In Situ Bioremediation and Crop Growth Promotion Using *Trichoderma* Microbial Inoculant (TMI) Ameliorate the Effects of Cu Contamination in Lowland Rice Paddies

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Soil contamination from mine tailings has impoverished the farming community of Mogpog, Marinduque, Philippines. Crop productivity has dwindled due to heavy metal (HM) contamination so the farmers’ incomes have consequently diminished. To help alleviate the constraints to crop productivity, a rice paddy experiment was conducted during the 2020 wet season in Mogpog, Marinduque to test the efficacy of using rice straw compost (RSC) and *Trichoderma* microbial inoculant (TMI) in increasing the yield and reducing the levels of soil copper and lead. The initial soil Cu content in the area was 379–512 mg kg\(^{-1}\), whereas the mean Pb concentration was 42 mg kg\(^{-1}\). Three treatments with three replicates each in a randomized complete block design were made: T0 (control), T1 (with RSC), and T2 (RSC + TMI). RSC was applied at 0.18 kg m\(^{-2}\). Mineral fertilizers were applied to all treatments at mean rates of 27.3 g m\(^{-2}\) for N and 16.17 g m\(^{-2}\) each for P and K fertilizers. After harvest, Cu, Pb, pH, organic matter (OM), and yield were measured. Data analysis showed that soil Cu levels had a significant, moderately negative correlation with yield. The combination of RSC and TMI in T2 significantly increased yield compared to the other treatments. Mean soil Cu and Pb concentrations in T2 were significantly lower than those in T1 and T0, respectively. Thus, using TMI together with RSC had a significant impact on yield accompanied by a reduction of total soil Cu and Pb concentrations. Possibly, TMI + RSC bioremediation and growth-promotion effects synergistically combined to produce large yield responses and remediation of HM in the soil, as discussed in the paper. Furthermore, rice grains harvested from these paddies have Cu content within the allowable limit for the element, whereas Pb was undetected in the rice grains.

Keywords: bioremediation, crop growth promotion, heavy metals, mine tailings, rice, *Trichoderma*

INTRODUCTION

Soil is an important non-renewable natural resource that requires proper management for sustainable use. It is the foundation of terrestrial ecosystems that provide benefits to human society, especially food production. Toxic levels of HM in soil cause serious damage to the system and persist in the environment. They can be biomagnified through transfer from one trophic level to another and are subject to bioaccumulation in the food that humans consume (Ahmad *et al*. 2015). They are known carcinogens and many, such as cadmium and lead, damage human organ systems (Wuana and Okieimen 2011). In addition, HM also negatively affect microorganisms and microconsumers, biological components of the
soil environment that provide ecosystem services such as nutrient cycling (Rodríguez-Eugenio et al. 2018). Although some HMs are micronutrients (like Zn, Cu, and Mo), they can have harmful effects on crops and cause yield reduction at concentrations above normal (Ahmad et al. 2015).

In the Philippines, mine tailings are the main source of HM pollution. From 1982–2007, there were about 20 recorded dam failures [Chronology of Tailings Dam Failures in the Philippines 1982–2007; http://www.piplinks.org/system/files/Tailings+dam+failures+(070722).doc]. Studies done a few years after the MARCOPPER mine tailings pond disasters provide the records of the environmental damage that resulted from mining accidents. David (2002) reported the HM contents of mine tailings from MARCOPPER that were released as follows: Cu (706–3,080 ppm), Mn (445–1,060 ppm), Pb (43–56 ppm), and Zn (131–276 ppm). Tailings rich in HM inundated agricultural lands are located downstream of the ponds.

Lindon et al. (2014) reported that 13 years after the MARCOPPER Maguila-guila dam collapse in 1993 that contaminated the Mogpog river valley and inundated the agricultural areas, these lands were still heavily polluted with silt and toxic tailings and suffering from the effects of acid mine drainage. Due to this pollution, these lands lost about 30 cavans (1.5 t ha⁻¹) rice yield per cropping.

Lindon et al. (2014) further discussed how governance affected the development legacy (good and bad) of mining in the Philippines, with the MARCOPPER mine disaster as their case study. Their paper concluded that poor governance and weak institutions, coupled with unsustainability and conflict, are possibly the most relevant cause of mining problems in the country. The study further stated that such a mining legacy is in direct contrast to the experience of Chile, Malaysia, and Botswana, where mining wealth was managed to ensure they contribute to human development.

The use of fungi – particularly *Trichoderma* – as remediating agents for HM in wastewater and soil has gained attention worldwide, as they are cost-effective and eco-friendly. Siddiquee et al. (2015) provided the different mechanisms by which fungi can be effective in reducing or detoxifying HM. Biosorption of the metal on the extensive cell wall of filamentous fungi is one process. This may involve ion exchange, complexation, adsorption, and precipitation and is dependent on the characteristics of the metal and the amount of fungal biomass available. It may also involve metal uptake with the use of a specific carrier molecule through the active transport of H⁺ gradient transport system. Once inside the cell, the metal can be immobilized by vacuolar compartmentalization or by complexation with SH peptides. The metal can also be transformed intracellularly by various chemical reactions or can be volatilized or may be transferred to other parts of the fungal mycelia. The metal can also be mobilized by chelation with fungal products such as citric or oxalic acids and produce insoluble oxalates. Filamentous fungal biomasses had a high percentage of cell wall materials with HM-binding properties and, thus, take considerable quantities of HM, even with the absence of physiological activity (Siddiquee et al. 2015). HM cause oxidative damage in plants grown in polluted soil, and Xu et al. (2018) showed that *Arabidopsis thaliana* inoculated with *Mucor circinelloides* and *Trichoderma asperellum* exhibited higher antioxidant activity compared to the uninoculated plants, which increased the plant’s tolerance to cadmium and lead.

The present work is a continuation of a series of studies on the bioremediation of lowland rice paddies inundated by mine tailings using RSC (Cuevas et al. 2014) and TMI (Cuevas et al. 2019). Both studies were conducted in Mankayan, Benguet, northern Luzon, where affected farms were contaminated by tailings from a dam failure accident that occurred in 1986. The studies showed that compost application significantly increased soil pH and rice yield. Copper concentration in rice roots was significantly correlated to soil copper concentration, but minimal copper was translocated to the straw. Rice grains had copper levels within the limit allowed by WHO. Cuevas et al. (2019) showed that RSC applied at the rate of 2 kg m⁻² can significantly reduce soil copper. It also demonstrated that all rice straw gathered in the same paddy composted using the pile method and combined with the use of TMI as seed coat significantly increased yield over that of the control. However, this 2019 study did not clearly show the capacity of TMI to lower soil copper concentration in contaminated paddies. At the same time, the paddy method of composting was not acceptable to the farmers due to the high labor inputs.

This present study aims to show that rice straw can be composted naturally by a simple scatter method (with less labor input) and, with the use of TMI as a seed coating, can increase the yield and reduce the soil copper and lead levels. If this method can be implemented by farmers every cropping, there will be a gradual phytobial remediation or microbial assisted phytoremediation (Tripathi et al. 2013) with a concomitant increase in rice yield. This practice can be adaptive and sustainable.

The study reported in this paper was conducted in Mogpog, Marinduque – an island province lying about 160 km south of the capital Manila in the Philippines. The agricultural lands in this municipality were badly damaged during the 1993 and 1996 dam failures of the MARCOPPER Mining Corporation (MMC) tailings pond (Lindon et al. 2014). Another mining corporation, Consolidated Mines

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Incorporated (CMI) also heavily contributed to land pollution. Medianista and Labay (2017) described the mined-out area of CMI in Barangay Capayang as almost barren with only 30% vegetation cover. Mante et al. (2019) compared the soil chemical properties in rehabilitated and unrehabilitated sites in the area. Results of this study showed soil pH of 4.13 and 4.80, %OM of 0.5 and 0.39%, and copper content of 314 and 620 mg kg$^{-1}$ respectively, for rehabilitated and unrehabilitated sites. The acidic pH, low OM content, and high concentration of soil copper are among the factors that make the area unfavorable for plant growth and development. Figure 1 shows the landscape view of the Capayang mined-out area in 2017.

The rice paddies were contaminated with copper (379–512 mg kg$^{-1}$), which is almost twice the level in the Mankayan, Benguet paddies. Furthermore, soil lead was also detected. The objective of this present research is to show the remediation capacity of TMI in lowering the concentration of copper and lead in soil, which was not clearly shown in the previous two studies. In addition, it aims to show the combined effect of compost and TMI in increasing the rice yield in heavily contaminated rice paddies.

MATERIALS AND METHODS

Study Site

Two mining companies operated in the province of Marinduque – namely, CMI and MMC. Figure 2 shows the map of Mogpog, Marinduque and the relative location of the extraction sites of the two companies, which were about 50 km apart. These companies were extracting gold and copper. The former company operated from 1968–1980, whereas the latter operated from 1967–1996. Both mining companies contributed to HM pollution of agricultural lands in Mogpog (communication with Mogpog Municipal Agriculture Officer). The Maguila-guila dam collapse in 1993 and the tailings dam breach in 1996 resulted in mine tailings inundating agricultural fields in five barangays along the Mogpog river. Rice fields in Brgy. Capayang received mine tailings fall out from CMI’s abandoned mined-out site.

The present study was done during the wet season (August–December 2020) when there was no moisture stress. Three farmer co-operators participated in the study. The rice paddies used are located below the mined-out area of the CMI (Figure 2) located in Barangay Capayang.

![Figure 1. CMI mined out area in Brgy. Capayang, Mogpog, Marinduque.](image-url)
Treatments

Three treatments were made as follows: T0 – control (no compost, no TMI), T1 – with rice straw composted in situ (RSC, no TMI), and T2 – with RSC and TMI seed-coated at a rate of 8.3 g kg\(^{-1}\) of seeds. A completely randomized block design was employed with each farmer’s three paddies representing one replicate block. Thus, there were three blocks involved. The three farmers have contiguous rice paddies, with Farmer 1 paddies located at N-13° 30.131’, E-121° 51.498’.

TMI used in this study was developed by the senior author. It consisted of two strains of *Trichoderma ghanense* Doi (formerly identified as *T. pseudokoningii* Rifai) and one strain of UV-irradiated *T. harzianum* Rifai mixed in equal proportions. These species of *Trichoderma* are naturally occurring free-living fungi that are components of soil and litter-root environments. The product is commercially produced by BIOSPARK Corp., UPLB Science Park, College, Laguna – a Filipino company that signed a licensing agreement with the University of the Philippines Los Baños (UPLB). It is sold in a 250-g pack; 1 g contains 4.8 \(\times\) 10\(^8\) cfu g\(^{-1}\).

The practice of *in situ* composting by scattering rice straws after the harvest had been implemented for two rainy seasons (2018 and 2019) by 20 farmer cooperators in Brgy. Capayang before this specific study was conducted. Intermittent light rain occurred on the site from June–July each year such that the straws remained moist. The condition was aerobic as straws remained exposed to air in as much as rainwater was not enough to flood the paddies. Land preparation cannot be started since the soil was not yet fully water-saturated. Irrigation water usually became available in August. Decomposed rice straws just before they were plowed back into the soil had the following properties: pH – 6.0, %C – 27.26, %OM – 46.9, %N – 0.55, P – 834 mg/kg, K – 5344 mg/kg, Cu – 53.0 mg/kg, Pb – 53 mg/kg (unpublished data). The difference of 6.3–10.8% C between fresh rice straw C content of 33.7–38.2% (IRRI Rice Knowledge Bank) with that of 27.4% of plowed-back rice straw indicated that decomposition took place. A pH of 6.0 for plowed-back straw showed that the decay process was aerobic.

For the *in situ* rice straw composting, rice straws from the previous cropping harvested in May 2020 were scattered in the paddy and allowed to decompose. About 0.18 kg m\(^{-2}\) straws were applied in T1 and T2. This was computed from the mean yield of the three farmers from the previous 2020 dry season cropping of 0.1818 kg m\(^{-2}\) (1:1 ratio of grain to straw – IRRI Rice Knowledge Bank). Wet season cropping started in August 2020, where a 3-mo interval between cropping allowed natural decomposition. One paddy from each farmer cooperator was left as control with *no in situ* composting since wet season 2018.

All three farmers performed all the treatments and planted the NSIC Rc300 rice variety developed by the Philippine Rice Research Institute for lowland rice cropping. Fifteen to 18-d-old rice seedlings were transplanted into the field and observed at a 10 cm × 10 cm planting distance. Farmers performed similar cultivation practices such as transplanting, weeding, control of irrigation water, and other cultural practices. F3 applied only N fertilizer at 12.7 g m\(^{-2}\), whereas F1 applied NPK at 53.0 g m\(^{-2}\) N and 42 g m\(^{-2}\) each for P and K. F2 applied NPK at 16.23 g m\(^{-2}\) N and 6.5 g m\(^{-2}\) each for P and K. Mean application by the three farmers for N was 27.3 g m\(^{-2}\) and 16.7 g m\(^{-2}\) each for P and K.
Soil Analysis and Yield Measurements

Soil samples were taken in May 2018 from the farmer cooperators in different barangays where rice fields have been affected by mine tailings as baseline data for our project’s different studies. The data presented here are from Brgy. Capayang of cooperators for this particular study. Samples were analyzed for pH, OM, P, K, and Cu contents. Pb was not analyzed then since only Cu was found high in other reports about the land pollution in the area. The results of these initial soil analyses are presented in Table 1. Repeat soil analyses were conducted a month after the present experiment was completed in January 2021. Samples were analyzed for pH, OM, N, P, K, as well as Cu and Pb contents. All soil Cu and Pb were analyzed as total Cu and Pb through the ICP-OES trace metal analysis method using microwave digestion with HNO_3-H_2O_2 at a ratio of 7:3 (v/v) for the extraction.

The methods of Recel and Labre (1988) were used in this study to analyze the soil pH (1:1 w/v of soil/water) and soil fertility parameters. Percentage OM was analyzed using the Walkley-Black method, available P through the Bray method 1, and the exchangeable K was extracted using 1N ammonium acetate with a pH of 7.0 using an orbital shaker for 30 min. All analyses were done at the Agricultural Systems Institute, Division of Soil Science, College of Agriculture and Food Science, UPLB. The yield was measured from harvested rice and expressed as kg of rice grains per m^2 of land area.

Statistical Analysis

Multiple analysis of variance test with Tukey’s test was conducted to compare data from the three treatments using the SSPS ver. 21 software. Correlation and stepwise regression analyses for the different soil parameters and crop yield were also done.

RESULTS AND DISCUSSION

Effects of Treatments on Crop Yield and Soil Parameters

The yield of rice. According to the Department of Agriculture, the average potential yield of NSIC Rc300 inbred variety of rice is 5.3 t ha\(^{-1}\) for direct-seeded rice (max of 9.0 t ha\(^{-1}\)) and 5.7 t ha\(^{-1}\) for transplanted rice (max of 10.4 t ha\(^{-1}\)). However, in the highly Cu-contaminated rice fields of Brgy. Capayang, Mogpog, the yield is reduced to less than half of the potential, as shown by the T0 yield of only 1.62 t ha\(^{-1}\) equivalent to 0.162 kg m\(^{-2}\) (see Figure 2). This observation has been corroborated by farmers since the start of the project in 2017 (unpublished survey results). Reincorporation of rice straw and allowing it to decompose naturally in the rice fields also did not help in increasing the yield, as shown by the T1 yield of 1.86 t ha\(^{-1}\) (0.186 kg m\(^{-2}\)). These values are actually similar to the Rc300 yield recorded during 2018 (1.47 t ha\(^{-1}\)).

Figure 2 also shows that T2 significantly increased the yield of rice (4.7 t ha\(^{-1}\)) by around 3 t ha\(^{-1}\), which is more than 100% over both T0 and T1, although this is still below the average potential yield of 5.7 t ha\(^{-1}\). This clearly shows the beneficial growth-promoting effects of TMI as reported in previous studies (Banaay et al. 2012; Cuevas 2006; Cuevas et al. 2019). This effect is consistent with 2018 unpublished data showing a 90%
increase in Rc300 yield over the control when *in situ* rice straw composting together with seed-coating with TMI is applied. However, the practice of returning rice straw to the field to be naturally decomposed may not be enough to ameliorate the yield reduction caused by HM contamination in the area. It is hypothesized that the reason why T1 is not as effective as T2 may be due to the levels of Cu in the soil and the specific action of TMI, as will be discussed shortly.

The huge increase in yield with *Trichoderma* application is not uncommon. Khadka and Uphoff (2019) have likewise documented a 2.85 t ha⁻¹ increase in the yield of rice with *Trichoderma* inoculation. The yield increase is made possible by the various physiological and growth responses of rice plants to *Trichoderma* that may include increases in carbohydrate metabolism, photosynthetic and respiratory rates, stomatal conductance, transpiration, internal CO₂ concentration, water use efficiency, plant height, leaf number, tiller number, panicle number, root length, root fresh weight, and chlorophyll a and b content (Doni et al. 2014; Harman et al. 2019). In addition to its plant growth-promoting effects, *Trichoderma* also functions as an agent of biocontrol, natural decomposition, bioremediation, and stress tolerance, as well as a biofertilizer by helping the rice plants take up nutrients from the soil (Debnath et al. 2020). All of these known activities of *Trichoderma* in the soil may result in the large yield response of crops.

Multiple correlation analysis showed that soil Cu is moderately negatively correlated with yield (*r* = −0.464 significant at *P* = 0.05), which means that the higher the mean soil Cu the lower the yield. Xu et al. (2006) stated that the effect of copper on rice yield was seen starting with a 10% yield reduction at 100 mg kg⁻¹ copper, a 50% yield reduction at 300–500 mg kg⁻¹ copper, and finally, a 90% yield reduction at 1,000 mg kg⁻¹ Cu concentrations. With soil Cu at 100–200 mg kg⁻¹, rice yield decreases due to the reduced number of spikelets per panicle (Xu et al. 2005). At levels of more than 400 mg kg⁻¹ soil Cu, considerable yield loss was recorded due to the decrease in the number of panicles caused by Cu stress. This also caused low recovery from transplanting, delayed tillering, and reduced tiller numbers. One mechanism by which toxic levels of soil Cu inhibit rice seedling growth is by inhibiting NO₃⁻ uptake and its upward translocation by modulating the expression level of NO₃⁻ transporter genes (Huo et al. 2020). Thus, rice seedlings supplied with NO₃⁻ had lower shoot biomass than those with NH₄⁺ under Cu stress. However, previous studies have shown that beneficial *Trichoderma* species are able to overcome the constraints posed by Cu toxicity through its bioremediation activities and growth-promoting effects such as increase in panicle numbers, increase in a number of tillers, and enhanced uptake of nutrients, as previously mentioned (Debnath et al. 2020; Doni et al. 2014; Harman et al. 2019).

It has been shown that the *Trichoderma* species used in this study enhance the decomposition process (Cuevas et al. 2019). Other studies have also shown that *Trichoderma* species are able to accelerate the decomposition of rice straw because of their ability to produce various lignocellulolytic enzymes (Chen et al. 2019) with degradation percentages of 12–44% observed within the first 2–4 wk. This percentage of release is achieved in 1–3 mo under normal uninoculated conditions with 55% C, 40% N, 80% P, and 95% K released during this time (Yan et al. 2019). The same paper by Yan et al. (2019) also determined that 35% of cellulose, 55% of hemicellulose, and 25% of lignin components of the rice straw are released during the first 3 mo of decomposition. According to Nakajima et al. (2016), aerobic decomposition of rice straw at 25 °C is 30–35% after 12 wk and 38–46% after 24 wk. Whereas straw decomposition rates vary between different soil types, moisture levels, temperatures, degree of aeration, and incubation times, the results presented by Yan et al. (2019) and Nakajima et al. (2016) show that there is already considerable decomposition of rice straw during the first 1–6 mo of the decomposition process, whether in flooded or non-flooded conditions. With the addition of microbial inoculants, this process is enhanced and accelerated. Thus, it is not surprising that the application of *Trichoderma* with RSC in this study would elicit a greater response than the application of RSC alone. *Trichoderma* species not only accelerate the decomposition process and, therefore, the release of nutrients, but they also cause systemic physiological changes in the host plants that lead to growth promotion.

*Trichoderma* species have long been known to promote plant growth in part through the provision of nutrients to host plants. Plants inoculated with beneficial *Trichoderma* strains have enhanced nutrient uptake facilitated by solubilization of nutrients in the soil – including, but not limited to, N, P, and Zn (Cuevas 2006). Yedidia et al. (2001) showed that inoculation of cucumber plants with *T. harzianum* significantly increased the uptake of Cu, P, Fe, Zn, Mn, and Na – as well as their concentration inside plant tissues. Harman et al. (2004) also reported that *Trichoderma* T-22 caused a generalized increase in the plant uptake of many other elements, aside from those mentioned above such as Ar, Co, Cd, Cr, Ni, Pb, V, Bo, and Al. The paper showed that T-22 reduced metallic ions to increase their solubility and also produced siderophores that chelate iron. This activity may explain, at least partially, the plant-growth-promoting ability of *Trichoderma* sp. and could provide the basis for its bioremediation capability.
It has been reported that the presence of several ATP-binding cassette (ABC) transporters may be responsible for the ability of *Trichoderma* to remediate HM from polluted soil (Harman et al. 2004). ABC transporters are apparently also responsible for the resilience of *Trichoderma* against other environmental toxicants such as fungicides and antibiotics from other soil microorganisms. *Trichoderma*-treated plants also display enhanced root development, increased root hair formation, deeper rooting, higher biomass, and increased yield. In the study by Yedidia et al. (2001), cucumber plants exhibited a 95 and 75% increase in the root area and cumulative root length, respectively, as well as significant increases in biomass and yield. In the Philippine setting, increased root length and significant growth promotion are also observed in crops treated with *Trichoderma* (Banaay et al. 2012). The enhanced root density and deeper root penetration observed may confer substantial benefits to crops in dry growing seasons, during nutrient insufficiency, in highly compacted soils, and under various stresses affecting the root system. It was also suggested that perhaps thicker and healthier roots are also more resistant to root pathogens hence the plant is able to increase productivity. The combined effects of increased uptake of nutrients and enhanced root development, together with other effects, lead to general plant growth promotion.

The *Trichoderma* spp. present in TMI as well as other *Trichoderma* spp. have been proven to promote the growth of plants leading to higher yield. Cuevas (2006) has shown that *Trichoderma pseudokoningii* (= *T. ghanense*, Banaay et al. 2012), a component species in TMI, enhances the mineralization of nutrients from OM. The inoculant also helped in making nutrients such as P and Zn more available to rice crops, hence increasing crop yield. Banaay et al. (2012) also demonstrated the growth-promoting activity of the inoculant in rice and biological control of aerobic rice damping-off and nematode pathogens. Khadka and Uphoff (2019) reported yield increases across *Trichoderma* treatments in the system of rice intensification management and conventional management of transplanted rice compared to that of untreated plots. Babu et al. (2014) showed that inoculation of *Trichoderma virens* PDR-28 in soil with mine tailings rich in HM significantly increased the dry biomass of maize roots and shoots compared to uninoculated soil. Chlorophyll, total soluble sugars (reducible and nonreducible), starch, and protein contents also significantly increased in inoculated treatments compared to the control (Babu et al. 2014).

**Soil parameters.** Initial analysis of soil from Brgy. Capayang in 2018 showed acidic pH (5.6), low %OM (2.4), low %N (0.1), normal P levels (2.3 mg kg⁻¹), normal K levels (0.2 cmol), and high soil Cu content (453 mg kg⁻¹) (Table 1). In this study conducted in 2020, soil parameters have not changed much as shown by the T0 soil parameter values shown in Table 2. In addition, soil Pb levels were within the normal range for tropical agricultural soils albeit at the upper limit of the range.

Table 2 also shows that the addition of *in situ* composted rice straw significantly increased pH relative to the control. This is a well-known effect of compost that may affect nutrient availability in the soil (Cuevas et al. 2014). The added OM (RSC) in this study could be instrumental in the observed increase in Cu in the soil. The RSC alone could not have caused a 40% increase in total Cu compared with the control setup (T1 vs. T0; see Table 2) because only 0.18 kg m⁻² of rice straw were added containing approximately 12 mg kg⁻¹ straw, which translates to 2.16 mg Cu m⁻². If 1 m³ of soil is equivalent to 1,200–1,700 kg (1,450 kg on the average), then Cu in the added RSC only amounts to 2.16 mg per 1,450 kg soil, which is 0.0015 mg kg⁻¹ soil. This is a negligible amount of copper that does not account for the huge increase in soil Cu observed in T1.

### Table 2. Comparison of soil parameters of samples from Barangay Capayang, Mogpog, Marinduque and good lowland soil.

<table>
<thead>
<tr>
<th>Soil samples and treatments</th>
<th>pHᵇ</th>
<th>OM (%)</th>
<th>N (%)</th>
<th>P (mg kg⁻¹)</th>
<th>K (cmol kg⁻¹)</th>
<th>Pb (mg kg⁻¹)ᵇ</th>
<th>Cu (mg kg⁻¹)ᵇ</th>
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<tr>
<td>Barangay,</td>
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<td>T0</td>
<td>4.4 b</td>
<td>3.3</td>
<td>0.1</td>
<td>0.8</td>
<td>0.1</td>
<td>41.8 a</td>
<td>565.9 ab</td>
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<td>Capayang,</td>
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<td>Mogpog, soil</td>
<td>5.1 a</td>
<td>3.6</td>
<td>0.1</td>
<td>0.9</td>
<td>0.3</td>
<td>39.9 ab</td>
<td>792.9 a</td>
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<td>T1</td>
<td>4.5 b</td>
<td>3.4</td>
<td>0.1</td>
<td>0.7</td>
<td>0.2</td>
<td>36.8 b</td>
<td>377.2 b</td>
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<td>T2</td>
<td>4.7</td>
<td>3.4</td>
<td>0.1</td>
<td>0.8</td>
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<td>39.5</td>
<td>578.7</td>
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<td>Mean</td>
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<td>Lowland soil in the</td>
<td>4.0–8.0</td>
<td>3–6</td>
<td>0.05–0.45</td>
<td>1.5–13.5</td>
<td>0.15–1.35</td>
<td>(15–40)</td>
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<td>Philippines and tropical</td>
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ᵃT0 – control (no compost, no TMI); T1 – with compost only (no TMI); T2 – with both compost and TMI
ᵇValues followed by the same letter are not significantly different at P < 0.05
ᶜData obtained from Banaay and Cuevas (2022)
Wierzbowska et al. (2018) suggested that organic fertilization, such as in the case of the addition of RSC in this study, may lead to weaker penetration of trace elements deep into the soil profile; hence, sampling in the upper 20-cm of the soil profile might generate higher levels of Cu as compared to when trace elements are free to leach deeper into the soil profile in the case of T0. Kashem et al. (2007) have shown that Cu in the soil exists mostly in the oxide-bound, organically-bound, and residual fractions – and less in the mobile (water-soluble, exchangeable, and carbonate-bound) fractions. With the addition of OM, a shift in the proportions of these fractions occurs. Previous studies have shown that the addition of OM such as compost to the soil increases Cu sorption due to the high affinity of this metal for organic compounds (Gusiatin and Kulikowska 2015). There is a shift from water-soluble fractions to those that are bound to OM. This means that a higher proportion of soil Cu is retained in the high OM layer of the soil, hence suggesting that less is leached down to the lower layers.

In the case of T2, where soil copper significantly decreased relative to T1, Trichoderma in TMI was able to accelerate the decomposition of organic materials, leading to more light fractions that can more easily leach than particulate OM and other larger complex organic substances. Wiatrowska and Komisarek (2019) discussed that although the light fractions in decomposed straw are low, they are highly metal-enriched and further decomposition progresses the mineralization process leading to a doubling of dissolved organic carbon with leaching) along the soil column. Addition of Trichoderma also increases plant uptake of Cu (Yedidia et al. 2001) but, together with the rice plant (Cui et al. 2019), keeps most of it in the roots; hence, the grains and shoots are not affected by the toxic levels (Table 3). Other reasons why T2 was able to decrease total soil Cu compared to T1 will be discussed in later paragraphs relating to the effect of Trichoderma.

One indication that strong Cu-OM complexes may have been formed with the addition of RSC at a slightly elevated pH is the observation that Cu in shoots is significantly lower in T1 than in T0 and T2 (Table 3). This indicates that bioavailable Cu in T1 is lower even if soil Cu is higher than the other treatments. Wuana and Okieimen (2011) and Wierzbowska et al. (2018) stated that Cu strongly complexes with OM in soil, and only a small fraction of copper is found in the solution. The addition of RSC could have facilitated greater retention of Cu-OM complexes in the upper layers of the soil, leading to a higher Cu concentration when the upper 20 cm was sampled.

It is difficult to exactly pinpoint specific causes of the observed changes in soil copper concentrations in this study because of confounding factors in the field that cannot be totally controlled in a natural farmer’s field setting. However, what can be done to tease-out the effects of confounding factors is to conduct controlled experiments in the laboratory or the greenhouse. These artificial environments cannot completely duplicate what is happening in the field, but at least some hypothesized interactions can be tested. These experiments are beyond the scope of this study so it is recommended for future research. It is also recommended that other Cu pools in the soil (e.g. dissolved, exchangeable, bioavailable, etc.) be quantified in addition to the “total” soil Cu concentrations in order to have an idea of variations and shifts within these pools.

Levels of soil Cu in T2 (377 mg kg\(^{-1}\)) were not significantly different from T0 (567 mg kg\(^{-1}\)) but significantly lower than T1 (793 mg kg\(^{-1}\)). T1 plots had significantly higher levels of Cu than T2 but were not significantly different from T0. This means that T1 (application of compost without the use of TMI) was not effective in remediating or immobilizing soil copper in the area. Hou et al. (2020) mentioned that the effect of soil OM in compost on HM bioavailability is complicated. Dissolved humic substances from soil OM can form soluble complexes with HM like cadmium, thereby increasing its bioavailability but may form stable complexes in HM like mercury resulting in low mobility. On the other hand, fulvic acids form mercury complexes that are more labile and more bioavailable. But in the

<table>
<thead>
<tr>
<th>Treatments*</th>
<th>Shoot Cu content (mg kg(^{-1}))(^{b})</th>
<th>Grain Cu content (mg kg(^{-1}))(^{c})</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>10.72 a</td>
<td>0.72</td>
</tr>
<tr>
<td>T1</td>
<td>7.21 b</td>
<td>0.66</td>
</tr>
<tr>
<td>T2</td>
<td>12.26 a</td>
<td>0.98</td>
</tr>
</tbody>
</table>

*T0 – control (no compost, no TMI); T1 – with compost only (no TMI); T2 – with both compost and TMI

\(^{b}\)In a column, means followed by a common letter are not significantly different at \(P = 0.05\)

\(^{c}\)According to the Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc of the National Academy of Sciences, the limit of daily dietary intake for Cu is 900 μg/d. The Cu content of grains (mean 0.79 mg/kg) in Capayang, Mogpog is well below the limit considering that at this average grain Cu concentration, 1 cup of cooked rice translates to only 52.6 μg.
presence of Trichoderma sp., HM may be taken up by both the fungus and the plant. This may also be the reason why the compost used in the previous study in Mankayan was successful in remediating Cu from the soil since it used a Trichoderma compost activator to speed up the composting process. Previous studies have shown that Trichoderma spp. are capable of bioremediating HM in the soil through biosorption, bioaccumulation, biovolatilization, and phytoremediation (also called microbe-assisted phytoremediation; Tripathi et al. 2013). They concluded that Trichoderma-assisted phytoremediation is highly beneficial especially for multiple heavy-metal contaminations, as this system utilizes various metal detoxifying properties. In the present study in Mogpog, the treatment with RSC + TMI inoculated in rice served as a phytoremediation system that either removed HM (both Cu and Pb) from the soil made them more stably immobile or leached the metals deeper into the soil profile and, at the same time, improved crop yield.

The bioremediation capability of different species of Trichoderma has also been reported by other researchers. Xu et al. (2018) reported that Mucor circinelloides and Trichoderma asperellum can strengthen Arabidopsis thaliana tolerance to cadmium and lead shown by higher root length and shoot fresh weight of plants compared to non-inoculated plants. HM, causes increased reactive oxygen radicals that cause oxidative damage to the plant. Fungi-inoculated plants showed significantly higher increased antioxidant activity compared to the uninoculated plants, reducing the toxic effects of the HM. The two fungi also had the capacity to immobilize Cd and Pb in soils and reduce the translocation of Cd and Pb from soils to aboveground tissues. Similarly, Babu et al. (2014) recommended that T. virens PDR-28 can be used as a soil inoculant to promote plant biomass production at abandoned mine sites. They concluded that the fungus also protects plants against the growth-inhibiting effects of HMs and, thus, is effective in phytostabilization of contaminated soil. Studies in Philippine rice fields contaminated by mine tailings (Cuevas et al. 2014, 2019), as previously mentioned, show the bioremediation potential of Trichoderma species in rice paddies.

The study by Yedidia et al. (2001) showed that Trichoderma harzianum increased the growth of host plants while also increasing micronutrient concentrations. Their study also showed that Cu concentrations significantly increased only in the roots of the plant and not in the shoots. A similar case may have happened in the Mogpog study, where TMI induced increased uptake of Cu from the soil but was retained only in the roots of the rice plants and not in the shoots and grains. This hypothesis may be supported by results from the study of Cui et al. (2019) as described earlier in this paper, where rice plants restrict the translocation of Cu in the shoots and instead maintain them in the roots. It is recommended for future studies that all rice plant tissues be analyzed and not just the edible portions in order to test the hypothesis presented. Furthermore, the role of iron plaques in restricting Cu and Pb translocation from roots to shoots, as shown in previous studies (Tripathi et al. 2014), may be explored and investigated in terms of its interaction with Trichoderma in the roots and the rhizosphere.

Soil Pb concentrations in Mogpog are not problematic since they are within the range that is considered as naturally-occurring (see Table 2). In addition, Pb in the soil is more stable, is less bioavailable, and exists more in the immobile fractions, and the addition of OM further enhances the immobility and low bioavailability of this HM in the soil (Kashem et al. 2007).

**HM Contents of Rice Grains**

A big health concern for crops grown in agricultural soils contaminated by mine tailings is the possibility that HM present in the tailings will be translocated to the edible portion of the crop. Results showed that there are no significant differences between treatments (T0, T1, and T2) in rice grains Cu content that ranged from 0.66–0.98 mg kg\(^{-1}\) (Table 3). This level is within the WHO allowable limit of 10 mg kg\(^{-1}\) for Cu (WHO 1982). This observation that minimal Cu is translocated from soil to grains is consistent with the findings of Cuevas et al. (2014) for rice crops grown in mine tailings-contaminated lowland agricultural soils in Mankayan, Benguet. Also, in the present study in Mogpog, Pb was undetected in rice grains from all treatments; hence, Pb is not really a concern in this area. Even in highly contaminated areas, Pb does not accumulate in rice grains, had a low transfer factor, and poses minimal health risks to consumers (Fan et al. 2017).

**CONCLUSION**

This study showed that the agricultural fields in Brgy. Capayang, Mogpog, Marinduque located downstream of the CMI mined-out site were heavily contaminated with copper reaching up to 512 mg kg\(^{-1}\), which negatively affected yield. This copper concentration can be reduced by in situ composted rice straw in combination with TMI applied as a seed coat in rice seeds before sowing. Application of RSC alone (without an activator to speed up the process of decomposition) is not advisable since it tends to increase the level of total copper in the upper soil layers. However, the use of TMI in combination with RSC significantly improved yield, reversed the effect of Cu toxicity on crop productivity, and decreased the soil Cu concentration – possibly through dissolution...
coupled with leaching and/or sequestration in the roots of the host plant. Pb was also present in the soil but did not reach toxic levels and was within the range that is naturally occurring in soils. Soil lead was also significantly reduced by combined RSC and TMI, possibly owing to the bioremediation activities of Trichoderma. The rice grains grown in the Mogpog rice paddies with Cu and Pb are safe to eat since the Cu level in the rice grains is within the allowable limit, whereas Pb was undetected in the rice grains.

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