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MINI-REVIEW

# Advantages and challenges of increased antimicrobial copper use and copper mining

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**Abstract** Copper is a highly utilized metal for electrical, automotive, household objects, and more recently as an effective antimicrobial surface. Copper-containing solutions applied to fruits and vegetables can prevent bacterial and fungal infections. Bacteria, such as *Salmonellae* and *Cronobacter sakazakii*, often found in food contamination, are rapidly killed on contact with copper alloys. The antimicrobial effectiveness of copper alloys in the health-care environment against bacteria causing hospital-acquired infections such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* O157:H7, and *Clostridium difficile* has been described recently. The use of copper and copper-containing materials will continue to expand and may lead to an increase in copper mining and production. However, the copper mining and manufacturing industry and the consumer do not necessarily enjoy a favorable relationship. Open pit mining, copper mine tailings, leaching products, and deposits of toxic metals in the environment often raises concerns and sometimes public outrage. In addition, consumers may fear that copper alloys utilized as antimicrobial surfaces in food production will lead to copper toxicity in humans. Therefore, there is a need to mitigate some of the negative effects of increased copper use and copper mining. More thermo-tolerant, copper ion-resistant microorganisms could improve copper leaching and lessen copper groundwater contamination. Copper ion-resistant bacteria associated with plants might be useful in biostabilization and phytoremediation of copper-contaminated environments. In this review, recent progress in microbiological and biotechnological aspects of microorganisms in contact with copper will be presented and discussed, exploring their role in the improvement for the industries involved as well as providing better environmental outcomes.

**Keywords** Antimicrobial copper · Copper mining · Phytoremediation · Microbial copper resistance

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## Introduction

Copper is an essential micronutrient element required by almost all living organisms, including humans, as it contributes to the function of numerous essential metabolic processes. However, copper ions at increased levels are toxic to most organisms due to their ability to generate reactive oxygen species and act as a strong soft metal, e.g., leading to a release of iron from Fe–S clusters (Macomber and Imlay 2009). Copper was the first metal used by human civilizations, presumably because it could be found in the native, metallic form which does not require smelting (Grass et al. 2011). The use of copper needles in ancient Chinese acupuncture is thought to have originated from the Yellow Emperor Huang-Ti and was recorded in the Internal Medical Classic, Nei Ching, around 2500 B.C. (Fields 1947). Copper was also an ingredient in ancient Chinese mineral preparations ("stone drugs"), which have been adapted over the past 2,000 years for treatment of specific diseases such as endemic fluorosis and Kaschin–Beck disease (Yu et al. 1995). An Egyptian medical text, written between 2600 and 2200 B.C., describes the application of copper to sterilize drinking water and chest wounds. The ancient Greeks of the pre-Christian era of Hippocrates (400 B.C.) discovered the sanitizing power of copper thousands of years ago, and they prescribed copper for pulmonary diseases and for purifying drinking water (Dollwet and Sorenson 1985).

However, humans were not the first to discover and use the antimicrobial properties of copper. Macrophages in the human and other mammalian immune systems concentrate copper to enhance killing by oxidative burst (White et al. 2009). Therefore, pathogens such as *Mycobacterium tuberculosis*, *Staphylococcus aureus*, or *Salmonella enterica* serovar Typhimurium able to survive in macrophages require unique copper resistance systems (Osman et al. 2010; Soutourina et al. 2010; Wolschendorf et al. 2011). In contrast, studies have shown that copper deficiency impaired the bactericidal activity of neutrophils and macrophages in vitro (Babu and Failla 1990a, b). Rice plants also appear to derive some protection from copper in their xylem as pathogens such as *Xanthomonas oryzae* pv *oryzae* need to induce copper redistribution to be able to proliferate and spread causing bacterial blight disease (Yuan et al. 2010). This might be a widespread antimicrobial mechanism in plants.

Copper also has a great environmental impact. Global estimates indicate the oceans are responsible for approximately half of the carbon dioxide fixed on Earth. Marine cyanobacteria such as *Proclorococcus* and *Synechococcus* as well as small eukaryotic algae are thought to be responsible for much of the surface water-mediated photosynthesis and subsequent $CO_2$ fixation. In a recent paper, Paytan et al. (2009) reported that Sahara aerosols can readily inhibit photosynthesis. Copper, a metal that can inhibit photosynthesis and other cellular functions, is thought to be a significant component of these aerosols. Stuart et al. (2009) studied the effects of copper shock in marine *Synechococcus* species indicating that some coastal water *Synechococcus* strains may have developed copper tolerance. While copper may be inhibitory to photosynthesis and other metabolic processes, copper-containing enzymes greatly influence the net flux of greenhouse gases to the atmosphere. Two catabolic enzymes have a major influence on greenhouse gas flux: nitrous oxide reductase, the terminal step of denitrification, and particulate methane monooxygenase (pMMO), for removal of methane from the atmosphere. Dupont et al. (2010) compared metal utilization in archaea, bacteria, and eukaryotes through analysis of protein structures and comparative genomics. Protein structures for binding of Cu and Zn did not evolve before the "Great Oxidation Events" of oceans and atmosphere and resulted in a simultaneous evolution of metal-binding electron transport proteins involved in redox transformations of C, N, S, and O necessary for biogeochemical cycling.

Copper can be introduced into soils via sewage sludge, mine effluents, agricultural irrigating water, and industrial waste. Many bacteria have developed a series of copper-resistance mechanisms to survive the adverse environment with high level copper concentrations (Rensing and Grass 2003; Teitzel and Parsek 2003; Waldron and Robinson 2009). Recently, an increased number of highly copper-resistant microorganisms have been isolated which show a remarkable resistance to a wide range of metal ions while surviving in mineral-rich environments (Dopson et al. 2003; Golyshina and Timmis 2005; Franke and Rensing 2007). Copper pollution in soil, water, and the atmosphere has become a global environmental problem because of mining, industrial, and agricultural practices (Nriagu and Pacyna 1988). In order to lessen the environmental impact caused by copper mining (Fig. 1), useful environmental biotechnological processes for mining and remediation are being developed. For example, copper ion resistance and acidic pH tolerance in bacteria are features that can be utilized not only to improve bioleaching but also for bioremediation of copper-contaminated soils.

## Recent developments in copper applications

Copper used in wiring, plumbing, cooling, and roofing account for most of the demand for copper products (Edelstein 2011). About 10% of copper consumption consists of consumer and general products used in agriculture, animal farming, water distribution systems, and healthcare settings. In these areas, copper applications are in most part for the control of microorganisms. The antimicrobial properties, microbial resistance, growth benefits, toxicity, and deficiency of copper have been described extensively in the literature. However, the recent re-assessment of the benefits of antimicrobial copper follows a proliferation of multiple antibiotic-resistant and disinfectant-resistant bacterial strains in the healthcare environment, copper-tolerant plant pathogens, and continued contamination of water and food supplies with human pathogens and subsequent disease outbreaks. Thus, the many re-discovered uses for copper in the war against pathogens may have advantages and could very well result in an increased demand for copper products and also provide some solutions to the existing challenges for already environmentally impaired copper mining sites.

**Fig. 1** Open pit copper mine at Morenci, Arizona, USA (courtesy of C Rensing)

## Use of copper in agriculture

Copper ion compounds have extensive applications in agriculture. Due to the antimicrobial efficacy of copper, its modern day use in bacteriocides and fungicides on fruits, vegetables, and plants can be traced back to its early use in the nineteenth century. French scientist Millardet showed in the 1880s that spraying of grapes and vines with a mixture of copper sulfate, lime, and water rendered them remarkably free of downy mildew. By 1885, his vintner's spray formulation was the fungicide of choice in the USA and was given the name "Bordeaux mixture" (Borkow and Gabbay 2005). Copper compounds used against various fungal diseases of plants were soon an established method, and copper fungicides have been indispensable as many thousands of tons are used annually all over the world to prevent plant diseases. One of the first copper-resistance systems described in plant pathogens was the copABCD operon in *Pseudomonas syringae* pv *tomato*. The protein products were found to increase with cellular copper accumulation (Mellano and Cooksey 1988; Cha and Cooksey 1991). Voloudakis et al. (1993) suggested the resistance genes transfer via plasmids between pseudomonads and xanthomonads. Indeed, homology was shown between the plasmid borne copper-resistance genes *pcoABCDRSE* of *E. coli* and the *copABCD* and *copRS* determinants of *P. syringae* pv. *tomato* and *Xanthomonas campestris* (Rensing and Grass 2003). Copper has been used effectively against *X. campestris* which causes canker in citrus and black rot disease in crucifers (Jiang et al. 2008), although copper resistance has been identified in some strains (Teixeira et al. 2008). Recently, we analyzed the genome sequence of *Sinorhizobium meliloti* CCNWSX0020 which was isolated from *Medicago lupulina* growing in mine tailings in China and identified several unique operons including one operon containing genes encoding a multicopper oxidase and a tyrosinase on an unusual plasmid (unpublished results). These results suggest that symbiotic bacteria, such as rhizobia, can be genetically adapted through horizontal gene transfer in order to make selected rhizobia competitive for survival and plant symbioses in contaminated soils.

Copper is also used extensively as a growth-promoting agent on farm animals. For example, copper has been used as growth promoters in pig diets for at least 45 years (Edmonds et al. 1985). In Europe and the USA, copper is added to the feed for piglets and cattle (Hasman et al. 2006; Amachawadi et al. 2010; Jacob et al. 2010). This practice has continued because pigs fed such supplemented diets exhibited improved average daily weight gain and food conversion efficiency. Copper supplementation is protective against *E. coli*-induced mastitis in dairy cattle (Scaletti et al. 2003), and copper deficiency can result in mineral disorders that affect livestock at pasture. A well-documented example is copper deficiency termed "swayback" in sheep from Australia. The condition appeared as a result of copper interacting with other dietary components, particularly molybdenum and sulfur, resulting in a decrease in copper absorption. Supplementing the diet of sheep with copper revealed that sheep were very susceptible to copper. Dietary supplements to prevent deficiency resulted in elevated hepatic copper concentrations, liver failure, and death of the animals (Ishmael et al. 1971). Brewer (2010) recently reviewed the dangers associated with human diet supplements containing copper, particularly in the elderly population. A higher level of "free" copper ions capable of causing oxidative damage may be involved in altered copper homeostasis in the body, which could result in increased oxidant stress and possible involvement in neurodegeneration as seen in diseases such as Alzheimer's, Parkinson's, and Huntington's. In contrast, Kessler et al. (2008) conducted a randomized, double-blind, placebo-controlled phase 2 clinical trial in patients with mild Alzheimer's disease (AD) and evaluated the effects of oral copper supplementation on cerebral–spinal fluid (CSF) parameters, such A$\beta$42 and Tau proteins which are CSF biomarkers for AD, as well as copper levels in CSF. Patients received 8 mg of copper daily in the form of Cu orotate for 12 months, and results showed that copper levels in the CSF samples after 12 months were not statistically different from the patients receiving placebos. In addition, copper did not have an effect on Tau and phosphorylated Tau levels, but a 10% decrease in A$\beta$42 levels was observed when compared to a 30% decrease in the placebo group. These results indicate that the oral intake of copper did not pose a significant risk to AD patients.

## Antimicrobial copper materials

### Copper in water distribution systems

Many diseases can be acquired through waterborne microorganisms, and the bactericidal properties of copper have been tested for their capacity as a water purifier. There have been many reported occurrences of pathogens, such as *Legionella*, in cooling towers and air conditioners located in public and industrial facilities, which impose a large public health risk (Landeen et al. 1989; Miyamoto et al. 1996; Lin et al. 1998). Lin et al. (1996, 1998) found silver and copper ions are representative examples of inorganic disinfectants which have no residual and corrosion problem. Copper was found to be one of the most toxic metals to heterotrophic bacteria in aquatic environments. The sensitivity to heavy metals of microflora in water was: Ag>Cu>Ni>Ba>Cr>Hg>Zn>Na>Cd (Albright and Wilson 1974). According to some reports published, drinking water is now disinfected by using copper–silver ionization or chlorine and silver–copper ionizations to control pathogenic microorganism (Abad et al. 1994; Rohr et al. 2000, Kim et al. 2004; Shrestha et al. 2009). The efficacy of 1:10 silver/copper combinations for inactivation of *Hartmannella vermiformis* amoebas and the ciliated protozoan *Tetrahymena pyriformis* in vitro was investigated by Rohr et al. (2000). In addition, many algae are highly susceptible to copper which led to the use of copper salts by water engineers to prevent the development of algae in potable water reservoirs. Copper is also employed to control green slime and similar algal scums in farm ponds, swimming pools, irrigation and drainage canals, rivers, lakes, and rice fields (Murray-Gulde et al. 2002). There is a potential for increased copper levels in soils and groundwater as a result of these treatments. In addition, algae may develop copper resistance as reported by Stuart et al. (2009) which would require higher concentrations of copper to be effective. On the other hand, copper ion-resistant algae could be utilized as bio-indicators or for removal of excess copper from water since many algae species have been identified as bioaccumulators of heavy metals (Karez et al. 1994; Andrade et al. 2006; Rajfur et al. 2010). However, high copper and other metals concentrations in drinking water are undesirable and need to be monitored and controlled. Brewer (2010) pointed out that an increased level of copper in drinking water can lead to an increase of "free" copper in the human body over time which may be a factor in neuro-degenerative diseases and decreased levels of cognition. Some of this speculation is based on a study by Sparks and Schreurs (2003) where rabbits consuming a high cholesterol diet were employed as the model group for the study of amyloid plaque development and cognition in AD. The copper level in their drinking water was at 0.12 ppm, reported as 10 times less than the EPA recommended limit for human consumption. However, since this study was utilizing high-cholesterol preconditioning of the animals, it may not necessarily have been copper in their drinking water that was responsible for the observed decline in cognitive ability and increased plaque development. Kessler et al. (2008) in their human clinical trials did not report a decrease in cognition in patients receiving copper dietary supplements in addition to the measured ≤2 mg/l copper levels in their home drinking water.

### Antimicrobial use of copper surfaces in the healthcare environment

During the past decade, the antimicrobial properties of copper have been tested experimentally in healthcare environments with good success. Preliminary studies with copper–silver ionization in hospital water distribution systems revealed that control of *Legionella* could be achieved (Stout and Yu 2003) and lower prevalence of septate molds and yeasts was seen (Pedro-Botet et al. 2007). New copper-based biocide formulations were effective against MRSA, glycopeptide-resistant *Enterococcus*, *Legionella pneumophilia*, and *Acinetobacter calcoaceticus/baumannii* (Gant et al. 2007). Reports of hospital trials utilizing copper alloy touch surfaces showed that the overall numbers of viable bacteria recovered was 66–99% lower than those isolated from their control equivalents (Casey et al. 2010; Mikolay et al. 2010). Mehtar et al. (2008) evaluated the effectiveness of copper alloys against clinical isolates of *Mycobacterium tuberculosis* and *Candida albicans.* Two drug-resistant *M. tuberculosis* strains were tested on solid copper and showed 88–98% growth inhibition in comparison to control surfaces, and *C. albicans* was completely deactivated within 60 min. Weaver et al. (2008) reported a decrease in viability of *Clostridium* *difficile* vegetative cells and spores on copper alloys. However, the inactive spores may need additional germination treatments for maximum efficacy of copper surfaces. Wheeldon et al. (2008) were able to show that by adding 1% sodium taurocholate a significant reduction in dormant spores and subsequent culturing of strains of *C. difficile* could be seen after 60 min on copper alloys and a 2.71 to 3.22 log reduction after 180 min. Archaea so far have rarely been implicated as pathogens in disease processes although they exist in close associations with macroorganisms. A study by Vianna et al. (2006) identified an increase in *Methanobrevibacter oralis* type species in infected root canals. Archaea would be a novel microbial group for the study of contact killing by copper alloys and which role they might play in anaerobic infectious processes in the body. The results of previous and ongoing studies suggest that rapid killing of microorganisms is achieved on contact with copper alloys with greater than 85% copper content. The antimicrobial properties are long lasting and do not diminish by intermittent cleaning or oxide formation on the surface (Grass et al. 2011). Microorganisms surviving on dry copper surfaces are still susceptible to killing by copper in solution, indicating that copper ion resistance would not be one of the survival mechanisms in this case (Espirito Santo et al. 2010). The suggested mechanisms of contact killing of microorganisms on copper alloys are discussed in the following paragraph.

## Mechanisms of contact killing on copper alloys

Genes involved in copper ion resistance can influence survival on copper alloys (Elguindi et al. 2009; Molteni et al. 2010). However, it could be shown that copper ion resistance can be overcome through contact killing on metallic copper surfaces (Table 1). Copper alloys rapidly deactivated copper ion-resistant strains of *E. coli* and *Enterococcus faecium* in laboratory testing (Elguindi et al. 2011). A prolonged survival of *E. faecium* strains on dry copper surfaces could be due to differences in the membrane structure between Gram-positive and Gram-negative bacteria and copper ion transport pathways and transformations which deserve further investigation. Copper ions released from copper surfaces are thought to be responsible for the cascading effect of copper toxicity in bacterial cells. Suggested mechanisms may involve an initial cell membrane compromise, followed by rapid accumulation of copper ions in the bacterial cells, and subsequent protein damage resulting in cell death and DNA degradation (Grass et al. 2011). A direct effect on bacterial survival due to a decreased level of copper ions ($Cu^{1+}/Cu^{2+}$) released from metallic copper surfaces could be observed when the corrosion inhibitor benzotriazole was applied. Lower copper corrosion rates increased survival of copper ion-resistant strains of *E. coli* in a direct inverse relationship (Elguindi et al. 2011). Moreover, the level of copper ions in buffer containing bacterial cells was found to be greater than

**Table 1** Examples of survival times for copper ion-resistant *E. coli*, *E. faecium*, *S. enterica* pv. Typhimurium, and *C. sakazakii* on 99.9% copper alloys under changing environmental conditions

| Strain | MIC (mM $CuCl_2$ in solid media) | Cells suspended in culture medium (moist surface) | Cells suspended in culture medium (dry surface) | Cells suspended in 0.8% NaCl (moist surface) | Cells after 30 days of desiccation, in culture medium (moist surface) | Cells after 30 days of desiccation, in culture medium (dry surface) |
|---|---|---|---|---|---|---|
| *Cronobacter sakazakii* MZ0685 | 10 | <10 min | <1 min | ND | <10 min | <1 min |
| *Salmonella enterica* pv. Typhimurium S9 | 14 | <30 min | <15 min | <3 min | ND | ND |
| *Escherichia coli* 77-30009-6 | 4 | <30 min | <15 min | <3 min | ND | ND |
| *Enterococcus faecium* 75-30704-5 | 10 | <60 min | >120 min | <30 min | ND | ND |
| *Pseudomonas aeruginosa* PAO1 | 2.0 | <15 min | ND | ND | ND | ND |
| *Pseudomonas aeruginosa cinR*::*lSlacZ/hah* | 0.5 | <1 min | ND | ND | ND | ND |

For comparison, *P. aeruginosa* PAO1 (WT) and a mutant with copper-resistance genes response regulator disruption (*cinR*::*lSlacZ/hah*) are shown

*MIC* minimal inhibitory concentration, *ND* not done in buffer alone suggestive of an increase in copper ions on copper surfaces as a result of bacterial metabolic activities (Espirito Santo et al. 2011). Excess of intracellular copper produces reactive oxygen species (ROS), which can be influenced by ROS quenchers and prolong bacterial survival on copper alloys (Espirito Santo et al. 2008). Rich culture media can prolong survival on copper surfaces due to binding of copper ions by organic molecules and decreasing copper bioavailability and subsequent ion influx (Noyce et al. 2006; Molteni et al. 2010; Elguindi et al. 2011). In addition, studies involving low moisture conditions on copper surfaces revealed that *E. coli* could be deactivated in less than 1 min in laboratory testing (Espirito Santo et al. 2011). *E. coli* cells were found to very rapidly accumulate copper ions reaching low molar concentrations of intracellular copper (Espirito Santo et al. 2011). However, the survival of microorganisms on dry copper surfaces, such as coins, may be attributed to bacterial adaptations which do not protect the isolates from copper toxicity in liquid medium (Espirito Santo et al. 2010). Microbes on dry copper may be in a dormant stage since few nutrients are available to them, as it is often the case in natural environments and to which environmental microorganisms have adapted. As suggested by Grass et al. (2011), dormancy and rapid killing on contact with copper are conditions not conducive to horizontal resistance gene transfer.

## Copper applications in food industries

Similar to the healthcare environment, the food production and processing industry is continuously challenged by microorganisms that can cause spoilage of foods and disease outbreaks among consumers (Mead et al. 1999). Recalls of contaminated products can result in dramatic financial losses for the companies involved. Copper-containing disinfectants and pesticides have been in use for decades in agriculture, food, and healthcare environments (Borkow and Gabbay 2005). In addition, food production often starts with the use of copper supplements and antibiotics in order to promote growth and supply disease-free animals (Aarestrup and Hasman 2004). As a result of this practice, copper ion-resistant strains of *Escherichia coli*, *Enterococcus faecium*, and *Salmonella* have been isolated from pigs and poultry (Hasman et al. 2006). In the USA, *Salmonella enteritidis* and *Salmonella typhimurium* contaminations in foods and food products accounted for 30% of salmonellosis cases reported from 1996 to 2006 (Centers for Disease Control and Prevention (CDC) FoodNet Reports). *E. coli* O157:H7 continues to cause food-related outbreaks, most recently in the Fall of 2010 in the USA due to contamination of cheese (Centers for Disease Control and Prevention (CDC) FoodNet Reports). *Cronobacter sakazakii* has been isolated from factory production lines of powdered infant formula and was associated with disease outbreaks in low birth weight neonates. The genomes of *C. sakazakii* strains implicated in neonatal meningitis cases were sequenced and copper resistance genes identified which may in part be responsible for the bacterium's ability to invade brain microvascular endothelial cells (Kucerova et al. 2010). *C. sakazakii* has been tested in biofilms associated with enteral feeding tubes in neonates, and Hurrell et al. (2009) reported that no differences were seen in capsule production and biofilm formation in polyvinylchloride, polyurethane, and silver-impregnated flexelene feeding tubes. Current results obtained in our laboratory showed that *C. sakazakii* and *Salmonella enterica* serovar Typhimurium were rapidly killed on copper alloys when suspended in various media, and no changes in survival times were observed with *C. sakazakii* recovered after 30 days of desiccation (Table 1). Copper alloys could be instrumental for improved food safety by reducing the microbial load in the food production and processing environment. Copper materials may also be useful as antimicrobial surfaces if used in animal feed lots, e.g., food containers, in the water supply for the animals, caging materials, and storage containers. Costs may be deflected by less use of antibiotics and decreased risk of selection for multiple antibiotic-resistant bacterial strains.

## Copper in air filtering

Microbes are everywhere, and the possibilities for copper materials to be useful in many aspects of consumer protection from pathogenic microorganisms are rapidly expanding. Emerging technologies with copper incorporated into other materials, such as developed by Cupron Scientific®, are being tested for their effectiveness against airborne microorganisms. One example is a N95 face mask design containing copper oxide particles which was compared to a regular N95 mask for filtration and deactivation of influenza viruses in a study by Borkow et al. (2010). Results showed a significant decrease in infectious titers of human influenza A virus (H1N1) and avian influenza virus (H9N2) among the viruses recovered from the copper containing vs. regular masks. N95 masks are personal respiratory protection devices designed to filter out 95% of small particles in the air which has important applications for industrial and healthcare environments (National Personal Protective Technology Laboratory 2011). Therefore, additional protection such as de-activation of potential pathogens by adding copper would be welcomed. However, the N95 masks have to be properly fitted to each person's face in order to be effective. Extensive facial hair can prevent a proper fit, and persons with respiratory impairment may not be able to tolerate the tight-fitting mask. Nevertheless, N95 masks could be easily distributed and fitted for military personnel as well as the general public in case of a threat of airborne pathogens. Copper coils in heating, ventilating, and air-conditioning systems (HVAC) are currently tested in US Army trials to evaluate their effect on the decrease and de-activation of airborne pathogens (Copper Air Quality Program USAMRMC 2011). Depending on the results obtained, the usefulness of copper alloys in manufacturing of HVAC systems could mean a substantial increase in copper materials application.

## Environmental technology aiming to lessen environmental impact of copper mining

Mining cutbacks and rising copper prices as well as high global demand are indicators for continued price growth in the near term (James and Beckman 2011). In addition, US copper mine production was expected to increase in 2011 due to expansions and restoration of cutbacks (Edelstein 2011). However, from an environmental aspect, it is becoming more critical for the copper mining and manufacturing industry to minimize the negative impacts on the environment. Curiously, some of the microorganisms associated with copper mining and existing wastelands could provide ways for the industry to compensate for the continuous pollution, when used in conjunction with the right biotechnology.

### Copper mining aspects and environmental problems

Bioleaching is the extraction of specific metals from their ores using living organisms, which has been widely and successfully used in industrial processes to extract copper and other metals. The bioleaching of low-grade ores is more economical and cleaner than the traditional mining procedures using cyanide because it does not require high amounts of energy and does not generate harmful emissions such as sulfur dioxide as opposed to roasting and smelting (Kishino et al. 2000; Jerez 2009a; Orell et al. 2010). Moreover, it can also be applied in some high-grade ores (around 1–2% Cu) or concentrates used in bioreactors (higher than 60% Cu). Therefore, this is an important biotechnological procedure used in several countries that generate several hundred thousand tons a year of metals such as copper (Jerez 2009b; Orell et al. 2010). Bioleaching can involve numerous ferrous iron- and sulfur-oxidizing bacteria, including Gram-negatives of the genera *Acidithiobacillus*, and *Leptospirillum* (Kelly and Wood 2000; Rohwerder et al. 2003; Olson et al. 2003; Rawlings 2005) as well as Gram-positives of the genera *Acidimicrobium*, *Ferromicrobium*, and *Sulfobacillus* (Clark and Norris 1996; Hallberg and Johnson 2001; Schippers 2007). The general principle of bioleaching process for copper is mainly based on the redox reaction of chemo-litho-autotrophic microorganisms as follows (Rawlings 2005; Jerez 2009a; Watlinga et al. 2010):

$$CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow 5\ FeSO_4 + CuSO_4 + 2S^0$$

In this process, insoluble metal sulfides (chalcopyrite) are oxidized by ferric iron to soluble metal sulfates ($CuSO_4$) which can be further purified and refined to yield copper. The role of microorganisms is the further oxidation of the ore and regeneration of the oxidant ferric iron from the generated ferrous iron (Olson et al. 2003; Rawlings 2005; Rohwerder and Sand 2007; Watlinga et al. 2010). Considering microbes used in biomining could stand low pH condition and resist high metal concentration, it would be appropriate to use them not only in biomining but also for bioremediation or removal of copper from contaminated soils. For example, by combining sulfur-oxidizing bacteria with sulfate-reducing bacteria, the sulfur-oxidizing bacteria would produce sulfuric acid to bioleach copper in the solid phase of the soil, and the sulfate-reducing bacteria generate hydrogen sulfide in a bioreactor to precipitate the copper leachate as insoluble metal sulfides (Jerez 2009a, b). Studies conducted with sulfate-reducing bacteria in laboratory bioreactors simulating this system were successful in producing metal sulfides except at very low pH of 4.0 or 3.0 (Cao et al. 2009). Sulfate-reducing bacteria, such as *Desulfovibrio* spp., could be genetically engineered to be more thermo-tolerant as well as acid and copper resistant in order to make the leaching process more efficient. The utilization of *Halothiobacillus* or anaerobic green and purple sulfur bacteria, which can use excess hydrogen sulfide, would reduce the release of the harmful gas into the environment. The resulting elemental sulfur could be re-oxidized and re-used for leaching. Microbial communities involved in copper leaching can be subjected to metagenomic analysis which would give additional data on the most efficient groups and would allow close monitoring for the effectiveness of any additions/changes made to the structural and functional genetic diversity in a particular microbial consortium. Moreover, copper resistance genes have been identified in extremophiles such as the archaeae *Ferroplasma acidarmanus* Fer1 (Baker-Austin et al. 2005) and *Sulfolobus solfataricus* P2 (Ettema et al. 2006). It is not without merit that copper-resistant and acidic pH-tolerant archaeal species may be useful in the bioleaching/bioremediation processes related to copper mining.

### Phytoremediation of contaminated mining sites

Phytoremediation is the use of plants to remove or reduce toxicity of hazardous substances in the environment, including phytostabilization and phytoextraction (Salt et al. 1998; Ghosh and Singh 2005; Pilon-Smits 2005). These processes have attracted attention in recent years because of the low cost of implementation and environmental benefits. Phytoextraction, as the most effective and most difficult in the technically strategy of phytoremediation, is mainly based on the use of hyper-accumulating plants to concentrate soil contaminants in their above-ground tissues, and then extract the metal from the plant biomass (Baker and Brooks 1989; Kramer 2005). Technologies such as chelator-assisted (citric acid, EDTA, CDTA, DTPA, NTA, etc.) phytoextraction (Huang et al. 1997; Cooper et al. 1999) and transgenic phytoremediation (Rugh et al. 1996; Ruiz et al. 2003) have been developed in order to increase contaminant uptake in the phytoremediator (up to 100-fold higher than the concentration in the soil) and produce more biomass for commercial use. Blaylock et al. (1997) used the soil-applied chelating agents to enhance the accumulation of lead in Indian mustard. Huang et al. (1997) studied the role of synthetic chelates in lead phytoextraction. Genetic engineering techniques to implant more efficient accumulator genes into other plants have been suggested by many authors (Brown et al. 1995; Cunningham and Ow 1996; Terry and Bañuelos 2000). Ruiz et al. (2003) and Bizily et al. (2000) introduced modified bacterial *merA* and *merB* genes into *Arabidopsis* and other plants to detoxify organomercurial compounds and subsequently volatilize elemental mercury. The plants used for phytostabilization could develop an extensive root system, create a cover of vegetation, prevent wind and water erosion, immobilize metals in mine tailings, and provide a rhizosphere wherein a flourishing microbial consortium assisting phytoremediation is supported (Mendez and Maier 2008, Kramer 2005, Wenzel 2009). The interactions between plants and beneficial rhizosphere microorganisms can enhance biomass production and tolerance of the plants to heavy metals in stress environments, rendering microorganisms an important component of phytoremediation technology (Glick 2003; Dell'Amico et al. 2005; Sheng and Xia 2006).

## Plant–microbe interactions for growth

Plant growth-promoting rhizobacteria (PGPR) can promote plant growth through synthesizing phytohormones and enzymes (ACC deaminase), producing siderophores which can solubilize or bind iron, fixing atmospheric nitrogen and solubilizing phosphorus and other nutrients (Verma et al. 2001; Passardi et al. 2004). In addition to interacting with microorganisms in the rhizosphere, plants are internally colonized by endophytic bacteria (Badri et al. 2009). Plant growth-promoting mechanisms of endophytic bacteria are similar to the mechanisms of rhizosphere microorganisms which involve nitrogen fixation (James 2000; Doty 2008), the production of plant growth regulators such as auxins, cytokinins, and gibberellins (Asghar et al. 2004; Bent et al. 2001; Garcia de Salamone et al. 2001), suppression of the production of stress ethylene by 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (Dell'Amico et al. 2005; Belimov et al. 2005), and alteration of sugar sensing mechanisms in plants (Goddijn and Smeekens 1998). Dary et al. (2010) studied in situ metal phytostabilization potential of *Lupinus luteus* inoculated with metal-resistant PGPRs in a polluted site. Sheng and Xia (2006) showed inoculants of cadmium-resistant, rhizosphere-competent bacterial strains improved root elongation, and root and shoot biomass production of *Brassica napus* grown on cadmium-polluted soil. Abou-Shanab et al. (2007) also showed root growth and proliferation in the polluted soils in the presence of ACC deaminase-producing bacteria. The Rhizobium–legume symbiosis has also been proposed as a tool for rhizo-remediation of arsenic and other heavy metals in soils (Sriprang et al. 2002; Carrasco et al. 2005; Pajuelo et al. 2007, 2008). Overall, both phytoextraction and phytostabilization combine stimulatory effects of rhizosphere microorganisms on plant growth with metal removal from the ground by keeping the soil structure intact (Haferburg and Kothe 2010). Phytoremediation of mine tailings is a promising remedial technology and also requires further research to identify factors affecting its long-term success by expanding knowledge of suitable plant species and mine tailings chemistry in ongoing field trials. In a recent study, Wei et al. (2009) reported on a heavy metal-resistant strain CCNWRS33-2 which was isolated from the root nodule of *Lespedeza cuneata*. The plant was found in gold mine tailings, and 108 bacterial strains were isolated from various legumes with *Agrobacterium tumefaciens* CCNWRS33-2 being the most resistant to copper, cadmium, lead, and zinc. 16S rRNA analysis showed very high similarity to *A. tumefaciens* LMG196. Results showed that bacterial growth rates of this strain were increased in the presence of 1 mM and 2 mM copper by 2.3- and 1.3-fold, respectively. This might indicate that systems such as the CusCBA RND system is constitutively expressed making it harder for essential metals such as Cu to get into the cytoplasm. The leguminous plant *Robinia pseudoacacia* is the dominant tree species in mine tailings and could be used to promote the nutrient availability of nitrogen and phosphorus in highly polluted soils. A copper-resistant strain of *Mesorhizobium amorphae* was isolated from root nodules of *R. pseudoacacia* in lead–zinc mining tailings in Gansu Province of China. Through pot experiments, we found this copper-resistant *M. amorphae* strain could help *R. pseudoacacia* survive in copper-contaminated soil due to its ability to establish a nitrogen-fixing symbiosis with leguminous plants and enhance growth in the presence of copper (Fig. 2). In addition, the utilization of transferrable


**Fig. 2** Inoculation with copper-resistant strain *Mesorhizobium amorphae* CCNWGS0123 (*middle*) resulted in a significant increase in growth of *Robinia pseudoacacia* seedlings when compared to the control (*left*) without inoculation. On the *right* side, seedlings are shown inoculated with copper-sensitive strain *M. amorphae* CCNWGS0300. All seedlings were grown in the presence of 300 mg/kg Cu (courtesy of GH Wei)

copper-resistance genes on mobile elements such as plasmids or transposons is a process which could be applied to endophytic, rhizosphere, and non-rhizosphere bacteria involved in phytoremediation of copper-contaminated soils. This approach appears to be the most promising biotechnology for successful phytostabilization.

## Future trends

The United States Environmental Protection Agency (EPA) has designated copper alloys as antimicrobial materials (2008), and preliminary studies worldwide have shown significant decreases of microbial populations on copper surfaces in hospital trials. These findings are an indication that copper in the healthcare environment may soon be the standard for common touch surfaces. Recurrent disease outbreaks due to food-associated pathogens may result in an increased need of copper surfaces in food production and processing. More research is needed to alleviate consumer fears of copper contamination in food products. The role of copper–molybdenum interaction should be investigated and its effects on copper toxicity in organisms. Copper in filters, pipes, solutions, fabrics, etc. will be evaluated for its efficacy in decreasing and killing microorganisms in a wide range of environments without the need for antibiotics or toxic chemicals. In addition, the continuing development of hybrid cars and information technologies will require more copper for electrical components. Copper is of course a limited resource but fortunately a very recyclable metal which should result in negligible copper waste. The historic and current practices of copper mining need to be re-evaluated in order to ameliorate the continuous and likely increasing production of mining waste. Copper ion-resistant bacteria exist in non-rhizosphere soils, rhizosphere soils, and as endophytes in plant tissues. Together with copper-tolerant plants, they can be useful in phytoremediation of current and future metal contaminated wastelands. Plant–microbe models can be established depending on the types of remediation needed, e.g., removal of contaminants through phytoextraction, re-vegetation of copper mine tailing soils with native plants for phytostabilization, or in phytostimulation for increased microbial activity and diversity in metal-contaminated soils.

## Conclusion

Copper is a unique metal known for its antimicrobial properties throughout millennia. The re-discovery of the usefulness of copper in the food and healthcare environments has some potential for an increase in copper mining and manufacturing as well as recycling of copper in the next decades. While this prospect is encouraging in terms of economic potential for nations and industries involved in copper production, there is also a downside due to environmental damage and pollution created by these industries. Interestingly, the copper ion-resistant microorganisms proliferating in many environments due to increased exposure to copper can also be rapidly killed on copper alloys. Bacteria capable of withstanding high concentrations of copper and very acidic environments may be useful in combination with copper-tolerant plants to help in the clean-up of copper- and other metal-contaminated sites. Re-vegetation of mine wastelands is an important task which should be an attainable goal in the near future. Addition of plants and microbes to contaminated soils would promote soil nutrient cycling, remove excess metals, improve soil texture and moisture, help prevent release of copper-containing aerosols, and keep excess copper from reaching aquifers. The previous and current research reviewed here is providing excellent examples of the success and versatility of copper materials and copper ion-resistant microorganisms.

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