has been extensively studied since they are extremely important in the development of rancid and undesirable flavors in food products.

The oxidation of lipids, more appropriately called lipid peroxidation, is the process in which free radicals 'steal' electrons from fatty acids within the lipid molecules causing damage. Polyunsaturated fatty acids are most vulnerable because they have many double bonds with intervening methylene ( $-\text{CH}_2-$ ) groups with especially reactive hydrogens. The process is a chain reaction event with an initiation, propagation, and termination.

Lipid peroxidation is initiated when a ROS combines with a hydrogen atom from a fatty acid to produce water and a fatty acid radical. This fatty acid radical is not a very stable molecule, so it reacts quickly with molecular oxygen to create a peroxy-fatty acid radical, which is also unstable. This reacts with another fatty acid to produce a new fatty acid radical and a lipid peroxide or a cyclic peroxide if it reacted with itself. The cycle continues with a chain reaction effect since a radical is always produced when a radical reacts with a non-radical. The chain reaction stops only when two radicals react to produce a non-radical, which only occurs when the concentration of radicals is extremely high. Termination may also occur if the radicals are caught by antioxidants such as vitamins E or C or the enzymes SOD, CAT, and peroxidase.

The damage to the membrane is a reduction in integrity and function, with cell death often following. In addition, toxic end-products can be mutagenic and carcinogenic.

## Protein oxidation

Oxidation of proteins results in changes in protein structure, which changes function. Fragmentation of the peptide chain, altered electrical properties, and increased susceptibility to proteolysis may also occur (Davies, 1987). Amino acids differ in their susceptibility to oxidation and the ROS differ in their effects on specific amino acids. Most changes in a protein due to oxidation are not reversible. DNA is particularly sensitive to oxidation since any change in the molecule can lead to enormous dysfunction. Deletions, mutations and other lethal changes in the DNA molecule occur because of oxidation. Both the sugar and the base portions of the molecule are susceptible to oxidation (Imlay and Linn, 1986).

## Air Pollution

Air pollution exists in many forms, all with potential impacts on plant growth and productivity. Most air pollutants are products of human activity including, but not limited to: (i) manufacturing; (ii) use of the internal combustion engine; and (iii) burning of organic materials including wood, coal and oil (Zeiger, 2010). Some pollutants resulting from human activity include  $\text{CO}_2$ , CO,  $\text{H}_2\text{S}$ , HF,  $\text{SO}_2$ , NO,  $\text{NO}_2^-$ ,  $\text{C}_2\text{H}_4$ , and particulates. Complex chemical reactions in the atmosphere between sunlight and certain of these substances lead to the production of ozone, peroxyacetyl nitrate (PAN) and  $\text{H}_2\text{O}_2$ . Exposure to any of these substances can negatively impact plant growth and development (Zeiger, 2010; Gheorghe and Ion, 2011) and the plant responses to these pollutants may be acute or chronic.

While over 3000 substances have been identified as air pollutants (Gheorghe and Ion, 2011) a more limited number have been confirmed to affect plant growth. Pollutants can have a direct, toxic effect on plants or they may have an indirect effect by altering soil pH and heavy metal availability in the soil (Gheorghe and Ion, 2011).

To understand the impact of these pollutants on plant growth and development, long-term studies are needed. Short-term studies subjecting plants to high levels of specific pollutants for short periods of times can produce abnormal results that would not reflect long-term exposure to lower levels of pollutants. Metabolism is altered long before visible symptoms appear. For example, photosynthesis

is inhibited with as little as 0.1 ml NO<sub>x</sub>/l while visible necrosis is not visible until levels reach 5 ml/l (Zeiger, 2010). When other pollutants are added to the mix, the threshold for metabolic disorder is even lower. Furthermore, weakened tissues are more susceptible to injury from other sources such as drought, heat or biotic pests.

### Particulates

While often not being directly toxic to plants, particulate pollutants such as dust generally reduce photosynthesis by reducing light interception by leaves and clogging stomata, lowering their conductance to CO<sub>2</sub> (Zeiger, 2010).

There are many forms of particulates that are considered potentially harmful to plants (Gheorghe and Ion, 2011) and many of them elicit their effects only on plants close to the point source of the pollutant. Dust from cement factories contains large amounts of calcium oxide which is alkaline so when it is dissolved in atmospheric moisture it can have a pH as high as 12 (Gheorghe and Ion, 2011). Other pollutant dusts include lime, gypsum, soot, magnesium oxide, boric acid, potassium, calcium and sodium chlorides, and sodium sulfate.

Pubescent leaves tend to trap more dust particles than leaves that are glabrous. In either type of leaf, dust particles may be quite abrasive and may erode a leaf's cuticle. In addition, when dust that has been deposited on a leaf's surface becomes wet and then dries, a crust may form which is difficult to remove.

### SO<sub>2</sub> and NO<sub>x</sub>

Both SO<sub>2</sub> and NO<sub>x</sub> (x = 1, 2 or 3, depending on specific situation) react with water to form acid rain. 'Pure' rain is slightly acidic with a pH of about 5.6, due to carbonic acid (H<sub>2</sub>CO<sub>3</sub>) resulting from dissolved CO<sub>2</sub>. NO<sub>x</sub> and SO<sub>2</sub> dissolved in rain decreases its pH to between 3 and 4. This acidity can remove minerals from plant tissues and affect soil pH which must be corrected with the addition of a buffering agent, usually limestone, in areas where the soil is low in calcium carbonate.

Both SO<sub>2</sub> and NO<sub>x</sub> are absorbed via the same route as CO<sub>2</sub> and when dissolved in water inside the leaf, may form NO<sub>2</sub><sup>-</sup>, which at high levels is toxic. Absorption of SO<sub>2</sub> can cause stomatal closure which reduces photosynthesis. In the cell, SO<sub>2</sub> dissolves to form bisulfite and sulfite ions.

Sulfite can be toxic at high concentrations. It can also be metabolized to sulfate, providing a source of sulfur to the plant.

Leaves exposed to SO<sub>2</sub> may exhibit irregular white, red, brown or black spots (Gheorghe and Ion, 2011). In general, younger leaves are more sensitive than older leaves to damage and seedlings are more vulnerable than older plants. If SO<sub>2</sub> concentrations are high enough, the thylakoid membrane in chloroplasts begins to swell and electron transport is interrupted. This ultimately leads to reduced photosynthesis. In addition, respiration increases with exposure. SO<sub>2</sub> can also affect protein and membrane structure. Visible injury symptoms can be acute or chronic. Acute symptoms appear as lesions on both sides of the leaves which start out as interveinal or marginal water-soaked regions which quickly turn white, tan or reddish brown depending on the time of year and species. Chronic injury symptoms are a general chlorosis and occasional bronzing on the underside of the leaves.

Some species particularly sensitive to SO<sub>2</sub> include alfalfa (*Medicago sativa*), barley (*Hordeum vulgare*), buckwheat (*Fagopyrum esculentum*), clover (*Trifolium* spp.), oats (*Avena sativa*), pumpkin (*Cucurbita* spp.), radish (*Raphanus sativus*), rhubarb (*Rheum rhabarbarum*), spinach (*Spinacia oleracea*), squash (*Cucurbita* spp.), Swiss chard (*Beta vulgaris*), and tobacco (*Nicotiana tabacum*). Species more resistant to SO<sub>2</sub> injury include asparagus (*Asparagus officinalis*), cabbage (*Brassica oleracea* Capitata Group), celery (*Apium graveolens*), corn (*Z. mays*), onion (*Allium cepa*), and potato (*Solanum tuberosum*).

Nitrogen dioxide (NO<sub>2</sub>) is a gaseous air pollutant which mainly affects younger plants and younger tissue, especially leaves. NO<sub>2</sub> induces the formation of crystalline structures in the stroma of the chloroplast which leads to swelling of the thylakoid membrane and a reduction in photosynthesis. Chlorosis and tip burn of leaves or needles are common symptoms.

### Ozone

Smog produced through complex interactions of sunlight with some of the aforementioned chemicals contains the well-known pollutants ozone (O<sub>3</sub>) and PAN. These pollutants can injure both plants and animals. One of the most noxious and phytotoxic pollutants is ozone (Heagle, 1989). It is a

highly reactive, irritating gas which can directly bind to membranes and interfere with metabolism. It also reacts with oxygen to produce ROS, which then impart their dysfunctions.

So how big of a problem is ozone pollution? Initially, the impact on crops and productivity was thought to be limited to areas near sources of the pollutant, such as power plants, urban centers, and industrial sites (Fiscus *et al.*, 2005). However, as industrialization continues, populations increase and automobile contributions to the ozone problem steadily increase, the problem has taken on a global scale (Krupa *et al.*, 1995). The average tropospheric ozone level worldwide is approximately 50 nmol/mol (the year 2000; Fiscus *et al.*, 2005), 25% above the level established as harmful to sensitive species (Fuhrer *et al.*, 1997). A huge problem is that many ozone precursors are emitted in automobile exhaust.

Ozone enters a leaf through stomata and then dissolves in the aqueous layer surrounding the cells producing hydroxyl and peroxy radicals along with superoxide, albeit very slowly (Heath, 1987). Additionally, ozone may react directly with membrane lipids, amino acid in membrane proteins or enzymes encountered in the cell wall (Fiscus *et al.*, 2005).

Products of these early ozone reactions are probably the messengers leading to the plant's response to ozone exposure. These initial messengers probably interact with other plant defense messengers such as ethylene, salicylic and jasmonic acid. Ethylene seems to promote ozone injury while jasmonate seems to help minimize injury from ozone (Fiscus *et al.*, 2005).

After the initial reactions with ozone in the apoplast, further oxidation occurs in the cytoplasm and subcellular components ultimately resulting in visible injury seen as lesions. If exposure to ozone is high, lesion formation is acute, with lesions becoming visible within hours of exposure. At lower doses, lesion formation occurs more slowly over a period of days or even weeks.

Ozone interferes with normal photosynthesis by interfering with electron transport, guard cell function and by reducing ribulose biphosphate carboxylase oxygenase (RuBisCO), levels and activity (Miller, 1987). Transport of photosynthates to sinks from the source leaf may also be impaired by ozone, further reducing photosynthesis via inhibitory feedback mechanisms. Ozone exposure generally has a more deleterious effect on yield when the

exposure occurs during the reproductive period compared with the reduction seen with exposure during vegetative growth.

While a general overall reduction in productivity is observed with exposure to ozone, the specific amount of reduction varies considerably among species, cultivars within species, length and level of exposure, environmental conditions during exposure and concomitant exposure to other pollutants. Specific environmental factors affecting the response to ozone include: (i) leaf temperature; (ii) water vapor pressure deficit between the leaf and air; (iii) photosynthetic photon flux density (PPFD); (iv) soil water availability; and (v) atmospheric CO<sub>2</sub> concentration.

The same mechanisms for dealing with oxidative stress are involved in ozone tolerance. In particular, ascorbic acid synthesized in the cytoplasm and transported to the apoplast seems to be quite important in detoxifying ozone and related ROS.

## PAN

PAN (peroxyacetylnitrate, C<sub>2</sub>H<sub>3</sub>NO<sub>5</sub>) is a photochemically produced secondary pollutant. It is a major component of smog and may cause injury at levels as low as 1 ppm. Symptoms on sensitive species appear as silvering or bronzing of lower leaves (Thomson *et al.*, 1965). As a toxin, PAN interferes with photosynthesis and cell wall metabolism.

## Fluorides

Fluorides are toxic pollutants produced in the manufacturing of brickwork, aluminum, glass, steel, ceramics, and phosphate fertilizer plants. Fluorides may be in the gaseous form (HF, SiF<sub>6</sub>, CF<sub>4</sub>, F<sub>2</sub>) or occur as particulates (Ca<sub>3</sub>AlF<sub>6</sub>, cryolite), CaF<sub>2</sub>, NH<sub>3</sub>F, AlF<sub>3</sub>, CaSiF<sub>6</sub>, NaF and Na<sub>2</sub>SiF<sub>6</sub>. Aerosols of NaF, NaAlF<sub>6</sub> and AlF<sub>3</sub> may also form (Gheorghe and Ion, 2011). The most injurious form of fluoride is HF. Particulate fluorides fall on plants and gaseous fluorides are absorbed by plants causing injury to both plants and animals. Cattle are particularly sensitive to ingested fluoride from particulate pollution of forage crops (Shupe, 1969).

Plants are sensitive to levels as low as 0.1 ppm HF, the main source of plant injury. If the source of fluorine is eliminated, many plants will recover from injury. Fluorides combine with the metallic component of enzymes and inhibit their functions. General cellular metabolism is impaired, and in

particular, photosynthesis, respiration, nucleic acid synthesis and energy production are affected. In leaves exposed to HF, ribosomes detach from the endoplasmic reticulum, the number of ribosomes decrease and mitochondria swell. Ultimately tissue necrosis occurs, particularly in leaf margins. Species particularly susceptible to fluoride injury include apricot (*Prunus armeniaca*), blueberry (*Vaccinium* spp.), peach (*Prunus persica*), gladiolus (*Gladiolus communis*), grape (*Vitis* spp.), plum (*Prunus* spp.), sweet corn (*Zea mays*) and tulip (*Tulipa* spp.). Resistant species include alfalfa (*M. sativa*), asparagus (*A. officinalis*), bean (*Phaseolus* spp.), cabbage (*B. oleracea* Capitata Group), carrot (*Daucus carota*), cauliflower (*Brassica oleracea* Botrytis Group), celery (*A. graveolens*), cucumber (*Cucumis sativus*), eggplant (*Solanum melongena*), pea (*Pisum sativum*), pear (*Pyrus* spp.), pepper (*Capsicum* spp.), potato (*S. tuberosum*), squash (*Cucurbita* spp.), tobacco (*N. tabacum*) and wheat (*Triticum aestivum*).

### Chlorine

Chlorine is a toxic gas that may cause plant injury. Injury is often limited to the area immediately surrounding the site of discharge. The two main sources of chlorine pollution are: (i) chlorine gas ( $\text{Cl}_2$ ); and (ii) hydrogen chloride (HCl), which quickly forms aerosols of hydrochloric acid (Gheorghe and Ion, 2011).

Older plants are generally more sensitive to injury from  $\text{Cl}_2$  than younger plants but tissue age within a plant does not matter. Symptoms of injury are quite variable but often include tip burn of leaves or needles. Many species can recover from chlorine injury if the source of chlorine is eliminated.

### Other atmospheric pollutants

Some other pollutants which may cause problems in limited situations include ammonia, volatile organic compounds, ethylene, methane, chlorofluorocarbons, hydrogen sulfide, bromine gas, carbon monoxide, iodine, and mercury vapor.

Ethylene is also a plant hormone, thus exposure to ethylene can cause a wide range of changes in plant metabolism. In general, plant growth is severely stunted with exposure to excessive amounts of ethylene. Leaf and flower abscission can also occur. Epinasty is also a common symptom of ethylene exposure.

## Soil-related Stress

Soil is a complex mixture of biotic and abiotic components. The productivity or lack thereof of a given soil is often related to the nutrients available to plants for growth. Both the quantity and the quality or form of nutrients is important to consider. While a number of chemical attributes of a soil are important for helping determine their productivity, one of the most important attributes of a soil directly affecting naturally occurring or producer-applied nutrient availability is pH.

### Soil pH

Soil pH is a key factor in determining the form, solubility, and availability of a nutrient in a soil and any problems that may be associated with its deficiency or excess. Soil pH is a measure of the acidity or alkalinity of a soil solution (the solution which results when a soil is wet) and may range from 0 to 14, 0 being acidic and 14 being basic. Low pH is associated with a high concentration of  $\text{H}^+$  ions and low concentration of  $\text{OH}^-$  ions in the soil solution. A high pH is just the opposite. The concentration of  $\text{H}^+$  ions helps determine the availability to the plant of nutrients in the soil if present. It is also associated with the overabundance of some nutrients which may be toxic (e.g. aluminum). Most soil-derived nutrients are readily available for plant use at a soil pH of between 5.5 and 6.5.

Soil pH changes over time. Soils will tend to become more acidic as rainwater, which itself is acidic, leaches basic ions such as calcium, magnesium, potassium and sodium out of the soil. Carbon dioxide from decomposing organic matter and root respiration can dissolve in soil water to form a weak acid. Stronger acids such as nitric and sulfuric can form from decaying organic matter or from oxidation of ammonium and sulfur fertilizers. To reverse the pH-lowering influence of these acids, lime is usually added to acidic soils on a regular basis. In addition to raising the soil pH, lime also provides calcium and magnesium. In certain situations, such as extremely arid environments, soil pH can be lowered by applying agricultural sulfur.

### Essential plant nutrients

All plants require certain elements to complete their life cycle. An essential element is required for most plants to complete their life cycle and no



other element can substitute for it. The element must also be either directly incorporated into a plant metabolite or be essential for enzyme function. Some plants utilize other elements, namely sodium, cobalt, vanadium, and silicon. They are not included on the list of essential elements because other elements can substitute for them if they are deficient or they are only used by some but not all plants.

There are 16 elements that have been identified as essential for plant growth: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, zinc, copper, boron, molybdenum, and chlorine (Table 6.2). Carbon, hydrogen and oxygen are derived from  $\text{CO}_2$  and  $\text{H}_2\text{O}$  and are not considered mineral elements. All of the other elements are supplied in the soil solution. Six of them (N, P, K, Ca, Mg, and S) are required in relatively large quantities and are therefore called macronutrients. The other seven (Fe, Zn, Mn, Cu, B, Mo, and Cl) are required in much lower amounts and are therefore called micro or minor nutrients (Hodges, 2003).

It is often helpful to remember the essential nutrients for their general, main function in plant physiology: (i) structure; (ii) energy metabolism; (iii) charge balance; (iv) enzyme activation; and (v) electron transport. The three structural elements are carbon, hydrogen, and oxygen. The nutrients involved in energy metabolism include nitrogen, sulfur, and phosphorus. Charge balance in a plant is maintained by potassium, calcium, and magnesium. Finally, enzyme activators and electron transporters include iron, manganese, zinc, copper, boron, molybdenum, and chlorine.

Nutrients are either mobile or immobile in both the soil and the plant. In the soil,  $\text{NO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{BO}_3^{2-}$ ,  $\text{Cl}^-$ , and  $\text{Mn}^{2+}$  are extremely soluble in water and are very mobile. Other soluble nutrients may be adsorbed to soil particles and are therefore considered less mobile. These nutrients include  $\text{NH}_4^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ , and  $\text{Cu}^{2+}$ . The other nutrients are either only slightly soluble or adhere tightly to soil particles. They include  $\text{H}_2\text{PO}_4^-$ ,  $\text{HPO}_4^{2-}$ , and  $\text{Zn}^{2+}$ .

Nutrient mobility in plants is important as it provides a hint as to where you should look for deficiency symptoms first, older or younger leaves. Nitrogen, phosphorus, and potassium are highly mobile and zinc is fairly mobile, thus symptoms will appear on older leaves first. Sulfur, iron, manganese, copper, molybdenum, and chlorine are less mobile and symptoms generally appear on older

leaves first. Calcium and boron are immobile and symptoms always appear on younger tissues first.

It is important to understand each nutrient's general function in plant physiology to understand why we pay so much attention to fertility when trying to optimize growing conditions for our crops. The following discussion provides a brief yet complete outline of the function of the major and minor plant nutrients as well as a general description of the symptoms of their deficiencies. Excesses are much less common, but certain common situations will be discussed. Toxic nutrients, primarily metals, will be covered later in this chapter.

When one or more essential nutrients are lacking, a mineral deficiency develops. While symptoms of the deficiency of a single element may be relatively easy to identify in many species, multiple deficiencies developing at once can be very difficult to identify. Additionally, symptoms of stress from other sources, including excesses of some nutrient(s), may confound the issue making identification of the problem even more difficult. A deficiency in one or more nutrients nearly always results in reduced yield, product quality, and sometimes plant death.

Identification of nutrient deficiencies (and excesses) is important for good horticultural management (Table 6.3). Remember that deficiencies of different nutrients often have the same symptoms, and plants are often under a continually changing environment which may mask or exacerbate problems.

### **Carbon (C)**

Carbon is one of the most plentiful elements in living organisms. The source of carbon for plant growth and development is  $\text{CO}_2$ . Carbon has four valence electrons allowing it to bond to as many as four other atoms. Carbon can donate or receive electrons in bonding, giving it both acidic and basic qualities. The valence electrons are oriented in such a way as to allow complex three-dimensional carbon-to-carbon bonding and complex molecular formations. Linear and ring molecules are possible. The bonds between two carbon atoms or a carbon and a hydrogen atom are high energy bonds. Thus complex molecules with carbon in them (e.g. carbohydrates) can store a tremendous amount of energy in them. This energy can be released in respiration.

**Table 6.2.** Essential plant nutrients, characteristics, and functions in plants.

Nutrient	Main absorbed form	Mobility	Major properties/functions
Carbon	CO <sub>2</sub>	–	Can donate or receive electrons in bonding; complex three-dimensional carbon to carbon bonding with complex molecular formations; linear and ring molecules possible; bonds between two carbon atoms or a carbon and a hydrogen atom are high energy bonds; complex molecules with carbon in them can store a tremendous amount of energy
Hydrogen	H <sub>2</sub> O	–	Energy transduction; ion movement; pH regulation
Oxygen	CO <sub>2</sub>	–	Powerful oxidizing agent; combines with many other elements; creates polar molecules, hydrogen bonds and acidic groups; important in chelate formation
Nitrogen	NO <sub>3</sub> <sup>–</sup> ; NH <sub>4</sub> <sup>+</sup>	Mobile	In ring molecules, acts as the center for redox reactions and the amine nitrogen is important in metal binding; can act as an electron donor; peptide bonds in proteins permits helix formation in nucleic acids; adds asymmetry to molecules allowing for important chemical properties such as basicity, charge, chemical reactivity and structure; major component of proteins (enzymes); part of the chlorophyll molecule
Phosphorus	H <sub>2</sub> PO <sub>4</sub> <sup>2–</sup>	Mobile	Integral part of proteins and lipids; important in energy metabolism (ATP); important for stress tolerance
Potassium	K <sup>+</sup>	Mobile	Catalyst and an enzyme activator for many reactions in protein and carbohydrate metabolism; important in stomatal function and water relations; improves disease resistance and quality of fruits and vegetables
Calcium	Ca <sup>2+</sup>	Immobile	Important component of cell walls, linking with pectins to enhance rigidity; important in membrane integrity; important signaling molecule
Magnesium	Mg <sup>2+</sup>	Mobile	Central atom in the chlorophyll molecule; enzyme activator especially those involved in ATP metabolism, nucleic acid synthesis and carbon fixation; important in membrane structure, especially organelle membranes
Sulfur	Mostly SO <sub>4</sub> <sup>2–</sup> from the soil; some SO <sub>2</sub> through the leaves	Slightly mobile	Major constituent of cysteine, cystine and methionine (amino acids); important in electron transfer reactions; sulfhydryl groups (SH) often the reactive sites of enzymes or coenzymes; important in protein conformation; important component of odor and flavor compounds in <i>Allium</i> and <i>Brassica</i>
Iron	Fe <sup>2+</sup> ; Fe <sup>3+</sup>	Immobile	Important catalyst in the formation of chlorophyll; important in oxidation–reduction reactions; location for electron transfer in many enzymes; important for electron transport in photosystem I of photosynthesis
Manganese	Mn <sup>2+</sup> ; Mn <sup>3+</sup>	Immobile	Enzyme activator; important in chlorophyll synthesis; a major function of manganese is the removal of electrons from water during photosynthesis; important in respiration and nitrogen metabolism; important in lignin production
Zinc	Zn <sup>3+</sup>	Immobile	Primarily an enzyme activator, acting as a cofactor for more than 200 enzymes
Copper	Cu <sup>2+</sup>	Immobile	Important for its oxidation–reduction properties; catalyst in chlorophyll formation; important part of several enzymes
Boron	H <sub>3</sub> BO <sub>3</sub>	Immobile	Important in pollen tube germination and sugar translocation across membranes
Molybdenum	MoO <sub>4</sub> <sup>2–</sup>	Mobile	Important as an electron carrier in nitrogen reduction from NO <sub>3</sub> <sup>–</sup> to NH <sub>4</sub> <sup>+</sup> ; required by <i>Rhizobium</i> for nitrogen fixation in legumes
Chlorine	Cl <sup>–</sup>	Mobile	Important in photosynthesis; may be important for maintaining an electrical equilibrium in the plant

**Table 6.3.** Quick general nutrient deficiency identification based on general symptoms for key nutrients that may often be deficient. Those nutrients that in general are not usually deficient are not included.

Worst symptoms on older or younger leaves?	General symptoms	Likely deficient nutrient
Older leaves	All leaves light green; older leaves uniformly chlorotic, some may be brown or dead; stems with greatly reduced terminal growth	Nitrogen
Older leaves	Most leaves dark green; stunted growth overall; abnormal red and purple leaves and stems; spindly growth	Phosphorus
Older leaves	Older leaves have interveinal chlorosis beginning at tips; margins may cup upwards; some leaves may be whitish	Magnesium
Older leaves	Leaves mottled; necrotic leaf tips and margins; crinkled leaves; abnormally short internodes; weak stems with brown streaking	Potassium
Newer leaves	Dead or burnt looking, deformed terminal buds	Calcium
Newer leaves	Light green leaves that are never whitish or chlorotic; veins lighter than interveinal tissue	Sulfur
Newer leaves	Leaves chlorotic with green veins	Iron

When carbon is deficient or limited in availability (such as in a closed greenhouse in the winter), growth is greatly reduced. Under normal growing conditions, carbon supply to the plant is limited only by stomatal closure.

### Hydrogen (H)

Hydrogen is powerful reducing agent (accepting electrons). It is the most plentiful element in the universe as far as we know. Free hydrogen is rather rare, but it is just about everywhere combined with other elements. It is supplied to the plant as water ( $H_2O$ ) and is split during photosynthesis to provide oxygen and the powerful reducing agent, the hydrogen ion ( $H^+$ ). The hydrogen cation is extremely important in plants for energy transduction, ion movement, and pH regulation.

Hydrogen is never deficient, thus there are no symptoms for hydrogen deficiency.

### Oxygen (O)

Oxygen is a powerful oxidizing (giving up electrons) agent. The most common form of oxygen is a gas,  $O_2$ . Other forms exist as ROS (as previously discussed in this chapter). Oxygen also combines with many other elements in both living and non-living things.

When oxygen forms covalent bonds with other atoms, it tends to draw electrons towards itself, creating polar molecules (molecules that have charged ends), hydrogen bonds, and acidic groups.

Oxygen is also important in chelate formation as it shares electrons with metals, keeping them held to the chelating molecule.

Nearly all of the oxygen in plants is derived from  $CO_2$  during photosynthesis.

If oxygen is deficient (as in waterlogged soils) aerobic respiration is curtailed, often resulting in anaerobic respiration and the production of toxic metabolites.

### Nitrogen (N)

After carbon, hydrogen and oxygen, nitrogen is the nutrient used in the largest quantities of any of the remaining essential nutrients. It is also usually the most deficient nutrient, especially in non-leguminous species. It is taken up by plants as nitrate ( $NO_3^-$ ) or ammonium ( $NH_4^+$ ).

It exists in nature primarily as  $N_2$ . Nitrogen has five electrons in its outer shell: three are valence electrons with two that are unshared, which makes positively charged ions possible. In ring molecules, nitrogen acts as the center for redox reactions and the amine nitrogen is important in metal binding, such as the iron in cytochrome or the magnesium in chlorophyll. In many reactions, nitrogen can act as an electron donor.

Peptide bonds in proteins are limited in how they can rotate which permits helix formation in nucleic acids. In addition, nitrogen adds asymmetry to molecules allowing for important chemical properties such as basicity, charge, chemical reactivity, and structure.

Most of the nitrogen found in plants is in the fully reduced state, while most of the nitrogen taken up from the soil by plants is in the fully oxidized state ( $\text{NO}_3^-$ ). Nitrate must be reduced after being taken up by the plant before it can be used in metabolism.

Nitrogen is a major component of proteins. Enzymes are proteins and enzymes run the show in biology. Nitrogen is also part of the chlorophyll molecule.

Nitrogen is a mobile nutrient, meaning that it can move in a plant after its initial fixation into a metabolic component. Since it is mobile, deficiency symptoms appear on older tissues first. Nitrogen deficient plants normally have uniformly yellowing older leaves and sometimes the veins and petioles take on a reddish hue. Under an extreme deficiency, leaves may turn nearly white. Even though younger leaves remain green due to nitrogen translocated from the older leaves, they become progressively smaller in size. Branching is greatly reduced and plants become rather spindly. Older leaves become more sensitive to drought stress and senesce quickly.

The “nice” thing about nitrogen deficiency is that it is easily and quickly reversed by adding nitrogen. Recovery occurs within days, and given enough time, most plants can completely recover.

Excess nitrogen can cause excessive vegetative growth at the expense of sexual reproduction. Thus if fruit is the desired commodity, yields are greatly reduced and maturity is delayed. Excessive vegetative growth can also lead to lodging. In addition, succulent growth resulting from excess nitrogen may also be more susceptible to pest attack.

### **Phosphorus (P)**

Phosphorus is taken up by the plant as the phosphate anion,  $\text{H}_2\text{PO}_4^{2-}$ . It is an integral part of proteins and lipids and is important in energy metabolism (ATP). Phosphorus is important for good root development and stress tolerance. Phosphorus is mobile, therefore the deficiency symptoms are observed first in older leaves.

Phosphorus deficient plants are often severely stunted but dark green, often mistaken for unstressed, younger plants. Some species develop a pronounced purplish color on the stems, petioles and underside of the leaves. With severe deficiency, the leaves often look bluish gray. Older leaves may also develop brown, netted veining. Seed formation may also be inhibited.

### **Potassium (K)**

Next to nitrogen, potassium is the nutrient in the greatest quantity by plants. Plants take up potassium as  $\text{K}^+$  and some plants may take up more than they really need and this is called ‘luxury consumption’. Potassium is dissolved in the cytoplasm and apoplast and is not part of any structural component in the plant.

Potassium is necessary in plants as a catalyst and an enzyme activator for many reactions in protein and carbohydrate metabolism. Potassium is also very important in stomatal function and water relations of plants. Potassium often improves disease resistance and can also improve the quality of fruits and vegetables.

Potassium is a mobile nutrient, thus deficiency symptoms appear on older leaves. Symptoms include a general interveinal chlorosis which progresses from the tip and margins towards the central vein of recently matured leaves. Necrosis may also occur. Veins normally remain green and leaves may appear crinkled and curled. There is normally a sharp delineation among green, yellow, and necrotic tissues. In some plants, the first symptoms are white speckles on leaf blades. Other symptoms of potassium deficiency include lodging in grains and accumulation of excess, non-protein nitrogen in forage crops.

The chlorosis caused by potassium deficiency cannot be reversed as with nitrogen deficiency.

### **Calcium (Ca)**

Calcium is often the most abundant cation in plants and is taken up by the plant as  $\text{Ca}^{2+}$ . Calcium is an important component of cell walls, linking with pectins to enhance rigidity. Calcium is also important in membrane integrity and calcium levels in the cytoplasm often change as hormonal signals induce developmental changes in cells. Thus calcium is an important signaling molecule or secondary messenger in plant development.

Since lime is often regularly applied to soils that might exhibit a calcium deficiency (i.e. soils with a low pH), field deficiencies are usually rare. Symptoms of calcium deficiency often do not become apparent since low soil pH which normally induces calcium deficiency usually induces other problems which would limit plant growth before calcium could reach deficient levels. Greenhouse and hydroponic culture is a different story. Calcium

deficiencies often occur in these situations, so care must be taken to monitor plants closely.

Calcium is immobile therefore deficiency symptoms appear in young tissue. A classic symptom of calcium deficiency is the failure of terminal buds to develop properly as meristems die. Margins of leaves also become necrotic as a result of calcium deficiency. Other classic calcium deficiencies include: (i) cat-facing and blossom end rot in tomatoes (*Solanum lycopersicum*); (ii) bitter pit and cork spot in apples (*Malus domestica*); (iii) black heart of celery (*A. graveolens*); (iv) leaf tip burn in cabbage (*B. oleracea Capitata* Group) and lettuce (*Lactuca sativa*); and (v) cavity spot of carrots (*D. carota*).

Many of these calcium deficiency problems are due to poor translocation of calcium to developing tissues rather than a low supply of calcium from the soil. In blossom end rot of tomatoes (*S. lycopersicum*), for example, the incidence of the malady is governed primarily by the supply of water, and therefore calcium, to the young developing fruit. If rapidly growing young fruit are suddenly subjected to a water deficit, the distal end of the fruit will become soft and rotten looking. This appearance is due to a lack of calcium pectate solidifying cell walls. An excess of water can also reduce the translocation of calcium and induce symptoms.

### **Magnesium (Mg)**

Magnesium is taken up by plants as  $Mg^{2+}$ . One major function of magnesium plants is that it is the central atom in the chlorophyll molecule, thus crucial in photosynthesis. Magnesium is also important as an enzyme activator, particularly in enzymes involved in ATP metabolism, nucleic acid synthesis, and carbon fixation. Magnesium is also important in membrane structure, especially organelle membranes.

Magnesium is mobile therefore deficiency symptoms appear on older leaves first. It is expressed as interveinal chlorosis with some puckering of the leaf blade. In grasses, whitish or yellow striping of leaves may occur. Magnesium and nitrogen deficiencies may appear similar; however, veins ultimately become chlorotic in nitrogen deficiency but not with magnesium deficiency.

### **Sulfur (S)**

Sulfur may be absorbed as sulfur dioxide ( $SO_2$ ) through the leaves, but most sulfur in plants is absorbed as sulfate ( $SO_4^{2-}$ ) from the soil. Sulfur is a

major constituent of three (cysteine, cystine, and methionine) of the 21 amino acids that form proteins in plants. Sulfur is important in electron transfer reactions and sulfhydryl groups (SH) are often the reactive sites of enzymes or coenzymes, as they are important in protein conformation. Sulfur is also an important component of odor and flavor compounds in various *Allium* and *Brassica* species.

Sulfur is slightly mobile with deficiency symptoms sometimes on older leaves but normally appearing on younger leaves. Plants appear uniformly chlorotic, weak and spindly. Sulfur deficient leaves are often narrow with their veins exhibiting more chlorosis than the lamina. Veins and petioles often show a distinct red color. Sulfur and nitrogen deficiency appear similar, however, sulfur deficiency is widespread over the entire plant while nitrogen deficiency is usually confined to older leaves. In addition, the red color often seen on the underside of leaves and on petioles is pinkish and less vivid than that which might be seen in nitrogen deficient leaves. Sulfur deficient leaves ultimately form necrotic spots near the petiole, become more erect, twisted and brittle.

### **Iron (Fe)**

Iron is a divalent or trivalent cation absorbed via the roots or foliarly. It is translocated in plants as a chelate since it precipitates readily. It is an important catalyst in the formation of chlorophyll. It is also important in oxidation–reduction reactions, the relatively easy transformation from  $Fe^{2+}$  to  $Fe^{3+}$  and vice versa. With this property, iron is the location for electron transfer in many enzymes. Iron is important for electron transport in photosystem I of photosynthesis.

Most iron deficiency occurs on high pH soils since iron is readily available to plants at a low pH. It is immobile, thus deficiency symptoms are first observed on younger tissue. Extensive interveinal chlorosis occurs, with veins remaining green. Under severe deficiency, leaves often turn white.

In excessive concentrations, iron is toxic to plants. This is because iron reacts with oxygen in what are called the Fenton reactions, producing toxic oxygen radicals.

### **Zinc (Zn)**

Zinc is taken up from the soil by plants as a trivalent cation,  $Zn^{3+}$ . It can be taken up foliarly as well.

Zinc is primarily an enzyme activator, acting as a cofactor for more than 200 enzymes.

Zinc is immobile and young tissues show the first symptoms of deficiency. Interveneal chlorosis is quickly followed by severe reduction in internode length, giving the plant a rosette appearance. Leaf margins often become puckered. Pitting may develop on the intervenal tissues of the upper surfaces of older leaves. Guttation is also apparent with zinc deficiency.

Zinc toxicity can also occur, especially at low soil pH. Much of the absorbed zinc in a plant is complexed with citric and malic acid in the xylem, thus unavailable to interfere with plant metabolism.

Zinc toxicity symptoms first appear in young tissue as a general chlorosis which may progress to reddening due to anthocyanin production. Toxic levels of zinc induce small, vertically oriented leaves. Growth of the main root is inhibited and lateral roots are fewer and shorter and may exhibit a yellow coloration. Elongation of cells in the stem is inhibited with high zinc levels.

Excess zinc can inhibit both photosystems I and II with a reduction in photosynthesis due to displacement of magnesium by zinc at the water-splitting site in photosystem II. In addition, RuBisCO activity is inhibited. The primary toxic action of zinc is the inhibition of ATP synthesis and therefore energy metabolism in plants. In addition, free radical generation increases along with the activity of enzymes which remove them from the plant.

### **Boron (B)**

Boron is absorbed as  $H_3BO_3$  and can be absorbed through the leaves. With many crops there is a fine line between deficiency, sufficiency, and toxicity with boron. Boron is important in pollen tube germination and sugar translocation across membranes.

Boron is immobile and young tissues show deficiency symptoms before older tissues. Terminal bud growth stops, and shoot growth may appear as a rosette at the terminal ends of shoots.

While boron is often a deficient nutrient in many soils, there are instances when the level of boron is elevated enough to be toxic, particularly in arid or semi-arid regions.

Much of the boron in soils is not readily available for uptake by plants. When in the soil solution, boron is predominantly boric acid ( $B(OH)_3$ ), which

is extremely mobile and is easily leached from the soil, taken up by plants or temporarily adsorbed to soil or organic matter.

Soils high in boron are often derived from: (i) marine evaporites or sedimentation; (ii) high levels of boron in irrigation water; or (iii) from wastes from mining, coal-fueled electricity generation, and chemical discharge from manufacturing. Sodium perborate is also a widely used household and industrial bleaching agent which can contaminate water discharged from waste-water treatment facilities.

Some of the most common symptoms of boron toxicity in plants are chlorotic and necrotic patches at the tips or along the margins of older leaves. However, there are species (*Prunus*, *Malus* and *Pyrus*) where boron is mobile in the phloem and is deposited in sinks (fruit), thus leaf symptoms do not appear. Roots do not normally exhibit symptoms of boron toxicity.

Species that are particularly sensitive to high soil boron levels include avocado (*Persea americana*), apple (*M. domestica*), and green bean (*Phaseolus vulgaris*). Moderately sensitive species include oat (*Avena sativa*), corn (*Zea mays*), and potato (*S. tuberosum*) while tolerant species include carrot (*D. carota*), alfalfa (*M. sativum*), and sugarbeet (*Beta vulgaris*). Other boron-tolerant species include: saltbush (*Atriplex* spp.), milkvetch (*Astragalus*), barley (*Hordeum*), wheat (*Triticum*), Indian mustard (*Brassica juncea*), and tall fescue (*Festuca arundinacea*). The general mechanism for high soil boron tolerance is reduced boron uptake and it is controlled by several additive genes.

### **Manganese (Mn)**

Manganese is absorbed by plants as di- or trivalent cations. It can also be absorbed through the leaves. Manganese is an important enzyme activator and is important in chlorophyll synthesis. A major function of manganese is the removal of electrons from water during photosynthesis. It is also important in respiration and nitrogen metabolism. Manganese is also important for plant defense against fungal infection as it is important in lignin production.

Manganese is immobile and young leaves develop yellow streaks. Deficiencies are rare since soils are generally adequate in manganese and the amount plants require is very low.

Toxicity may occur, especially when soil pH is low or soil levels of manganese have been elevated

by human activity. Manganese is transported through the xylem as a free ion.

Toxicity symptoms first appear as necrosis on leaves, petioles and stems on older tissue. There may be general leaf bronzing accompanied by a shortening of internode length. Younger leaves may appear crinkled and terminal buds may die. Roots may turn brown or crack.

Excess manganese may reduce chlorophyll levels in leaves which could lead to decreased photosynthesis. Loss of stomatal function might also occur. Fewer cells form per leaf, resulting in smaller leaves.

### **Molybdenum (Mo)**

Molybdenum is absorbed as  $\text{MoO}_4^{2-}$ . It is important as an electron carrier in nitrogen reduction from  $\text{NO}_3^-$  to  $\text{NH}_4^+$ . Molybdenum is required by *Rhizobium* for nitrogen fixation in legumes.

Molybdenum deficiency appears as nitrogen deficiency, for that in fact is what molybdenum's deficiency causes. In broccoli (*Brassica oleracea* Italica Group) and cauliflower (*B. oleracea* Botrytis Group) molybdenum deficiency results in a classic whiptail leaves, resulting from lack of lamina and vascular differentiation. Molybdenum can be toxic and is easy to spot since leaves turn bright orange.

### **Copper (Cu)**

Copper is taken up by plants as  $\text{Cu}^{2+}$  and can be absorbed through leaves. It is important for its oxidation–reduction properties. It is a catalyst in chlorophyll formation and is an important part of several enzymes.

Deficiency symptoms are rare, but when they do occur they originate in young tissue since copper is immobile. Leaves are often yellow and stunted and soon develop a bluish-green tint. Petioles curl and leaves turn downwards.

Copper may also be toxic. Organic matter in the soil tends to keep excess copper sequestered from plants. Copper is transported through the plant mostly through the xylem. There, copper is almost 100% sequestered to amino acids making it unavailable for involvement in plant maladies. However, if levels are high enough or soil organic matter is low, excesses can occur.

A common symptom of excess copper is interveinal chlorosis which may take the form of white or cream-colored spots or lesions. Leaf margins and tips can become necrotic and foliage may

turn reddish purple. Radicles of emerging seedlings may be short and have blunt tips with necrosis with a predisposition to fungal attack. Root hair production is also inhibited.

Copper can substitute for magnesium in the chlorophyll molecule, thus in excess copper conditions, chlorophyll content is reduced which results in a reduced photosynthetic capacity. Photosystem II is inhibited, increased breakdown of carotenoids occurs and free radical production increases. With increased free radical activity, leaf senescence is accelerated. Excessive copper also inhibits ATPase activity of the plasma membrane, reduces plasma membrane integrity, and increases potassium leakage from the cell. Both cell division and elongation are reduced.

### **Chlorine (Cl)**

Chlorine is taken up by plants as the chloride ion,  $\text{Cl}^-$ . It is important in photosynthesis and may be important for maintaining an electrical equilibrium in the plant.

Deficiency symptoms are very rare, but may include abnormally shaped leaves with interveinal chlorosis and wilting of young leaves. Older leaves may cup downwards and in severe cases, bronzing of the upper side of mature leaves may appear. Excess can lead to poor storage of potatoes (*S. tuberosum*) and reduced smoking quality of tobacco (*N. tabacum*). Some species (*Persea*, *Prunus*, *Vitis*) are particularly sensitive to even low to moderate chloride levels in the soil.

### **Mineral toxicity**

Certain elements, especially metals, including aluminum, manganese, lead, and cadmium are particularly toxic to plants and are often most available (soluble) when the pH is less than 6. These toxic elements are often present in soils that are productive but they do not cause harm to plants because at productive soil pHs, the normally toxic elements are not soluble in the soil solution. In other cases, these metals are present at particularly toxic levels due to contamination of the soil or groundwater by human activity.

The general plant response to metal toxicity is reduced productivity. Ultimately this reduction is traced back to abnormal metabolism induced by one or more toxic metals. For example, photosynthesis is reduced with copper toxicity because

copper atoms replace magnesium in chlorophyll molecules. These chlorophyll molecules are photosynthetically ineffective and ROS begin to accumulate causing premature senescence of leaves. Zinc is also known to inhibit photosynthesis by replacing magnesium at the site of water splitting in photosystem II. Heavy metals also interfere with enzyme systems leading to reduced productivity. Mitosis is generally inhibited by toxic metals, plasma membrane integrity is reduced, and ion uptake from the soil solution is compromised.

### Aluminum

Aluminum (Al) is found in all soils and is often extremely toxic to plants at levels as low as 2–3 ppm at a pH of 5.5 or lower (Rout *et al.*, 2001). Aluminum is especially toxic to seedlings and may be hard to identify. Plant symptoms resemble phosphorus deficiency and include: (i) general stunting; (ii) small, dark green leaves; (iii) purplish stems and leaves; and (iv) death of leaf tips. Sometimes symptoms are due to an aluminum-induced calcium deficiency which may include curling young leaves or death of growing points. In any case, the most widely seen effect of excessive aluminum is an inhibition of root growth, particularly an inhibition of cell division in root tips and lateral roots. New root growth is inhibited while lateral roots become thickened and brown. The root system forms many stubby roots and lacks branching.

The inhibition of cell division may be due to aluminum binding to the DNA molecules which increases the rigidity of the double helix which in turn decreases DNA replication. Aluminum also increases cell wall rigidity by cross-linking pectins, making the cell wall less flexible. In addition, aluminum causes a decrease in root respiration, interferes with sugar phosphorylation, and the deposition of polysaccharides in cell walls.

Aluminum also interferes with phosphorus nutrition by fixing phosphorus in an unavailable form in the soil or on the surface of plant roots. Aluminum also interferes with uptake, translocation and utilization of the essential nutrients phosphorus, calcium, potassium, magnesium, and iron. Some plants are tolerant of high levels of soil aluminum, attributed to uptake and utilization of calcium and phosphorus even in the presence of excessive aluminum. This tolerance seems to be controlled by a single gene in many species. Resistance to aluminum toxicity may result from a

plant's ability to sequester excess aluminum by binding it to specific proteins.

The biochemical effects of aluminum on plants are associated with the alteration in structure and function of root cell membranes. Aluminum can bind to either the proteins or lipids in a membrane, depending on pH and other conditions, thereby reducing the membrane's fluidity. Aluminum causes phosphate anions to bind to the cell wall while preventing calcium from doing so.

Aluminum toxicity is also related to nitrogen metabolism. Nitrate ( $\text{NO}_3^-$ ) uptake is reduced while ammonium uptake increases under high aluminum levels. Excessive aluminum can also interfere with the symbiotic relationship between *Rhizobium* and nitrogen-fixing species by reducing bacterial multiplication and nodule formation.  $\text{NO}_3^-$  uptake by soybean decreased when aluminum concentration in solution increased from 10 to 50 mM. Aluminum increased ammonium uptake and  $\text{H}^+$  release in aluminum-sensitive sorghum cultivars.

At the subcellular level, aluminum interferes with ATP production by inhibiting ATPase activity. Drastic decreases in the ATP pool have been observed under high aluminum levels.

### Arsenic

Arsenic is an extremely toxic element (Meharg and Hartley-Whitaker, 2002). It is often associated with mining and can be found contaminating groundwater, crops irrigated with contaminated water, and livestock which ingest contaminated feed and water. In addition, arsenic was an ingredient in many pesticides which lead to contamination of prime agricultural land.

Arsenic exists in the environment as inorganic arsenate,  $\text{As(V)}$ , and arsenite,  $\text{As(III)}$ , with both forms available to plants in the soil solution. Microbes in the soil, mammals, and invertebrates may metabolize inorganic arsenic to organic forms.

Arsenate is the predominant form of arsenic found in most soils and it competes with phosphate for uptake and utilization by the plant. Some species are resistant to arsenic and this resistance is generally due to reduced uptake and is controlled by a single gene. Reduced arsenic uptake can be induced by increasing the available phosphate in the soil solution. Even with resistance, species often accumulate appreciable levels of arsenic in their tissues. These species must compartmentalize or transform the arsenic to a less toxic form.



Arsenic toxicity to plants was first studied in the USA through pesticide residues in rice (*Oryza sativa*) grown on land previously devoted to cotton (*Gossypium* spp.) production. The pesticide monomethyarsenate (MMA) was used extensively in cotton production. Rice grown on old cotton land show symptoms of a disorder called straighthead disease, a disease associated with decreased flower fertility, which is caused by exposure to arsenic. Rice cultivars varied considerably in their susceptibility to arsenic toxicity.

Arsenic poisoning in plants causes symptoms ranging from reduced root growth to death. Arsenic competes with phosphate in the formation of ATP. Exposure to arsenic results in the production of ROS as arsenate is converted to arsenite. Additional ROS are generated when the arsenite is methylated.

As a major mechanism for arsenic tolerance, plants synthesize phytochelatins (PCs, [ $\gamma$ -glutamate-cysteine] $_n$ -glycine) when exposed to inorganic arsenic. The PC and arsenic form a complex rendering the arsenic non-toxic as long as the pH is acidic. The AS-PC complex is not stable at higher pH and arsenic can re-oxidize and become toxic.

Plants which grow on arsenic-contaminated soils are often mycorrhizal. Mycorrhizal fungi assist plants in acquiring phosphorus, thus it must be considered that the fungi might also increase arsenic uptake as well. Infection of both tolerant and intolerant plants by mycorrhizal fungi increases arsenic resistance.

### **Cadmium**

Cadmium never exists as an isolated metal in nature, but rather as an integral part of lead:zinc mineralization. Cadmium finds its way into the environment as waste products from human industrial activity including power generation, metal working, electroplating, and the manufacturing and/or inappropriate disposal of nickel-cadmium batteries. It is a most dangerous metal: it is extremely soluble in water, extremely mobile and it takes very little cadmium to induce toxicity (Das *et al.*, 1997).

Exact effects of cadmium on plants are not well understood, but cadmium can alter the uptake and utilization of other minerals (phosphorus, manganese, iron, calcium, magnesium, potassium) by interfering with them or through cadmium's deleterious effects on soil microbes that make nutrients available. Most of the cadmium absorbed by plants remains in the roots and is not translocated.

Some of the classic symptoms of cadmium poisoning in plants are leaf rolling, chlorosis, and general growth stunting. Cadmium has been shown to have many metabolic effects on plants including: (i) reduced absorption of nitrate; (ii) reduced nitrogen fixation in nodules of nitrogen-fixing species; (iii) decreased plasmalemma permeability and reduced water content of cells; (iv) decreased ATP formation; (v) enhanced lipid peroxidation and reduced membrane functionality; (vi) inhibited chlorophyll synthesis; and (vii) reduced carbon fixation. Cadmium probably causes these abnormalities by inducing free radical production and thereby inducing oxidative stress or by interfering with stress-coping mechanisms.

One mechanism plants utilize for minimizing cadmium damage is to sequester the ion in its roots via chelation by components of the cell wall. Another line of defense for the plant would be via immobilization by the plasma membrane. Cadmium could also be chelated by metallothioneins (MT) or PCs, both polypeptides, and stored in the vacuole, rendering it ineffective in causing damage.

### **Chromium**

Chromium is widely used in industry, particularly the leather-processing industry. It is found in water, air and soil and is a serious environmental hazard to living organisms (Shanker *et al.*, 2005). Hexavalent chromium (VI) is extremely toxic while trivalent chromium (III) is slightly less toxic. Hexavalent chromium usually occurs as chromate ( $\text{CrO}_4$ ) or dichromate ( $\text{Cr}_2\text{O}_7$ ) oxyanions and is a very potent oxidizing agent. Much of chromium's damage comes from its generation of ROS. Trivalent chromium is usually bound to organic matter and is therefore usually less toxic.

Chromium uptake by a plant is via uptake carriers for essential plant nutrients such as sulfur, iron, and phosphorus. Hexavalent chromium uptake by plants is an active process while uptake of trivalent chromium is passive. Movement is primarily in the xylem, although most absorbed chromium stays in the roots.

Seed germination is greatly reduced in the presence of chromium. Stem and root growth are also diminished on exposure to chromium. Leaf number may be only half of normal when a plant is exposed to chromium, and this leads to a great reduction in yield.

On a metabolic level, chromium can reduce photosynthesis as much as 95%! This is amazing since most of the chromium remains in the roots and the disruption occurs in the leaves. The inhibition of photosynthesis is due to disruption of the organization of chloroplast ultrastructure, inhibition of electron transport or by negative effects on the enzymes of the Benson-Calvin cycle. Other metabolic abnormalities like those described for the other heavy metals also occur with exposure to chromium.

### **Mercury**

Mercury toxicity to plants arises from mercury pollution (Patra and Sharma, 2000). About two-thirds of the mercury pollution arises from natural causes while a third is generated by humans. In agricultural soils, much of the mercury comes from sludge, manures and fertilizers as well as seed-coating pesticides.

Even though mercury toxicity to plants is a problem, it is not that widespread, since mercury is not very available for plants to take up. Mercury tends to accumulate in the roots, thus any mercury found in the shoots comes from foliar absorption. With respect to foliar absorption, C3 species absorb five times more mercury than C4 plants. Most of the mercury in plants responsible for toxicity is from airborne sources.

Mercury may cause toxicity by altering membrane permeability, reacting with phosphate groups of ADP and ATP, thereby altering energy metabolism, or by replacing major cations in key metabolic processes. Mercury can replace magnesium in chlorophyll thereby inhibiting photosynthesis.

Transgenic plants capable of cleaving mercury ions from methyl-mercury complexes and then reducing them to the less toxic metallic form have been developed. Such plants which include *Arabidopsis thaliana*, *Brassica* (mustard), *N. tabacum* (tobacco) and *Liriodendron tulipifera* (tulip poplar) can be used in phytoremediation projects to remove mercurial contaminants from the soil.

### **Selenium**

While some plants tolerate high levels of selenium in the soil and may be useful in phytoremediation of selenium-contaminated soil, most plants are selenium sensitive (Terry *et al.*, 2000). Selenium has been considered by some a micronutrient and the range between sufficient and toxic is narrow.

Selenate is absorbed via active transport while selenite is absorbed passively. Plants can also absorb selenium from the air through their leaves.

When sensitive plants are exposed to excess selenium, symptoms of injury include: (i) stunted growth; (ii) chlorosis; (iii) withering of leaves; (iv) decreased protein synthesis; and (v) eventually premature death of the plant.

The major mechanism of selenium toxicity is through incorporation into proteins as selenium cysteine and selenium methionine in place of cysteine and methionine, respectively. This leads to altered form and function of the resultant proteins and an aberration in metabolism. In addition, selenium inhibits chlorophyll synthesis, nitrate reduction in leaves and inhibits the production of free radical scavengers.

### **Lead**

Lead is a dangerous environmental pollutant, generally accumulating in the top layers of the soil (Sharma and Dubey, 2005). Most of our lead contamination comes from human activity: (i) lead from mining and smelting; (ii) lead-based paints and batteries; and (iii) leaded gasoline. Further contamination of agricultural lands comes from application of municipal sewage sludge as fertilizer. Some plants tolerate high levels of lead and/or accumulate high levels of lead in their roots. These plants can be used for phytoremediation of polluted sites.

Lead is easily absorbed by plants acting as a slow rather than acute poison. While high levels of lead can occur in both roots and shoots, lead tends to accumulate in the roots following absorption. Lead availability increases with pH between 3.0 and 8.5. Between pH 5.5 and 7.5 lead is generally not available for plant uptake due to precipitation with soil phosphates and carbonates. Lead in soils is usually tightly bound to colloids or organic matter. Furthermore, lead binds to mucilage on the soil roots preventing its uptake into the plant. Lead which makes it into the root is often bound to the cell wall. Some lead may be transported through the plasma membrane into the cell. Further transport through the root may be blocked by the endodermis. In general, tissues furthest away from the root are lowest in lead content.

The initial symptoms of lead toxicity are a rapid inhibition of root growth, general stunted plant growth, and chlorosis. Lead toxicity leads to many

physiological anomalies: (i) inhibition of sulfhydryl-containing enzymes by alteration of tertiary structure; (ii) inhibition of seed germination; (iii) mitotic irregularities in root cells due to perturbation of microtubules; (iv) leakage of potassium from root cells, indicating interruption of membrane integrity; (v) reduced protein content and altered lipid content; and (vi) reduced synthesis of DNA and RNA. Lead also enhances the activity of certain enzymes, particularly those involved in hydrolytic activities and antioxidative enzymes.

Photosynthesis is greatly perturbed by lead toxicity. Chlorophyll, carotenoid and plastoquinone synthesis are inhibited and chloroplast ultrastructure and membrane composition is distorted. Enzymes of the Benson-Calvin cycle are inhibited as is electron transport. CO<sub>2</sub> deficiency occurs due to stomatal closure.

At high levels of lead exposure, respiration is decreased due to aberrations in electron flow in the electron transport system. At lower levels of exposure, respiration seems stimulated. RuBisCO is inhibited by lead exposure whereas oxygenase is unaffected. Thus under lead toxicity conditions, photosynthesis is inhibited while photorespiration is not. This might explain higher respiration and ATP levels under lead toxicity stress.

Lead generally blocks the uptake of potassium, calcium, magnesium, manganese, zinc, copper, iron, and nitrates into the roots by physically blocking sites of entry into the cells. Another mechanism for ion blocking by lead is enzyme and membrane changes caused directly by lead toxicity.

Transpiration declines with exposure to lead. Both leaves and guard cells are smaller in lead-stressed plants. Lead also induces an increase in abscisic acid, which causes stomatal closure.

### **Boron**

Boron deficiency is more prevalent than an excess of boron. However, when it occurs, excessive boron can lead to many problems in plant production (Nable *et al.*, 1997). Much of the boron in soil comes from the evaporation of seawater and much of that boron is in a fixed form unavailable for uptake by plants. Excessive levels of boron in the soil usually come from irrigation water that has a high boron content and occurs mostly in arid and semi-arid environments. Boron in the soil solution occurs primarily as boric acid, B(OH)<sub>3</sub>, which is mobile in the soil and easily leached. Boric acid in

the soil may also be adsorbed to organic matter or soil particles or absorbed by plants.

It is difficult to predict the potential for boron toxicity based on soil analysis since the total boron content may not reflect the plant-available boron. The highest boron concentrations in plant tissues tend to be in tissues at the end of the translocation stream, in particular, leaf tips and margins. Leaf analysis is also not a very good predictor of injury since different portions of the leaf may contain different levels of boron in them which may or may not indicate injurious levels. The range for plant-tissue boron levels that exhibit injury symptoms is often very wide.

General toxicity symptoms include leaf margin or tip burn in older leaves. In species where boron is phloem mobile, symptoms may appear in sinks such as fruits as gummosis or necrosis. Bark necrosis may also occur with excessive boron. Symptoms do not usually appear in roots. Other general symptoms of boron toxicity include: (i) decreased chlorophyll content; (ii) reduced growth; (iii) loss of leaf area; and (iv) leaf cupping.

### **Copper**

While pH often regulates the amount of bioavailable metals in the soil solution, the amount of bioavailable copper is more closely related to the amount of organic matter in the soil than to pH (Reichman, 2002). Copper is strongly adsorbed to organic matter rendering it unavailable for plant uptake.

An initial symptom of copper toxicity is interveinal chlorosis in leaves. The initial chlorosis may be a reflection of iron deficiency induced by excess copper. The chlorosis often becomes cream colored or whitish. If the leaves also exhibit redness, there may be a copper-induced zinc deficiency as well. As the toxicity intensifies leaf tips and margins become necrotic. Eventually the entire leaf will become necrotic. In cases of acute toxicity, leaves often wilt quickly then become necrotic. In some species foliage becomes purplish. Copper toxicity also affects the roots, often long before shoot symptoms are visible. Root growth is often stunted and discolored and may be prone to fungal infection. Root hair production may also be inhibited (Reichman, 2002).

### **Manganese**

Manganese becomes more soluble in the soil solution as pH is lowered making the possibility of

manganese toxicity more likely in acid soils (Reichman, 2002). Soils high in organic matter may generally exhibit less manganese toxicity than highly mineral soils. However, in soils high in other cations, manganese cannot readily form organic complexes, thus toxicity of manganese is more related to pH than to soil organic matter content. Waterlogged and compacted soils tend to exacerbate manganese toxicity problems by inducing the formation of the  $Mn^{2+}$  ion which is highly soluble and thus available for inducing toxicity.

Manganese toxicity is often characterized by brown spots on leaves, petioles and stems which ultimately become necrotic. Symptoms begin on older leaves and progress to younger ones eventually causing death if severe enough. Other symptoms may include general leaf bronzing or crinkling of leaf blades. Leaf crinkling may actually be manganese-induced calcium deficiency. Roots may be brownish and sometimes display cracking. Manganese toxicity may also induce iron or magnesium deficiencies (Reichman, 2002).

### **Zinc**

Zinc is more soluble at a low pH making zinc toxicity a possibility in acid soils (Reichman, 2002). High levels of organic matter in soils helps form complexes with zinc making it unavailable for plant uptake, thus leading to reduced zinc toxicity.

Zinc toxicity is often characterized by a general chlorosis of younger leaves. Anthocyanin production may increase with zinc toxicity, thus leaves may become red. Zinc toxicity also leads to smaller leaves which may be vertically oriented. Leaves may also become necrotic. Root growth is reduced by zinc toxicity with a shorter primary root and fewer lateral roots which may become yellow. Zinc toxicity may also induce an iron deficiency and interfere with phosphorus metabolism in the plant (Reichman, 2002).

### ***Alleviating soil mineral toxicity***

In order to reduce economic and aesthetic damage caused by toxic mineral elements, a number of different strategies have been developed for alleviating soil mineral toxicity. With water-soluble toxins, high levels can sometimes be leached out of the soil via overhead irrigation. Another approach is to change the soil pH to change the toxin's solubility, forcing it to become adsorbed to soil particles or

organic matter or to precipitate out. Another approach is to remove the toxin by growing tolerant species that absorb large quantities of the toxin and harvesting the plants. The problem though then becomes of what to do with the removed plant material. A final approach is to develop toxin-resistant cultivars that are able to produce acceptably on otherwise toxic soils. Resistant cultivars may exclude toxic metals from uptake, excrete excessive amounts of specific metals back into the soil, or sequester excess metals in the vacuole.

### **Soil salinity and sodicity**

Around 6% of the earth's land is saline or sodic. Saline soils have enough soluble salts in them so that they can inhibit plant growth and development. Sodic soils are soils where the negative sites of clay particles are occupied by sodium ions. Salinity is measure by electrical conductivity (EC) and a saline soil is one with an EC of 4 dS/m or more. Many crops are affected by soils with an EC lower than 4 dS/m (Munns, 2012).

### ***Soil salinity***

There are two main types of salinity: (i) natural; and (ii) human induced. Natural salinity in soils is the result of either natural weathering of parent material which releases salts into the soil or deposition of salt from seawater by wind and rain. Salts of natural salinity include chlorides of sodium, calcium, and magnesium, as well as sulfates and carbonates. Sodium chloride is the principle salt from seawater.

While you might think that deposition from seawater is minimal, but it can actually be significant, especially at locations close to the water source (usually an ocean). Most wind/rain-deposited seawater has from 6 to 50 mg/kg of salt in it. If we use 10 mg/kg as a general average, 10 kg/ha of salt would be deposited for each 100 mm of rainfall/year. This salt accumulation is constant, so over time the salt added to the soil is significant. Clay soils would retain much of that salt while sandy soils would not, both retention rates depending on annual rainfall.

Human-induced salinity arises after land is cleared and planted with annual crops. Natural perennial vegetation often relies on a deep water table while annual crops usually require frequent irrigation in most parts of the world. The irrigation

of annual crops most often supplies excess water, thereby raising the water table and mobilizing salts deep in the subsoil. These salts are brought to the surface. Eventually, the soil becomes salty enough to be considered saline.

A second human-induced source of salinity is through irrigation which uses water rich in salts or is on insufficiently drained land. Even with good quality irrigation water ranging from only 200 to 500 mg/kg of soluble salt, irrigation of 1 ha with water of 500 mg/kg salt content, would deposit (assuming 6000–10,000 m<sup>3</sup> of water/ha/year) 3–5 t of salt. Crops remove very little of this salt, thus it will accumulate in the root zone. Eventually it will reach levels considered saline. Insufficiently drained soil does not allow excess salt, either natural or human deposited, to be leached from the soil and the soil is eventually considered saline.

Soil salinity is easily determined by measuring electrical conductivity (EC = dS/m). While we can measure a soil's EC, we also often measure the EC of irrigation water, rainwater and fertilizer solutions in hydroponic and greenhouse culture. Pay attention to the units used in reporting EC as it is often reported as deciSiemens per centimeter (dS/cm) (1000 dS/m) rather than deciSiemens per meter. Quality of water is often expressed as total soluble salts (TSS) with the international convention of 1 dS/m equal to 640 mg/l TSS (Munns, 2012).

On a large scale, soil salinity is often mapped using an electromagnetic (EM) meter which estimates the bulk EC of the soil. A transmitter coil sends out an impulse of alternating current which generates a primary magnetic field in the soil which causes a secondary magnetic field to develop which depends on soil conductivity. The primary and secondary magnetic fields are detected by a receiver and the readings generated into a reading of soil salinity.

### Soil sodicity

Sodic soils are low in soluble salts but very high in exchangeable sodium (Na<sup>+</sup>) which binds to negative charges on soil particles, particularly clay and organic matter. Sodium causes soil structure degradation. Soil sodicity is defined as an exchangeable sodium percentage (ESP) which imparts soil degradation. Sodic soils are also generally very alkaline with a pH of 8.5–10.

Normally, soil particles are held together by divalent cations such as Ca<sup>2+</sup>. When a monovalent cation such as Na<sup>+</sup> replaces the divalent cation in

low-soluble salt soils, the soil aggregates swell and clay particles separate from each other, thereby destroying the structure of the soil. Globally, soils are considered sodic if the ESP is from 6 to 15. The ESP that is considered sodic varies, since the destruction of soil structure depends on the soil's divalent cation content. In areas where the divalent cation content is naturally low, the ESP needed for damage is lower than regions with a higher divalent cation content.

Sodic soils develop over a long time. Naturally occurring salts or salts deposited by irrigation or rainwater eventually cause the clay particles to become saturated with Na<sup>+</sup>. At the same time, irrigation often leaches divalent cations from the soil, exacerbating the situation. Eventually the clay particles that have dispersed due to the sodium begin to settle deeper in the soil profile and clog pores, impeding water drainage. The soil becomes poorly drained and waterlogged.

### Salinity and plants

Soil salinity inhibits plant growth in two major ways: (i) osmotic stress; and (ii) direct salt injury (Munns, 2002; Munns and Tester, 2008). The decreased osmotic potential of the soil solution with a high concentration of salts induces osmotic stress. Plants have a difficult time absorbing adequate amounts of water from the soil. The water that is absorbed has high levels of sodium and chloride ions in it, which can directly injure cells, especially in leaves.

Species differ greatly in their level of salt tolerance. Barley (*H. vulgare*), for example, is quite salt tolerant while rice (*O. sativa*) is sensitive. Salt tolerance or sensitivity is determined based on the salt level needed to reduce biomass production or yield over time. The lower the level of salt needed for injury or decreased yield, the more sensitive the species.

Salt-induced osmotic stress results in reduced root and leaf growth as well as a decrease in stomatal conductivity, which in turn reduces photosynthesis. These responses and others resemble those observed during drought stress (see Chapter 8, this volume).

A direct effect of absorbed salt which leads to very high levels of Na<sup>+</sup> and Cl<sup>-</sup> in leaves, especially older ones, is an increase in senescence of older leaves. Salt does not directly cause a reduction in the production of new leaves. If fewer new leaves

are produced relative to senescing leaves, the plant cannot photosynthesize enough to support a crop.

There is little genotypic variation in plant responses to salt-induced osmotic stress. Variation in resistance to direct salt injury is observed as differences in the ability to regulate the uptake of  $\text{Na}^+$  and  $\text{Cl}^-$ . Salt-tolerant species take up little  $\text{Na}^+$  and  $\text{Cl}^-$  while sensitive species absorb large quantities of  $\text{Na}^+$  and  $\text{Cl}^-$ . Most crops can only tolerate a maximum of about 100 mM NaCl or 10 dS/m EC before a significant reduction in growth or yield occurs. In both sensitive and tolerant species, enzymes do not adapt to high salt levels in the cytoplasm, thus salt must be sequestered in the vacuole, away from enzymes.

Halophytes are a specific group of salt-tolerant plants. Their salt tolerance is not due to any special metabolic adaptations to high levels of  $\text{Na}^+$ , but rather their ability to compartmentalize very large amounts of  $\text{Na}^+$  in vacuoles.

### ***Alleviating stress***

A number of approaches may be taken in an attempt to reclaim saline or sodic soils. In irrigated soils that have become saline or sodic, large quantities of water are applied at once in an attempt to remove the salt left behind by irrigation. This process called reclamation is slow, expensive, and requires good water drainage.

In sodic soils, sodium ions can be replaced with calcium ions by applying large quantities of gypsum (calcium sulfate) to the soil, followed by excessive water application to leach the sodium out of the soil. Gypsum is preferred to limestone as a calcium source since it has little effect on soil pH.

When saline or sodic soils interfere with seed germination and crop establishment, the top layer of soil is removed along with its excessive amounts of salt to enhance seed germination and seedling growth. Another approach is to irrigate with high quality water just prior to seeding, perhaps combined with ridges, to remove salts from the top layer of soil. Raised beds or ridges help minimize salinity effects. If ridging is not feasible, the entire field can be flooded prior to planting with good quality water. Just before all of the water has percolated into the soil, the seeds can be sown allowing them to settle into the mud by gravity.

Other management practices that can help alleviate problems associated with saline soils include: (i) mulching; (ii) deep tilling; and (iii) the incorporation of organic matter. Mulching reduces evaporation of water from the soil and its concomitant elevation of surface-soil salt levels. Additionally, mulching reduces irrigation frequency. Deep tilling involves mixing the surface layer of soil where much of the salt is into the deeper layer of soil, thereby diluting its concentration. Adding organic matter to the soil improves soil structure and drainage.

If alleviating the stress caused by salt or sodium in the soil is not possible, salt-tolerant species or cultivars can be utilized. Reclamation of abandoned salinized land with halophytes is useful in returning the land to productivity. Tallwheat grass (*Thinopyrum ponticum*) and saltbush (*Atriplex amnicola*) are halophytes that can be grown on salinized land as fodder. Once the salt has been removed from the soil, other less tolerant species can be re-introduced.