

#### **Oxidation Reduction Potential**

Many research studies now confirm the involvement of redox regulation responses in various physiological pathways in plant development. These include the production of oxidase enzymes when plants encounter stress events. Oxidized tissue of the cell wall is well known to be involved in plant wound healing and pathogen attack responses as triggered by cell wall-stiffening events and defense gene expression. High levels of amino acid-based oxidases are expressed in plants undergoing stress—known or unknown to the grower. By monitoring the ORP of the plant, growers can monitor the sum total of oxidation that is occurring in the plant. ORP is an effective qualitative indicator of plant stress.

When plants encounter root expansion limitations due conditions such as heat or drought stress, nutrientdeficiencies, or soil compaction, the plant's root system cannot keep up with the needs of the aboveground growth. When leaves begin to be irradiated by excess light energy, reactive oxygen species (ROS) are produced and the chloroplasts that carry out photosynthesis can easily suffer oxidative stress and stop photosynthesizing.

#### **Total Phenols**

Phenols in plant leaves are generally considered vital defense compounds when stresses lead to an increased production of free radicals and other oxidative species in plants. These stresses include high light or UV radiation, low temperatures, pathogen infection, herbivory, heavy metals, and nutrient deficiency. Phenols display antimicrobial and antioxidant properties, which help the plant evade pathogenic infections and protect vital tissues from the toxic effects of oxidation. If plant phenol levels are observed to be rising during the growing season, the plant is coming under increased stress due to one or more influences. However, if plant phenol levels rise then drop suddenly while a stress event is still present, this indicates the plant lacks sufficient resources to continue to fight the infection and may die as a result.

### **Total Protein**

Many of the essential proteins in the plant are present in leaf sap, indicating an essential fundamental role of these proteins in maintaining plant function and productive capacity. Proteins also contribute to intercellular communication, coordinating the growth and development of many plant organs. Overproduction of plant proteins is usually in response to compromised plant tissue due to insect and/or disease pressure or other induced stress events. Monitoring plant protein content can help growers identify and mitigate stress response patterns and is essential for crop success.

#### Free Amino Nitrogen (FAN)

Excessive FAN can cause osmotic stress, creating an overexpression of cellular protein response as the plant tries to maintain cellular integrity. Osmotic stress is caused by a sudden change in the solute concentration around a cell, which leads to a rapid change in the movement of water across the cell membrane. Under conditions of high salts, water is drawn through the cell walls too rapidly, causing a loss of cell integrity due to rapidly shifting pressure gradients in and around the cell wall.

Nitrogen stress: plants under stress accumulate excess carbohydrates, generally leading to an imbalance in the relative concentrations of carbohydrates and nitrogen. This can cause premature lignification and rapid onset of flower production. If FAN is high, good amino N is present to avoid the onset of flower productionby nitrogen stress.



Fermentation potential (Wine grape growers): many grape varieties are deficient in available nitrogen, and the type of nutrient used and the timing of addition are crucial in the outcome of alcoholic fermentation. It is known that organic nutrients with amino acids and/or peptides and proteins added at the beginning of fermentation are a strategy for achieving complete fermentation and maximizing the flavor potential of the wine.

Fermentation potential (Fresh Fruit Growers): low FAN can be caused by heat stress, and leads to low available N, osmotic stress, and a rapid lignification of the plant due to an increase in the C:N ratio. When FAN is too high, more N is available for assimilation by molds and storage rot, increasing the need to rapidly transfer crops to the fresh market or the end-user.

Amino acid shortage/excess: The level of FAN is directly related to the plant's ability to replicate and create new cells. When FAN is high, rapid plant growth can occur, leading to increased replication of cells and larger fruits. Short-term high FAN levels due to peptide and amino acid translocation into developing plant organs can lead to increased production during crucial growth stages. However, if other nutrient deficiencies inhibit growth, FAN will over accumulate, leading to susceptibility to osmotic stress.

Insect Food Supply: the FAN content of the blood of insects is exceptionally high. When FAN levels of insectstressed plants are compared to non-insect stressed plants, they generally show higher levels of FAN.

### Ethanol

Ethanol affects the activity of a range of intracellular cytoplasmic proteolytic enzymes. Increasing ethanol concentrations generally increase proteolytic enzyme secretion and reduce amino acid and peptide absorption. This leads to a loss of cell integrity and susceptibility to abiotic and biotic stress. In other words, an increase in ethanol degrades cellular proteins and allows subsequent predation of plant matter by insects and/or disease.

## Nickel - (Ni)

Nickel has positive effects on plant growth at low concentrations (0.01 to 10 mg/kg soil), whereas levels above 10 mg/kg tend to cause toxicity. When plants are Ni deficient, they cannot process urea, disrupting nitrogen assimilation and causing urea to accumulate to toxic levels--this often presents as leaf-tip necrosis on pale green leaves. Ni deficiency also induces iron deficiency. Proper Ni nutrition is essential for plant response to disease; Ni application has been shown to reduce fungal infections by 50%. Ni is essential for proper root nodule growth and seed yield in leguminous plants like soybean. Ni deficiency can be induced via excessive fertilization with competing ions (Zn2+, Cu2+, Mg2+, Mn2+, Fe2+, Ca2+). Excessive Ni can cause symptoms similar to other heavy metal toxicities. Nickel toxicity can cause altered leaf morphology, increased cell wall permeability and ion leakage, reduced transpiration, disrupted photosynthesis and increased oxidative stress. Excessive Ni may also inhibit the uptake of other essential ions, such as Fe2+ and Mg2+. Nickel can directly inhibit enzymes by interacting with sulfhydryl (SH) groups on proteins. When Ni binds to an SH-group, it causes conformational changes that can deactivate enzymes and proteins. Like other heavy metals, the oxidative stress produced by excess Ni can cause chromosomal damage and mutations to cell nuclei.



### Lead - (Pb)

Lead takes second on the environmentally toxic heavy metals list and represses plant growth at concentrations beyond 30 mg/kg. Bioaccumulation of Pb during plant growth affects total chlorophyll content, fresh weight, root, and shoot length. Pb tends to accumulate more readily in plant roots than other parts of the plant–traces of Pb will translocate to other regions of the plant, including the fruits and shoots. Plant stress brought on by high concentrations of Pb will activate critical enzymes within the plant to eliminate reactive oxygen species (ROS): ascorbate peroxidase (APX– 25mg/kg), superoxidase dismutase (SOD–50mg/kg), catalase (CAT–100mg/kg). Exposure to Pb changes the expression of the proteins responsible for regulating ROS signaling–heightened protein production is monumental in regulating cellular metabolic processes. Mitogen-activated protein (MAP) kinase pathways supervise the plant's signaling system–aiding to combat oxidative stress, operating through a series of reactions, thereby modifying gene expression and protein synthesis.

### Titanium - (Ti)

Titanium is not a required nutrient but can be beneficial for plant growth at low levels. Ti is similar to iron at an atomic level and is taken up by the plant by iron transporter proteins. The redox capabilities of Ti can enhance photosynthetic activity and other enzymatic reactions, leading to increased biomass. Ti has been shown to increase iron uptake, so titanium's beneficial impact on plant growth may be most pronounced when iron is deficient. Low concentrations of Ti have been shown to help plants cope with abiotic stresses, such as drought, cold and heat stress, heavy metal toxicity, and low N and P. However, concentrations of Ti >50 mg/kg in the soil may cause toxicity in plants. This toxicity is primarily caused by an overabundance of Ti in the cell competing with iron and other metals for binding sites, inhibiting the function of enzymes.

### Vanadium - (V)

Vanadium may be beneficial for plant health at trace levels, but levels as low as 2 mg/kg can be damaging. Vanadium commonly occurs as vanadate, which is structurally similar to phosphate. It can be mistakenly substituted for phosphate and taken up by the plant, where it can inhibit vital metabolic processes and generate high levels of reactive oxygen species, which can damage cellular components; vanadium can even damage chromosomes. However, most vanadium that enters the plant is deposited in the roots, and little V is transported to above-ground tissues.

### Tin - (Sn)

The toxicity of tin depends on whether it is organic (organotin) or inorganic salt. Tin salts are poorly adsorbed, although they can accumulate in root tissues. Soil concentrations above 10 mg/kg may cause toxicity in plants. Organotin compounds are typically more toxic than tin salts. They often enter the environment through human activities but have relatively short half-lives. Organotin compounds are hydrophobic and adsorb to metal oxides and organic matter like Humic acids and clay. 10 mg/L dissolved organic matter significantly reduces the bioavailability of organotin compounds. At lower pH values, cation exchange and adsorption of tin increases, reducing the amount of dissolved tin species.

### Silver - (Ag)

Silver has been reported to have positive effects at low concentrations, attributed to its ability to exchange electrons with Fe and other essential ions, thereby improving biological redox conditions. Like many other metals, it can cause oxidative stress and degrade cellular components at high concentrations.



## Chromium - (Cr)

Chromium is a naturally occurring heavy metal contaminant. It is the 7th most abundant element and one of the more significant carcinogens. Hexavalent chromium (VI) and trivalent chromium (III) are the predominant states of chromium present in the soil, but Cr (VI) has a higher biotoxicity than Cr (III) and both are readily taken up by plants.

Cr (VI) is transported into plants cells via sulfate chaperones, while Cr(III) enters the plant cells through passive cation exchange sites among the cell wall. Unmitigated high concentrations of Cr inhibit morphological, physiological, and metabolic activities within the plant, ultimately resulting in plant death.

Leaf photosynthesis is reduced up to 50% as Cr is transported to the upper plant parts. Cr toxicity can cause leaf-wilt and interveinal chlorosis throughout the plant. Furthermore, plants undergo ultrastructural change throughout subcellular compartments leading to root cell damage, reduction in total pigment content, disturbance in the water and mineral nutrient balance, enzymatic deactivation, and degenerative cell division. Cr inhibitory effects limit mitochondrial electron transport causing an increase in reactive oxygen species (ROS), perpetuating oxidative stress in the plant.

## Arsenic - (As)

Accumulation of As in soil from irrigation water presents as either inorganic arsenate or arsenite, which are heavily affected by soil pH, redox potential, and soil organic matter (SOM) (Brammer and Ravenscroft, 2009). Mobility of As in soil is dependent on the soil characteristics; soils rich in Al or Fe oxides bind As strongly; thus, sandy soil has the lowest As retention, while silt soil tends to have a high As concentration retained by the clay. All bioavailable As in the soil is not phytoavailable; uptake depends on plant type and species, plant ability to incorporate and translocate As, and the predominant As species. Arsenic accumulation in plants is usually in the order: roots > stem > leaves > edible parts. In plants, low concentrations of As have been reported to increase growth, but As is a metabolic inhibitor, negatively impacting the plant at high concentrations. Excessive As inhibits germination, reduces root and shoot growth, lowers the yield, produces reactive oxygen species (ROS), affects photosynthesis mineral nutrition, and causes necrosis, thus posing a threat to agricultural productivity.

### Cadmium - (Cd)

When Cd accumulates in the plant, it causes widespread damage. Cd toxicity causes oxidative stress and can potentially cause damage to DNA strands. Visible symptoms include leaf chlorosis, leaf rolling, necrosis, and growth inhibition; Cd toxicity is associated with inhibited root elongation and disrupted photosynthesis. Cd also disrupts the plant's ability to take up other nutrients, exacerbating the stress.



## Soil Bicarbonates

In arid soil and overcultivated soils, Bicarbonate can be a major problem, that is unknown tot eh grower as it enters the system from the irrigation water. As Bicarbonate increases in the soil, it increases soil pH while DECREASING the availability of Calcium. It tends to cause shallow soil horizon leaching of positively charged ions, specifically calcium, iron, magnesium. It does this in a way that pulls calcium off the clay colloid, leaches it and replaces the exchangeable Calcium previously available for a salt-based molecule, usually Sodium and/or Potassium. This makes plant available Calcium low, and decreases over time while making plant available Sodium increase, and grow higher over time. This leads to a lower quality of crop production and less viable bioregions year over year. It also reduces the soil availability of Magnesium, Iron, Manganese leading to loss of germination potential of all native species and decreased potential of any cultivated species. Soils with high Bicarbonates usually require a steady diet of carbon and organic acids to release minerals bound to the soils.

## Soil Phenols

Phenols in soils are generally considered indicative of Humic acid production via fungal digestion of root exudates. By tracking and analyzing phenol production in the soil, growers can begin to assess the rate at which they are building stable organic matter throughout the season. Humic acid production and/or availability for stable microbial communities is a key driver of soil health and carbon sequestration via root zones. As total phenols increase in the soil, other key health benefits are transferred to the crops such as the ability to enzymatically access SIR immunity to stress, insects and disease. Additionally, many phenols are suppressive to weed seed germination, soil pathogen proliferation leading to ability to assess long term weed potential and soil-borne disease suppression.

## **Soil Total Protein**

Soil organic matter contains nitrogen primarily in the form of protein, which is a source of available N exclusively for microbes and animals, made available during the growing season via slow-release mineralization. Plants take up organic nitrogen compounds of low molecular mass, including amino acids via membrane transporters into root cells and also as peptides and proteins. Soils that maintain N in SOM, are usually high in soluble C:N ratio, microbially active, and minerally abundant in residue from crops, cover crops, and degrading plant matter. Soil Protein is i ndicative of total biomass as cycled through living organisms such as plants, animals, insects and other form of life. It is critically important as it is a major source of value to growers in the form of Carbon: Nitrogen ratio and the stable nature of these 2 in soil Protein molecules. Cycling biologically active soil with large reserves of decomposing plant material in organic form provides N over time as opposed to applying soluble forms of N that are more easily lost.

Decomposition by microbial biomass builds up soil Organic Matter as plant residues and other C containing materials decompose in the soil, binding to soil mineral colloids creating long term stable soil aggregates—influencing water storage, runoff reduction and more.

### Free Amino Nitrogen (FAN)

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Amino acid shortage/excess: The level of FAN is directly related to the plant's ability to replicate and create new cells. When FAN is high, rapid plant growth can occur, leading to increased replication of cells and larger fruits. Short-term high FAN levels due to peptide and amino acid translocation into developing plant organs can lead to increased production during crucial growth stages. However, if other nutrient deficiencies inhibit growth, FAN will over accumulate, leading to susceptibility to osmotic stress.



## Ethanol

Ethanol affects the activity of a range of intracellular cytoplasmic proteolytic enzymes. Increasing ethanol concentrations generally increase proteolytic enzyme secretion and reduce amino acid and peptide absorption. This leads to a loss of cell integrity and susceptibility to abiotic and biotic stress. In other words, an increase in ethanol degrades cellular proteins and allows subsequent predation of plant matter by insects and/or disease.

## Nickel - (Ni)

Nickel is found in soils at an average of 50 mg/kg. Soil Ni commonly ranges from 5 to 500 mg, and serpentine soils have higher natural levels of Ni. Elevated Ni levels may occur due to industrial activity or from the agricultural application of heavy metal-contaminated sewage sludge. Nickel can be found in oxidation states ranging from -1 to +4, but Ni(II) is the most common state in biological systems. Available nickel may be considerably lower than total nickel concentration, but true nickel deficiencies are rare. Ni deficiency can be induced via excessive fertilization with competing ions (Zn2+, Cu2+, Mg2+, Mn2+, Fe2+, Ca2+). Above pH 6.5, Ni forms insoluble hydroxide complexes with iron, aluminum, and manganese. Below pH 6.5, most Ni compounds are relatively soluble. Organic soils have been shown to adsorb more Ni than sandy soils. However, amino acids and other organic acids can chelate Ni and mobilize it. Thus, acidic, carbon-rich soils may promote Ni uptake. Liming has been demonstrated to reduce Ni uptake in acidic soils.

## Lead - (Pb)

The transfer of metals and chemicals in food chains is commonly represented by bioconcentration factors (BCFs) and bioaccumulation factors (BAFs). These factors are founded on the assumption of linear relationships between metals in soil and plants, earthworms, mammals, and other target organisms. BCF is generally defined as the ratio of the test chemical concentration in an organism to the concentration in water or soil in a constant state. BAF is the ratio of the test chemical concentration in an organism to the concentration in its food at a constant state. BCFs are generally used for plants and invertebrates and are expressed in the wet weight of tissue and dry weight of soil, whereas BAFs are typically used for accumulation by birds and mammals and are conveyed on a wet weight basis only. Plants can sequester metal ions that harm other organisms due to the higher toxic concentrations. Heavy metal acquisition and accumulation are controlled by rates of complex transport interactions and chelating activities within the plant. Such processes remove the metal-contaminants from the soil; however, crops for human consumption or animal feed grown on contaminated soils potentiates the introduction of said metal-contaminants into the food chain. Quantifying the synergism between vegetable Pb accumulation and soil Pb content is challenging-generally, subpar levels of soluble and bioavailable Pb are straightforwardly contingent on the total soil Pb load. Instead, the relationship between vegetable Pb concentration and Pb soil concentration is subjugated via strict soil properties, including pH, soil organic matter (SOM), and dissolved organic matter (DOM). Generally, plant-uptake noted for Pb is considerably low unless soils are strongly acidic due to the strong proclivity for Pb remaining quiescent in neutral to higher-pH soils via adsorption and precipitation reactions.

### Titanium - (Ti)

Titanium - Titanium (Ti) is relatively abundant, but the minerals that contain it are not very soluble in water, and mineralized titanium is thus considered inert. The most stable oxidation state is +4, and most titanium in minerals is found as TiO2 or bound in iron-titanium oxides. The primary factor determining the

bioavailability of Ti is pH, and titanium is more soluble at pH values below 4. Ti is not a required nutrient but can benefit plant growth at low levels. Ti is similar to iron at a molecular level and is taken up by the plant by iron transporter proteins. The redox capabilities of Ti can enhance photosynthetic activity and other enzymatic reactions, leading to increased biomass.



# Titanium (continued)

Ti has been shown to increase iron uptake,

so titanium's impact on plant growth may be most pronounced when iron is deficient. Low concentrations of Ti have been shown to help plants cope with abiotic stresses, such as drought, cold and heat stress, heavy metal toxicity, and low N and P. However, concentrations of Ti >50 mg/kg in the soil may cause toxicity in plants. This toxicity is primarily caused by an overabundance of Ti in the cell competing with iron and other metals for binding sites, restricting many enzymes from functioning correctly.

## Vanadium - (V)

Vanadium is a transition metal found naturally in small amounts. Vanadium pentoxide (V2O5) is the most common usable form of vanadium; however, it can also be found as ammonium metavanadate (N44VO3), sodium metavanadate (NaVO3), and sodium orthovanadate (Na3VO4). Vanadium can range in oxidation state from +2 to +5. It is found as Vanadium (IV/+4) (or vanadyl) in reducing environments. Vanadium (V/+5) prevails in aerobic environments, forming vanadate (H2VO4-) above pH 4 and VO2+ below pH 4. The sorption strength of the soil is the primary determining factor of the toxicity of vanadium. Vanadium adsorbs strongly to iron and aluminum oxides and hydroxides and can form complexes with organic matter like clays. When bound to organic matter, vanadium (V) can be reduced to less mobile Vanadium (IV). Vanadium adsorbed to these compounds is not bioavailable, so the total V content of the soil is not a good indicator of V toxicity. The amount of V in soil solution is a more accurate indicator that may benefit plant health at trace levels, but levels as low as 2 mg/kg can be damaging. Vanadium commonly occurs as vanadate, which is structurally similar to phosphate. It can be mistakenly substituted for phosphate and taken up by the plant, where it can inhibit vital metabolic processes and generate high levels of reactive oxygen species, which can damage cellular components; Vanadium can even damage chromosomes. However, most vanadium that enters the plant is deposited in the roots, and little V is transported to above-ground tissues.

## Tin - (Sn)

The average concentration of tin in soils has been reported to be approximately 1-10 mg/kg but can vary greatly depending on the parent rock. Tin may occur in a +2 or +4 oxidation state. Tin(II) is less stable than tin(IV) and can be oxidized to tin(IV) easily. Ionic tin forms anions at high pH values and neutral or positive cations at lower pH values. Sn2+ is only stable at pH values below 4. The toxicity of tin depends on whether it is organic (organotin) or inorganic salt. Tin salts are poorly adsorbed, although they can accumulate in root tissues. Soil concentrations above 10 mg/kg may cause toxicity in plants. Organotin compounds are typically more toxic than tin salts. They often enter the environment through human activities but have relatively short half-lives. Organotin compounds are hydrophobic and absorb metal oxides and organic matter like Humic acids and clay. Cation exchange and adsorption increase at lower pH values, reducing the amount of dissolved tin. 10 mg/L dissolved organic matter significantly reduces the bioavailability of organotin compounds.

## Silver - (Ag)

Silver is a minor constituent in the soil. Silver nanoparticles are widely used industrially and accumulate in sewage sludge, often used as an agricultural amendment. Ag preferentially binds to thiol groups, forming silver sulfide (Ag2S) nanoparticles poorly soluble in water. Silver may also form AgCl, metallic Ag (Ag0), and other species. Biological reactions in the soil may produce free Ag+ ions. High pH values and high cation exchange capacity create negatively charged binding sites for the positively charged Ag+, increasing Ag sorption. In addition, soils with more organic matter, finer soil texture, and more iron oxides adsorb more Ag than coarse, sandy soils. Dissolved Ag can be complexed by Humic and fulvic acids, significantly decreasing bioavailability. Ag mobility has been shown to decrease significantly in anaerobic conditions than in aerobic.



# Chromium - (Cr)

Chromium- (Cr) is a naturally occurring heavy metal contaminant--the 7th most abundant element and one of the more significant carcinogens--found in the environment throughout the planet Dual-valent chromium (VI) and trivalent chromium (III) are the predominant states of chromium present in the soil , while Cr (VI) has a higher biotoxicity than Cr (III); however, both are readily taken up by plants. Cr (VI) is transported into plants cells via sulfate chaperones; Cr(III) enters the plant cells through passive cation exchange sites among the cell wall. If left unmitigated, high concentrations of Cr inhibit morphological, physiological, and metabolic activities within the plant, ultimately resulting in plant death.

The distribution of Cr(III) and Cr(VI) containing compounds in the environment depends on the presence of oxidizing or reducing compounds, redox potential, the formation of Cr(III) complexes or insoluble Cr(III) salts, the kinetics of the redox reactions, pH, and the total chromium concentration. Both natural and anthropogenic sources contribute to total Cr toxicity in the environment. Mineral leaching accounts for the natural origin of Cr in groundwater that is dominated by Cr(VI). However, above 70% of total Cr in the environment is due to the anthropogenic pollutants from effluent streams from paper and pulp mills, non-ferrous metal smelters, leather tanning industries, refineries, releases from thermal generating stations, and urban stormwater runoff. In nature, Cr(III) predominates in soil and occurs in small amounts in rocks. Worldwide, the average concentration of 60 mg/kg. Biomass and root shoot length are reduced when high concentrations of Cr are taken from the soil. While Cr-induced alterations cause damage to roots, a worse outcome is insufficient water and nutrient uptake, that can lead to long-term damage to roots.

## Arsenic - (As)

Arsenic-Groundwater abundance and natural availability allow farmers to utilize groundwater irrigation and free farmers of rainwater dependence. Furthermore, rainwater cannot meet the high demands of present-day agriculture and the growing population. Although there are no recommended limits for vegetables, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have set thresholds for As in drinking water (10 ug/L) and irrigation water (100 ug/l). Although irrigation water is limited to 100 ug/L, As can still build up in crop soil which, in turn, increases the As density within the plant. Ultimately, the As build-up in the plant reaches the consumer's plate and contributes to dietary health hazards. Hyperaccumulation of As in the soil potentiates a change in overall soil quality, and these trace elements in soil and plants alter food quality and safety.

## Cadmium - (Cd)

Cadmium enters the soil primarily through anthropogenic inputs. Industrial contaminants, mining wastes, sewage sludge, and even manure may contain significant amounts of Cd. In addition, specific sources of soft rock phosphate fertilizers have been shown to contain 50-100 ppm Cd. The toxicity of Cd is determined by soil characteristics such as pH, organic matter content, clay content, and cation exchange capacity (CEC). pH is considered to be the most important of these factors. Cd uptake is facilitated by lower pH values, whereas high pH values can buffer Cd toxicity by favoring adsorption of Cd to soil colloids. Organic matter in the soil increases the CEC, providing more sites for Cd adsorption. Adding organic materials to the soil may decrease Cd availability. In a study of wheat, the authors found that a 5.0% amendment of biochar decreased Cd uptake and significantly reduced the accumulation of Cd in shoots by 47%.