

Alleviation of Phyto-toxicity of Copper on Agricultural Plants

KKIU Arunakumara · Buddhi Charana Walpola · Min-Ho Yoon

Received: 31 May 2013 / Accepted: 13 September 2013 / Published Online: 31 October 2013

© The Korean Society for Applied Biological Chemistry and Springer 2013

Abstract Copper (Cu), a redox-active transition metal, is known to be involved in protein metabolism, photosynthetic and respiratory electron transport, cell wall metabolism, antioxidant activity, nitrogen fixation, ion metabolization, and hormone perception, among others in plants. Though Cu has been listed among the essential elements, it could potentially result in complete inhibition of plant growth and development at excess concentrations. Measures available for alleviating Cu toxicity in plants are discussed in the present paper. Exogenous application of nitric oxide through up-regulating the components of antioxidant defense system [catalase (CAT-EC 1.11.1.6), peroxidase (POD-EC 1.11.1.7), superoxide dismutase (SOD-EC 1.15.1.1), and ascorbate peroxidase (APX-EC 1.11.1.11) activities] and stimulating the enzyme P5CS (D1-pyrroline-5-carboxylate synthetase), which catalyzes proline biosynthesis, has been proved to stand against the adverse impacts of Cu toxicity. Addition of cations (such as Ca^{2+} and Mg^{2+}) through stimulating site-specific competition for metal ions could also prevent excess accumulation of Cu in cell interior. Silicon application, through nutrient balancing and physically blocking the apoplastic bypass flow has also been recognized to be effective in alleviating Cu toxicity. Addition of organic amendments and use of arbuscular mycorrhizal fungi as soil inoculants have also proved successful in amelioration of Cu-contaminated soils. Though molecular and physiological mechanisms associated with Cu toxicity have been substantially investigated, information on the regulation of the expression of stress-related genes in key agricultural plant species is still lacking. Additional research efforts focusing at field validation of the toxicity alleviation methods are also equally important.

Keywords agricultural plants · alleviation · copper · field validation · phyto-toxicity

Introduction

Contamination of soils with heavy metals has become one of the most significant environmental problems today (Chand et al., 2012; Olteanu et al., 2013). Metal uptake by crop plants and subsequent accumulation in edible parts pose strong adverse impacts on human health through the food chain (Sadon et al., 2012; Yeh and Pan, 2012). Apart from the metals with unknown biological functions (Cd, Cr, Pb, Co, Ag, Se, Hg), essential elements (Fe, Mn, Zn, Cu, Mg, Mo, Ni) also continue to accumulate in agricultural soils through natural sources, wastewater irrigation, animal manures, and sewage sludge application, use of fertilizer and agrochemicals, mine exploration, metallurgy industry, and paint pigments among others (Thomas et al., 2012). The essential elements also can be hazardous, because they are toxic to plants at higher concentrations.

Copper (Cu) a redox-active transition metal essential for protein metabolism, photosynthetic and respiratory electron transport, cell wall metabolism, antioxidant activity, biogenesis of molybdenum cofactor, nitrogen fixation, ion metabolization, hormone perception, and ethylene sensing (Yruela, 2005; Kamali et al., 2012; Olteanu et al., 2013), could potentially result in complete inhibition of plant growth and development at elevated concentrations (Wang et al., 2012a). Generally, plant tissues contain 10 $\mu\text{g/g}$ (dry weight) of Cu (Ducic and Polle, 2005). Due to increased use of agrochemicals such as pesticides, bactericides, and fungicides, which contain Cu as an active component, Cu toxicity to agricultural plants is often reported (Chen et al., 2013; Olteanu et al., 2013). The problem is much severe in greenhouse industry, where use of electrolytically generated Cu to control diseases and algae is quite common (Zheng et al., 2004). Plants with excess Cu are reported to experience molecular damage mainly due to

K. Arunakumara
Department of Crop Science, Faculty of Agriculture, University of Ruhuna,
Kamburupitiya, Sri Lanka

B. C. Walpola · M. -H. Yoon (✉)
Department of Bio-Environmental Chemistry, College of Agriculture and
Life Sciences, Chungnam National University, Daejeon, 305-764, Republic
of Korea
E-mail: mhyoon@cnu.ac.kr

production of reactive oxygen species (ROS) and free radicals, which could lead to oxidation of biomolecules such as lipids, proteins, nucleic acids, and carbohydrates (Olteanu et al., 2013). Therefore, excessive concentrations of Cu may cause difficulties in nitrogen and protein metabolisms and may result in chlorosis of leaves, bronzing, and necrosis (Wei et al., 2008; Zou et al., 2012). Cu toxicity disturbs biosynthesis of photosynthetic machinery, leading to poor carbon assimilation rate (Qian et al., 2005), alters the hormonal status, and inhibits cell division (Lequeux et al., 2010). In addition, the toxicity may pose disturbances in the uptake and metabolism of other mineral elements (Lequeux et al., 2010).

It is well known that toxicity of a metal depends on the bioavailability of the metal in the soil and relative concentrations of other elements, which moderate toxicity responses (Wang et al., 2012b). Therefore, several remediation techniques including liming with different materials such as limestone (CaCO_3), burnt lime (CaO), slaked lime [$\text{Ca}(\text{OH})_2$], dolomite [$\text{CaMg}(\text{CO}_3)_2$], and slag (CaSiO_3), which could elevate soil pH, rendering metals less bioavailable for plant uptake. Zn fertilization, use of organic amendments, crop rotation, and water management among others, have been successfully employed in reducing metal uptake by crop plants (Arunakumara et al., 2013). Copper, in this context, is generally found in the soil as bound forms with the soil solids; thus, the available content is comparatively low (Luo et al., 2006). The available form is known to be Cu^{2+} , whereas the remaining species bound with inorganic (e.g., CO_3^{2-} , OH) or organic ligands depending on the soil pH. However, the speciation of Cu is not yet elucidated fully, though the functions and structural details are available for many Cu-dependent enzymes and Cu chaperones (Pilon et al., 2006). The continuous exposure of agricultural plants to excess Cu apparently carries an inherent risk of causing Cu toxicity, which is needed to overcome through better controlling Cu uptake, utilization, and detoxification (Yuan et al., 2013). As of present knowledge, it is apparent that due to the complex nature associated with soil-metal complexation, some aspects of Cu toxicity remain unknown. Moreover, the practical applicability of toxicity alleviation techniques is vastly untested under field conditions. The objective of the present paper is to discuss the alleviation measures available for phyto-toxicity of Cu.

Exogenous Application of Nitric Oxide (NO)

It has been widely recognized that gaseous free radical nitrous oxide is actively involved in resistance mechanism of abiotic-stress in plants (Hasanuzzaman et al., 2010; Marciano et al., 2010; Siddiqui et al., 2011). NO could act as a signaling molecule enabling it to mediate some physiological and biochemical functions in plant cells (Gao et al., 2012). Despite many aspects of stress alleviation in plants remain to be elaborated with details, several reports are available on the contribution of nitric oxide towards the plant's response to salinity (Zhao et al., 2004; Song et

al., 2009), water deficit (Neill et al., 2002; Hao et al., 2008; Arasimowicz-Jelonek et al., 2009), metal toxicity (Wang and Yang, 2005; Xiong et al., 2010), UV radiation (An et al., 2005), mechanical wounding (Corpas et al., 2008), and ozone (Ahlfors et al., 2009).

Several mechanisms have been proposed in explaining the role of NO in the defense system against the adverse impacts of heavy metals (Xiaong et al., 2010). Hsu and Kao (2004) suggested that NO might be involved in increasing the antioxidant content and antioxidative enzyme activity in plants, which was later confirmed by Tewari et al. (2008); they observed exogenous application of NO up-regulate the components of the antioxidant defense mechanism enabling the adventitious root of *Panax ginseng* to cope with Cu toxicity. Analogous to this, Cui et al. (2010) reported that exogenous application of NO could exert its protective mechanism through the activation of some antioxidative enzymes in tomato plants affected by excess Cu. It has been well documented that copper-induced oxidative stress is linked with the generation of harmful ROS such as singlet oxygen ($\text{O}_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\cdot\text{OH}$), all of which could damage biological molecules by lipid peroxidation (Demirevska-Kepova et al., 2004; Contreras et al., 2009). As reported by Drażkiewicz et al. (2007) and Posmyk et al. (2009), antioxidative enzymes, such as catalase (CAT-EC 1.11.1.6), peroxidase (POD-EC 1.11.1.7), superoxide dismutase (SOD-EC 1.15.1.1), and ascorbate peroxidase (APX-EC 1.11.1.11) could minimize ROS damage or oxidative bursts.

Zhang et al. (2009) studied the effect of NO on tomato plants affected by excess Cu, suggesting that application of NO could protect the cells through promoting the conversion from singlet oxygen into H_2O_2 and O_2 . In fact, SOD is known to catalyze the dismutation of superoxide into H_2O_2 and O_2 . Therefore, as observed by Yu et al. (2005) in rice leaves, conversion from singlet oxygen into H_2O_2 and O_2 may be attributed to the Cu-induced increase in SOD specific activity. Production of H_2O_2 is recognized to be an early response to Cu toxicity. According to Shi et al. (2005a), generated H_2O_2 should be scavenged efficiently, before it could interact with singlet oxygen to form highly reactive hydroxyl radicals, the key responsible agent for oxygen toxicity in the cell. Zhang et al. (2009) reported that application of NO greatly induced the H_2O_2 -scavenging enzymes POD and APX in tomato plants under Cu stress, which is similar to the findings of Yu et al. (2005), who observed increased tolerance in rice leaves to excess Cu. Their results provide clear evidence for the alleviation of membrane lipid oxidative injuries caused by elevated active oxygen species under Cu stress by NO-enhanced activities of antioxidative enzymes. In fact, lipid peroxidation is known to be closely related to the accumulation of ROS, and under changing environmental conditions, the lipid process is considered to be an important factor exerting an effect on ATPase (Veselov et al., 2002). Therefore, ATPase and PPase proteins could be affected by excess Cu due to lipid peroxidation. Accordingly, Zhang et al. (2009) concluded that copper toxicity in tomato plants could be

mitigated by exogenous application of NO, because it decreases lipid peroxidation, resulting in higher ATPase activity. Zhang et al. (2008) reported that exogenously applied NO could stimulate proline accumulation in the cells of *Chlamydomonas reinhardtii*, which are under Cu stress. As reported by Chen et al. (2001) and Siripornadulsil et al. (2002), proline biosynthesis in plants is catalyzed by the enzyme P5CS (D1-pyrroline-5-carboxylate synthetase). NO through stimulation of P5CS activity and up-regulation of the expression of P5CS could thus play a vital role in proline metabolism under heavy metal stress (Zhang et al., 2008). According to Yu et al. (2005), exogenous application of NO could reduce accumulation of NH₄⁺, which contributes to the alleviation of Cu toxicity in rice. The role of NO as a signaling molecule leading to changes in gene expression under heavy metal stress is supported by several research evidences (Lamattina et al., 2003; Pagnussat et al., 2003; Wilson et al., 2009).

With regards to the other metals, use of NO for prevention of cadmium and zinc-induced oxidative stress was reported by Bartha et al. (2005) and Rodriguez-Serrano et al. (2006), respectively for *Brassica juncea* and *Pisum sativum*. Al-induced inhibition of root growth was found to be prevented by NO in *Oryza sativa* (Marciano et al., 2010), *Cassia tora* (Wang and Yang, 2005), *Phaseolus vulgaris* (Wang et al., 2010), and *Citrus grandis* (Yang et al., 2012). Xiong et al. (2009) suggested that NO might increase heavy metal accumulation in root cell walls, where as decreasing the metal contents in the soluble fraction of leaves in plants. However, as reported by Cui et al. (2010), Cu uptake and transport in tomato plants were not affected by the exogenous application of NO. A comparative description of several recently published reports on alleviation of metal toxicity by exogenous application of NO in agricultural plants is given in Table 1.

Table 1 Comparative summary of the several reports on the effects of exogenous NO in alleviating heavy metal toxicity in agricultural plants

Metal	NO donor	Plant species	Experimental conditions	Mechanism/s involved	Reference
CuSO ₄ (1 μM)	Sodium nitroprusside (SNP) (100 μM)	Tomato (<i>Lycopersicon esculentum</i> Mill. cv. No. 4 Zhongshu) seedlings (3 weeks old)	Nutrient solution (pH 6.0–6.5), Ex. Period-6 days, Under natural light, T ⁰ 25–30°C (day) and 18–25°C (night), RH 65–75%	Induced transcription and increased activity of antioxidant enzyme (CAT, POD, SOD and APX), Increased MT accumulation, Reduced H ₂ O ₂	Wang et al. (2010)
CuSO ₄ (10 mM)	SNP (100 μM)	Rice (<i>Oryza sativa</i> L., cv. Taichung Native), detached leaves of hydroponically grown 10 days old plants	Apical 3 cm of the leaf segments, Floated in a Petri dish containing 10 mL of test solution, Ex. Period-24 hrs, T ⁰ 27°C in the light (40 μmol/m ² /s)	Promoted antioxidant enzyme (SOD and POX), Reduced early increase in H ₂ O ₂ content and lipid peroxidation, Reduced NH ₄ ⁺ accumulation	Yu et al. (2005)
CuCl ₂ (50 μM)	SNP (100 μM)	Tomato (<i>Lycopersicon esculentum</i> Mill. cv. Meigu) (3 weeks old)	Nutrient solution (pH 5.0), Ex. Period-8 days, Under natural light, T ⁰ 16°C (day) and 25°C (night), RH 90%	Promoted antioxidant enzyme (POD and APX), Adjusted the activity of H ⁺ -ATPase and H ⁺ -PPase in plasma membrane or tonoplast, Reduced malondialdehyde (MDA) and H ₂ O ₂ contents	Cui et al. (2010)
CuCl ₂ (50 μM)	SNP (100 μM)	Tomato (<i>Lycopersicon esculentum</i> Mill. cv. Meigu) (3 weeks old)	Nutrient solution (pH 5.0), Ex. Period-8 days, Under natural light, T ⁰ 25–32°C (day) and 19–26°C (night)	Promoted ROS-scavenging enzymes (POD and APX), Reduced H ₂ O ₂ content, Alleviated inhibition of H ⁺ -ATPase and H ⁺ -PPase in plasma membrane or tonoplast	Zhang et al. (2009)
CdCl ₂ (0.2 mM)	SNP (0.5 mM)	Rice (<i>Oryza sativa</i> L., cv. Zhonghua 11) seedlings of hydroponically grown (4 weeks old)	Nutrient solution (pH 4.5), Ex. Period-10 days, Under green house conditions, T ⁰ 30°C (day) and 24°C (night), RH 80%	Increased pectin and hemicellulose content and Cd accumulation in the cell wall of roots, Decreased Cd accumulation in soluble fraction of leaves	Xiong et al. (2009)
CdCl ₂ (0.1 mM)	SNP (0.5 mM)	Rice (<i>Oryza sativa</i> L., cv. Xiushui63) seedlings (3 weeks old)	Nutrient solution (pH 5.0), Ex. Period-8 days, Under natural light, T ⁰ 25–32°C (day) and 19–25°C (night)	Altered the expression of proteins (involved in photosynthesis, carbohydrate metabolism, nitrogen metabolism, oxidative phosphorylation, oxidative stress responses, signal transductions and cell division), Decreased H ₂ O ₂ and MDA contents	Zhao et al. (2012)
CdCl ₂ (5 mM)	SNP (100 μM)	Rice (<i>Oryza sativa</i> L., cv. Taichung Native 1), detached leaves of 12 days old plants	Apical 3 cm of the third leaf floated in test solution, T ⁰ 27°C in the light (40 μmol/m ² /s) Ex. Period-24 h	Decreased the specific activities of antioxidant enzymes (SOD, APX, GR, CAT, and POX) and PAL, Decreased NH ₄ ⁺ accumulation, Prevented accumulation of H ₂ O ₂ and MDA	Hsu and Kao, (2004)

Table 1 Continued

Metal	NO donor	Plant species	Experimental conditions	Mechanism/s involved	Reference
CdCl ₂ (100 μM) PbCl ₂ (1500 μM)	SNP (10 μM)	Yellow lupin (<i>Lupinus luteus</i> L. cv.Ventus)	Seedlings incubated in 10 μM SNP for 24 h followed by metal treatment for 48 h	Increased activity of antioxidant enzymes (specially SOD), Reduced the amount of superoxide anion	Kopyra and Gwóz' dz', (2003)
AlCl ₃ (75 μM)	SNP (25 μM)	Rice (<i>Oryza sativa</i>) seedlings cultured in CaCl ₂ solution (4 days old)	Nutrient solution (pH 4.5), Ex. Period-24 h, Under photon flux density of 120 μmol/m ² /s, Mean T ⁰ 25°C	Decreased the contents of pectin and hemicellulose, Increased the degree of methylation of pectin, Decreased Al accumulation in root tips and cell walls	Zhang et al. (2011)
AlCl ₃ (50 μM)	SNP (2.5 mM)	Rye (<i>Secale cereal</i> L. cv King) and Wheat (<i>Triticum aestivum</i> L. cv Jinmai47) (4 days old)	CaCl ₂ solution (pH 4.5), Ex. Period-24 h, Cultured in self-regulating conditions, T ⁰ 25°C (day) and 20°C (night)	Regulated hormonal equilibrium (increasing GA content and decreasing the values of IAA/GA and IAA/ZR), Reduced Al accumulation in the root apices	He et al. (2012)
AlCl ₃ (10 μM)	SNP (0.4 mM)	<i>Cassia tora</i> L. (3 days old)	CaCl ₂ solution (pH 4.5), Ex. Period-12 hrs, Under fluorescent light (100 μmol/m ² /s), Mean T ⁰ of 22°C	Increased activities of antioxidant enzymes (CAT, POD, and SOD), Decreased the activity of lipoxigenase (LOX), Decreased Al accumulation in roots	Wang and Yang (2005)
AlCl ₃ (30 μM)	SNAP (30 μM)	Rice (<i>Oryza sativa</i>) seedlings (2 weeks old)	Nutrient solution (pH 4.5), Ex. Period-5 days, Under fluorescent light (200 μmol/m ² /s), T ⁰ 30°C (day) and 25°C (night), RH 75–80%	Increased activities and protein expression of antioxidant enzymes and S-nitrosoglutathione reductase (GSNOR), Altered the expression of proteins associated with cell wall synthesis, cell division and cell structure, calcium signaling and defense responses	Yang et al. (2013)
Sodium arsenate (As) (50 μM)	SNP (50 μM)	Rice (<i>Oryza sativa</i> L. var. No. 3) seedlings (4 days old)	Nutrient solution (pH 4.5), Ex. Period-24 hrs, Under fluorescent light (240 μmol/m ² /s), T ⁰ 30°C (day) and 25°C (night), RH 76%	Decreased As-induced MDA, superoxide ion, root oxidizability and H ₂ O ₂ content, Decreased in As-induced increase in the specific activities of antioxidant enzymes (SOD, APX, CAT and GPX)	Singh et al. (2009)
Sodium arsenate (As) (25 μM)	SNP (100 μM)	Tall fescue (<i>Festuca arundinacea</i> cv. Arid 3) (3 weeks old)	Nutrient solution (pH 5.0), Ex. Period-8 days, Under fluorescent light (100 μmol/m ² /s), T ⁰ 25°C (day) and 20°C (night), RH 70%	Increased activities of antioxidant enzymes (SOD, CAT, and APX), Reduced the levels of H ₂ O ₂ and superoxide radicals	Jin et al. (2010)
NiCl ₂ (500 μM)	SNP (100 μM)	Tomato (<i>Lycopersicon esculentum</i> Mill., cv. Urbana VF) (25 days old)	Nutrient solution (pH 6.5), Ex. Period-10 days, Under fluorescent light (190 μmol/m ² /s), T ⁰ 26°C (day) and 22°C (night), RH 65%	Increased activities of antioxidant enzymes (GPX, CAT, and APX), Reduced the levels of H ₂ O ₂ and lipid peroxidation, Deceased proline accumulation	Kazemi (2012)
NiCl ₂ (0.2 mM)	SNP (0.3 mM)	Bean (<i>Phaseolus vulgaris</i> L.) seedlings (3 days old)	Nutrient solution (pH 4.5), Ex. Period-4 days, Under fluorescent light (150 μmol/m ² /s), T ⁰ 25°C (day) and 18°C (night), RH 70%	Increased activity of antioxidant enzyme (POD and CAT), Decreased in proline and soluble protein accumulation	Mihailovicand Drazic (2011)

Use of Cations

The concept of biotic ligand model (BLM) has recently been proved to be applicable in predicting metal toxicity to terrestrial plants (Kopittke et al., 2011; Chen et al., 2013), in addition to its wide application in aquatic environment (Wu et al., 2013). The module is based on the main assumption that the toxicity of a metal is basically determined by the degree of binding to the site

of action, specifically the plasma membrane (Paquin et al., 2000). Thus, addition of cations (such as Ca²⁺ and Mg²⁺) could stimulate the competition of heavy metal for the active sites (Borgmann et al., 2005), resulting in alleviation of the phyto-toxicities of metals (Paquin et al., 2002).

The BLM approach was employed in assessing the alleviation effects of magnesium on Cu toxicity using a hydroponic experiment with Kyoho grapevine (*Vitis vinifera* L.) cuttings (Chen et al.,

2013). The cuttings were exposed separately to 0 (control), 1, 5, 10, 15, and 25 μM of Cu^{2+} as CuSO_4 , while keeping the Mg^{2+} concentrations at 0.2, 2, 4, and 8 mM, respectively. They observed decreased Cu^{2+} concentration in root with increased Mg^{2+} concentration. Consequently, the effective activity of Cu^{2+} , resulting in a 50 % reduction of root elongation (EA_{50}), linearly increased with increased Mg^{2+} activity (up to about 4.0 mM of Mg^{2+}). Similar positive linear relationships between EA_{50} versus Mg^{2+} concentrations have been reported within the ranges of $\{\text{Mg}^{2+}\}$ up to 2.5 mM for barley (Lock et al., 2007) and up to 1.5 mM for wheat (Luo et al., 2008). Based on the finding, they concluded that the amelioration effects of Mg on Cu toxicity and accumulation in roots could be due to competition between Mg^{2+} and Cu^{2+} on the binding sites. Kopittke et al. (2011) investigated the alleviation of Cu toxicity by Al^{3+} , Ca^{2+} , H^+ , Mg^{2+} , and Na^+ with *Vigna unguiculata* seedlings in simple nutrient solutions and reported that the cations could depolarize the plasma membrane (PM) by reducing the negativity of electrical potential at the outer surface of the PM, which could subsequently decrease the activity of Cu^{2+} at the outer surface of the PM. Though the addition of Na^+ had no apparent influence on the toxicity of Cu, the addition of 10 mM Mg^{2+} , 20 mM Ca^{2+} , 25 μM H^+ or 10 μM Al^{3+} resulted in decreases in the toxicity of Cu remarkably. Significant reduction in Cu toxicity through the addition of Al^{3+} was also reported by Fortunati et al. (2005), who studied rhizotoxicity of Al^{3+} , Mn^{2+} , and Cu^{2+} on wheat (*Triticum aestivum*) seedlings grown in well-defined nutrient solutions, with these elements present singly or in combination. Luo et al. (2008) assessed the extent of Cu rhizotoxicity alleviation by Ca^{2+} , Mg^{2+} , Na^+ , K^+ , and H^+ independently with wheat seedlings grown in nutrient solutions. They observed a liner relationship between increasing activities of Ca^{2+} and Mg^{2+} (but not Na^+ , K^+ and H^+) and 2 d EC_{50} (as Cu^{2+} activity), supporting the concept that some cations can compete with Cu^{2+} for binding to the active sites at the terrestrial organism-solution interface; the 2 d EC_{50} for root growth was shown to have a 4-fold increase when Ca^{2+} (0.46) or Mg^{2+} (0.34) was introduced to the medium. Kinraide et al. (2004) also reported mitigating effects of Ca^{2+} and Mg^{2+} on Cu toxicity to wheat grown in nutrition solution. However, they further stated that though the differences in the ameliorative effectiveness of Ca^{2+} and Mg^{2+} were apparent, H^+ was shown to be more effective (70 times) than Ca^{2+} and Mg^{2+} in alleviating Cu rhizotoxicity, which was in line with Di Toro et al. (2001). According to Vulkan et al. (2004), amount of adsorbed Cu^{2+} to the plasma membrane of wheat root decreased with increasing concentrations of Ca^{2+} , K^+ , and La^{3+} . Further elaborating their findings, Luo et al. (2008) suggested that the competitions between toxic metal ions and cations for binding sites vary with the plant species and toxicants concerned. In this context, the deviant effects of high concentration of competitive cations also come into account as observed by Lock et al. (2007) from their study with *Hordeum vulgare*, where Mg^{2+} activity resulted in a twofold increase in EC_{50} value of Cu^{2+} , whereas no significant effect was found from Na^+ , K^+ , and H^+ activities.

Electrostatic model (ESM), another approach applicable in assessing ameliorative effects of metal toxicity by coexistent cations, is presently being discussed among researchers (Kopittke et al., 2011; Wang et al., 2011). According to the new concept, the negativity of electrical potential of the plasma membrane is lowered by the addition of coexistent cations, resulting in diminished metal activity on the plasma membrane. Commenting on these models, Wang et al. (2012a) suggested that the integration of BLM and ESM could be a better approach in predicting metal toxicity thresholds. However, it is well understood that the metal toxicity and uptake are governed by several factors including the available quantity of the concerned metal in soil (both soluble and replenished metals) and plant species. Therefore, before field validation of cation-based metal toxicity alleviation tactics, factors such as contribution of replenishment of metal from soil solids to phyto-toxicity should be investigated with different metal species and soils as previously reported by Singh and Agrawal (2010) and Juang et al. (2012).

Not only for alleviating Cu toxicity, the literature is rich with the reports on alleviation of toxicity of other metals also by cations. According to Voigt et al. (2006), rhizotoxicity of both Cd and Cu to *Lactuca sativa* in soil extracts can be inhibited by the actions of Ca^{2+} and H^+ . Thakali et al. (2006) reported that Ca^{2+} and Mg^{2+} could alleviate the rhizotoxicity of nickel to plants. As reported by Weng et al. (2004), Ni^{2+} binding to the active sites of *Avena sativa* L. was affected by H^+ . In the case of Zn^{2+} , Pedler et al. (2004) reported that Mg^{2+} and K^+ in solution could alleviate rhizotoxicity to *Triticum aestivum* and *Raphanus sativus*.

Role of Silicon

The beneficial effects of silicon (Si) on growth and development of plants have long been discussed (Oliva et al., 2011; Ali et al., 2012). The ability of Si to alleviate abiotic and biotic stresses in plants has been often investigated (Ma, 2004; Datnoff et al., 2007). Possible mechanism through which Si enhances plant resistance to various stresses has been extensively studied over the years (Bockhaven et al., 2013). One of the key attributions of Si in plant tissues is recognized to be nutrient balancing (Mateos-Naranjo et al., 2013), which in fact is executed through discouraging uptake and translocation of some nutrients, such as manganese (Li et al., 2012) or through the enhancement of uptaking other metals, such as Zn (Epstein and Bloom, 2005).

Li et al. (2008) studied the effects of Si on Cu detoxification with the model plant, *Arabidopsis thaliana*. They reported that elevated Si treatment (1.5 mM) could alleviate the reduction of shoot and root fresh weight caused by elevated Cu (30 μM). The treatment was also found to reduce leaf chlorosis caused by Cu toxicity. Similar results were reported by Frantz et al. (2011), who observed Si-mediated alleviation of Cu toxicity symptoms in *Zinnia elegans* (Si accumulator) and *Antirrhinum majus* (Si non-accumulator) with species-dependent variations. Oliva et al. (2011)

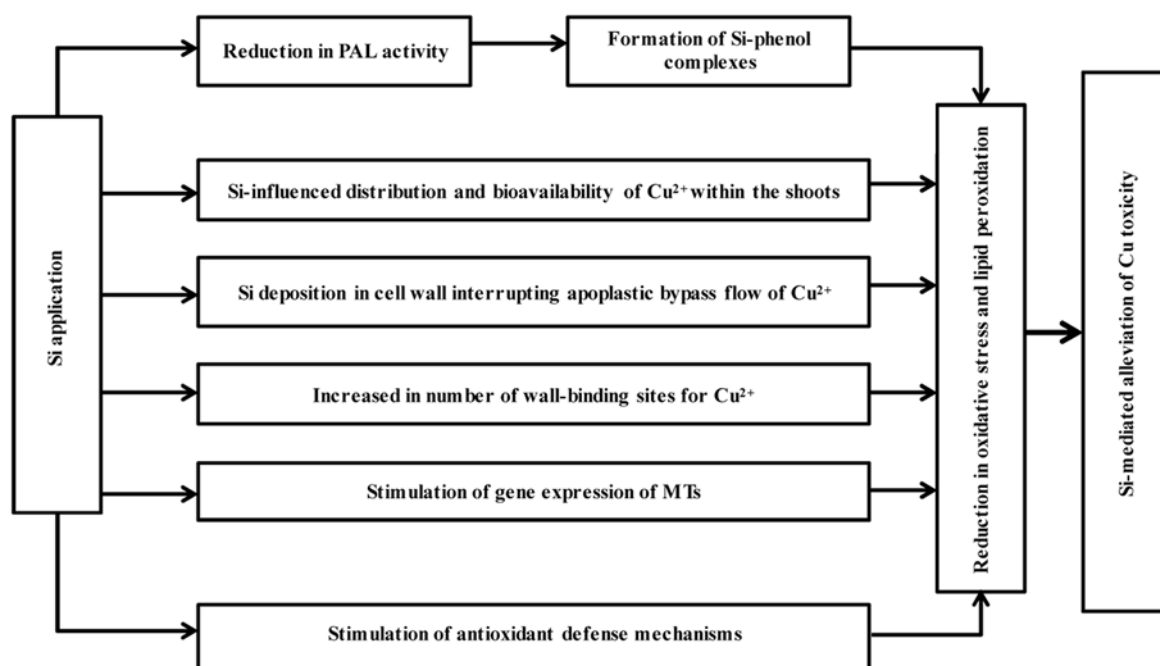


Fig. 1 Schematic diagram showing Si-mediated alleviation of Cu toxicity in plants.

also reported that Si supplementation alleviates Cu-induced growth inhibition and toxicity symptoms in *Erica andevalensis*. However, as reported by Epstein and Bloom (2005) for other species, Si-mediated growth enhancement of *Erica* plants could not be observed if the plant receives optimal concentrations of Cu. Analogous to these findings, Si-mediated growth promotions have been reported with other metals as well (Liang et al., 2005).

Further explaining their results, Li et al. (2008) reported that growth enhancement and suppression of visual toxicity symptoms in Si-treated plants might be due to Si-influenced distribution or bioavailability of Cu within the leaves, because such a distribution could avoid increased metal accumulations in a particular group of cells. However, Oliva et al. (2011), observed inhibition in Cu translocation from roots to shoots, thus claimed that growth promotion of *Erica* is due to Si-mediated reduction in Cu concentration in shoots. Though Li et al. (2008) denied that growth promotion in Si-treated plants is enhanced by the reduced uptake and translocation of Cu, the possibility for such attributions was confirmed by Li et al. (2012), who studied Si-mediated alleviation of Mn toxicity in *Oryza sativa*. Similar reductions in uptake and root-to-shoot transport of metals in *O. sativa* seedlings was also reported by Shi et al. (2005b) for Cd and Guo et al. (2005) for arsenate. As reported by Ma et al. (2006) apoplastic bypass flow of metals can be interrupted by the added Si, because it could subsequently be deposited in plant cell walls. Shi et al. (2005b) also reported Si-mediated reduction in the apoplastic transport of Cd through physically blocking the apoplast bypass flow across the roots.

Li et al. (2008) observed a reduction in stress-induced enzyme,

phenylalanine ammonia lyase (PAL-EC 4.3.1.5) activity in the shoots of Si-supplemented *Arabidopsis* plants enabling them to avoid Cu-induced stress. It was also confirmed by Frantz et al. (2011); however, they noticed reduced PAL activity not only in shoots but in roots as well. In fact, plants are benefited by the downstream products of PAL activity, which make sure better preparation of plants against adverse environmental stress. The mechanism by which reduced PAL activity alleviates stress may be attributed to the formation of Si-phenol complexes (Rogalla and Römeheld, 2002) and their subsequent cross linkage with wall polymers. Kidd et al. (2001) observed fifteen times more phenolics in Si-treated maize plants than in untreated plants, suggesting that Si could enhance the tolerance to Al toxicity by mediating the metabolism of phenolic compounds. As reported by Maksimovic et al. (2007) for Mn, Si supplementation could result in decreased concentration of plant-borne substrates of anti-oxidative enzymes, such as ferulic acid and coniferyl alcohol. Reduction in these phenolic compounds could down-regulate PAL activity, resulting in reduced oxidative stress. According to Li et al. (2008), addition of Si could increase the number of wall-binding sites for Cu, which was analogous to the findings of Maksimovic et al. (2007). Furthermore, Khandekar and Leisner (2011) reported that instead of just providing extensions of cell wall-binding sites for Cu, Si could also be involve in promoting gene expression of metallothioneins to maintain low levels of soluble Cu within the cell. However, not like most shoots, plant roots generally consist of endodermis, which regulates nutrient uptake into the vascular system. Therefore, as reported by Kirkham (2006) for Cd, once deposited in the endodermal layer, Si could restrict shoot uptake

of Cu by functioning as a natural barrier. This would ultimately result in increased accumulation of Cu in epidermal and cortical cells, inducing stress responses such as increased PAL activity. Khandekar and Leisner (2011) examined the expression of genes responsible for PAL activity, and they observed increased expression of the genes in response to high Cu, however decreased when the plants were also treated with increased Si.

Frantz et al. (2011) also observed Si-mediated reduction in POD activity, which was elevated by increased Cu in leaves and roots. Increase in POD activity due to Cu toxicity was previously reported by Mocquot et al. (1996) in maize (*Zea mays*) seedlings. These reports indicate that Si may play a role in minimizing active oxygen species production, thereby reducing POD expression. According to Shi et al. (2005a), Si supplementation could result in reduced membrane lipid peroxidation and increased antioxidants contents induced by excess Mn. Li et al. (2008) further reported that RNA levels of two *Arabidopsis* copper transporter genes, copper transporter I (*COPT1*) and heavy metal ATPase subunit 5 (*HMA5*) were significantly decreased when Si levels were elevated. Therefore, they concluded that Si-mediated resistance of *Arabidopsis* to Cu stress is attributed to several factors ranging from physiological changes to alterations of gene expression. This conclusion was strongly supported by the results of Khandekar and Leisner (2011), who investigated Si-mediated expression of *Arabidopsis thaliana* genes involved in Cu toxicity. Schematic representation on Si-mediated alleviation of Cu toxicity in plants is shown in Fig. 1. Based on all above results, Si as an important element should be included in fertilizer mixtures, which could ensure successful re-vegetation of Cu contaminated soils. However, such initiatives must move forward cautiously, because some detrimental effects could be witnessed as reported by Kamenidou et al. (2008) for excess supplementations of Si.

Addition of Organic Amendments

Addition of different types of organic amendments (such as compost, manures, biosolids, and wastes) has been proved successful in reducing the mobility of metals and amelioration of contaminated soils (Clemente et al., 2005; Clemente et al., 2006; Mench et al., 2010). However, the effectiveness of organic amendments in altering the bioavailability of heavy metals depends on several factors including the nature of the organic amendment, their microbial degradability, soil type, soil pH, redox potential, and metals concerned (Walker et al., 2003; 2004).

Campbell (2010) reported that organic amendments can effectively be used to combat Cu toxicity in plants, because such amendments can induce chelation reactions and form a soluble complex through which much of the Cu can leach out of the soil profile or be taken up safely by plants. The chelation reaction and the effectiveness of organic amendment in the chelating process are, however, dependent upon soil pH, thus lime may also be added simultaneously in order to raise the pH and aid the process

by which Cu is removed. According to them, organic acids such as citric, fulvic, and malic acids are contained in organic amendments, and complex well with Cu. The role of the organic amendment in improving plant growth under Cu stress could, in part, be ascribed to the formation of metal-citrate complexes (Bolan and Duraisamy, 2003). The enhancement of growth through improved nutrition could also be recognized as a major role of organic amendments in ensuring the Cu tolerance in plants (Caravaca et al., 2004).

Angelova et al. (2010) studied the effect of organic additives (peat, compost, and vermicompost) on the quantity of mobile forms of lead, zinc, cadmium, and copper and uptake by potato (*Solanum tuberosum L.*) plants. The organic amendments led to the increase in tuber yield, starch yield, and absolute dry substance and to decrease in reducing sugars in potatoes. The amendments increased effective immobilization of Pb, Cu, Zn, and Cd, and their phyto-accessible forms in soil, whereas decreasing the metal content in potato tubers. According to them, the results were best expressed with 10% compost or 10% vermicompost. Huang et al. (2011) studied the effects of rice straw ash (RSA) amendment on the solubility and distribution of Cu in contaminated paddy soils with flooding incubation and reported that addition of RSA could suppress the release of Cu into the soil solution. RSA amendment increased soil pH and decreased redox potential, encouraging the transformation of Cu into less soluble forms. Furthermore, RSA possessed high metal-binding capacity. Therefore, their results suggested that the RSA can retard the bioavailability and movement of Cu in the contaminated soil.

Biochar, which has been shown to have soil-conditioning properties, were identified as a potential soil amendment, resulting in improved physico-chemical characteristics of soil (Beesely et al., 2010). The application of biochar has been further encouraged due to its high organic carbon contents, which can be as high as 90%, depending on the source of origin (Yin Chan and Xu, 2009). Increases in soil pH and key soil macro-elements, reductions in heavy metals in leachates (Novak et al., 2009), and adsorption of dissolved organic carbon (Pietikainen et al., 2000) have been reported as the key potential benefits of biochar application. In addition, the effects of biochar could remain in the soil for a longer period of time, and thus could reduce the possibility of heavy metal accumulation associated with repeated applications of other amendments (Lehmann and Joseph, 2009) such as sewage sludge. Buss et al. (2012) investigated the ameliorating properties of a forestry-residue biochar with young quinoa plants (*Chenopodium quinoa*) with Cu toxicity. Under green house conditions, the plants without biochar showed severe stress symptoms, followed by reduced growth shortly after Cu application at 50 µg/g, and died at 200 µg/g. Increasing concentrations of biochar in the growth medium significantly increased the plant performance, irrespectively of the severity of Cu toxicity. In the presence of biochar, absorption of Cu was found to be decreased, resulting in reduced concentrations of Cu in the roots, shoots, and leaves. Based on these results, biochar could be employed as an

amendment to combat Cu toxicity in plants, particularly in sandy soils. Furthermore, the ameliorative effects are attributed to the increased adsorption of Cu to the negatively charged biochar surfaces. As stated by Brown et al. (2003) and Clemente et al. (2006), Cu has a high affinity for organic matter, though their stability in soils generally decreases with increasing acidity (Alvarenga et al., 2008a;b). Karami et al. (2011) assessed green waste compost and biochar amendments for their assistance in regulating the mobility of Cu and Pb and the resultant uptake by ryegrass (*Lolium perenne* L. var. Cadix). Biochar was more effective than green waste compost in reducing Cu concentrations in pore water as well as in ryegrass shoots. Biochar application did not result in significantly high concentrations of dissolved organic carbon (DOC) compared to the untreated soil; however, combine application of green waste compost and biochar increased DOC significantly, which is in line with report of Beesley et al. (2010). DOC could play a significant role in the immobilization of metals (including Cu) by forming metal-DOC complexes, thereby decreasing metal phytotoxicity (Bolan et al., 2003). Karami et al. (2011) did not observe considerable alteration in soil pH due to the addition of biochar, though green waste compost raised soil pH significantly. On the contrary, Beesley et al. (2010) observed significant increase in soil pH along with more than 30-fold increments of Cu and As in soil pore water after addition of both amendments together. According to them, addition of amendments to multi-element contaminated soils can have contradictory effects on the mobility, bioavailability, and toxicity of specific elements, depending on the amendment used.

Use of Arbuscular Mycorrhizal Fungi (AMF)

AMF, a well-known metal tolerance group of microorganisms, are naturally found in almost all habitats and climates (Del Val et al., 1999). They form mycorrhizal symbioses with many plant species and act as a dominant population associated with their rhizosphere (Ferrol et al., 2009). The AMF colonization could enhance metal tolerance, whereby ensuring the survival of plants in metal contaminated soils (Carvalho et al., 2006; Hildebrandt et al., 2007; Meier et al., 2012). The enhanced tolerance is reported to be attributed to the immobilization of metals within or near the roots, reduced uptake, restricted translocation of metals to the shoots, and to the improved nutritional status of the plants (Leyval et al., 1997; Göhre and Paszkowski, 2006; Reinhardt, 2007; Meier et al., 2011). In contrast, AMF could also enhance the metal translocation to shoots (Davies et al., 2001; Trotta et al., 2006). In addition, biosorption of metals to the cell wall and sequestration (Zafar et al., 2007) and exuding enzymes such as acid phosphatase (Carrasco et al., 2011) have also been discussed as possible mechanisms through which AMF could alleviate heavy metal stress. As reported by Gonzalez-Chavez et al. (2004) and Cornejo et al. (2008), AMF are capable of improving soil structure through the actions of external mycelium and the production of a

glycoprotein known as glomalin, which also has a possible contribution to sequestration of metals.

Meier et al. (2011) evaluated the applicability of AMF inoculation and/or sugar beet agro waste (SB) to combat Cu toxicity in *Oenothera picensis*. Soils with different concentrations of Cu (0, 100, and 500 mg/kg), either with or without SB and inoculated with Cu-adapted Glomeromycota fungi (GA), non-adapted *Glomus claroideum* (GC) or no fungus were used for growing the plants. SB amendment led to the significantly increased shoot biomass compared to the unamended soils, and allowed the survival of non-mycorrhizal and GC-inoculated plants, even at the highest Cu level. GA colonization was also found to be enhanced by the SB amendments particularly at the highest Cu level. Their results thus provided valuable evidence for the role of Glomeromycota in the alleviation of Cu toxicity. Similar responses of metal tolerant AMF have been reported by Hildebrandt et al. (2007) and Azcon et al. (2009). Two metallophytes (*Oenothera picensis* and *Imperata condensata*) and a crop plant (*Helianthus annuus*) were employed in a pot culture experiment to assay the behavior of different AMF inocula on plant growth and Cu uptake (Meier et al., 2012). The plants were exposed to different levels of Cu (0, 150, 300 or 450 mg/kg), with or without inoculation of Cu-adapted AM fungi (GA) or Cu non-adapted strain GC. As revealed by the results, biomass production in metallophyte plants was not benefited by AMF, whereas led to enhanced shoot growth of *H. annuus*, especially at higher Cu levels. Moreover, biomass production in inoculated and uninoculated plants was found to be different depending on the AMF inocula and the Cu level. Differences in AM fungal parameters (root colonization, spore number, and glomalin production) were also dependent on the Cu level and the AMF inoculums. As reported by Hildebrandt et al. (2007) and Ferrol et al. (2009), the host plant may regulate AMF colonization as a protective role of fungi against the metal toxicity. Meier et al. (2012) further observed significantly higher glomalin accumulation and Cu-bound to glomalin in AMF colonized *H. annuus* plants, which could be considered as a special exclusion mechanism developed by AMF to cope with high Cu levels in the soil. Similarly, Cornejo et al. (2008) also reported a good positive correlation between Cu concentration in soil and glomalin production. Gonzalez-Chavez et al. (2004), based on their findings under *in vitro* conditions, reported that 1 g of glomalin was able to sequester 28 mg of Cu. Therefore, proven capacity for producing glomalin and subsequent sequestering of metals deserve further studies in order to use AMF effectively in phytostabilization programs (Cornejo et al., 2008; Vodnik et al., 2008). They reported that the effects of AMF may vary with the host plant species and AMF strain. Therefore, as suggested by Dodd et al. (2000), certain compatibility between AMF and host plant species may exist.

Carrasco et al. (2011) concluding their findings reported that inoculation of autochthonous AMF was found to be effective in stimulating growth and alleviating heavy metal toxicity for plants growing on a soil contaminated by multiple heavy metals. They

observed decreased Zn and Cu contents in the roots of *Coronilla juncea* L. seedlings grown in a polluted soil inoculated with a native AMF, *Glomus mosseae* (Nicol. and Gerd.) The effect of indigenous AMF versus non-adapted fungus in promoting plant establishment and survival on contaminated soil has been reported for soils polluted with Zn (Hildebrandt et al., 2007), Pb (Sudová and Vosátka, 2007), and Cu (Leung et al., 2006). According to Hildebrandt et al. (2007), native AMF adapted to Cu-polluted soils may have developed a differential mechanism that improves their tolerance to a wide range of metal concentrations in soil. González-Chávez et al. (2002) reported that different AMF isolates from the same polluted soil could have different metal tolerance abilities, which was in line with Del Val et al. (1999), who provided further evidence for functional diversity within AMF populations in Cu-polluted soils. Wang et al. (2007) conducted a pot culture experiment to study the growth and Cu uptake by maize (*Zea mays*) inoculated with or without AMF, *Acaulospora mellea*. Compared with nonmycorrhizal controls, Cu uptake efficiency in mycorrhizal plants was higher when no Cu was added, to the culture medium, but lower at other levels (100, 200, 400, 800 mg/kg), and Cu translocation efficiency was lower at all Cu addition levels. Based on the results, they claimed that *A. mellea* showed a potential role in phytostabilization of soil moderately polluted with Cu.

Use of Grafted Plants

Cucumber plants (*Cucumis sativus* L. cv. Akito), either ungrafted or grafted onto the commercial rootstock ‘Shintoza’ (*Cucurbita maxima* Duchesne × *Cucurbita moschata* Duchesne) were employed in evaluating the effectiveness of grafting on Cu tolerance (Rouphael et al., 2008). Plants grown under greenhouse conditions were provided with nutrient solution containing different concentrations of Cu, and the growth, yield, fruit quality, leaf gas exchange, chlorophyll and carotenoid contents, electrolyte leakage, mineral composition, and assimilated partitioning were measured. Concentration-dependent reduction in yield, shoot, and root biomass, as well as fruit quality was observed in Cu-treated cucumber plants. However, reductions in the yield, shoot, and root biomass were significantly lower in grafted plants than those of the ungrafted plants. Cu-induced reductions in net assimilation, stomatal conductance, chlorophyll and carotenoid content were more severe in ungrafted plants than those of grafted plants. The result showed that the improved crop performance of grafted plants was attributed to their strong capacity to inhibit Cu accumulation in the aerial parts of the plants and to the ability to maintain a better plant nutritional status. Similar results were reported by Savvas et al. (2009), who observed lower translocation of copper, ferrous, and magnesium to the leaves of tomato plants grafted onto ‘He-Man’ variety in comparison with the self-grafted plants. Lower concentrations of copper, manganese, zinc, boron, ferrous, and molybdenum in tomato plants grafted onto pepper

rootstock were observed by Kawaguchi et al. (2008). However, the reductions in the concentrations of Cu, Fe and Mn were not significant compared to the control plants. As reported by Martínez-Ballesta et al. (2010), reductions in the metal contents in grafted plants could be explained by the smaller root system and restricted xylem hydraulic conductivity from the rootstock to the scion. Recently, Zhang et al. (2013) conducted a greenhouse experiment to elaborate the effects of grafting on Cu toxicity to cucumber plants. Cucumber seedlings (cv. Xintaimici) either ungrafted or grafted onto the rootstock (*Cucurbita ficifolia*) were treated with 40 μmol/L CuSO₄·5H₂O. They observed Cu-induced growth inhibition and accumulation of H₂O₂ in roots. Moreover, the ROS-scavenging enzyme activities in roots of grafted seedlings were significantly higher than those of ungrafted plants. The activities of H⁺-ATPase, H⁺-PPase, and Ca²⁺-ATPase in plasma membrane and/or tonoplast in grafted cucumber seedlings under Cu stress were also higher than those in ungrafted plants. Therefore, they concluded that grafting could expulse the excess H⁺, promote the cytoplasm alkalinization, regulate the intracellular Ca²⁺ concentration, and bring the cytoplasm concentration of free Ca²⁺ to extremely low level under Cu stress. As reported by Edelstein and Ben-Hur (2007), the concentrations of boron, zinc, strontium, manganese, copper, titanium, chromium, nickel, and cadmium in fruits from grafted melon plants (cv. ‘Arava’) treated to the commercial cucurbita rootstock ‘TZ-148’ were lower than those from ungrafted plants. The lower element concentrations in fruits were ascribed mainly to differences in characteristics of the root systems between the two plant types. The response of grafted plants to heavy metals stress is different from that of the self-rooted plants. According to Savvas et al. (2010), the differences vary mainly with the rootstock genotype. Many of the above rootstocks are capable of checking the uptake and/or the transport of heavy metals to the shoots, thereby weakening the severity of the damage caused by excessive external concentrations. Savvas et al. (2010) recognized other possible contributions such as external detoxification of harmful elements and hormonal signals modifying gene expression in the scion to the amelioration effects of grafting on metal toxicity.

Apart from the topics discussed above, few other reports with the potential exploitation to combat Cu toxicity are available in the literature. Fan et al. (2012) conducted a greenhouse study in assessing the potential of calcium water treatment residue (Ca-WTR) to ameliorate the toxicity and uptake of Cu by ryegrass (*Lolium perenne* L.) and lettuce (*Lactuca sativa* L.). They used Alfisol and Spodosol amended with different levels of Ca-WTR (5–100 g/kg soil). At the end of 8-week growing period, the biomass yields were found to be increased with WTR application at the rates of 5–20 g/kg for Alfisol (pH 5.45) and 5–50 g/kg for Spodosol (pH 4.66), and decreased at high rates (>20 g/kg for Alfisol and >50 g/kg for Spodosol). With increased rate of Ca-WTR, Cu concentration in ryegrass shoots was found to decrease significantly, which also occurred in lettuce at Ca-WTR rates over 50 g/kg. Fan et al. (2011), through laboratory incubation and

column leaching experiments, estimated the optimal rate of Ca-WTR for remediating Cu-contaminated soils. As revealed by the results, Ca-WTR amendment could significantly raise soil pH and decrease water soluble and exchangeable Cu by 62–90% in the contaminated soils. The content of Cu in the leachate after 10 leaching events was reduced by 80 and 73%, respectively for the two tested soils at the Ca-WTR rate of 20 g/kg for Alfisol and 100 g/kg for Spodosol. They further stated that a pH value of 6.5 was critical for lowering Cu availability in the soils. Moreover, Ca-WTR could convert the bioavailable Cu into more stable Cu fractions, i.e. oxides-bound and residual Cu.

Stuckey et al. (2009) exploited the ability of highly charged swelling mica (Na-2-mica) to reduce the absorption of Cu by plants grown in contaminated soils. Ryegrass (*Lolium perenne* L.) was grown in Cu-contaminated acid soils (pH 4.2) amended with 0.5 and 1% (w/w) mica, and 1% (w/w) montmorillonite. The addition of mica led to enhanced root growth and reduced Cu content in roots, thus exhibited the ability to reduce Cu bioavailability in Cu-contaminated surface soils.

Conclusion

Although several research publications on Cu toxicity and alleviation tactics are available, adequate and complete explanation on as to how de-toxifying agents are involved in the regulation of the expression of stress-related genes in economically important crop species is still found to be lacking. Furthermore, published contradictory findings (possibly due to different experimental conditions such as different concentrations, plant species and their growth stages, experimental duration, and method used) urge to further elucidate certain aspects of Cu toxicity and alleviation tactics. Further studies are needed for field validation of toxicity alleviation methods with agricultural plants. Another area that may be worth investigating is alteration of fertilizer mixtures with possible inclusion of among others, Si and Mg, in particular, for the crops grown in Cu contaminated soils.

References

- Ahlfors R, Brosche M, Kollist H, and Kangasjarvi J (2009) Nitric oxide modulates ozone induced cell death, hormone biosynthesis and gene expression in *Arabidopsis thaliana*. *Plant J* **58**, 1–12.
- Ali A, Basra SMA, Hussain S, Iqbal J, Ahmad AHABM, and Sarwar M (2012) Salt stress alleviation in field crops through nutritional supplementation of silicon. *Pak J Nutr* **11**, 637–55.
- Alvarenga P, Goncalves AP, Fernandes RM, de Varennes A, Vallini G, Duarte E et al. (2008b) Evaluation of compost and liming materials in the phytostabilization of a mine soil using perennial ryegrass. *Sci Total Environ* **406**, 43–56.
- Alvarenga P, Palma P, Goncalves AP, Baião N, Fernandes RM, de Varennes A et al. (2008a) Assessment of chemical, biochemical and ecotoxicological aspects in amine soil amended with sludge of either urban or industrial origin. *Chemosphere* **72**, 1774–81.
- An LZ, Liu YH, Zhang MX, Chen T, and Wang XL (2005) Effects of nitric oxide on growth of maize seedling leaves in the presence or absence of ultraviolet-B radiation. *J Plant Physiol* **162**, 317–26.
- Angelova V, Ivanova R, Pevicharova G, and Ivanov K (2010) Effect of organic amendments on heavy metals uptake by potato plants. pp. 84–7, Soil Science, Australia.
- Arasimowicz-Jelonek M, Floryszak-Wieczorek J, and Kubis J (2009) Involvement of nitric oxide in water stress-induced responses of cucumber roots. *Plant Sci* **177**, 682–90.
- Arunakumara KKIU, Walpola BC, and Yoon MH (2013) Agricultural methods for toxicity alleviation in metal contaminated soils. *Korean J Soil Sci Fert* **46**, 73–80.
- Azcon R, Peralvarez MC, Biro B, Roldan A, and Ruiz-Lozano JM (2009) Antioxidant activities and metal acquisition in mycorrhizal plants growing in a heavy-metal multicontaminated soil amended with treated lignocellulosic agrowaste. *Appl Soil Ecol* **41**, 168–77.
- Bartha B, Kolbert Z, and Erdei L (2005) Nitric oxide production induced by heavy metals in *Brassica juncea* L. Czern. and *Pisum sativum* L. *Acta Biol Szeged* **49**, 9–12.
- Beesley L, Moreno-Jiménez E, and Gomez-Eyles JL (2010) Effects of biochar and green waste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. *Environ Pollut* **158**, 2282–7.
- Bockhaven JV, Vleeschauwer DD, and Höfte M (2013) Towards establishing broad-spectrum disease resistance in plants: Silicon leads the way. *J Exp Bot* **64**, 1281–93.
- Bolan NS, Adriano DC, Mani S, and Khan AR (2003) Adsorption, complexation and phytoavailability of copper as influenced by organic manure. *Environ Toxicol Chem* **22**, 450–6.
- Bolan NS and Duraisamy VP (2003) Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: a review involving specific case studies. *Aust J Soil Res* **41**, 533–55.
- Borgmann U, Nowierski M, and Dixon DG (2005) Effect of major ions on the toxicity of copper to *Hyalella azteca* and implications for the biotic ligand model. *Aquat Toxicol* **73**, 268–87.
- Buss W, Kammann C, and Koyro H (2012) Biochar reduces copper toxicity in *Chenopodium quinoa* Willd. in a sandy soil. *J Environ Qual* **41**, 1157–65.
- Campbell B (2010) Organic matter application can reduce copper toxicity in tomato plants. *Natural Sciences Education* **39**, 45–8.
- Caravaca F, Alguacil MM, Vassileva M, Díaz G, and Roldán A (2004) AM fungi inoculation and addition of microbially-treated dry olive cake-enhanced afforestation of a desertified Mediterranean site. *Land Degrad Dev* **15**, 153–61.
- Carrasco L, Azcón R, Kohler J, Roldán A, and Caravaca F (2011) Comparative effects of native filamentous and arbuscular mycorrhizal fungi in the establishment of an autochthonous, leguminous shrub growing in a metal-contaminated soil. *Sci Total Environ* **409**, 1205–9.
- Carvalho L, Cacador I, and Martinis-Loucao M (2006) Arbuscular mycorrhizal fungi enhance root cadmium and copper accumulation in the roots of the salt marsh plant *Aster tripolium* L. *Plant Soil* **285**, 161–9.
- Chand S, Pandey A, and Patra DD (2012) Influence of nickel and lead applied in combination with vermicompost on growth and accumulation of heavy metals by *Mentha arvensis* Linn cv Kosi. *Indian J Nat Prod Resour* **3**, 256–61.
- Chen BC, Ho PC, and Juang KW (2013) Alleviation effects of magnesium on copper toxicity and accumulation in grapevine roots evaluated with biotic ligand models. *Ecotoxicol* **22**, 174–83.
- Chen CT, Chen LM, Lin CC, and Kao CH (2001) Regulation of proline accumulation in detached rice leaves exposed to excess copper. *Plant Sci* **160**, 283–90.
- Clemente R, Almela C, and Bernal MP (2006) A remediation strategy based on active phytoremediation followed by natural attenuation in a soil contaminated by pyrite waste. *Environ Pollut* **143**, 397–406.
- Clemente R, Waljker DJ, and Bernal MP (2005) Uptake of heavy metals and As by *Brassica juncea* grown in a contamination soil in Arnalcollar

- (Spain): The effect of soil amendments. *Environ Pollut* **136**, 46–58.
- Contreras L, Mella D, Moenne A, and Correa JA (2009) Differential responses to copper-induced oxidative stress in the marine macroalgae *Lessonia nigrescens* and *Scytosiphon lomentaria* (Phaeophyceae). *Aquat Toxicol* **94**, 94–102.
- Comejo P, Meier S, Borie G, Rillig M, and Borie F (2008) Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. *Sci Total Environ* **406**, 154–60.
- Corpas FJ, Chaki M, Fernández-Ocaña A, Valderrama R, Palma JM, Carreras A et al. (2008) Metabolism of reactive nitrogen species in pea plants under abiotic stress conditions. *Plant Cell Physiol* **49**, 1711–22.
- Cui XM, Zhang YK, Wu XB, and Liu CS (2010) The investigation of the alleviated effect of copper toxicity by exogenous nitric oxide in tomato plants. *Plant Soil Environ* **56**, 274–81.
- Datnoff LE, Elmer WH, and Huber DM (2007) In *Mineral nutrition and plant disease*. APS Press, USA.
- Davies FT, Puryear JD, Newton RJ, Egilla JN, and Saraiva Grossi JA (2001). Mycorrhizal fungi enhance accumulation and tolerance of chromium in sunflower (*Helianthus annuus*). *J Plant Physiol* **158**, 777–86.
- Del Val C, Barea JM, and Azcón-Aguilar C (1999) Assessing the tolerance to heavy metals of arbuscular mycorrhizal fungi isolated from sewage sludge-contaminated soils. *Appl Soil Ecol* **11**, 261–9.
- Demirevska-Kepova K, Simova-Stoilova L, Stoyanova Z, Holzer R, and Feller U (2004) Biochemical changes in barley plants after excessive supply of copper and manganese. *Environ Exp Bot* **52**, 253–66.
- Di Toro DM, Allen HE, Bergman HL, Meyer JS, Paquin PR, and Santore RC (2001) Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ Toxicol Chem* **20**, 2383–96.
- Dodd J, Boddington C, Rodríguez A, Gonzalez-Chavez C, and Mansur I (2000) Mycelium of arbuscular mycorrhizal fungi (AMF) from different genera: form, function and detection. *Plant Soil* **226**, 131–51.
- Dražkiewicz M, Skórzyńska-Polit E, and Krupa Z (2007) The redox state and activity of superoxide dismutase classes in *Arabidopsis thaliana* under cadmium or copper stress. *Chemosphere* **67**, 188–93.
- Ducic T and Polle A (2005) Transport and detoxification of manganese and copper in plants. *Braz J Plant Physiol* **17**, 103–12.
- Edelstein M and Ben-Hur M (2007) Preventing contamination of supply chains by using grafted plants under irrigation with marginal water. In *Proceedings of the International Symposium on Water Resources Management*, pp. 150–4, Wilson, USA.
- Epstein E and Bloom AJ (2005) In *Mineral nutrition of plants: Principles and perspectives*, Sinauer, USA.
- Fan J, He Z, Ma LQ, Yang Y, Yang X, and Stoffell PJ (2011) Immobilization of copper in contaminated sandy soils using calcium water treatment residue. *J Hazard Mater* **189**, 710–8.
- Fan J, He Z, Ma LQ, Nogueira TAR, Wang Y, Liang Z et al. (2012) Calcium water treatment residue reduces copper phytotoxicity in contaminated sandy soils. *J Hazard Mater* **199–200**, 375–82.
- Ferrol N, Gonzalez-Guerrero M, Valderas A, Benabdellah K, and Azcón-Aguilar C (2009) Survival strategies of arbuscular mycorrhizal fungi in Cu-polluted environments. *Phytochem Rev* **8**, 551–9.
- Fortunati P, Lombi E, Hamon RE, Nolan AL, and McLaughlin MJ (2005) Effect of toxic cations on copper rhizotoxicity in wheat seedlings. *Environ Toxicol Chem* **24**, 372–8.
- Frantz JM, Khandekar S, and Leisner S (2011) Silicon differentially influences copper toxicity response in silicon-accumulator and non-accumulator species. *J Amer Soc Hort Sci* **136**, 329–38.
- Gao Z, Lin Y, Wang X, Wei M, Yang F, and Shi Q (2012) Sodium nitroprusside (SNP) alleviates the oxidative stress induced by NaHCO₃ and protects chloroplast from damage in cucumber. *Afr J Biotechnol* **11**, 6974–82.
- Göhre V and Paszkowski U (2006) Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. *Planta* **223**, 1115–22.
- González-Chávez C, D'Haen J, Vangronsveld J, and Dodd JC (2002) Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. *Plant Soil* **240**, 287–97.
- Gonzalez-Chavez M, Carrillo-Gonzalez R, Wrigth S, and Nichols K (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Environ Pollut* **130**, 317–23.
- Guo W, Hou YL, Wang SG, and Zhu YG (2005) Effect of silicate on the growth and arsenate uptake by rice (*Oryza sativa* L.) seedlings in solution culture. *Plant Soil* **272**, 173–81.
- Hao GP, Xing Y, and Zhang JH (2008) Role of nitric oxide dependence on nitric oxide synthase-like activity in the water stress signaling of maize seedling. *J Integr Plant Biol* **50**, 435–42.
- Hasanuzzaman M, Hossain MA, and Fujita M (2010) Physiological and biochemical mechanisms of nitric oxide induced abiotic stress tolerance in plants. *Am J Plant Physiol* **5**, 295–324.
- He HY, He LF, Gu MH, and Li XF (2012) Nitric oxide improves aluminum tolerance by regulating hormonal equilibrium in the root apices of rye and wheat. *Plant Sci* **183**, 123–30.
- Hildebrandt U, Regvar M, and Bothe H (2007) Arbuscular mycorrhiza and heavy metal tolerance. *Phytochem* **68**, 139–46.
- Hsu YT and Kao CH (2004) Cadmium toxicity is reduced by nitric oxide in rice leaves. *Plant Growth Regul* **42**, 227–38.
- Huang JH, Hsu SH, and Wang SL (2011) Effects of rice straw ash amendment on Cu solubility and distribution in flooded rice paddy soils. *J Hazard Mater* **186**, 1801–7.
- Jin JW, Xu YF, and Huang YF (2010) Protective effect of nitric oxide against arsenic-induced oxidative damage in tall fescue leaves. *Afr J Biotechnol* **9**, 1619–27.
- Juang KW, Ho PC, and Yu CH (2012) Short-term effects of compost amendment on the fractionation of cadmium in soil and cadmium accumulation in rice plants. *Environ Sci Pollut Res* **19**, 1696–708.
- Kamali M, Pour MS, and Moud AAM (2012) Copper effects on growth parameters of hollyhock (*Althaea rosea* L.). *J Ornamental Horticult Plants* **2**, 95–101.
- Kamenidou S, Cavins TJ, and Marek S (2008) Silicon supplements affect horticultural traits of greenhouse-produced ornamental sunflowers. *HortScience* **43**, 236–9.
- Karami N, Clemente R, Moreno-Jiménez E, Lepp NW, and Beesley L (2011) Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. *J Hazard Mater* **191**, 41–8.
- Kawaguchi M, Tajiri A, Backhouse D, and Oda M (2008) Anatomy and physiology of graft incompatibility in solanaceous plants. *J Horticult Sci Biotechnol* **83**, 581–8.
- Kazemi N (2012) Effect of exogenous nitric oxide on alleviating nickel-induced oxidative stress in leaves of tomato plants. *Int J AgriSci* **2**, 799–809.
- Khandekar S and Leisner S (2011) Soluble silicon modulates expression of *Arabidopsis thaliana* genes involved in copper stress. *J Plant Physiol* **168**, 699–705.
- Kidd PS, Llugany M, Poschenrieder C, Gunse B, and Barcelo J (2001) The role of root exudates in aluminum resistance and silicon-induced amelioration of aluminum toxicity in three varieties of maize (*Zea mays* L.). *J Exp Bot* **52**, 1339–52.
- Kinraide TB, Pedler JF, and Parker DR (2004) Relative effectiveness of calcium and magnesium in the alleviation of rhizotoxicity in wheat induced by copper, zinc, aluminum, sodium, and low pH. *Plant Soil* **259**, 201–8.
- Kirkham MB (2006) Cadmium in plants on polluted soils: Effect of soil factors, hyperaccumulation, and amendments. *Geoderma* **137**, 19–32.
- Kopitke PM, Kinraide TB, Wang P, Blamey FPC, Reichman SM, and Menzies NW (2011) Alleviation of Cu and Pb rhizotoxicities in cowpea (*Vigna unguiculata*) as related to ion activities at root-cell plasma membrane surface. *Environ Sci Technol* **45**, 4966–73.
- Lamattina L, Garcia-Mata C, Graziano M, and Pagnussat G (2003) Nitric

- oxide: the versatility of an extensive signal molecule. *Annu Rev Plant Biol* **54**, 109–36.
- Lehmann J and Joseph S (2009) Biochar for Environmental Management. Earthscan, USA.
- Lequeux H, Hermans C, Lutts S, and Verbruggen N (2010) Response to copper excess in *Arabidopsis thaliana*: Impact on the root system architecture, hormone distribution, lignin accumulation and mineral profile. *Plant Physiol Bioch* **48**, 673–82.
- Leung H, Ye Z, and Wong M (2006) Interactions of mycorrhizal fungi with *Pteris vittata* (As hyperaccumulator) in As-contaminated soils. *Environ Pollut* **139**, 1–8.
- Leyval C, Turnau K, and Haselwandter K (1997) Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza* **7**, 139–53.
- Li J, Leisner SM, and Frantz J (2008) Alleviation of copper toxicity in *Arabidopsis thaliana* by silicon addition to hydroponic solutions. *J Am Soc Hort Sci* **133**, 670–7.
- Li P, Song A, Li Z, Fan F, and Liang Y (2012) Silicon ameliorates manganese toxicity by regulating manganese transport and antioxidant reactions in rice (*Oryza sativa* L.). *Plant Soil* **354**, 407–19.
- Liang YC, Wong JWC, and Wei L (2005) Silicon-mediated enhancement of cadmium tolerance in maize (*Zea mays* L.) grown in cadmium contaminated soil. *Chemosphere* **58**, 475–83.
- Lock K, Criel P, Schampelaere KAD, Van Eeckhout H, and Janssen CR (2007) Influence of calcium, magnesium, sodium, potassium and pH on copper toxicity to barley (*Hordeum vulgare*). *Ecotox Environ Safe* **68**, 299–304.
- Luo XS, Li LZ, and Zhou DM (2008) Effect of cations on copper toxicity to wheat root: Implications for the biotic ligand model. *Chemosphere* **73**, 401–6.
- Luo XS, Zhou DM, and Wang YJ (2006) Free cupric ions in contaminated agricultural soils around a copper mine in eastern Nanjing City, China. *J Environ Sci* **18**, 927–31.
- Ma JF (2004) Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. *Soil Sci Plant Nutr* **50**, 11–8.
- Ma JF, Tamai K, Yamaji N, Mitani N, Konishi S, Katsuhara M et al. (2006) A Si transporter in rice. *Nature* **440**, 688–91.
- Maksimovic JD, Bogdanovic J, Maksimovic V, and Nikolic M (2007) Silicon modulates the metabolism and utilization of phenolic compounds in cucumber (*Cucumis sativus* L.) grown at excess manganese. *J Plant Nutr Soil Sci* **170**, 739–44.
- Marciano DPDR, Ramos FT, Alvim MN, Magalhaes JR, and França MGC (2010) Nitric oxide reduces the stress effects of aluminum on the process of germination and early root growth of rice. *J Plant Nutr Soil Sci* **173**, 885–91.
- Martínez-Ballesta MC, Alcaraz-López C, Muries B, Mota-Cadenas C, and Carvajal M (2010) Physiological aspects of rootstock–scion interactions. *Sci Hort* **127**, 112–8.
- Mateos-Naranjo E, Andrades-Moreno L, and Davy AJ (2013) Silicon alleviates deleterious effects of high salinity on the halophytic grass *Spartina densiflora*. *Plant Physiol Biochem* **63**, 115–21.
- Meier S, Azcon R, Cartes P, Borie F, and Cornejo P (2011) Alleviation of Cu toxicity in *Oenothera picensis* by copper-adapted arbuscular mycorrhizal fungi and treated agrowaste residue. *Appl Soil Ecol* **48**, 117–24.
- Meier S, Borie F, Curaqueo G, Bolan N, and Cornejo P (2012) Effects of arbuscular mycorrhizal inoculation on metallophyte and agricultural plants growing at increasing copper levels. *Appl Soil Ecol* **61**, 280–7.
- Mench M, Lepp N, Bert V, Schwitzguébel JP, Gawronski SW, Schöder P et al. (2010) Successes and limitations of phytotechnologies at field scale: outcomes, assessment and outlook from COST Action 859. *J Soil Sed* **10**, 1039–70.
- Mihailovic N and Drazic G (2011) Incomplete alleviation of nickel toxicity in bean by nitric oxide supplementation. *Plant Soil Environ* **57**, 396–401.
- Mocquot B, Vangronsveld J, Clijsters H, and Mench M (1996) Copper toxicity in young maize (*Zea mays* L.) plants: Effects on growth, mineral and chlorophyll contents and enzyme activities. *Plant Soil* **182**, 287–300.
- Neill SJ, Desikan R, Clark A, and Hancock JT (2002) Nitric oxide is a novel component of abscisic acid signaling in stomatal guard cells. *Plant Physiol* **128**, 13–6.
- Novak JM, Busscher WJ, Laird DL, Ahmedna M, Watts DW, and Niandou MAS (2009) Impact of biochar amendment on fertility of a southeastern coastal plain soil. *Soil Science* **174**, 105–12.
- Oliva SR, Mingorance MD, and Leidi EO (2011) Effects of silicon on copper toxicity in *Erica andevalensis* Cabezudo and Rivera: A potential species to remediate contaminated soils. *J Environ Monit* **13**, 591–6.
- Olteanu Z, Elena T, Lacramioara O, Maria MZ, Craita MR, and Gabriela V (2013) Copper-induced changes in antioxidative response and soluble protein level in *Triticum aestivum* cv. beti seedlings. *Rom Agric Res* **30**, DII 2067-5720 RAR 2012–190.
- Pagnussat GC, Lanteri ML, and Lamattina L (2003) Nitric oxide and cyclic GMP are messengers in the indole acetic acid-induced adventitious rooting process. *Plant Physiol* **132**, 1241–8.
- Paquin PR, Gorsuch JW, Apte S, Batley GE, Bowles KC, Campbell PG et al. (2002) The biotic ligand model: a historical overview. *Comp Biochem Physiol C* **133**, 3–35.
- Paquin PR, Santore RC, Wu KB, Kavvas CD, and Di Toro DM (2000) The biotic ligand model: a model of the acute toxicity of metals to aquatic life. *Environ Sci Policy* **3**, 175–82.
- Pedler JF, Kinraide TB, and Parker DR (2004) Zinc rhizotoxicity in wheat and radish is alleviated by micromolar levels of magnesium and potassium in solution culture. *Plant Soil* **259**, 191–9.
- Pietikainen J, Kiikkilä O, and Fritze H (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos* **89**, 231–42.
- Pilon M, Abdel-Ghany SE, Cohu CM, Gogolin KA, and Ye H (2006) Copper cofactor delivery in plant cells. *Curr Opin Plant Biol* **9**, 256–63.
- Posmyk MM, Kontek R, and Janas KM (2009) Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. *Ecotoxicol Environ Safe* **72**, 596–602.
- Qian M, Li X, and Shen Z (2005) Adaptive copper tolerance in *Elsholtzia haichowensis* involves production of Cu-induced thiol peptides. *Plant Growth Regul* **47**, 66–73.
- Reinhardt D (2007) Programming good relation development of the arbuscular mycorrhizal symbiosis. *Plant Biol* **10**, 98–105.
- Rodríguez-Serrano M, Romero-Puertas MC, Zabalza A, Corpas FJ, Gomez M, Del Río LA et al. (2006) Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots: Imaging of reactive oxygen species and nitric oxide accumulation in vivo. *Plant Cell Environ* **29**, 1532–44.
- Rogalla H and Römeheld V (2002) Effects of silicon on the availability of boron: Possible effects on the phenol pathway and on the redox status in *Cucumis sativus* L. In *Boron in plant and animal nutrition*, Goldbach HE, Rerkasem B, Wimmer MA, Brown PH, Thellier M, and Bell RW (eds.), pp. 205–13, Kluwer Academic/Plenum, USA.
- Rouphael Y, Cardarelli M, Rea E, and Colla G (2008) Grafting of cucumber as a means to minimize copper toxicity. *Environ Exp Bot* **63**, 49–58.
- Sadon FN, Ibrahim AS, and Ismail KN (2012) An overview of rice husk applications and modification techniques in wastewater treatment. *J Purity Utility Reaction Environ* **1**, 308–34.
- Savvas D, Colla G, Rouphael Y, and Schwarz D (2010) Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. *Sci Horticulturae* **127**, 156–61.
- Savvas D, Papastavrou D, Ntatsi G, Ropokis A, Olympios C, Hartmann H et al. (2009) Interactive effects of grafting and manganese supply on growth, yield, and nutrient uptake by tomato. *HortScience* **44**, 1978–82.
- Shi QH, Bao ZY, Zhu ZJ, He Y, Qian QQ, and Yu JQ (2005a) Silicon mediated alleviation of Mn toxicity in *Cucumis sativus* in relation to activities of superoxide dismutase and ascorbate peroxidase. *Phytochem* **66**, 1551–9.
- Shi XH, Zhang CC, Wang H, and Zhang FS (2005b) Effect of Si on the distribution of Cd in rice seedlings. *Plant Soil* **272**, 53–60.
- Siddiqui MH, Al-Whaibi MH, and Basalah MO (2011) Role of nitric oxide in tolerance of plants to abiotic stress. *Protoplasma* **248**, 447–55.

- Singh HP, Kaur S, Batish DR, Sharma VP, Sharma N, and Kohli RK (2009) Nitric oxide alleviates arsenic toxicity by reducing oxidative damage in the roots of *Oryza sativa* (rice). *Nitric Oxide* **20**, 289–97.
- Singh RP and Agrawal M (2010) Variations in heavy metal accumulation, growth and yield of rice plants grown at different sewage sludge amendment rates. *Ecotox Environ Safe* **73**, 632–41.
- Siripornadulsil S, Traina S, Verma DPS, and Sayre RT (2002) Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. *Plant Cell* **14**, 2837–47.
- Song J, Shi G, Xing S, Chen M, and Wang B (2009) Effects of nitric oxide and nitrogen on seedling emergence, ion accumulation, and seedling growth under salinity in the ehalophyte *Suaeda salsa*. *J Plant Nutr Soil Sci* **172**, 544–9.
- Stuckey JW, Neaman A, Ravella R, Komameni S, and Marty'nez CE (2009) Highly charged swelling mica reduces Cu bioavailability in Cu-contaminated soils. *Environ Pollut* **157**, 12–6.
- Sudová R and Vosátka M (2007) Differences in the effects of three arbuscular mycorrhizal fungal strains on P and Pb accumulation by maize plants. *Plant Soil* **296**, 77–83.
- Tewari RK, Hahn EJ, and Paek KY (2008) Modulation of copper toxicity induced oxidative damage by nitric oxide supply in the adventitious roots of *Panax ginseng*. *Plant Cell Reports* **27**, 171–81.
- Thakali S, Allen HE, Toro DMD, Ponizovsky AA, Rooney CP, Zhao FJ et al. (2006) A terrestrial biotic ligand model: Development and application to Cu and Ni toxicities to barley root elongation in soils. *Environ Sci Technol* **40**, 7085–93.
- Thomas EY, I Omueti JA, and Ogundayomi O (2012) The effect of phosphate fertilizer on heavy metal in soils and *Amaranthus caudatu*. *Agr Biol J N Am* **3**, 145–9.
- Trotta A, Falaschi P, Cornara L, Minganti V, Fusconi A, Drava G et al. (2006) Arbuscular mycorrhizae increase the arsenic translocation factor in the As hyperaccumulating fern *Pteris vittata* L. *Chemosphere* **65**, 74–81.
- Vodnik D, Grčman H, Mačec I, van Elteren JT, and Kovačević M (2008) The contribution of glomalin related soil protein to Pb and Zn sequestration in polluted soil. *Sci Total Environ* **392**, 130–6.
- Voigt A, Hendershot WH, and Sunahara GI (2006) Rhizotoxicity of cadmium and copper in soil extracts. *Environ Toxicol Chem* **25**, 692–701.
- Vulkan R, Yermiyahu U, Mingelgrin U, Rytwo G, and Kinraide TB (2004) Sorption of copper and zinc to the plasma membrane of wheat root. *J Membrane Biol* **202**, 97–104.
- Walker DJ, Clemente R, and Bernal MP (2004) Contrasting effects of manure and compost on soil pH, heavy metal availability and growth of *Chenopodium album* L. in a soil contaminated by pyritic mine waste. *Chemosphere* **57**, 215–24.
- Walker DJ, Clemente R, Roig A, and Bernal MP (2003) The effect of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environ Pollution* **22**, 303–12.
- Wang FY, Lin XG, and Yin R (2007) Inoculation with arbuscular mycorrhizal fungus *Acaulospora mellea* decreases Cu phytoextraction by maize from Cu-contaminated soil. *Pedobiologia* **51**, 99–109.
- Wang HH, Huang JJ, and Bi YR (2010) Nitrate reductase-dependent nitric oxide production is involved in aluminum tolerance in red kidney bean roots. *Plant Sci* **179**, 281–8.
- Wang P, Kopittke PM, De Schampelaere KAC, Zhao FJ, Zhou DM, Lock K et al. (2011) Evaluation of an electrostatic toxicity model for predicting Ni(2+) toxicity to barley root elongation in hydroponic cultures and in soils. *New Phytol* **192**, 414–27.
- Wang P, Menzies NW, Wang YM, Zhou DM, Zhao FJ, and Kopittke PM (2012a) Identifying the species of copper that are toxic to plant roots in alkaline nutrient solutions. *Plant Soil* **361**, 317–27.
- Wang X, Hua L, and Ma Y (2012b) A biotic ligand model predicting acute copper toxicity for barley (*Hordeum vulgare*): Influence of calcium, magnesium, sodium, potassium and pH. *Chemosphere* **89**, 89–95.
- Wang YS and Yang ZM (2005) Nitric oxide reduces aluminum toxicity by preventing oxidative stress in the roots of *Cassia tora* L. *Plant Cell Physiol* **46**, 1915–23.
- Wei L, Luo C, Li X, and Shen Z (2008) Copper accumulation and tolerance in *Chrysanthemum coronarium* L. and *Sorghum sudanense* L. *Arch Environ Contam Toxicol* **55**, 238–46.
- Weng LP, Wolthoorn A, Lexmond TM, Temminghoff EJ, and Van Riemsdijk WH (2004) Understanding the effects of soil characteristics on phytotoxicity and bioavailability of nickel using speciation models. *Environ Sci Technol* **38**, 156–62.
- Wilson ID, Ribiero DM, Bright J, Harrison J, and Desikan R (2009) Role of nitric oxide in regulating stomatal apertures. *Plant signaling behavior* **4**, 467–9.
- Wu F, Mu Y, Chang H, Zhao X, Giesy JP, and Wu KB (2013) Predicting water quality criteria for protecting aquatic life from physicochemical properties of metals or metalloids. *Environ Sci Technol* **47**, 446–53.
- Xiong J, An L, Lu H, and Zhu C (2009) Exogenous nitric oxide enhances cadmium tolerance of rice by increasing pectin and hemicelluloses contents in root cell wall. *Planta* **230**, 755–65.
- Xiong J, Fu G, Tao L, and Zhu C (2010) Roles of nitric oxide in alleviating heavy metal toxicity in plants. *Arch Biochem Biophys* **497**, 13–20.
- Yang L, Tian D, Todd CD, Luo Y, and Hu X (2013) Comparative proteome analyses reveal that nitric oxide is an important signal molecule in the response of rice to aluminum toxicity. *J Proteome Res* **12**, 1316–30.
- Yang LT, Jiang H, Qi YP, and Chen LS (2012) Differential expression of genes involved in alternative glycolytic pathways, phosphorus scavenging and recycling in response to aluminum and phosphorus interactions in Citrus roots. *Mol Biol Rep* **39**, 6353–66.
- Yeh TY and Pan CT (2012) Effect of chelating agents on copper, zinc, and lead uptake by sunflower, Chinese cabbage, cattail, and reed for different organic contents of soils. *J Environ Anal Toxicol* **2**, 145–8. doi:10.4172/2161-0525.1000145.
- Yin Chan K and Xu Z (2009) Biochar: nutrient properties and their enhancement. In *Biochar for Environmental Management*, Lehmann J and Joseph S (Eds.), Earthscan, USA.
- Yruela I (2005) Copper in plants. *Braz J Plant Physiol* **17**, 145–6.
- Yu CC, Hung KT, and Kao CH (2005) Nitric oxide reduces Cu toxicity and Cu-induced NH₄⁺ accumulation in rice leaves. *J Plant Physiol* **162**, 1319–30.
- Yuan HM, Xu HH, Liu WC, and Lu YT (2013) Copper regulates primary root elongation through PIN1-mediated auxin redistribution. *Plant Cell Physiol* **54**, 766–78. doi:10.1093/pcp/pct030.
- Zafar S, Aqil F, and Ahmad I (2007) Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. *Bioresour Technol* **98**, 2557–61.
- Zhang LP, Mehta SK, Liu ZP, and Yang ZM (2008) Copper-induced proline synthesis is associated with nitric oxide generation in *Chlamydomonas reinhardtii*. *Plant Cell Physiol* **49**, 411–9.
- Zhang Y, Han X, Chen X, Jin H, and Cui X (2009) Exogenous nitric oxide on antioxidative system and ATPase activities from tomato seedlings under copper stress. *Sci Hortic* **123**, 217–23.
- Zhang Z, Wang H, Wang X, and Bi Y (2011) Nitric oxide enhances aluminum tolerance by affecting cell wall polysaccharides in rice roots. *Plant Cell Rep* **30**, 1701–11.
- Zhang ZK, Li H, He HJ, and Liu SQ (2013) Grafting raises the Cu tolerance of cucumber through protecting roots against oxidative stress induced by Cu stress. *J Integrat Agric* **12**, 815–24.
- Zhao LQ, Zhang F, Guo JK, Yang YL, Li BB, and Zhang LX (2004) Nitric oxide functions as a signal in salt resistance in the calluses from two ecotypes of reed. *Plant Physiol* **134**, 849–57.
- Zhao X, Ding C, Chen L, Wang S, Wang Q, and Ding Y (2012) Comparative proteomic analysis of the effects of nitric oxide on alleviating Cd-induced toxicity in rice (*Oryza sativa* L.). *Plant Omics J* **5**, 604–14.
- Zheng Y, Wang L, and Dixon M (2004) Response to copper toxicity for three ornamental crops in solution culture. *HortScience* **39**, 1116–20.
- Zou T, Zheng LP, Yuan HY, Yuan YF, and Wang JW (2012) The nitric oxide production and NADPH-diaphorase activity in root tips of *Vicia faba* L. under copper toxicity. *Plant Omics J* **5**, 115–21.