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COPPER

NUTRIENT AND TOXIN IN PLANTS

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Abstract

Copper is a heavy metal playing a role in several processes. Till 1930 there was no evidence of its importance to plants. After this, it had become an official essential element to plants. Although the concentration needed of copper is very small, it is present in various enzymes and acts out multiple different roles of action. What happens when there is too much or too little of the metal? All plants react differently to a deficiency of copper. But there are some overall effects. Copper toxicity is becoming a real problem in areas where there is copper pollution. Due to excessive fertilization or use of fungicides, a buildup of copper is occurring in the agricultural soil. This leads in some cases to food crops with more copper in their systems, but still the concentration of copper in the plants is not correlated with the concentration in the soil. Toxicity can lead to a decrease in biomass production and photosynthetic capacity. To get rid of excessive amount of copper we can use phytoremediation.

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Introduction

"The stimulating effect of copper on plant growth was noted early in the use of copper salts as fungicides. A few years ago Felix, 1927 obtained improvement in the growth of certain plants on several peat soil by the application of copper sulfate, both to the soil and in solution to the leaves. Allison, Bryan and Hunter were able, by the use of copper sulfate, to produce crops on certain otherwise unproductive peats of the Florida everglades. Bryan, 1927 also obtained greening in chlorotic leaves of plants grown in this soil by treating them with solutions of copper sulfate. These investigations do not furnish final proof, however, that copper is essential to plant growth. The work reported in this paper provides additional evidence on this point." (Sommer 1930).

A. L. Sommer was the first one to distinguish copper as a micronutrient for plants. The element is essential for plant growth and photosynthesis, but they need a very small amount.

Image 1: Experimental design A. L. Sommer.

For a long time it wasn't clear to researchers what copper has to offer for plants and how they process it. This bachelor thesis will include how plants take up copper, and use it in their system.
Pollution is not something new. Industrial areas, agriculture, and areas under anthropogenic pressure have a great deal in pollution numbers. Contamination with, for instance, heavy metals can be dangerous to human and animal life and that's where we have to be careful. How is copper present in the soil, and how did it get there in such high concentrations. Earlier there was not much copper in the soil. That is when plants adapted to have copper taken up as efficiently as possible. Now, when there is plenty of copper, due to pollution, plants are rather fast intoxicated with the heavy metal. What are the effects of copper toxicity?
Still there are soils where copper has completely weathered out of the upper layers. This is where deficiency takes place. What are the effects on plants when there is a deficiency of copper? And can we do something about this ongoing buildup of copper in agricultural and industrial areas.

How do plants process copper and which functions does it perform?

What are the key enzymes in which copper is present?

What are the effects of pollution? And what are the effects of toxicity of copper on plants?

How can we treat copper pollution?

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Main contributions of copper in plants

Copper is one of the oldest known metals. The use of copper dates back to 5000 BC and it occurs naturally in the cuprous (Cu^{+}) and cupric (Cu^{2+}) valence states. The ionization state depends on the physical environment, the solvent and the concentrations of ligands present. The cuprous state is more commonly found in minerals at considerable depth, whereas the cupric state is found closer to the earth's surface (Cotton et al. 1999).

The metal was identified as a plant nutrient in the 1930's by A.L. Sommer. So despite copper being essential to the plant, it can definitely be toxic in higher concentrations (Delas 1963). The uptake of copper depends on several circumstances. Namely the pH of the soil, the concentrations of copper itself present and the prevailing chemical species. Once the copper is inside the plant, it is mostly immobile (Shahbaz. 2011).

Uptake and transport

The uptake rate of copper is the lowest of all essential elements (Kabata et al. 2001). Copper is mainly inaccessible due to presence predominantly in insoluble form. It is available at a pH of 6.0 or lower, because of the increase of Cu^{2+} ions in the soil under more acidic circumstances. Most sources report availability of copper in soils to decrease above pH 7.0. This is due to copper binding way stronger to soil materials in a higher pH environment (Adriano 1986). Copper has limited transport in plants, therefore the highest concentrations are found in root tissue. It most likely enters the roots in dissociated forms, but it is present in the roots as a complex (Liu et al. 2001). Here it is bound to the cell walls due to its affinity with carboxylic, carbonylic, phenolic and sulfhydryl groups, ionic bonds with negatively charged sites and N, O and S bonds in cells.

The movement of copper among various parts of plants plays a predominant role in the plant's utilization of copper. The strong capability of root tissue to hold copper against the transport to shoots had been observed. The direct mechanism is yet not clearly understood, but is can be concluded that the excretion of copper form root cells into the xylem and phloem saps, where copper occurs in mobile forms is a key process in the copper nutrition of plants. The copper mobility within plant tissues strongly depends on the level of copper supply. Only small amounts move to young organs of the plants, this is also the place where deficiency if first discovered (Kabata et al. 2001). The distribution of copper within plants is highly variable. In roots, it is mostly immobile and with cell walls. In leaf and shoot tissue the concentrations are highest in phases of growth. In the green tissue of the plant most copper is present in plastocyanin. Plants also tend to have a copper supply in reproductive organs, but this varies widely among plants (Kabata et al. 2001).
The uptake and transport of copper in plants and its subcellular distribution and homeostasis are mediated by high affinity Cu transporters (COPT Transporters, other metallotransporter proteins Cu-transporting P-type ATP-ases, and chaperone proteins such as ATX, CCS, and COX17-like chaperones.) The translocation of Cu to above-ground parts seems to be well regulated as it is not correlated to the Cu concentration in the root environment (Shahbaz 2011).

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Metabolic processes

The amount of copper accumulated in plants is in no way correlated with the concentration in the soil. Every species has a different rate of copper levels. Accumulation of copper can be influenced by many competing elements. Copper uptake in lettuce in nutrient solution culture was affected by free copper ion activity, pH of the solution and the concentration of Ca^{2+} (Cheng et al. 2001).
Iron and copper metabolism seem to be associated in plants and in yeast. Ferric-chelate reductase is expressed on the root surface of plants and the plasma membrane of yeast under conditions of iron deficiency. Ferric reductase reduces Cu^{2+} in yeast and this may be involved in copper uptake (Lesuisse et al. 1992). Increases in manganese, magnesium, and potassium accumulation were associated with iron deficiency in pea, suggesting that plasma reductase may have a regulatory function in root ion-uptake processes via their influence on the oxidation-reduction status of the membrane. Evidence of this was found in a copper sensitive mutant of the mouse-ear cress, suggesting that defects in iron metabolism may influence copper accumulation in plants (Vliet et al. 1995).

Copper deficiency

Deficiencies in copper are mainly found on sandy soils that have been highly weathered, on mineral soils with high organic matter and on calcareous mineral soils (Kopsell et al. 2006). Deficiencies of micronutrients have increased in some crop plants due to increases in nutritional demands from high yields, use of high analysis fertilizers with low micronutrient quantities, and decreased use of animal manure applications. Copper deficiency symptoms appear to be species specific and often depend on stage of deficiency. In general, the terminal growing point of the plants begin to show deficiency symptoms first. This is a result of the immobility of copper in plants under low concentrations. Most plants will exhibit rosetting, necrotic spotting, leaf distortion and terminal dieback (Reuther et al. 1966). Many plants will also show a lack of turgor and discoloration of certain tissues. It limits the activity of many plant enzymes, including ascorbate oxidase, phenolase, cytochrome oxidase, plastocyanin and superoxide dismutase. Oxidation-reductions reactions are as earlier encountered very important. Copper deficiency also depresses carbon dioxide fixation and electron transport (Kopsell et al. 2006).

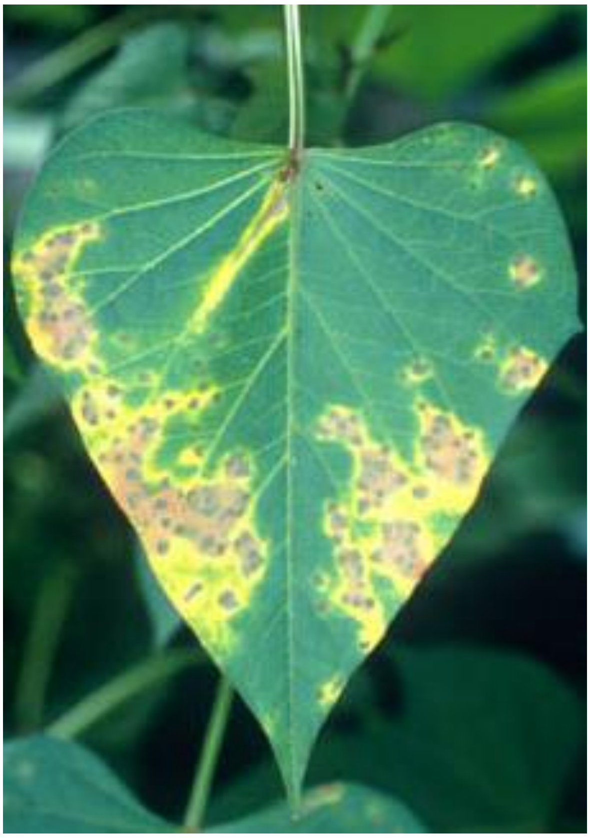

Image 2: Visible effects of copper deficiency.

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Enzymes in which copper is present

Copper is essential for plant growth and activity of many enzymes. But it has the lowest requirement among all elements. Cu is a component of photosynthetic electron transport (plastocyanin) mitochondrial respiration (cytochrome-C-oxidase), cell wall metabolism (amine oxidase), hormone signaling, thylakoids, and reactive oxygen metabolism (superoxide dismutase). The reversible oxidation-reduction of Cu makes it very valuable as a cofactor in enzymes. The Cu needs a way to get to the enzymes, this is done by copper chaperones. Every protein has its own chaperone for delivery of copper.

PLASTOCYANIN

This is a copper-containing protein, or called a blue copper protein, involved in electron transfer. It belongs to the plastocyanin family of copper-binding proteins. It is present in different organisms, like plants, algae and cyanobacteria. Plastocyanin is a monomeric molecule, with a molecular weight of 10.500 Da. This molecule differs among the organisms in which it is present, but the copper-binding site is conserved most of the time (Freeman et al. 2001). In photosynthesis, the molecule functions as an electron transfer agent between cytochrome F from photosystem II and P700+ from photosystem I. These are both membrane bound proteins with exposed residues on the lumen-side of the thylakoid membrane of chloroplasts. Cytochrome F acts as an electron donor and P700+ accept the electrons from reduced plastocyanin (Freeman et al. 2001). The molecular surface of plastocyanin differs for plants, algae and cyanobacteria, but the structure of the site, which binds copper is conserved throughout the kingdoms. The shape is described as 'distorted trigonal pyramidal' which is shown in the image below. The surface of the protein will vary slightly, but all plastocyanin types have a hydrophobic surface surrounding the exposed histidine in the copper-binding site.

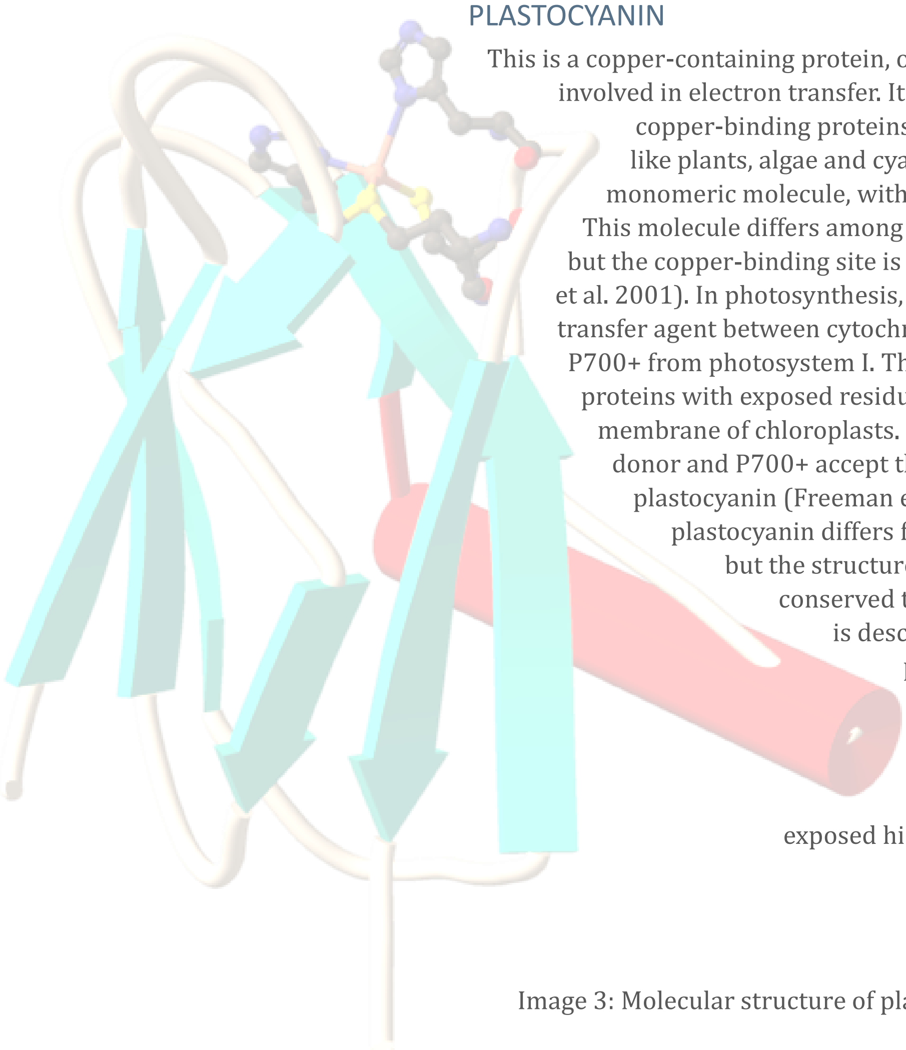

Image 3: Molecular structure of plastocyanin.

SUPEROXIDE DISMUTASE

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In general superoxide dismutase is an enzyme that catalyzes dismutation of oxygen radicals into normal ordinary oxygen. These radicals, O_2- (Superoxide) are a byproduct of the oxygen metabolism and cause major damage to all cell types. So superoxide dismutase is a very important antioxidant defense in nearly all living cells exposed to oxygen(Hayyan et al. 2016).

Superoxide dismutases were previously known as a group of metalloproteins with unknown function. Later on there were three families distinguished. Depending on protein fold and the metal cofactor: Cu/Zn, Fe/Mn and Ni. The first one has copper as a cofactor and will be further investigated.

Image 4: The molecular structure of Cu/Zn SOD.

The Cu/Zn type is most commonly used by eukaryotes. All the cytosols of eukaryotic cells contain a superoxide dismutase containing copper and zinc (Corpas et al. 2006). They act as antioxidants and protect cellular components from oxidation by 'reactive oxygen species' ROS. ROS can form due to drought, injuries, ozone, nutrient deficiencies, toxic metals and UV radiation. Superoxide can fragment DNA, denature enzymes and oxidize many lipids (Smirnoff 1993). When this oxidative stress rises, the levels of SOD follow. Because plants have many types of SODs, they can very effectively treat this stress. The Cu/Zn SODs are mainly concentrated in the chloroplasts and the chloroplasts and cytosol, but they provide less protection than FE SODs (Smirnoff 1993). Cu/Zn SOD is responsible for the rapid two-step dismutation of superoxide to molecular oxygen and hydrogen peroxide. The enzyme uses alternate reduction of the active-site copper (Tainer et al. 1983). The metalloprotein contains copper and zinc ions in each subunit. The subunits are composed of beta strands. Cu/Zn takes two molecules of superoxide, gets the extra electron from the other. In this scenario, you'll end up with an electron less, forming normal oxygen. And the other with the extra electron will pick up two hydrogen ions, to form hydrogen peroxide. The last is also dangerous, but is detoxified by the enzyme catalase (Smirnoff 1993). This enzyme uses CCS as a copper chaperone. The activity of SOD is thus not influenced by the availability of copper, but by the ability of CCS to have copper transported (Nevitt et al. 2012).

OTHER PROTEINS CONTAINING COPPER

Amine oxidase

These enzymes belong to a family of amine oxidase enzymes, which includes both primary-amine oxidase and diamine oxidase. They catalyze the oxidation of a wide range of bioorganic amines including neurotransmitters, histamine and xenobiotic amines. Copper-containing amine oxidases are found in bacteria, fungi, plants and animals and it participates in 8 metabolic pathways. It has 2 co-factors copper and TPQ (topa quinone). Some of the biological processes in which the protein is present are cell differentiation, wound healing, detoxification and signaling (Guss et al. 1996).

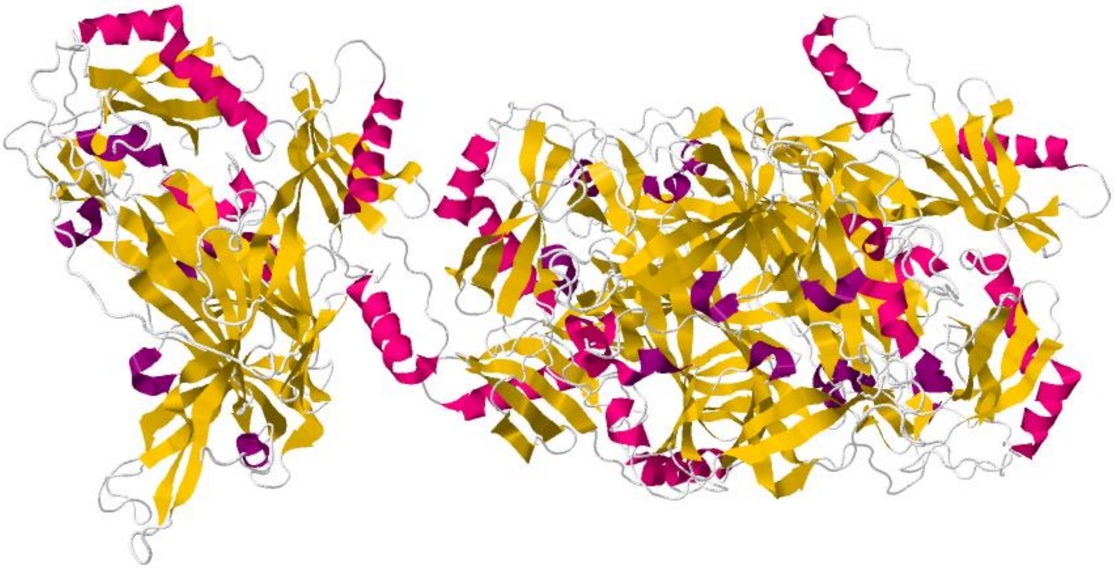

Image 5: The molecular structure of amine oxidase.

Cytochrome C oxidase

This is a mitochondrial metalloenzyme acting as the terminal enzyme of the mitochondrial respiratory chain. It is placed on the membrane of mitochondria. The catalytic core of COX is formed by three mitochondrial-encoded subunits and contains three copper atoms. In this enzyme the copper is an electron acceptor. Copper gets to the enzymes with a copper chaperone, COX17P.

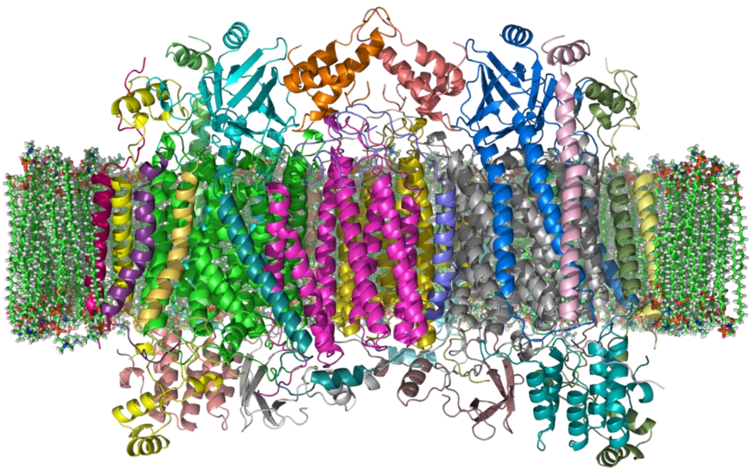

Image 6: The molecular structure of cytochrome C oxidase.

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Ecological problems concerning copper

Extensive use of copper-containing fungicides in areas and contamination due to mining activities have led to problems of toxicity in some agricultural regions. Because of this problem, remediation of copper and identification of tolerant species are receiving increased attention (Kopsell et al. 2006).

COPPER POLLUTION

Metal contamination issues are becoming increasingly common in India and elsewhere, with many documented cases of metal toxicity in mining industries, foundries, smelters, coal-burning power plants and agriculture. Heavy metals such as cadmium, lead, chromium, mercury, and copper are major environmental pollutants, particularly in areas with high anthropogenic pressure. Heavy metal accumulation in soils is of concern in agricultural production due to the adverse effect of food safety, crop growth due to phytotoxicity and environmental health of soil organisms. The influence of plants and their metabolic activities affects the geological and biological redistribution of heavy metals through pollution of the air, water and soil.

Before we can discuss the topic of copper pollution, we have to know how the element is distributed in the soil. Copper is one of the most versatile in its ability to interact with soil mineral and organic components. Copper can occur as ionic and complexed copper in soil solution, as a cation or absorbed ion, complexed in organic matter, occluded in oxides and in minerals (Barber 1995).

Copper exists mainly as Cu I and Cu II, but it also can occur in the metallic form or in ores. In minerals it is present as sulfide minerals, silicates, sulfates and carbonates. The most abundant mineral is chalcopyrite. (Kabata et al. 2001) because copper ions readily precipitate with sulfide, carbonate and hydroxide ions, it is rather immobile in the soil. For most agricultural soils, the bioavailability of Cu^{2+} is controlled by adsorption-desorption processes. Variable-charge minerals such as iron, manganese and aluminum oxides can carry varying degrees of positive or negative charges depending on soil pH. Therefore, adsorption of Cu^{2+} in variable-charged soils is pH dependent (Kopsell et al. 2006). Cu^{2+} dominate below a pH of 7.3, where $CuOH^{+}$ is most common at pH 7.3. The concentration of total soluble copper in the soil solution influences mobility, but the concentration of free Cu^{2+} determines the bioavailability of copper to plants and microorganisms (Bolan et al. 2003).

Adsorption mechanisms of Cu have been extensively studied by many scientists and there are a lot of papers available. Nonspecifically adsorbed Cu due to ionic bond formation is an easily exchangeable cation. The specific sorption of Cu is related to the reaction with electron-pair donors and forms rather strong bonds of a high covalency (Kabata et al. 2001). The greatest amounts of adsorbed copper have always been found for Fe and Mn oxides, amorphous Fe and Al hydroxides and several forms of clay. Occlusion, coprecipitation and substitution are involved in nonspecific adsorption on copper, but chelation and complexing are the key reactions regarding copper behaviour in most soils. Chelation is a type of bonding of ions and molecules to metal ions. It involves the present of two or more separate coordinate bonds between a polydentate ligand and a single central atom (IUPAC definition of Chelation). To conclude, organic matter modifies multiple reactions of copper with inorganic soil components. Organic complexing of copper has a prominent practical implication in governing the bioavailability and the migration of copper in the soil. The bioavailability of soluble forms of copper depends mostly on the molecular weights and the amounts present. In summary, the concentrations of copper in soil solutions are principally controlled by both the reactions of copper with specific substances and its own concentration (Kabata et al. 2001).

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USE OF COPPER IN FERTILIZERS

The addition of copper to cultivated soils with applied fertilizers, chemical and wastes has recently been extensively investigated. In the surface soils of vineyards has an extreme buildup of copper arisen. After 100 year of use of the Bordeaux mixture, a copper based fungicide (Kabata et al. 2001).
Application of copper to agricultural land is not necessary every year and residual effects of copper have been reported up to 12 years after application. Contamination of soils with copper happens mostly when there is too much fertilizer used. Besides that, copper hydroxide is the most used fungi- and bactericide for tomato diseases. Further on I will discuss this phenomenon.

Bordeaux mixture

The also called 'Bordo Mix' is a mixture of copper sulfate and slaked lime. It was used for treating mildew, and thus a fungicide. The mixture was invented in the region of Bordeaux in the late 19th century to treat vineyards, fruit-farms and gardens.
It achieves effect by means of the copper ions, they affect enzymes in the fungal spores to affect germination. It can be seriously harmful to fish, livestock and earthworms. It can be seen that not only the Bordeaux mixture is a possible risk in pollution, but also other fungicides containing copper. The ions of copper are the ones that treat the plants but also the ones that can be harmful. They are washed of the leaves by rain for instance and then reach the soil (Pears et al. 2005).

Image 6: Use of Bordeaux mixture on grapevines.

TOXICITY OF COPPER

Plants like all other organisms possess homeostatic mechanisms to maintain the correct concentration of essential elements in different cellular compartments and to minimize the damage from exposure to enhanced metal ions (Shahbaz 2011). Once inside the cell, the right amounts of copper have to be transported to the cellular compartments and to proteins. Higher levels of metal ions have to be rendered innocuous either by timely secretion, compartmentation or complexation to avoid interference with normal cellular metabolism (Hall 2002).

The toxicity of copper depend on the concentrations of Cu, growth stage and the duration of the exposure. In living organisms, the redox active metal is present both in Cu^{+} and Cu^{2+}. Excessive free Cu ions may catalyze the formation of highly toxic hydroxyl radicals or superoxide anions. These reactions can cause oxidative stress, lipid peroxidation, protein denaturation and DNA mutation (Shahbaz 2011). Excessive Cu ions can be phytotoxic and generally result in stunted root growth and shoot development and leaf chlorosis (Kopsell et al. 2006). The phytotoxicity of Cu is mostly due to its possible reaction with thiol groups of proteins and glutathione. Toxicity of copper can be caused by limited uptake of other essential elements, such as Fe. Higher levels of Cu can lead to injured plasma membranes, resulting in ion leakage from the roots (Hall 2002).

When an elevated concentration of copper in the roots is combined with UV-radiation, negative effects will show quickly. In Chinese cabbage this lead to a more rapid and stronger decrease in plant biomass production and pigment content. The enhanced Cu toxicity in the present of UV was largely due to a UV-induced enhanced accumulation of Cu in both root and shoots. An enhanced Cu content strongly affected the assimilation of sulfur in plants. It is suggested that high Cu tissue levels may interfere with signal compounds involved in the regulation of expression and activity of sulfate transporters (Shahbaz et al. 2012).

Ways of getting rid of built-up copper

It is in high favor of everyone to keep looking for ways to get rid of excess buildup copper in the soil. Scientists are currently researching many different copper removal methods. Some publications suggest that microorganisms assisted phytoextraction, using plants and bacteria to actively extract copper, is most promising. Still it remains a challenge to find plants that primarily accumulate copper in their shoots, which is a necessity when looking at time and money to treat the pollution.

Remediation efforts for heavy metals can be achieved in five ways: immobilization through pH alterations or manure addition, removal, sequestration, active mixing and plant uptake or phytoextraction (Mackie et al. 2012).
Altering pH to immobilize copper, called phytostabilization is often suggested in literature. This method requires addition of lime, and it is a temporary solution for the problem. Also manure can be used to limit free Cu^{2+} ion activity, but this increases bioavailability due to forming complexes with organic matter. Next, removal through washing with acids are not practical, either financially or logistically, for large areas of land that continue to be used for crops. Third, sequestration through exo-polymers has until now only been achieved under laboratory circumstances and it not ready to be used on large plots of land. Fourth, active mixture of the soil, looks like a viable solution, but can definitely not be used in vineyards, and fruit-farms, because it completely destroys the soil structure (Mackie et al. 2012).
Last, phytoextraction. This is the process of uptake of heavy metals by plants and seems most likely to be used in large quantities. After sufficient growth and metal accumulation in roots and shoots, plants are harvested. This is non-invasive and it retains soil fertility, is inexpensive and it utilizes the natural ecosystem (Mackie et al. 2012).

This kind of extraction requires hyper-accumulating plants(Pochenrieder et al. 2001). The plants itself can increase bioavailability of copper in many ways including, acidification of the soil and modification of redox potential. This can also be done in corporation with microorganisms. For this way of coper extraction to work, as many copper as possible has to be mobilized. This is then done by chelating assist and microorganisms (Mackie et al. 2012). *Brachiaria decumbens* is a high biomass plant with great potential for phytoremediation of copper-polluted soils. It was tested on vineyards soils and soils contaminated with copper mining waste. The plant exhibited high capacity copper phytoextraction in a vineyard soil and copper mining waste by the entire plant. This species showed no substantial depletion on macronutrient uptake or nutrient deficiency after growth in contaminated soils. Also there was shown greatest phytostabilization characteristics such as high shoots and root biomass production, and high copper bioaccumulation in the roots. For all characteristics summarized, *Brachiaria decumbens* can be an important tool for copper phytoremediation in copper contaminated areas and by this contribute to protect water quality and environment (Andreazza et al. 2013).

Conclusion and discussion

This bachelor thesis dealt with copper as an essential plant nutrient and how it behaves in the soil. What is does when it is inside the plant and in what enzymes it occurs. After that there was room for the cause and solution for copper pollution and how plants deal with toxicity and deficiency of copper.

The bioavailability of copper depends on its mobility in the soil. The mobile copper is available and can be used by the plant. Copper has limited transport in plants and is thus mostly found in root tissue, but the mobility in the plant also depends on the concentration and is by that also found in leaf and shoot tissue under circumstances of fast growth. When a plant experiences deficiency, the effects differ from species to species.
There are multiple enzymes which incorporate copper, thus copper has a major role in metabolic processes and reaction in the cell. And by that it is essential for a plants being, also because of the antioxidant enzyme (superoxide dismutase) in which copper is present.
Due to excessive use of fungicides, pollution through industries and agriculture in some areas, the soil contains a very high concentration of copper. The concentration of free copper in the soil is responsible for its bioavailability and thus to its risk to get phytotoxic for plants. This can be dangerous for animals and plants and can lead to copper toxicity. The major effect of this toxicity result in stunted root growth and shoot development and leaf chlorosis. This can lead to complete die off of the plant.
Pollution with copper is a serious problem in agricultural areas, vineyards and fruit-farms. Due to the toxicity effects is it intensively researched how to get rid of copper in the soil. Phytoextraction seems to be the best option out of five. With this extraction method there is no need to have the soil completely mixed up, which is also impossible in vineyards and fruit-farms, because of the complexity of the soil. Phytoextraction or phytoremediation is based on the use of plants that hyper accumulate heavy metals and are harvested after growth. This is the method, which is the least invasive and uses the natural ecosystem. *Brachiaria decumbens* is a possible model organism when looking at a species to hyper accumulate copper, and thus a non-invasive and rather cheap way to get rid of copper in polluted soil.

Image 7: *Brachiaria decumbens.*

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