Growth and nutrient uptake of Paraserianthes falcataria (L.) as affected by carbonized rice hull and arbuscular mycorrhizal fungi grown in an artificially copper contaminated soil

Article · January 2017

Reuben James Cillo Rollon
Caraga State University

Leo Jude D. Villasica
Caraga State University

Growth and nutrient uptake of *Paraserianthes falcataria* (L.) as affected by carbonized rice hull and arbuscular mycorrhizal fungi grown in an artificially copper contaminated soil

Reuben James C. Rollon, June Emmanuel V. Galleros, Gidel R. Galos, Leo Jude D. Villasica, Chime M. Garcia

Caraga State University, College of Agricultural Sciences and Natural Resources, Ampayon, Butuan City, Philippines. Corresponding author: R. J. C. Rollon, rollonreubenjames@gmail.com

Abstract. Potential heavy metal soil-plant transfer due to the disposal of high metal wastes, land application of fertilizers and pesticides and mining activity posed a serious threat to both the environment and human health. Hence, there is a need to limit heavy metal transfer from soil to plant through phytoremediation. This study examines the potential of arbuscular mycorrhizal fungi (AMF) and carbonized rice hull (CRH) application on selected soil chemical properties, plant growth, biomass production, P and Cu uptake of *Paraserianthes falcataria* grown on soils artificially polluted with Cu. A pot experiment with a total of eight treatment combinations from four different levels of Cu (0, 50, 100, 200 ppm) and two amendment types (unamended, amended (2% CRH + AMF)) was conducted. Results revealed that CRH and AMF inoculation significantly ($p < 0.01$) increased soil organic carbon (OC) by 17% and soil K by 28% while soil pH reduced by 8% compared to the unamended pots. Shoot and total dry matter production increased by 18% and 13% respectively in plants amended with CRH and AMF under increasing Cu rates. Plant dry biomass in CRH and AMF amended pots were positively correlated with soil pH ($p < 0.05$; $r = 0.976$) and tissue P uptake ($p < 0.01$; $r = 0.992$). Tissue P concentration significantly reduced by 17% with CRH and AMF inoculation while tissue P uptake was comparable across treatments. Cu concentration and Cu uptake in CRH and AMF in plants were reduced by 23% and 12% respectively, over unamended Cu-treated pots. Results demonstrated the potential of CRH and AMF to improve plant health even if grown under the Cu-stressed soil.
Key Words: *P. falcataria,* amendment, mycorrhizal symbiosis, heavy metal pollution, phytoremediation.

Introduction. Potential heavy metal soil-plant transfer posed a serious threat to both the environment and human health. Heavy metal accumulates in soil near mining sites, landfills, industrial sites and farm areas where fertilizer and pesticide had been applied. Heavy metals are non-biodegradable, persistent and tend to accumulate in soils and sediments which posed a great danger to living organisms (Tangahu et al 2011). Risk can be even higher if heavy metal contaminated soils were utilized for food crop production and enter the food chain. Hence, there is a need to limit heavy metal transfer from soil to plant.

A new promising approach to contain, stabilize or limit heavy metal movement in the soil is through phytoremediation (Vidali 2001). This technology utilizes metal-tolerant plant species which serve as cover on the surface of metal contaminated soil, thereby, preventing heavy metal from spreading into the soil environment (Bolan et al 2011). Plant roots immobilize heavy metal within the root zone through root surface adsorption, accumulation within roots and precipitation within the soil (Gohre & Paszkowski 2006). Thus, reducing heavy metal uptake and further off site-contamination. Grass species are widely been used as host plant because of its high tolerance to metals, produce large biomass and its ability to survive even in harsh soil condition (Zhang et al 2010; Olatunji et al 2014; Garba et al 2016). However, relying on plants alone to extract metal in the soil is a slow process and sometimes inefficient. Thus, maximizing plant's phytoremediation potential through inoculation of arbuscular mycorrhizal fungi (AMF) (plant root-fungi symbiosis) to remove, degrade or contain chemical contaminants offers more promising remediation technology.

The role of AMF in plant nutrition is well documented (Habte & Osorio 2008). AMF increases plant access to immobile nutrients particularly phosphorus (P), copper (Cu) and zinc (Zn) from the soil (Gosling et al 2006). AMF enhanced plant nutrition by increasing the solubility of plant nutrients and the volume of soil explored of AMF-infected roots. Moreover, AMF promotes plant health and offers protection of plants from heavy metal stress by reducing plant absorption and translocation of metal from the soil (Leyval et al 2002; Emamverdian et al 2015). AMF immobilize metal within the rhizosphere through adsorption on fungal cell walls and metal chelations with glomalin, an insoluble protein produced by AMF which binds heavy metal into unavailable form (Chibuike 2013). In addition, AMF ability to extract and immobilize heavy metals can be further enhanced with the charcoal application.

The use of a charcoal material as a soil amendment to correct soil fertility problem has been well recognized (Lehman et al 2003; Biederman & Harpole 2013). Charcoal addition has shown to reduce soil acidity, adds nutrients directly into the soil and improves soil water and nutrient retention. Apart from improving soil properties, charcoal can sorb a large amount of heavy metal, thereby, influencing its movement in the soil. Several studies had been reported that charcoal application can reduce metal concentration in the soil (Uchimiya et al 2010; Nigussie et al 2012; Kim et al 2015). This reduction was attributed to charcoal alkaline pH which promotes metal precipitation. Moreover, charcoal addition in soil increases cation exchange capacity, thus, offering new sites for metal sorption (Ogbonnaya & Semple 2013).

At present, there has been limited knowledge on plant-AMF-charcoal interaction on heavy metal decontamination. Moreover, there have been no extensive studies conducted to explore the possibility of using legumes as a potential host plant for phytoremediation. Most of the studies conducted on phytoremediation were focused on grasses and other agricultural crops, with a very little understanding on fast growing legumes such as *Paraserianthes falcataria*. This legume is associated with nitrogen (N) fixing bacteria which can further enhance N content in the soil. Application of charcoal material in soil can potentially reduce heavy metal concentration while AMF can reduce heavy metal uptake in the plant. Thus, application of both charcoal and AMF on Cu-contaminated soil would likely reduce Cu movement from soil to plants.

This study was conducted to assess the potential of *P. falcataria* applied with carbonized rice hull (CRH) and inoculated with AMF for phytoremediation, specifically, it aimed to: 1) determine the residual effects of CRH application, AMF inoculation and different Cu concentrations on selected soil chemical properties (pH, organic carbon (OC), N, P, potassium (K) and Cu); 2) determine the effects of CRH application and AMF inoculation on plant growth, biomass production, P and Cu uptake of *P. falcataria* at different Cu concentration.

Material and Method

Experimental design. A 4 x 2 factorial experiment was laid out in a randomized complete block design with three replicates under screen house condition of the College of Agricultural Sciences and Natural Resources (CASNR), Caraga State University (CSU), Ampayon, Butuan City, Philippines, from December 2016 to February 2017. There were eight treatment combinations from four levels of Cu (0, 50, 100 and 200 ppm) and two amendment types (unamended and amended (2% CRH + AMF)). The treatment combinations were designated as follows: T_1 = control; T_2 = 50 ppm Cu; T_3 = 100 ppm Cu; T_4 = 200 ppm Cu; T_5 = 2% CRH + AMF; T_6 = 50 ppm Cu + 2% CRH + AMF; T_7 = 100 ppm Cu + 2% CRH + AMF; T_8 = 200 ppm Cu + 2% CRH + AMF.

Soil collection and preparation. A bulk soil sample (0-20 cm depth) used in the experiment was collected from CASNR Experimental area, CSU. The soil was air dried for one week, pulverized and sieved in a 2-mm wire mesh. Subsamples were subsequently taken for chemical analyses (pH, OC, total N, extractable P, exchangeable K and available

Cu) and the rest was prepared for bagging. Soil analysis was done at the Regional Soils Laboratory of the Department of Agriculture, Taguibo, Butuan City.

A total of 24 polyethylene bags (14.5 cm diameter x 27.0 cm in height) were used in this study. Each pot was filled with 5 kg of non-sterilized soil on oven dry weight basis. Cu was applied on the soil as a diluted solution. Different concentrations were obtained by adding 200 mL of aqueous solution of $CuSO_4$ containing 0, 50, 100 and 200 ppm Cu. The medium was allowed to stabilize for 20 days before using. Every five days, the medium was mixed to ensure that the Cu solution was mixed thoroughly with the soil.

Carbonized rice hull production, characterization, and application. CRH was produced using an open-type fabricated carbonizer developed by PhilRice Research Institute in the Municipality of Remedios T. Romualdez, Agusan del Norte. Prior to carbonization process, rice hulls were sun-dried for three days. Rice hull was carbonized for 6 h. The CRH was sifted using a 2-mm mesh sieve. Subsamples were taken and submitted at Regional Soil Laboratory for chemical analysis (pH, total OC, N, P, K, Ca, Mg and Na). The sieved CRH were thoroughly mixed with soil at 2% concentration (w/w), 20 days prior to planting. The CRH amended and unamended soils were added with tap water up to 6 % moisture content of field capacity.

Preparation of planting materials and inoculation. Seeds of *P. falcataria* were allowed to grow in pre-germinating trays inoculated with AMF using mycovam, a commercial mycorrhizal inoculant produced by Biotech of the University of the Philippines at Los Baños, Laguna. The inoculant is a soil based containing spores of AMF *Glomus etunicatum, G. macrocarpum,* and *Gigaspora margarita* and infected roots of bahia grass (*Paspalum notatum*) as trap plant. Approximately 30 grams of mycovam was applied in the middle part of pre-germinating trays where seeds were sown. Five seeds were sown in each container and later thinned to one seedling per hole two days after germination. Seeds for the control treatment were sown in different trays to avoid cross contamination.

Transplanting. Twenty-five days after sowing, seedlings of mycorrhizal inoculated and non-inoculated with similar height were transplanted in polyethylene bags with holes sealed at the bottom treated with different Cu concentrations. One seedling was planted in each pot and was allowed to grow for 12 weeks. Moisture content in each pot was maintained at 60% of its field capacity. Weeds in each pot were removed manually, immediately after the emergence of weeds. Insects were also removed by handpicking.

Harvesting. Plants were harvested at 12 weeks after transplanting. Prior to harvest, plant height and stem diameter were determined. Plant height was measured one inch above the soil surface up to the tip of the longest leaf. Harvesting was done by cutting each plant close to the soil surface. The soils adhering to the roots were removed carefully. The shoot and roots were washed with tap water, rinsed with distilled water and blot-dried using a paper towel. The different plant parts were air-dried prior and then oven dried for three days in a forced draft oven set at 70^oC. Plant dry weights were obtained by weighing the shoots and roots separately after oven drying. The dry weight of shoots and roots were combined to obtain the total dry matter yield per plant.

Plant tissue and soil analysis. The oven dried plant samples were sent to Regional Soil Laboratory for analyses of P and Cu concentration. The amount of P and Cu uptake was determined by taking the product of total dry matter yield and their respective P and Cu contents. On the other hand, soil samples after harvest were air-dried and sieved (2-mm). About 300 g of each soil sample was sent to the Laboratory for soil pH, TOC, total N, extractable P, exchangeable K and available Cu analyses.

Statistical analysis. All data gathered were subjected to analyses of variance using STAR (v2.0.1) program. Comparison of treatment means was done using Tukey's Honest

Significant Difference Test at $p < 0.05$. Simple correlation analysis was also performed in order to analyze the relationship between the selected variable.

Results and Discussion

Initial chemical properties of soil. Initial chemical analysis of soil used as a potting medium is shown in Table 1. Chemical analysis showed that the soil used was slightly alkaline (pH = 7.34) with very low OC (0.58%), low total N (0.11%) with sufficient P (58 mg kg^{-1}) and K (291 mg kg^{-1}). Concentration of basic cations such as Mg and Na were high except Ca. While initial Cu content was considered above the average limit.

Table 1

Initial chemical properties of soil before experiment

Property	*Soil*
pH (1:5 soil to H_2O)	7.34
OC (%)	0.58
Total N (%)	0.11
Extractable P (mg kg^{-1})	58.00
Exchangeable (mg kg^{-1})	
K	291.00
Ca	Trace
Mg	19,600.00
Na	600.00
Cu	18.00

Chemical properties of carbonized rice hull. The properties of CRH are shown in Table 2. The derived CRH had a strongly alkaline pH (8.89) with very high OC (28.03%) which constitutes more than > 25% of the total elemental content of CRH. Moreover, the CRH had high total N (0.55%), P (0.07%) and K (0.48%) with low concentrations of Ca, Mg and Na.

Table 2

Chemical characteristics of CRH used for the experiment

Property	*CRH*
pH (1:5 soil to H_2O)	8.89
OC (%)	28.03
Total (%)	
N	0.55
P	0.07
K	0.48
Ca	Trace
Mg	0.01
Na	0.03

Effects of AMF inoculation, CRH and Cu addition on selected soil chemical properties. Table 3 shows the effects of Cu application, CRH and AMF inoculation on selected chemical properties of soil after harvest, plant growth and nutrient uptake of *P. falcataria*. Significant differences in pH, OC and exchangeable K between unamended and amended Cu-contaminated soil were detected. Mean soil pH at harvest was 6.79 in CRH and AMF amended pots and 7.40 in unamended pots (Table 4). The addition of 2% CRH and AMF inoculation significantly ($p < 0.01$) reduced soil pH by 8% over unamended pots. Production of organic acids during oxidation on the surfaces of charcoal and the release of protons (H^+) from charcoal's exchange sites might have contributed to the reduction in soil pH (Shenbagavalli & Mahimairaja 2012). Soil pH reduction with a charcoal application on alkaline soil has been reported also by Abrishamkesh et al (2015).

Table 3

Effects of Cu levels, CRH and AMF inoculation and their interaction on soil chemical properties, biomass, growth parameters, P and Cu concentration and uptake of *P. falcataria*

Parameter	*Amendment type (Factor A)*	*Cu level (ppm) (Factor B)*	*A x B*
pH	0.000**	0.108^{ns}	0.510^{ns}
OC	0.000**	0.862^{ns}	0.969^{ns}
Total N	0.091^{ns}	0.305^{ns}	0.737^{ns}
Extractable P	0.499^{ns}	0.409^{ns}	0.285^{ns}
Exchangeable K	0.000**	0.710^{ns}	0.826^{ns}
Available Cu	0.553^{ns}	0.000**	0.482^{ns}
Root biomass	0.277^{ns}	0.248^{ns}	0.030*
Shoot biomass	0.016*	0.050*	0.045*
Total Biomass	0.062^{ns}	0.058^{ns}	0.030*
Plant Height	0.950^{ns}	0.597^{ns}	0.705^{ns}
Tissue P conc.	0.009**	0.285^{ns}	0.324^{ns}
Tissue Cu conc.	0.035*	0.017*	0.391^{ns}
Tissue P uptake	0.426^{ns}	0.784^{ns}	0.067^{ns}
Tissue Cu uptake	0.243^{ns}	0.024^{ns}	0.190^{ns}

ns= not significant; * = significant at p <0.05; ** = significant at <0.01.

Soil total OC in the Cu-contaminated soil after harvest significantly differed between unamended and amended pots. The addition of 2% CRH significantly increased (p <0.01) soil OC by 17% compared to the unamended pots. The observed increase in soil OC with CRH addition was attributed to the inherently high carbon content of CRH as shown in Table 2. This increase of soil OC in the presence of CRH is in agreement with the earlier finding of Nigussie et al (2012) who reported an increase in OC in Cr polluted soil treated with charcoal. Simmilarly, Koyama et al (2016) observed an increase in carbon content in soil following rice husk charcoal application at 2%. In contrast, the addition of CRH and AMF in Cu-contaminated soil did not significantly influence total N and extractable P.

CRH addition at 2% and AMF inoculation significantly increased (p < 0.01) soil exchangeable K at harvest. On the average, soil K in CRH amended pot was 150.17 mg K kg^{-1} soil which is 28% higher compared to the average soil K (117.33 mg K kg^{-1} soil) in the unamended Cu-contaminated pot. The increase in soil K with CRH addition could have been attributed to the direct addition of K from charcoal ash during mineralization. According to Glaser et al (2002) ash in charcoal rapidly releases free bases such as K ions into the soil solution thereby providing readily available nutrient for plant growth. It is noteworthy to mention also that the charcoal used in this study contains a considerable amount of K (Table 2). Simmilarly, Major et al (2010) reported higher K availability in the soil following charcoal application. Such increase was attributed to the direct addition of nutrients from the charcoal material as well as the increase in nutrient retention in the soil.

The amount of soil extractable Cu after harvest steadily increased with increasing level of added Cu as shown in Table 4. The values ranged from 7.60 ppm without Cu application to 81.15 ppm with the application rate of 200 ppm in unamended pots. On the other hand, Cu concentration in CRH and AMF amended pots ranged from 7.25 ppm to 74.28 ppm. The average Cu concentration in CRH and AMF inoculated pot was slightly higher by 8% compared to the average Cu concentration in the unamended pot. The amount of Cu extracted from the soil after harvest was lower than the amount of Cu added to the soil. The decrease in Cu concentration could have been attributed to the adsorption on soil exchange sites. The Cu concentration in the non-contaminated soil which ranged from 7.25-7.60 ppm was within the typical range for most soils. While Cu concentration in Cu-contaminated which ranged from 28.08- 81.15 ppm was considered above the average limit. The slight increase in soil Cu concentration in amended pots could have been attributed to CRH application. According to Carter et al (2013) rice husk charcoal contains elevated levels of trace metals such as Cu which could potentially increase Cu content once added in the soil. Our findings concur with the result of Yachigo & Sato (2013) who reported an increase in heavy metal (Cu) concentration in the soil following charcoal application.

Table 4

Soil chemical parameters (pH, OC, total N, extractable P, exchangeable K and available Cu) as affected by different soil amendments

Amendment type	*Cu (ppm)*	*pH*	*OC (%)*	*Total N (%)*	*Extractable P (mg kg^{-1})*	*Exchangeable K (mg kg^{-1})*	*Available Cu (ppm)*
Unamended	0	7.38	1.01	0.14	26.67	127.67	7.60
	50	7.47	0.97	0.12	26.00	113.00	28.08
	100	7.49	1.01	0.13	26.00	115.67	41.02
	200	7.24	0.97	0.13	24.00	113.00	81.15
Mean		7.40a	0.99b	0.13	25.67	117.33b	39.46
Amended (2% CRH+AMF)	0	6.60	1.08	0.14	24.33	134.00	7.25
	50	7.08	1.18	0.15	27.00	158.33	31.48
	100	6.79	1.18	0.14	27.67	146.67	57.73
	200	6.70	1.18	0.13	26.00	161.67	74.28
Mean		6.79b	1.16a	0.14	26.25	150.17a	42.69
P value		**	**	ns	ns	**	ns

Means in a column within each experiment followed by the common letters are not significantly different at 5% level of significance; ns = not significant; * = significant at $p < 0.05$; ** = significant at < 0.01.

Effects of AMF innoculation, CRH and Cu addition on plant biomass production. Significant interaction effects ($p < 0.05$) between Cu rates, CRH addition, and AMF inoculation were detected on the root, shoot and total dry weight (Table 3). In pots applied with Cu at increasing rates without CRH and AMF added, root dry weights did not significantly differ (Figure 1). However, when CRH and AMF were applied, root dry weights significantly varied with Cu rates. The highest root dry weight recorded was in pots artificially contaminated with 50 ppm Cu amended with CRH and AMF. In contrast, plants grown in pots applied with 200 ppm Cu had the lowest root dry weight recorded.

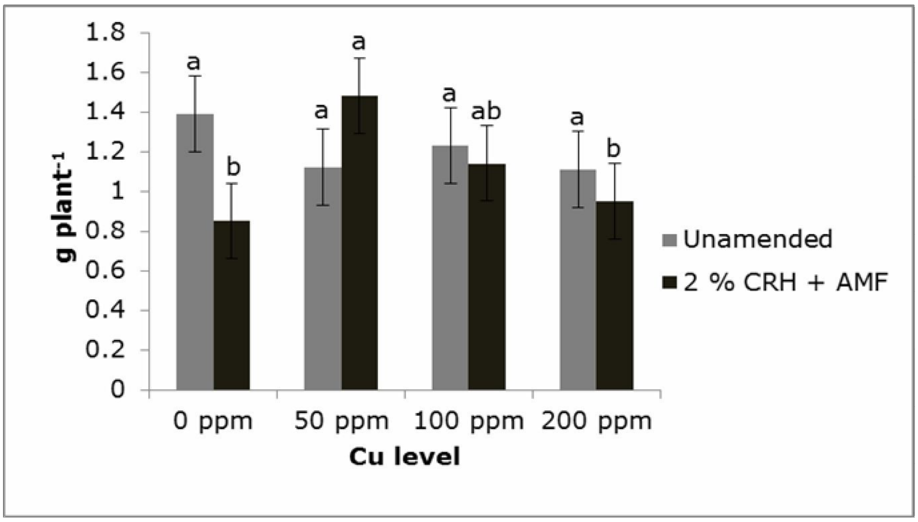

Figure 1. Root biomass of unamended and CHR + AMF amended *P. falcataria* at different Cu levels (0-200 ppm).

Similarly, in the absence of CRH and AMF, shoot dry weights of plants were comparable under increasing Cu stress (Figure 2). However, when CRH and AMF were added on Cu-contaminated soil, shoot dry weight was significantly improved. Mean shoot dry weight in plants amended with CRH and AMF under increasing Cu rates increased by 18% over the unamended Cu-contaminated soil. Plants that received 50 ppm Cu amended with CRH and AMF had the highest shoot dry weights while plants exposed to 200 ppm Cu level without CRH and AMF addition had the lowest shoot biomass recorded. When plants are exposed to lower Cu levels at 50 ppm, shoot dry weight was improved. However, when Cu level increases, a corresponding decrease in shoot weight was observed. Another interesting result of the study is that plants grown in CRH and AMF amended pots have heavier shoots than in plants grown in Cu-contaminated soil alone.

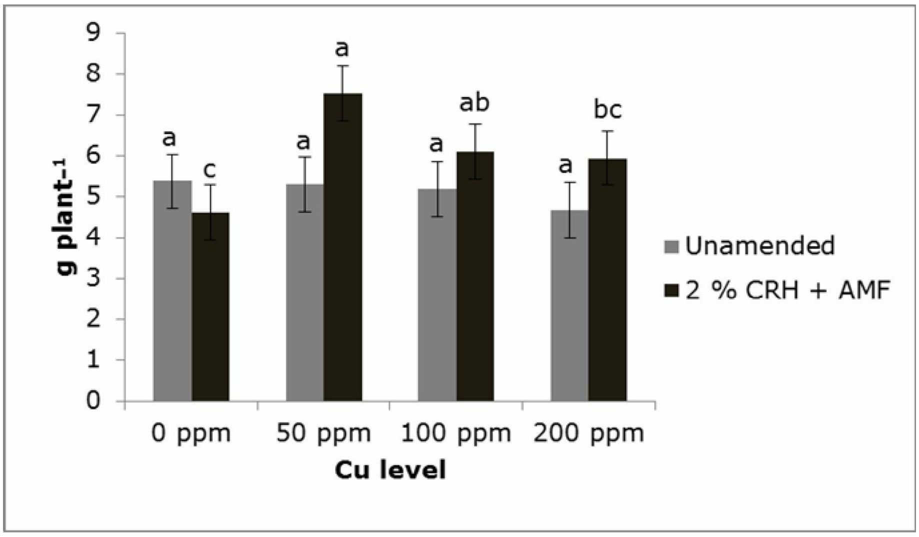

Figure 2. Shoot biomass of unamended and CHR + AMF amended *P. falcataria* at different Cu levels (0-200 ppm).

A similar trend was also noted in the total dry matter where the addition of CRH and AMF on Cu-contaminated soil enhanced dry matter production with the highest increase being observed at the 50 ppm Cu level (Figure 3). Mean total dry matter in plants amended with CRH and AMF was 7.16 g plant^{-1} which is higher by 13% compared to unamended pots (6.33 g plant^{-1}). Also, correlation analysis showed a positive and significant relationship between total dry matter and soil pH ($p < 0.05$; $r = 0.976$) and tissue P uptake ($p < 0.01$; $r = 0.992$) (Table 5). This implies that plant dry matter production in CRH and AMF amended pot is dependent on soil pH and tissue P uptake. Hence, an increase in soil pH and tissue P uptake at some certain point will result in an increase in plant dry matter production.

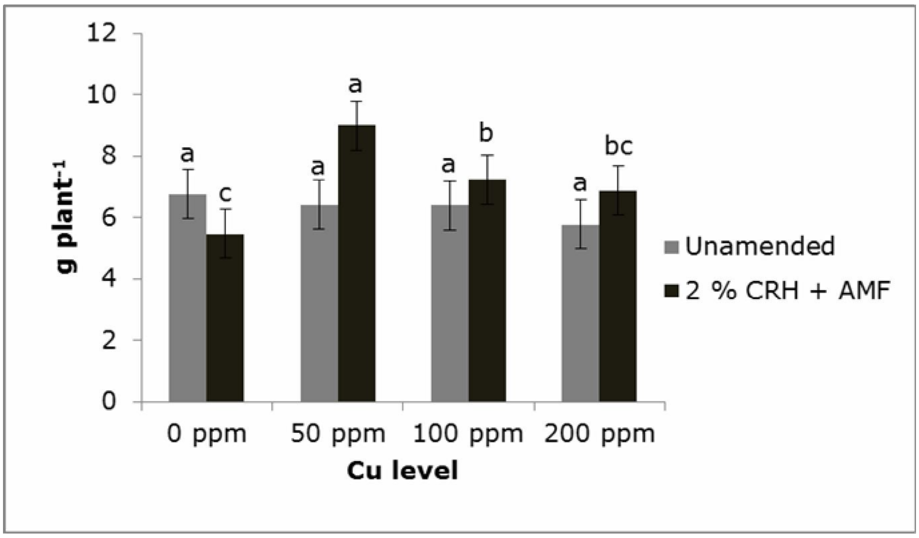

Figure 3. Total biomass of unamended and CHR + AMF amended *P. falcataria* at different Cu levels (0-200 ppm).

The observed increase in the root, shoot and total dry weights with CRH and AMF inoculation indicates the potential of both amendments to improve plant health even if grown under Cu-stressed soil. The findings of the present study also agree with that of Houben et al (2013) who reported an increase in plant biomass production following charcoal application. In contrast to the significant effects on plant biomass production, plant height at harvest was not significantly affected by CRH and AMF application (Table 3).

Effects of AMF inoculation, CRH and Cu addition on plant P and Cu concentration and uptake. The effects of Cu, CRH application and AMF inoculation on tissue P and Cu concentration and uptake are given in Table 5. A significant difference between unamended and amended Cu-contaminated soil was only detected in tissue P and Cu concentration. P concentration in unamended pots ranged from 0.25 to 0.34% while P concentration in amended pots ranged from 0.23 to 0.26%. Application of CRH and AMF on Cu polluted soil significantly ($p < 0.01$) reduced plant P concentration by 17%. In contrast, tissue P uptake was comparable in pots amended with CRH and AMF over unamended pots.

Similarly, Cu concentration significantly ($p < 0.05$) reduced with CRH and AMF inoculation. The reduction in Cu concentration in CRH and AMF treated pots was 23% over unamended Cu-treated pots. Likewise, Cu uptake also was reduced by 12% with CRH and AMF inoculation. Although the observed reduction in Cu uptake was not statistically significant. However, it was clear that with CRH and AMF inoculation both Cu concentration and uptake of plants were reduced. This reduction might have also contributed to the increase in plant dry matter production in CRH and AMF amended pots. According to Bano & Ashfaq (2013), AMF hyphae secretes glomalin, a protein which binds heavy metal forming a complex that may not be absorbed by plants, thereby, decreasing metal concentration in the plant tissue. Another possible reason for the reduced P and Cu concentration in the CRH and AMF amended pot is the dilution effect as a consequence of increasing plant biomass.

Table 5

Concentration and uptake of P and Cu in *P. falcataria* as affected by different concentration of Cu and soil amendments

Amendment type	*Cu (ppm)*	*Concentration (%)*		*Uptake (mg plant^{-1})*	
		P	*Cu*	*P*	*Cu*
Unamended	0	0.29	0.29	19.26	19.67
	50	0.25	0.28	15.96	17.81
	100	0.29	0.52	18.51	33.38
	200	0.34	0.47	19.22	26.47
Mean		0.29a	0.39a	18.24	24.33
Amended	0	0.26	0.25	14.15	13.86
(2% CRH+AMF)	50	0.23	0.26	20.63	23.44
	100	0.24	0.33	17.86	23.28
	200	0.24	0.36	16.34	24.66
Mean		0.24b	0.30b	17.25	21.31
P value		**	*	ns	ns

Means in a column within each experiment followed by the same letters are not significantly different at 5% level of significance; ns = not significant; * = significant at $p < 0.05$; ** = significant at < 0.01.

Conclusions. Results of the study revealed that application of 2% CRH and inoculation of AMF on artificially Cu-contaminated soil significantly increased soil OC and exchangeable K and reduced soil pH. Application of CRH and AMF under increasing Cu rates enhanced root, shoot and total dry matter production of *P. falcataria* with the highest biomass production recorded at 50 ppm Cu-treated pots. Moreover, tissue P and Cu concentration in *P. falcataria* were significantly reduced with CRH and AMF application. This study demonstrates the potential of CRH and AMF to promote plant health even when grown in Cu-contaminated soil.

Acknowledgements. We deeply thank the PhilRice Research Institute for allowing us to use their open-type fabricated carbonizer for CRH production. We also thank the Regional Soils Laboratory of the Department of Agriculture for the analysis of soil, CRH and plant tissue.

References

Abrishamkesh S., Gorji M., Asadi H., Bagheri-Marandi G. H., Pourbabaee A. A., 2015 Effects of rice husk biochar application on the properties of alkaline soil and lentil growth. Plant Soil Environ 61(11):475-482.

Bano S. A., Ashfaq D., 2013 Role of mycorrhiza to reduce heavy metal stress. Natural Science 5(12):16-20.

Biederman L. A., Harpole W. S., 2013 Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. Bioenergy 5:202-214.

Bolan N. S., Park J. H., Robinson B., Naidu R., Huh K. Y., 2011 Phytostabilization: a green approach to contaminant containment. Advances in Agronomy 112:145-204.

Carter S., Shackley S., Sohi S., Suy T. B. Haefele S., 2013 The impact of biochar application on soil properties and plant growth of pot grown lettuce (*Lactuca sativa*) and cabbage (*Brassica chinensis*). Agronomy 3:404-418.

Chibuike G. U., 2013 Use of mycorrhiza in soil remediation: a review. Academic Journals 8(35):1679-1687.

Emamverdian A., Ding Y., Mokhberdoran F., Xie Y., 2015 Heavy metal stress and some mechanisms of plant defense response. The Scientific World Journal 2015:1-18.

Garba S. T., Idi A. M., Baba A., 2016 Assessing the phytoremediation potential of the grass *Chrysopogon aciculatus* for the heavy metals: Cr, Co, Cd, Cu, Pb, Zn, Ni and Mn. Global Journal of Science Frontier Research 16(2):1-8.

Glaser B., Lehmann J., Zech W., 2002 Ameliorating physical and chemical properties of highly weathered soils in the tropic with charcoal-a review. Biology and Fertility of Soils 35:219-230.

Gohre V., Paszkowski U., 2006 Contribution of the arbuscular mycorrhizal symbiosis to heavy metal phytoremediation. Planta 223:1115-1122.

Gosling P., Hodge A., Goodlass G., Bending G. D., 2006 Arbuscular mycorrhizal fungi and organic farming. Agriculture, Ecosystems and Environment 113:17-35.

Habte M., Osorio N. W., 2001 Arbuscular mycorrhizas: producing and applying arbuscular mycorrhizal inoculum. CTAHR, University of Hawaii, Honolulu, pp. 1-47.

Houben D., Evrard L., Sonnet P., 2013 Beneficial effects of biochar application to contaminated soils on the bioavailability of Cd, Pb and Zn and the biomass production of rapeseed (*Brassica napus* L.). Biomass and Bioenergy 57:196-204.

Kim H. S., Kim K. R., Kim H. J., Yoon J. H., Yang J. E., Ok Y. S., Owens G., Kim K. H., 2015 Effect of biochar on heavy metal immobilization and uptake of lettuce (*Lactuca sativa* L.) in agricultural soil. Environmental Earth Sciences 74(2):1249-1259.

Koyama S., Katagiri T., Minamikawa K., Kato M., Hayashi H., 2016 Effects of rice husk charcoal application on rice yield, methane emission, and soil carbon sequestration in andosol paddy soil. JARQ 5(4):319-327.

Lehmann J., da Silva J. P., Steiner C., Nehls T., Zech W., Glaser B., 2003 Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant and Soil 249: 343-357.

Leyval C., Joner E. J., Del Val C., Haselwandter K., 2002 Potential of arbuscular mycorrhizal fungi for bioremediation. In: Mycorrhizal technology in agriculture. CRC Press, New York. pp. 175-186.

Major J., Rondon M., Molina D., Riha S. J., Lehmann J., 2010 Maize yield and nutrition during 4 years after biochar application to a Columbian savanna oxisol. Plant and Soil 333:117-128.

Nigussie A., Kissi E., Misganaw M., Ambaw G., 2012 Effect of biochar application on soil properties and nutrient uptake of lettuce (*Lactuca sativa*) grown in chromium polluted soils. American-Eurasian J Agric & Environ Sci 12(3):369-376.

Ogbonnaya U., Semple K. T., 2013 Impact of biochar on organic contaminants in soil: a tool for mitigating risk? Agronomy 3:349-375.

Olatunji O. S., Ximba B. J., Fatoki O. S., Opeolu B. O., 2014 Assessment of the phytoremediation potential of *Panicum maximum* (guinea grass) for selected heavy metal removal from contaminated soils. African Journal of Biotechnology 13(19):1979-1984.

Shenbagavalli S., Mahimairaja S., 2012 Characterization and effect of biochar on nitrogen and carbon dynamics in soil. International Journal of Advanced Biological Research 2(2):249-255.

Tangahu B. V., Abdulla S. R. S., Basri H., Idris M., Anuar N., Mukhlisin M., 2011 A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. International Journal of Chemical Engineering 2011:1-31.

Uchimiya M., Lima I. M., Klasson K. T., Wartelle L. H., 2010 Contaminant immobilization and nutrient release by biochar soil amendment: roles of natural organic matter. Chemosphere 80:935-940.

Vidali M., 2001 Bioremediation: an overview. Pure and Applied Chemistry 73(7):1163-1172.

Yachigo M., Sato S., 2013 Leachability and vegetable absorption of heavy metals from sewage sludge biochar. In: Soil processes and current trends in quality assessment. Hernandez Soriano M. C. (ed), InTech, Croatia, pp. 399-416.

Zhang X., Xia H., Li Z., Zhuang P., Gao B., 2010 Potential of four forage grasses in remediation of Cd and Zn contaminated soil. Bioresource Technology 101:2063-2066.

Received: 30 July 2017. Accepted: 22 August 2017. Published online: 20 August 2017.
Authors:
Reuben James Cillo Rollon, Caraga State University, College of Agricultural Sciences and Natural Resources, Department of Agricultural Sciences, Ampayon, Butuan City 8600, Philippines, e-mail: rollonreubenjames@gmail.com
June Emmanuel Vidal Galleros, Caraga State University, College of Agricultural Sciences and Natural Resources, Department of Agricultural Sciences, Ampayon, Butuan City 8600, Philippines, e-mail: galleros.june@gmail.com
Gidel Rosquites Galos, Caraga State University, College of Agricultural Sciences and Natural Resources, Department of Agricultural Sciences, Ampayon, Butuan City 8600, Philippines, e-mail: girylle17@gmail.com
Leo Jude Dayot Villasica, Caraga State University, College of Agricultural Sciences and Natural Resources, Department of Agricultural Sciences, Philippines, Ampayon, Butuan City 8600, e-mail: leojudev@gmail.com
Chime Mora Garcia, Caraga State University, College of Agricultural Sciences and Natural Resources, Department of Agricultural Sciences, Ampayon, Butuan City 8600, Philippines, e-mail: chimemora@gmail.com

How to cite this article:
Rollon R. J. C., Galleros J. E. V., Galos G. R., Villasica L. J. D., Garcia C. M., 2017 Growth and nutrient uptake of *Paraserianthes falcataria* (L.) as affected by carbonized rice hull and arbuscular mycorrhizal fungi grown in an artificially copper contaminated soil. AAB Bioflux 9(2):57-67.