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COPPER: ITS BIOLOGICALROLE AND TOXICITY

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Universally the demand for copper is one of the largest next only to iron and aluminium. Copper occurs in the soil mostly in divalent form. The Cu ion is present as inorganic and organic positively charged groups and is dissolved in the soil system as Cu2+ and organic Cu complexes. The divalent Cu ion has a strong affinity to soil organic matter compared with other divalent cationsin order of following sequence: Cu > Ni >Pb> Co > Ca > Zn >Mn> Mg. The Cu2+ concentration in the soil solution are governed by Cu adsorption to organic and inorganic soil particles. Copper can be released into the environment by both natural sources and human activities. When copper ends up in soil it strongly attaches to organic matter and minerals. Copper can seriously influence the productivity of certain farmlands, depending upon the acidity of the soil and the presence of organic matter. Under physiological conditions, the metal Cu can be converted to two common forms, the reduced Cu (I) state and the oxidized Cu (II) state. Mechanisms of Cu2+ion toxicity with relation to proteins, enzymes, nucleic acids and metabolites leads to significant changes in them and decreased feasibility.Similarly, an effect of toxicity is also observed in respiration. Copper is mandatory for the growth and metabolism of microorganisms since it is a cofactor for numerous enzymes. Under anaerobic conditions, the conversion of Cu2+ to Cu+ can be responsible for decreased survival of bacterial species. The plants grown under copper deficiency also show impairment in the photosynthetic transport chain. Copper toxicity can occur from the repeated use of fungicides that contain copper. The symptoms of copper deficiency are cupping and slight chlorosis of either the whole leaf or between the veins of the new leaves. Recently, synthesis of copper nanoparticles is being carried out using chemical and biological methods. The impact of copper nanoparticles depends on size, component and have some adverse result. However, the potential risk of use of copper nanoparticles must be assured before releasing them.

Keywords: Copper, Bioremediation, Toxicity

Copper is naturally available in trace amount in different sources like oceans, lakes and rivers all over the earth.Routine function of plants, animal and water organisms require copper(Rogers *et al*. 1985).Copper isan essential element for the human beings for their growth and metabolism.

India ranks 20th in the world, as a major producer. Copper in India is mostly used in telecom and electric division and also its demand is in building and construction, engineering, transport and consumer durables sectors (Kabwe and Yiming 2015).Due to the industrial activities copper becomes hazardous in environment (Chaney *et al*. 1997).Approximately 5-25 mg/kg copper is acceptable amount in plants and their tissues. Copper toxicity inhibits iron metabolism and the activities of many enzymes (Hou *et al.* 2007).

Environmental forms of copper

Copper is present in the soil in Cu^{+2} form. Copper is present normally as a stone lattice or in a mineral form. Soil organic matters bound the Cu in high amount. The Cu ion can bind within organic and organic form with negatively charged groups and is soluble as Cu^{2+} and Cu form in soil. Copper is normally bound with carbonates, organic matter, silicates and oxides of Al, Fe, and Mn (Reed and Martens 1996).The amount of Cu^{2+} in the soil leachate decreases sharply when the pH increases, however the organic Cu complex in the soil are not much dependent on soil pH. An organic Cu complex decreases when pH is very high. The Cu^{+2}present in organic matter of soil with other divalent cations is found in the following sequence: Cu > Ni >Pb> Co > Ca > Zn >Mn> Mg (Schnitzer and Skinner 1965, 1967).Copperis bound with chelate form to organic matter by covalent and coordinative bonds. Copper ion is bound with coordinative bond atom in S >N > O. As N is higher in amount in organic soil than S, N atoms can take part in quantitative terms to chelate Cu^{2+}.The large quantity of Cu^{2+} in organic soil is bound with covalently to carboxylic groups to make

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humic and fulvic acids.The Cu^{2+}chelate ion is stronger in comparison with other monodentate binding of Cu^{2+} and divalent cations (Carter, 1989).The bonds formed by $Cu^{2+}$are by far the strongest than any other divalent cations or carboxylate group (Schnitzer1965 and Skinner, 1967).

Cu is maintained in the soil by soluble Cu^{2+} salts like carbonates and oxides which are present in large amount in the soil. The Cu adsorption to organic and inorganic soil particles is directly dependent on Cu^{2+} concentration in the soil solution (Carter 1989). Cu is immobile in soils and not present in deeper soil layers and hence restricted to upper layer of soil horizon (Marcel and De La Salle, 1956).Thisleads to decrease in Cu concentration in soil profile. Copper can be reduced by strong acid or organic matter which can bind the Cupric salts. KCN is a stronger chemical for extracting Cu from soil compared to other chemicals because it can make complex with Copper in soil (Berringer 1963).

In the environment Copper can occur naturally like wind-blown dust, decaying vegetation, forest fires and sea spray and due to anthropogenic activities such as mining, metal production and phosphate fertilizer production (Hou *et al.* 2007, Demirevska-kepovaet al. 2004).Mostly copper settles in water or soil through binding or sedimentation. When soluble copper is released in the environment after its use in agriculture it directly or indirectly affects the human health (Davenport and Prusak 1998).When copper is released in soil it binds with organic matter and minerals present in soil, so it does not travel to groundwater but in surface water it can travel large distances, suspended in sludge particles or as free ions (Carter 1989).Copper does not degrade in the environment and it can be taken up by plants and animals from soils. In the copper-rich soils, vegetation does not survive (Davenport and Prusak 1998)and plant diversity is not found near copper-using factories. High amount of copper in farmlands may cause serious threat to plant survival depending on level of soil acidity and organic material present. Despite such threat, copper-containing manures are still applied (Carter 1989).

Biochemistry of Copper

Copper is absorbed in huge amount by plant root and negligible amount by shoots and leaves (Mattioni *et al.* 1997).Copper is an essential micronutrient for plants (Davenport and Prusak 1998) and plays important role in CO_2 assimilation and ATP synthesis. Cu is an essential component of various proteins like plastocyanin of photosynthetic system and cytochrome oxidase of respiratory electron transport chain (Shan *et al.* 2012).

In physiological State, Cu exists usually in two forms, the reduced Cu (I) state and the oxidized Cu (II) state. Reduced form of Cu attaches with sulphurcompounds like a thiol or a thioether group, and oxidized form of cubinds with oxygen or imidazole nitrogen groups (Cohu and Pilon 2010).This dual state of Cu allows it to combine with a huge range of molecules, in fastidious proteins, for biochemical reaction or for stabilizing structural features (Festa and Thiele 2011). Redox form of free Cu can directly bind to reactive oxygen species (ROS) by Fenton chemistry breaking down proteins, DNA and other bio molecules (Hänsch and Mendel 2009).

Cu^{2+} toxicity is connected with ions, proteins, enzymes, nucleic acids, metabolites and reducesthe possibilityof respiration.Most of the reduction in raw nutrient is a conversion in the presence of $CuSO_4$ (Gibbs *et al.* 2000).

The standard oxidation states of copper are (Hänsch and Mendel 2009):

Copper (0): metallic copper; Cu°.

Copper (I): cuprous ion; Cu+, unstable at neutral pH, oxidized to Cu^{2+} by air.

Copper (II): cupric ion; Cu^{2+}, stable form Cu (OH) 2 in water at alkaline pH.

Microbial Interactions with Copper

Microbial interactions with copper in

environment, exist from time immemorial. In medicine metallic copper was used, because of antibacterial properties, till the antibiotics became commercially available. In environment bacteria is responsible for bio-corrosion and leads to mechanical failure of metals in many applications(Haas *et al.* 2018).

Copper is cofactor for numerous enzymes and minute concentrations are necessary for the growth of microorganisms. Copper containing Proteins are electron carriers and Azurin plays an important role in the oxidation of iron (Fe) in *Thiobacillus ferrooxidans.* The blue copper protein of *Thiobacillus versutus* is an electron carrier between methylamine dehydrogenase and cytochrome. Methane mono-oxygenase, the enzyme responsible for the conversion of methane to methanol in *Methylosinus cycloclastes,* has one Fe and one Cu atom per enzyme moleculeand the superoxide dismutase of the marine *Photobacterium leiognathida* contains a copper atom. Copper is essential in microbial cells in minute amount; whereas higherconcentrations has a toxic poisonous effect (Haas *et al.* 2018).

The toxic form of copper is generally Cu^{2+}. In anaerobic conditions, Cu^{2+} is converted into Cu+decreasing the growth of bacterial species (MacLeod *et al*. 1967).The pH, redox potential (Eh), moisture, temperature, copper binding to environmental constituents and interactions with other ions are the main factors for the copper toxicity (Ashish *et al.* 2013).Copper toxicity is not limited to areas of high copper pollution. The presence of 0.006 mg Cu^{2+}/ml distilled water caused decreased feasibility of growth of *Aerobacter aerogenes* (Neelima and Reddy 2002) and *Klebsiella aerogenes* and survival was inhibited at 10-8 to 10-6 M Cu^{2+} (Haas *et al.* 2018).

Bioremediation is used for the removal of contaminants from environment and eco system. Bioremediation of copper using microbes is an important approach. In bioremediation, microbes employ the biological mechanisms and eliminate hazardous pollutants and renovate the ecosystem to its original condition (Neelima and Reddy 2002).The siderophores are iron-chelating compound which develop the mobility and reduce bioavailability of copper and its subsequent removal from soil. Biosorptive abilities of microbial biomass vary within groups but the biosorption competence of each biosorbent is affected by prehistory and pre-treatment, as well as the experimental conditions. Gram-positive bacteria cell walls contain peptidoglycan layers which have the amino acids alanine and glutamic acid, also meso-di-aminopimelic acid and teichoic acid. Gram-negative bacteria cell walls contain enzymes, glycoproteins, lipopolysaccharides, lipoproteins and phospholipids (Gupta *et al.* 2016).The amino acids and enzymes of the cell wall are the active sites for binding processes in bacteria (Haas *et al.* 2018)and act as legends for binding metal ions, remediate the contaminated environments. Microbial remediation is explained as the use of microorganisms to perform the absorption, precipitation, oxidation, and the reduction of metals like copper in the soil (Gupta *et al.* 2016). Microorganisms contain in conceivable metabolic pathways which use various toxic compounds as a source of energy for growth and development, during respiration, fermentation, and co-metabolism. Due to their characteristic derivative enzymes for a particular contaminant, they have evolved diverse mechanisms for maintaining homeostasis and resistance to copper, in order to adapt to toxic copper in the ecosystem (Hass *et al.* 2018).

Copper : Everyday Uses

In a normal state, copper does not exist inpure form (Ashish *et al.* 2013).This metal is highly conductive for electricity and heat. Many electronic parts and wiring are made up of copper. Copper is also used for making traditional cooking pots. This metal is resistant from corrosion and has found manifold applicationsas bronze and brass (Neelima and Reddy 2002). The metal is also used along with silver and gold, because of its properties.

Table 1: Different alloys of of copper (Mengel *et al*, 2018)

Copper(70%)+ Zinc(30%)= Brass	Brass is used where low friction is required such as locks, gears, bearings, door knobs, ammunition casings and valves; as well as for plumbing and electrical applications like water tap, showers; and extensively in brass musical instruments such as horns and bells
Copper(90%)+ tin (6%) + zinc(4%) = Bronze	Bronze is using in making of sculpture, musical instrument, cookware, food serving plates, coins and medals.
Copper(90%) + Nickel (10%)= Cupronickel	Cupronickel is widely used in Marin because it is resistant to seawater corrosion, low macro fouling rates, and better fabric ability to work in sea water.
Copper(89%) + Aluminium(11%) = Aluminium Bronze	Aluminium Bronze is also use as Bronze in making of sculpture, mirrors, musical instrument, coins and medals.
Copper(99%)+Arsenic(0.5%)=Arsenical Copper	Arsenical Copper imparts higher tensile strength and reduced tendency to scaling.
Copper(30%) + Tungsten(70%) = Copper Tungsten	Copper Tungsten has higher thermal conductivity, low thermal expansion and high arc resistance combined with good electrical conductivity.
Copper(88%) + Tin(8-10%) + Zinc(2-4%) = Gunmetal	As its name gunmetal is originally used chiefly for making guns, it has largely been replaced by steel. Gunmetal, which casts and machines well and is resistant to corrosion from steam and salt water, is used to make steam and hydraulic castings, valves, gears, statues, and various small objects, such as buttons.

Copper has a variety of application ranging from coins to pigments and its demand is high, especially in industrialized area (Ashish *et al.* 2013). 60% copper is used for making electrical tools, 20% for roofing and plumbing and in industrial machinery, 15% in heat exchangers and 5% in alloys. The important copper alloys are bronze, brass (a copper-zinc alloy), gun-metal (copper-tin-zinc) which is strong enough to make guns and cannons, and (copper and nickel) cupronickel used for low-denomination coins. Copper is perfect for electrical wiring because it can easily be drawn into fine wire and has a high electrical conductivity (Haas et al. 2018).

The human beings have about 70 to 80 mg of copper. Copper is macro nutrient forhuman being and it helps the iron metabolism and the formation of red blood cells (Stern et al. 2007).Two copper-containing enzymes, ceruloplasmin (feroxidase I) and (feroxidase II) have the capability to oxidize iron(Fe^{2+}) to iron (Fe^{3+}), which are connected to the protein transfer in for transportation to the red blood cells and blood formation. There is heredity link of copper levels in children and their parents (Stern *et al.* 2007).Another copper enzyme, lysyl oxidase, participates in cross-linking of collagen and elastin, which form the connective tissue. The lysyl oxidase helps in maintaining the integrity and elasticity of connective tissue in the heart and blood vessels and also plays a role in bone formation (Stern *et al.* 2007).

Copper enzymes catalyze the enzymatic reactions for the proper functioning of the brain and the nervous system (Singh and Tewari 2003. Dopamine-beta-mono oxidise catalysed the conversion of the neurotransmitter dopamine into nor-epinephrine (Stern *et al.* 2007).Monoamine oxidase (MAO) takes part in the metabolism of the neurotransmitters norepinephrine, epinephrine and dopamine. MAO utilise in the breakdown of the neurotransmitter serotonin, which justifies the use of MAO inhibitors as antidepressants (Romero *et al.* 2002). Phospholipids made Myelin sheath which synthesis the activity of the cytochrome-c oxidase copper enzyme. Copper enzyme tyrosinase catalyres the formation of the melanin pigment. Melanin is produced in cells, called melanocytes, and plays a role in the pigmentation of hair, skin and eyes (Singh and Tewari 2003).

Role of Copper in Plant Biology

In the environment, oxygen can increase due to the rise of photosynthetic organisms. This oxidative atmosphere may lead to decreased solubility of iron and the formation of iron oxides and to the continuous liberation of the soluble Cu (II) from insoluble Cu sulphide salts (Burkhead *et al.* 2009). Iron substituted by Cu can perform functions similar to iron. This explains why many Cu-proteins have a purposeful counterpart that uses Fe as cofactor and why growth on a substrate with a toxic Cu level is commonly linked to a decreasein Fe-content in roots and leaves (Burkheadet al. 2009). Plant phenotypes associated with Copper toxicity appear similarto Fe-deficiency, such as leaf chlorosis, decreased leaf chlorophyll content and enhanced oxidative tension (Pätsikkä *et al.* 2002).However, copper deficient plants develop chlorotic symptoms that show first at the tip of the young leaves followed by necrotic lesions (Singh and Tewari 2003). Copperdeficient plantsexplain destruction in the photosynthetic transport chain and a reduction in non-photochemical extinguish, and lack of plastocyanin (PC) function (Ghany and Pilon 2008).The main functions of copper are the transport of electrons in mitochondria and chloroplasts. However, in microorganisms the most profuse copper protein is plastocyanin, a photosynthesis-related protein mixed up in the transfer of electrons from cytochrome f to P700+, they have power over of the cellular redox state (a major Copper-binding protein is the Copper/Zinc superoxide dismutase) and remodelling of the cell wall (Cohu and Pilon 2010).

Toxicity of Copper

In metallic state Copper is not toxic, however, some of its salts are toxic. The most common salts of copper are the sulphate or the blue vitriol and the sub-acetate or Verdigris(Stern *et al.* 2007). Copper sulphate is a crystalline salt with blue colour and metallic taste in a small dose of 0.5 g it acts as an emetic but in large doses, as nuisance toxin produces gastric and intestinal frustration(Singh and Tewari 2003). At the time of cooking in Copper vessels it reacts with the vegetable acid and forms the blue-green salt. Copper compounds of Arsenic include Scheele's green (Copper arsenite), Paris green or emerald green (Copper Aceto-arsenite) (Kasana *et al.* 2017).High concentration of Copper is a strong inhibitor of enzymes. Poisoning effect of copper occurs within 15-30 minutes (Ashish *et al.* 2013).Copper toxicity affected the growth of Alyssum montanum (Ouzounidou 1994) cucumber (Moreno-Caselles *et al.* 2000) and Brassica juncea (Singh and Tewari 2003). Copper and Cdaffect the germination, plantlet length and number of lateral roots in Solanum melongena adversely (Neelima and Reddy 2002).

Soil cannot produce higher amount of copper but due to repeated use of fungicides copper toxicity in soilincreases (Ouzounidou 1994). A symptom of copper toxicity includes very small plants, bluish in colour, and ultimately turn yellow or brown. Copper Toxicity reduces the seed germination, plant vigour, and iron intake. It is a difficult task to decrease or neutralize the copper toxicity in soil because it is the insoluble metal in soil, which makes the soil unproductive for many years (Ouzounidou1994).

Copper Deficiency

Cu deficiency occurs because soils bind to Cu^{2+} strongly present in humus and engage the free Cu (Haas *et al.* 2018).Copper is a stationary (immobile) metal, deficiency symptoms can hence be seen in new leaves. Symptoms vary in different crops. Typically, the symptoms are cupping and a slight chlorosis in whole leaf or between the veins of the new leaves (Pherson *et al.* 2010). Within the chlorotic areas of the leaf and on the leaf margins small necrotic spots form and the new leaves become smaller in size, lose their sheen and in some cases the leaves may drop, the apical meristems turn into necrotic and die, the growth of lateral branches is inhibited (Kasana *et al.* 2017). Plants typically have a compressed emergence as the stem length linking the leaves becomes small. Colour of flower is repeatedly lighter than regular(Moreno-Caselles *et al.* 2000).Copper deficiency also occurs due to excess amount of potassium, phosphorus or other micronutrients present in soil(Rolff *et al.* 2008). Higher pH of the growing medium makes the uptake of Cu^{2+}hard by plants (Moreno-Caselles *et al.* 2000.

The implications of copper deficiency in the human beings are very diverse. The most common effects are anaemia, leucopoenia, bone lesions, and vesicle diverticulitis. In kids, some normally noted deficiency is hypotonic, psychomotor retardation and hypothermia (Keen *et al.* 1998).Iron deficiency is associated with copper deficiency like anaemia, which may be corrected by the copper supplements(Keen *et al.* 1998).Copper deficiency damages iron assimilation, decrease shemosynthesis and boosts the iron build up in storage spacetissues (Shibazaki *et al.* 2017). These processes are reliant on Ceruloplasmin in enzyme of copper. A constant copper deficiency can convert in hemosiderosis and iron accumulation in body tissues because hemoglobin iron cannot be reutilized (Pherson*et al.* 2010). Hemosiderosis diseases occur like malignancies, inflammatory disorders and the rheumatoid arthritis (Kumar *et al.* 2004).

The cytopaenia and neuropathy can occur due to lack of copper and treated by copper supplementation (Shibazaki *et al.* 2017).Copper deficient animals are characterized by increased small tissue copper concentrationsand researchers have studied that the female sheep should be given supplemental copper during beginning ofpregnancy. Other diseases and disorders like parallel neonatal ataxia and brain abnormalities have been reported in newborn copper-deficient goats, swine, guinea pigs, and rats and they also show smaller brain than normal one, collapsed cerebral hemispheres, shallow convolutions, and are hypomyelinated(Rogers *et al.* 1985).

Future prospects

In agriculture, nano technology has found wider applications in post-harvest storage of food products, but its application in improving crop productivity is recent one. At present attention is being paid to increase the crop yield through nano technological interventions by enhancing plant growth and reducing the impact of abiotic and biotic stresses. Copper, an essential micronutrient being incorporated into many proteins and enzymes, has a significant role in the health and nutrition of plants. Copper nano particles have improved properties compared to the bulk copper material and find applications like gas sensors, heat transfer fluids, catalysis, solar energy and batteries. Antibacterial and antifungal activities of copper nano particles have also found applications in the agriculture sector. The antimicrobial activity shown by copper nano particles against broad range of pathogenic micro-organisms both bacteria and fungi has created interest in assessing the role of nano particles for potential use in agriculture. Impact of nano particles on plants is dependent on many parameters starting from composition, concentration, size and physical to chemical properties of nano particles as well as plant species (Shalaby *et al.* 2016). Bacterium belonging to the genusSerratia isolated from an insect Stibara species was first micro-organism reported for synthesis of copper nanoparticles of 10–30 nm using 5 mM copper sulphate (Hasan *et al.* 2008) Metal and metal oxide-based nonmaterial act as mediators of DNA damage in living organisms, but the molecular mechanisms by which damage occurs are poorly studied (Athaet al. 2012). Spherical copper nanoparticles of 8–15 nm were produced by Pseudomonas stutzeri (Varshney *et al.* 2010), whereas quasi-spherical nanoparticles ranging from 10 to 40 nm were produced by Escherichia coli and Morganellamorganii (Singh *et al.* 2010; Ramanathan *et al.* 2013).

Application of copper nanoparticles ranging from 0.2 to 1.0 ppm blended in Murashige and

Skoog medium resulted in significant increase in various agronomic parameters of wheat, but concentration above 2 ppm showed deleterious effects. However, an enhanced growth and yield was observed by soil application of copper nano particles at concentrations ranging from 10 to 30 ppm in pots (Hafeez *et al.* 2015). On the other hand, exposing germinating seedlings of chickpea and Oryza sativa to copper oxide nano particles above 50 mg/l resulted in toxicity (Nair and Chung 2015, Costa and Sharma 2016). Studies carried on in vitro antifungal activity of copper nanoparticles reported the maximum antifungal activity against Curvularialunata MTCC 2030 followed by Alternariaalternata MTCC6572, Fusarium oxysporumMTCC1755 and Phoma destructive DBT66 (Kanhed *et al.* 2014). Copper nanoparticles of 79.295 nm were produced by using copper sulphate and extracellular metabolites from Penicillium aurantiogriseum, P. citrinum and P. waksmanii (Honary *et al.* 2012). However, the careless use of nanoparticles may have detrimental environmental effects. Despite many valuable uses of nanoparticles, the fate and effect of nanoparticles on environment are a matter of concern (Schilling *et al.* 2010, Song *et al.* 2015). The potential risks associated with production and application of copper nanoparticles therefore need to be investigated before releasing them into public domain.

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