Journal of Agricultural Research, Development, Extension and Technology, 1(1), 39-47 (2019) Full Paper

Growth performance and inorganic mercury uptake of Vetiver (*Chrysopogon zizanoides* Nash) inoculated with arbuscular mycorrhiza fungi (AMF): its implication to phytoremediation

Bryan Lloyd P. Bretaña[1], Sheimarie G. Salcedo[1], Lothy F. Casim[1] and Rhodora S. Manceras[2]

[1]Department of Biological Sciences, College of Arts and Sciences, University of Southern Mindanao
[2]Department of Plant Breeding and Genetics, College of Agriculture, University of Southern Mindanao

Received: 31 May 2019
Accepted: 15 July 2019

Abstract

The study was conducted to assess the growth performance and inorganic mercury uptake of vetiver grass (*Chrysopogon zizanoides* Nash) inoculated with arbuscular mycorrhizal fungi (AMF). Important growth parameters such as number of leaves, plant height, fresh weight and dry weight as well as mercury uptake were determined for one month duration. The experimental plants were applied with varying concentrations (0 ppm, 2 ppm, 4 ppm and 6 ppm) of mercuric chloride ($HgCl_2$). Different $HgCl^2$ treatments showed no significant difference in terms of number of leaves, plant height and dry weight in *C. zizanoides*, while those inoculated with AMF showed significant difference in fresh weight and percentage root colonization. In terms of mercury uptake, the mean uptake ranges from 0.001 ppm to 0.98 ppm which correspond to 0.13% to 1.86% uptake. Furthermore, mercury uptake in inoculated plants and *Glomus sp*. and Mykovam™-inoculated plants showed no significant difference at lower mercury concentrations but uptake significantly reduced in uninoculated plants at highest mercury concentration (6 ppm). It can be deduced that uptake rate of vetiver increased with AMF inoculation even at high mercury concentrations.

Keywords - mercury uptake, mychorriza, phytoremediation potential, plant-fungal associations

Introduction

Mining activities are known to release substantial amounts of toxic metals such as lead, arsenic and mercury (Keane, 2010) into the environment (Toxic Release Inventory, 2007). The presence of these toxic metals in the environment causes environmental impacts including habitat loss for wild life and fisheries, changes in water quality, sedimentation, toxins in tailings ponds and effluent, and acid generation. (Bacsujlaky, 2004; Burke, 2006).

However, chemical, physical or biological techniques can be employed to remediate contaminated soils (McEldowney et al., 1993). Living organisms such as plants can be used to alter environmental contaminants into non-hazardous or less hazardous substances (Leung, 2004). Established plants usually absorb heavy metals through their roots and are able to translocate these to the above-ground shoots for accumulation. Once sufficient plant growth and metal accumulation are attained, the above-ground portions of the plant are harvested and removed which results in the permanent removal of metals from the site (Kumar et al., 1995). In this context, phytoremediation or the use of plants to remove or reduce environmental contaminants such as heavy metals is recently being considered as a highly promising and cost-effective technology for the remediation of polluted sites (Cunningham & Berti, 1995).

In addition, a comparatively new approach to restore degraded land and protect against desertification is to inoculate the soil with arbuscular mycorrhiza fungi (AMF) with the re-establishment of vegetation (Bangoy, 2011). Arbuscular mycorrhiza fungi are obligate symbionts of most plants in their natural habitats (Habte & Osorio, 2010). Plants capable of forming association with arbuscular mycorrhiza fungi have been shown to accumulate significant amount of trace metals (Burke et al., 2000). Vetiver grass (*Chrysopogon zizanoides*) is one

Corresponding author email: Bryan Lloyd P. Bretaña *bryanlloydbretana@gmail.com*

of these successful plants to form associations with AMF and further accumulate metals (Burke et al., 2000; Karagiannidis & Nikolaou 2000).

Thus, this study was conducted to explore the growth performance and mercury uptake capacity of vetiver grass (*C. zizanoides*) associated with arbuscular mycorrhiza fungi (AMF). Specifically, this study aimed to elucidate the effect of AMF on the growth of vetiver grass in terms of number of leaves, height of plant, and biomass, compare the accumulation activity of vetiver grass and mycorrhiza association with varying concentrations of mercury (Hg) and to determine which AMF and vetiver grass association shows highest uptake capacity.

Methodology

Preparation and Sterilization of soil

A garden (loam) soil was used, obtained from an orchard at the University of Southern Mindanao Agricultural Research Center (USMARC), Kabacan, Cotabato, Philippines. The soil was sieved and sterilized using conventional method of sterilization (sterilization of soil using pan for 2 hours or until moist was vanished) then re-sterilized at 180°C for 2 hours using an oven. Each two kilograms of sterilized soil was placed in 11-inches diameter plastic pots.

Mercury Application into the Soil

Two ml of inorganic mercury chloride ($HgCl_2$) was added in liquid form to the soil at concentrations of 0, 2, 4 and 6 ppm per 2 kg soil placed in 11- inch plastic pots one week after transplantation. The mixed solution of mercury chloride ($HgCl_2$) and soil were allowed to stabilize for one week.

Treatments and experimental design

Randomized Complete Block Design (RCBD) was employed as the experimental design with four treatments replicated three times. The control has two kilograms of soil with no mercury chloride ($HgCl_2$) (0 ppb $HgCl_2$/2 kg soil) served as a control. Other treatments were 2 ppm $HgCl_2$/2 kg soil), 4 ppm $HgCl_2$/2 kg soil, and 6 ppm $HgCl_2$/2 kg soil (Figure 1). Each treatment was inoculated with two commercially available fungi spores (Mykovam™ and *Glomus* sp). Pots with no AMF served as a control.

Propagation of Test Plant and Inoculation of Arbuscular Mycorrhiza Fungi

The test plants were propagated using vegetative propagation technique of Chomchalow (2000) which was done by splitting mature tillers from vetiver clums or mother plants. Five grams of arbuscular mycorrhizal fungi spores was then inoculated in each 2 kg sterilized soil. The amount of inoculum was based on the recommendations of the manufacturer. The AMF spores were introduced at 2 cm below the soil surface. Before transplanting, roots were surface-sterilized with 0.5% sodium hypochlorite solution for one minute. The plants were maintained under screen house conditions.

Determination of Plant Growth

The number of leaves and plant height were initially noted before the start of the experiment. These parameters were first measured one week after the application of mercuric chloride ($HgCl_2$). Plant growth parameters were obtained every week for one month. Fresh weight and dry weight of the plant were measured to evaluate its biomass at the end of the observations.

Root Colonization of AMF

For assessing AMF colonization, plants were uprooted and 5 g of fresh roots were collected from the plants sampled at each randomly selected pot after 4 weeks. The root samples were cut into approximately 1 cm pieces, and were stained using the methods of Kormanik et al. (1980). The stained root samples were microscopically examined to assess the percentage of mycorrhizal colonization using the grid-line intersect method (Giovannetti & Mosse, 1980). The percentage of root colonization was calculated using the following formula:

% colonization= (total number of colonized root pieces)/(total number of root pieces examined) x 100

Determination of Mercury Uptake

Mercury uptake of the test plant was determined using the Atomic Absorption Spectrometry (AAS) at the Davao Analytical Laboratory Inc., Davao City, Philippines.

Statistical Analysis

Analysis of data was performed using the

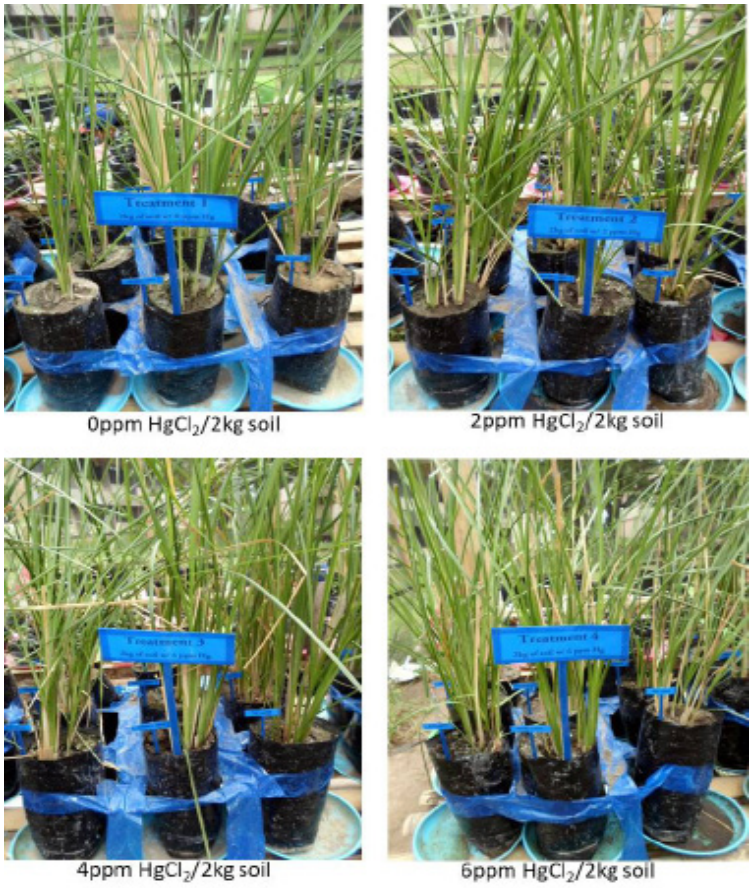

Figure 1. Experimental layout for the growth performance and mercury uptake of vetiver (*Chrysopogon zizanoides* Nash) inoculated with arbuscular mycorrhizal fungi.

STATISTICA software. The data was analyzed using three-way Analysis of Variance (ANOVA) with interaction and Tukey HSD was employed to detect statistical significant differences between means.

Results and Discussion

Growth of Vetiver Grass (*Chrysopogon zizanoides*) Applied with Inorganic Mercury and Inoculated with Arbuscular Mycorrhizal Fungi (AMF)

The mean number of leaves of vetiver as affected by inorganic mercury application and arbuscular mycorrhizal fungi (AMF) inoculation from one to four weeks experimental period is shown in Figure 2. The mean number of leaves increased significantly from week one to four ($p<0.05$). The mean number of leaves differ significantly between AMF inoculated plants ($p<0.05$). The *Glomus* sp. inoculated plants increased their leaves from 12 to 30 in four weeks even at highest inorganic mercury concentration (6 ppm) while both Mykovam™-inoculated and un-inoculated plants had highest leaf count at 0 ppm $HgCl_2$ (25 and 24 leaves, respectively). Vetiver grass inoculated with *Glomus* sp.-inoculated plants differ significantly in the number of leaves with Mykovam™-inoculated plants but not with the control plants. In terms of the effect of different inorganic mercury concentrations to the number of leaves, plants grown in 0 ppm $HgCl_2$ had significantly higher leaf counts compared to other treatments ($p<0.05$) while those grown in soil with different $HgCl_2$ concentrations showed no significant difference ($p>0.05$).

Although, significant difference was detected, inorganic mercury-free plants had only a difference of up to 3 leaves from the treated plants, showing tolerance to increasing heavy metal concentrations. *Chrysopogon zizanoides* is a special gramineous plant with an extremely strong ability to adjust to the environment, with well-developed root system could grow rapidly and resistant to metal contaminants (Gao, 2006). The AMF-colonized plant grown on heavy metal concentrations demonstrates survival strategy such as chelation of heavy metals through the formation of organic complexes as a result of the

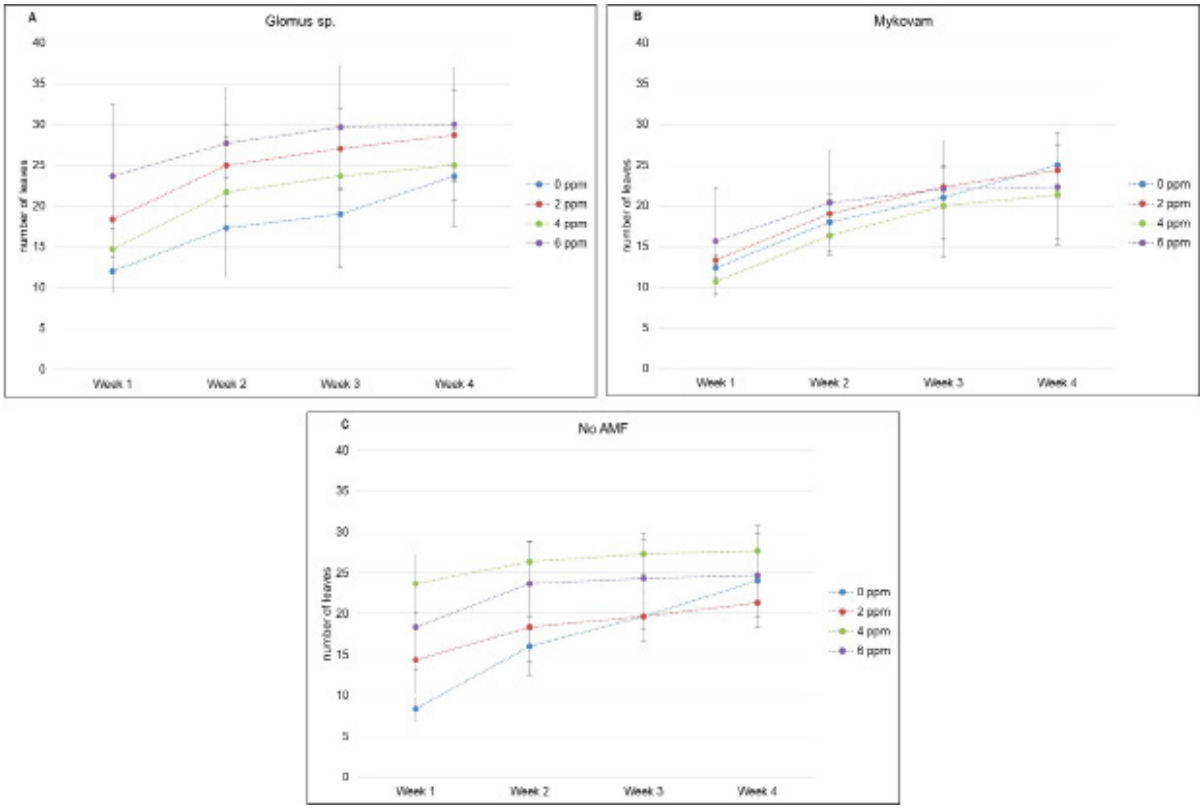

Figure 2. Mean number of leaves of *Chysopogon zizanoides* Nash inoculated with (a) *Glomus sp*., (b) Mykovam™ and (c) Uninoculated plants with different mercury concentrations in 4 weeks.

symbiotic relationship (Leyval et al., 1997). In this study, plants inoculated with AMF exhibited increased growth compared to the control plants. Siddiqui and Pichtel (2008) indicated that mycorrhizae forms symbiotic relationships with host plants and play a crucial role in plant growth. Arbuscular mycorrhiza AMF may influence plant by enhancing growth through vitamins and hormone production (Barea, 1997). Mycorrhizae are also recognized to manufacture growth-stimulating substances for plants, and as a result, boosting mineral nutrition and improved growth and biomass needed for phytoremediation (Khan et al., 2004).

The mean height of vertiver was significantly affected by the duration of the experiment and AMF inoculation ($p<0.05$) but not by inorganic mercury application ($p>0.05$; Figure 3). The mean plant height in all treatments increased from week one to week four, with significantly higher increase in week two to four compared to week one ($p<0.05$). The AMF-inoculated plants also showed significant difference in plant height ($p<0.05$), with plants Mykovam™-inoculated plants showing the highest mean height ($p<0.05$) while *Glomus* sp. inoculated and the control showed no significant difference ($p>0.05$). Although no significant difference was detected as an effect of inorganic mercury application, the interaction of mercury and AMF showed significant difference. Plant height was consistently high in Mykovam™-inoculated plants with highest height (106.38 cm) at week four with 0 ppm $HgCl_2$. In addition, Mykovam™-inoculated plants showed to increase plant height even at higher $HgCl_2$ concentrations (4 ppm and 6 ppm) while *Glomus* sp. inoculated plants had the highest height in lower $HgCl_2$ concentration.

The *C. zizanoides* associated with AMF applied with different mercury concentrations, may have supported plant survival in soils that were considerably polluted with toxic metals. The mycorrhizal association has established an alternative solution for growing plants in toxic soil (Leung, 2004). Thus, the result signifies that the host plant's ability to tolerate heavy metals may be credited to the unaltered heavy metal uptake system, and that *C. zizanoides* appears to depend mainly on their symbiotic relationship with AMF while it was exposed to heavy metals (Sharples et al., 2000a). Furthermore, AMF establishment in vetiver provides a role in decreasing exposure

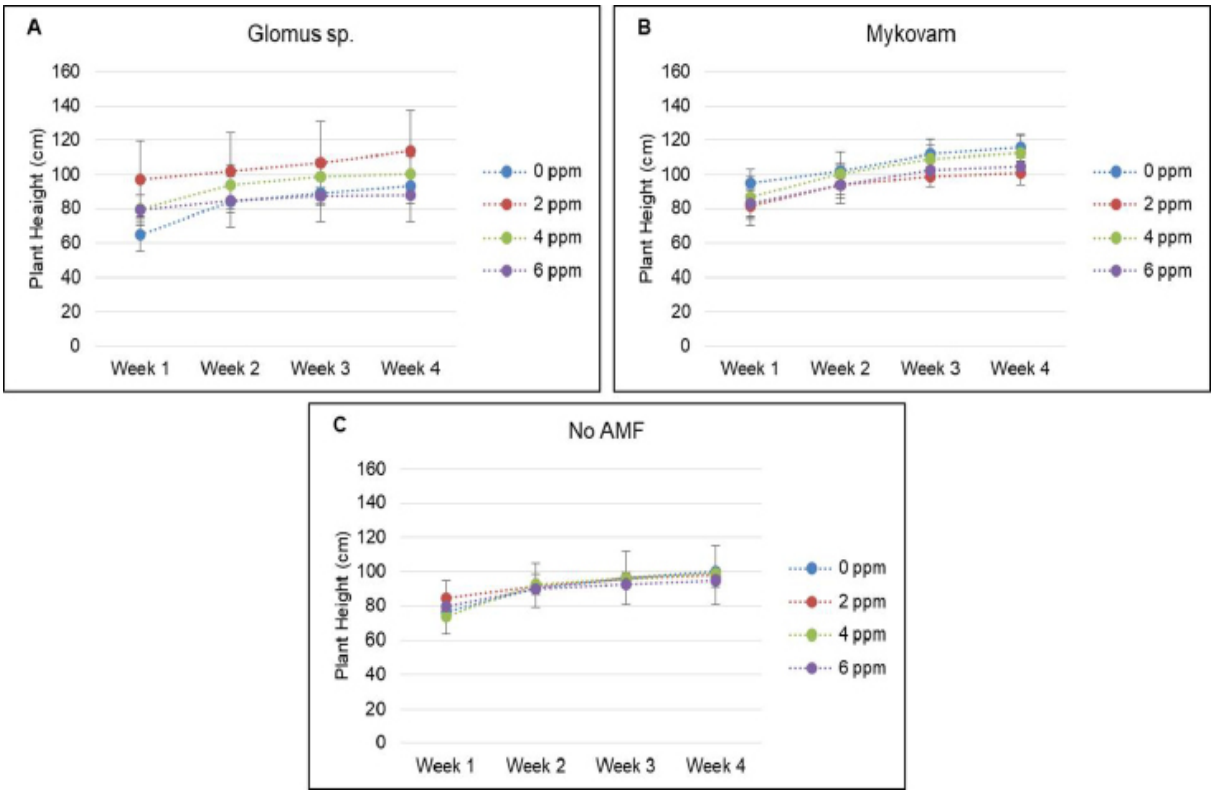

Figure 3. Mean height (cm) of *Chysopogon zizanoides* Nash inoculated with (a) *Glomus* sp., (b) Mykovam™ and (c) Uninoculated plants with different mercury concentrations in 4 weeks.

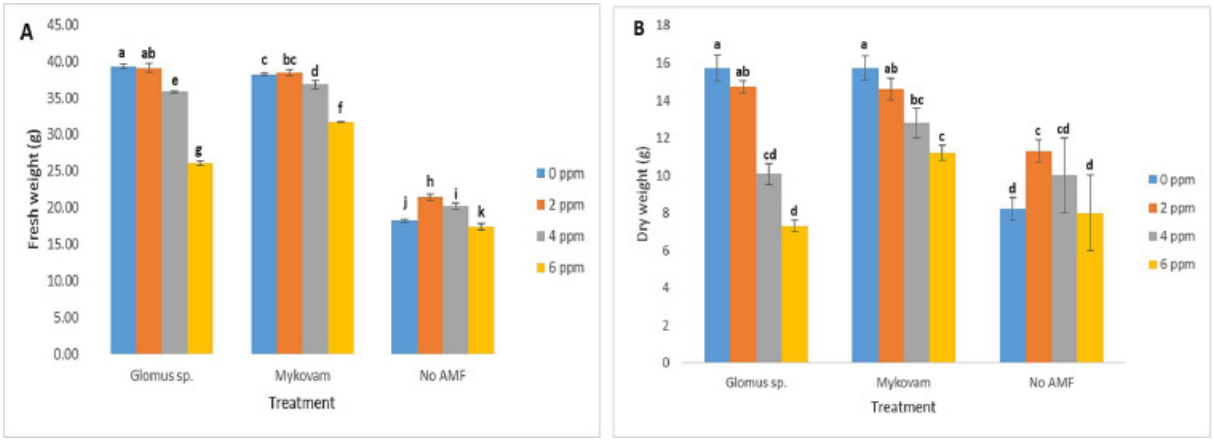

Figure 4. Fresh weight (A) and dry weight (B) of *Chysopogon zizanoides* Nash inoculated with arbuscular mycorrhizal fungi (AMF) applied with different inorganic mercury concentrations at 4 weeks after transplanting.

of the host to metals and preserving the supply of nutrients for growth in the nutrient-restricted and heavy metal contaminated soil (Sharples et al., 2000b).

In terms of fresh weight and dry weight (Figure 4), significant differences were observed for the different inorganic mercury concentrations and arbuscular mycorrhizal fungi ($p<0.05$), with highest fresh weight (39.3 g) in 0 ppm $HgCl_2$ inoculated with *Glomus* sp. while lowest was in 6 ppm $HgCl_2$ uninoculated with AMF. On the other hand, Mykovam™ inoculated plants showed the highest dry weight (15.73 g) while lowest dry weight was recorded in *Glomus* sp. inoculated plants at 6 ppm $HgCl_2$. In this study, *C. zizanoides* inoculated with AMF even with application of inorganic mercury still increased both in fresh weight and dry weight.

The fact that inoculation of AMF increased the overall plant weight more than the uninoculated

plants and indicated that the inoculation of AMF on *C. zizanoides* was successful under different $HgCl_2$ concentrations. In a different study, Wong et al. (2007) observed that shoot weight decreased as metal concentrations increased. *Chrysopon zizanoides* was reported to tolerate extreme soil conditions and climatic variations, including heavy metals, and has the potential for phytoremediation. Several studies reported the potential of using vetiver to remove heavy metals from soil garbage leachate (Xia et al., 1999; Roongtanakiat et al., 2007), wastewater (Roongtankiat et al., 2007) and mine tailings (Yang et al., 2003; Roongtanakiat et al., 2007).

Percentage Colonization of Arbuscular Mycorrhizal Fungi (AMF) in *Chrysopogon zizanoides*

Figure 5 shows the % colonization of AMF in the roots of *C. zizanoides*. A relatively high % colonization of AMF was observed which ranges from 42% to 68.67% with highest colonization in Mykovam™ at 0 ppm $HgCl_2$ while lowest in *Glomus sp*. at 6 ppm $HgCl_2$. Overall statistical analysis showed significant difference in % colonization ($p<0.05$). Furthermore, Mykovam™ showed better colonization than Glomus even at highest concentration, with 57% colonization compared to 42% in *Glomus* sp. at 6 ppm $HgCl_2$.

From the growth ability of *C. zizanoides, Glomus* sp. and Mycovam™ almost have the same capacity of promoting plant nutrition, compared to plants uninoculated with AMF. AMF are known to encourage morphological alterations in the host plant root system and a more branched and enhanced root system has been observed in mycorrhizal plants of various herbaceous and woody species (Berta et al., 1995). Other studies have also shown that heavy metals can negatively affect mycorrhizal colonization as shown in *Glomus* sp. at 6ppm $HgCl_2$. Chao and Wang (1990) described a decrease in the AMF colonization on plants as an effect of the addition of mercury, amongst other heavy metal contaminants. However, in some cases, the consequence of heavy metal presence on mycorrhizal colonization can also be neutral (Diaz et al., 1996).

Furthermore, Mykovam™ is a soil-based biofertilizer containing effective species of mycorrhizal (VAM) AMF. It is a soil inoculant containing spores and mycorrhizal propagules commonly used in nursery-grown seedlings (BIOTECH 1995, Lapitan & Garcia, 1994). It assists in the uptake of water and essential nutrients from the soil and can be used for agricultural crops, fruits trees and forest trees. Moreover, the presence of mycorrhizae affects the efficiency of roots as they increase water and nutrient uptake, enhance resistance to drought and root disease and survival of newly planted trees (Darr, 1996). Mykovam™ is composed of a cocktail of 3 arbuscular mycorrhizal AMF of *Glomus etunicatum*, *Glomus macrocarpus* and *Gigaspora margarita* (Zarate et al., 1999).

Mercury Uptake of *Chrysopogon zizanoides* Nash

The mercury uptake of *C. zizanoides* is shown in Figure 6. In general, the mercury uptake by *C. zizanoides* was observed to be very minimal ranging from 0.06 ppm to 0.11

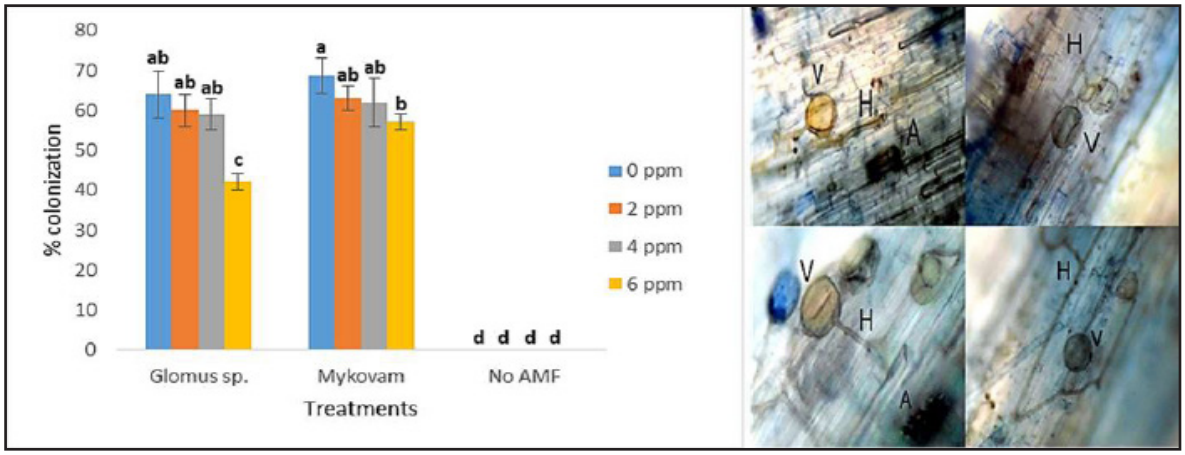

Figure 5. Percentage root colonization of AMF in *Chysopogon zizanoides* Nash applied with different inorganic mercury concentrations at 4 weeks after transplanting. Important structures such vesicle (V), hyphae (H) and arbuscule (A) are also shown.

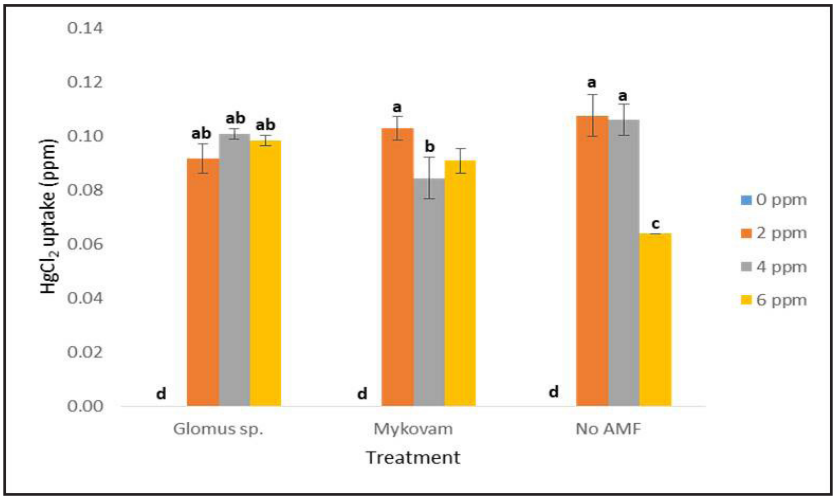

Figure 6. Inorganic mercury uptake of *Chysopogon zizanoides* Nash inoculated with arbuscular mycorrhizal fungi (AMF) at 4 weeks after transplanting.

ppm which correspond to 1.06% to 5.38% uptake from soil. As observed, highest mercury absorption were noted in uninoculated plants at 2 ppm to 4 ppm concentration but lowest at 6 ppm while *Glomus* sp. and Mykovam™-inoculated vetiver showed almost comparable uptake. As a whole, however, statistical analysis showed no significant difference ($p>0.05$) in the uptake of plants with or without AMF inoculation.

The result illustrated that plants uninoculated with AMF showed the highest mercury uptake. This can be explained primarily due to the specific bioaccumulation characteristic of heavy metals and consequently will accumulate differently in the ecosystem (Githongo, 2010). Species and or strains of AMF vary in their ability of tolerance to physical and chemical properties of soil and may also differ in their effectiveness in improving plant growth. AMF uptake of heavy metals from the soils changes the accumulation of heavy metals in soils and as a result, reduce the heavy metals accessible for plant uptake. This in turn decreases biomagnifications (Githongo, 2010).

Heavy metals in contaminated soils could be reduced by the establishment of arbuscular mycorrhiza (AM) with obligate symbiotic AMF (Heggo et al., 1990, Weissenhorn et al.,1995, Chen et al., 2004). On the other hand, heavy metal stress in plants can be regulated by use of AMF (Hildebrandt et al., 1999). AMF that colonize plant roots and significantly decrease the uptake of heavy metals into plant cells may be one (Eason et al., 2001). A positive growth response to AMF symbiosis results into reduced heavy metal concentrations in plant shoots can be due to the biomass dilution effect (Harley and Smith, 1983). According to Huang et al. (2006), mycorrhizal colonization may lead to an upsurge in the accumulation of heavy metal in roots while a decrease in the shoots. Pawlowska et al. (2004) showed that plants colonized with the AMF, *Glomus* intraradices grew noticeably better than non-mycorrhizal controls. Evidences showed that AMF can significantly filter out contaminants such as toxic heavy metals and as a consequence, keep them away from the plants. However, high concentrations of heavy metals adversely alter the activity of AMF populations in soil (Rajendran et al., 2003), thus we can assume that mercury uptake of vetiver grass inoculated with AMF increases as heavy mercury concentrations increases.

Conclusion

The study highlights the effect of the arbuscular mycorrhiza inoculation to inorganic mercury uptake and growth performance of *C. zizanoides*. Results revealed that AMF inoculation significantly affects the growth of *C. zizanoides*. In addition, AMF inoculation enhances mercury uptake by the plant even at high concentrations. *C. zizanoides* in general was able to uptake minimal inorganic mercury from the soil. Therefore, it can be inferred that vetiver is not a hyperaccumulator but its potential as a good candidate for phytoremediation cannot be ignored. Future studies on the use of other forms of mercury such as organic, elemental and other heavy metals are highly recommended to fully elucidate the vertiver uptake capacity.

Acknowledgement

The authors would like to thank Department of Science and Technology-Philippine Council for Industry, Energy and Emerging Technology Research and Development (DOST-PCIEERD) for the research fund.

References

Bacsujlaky, M. (2004). *Examples of modern mines that damaged rivers and fisheries*. Retrived March 2, 2018, from https://wman-info.org/wp-content/uploads/2012/08/MinesRiversFish.pdf

Bangoy, J. (2011). *Arbuscular mycorrhiza contribution to the growth performance and lead uptake of pinto peanut (Arachis pintoi)*. Unpublished Manuscript, University of Southern Mindanao.

Barea, J.M. (1997). Mycorrhiza/bacteria interaction on plant growth formation. In A. Ogoshi, A. Kobayashi, Y. Homma, F. Kodama, N. Kondon, & S. Akino (Eds), *Plant growth promoting rhizobacteria, present status and future prospects* (pp. 150–158), Paris, France: OECD.

Berta, G., Trotta, A., Fusconi, A., Hooker, J., Munro, M., Atkinson, D., Giovannetti, M., Marini, S., Fortuna, P., Tisserant, B., Gianinazzi-Pearson, V. & Gianinazzi, S. (1995) Arbuscular mycorrhizal induced changes to plant growth and root system morphology in *Prunus cerasifera* L. *Tree Physiology*, *15*, 281–293.

BIOTECH (1995). *Mykovam: inoculant for agriculture fruit trees and forest tree crops*. Laguna, Philippines: National Institute of Molecular Biology and Biotechnology, Ext. Com. Unit University of the Philippines.

Burke, G. (2006). Opportunities for environmental management in the minings in Asia. *The Journal of Environment and Development*, *15*(2), 224-235.

Chao, C.C. & Wang, Y.P. (1990). Effects of heavy metals on the infection of vesicular arbuscular mycorrhizae and the growth of maize. *Journal of the Agricultural Association of China*, *152*, 34–45.

Chen B.D., Liu, Y., Shen, H., Li, L.I. & Christie, P. (2004) Uptake of cadmium from an experimentally contaminated calcareous soil by arbuscular mycorrhizal maize (*Zea mays* L.). *Mycorrhiza*, *14*, 347–354.

Chomchalow, N., (Editor) (2000). *Manual of the International Training Course of the Vetiver System*. Retreved March 2, 2018, from http://www.vetiver.org/PRVN_TCM.pdf

Cunningham, S.D., Berti, W.R. & Huang, J.W. (1995). Phytoremediation of contaminated soils. *Trends in Biotechnology*, *13*, 393-397.

Darr, S. (1996). *Tree Fertilization Reasons and Methods*. Arbor Age Magazine.

Diaz, G., Azconaguilar, C. & Honrubia, M. (1996). Influence of arbuscular mycorrhizae on heavy metal (Zn and Pb) uptake and growth of *Lygeum spartum* and *Anthyllis cytisoides*. *Plant Soil*, *180*, 241–249.

Eason W.R, Webb, K.J., Michaelson-Yeates, T.P.T., Abberton, M.T., Griffithe, G.W., Culshaw, C.M., Hooker, J.E. & Dhanoa, M.S. (2001). Effect of genotype of *Trifolium repens* on mycorrhizal symbiosis with *Glomus mosseae*. *Journal of Agricultural Science*, *137*, 27–36.

Gao, L. (2006). *Study on AMF in rhizosphere of vetiver grass in field nursery*. Retrieved March 2, 2019, from https://www.vetiver.org/ICV4pdfs/EB03.pdf

Giovannetti, M. & Mosse, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytologist*, *84*, 489–500.

Githongo, M. (2010). Effect of sewage wastewater irrigation on soil biodiversity and heavy metals accumulation in soils and selected crops. *Journal of Tropical and Subtropical Agrosysytems*, *12*, 1–63.

Habte, M. & Osorio, N.W. (2010). Arbuscular mycorrhizas: producing and applying arbuscular mycorrhizal inoculum. College of Tropical Agriculture and Human Resources, University of Hawaii, 15–23.

Harley, J.L. & Smith, SE. (1983). *Mycorrhizal Symbiosis*. London, UK: Academic Press.

Heggo, A., Angle, J.S. & Chaney, R.L. (1990). Effects of vesicular–arbuscular mycorrhizal fungi on heavy metal uptake by soybeans. *Soil Biology and Biochemistry*, *22*, 865–869.

Hildebrandt, U., Kaldorf, M. & Bothe, H. (1999). The zinc violet and its colonization by arbuscular mycorrhizal fungi. *Journal of Plant Physiology*, *154*, 709–711.

Huang, H., Zhang, S., Chen, B.D., Wu, N., Shan, X.Q. & Christy, P. (2006). Uptake of atrazine and cadmium from soil by maize (*Zea mays* L.) in association with the arbuscular mycorrhizal fungus *Glomus etunicatum*. *Journal of Agricultural Food Chemistry*,

54(25), 9377–9382.

Karagiannidis, N. & Nikolaou, N. (2000). Influence of arbuscular mycorrhizae on heavy metal (Pb and Cd) uptake, growth, and chemical composition of *Vitis vinifera* L. (cv. Razaki). *American Journal of Enology and Viticulture*, *51*(3), 269–275.

Keane, C. (2010). Mining and Toxic Metals: a case study of the proposed Donlin Creek mine. Resisting Environmental Destruction in Indigenous Lands: an Alaska Native Grassroots network, 1–3.

Khan, F.I., Husain, T. & Hejazi, R. (2004). An overview and analysis of site remediation technologies. *Journal of Environmental Management*, *71*(2), 95–122.

Kormanik, P.P., McGraw, A.C. & Shultz, R.C. (1980). Procedures and equipment for staining large numbers of plant roots for endomycorrhizal assay. *Canadian Journal of Microbiology*, *26*, 536–538.

Kumar P.B.A.N., Dushenkov, V., Motto, H. & Raskin, I. (1995). Phytoextraction: the use of plants to remove heavy metals from soils. *Environmental Science and Technology*, *29*, 1232–1238.

Lapitan, P.G. & Garcia, M.U. (1994). Application of Biotechnology in Forestry: Prospects and Progress. A paper presented during the continuing Professional Education for foresters Program. *A short course on Recent Developments in Forestry and Environments*. IFC College of Forestry, UPLB College, Laguna.

Leung, M. (2004). Bioremediation: techniques for cleaning up a mess. *Journal of Biotechnology*, *2*, 18–24.

Leyval, C., Turnau, K. & Haselwandter, K. (1997). Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza*, 7, 139–153.

McEldowney, S., Hardman, D.J. & Waite, S. (1993). Treatment Technologies. In S. McEldowney, J. Hardman, & S. Waite, (Eds.), *Pollution Ecology and Biotreatment Technologies*. Singapore: Longman Publishers.

Pawlowska T.E., Chaney R.L., Chin, M. & Charvat, I. (2001). Effects of metal phytoextraction practices on the indigenous community of arbuscular mycorrhizal fungi at a metal-contaminated landfill. *Applied and Environmental Microbiology*, *66*(6), 2526–2530.

Rajendran, P., Muthukrishnan, J. & Gunasekaran, P. (2003). Microbes in heavy metal remediation. *Indian Journal of Experimental Biology*, *41*(9), 935–44.

Roongtanakiat, N., Tangruangkiat, S. & Meesat, R. (2007). Utilization of vetiver grass (*Vetiveria zizanoides*) for removal of heavy metals from industrial wastewater. *Science Asia*, *33*, 397–403.

Sharples, J.M., Meharg, A.A., Chambers, S.M. & Cairney, J.W.G. (2000). Mechanism of arsenate resistance in the eroid mycorrhizal fungi *Hymenoscyphus ericae*. *Plant Physiology*, *124*, 1323–1327.

Sharples, J.M., Meharg, A.A., Chambers, S.M. & Cairney, J.W.G. (2000). Symbiotic solution to arsenic contamination. *Nature*, *404*, 952–961.

Siddiqui, Z.A. & Pichtel, J. (2008). Mycorrhizae: an overview. Sustainable agriculture and forestry, 1-35.

Toxic Release Inventory. (2007). *Environmental Protection Agency*. Retrieved March 9, 2018, from http://www.epa.gov/triexplorer.

Weissenhorn, I., Leyval, C., Belgy, G. & Berthelin, J. (1995). Arbuscular mycorrhizal contribution to heavy metal uptake by maize (*Zea mays* L.) in pot culture with contaminated soil. *Mycorrhiza*, *5*, 245–251.

Wong, C.C. (2007). The role of mycorrhizae associated with vetiver grown in Pb-/Zn-Contaminated Soils: Greenhouse Study. *Restoration Ecology, 15*(1), 60–67.

Xia, H.P., Wang, Q.L. & Kong, G.H. (1999). Phyto-toxicity of garbage leachates and effectiveness of plant purification for them. *Acta Phytoecologica Sinica*, *23*(4), 289–301.

Yang, B., Shu, W.S., Ye, Z.H., Lan, C.Y. & Wong, M.H. (2003). Growth and metal accumulation in vetiver and two Sesbania species on lead/zinc mine tailings. *Chemosphere*, *52*, 1593-1600.

Zarate, J.T., Dela Cruz, R.E. & Iringan, D.S. (1999). Response of six Acacia species to arbuscular mycorrhizal fungal inoculation. In R.E. Dela Cruz, M. Follosco, K. Ishii, & K. Susuki, (Eds.), *Proceedings of Manila Workshop* (pp 90–97). Manila, Philippines: BIO-REFOR.