Alleviating the yield constraints posed by copper contamination in lowland rice and vegetable crop areas using *Trichoderma* technologies

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ABSTRACT

The farming community of Mogpog, Marinduque, Philippines has been beset by problems caused by soil contamination from mine tailings since the 1990s. Crop productivity declined and farmer incomes have diminished. This study aims to alleviate constraints to crop yield through the use of *Trichoderma* Microbial Inoculant (TMI), and *Trichoderma*-activated rice straw compost (RSC). There were 102 and 79 rice farmers who participated in the 2018 dry and wet seasons, respectively; and 24 vegetable growers during the 2018 growing seasons. Soil sampling in representative areas in participating barangays revealed severe, moderate, and low Cu-contamination levels. In severely contaminated areas, high Cu levels were observed up to one-meter depth and possibly deeper. Preliminary analysis of a limited data-set showed that rice yield is reduced under high soil-Cu levels. Field experiments covering 40 ha of rice fields were conducted to compare crop productivity between control and treated set-ups. Rice productivity significantly increased by 12-28% relative to the control. Higher yield was attributable to more filled-up grains, productive tillers, and greener flag leaves in treated versus control set-ups. Vegetable productivity was higher by 20-37% in the treatment with TMI compared with control plants. Vegetables treated with TMI were harvested 3-7 days earlier than the control. The application of TMI can contribute to increased productivity within a shorter time period. Furthermore, low amounts of Cu were detected in rice grains and vegetables. This indicates that minimal Cu was translocated to the edible portions of the crops that are within the threshold levels for food safety.

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KEYWORDS

Copper contamination, rice and vegetable production, *Trichoderma*, plant growth promotion

INTRODUCTION

Rice is one of the most important commodities in the Philippines since it is the staple food for Filipinos. It also provides livelihood for millions of farmers nationwide. The rice farmer contributes to food security and the economy of the whole nation yet they are the poorest in our country. In Mogpog, Marinduque, two major mining disasters that occurred more than 25 years ago have devastated the area and further impoverished the farming and fishing communities. The Maguila-Guila mine tailings dam collapsed in 1993 flooding the Mogpog River valley followed by the bursting of the drainage tunnel linking the Tapian mine tailings pit to the Boac River in 1996 (Lindon et al., 2014). The disasters spilled contaminated water and debris downstream. This has led to the heavy metal (particularly Cu) contamination of soil due to inundation of farmlands with mine tailings. Rice production was reduced to half and farmers' income diminished (unpublished farmer survey results). In a 2008 community-based monitoring survey, almost half (48.5%) of the households were classified as income poor with 30.7% suffering from food poverty (Lindon et al., 2014). The problem on heavy metal contamination of the soil needs to be addressed accordingly in order to help increase the local source income of the community.

Previous studies have shown that excessive soil Cu concentrations reduce rice yield by 10-90% depending on the levels of soil Cu (Xu et al., 2006). Yield reduction was attributed to effects of Cu toxicity on number of spikelets per panicle, number of panicles, and number of tillers (Xu et al., 2005). Furthermore, Cu stress leads to decreases in uptake of nitrate by rice plants (Huo et al., 2019). However, constraints to crop growth posed by heavy metal toxicity can be mitigated by beneficial plant-fungi interactions. Studies have shown that beneficial fungi inoculated either in plants or on the soil are capable of promoting plant growth despite high levels of heavy metals (Adams et al., 2008; Babu et al., 2014; Xu et al., 2018). These studies show the ability of *Trichoderma* to aid phytostabilization of contaminated soil.

The biofertilizer-biocontrol *Trichoderma* microbial inoculant (TMI) and *Trichoderma*-mediated rapid composting technologies of UPLB were developed from local strains of *T. ghanense* and *T. harzianum*. They have the capacity to alleviate the constraints posed by soil heavy metal contamination as seen in previous studies in Mankayan, Benguet which suffered a similar mine tailing dam disaster as Mogpog (Cuevas et al., 2014, 2019). In the study in Mankayan, crop productivity was increased despite high levels of Cu in the soil. Use of TMI and *Trichoderma*-activated compost has the capacity to remediate heavy metals from the soil and increase crop productivity. TMI strains have likewise shown activities for plant growth-promotion, biocontrol of soil-borne pests and diseases, and induction of systemic resistance in rice plants (Banaay et al., 2011, 2012, 2013). The same benefits may be derived for the farmers of Mogpog. If agricultural fields in Mogpog become productive again, farmers will have higher income, and this can help alleviate poverty in the area. This study specifically aims to determine the baseline soil Cu levels in selected barangays in Mogpog, Marinduque, and to determine the effectivity of TMI and *Trichoderma*-activated rice straw compost (RSC) in increasing crop yields relatively to untreated control set-ups given the soil Cu concentrations present in the area.

MATERIALS AND METHODS

Soil Analysis

Soil samples were taken from the upper 20 cm of soil along 31 representative points within 13 participating barangays in Mogpog, Marinduque at the start of the cropping season. In addition, samples were taken from deeper portions of the soil profile (0.5 m and 1.0 m deep) in 11 selected sampling points in 4 barangays. A total of 42 soil samples were sent for analysis of pH, organic matter, N, P, K, and Cu to the Soil Science Analytical Services Laboratory, UP Los Baños (UPLB). 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 pH of 7.0, using an orbital shaker for 30 minutes. Results of the analyses serve as baseline data for soil parameters.

Field Studies to Evaluate Yield Effects

Field studies were conducted with 102 co-operator farmers (totalling 40.39 ha) during the dry season of 2018 (DS 2018) and 79 co-operator farmers (totalling 41.2 ha) during the wet season of 2018 (WS 2018). There were only two treatments, namely – Treated with TMI + RSC, and Farmer's Practice or the Control (without TMI and RSC). TMI and *Trichoderma* compost activator were applied according to the package instructions. For TMI application, the rice seeds were first soaked in water for 24 hours after which TMI was applied as seed-coat at a rate of 250 kg TMI per 20 kg of hybrid rice or 50 kg of in-bred rice. The mixture was incubated for 10 hours before sowing. For the RSC, *in situ* composting was done as follows: three weeks before planting, cut rice straw from the previous harvest was added to the field at a rate of approximately 1.8 t ha^{-1} (based on previous harvests), soaked in 5 cm of water in the field with NPK (14-14-14) added at a rate of 10 kg ha^{-1} and *Trichoderma* compost activator at a rate of 400 g ha^{-1}. The straws were allowed to decompose aerobically for two weeks, after which, rice straw compost was plowed under for incorporation into the soil one week prior to planting. Farmers were allowed to use the usual rice/vegetable variety that they plant and continue their usual cultivation practices as control. The only variable introduced for each farmer's field is the addition of TMI and compost.

Harvest data was obtained from all rice farmers and some yield parameters (productive tillers, filled grains, and unfilled grains) were obtained from 1 x 1 quadrats from 9 selected farmer fields. Leaf color chart (LCC) readings were taken from rice flag leaves during the grain maturation stages according to the procedure recommended by the International Rice Research Institute (IRRI).

A total of 24 farmers were involved in the field test for TMI in fruits and vegetables. The crops cultivated were pechay, pakwan, talong, kamatis, ampalaya, okra, and sitaw (see Table 3). Two set-ups for each farmer were used, namely treated (with TMI) and control (no TMI). Only the harvest data was obtained from the farmers after the cropping period. No rice straw compost was included in these set-ups since the RSC was a recommendation specific for crop residue management in rice paddies.

Percentage change in crop harvest was computed as follows:

$$\%\,change = \frac{|Treated - Control|}{Control} \times 100$$

Copper Content Analysis of Crops

To answer the question of whether Cu in soil is transported to portions of the plant that are eaten by humans, several selected crops (root crops, vegetables and rice grains) were analyzed for extractable Cu content through 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. Samples were taken from selected farmers' fields from 6 barangays and sent to an analytical services laboratory in UPLB for analysis. This serves as baseline data for Cu content in crops that was requested by the Mogpog LGU.

Statistical Analyses

Statistical analysis was conducted using paired t-Test for the yield data, 2-sample t-Test for determination of differences between yield in normal and Cu-contaminated fields, and Chi Square test for association for the LCC readings. Bioconcentration Factor (BCF) was computed to measure the ability of crops to take up and transport metals to specific plant tissues according to the formula presented by Kacprzak et al. (2014) as follows:

$$BCF = \frac{metal\ concentration\ in\ plant\ tissues\ (ppm)}{metal\ concentration\ in\ the\ soil\ (ppm)}$$

RESULTS AND DISCUSSION

Soil analysis

Initial soil analysis revealed that there are areas that are severely, moderately, and minimally Cu-contaminated (see Figure 1 and Table 1) based on background soil Cu levels (Oorts 2013, Yaron et al. 2012) and increasing severity of published effects on rice (Xu et al. 2005, 2006). The rest have Cu concentrations that are within the normal range. Among 42 soil samples, 28% had normal Cu (<50 ppm), 9% had low Cu contamination (50-100 ppm), 23% were classified as moderately Cu-contaminated (101-200 ppm), while 40% had severe Cu contamination (>200 ppm). Those plots that have moderate and severe contamination are those near the CMI pit, except for the one in Janagdong. This particular plot is queer because the severely contaminated plot is right next to the uncontaminated plot (see lower left hand corner of the map shown in Figure 1A). A larger view of the site map (Figure 1B) shows that the contamination may be due to inundation with river waters contaminated by mine tailings from dams and pits located further upstream. Furthermore, repeat sampling and analysis of areas that are severely contaminated showed that the high Cu concentrations extend up to 1 meter depth and possibly even deeper. The soil Cu concentrations in Mogpog are higher than those observed in Mankayan (Cuevas et al., 2014) which has a similar history of heavy metal contamination due to mine tailings dam failures. The contamination is widespread owing to the spillage of mine tailings through the rivers whose waters overflowed to the adjacent agricultural fields hence covering a large portion of the lowland areas (Lindon et al., 2014; Regis, 2006). Furthermore, multiple dam and pipe ruptures occurred in the past, coupled with intermittent spillage/seepage especially during heavy rains. A study by David (2003) and a report by Regis (2006) have previously determined that Cu is the primary contaminant in the area.

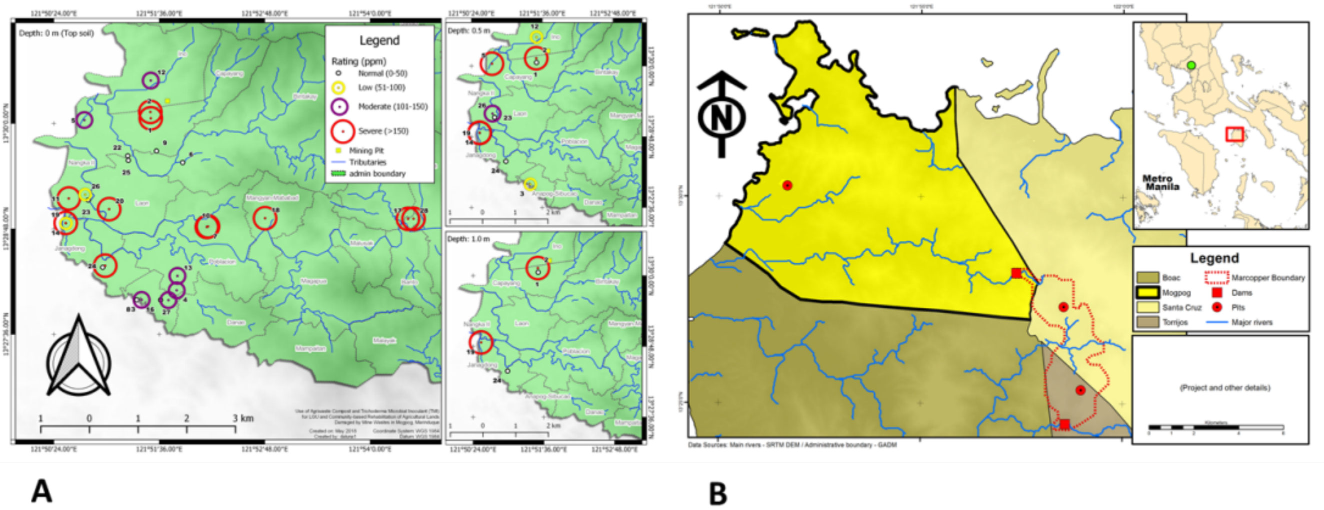

Figure 1: Map of Mogpog showing the (A) location of soil sampling areas and their respective Cu-contamination level and (B) site map showing location of mine tailing dams and pits from previous mining operations of Marcopper and CMI.

Table 1: Cu-contamination categories used in this study.

Cu-contamination category	Range of values (in ppm or mg kg^{-1})	Remarks	References
Normal	≤ 50	Background levels in soil	Oorts, 2013 Yaron et al., 2012
Minimal contamination	51-100	Significant effects on grain yield (and 1 of 4 yield components), tiller dynamics after transplanting, total biomass	Xu et al., 2005 Xu et al., 2006
Moderately contaminated	101-200	Significant effects on grain yield (and 1 of 4 yield components), tiller dynamics with obvious toxic symptoms after transplanting, total biomass	
Severely contaminated	>200	Significant effects on plant height, leaf number, number of elongated internodes, heading date, grain yield (and 4 of 4 yield components), tiller dynamics with obvious toxic symptoms after transplanting, total biomass	

Other measured soil parameters indicate that, on the average, Mogpog soil is acidic, has low organic matter (OM), low nitrogen, but normal phosphorus and potassium levels (Table 2). The pH and N levels, however, are common to lowland soils in the Philippines and in tropical Asia and could be attributed to the parent material, climate, and environment of lowland soils. A

Table 2: Comparison of soil parameters of Mogpog soil and good lowland soil.

Soil sample	Mean values of measured soil parameters					
	pH	Organic Matter (%)	N (%)	P (ppm)	K (cmolc/kg)	Cu (ppm)
Mogpog, Marinduque soil (range of values for all study sites; standard deviation)	5.54 (4.3-7.3; SD=0.76)	2.29 (0.28-5.08; SD=1.43)	0.12 (0.03-0.22; SD=0.06)	2.99 (0.6-9.0; SD=2.10)	0.23 (0.07-0.69; SD=0.15)	154.03 (11-512; SD=131.49)
Lowland soil in the Philippines and tropical Asia (good lowland soil standard)*	4.0-8.0 (5.5-6.5)	3-6 (≥4)	0.05-0.45 (>0.2)	1.5-13.5 (≤15)	0.15-1.35 (>0.2)	(<50)

**data obtained from Miura et al. (1997), Kyuma (1985), and Ponnamperuma (1984)*

previous study by Regis (2006) also showed that the soil in Mogpog is acidic (even as low as pH 2-3 in some areas) and the acidity is because of the acid mine drainage phenomenon caused by the inundation of mine tailings.

Yield and soil Cu content

A two-sample t-Test was conducted to determine if yield is affected by soil Cu content (in untreated plots with three replicates each). This is only a preliminary assessment since yield data was limited to two farmers only who are located in the same barangay (paddies are near each other in barangay Capayang), using the same rice variety (NSIC 218), and having different soil Cu levels (one is normal and the other is severely contaminated). Analysis from DS and WS 2018 data revealed that there is a significant ($P<0.05$) difference between rice yield under normal mean soil Cu levels of 24 ppm (5.1 t ha^{-1}) and yield under severe mean Cu contamination levels of 389 ppm (1.71 t ha^{-1}). The difference is equivalent to a 66.5% decrease in crop yield. This agrees with existing literature stating 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 90% yield reduction at 1,000 mg kg^{-1} Cu concentrations (Xu et al., 2006). Copper toxicity leads to reduced number of spikelets per panicle, reduced number of panicles, and inhibition of nutrient uptake (Huo et al., 2019; Xu et al., 2005, 2006).

Comparison of yield between treatments

Generally, weight of harvested rice grains was greater in plots with *Trichoderma* (treated) than in plots without *Trichoderma* (control). Figure 2 shows the mean yield of treated and untreated crops during the DS and WS 2018 cropping periods. On the average, regardless of Cu-contamination levels, there was a 12.3% increase in yield over the control during the 1st cropping and a 28.4% increase in yield over the control during the 2nd cropping. The differences were significant based on the results of paired t-Test ($P<0.05$ in the 1st cropping and $P<0.01$ in the 2nd cropping). All in all, based on two seasons of cropping, plots treated with TMI and RSC had significantly greater yield than control plots.

The observed higher yield in treated versus control plants may be related to the higher filled grain weight and higher number of productive tillers in treated versus control plants. Statistical analyses showed that filled grain weights were higher in treated than in control plants in both cropping seasons while number of productive tillers was higher in treated plants during the 2nd cropping season (see Table 3 for summary data on yield parameters). This indicates that the effect of treatment with *Trichoderma* is to enhance the ability of plants to fill up the individual grains and support development of productive tillers.

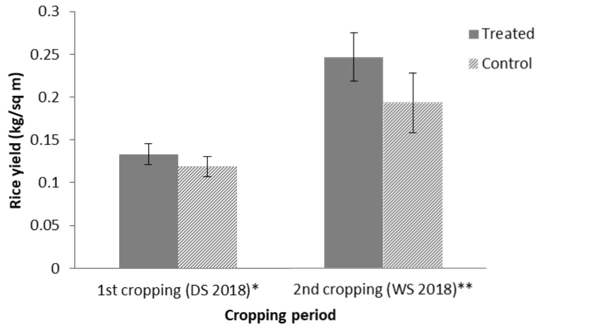

Figure 2: Yield data of rice plants with and without *Trichoderma* treatment during the 1st cropping period of the 1st year of the study (means are significantly different based on Paired t-Test at $P<0.05$).*significant, **highly significant

This is most probably related to the observed greenness of the flag leaves during the reproductive stage (Figure 3 and Table 4). The Leaf Color Chart (LCC) readings were significantly associated to the treatments applied as shown by significant Chi Square test results. It was observed that plants treated with *Trichoderma* were able to maintain greener flag leaves (LCC readings between 3 and 4) which are directly responsible for the delivery of 50% of photosynthates to the grains (Acevedo-Siaca et al. 2021, Li et al. 1998) thus enhancing grain yield and productivity. On the other hand, control plants had lighter green color of the flag leaf (LCC readings between 2 and 3) compared to the treated plants and seem to be shorter and less expanded. These observations imply that the flag leaves in control plots have lower chlorophyll content, hence lower photosynthetic activity and therefore less photosynthates are delivered to the grains during grain maturation leading to lower grain weight. Further, shorter and less expanded leaves (smaller leaf area) are correlated to reduced yield parameters (Rahman et al. 2013) that are probably due to reduced light interception contributing to lower photosynthetic rates.

The results of this study showing increased productivity, yield parameters, and greenness of leaves are consistent with those in existing literature. As mentioned in the Introduction, Banaay et al. (2012) and Cuevas (2006) have shown the growth-promoting and yield-increasing effects of TMI on rice in areas not contaminated by heavy metals in the Philippines. Harman et al. (2019) has shown that *Trichoderma* spp. are able to increase the photosynthetic efficiency of plants by up-regulating the genes involved in pigment production hence increasing light interception and harvesting and consequently improving photosynthetic rates. Various *Trichoderma* spp. are able to up-regulate genes related to increasing the efficiencies of the carbon-fixation process as well as the electron transport chain in

Table 3: Some rice yield parameters measured from selected farmer fields during the 1st and 2nd cropping periods.

Treatments	Mean values of measured yield parameters				
	Filled Grains* (g)	Unfilled Grains (g)	% filled grains	%unfilled grains	Number of productive tillers**
1st cropping period (DS 2018)					
Control (without *Trichoderma*)	836.44	40.89	94.88	5.12	586.44
Treated (with *Trichoderma* - TMI and compost activator)	924.94	10.50	98.88	1.12	581.44
2nd cropping period (WS 2018)					
Control (without *Trichoderma*)	473.40	192.20	70.50	29.50	477.89
Treated (with *Trichoderma* - TMI and compost activator)	543.80	196.60	71.75	28.24	545.89

**significantly different means at P<0.01 in both cropping periods*
***significantly different means at P<0.01 during the 2nd cropping period*

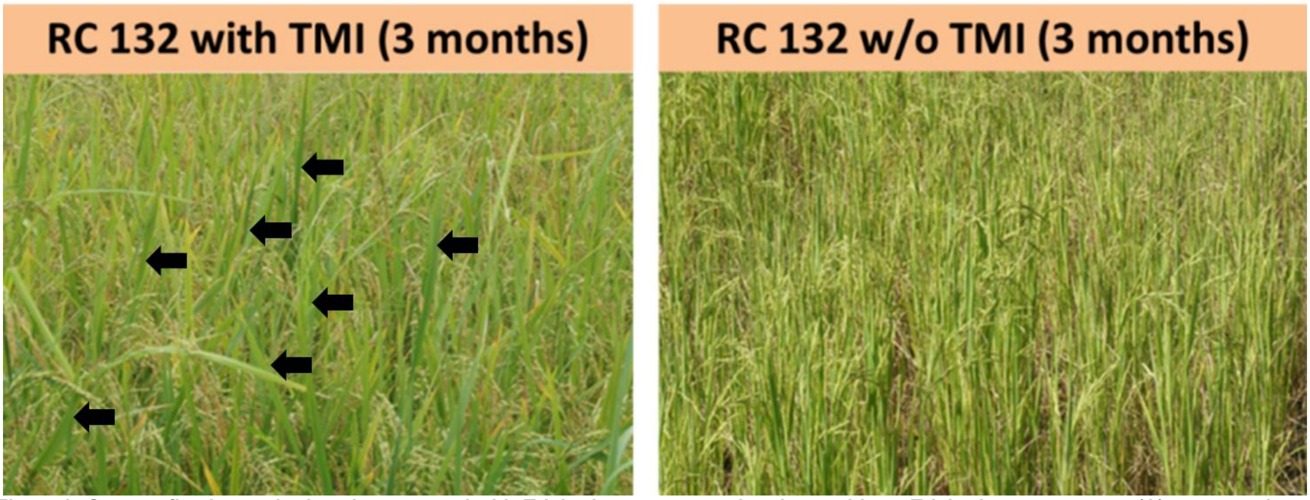

Figure 3: Greener flag leaves in rice plants treated with *Trichoderma* compared to those without *Trichoderma* treatment. (A) arrows point to the green fully expanded flag leaves in treated plants, (B) flag leaves are lighter green and not yet as fully expanded and seems shorter in the control compared to treated plants. Pictures were taken at the same time from contiguous paddies.

Table 4: Average leaf color chart (LCC) reading of the rice flag leaf during grain-filling stage in TMI and compost- treated plots vs. control plots during the DS 2018.

Barangay where the rice fields monitored were located	Average LCC reading of rice flag leaf in different treatments*	
	with *Trichoderma*	without *Trichoderma*
Anapog-Sibucao	3.50	2.33
Ino	3.67	2.17

**Chi square test for association shows significant association (P<0.05) between treatments and greenness of flag leaves with plants treated with Trichoderma being more green than control plants.*

photosynthesis. Studies by Debnath et al. (2020), Doni et al. (2014 and 2017), and Shoresh and Harman (2008a and 2008b) show that *Trichoderma* species elicit various physiological and growth responses from plants that include increases in carbohydrate metabolism, photosynthetic and respiratory rates, stomatal conductance, transpiration, internal CO_2 concentration, water-use efficiency, and chlorophyll *a* and *b* content. A study by Rahul et al. (2016) has shown that various *Trichoderma* spp. are able to increase the length of rice flag leaves thus contributing to plant growth and productivity. In addition, the effects mentioned result to large changes in growth parameters such as increase in plant height, leaf number, tiller number, panicle number, and root length, that all contribute to crop productivity and large crop yield responses.

Yield data on fruits and vegetables

Fruits and vegetables such as pakwan, kamatis, okra, ampalaya and sitaw treated with TMI exhibited significant increase in yield ranging from 20 to 49.4% (Table 5). The highest yield increase in terms of weight of harvested produce was seen in pakwan (watermelon) at 49.4%. Ampalaya (bitter gourd) and sitaw (string bean) showed 20-21% increase while kamatis (tomato) and okra showed 45% and 36% increase, respectively. The average increase for all the crops treated with TMI was 28.85% over that of the non-treated ones. In addition, crops treated with TMI developed faster and were therefore harvested earlier than the untreated ones (Figure 4). Vegetables treated with TMI and inorganic fertilizers were harvested an average of 3 days earlier than those without TMI but with the same amount of inorganic fertilizers. On the other hand, vegetables treated with TMI and organic fertilizers were harvested an average of 6.7 days earlier than those without TMI but with the same amount of fertilizers. The effect of addition of TMI is to increase crop productivity and shorten the cropping period. The earlier harvest may be due to the faster growth of the plants compared to the control. This is consistent with the growth-promoting effect of *Trichoderma* sp. (Harman et al., 2019) and is an expected effect of TMI as shown in previous studies (Cuevas, 2006; Banaay et al., 2012).

Copper Analysis of Crops

Baseline data on copper content of edible portions of crops planted in

Table 5: Mean yield of fruits and vegetables in treated (with *Trichoderma*) and control (no treatment) plots.

Crop	Mean yield (g m^{-2}) in treatments	
	Control (farmer's practice, no *Trichoderma*)	Treated (with *Trichoderma*)
Pechay (*Brassica rapa*)	24.95	32.65
Pakwan (*Citrullus lanatus*)*	107.50	171.75
Pakwan (*Citrullus lanatus*) organic*	583.92	872.61
Talong (*Solanum melongena*)	24.76	34.25
Talong (*Solanum melongena*) organic	43.06	61.11
Kamatis (*Solanum lycopersicum*)	34.16	34.25
Kamatis (*Solanum lycopersicum*) organic*	29.47	42.83
Ampalaya (*Momordica charantia*)*	13.94	17.45
Okra (*Abelmoschus esculentus*)	24.09	30.58
Okra (*Abelmoschus esculentus*) organic*	16.98	23.16
Sitaw (*Vigna unguiculata*)*	64.91	96.83

significantly different means based on Paired t-Test at P<0.05

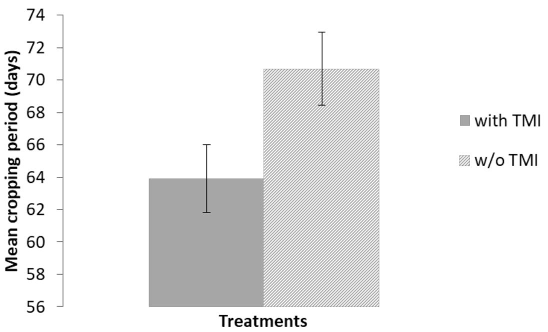

Figure 4: Mean cropping period of fruits and vegetables with and without *Trichoderma* treatment.

high soil Cu- containing paddies in Mogpog are shown in Tables 6 and 7. This data is presented here only as a reference for the safe consumption of the crops grown in Cu-contaminted areas and does not reflect the effect of TMI on plant Cu concentrations since the samples were taken from non-treated plants.

Root crops and vegetables have 10-12 ppm Cu while rice grains contain only 3-4 ppm (mg/kg) Cu. The recommended dietary allowance (RDA) of copper for humans is 900 µg per day. The level of Cu in milled rice grains of Mogpog is only an average of 2.97 ppm (mg/kg) of uncooked rice grains. If we consider that 1 kg of uncooked rice is equivalent to 3 kg of cooked rice and if 1 cup of cooked rice is roughly 200g, then the dietary intake of Cu from 1 cup of cooked rice is approximately 0.198 mg which is equivalent to 198 µg. If a person eats 3 cups a day, that only amounts to 594 µg per day and therefore will not exceed the RDA. Even if a person exceeds the RDA for Cu, the maximum tolerable limit of Cu intake for adults is 10 mg (10,000 µg) per day (Institute of Medicine Panel on Micronutrients, 2001) so there is still a large allowance for dietary intakes without reaching the critical amount beyond which adverse effects on internal organ functions can be observed. This means that it is safe to eat rice grown in Mogpog paddies even if soil copper is

Table 6: Baseline data on level of copper (ppm) of different root crops taken from selected barangays in Mogpog, Marinduque.

Barangay	Rootcrop	Sample	Cu (ppm)*	Soil Cu (ppm)	BCF**
Capayang	Kamote (sweet potato)	*R1*	12	350	0.03
		R2	9		
Nangka II	San Fernando	*R1*	12	188	0.06
		R2	11		
Sumangga	Labanos (radish)	*R1*	6	238	0.03
		R2	6		
Janagdong	Uraro (arrow root)	*R1*	10	290	0.04
		R2	12		
Butansapa	San Fernando	*R1*	16	182	0.09
		R2	15		
Mangyan-Mababad	San Fernando	*R1*	8	158	0.05
		R2	7		

*Recommended Dietary Allowance for adults = **900 µg/day** for copper (Institute of Medicine Panel on Micronutrients, 2001).
**Bioconcentration factor – a measure of the ability of the plant to take up metals and translocate it from the soil to the shoots

Table 7: Baseline data on level of copper (ppm) of rice and vegetables taken from selected barangays in Mogpog, Marinduque.

Barangay	Sample	Cu (ppm)*	Soil Cu (ppm)	BCF**
Capayang	Rice (milled)	3.6	350	0.01
Capayang	Rice (milled)	2.9	350	0.01
Janagdong	Rice(milled	2.4	290	0.01
Capayang	Rice (unmilled)	2.8	350	0.01
Janagdong	Rice (unmilled)	3.1	290	0.01
Capayang	Rice (unmilled)	3.8	350	0.01
Sumangga	Sili (*Capsicum*)	11.1	238	0.05
Janagdong	Talong (*Solanum melongena*)	12.6	290	0.04
Capayang	Okra	11.1	350	0.03

*Recommended Dietary Allowance for adults = **900 µg/day** for copper (Institute of Medicine Panel on Micronutrients, 2001).
**Bioconcentration factor – a measure of the ability of the plant to take up metals and translocate it from the soil to the shoots

several times higher than the normal level of 50 ppm for highly mineralized soil.

Copper content of crops grown in Cu-contaminated soil ranges from 2.4 – 16 ppm. Rice grains showed the least Cu content among all crops tested with only 2.4 – 3.8 ppm, which is well within the recommended daily dietary intake as described earlier. Root crops contained 6 – 16 ppm Cu while the vegetables sili, talong, and okra, have 11.1-12.6 ppm Cu. These are not consumed every day and intakes are usually only in small amounts. In general, considering the amounts eaten per day, the crops analysed were safe to eat based on Cu content.

The bioconcentration factors (BCF) calculated also shows that the crops have naturally low ability to take up and translocate the Cu in soils to the aerial shoots that are eaten by humans. The values presented are 10x lower than in other plants evaluated in a study by Kacprzak et al. (2014) and similar to the ones obtained by Cui et al. (2019). Therefore, the chosen crops pose no threat to the health of persons consuming the harvested produce grown in Cu-contaminated areas.

CONCLUSION

Baseline soil analysis data shows that some areas in Mogpog are severely contaminated by Cu, others are moderately contaminated, while a few are only slightly contaminated. There are also areas that show normal levels of Cu in the soil. Furthermore, Cu contamination in severely contaminated areas extends up to 1 meter depth and possibly deeper. Preliminary analysis shows that higher soil copper concentrations are associated with lower rice yields.

Addition of TMI and RSC resulted to increased productivity of rice in all areas regardless of level of Cu contamination. Increase in harvest is due to more filled up grains and greater number of productive tillers. The higher filled grain weights may be due to greener flag leaves contributing more photosynthates to the grain compared to the untreated plants. Application of TMI and compost in fruits and vegetables likewise increased the yield and caused faster development of crops leading to earlier harvest.

Baseline data shows that Cu content of rice and selected root crops and vegetables in the severely Cu-contaminated areas in Mogpog are minimal and do not pose a threat to persons consuming the produce.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest in the conduct of this study.

REFERENCES

Acevedo-Siaca LG, Coe R, Quick WP, Long SP. Variation between rice accessions in photosynthetic induction in flag leaves and underlying mechanisms. J Experimental Botany 2021; 72(4): 1282-1294.

Adams P, De-Leij FAAM, Lynch JM. *Trichoderma harzianum* Rifai 1295-22 Mediates Growth Promotion of Crack Willow (*Salix fragilis*) Saplings in Both Clean and Metal-Contaminated Soil. Microb Ecol 2007; 54: 306–313.

Babu AG, Shim J, Bang KS, Shea PJ, Oh BT. *Trichoderma virens* PDR-28: a heavy metal-tolerant and plant growth-promoting fungus for remediation and bioenergy crop production on mine tailing soil. J Environ Manage 2014; 132:129-34.

Banaay CGB, Vera Cruz CM, Cuevas VC. *Trichoderma ghanense* CDO induces resistance against blast and sheath blight pathogens in aerobic rice and selected traditional upland rice varieties. J Trop Plant Pathol 2011; 47:42-55.

Banaay CGB, Cuevas VC, Vera Cruz CM. *Trichoderma ghanense* promotes plant growth and controls disease caused by *Pythium arrhenomanes* in seedlings of aerobic rice APO variety. Phillip Agric Sci 2012; 95(2):54-63.

Banaay CGB, Vera Cruz CM, Cuevas VC. Effect of organic matter amendment on the rhizosphere microbial community and root-infecting pathogens of aerobic rice variety Apo. Philipp Sci Lett 2013; 6(1): 107-118.

Cuevas VC. Soil inoculation with *Trichoderma pseudokoningii* Rifai enhances yield of rice. Philipp J Sci 2006; 135(1): 31–37.

Cuevas VC, Orajay JI, Lagman CA Jr. Rice Straw Compost as Amendment to Reduce Soil Copper Toxicity in Lowland Rice Paddy Field. Philipp Sci Lett 2014; 7(2): 350–355.

Cuevas, VC, Lagman, CA. Jr, Anupo X., Orajay JI, & Malamnao, FGE. Yield improvement with compost amendment and *Trichoderma* microbial inoculant (TMI) in rice paddies inundated by copper-rich mine tailings. Philipp Sci Lett 2019; 12(1): 31-38.

Cui J, Zhao Y, Lu Y, Chan T, Zhang L, Tsang DCW, Li X. Distribution and speciation of copper in rice (*Oryza sativa* L.) from mining-impacted paddy soil: Implications for copper uptake mechanisms. Environ Int 2019; 126: 717-726.

David CPC. Establishing the impact of acid mine drainage through metal bioaccumulation and taxa richness of benthic insects in a tropical Asian stream (The Philippines). Environ Toxicol Chem 2003; 22(12): 2952-9. doi: 10.1897/02-529.

Debnath S, Chakraborty G, Dutta SS, Chaudhuri SR, Das P, Saha AK. Potential of *Trichoderma* species as biofertilizer and biological control on *Oryza sativa* L. cultivation. Biotechnol Veg 2020; 20(1): 1-16.

Doni F, Isahak A, Zain CRCM, Yusoff WMW. Physiological and growth response of rice plants (*Oryza sativa* L.) to *Trichoderma* spp. inoculants. AMB Express 2014; 4: 45.

Doni F, Zain CRCM, Isahak A, Fathurrahmann F, Anhar A, Mohamad WNW, Yusoff WMW, Uphoff N. A simple, efficient, and farmer-friendly *Trichoderma*-based biofertilizer evaluated with SRI Rice Management System. Org Agr 2017; DOI 10.1007/s13165-017-0185-7.

Harman GE, Doni F, Khadka RB, Uphoff N. Endophytic strains of *Trichoderma* increase plants' photosynthetic capability. J Appl Microbiol 2019; 130: 529-546.

Huo K, Shangguan X, Xia Y, Shen Z, Chen C. Excess copper inhibits the growth of rice seedlings by decreasing uptake of nitrate. Ecotoxicol Environ Saf 2019; doi: 10.1016/j.ecoenv. 110105. Epub 2019 Dec 26. PMID: 31884325.

Institute of Medicine Panel on Micronutrients. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington (DC): National Academies Press (US), 2001; 7, Copper. Available from: https://www.ncbi.nlm.nih.gov/books/NBK222312/

Kacprzak MJ, Rosikon K, Fijalkowski K, Grobelak A. The effect of *Trichoderma* on heavy metal mobility and uptake by *Miscanthus giganteus*, *Salix* sp., *Phalaris arundinacea*, and *Panicum virgatum*. Appl Environ Soil Sci 2014: https://doi.org/10.1155/2014/506142

Kyuma K. Fundamental characteristics of wetland soils. In: International Rice Research Institute, Wetland Soils: Characterization, Classification, and Utilization. Los Banos: IRRI, 1985: 191-206.

Li Z, Pinson SRM, Stansel JW, Paterson AH. Genetic dissection of the source-sink relationship affecting fecundity and yield in rice (*Oryza sativa* L.). Molecular Breeding 1998; 4: 419-426.

Lindon JG, Canare TA & Mendoza RU. Corporate and public governance in mining: lessons from the Marcopper mine disaster in Marinduque, Philippines. Asian J Bus Ethics 2014; 3:171–193.

Miura K, Badayos RB, Briones AM. Characteristics of soils of lowland areas in the Philippines with special reference to parent materials and climatic conditions. JIRCAS Journal 1997; 5:31-42.

Oorts K. Copper. In: Alloway B. (eds) Heavy Metals in Soils. Environmental Pollution, vol 22. Springer, Dordrecht 2013; https://doi.org/10.1007/978-94-007-4470-7_13

Ponnamperuma FN. Soil health of the IRRI farm. Thursday seminar report, March 24, 1984. International Rice Research Institute, Los Baños, Laguna, Philippines.

Rahman MA, Haque ME, Sikdar B, Islam MA, Matin MN. Correlation analysis of flag leaf with yield in several rice cultivars. J Life Earth Sci 2013; 8: 49-54.

Rahul SN, Khilari K, Prasad CS, Singh R, Mishra P, Tomar A. Effect of native *Trichoderma* isolates on the plant growth of rice (*Oryza sativa* L.) plant. South Asian J Food Technol Environ 2016; 2(2): 408-412.

Recel MR, Labre ZM (eds). Methods of soil, plant, water and fertilizer analysis for research. Manila, Philippines: Bureau of Soils and Water Management. 1988: 199 p.

Regis. E. G. Assessment of the effects of acid mine drainage on Mogpog river ecosystem, Marinduque, Philippines, and possible impacts on human communities. Melbourne: Oxfam Australia, 2006:

Shoresh M, Harman GE. The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: A proteomic approach. Plant Physiol 2008a; 147:2147–2163.

Shoresh M, Harman GE. The relationship between increased growth and resistance induced in plants by root colonizing microbes. Plant Signal Behav 2008b; 3(9):737–739.

Xu J, Yang L, Wang Z, Dong G, Huang J, Wang Y. Effects of soil copper concentration on growth, development, and yield formation of rice (*Oryza sativa*). Rice Sci 2005; 12(2): 125-132.

Xu J, Yang L, Wang Z, Dong G, Huang J, Wang Y. Toxicity of copper on rice growth and accumulation of copper in rice grain in copper contaminated soil. Chemosphere 2006; 62 (4): 602-607.

Xu Z, Xinxin L, Yang H, & Cuia Z. Biochemical mechanism of phytoremediation process of lead and cadmium pollution with *Mucor circinelloides* and *Trichoderma asperellum* Ecotoxicol Environ Saf 2018; 157: 21-28.

Yaron B, Calvet R, Prost R. Soil Pollution: Processes and Dynamics. Springer 2012; 315 pp.