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Research Article

Influence of Arbuscular Mycorrhizal Fungi on Growth and Heavy Metal (Cd & Hg) Uptake of Pinto Peanut *(Arachis pintoi)*

Ranjiv E. Baleva[1, 2], Cyrelle M. Besana[2], May Eva Z. Garcia[2]

[1]Department of Math and Science, University of Mindanao, Matina, Davao City
[2]Department of Biological Sciences, University of Southern Mindanao, Kabacan, Cotabato
Corresponding author email: rebbaleva@gmail.com

The pollution of the ecosystem by heavy metals is a real threat to the environment because metals cannot be naturally degraded like organic pollutants and persist in the ecosystem having accumulated in different parts of the food chain. Due to the acute toxicity of heavy metal contaminants, an urgent need to develop low-cost, effective, and sustainable methods to remove or detoxify them from the environment. A study to investigate the growth and heavy metal (Cd and Hg) absorption capacity of Arachis pintoi associated with arbuscular mycorrhizal fungi (AMF) was conducted. The heavy metal (Cadmium and Mercury) uptake of A. pintoi inoculated with arbuscular mycorrizal fungi was also determined and compared by quantifying the heavy metal absorbed by the plants through Atomic Spectrophotometry. Randomized Complete Block Design (RCBD) was used as the experimental design with four treatments replicated three times each for Cadmium and mercury was made. The analysis of variance suggests a highly significant difference in the main effect of treatments, the main effect in weeks and their interaction in cadmium and mercury set-up. The results have found out that Treatment 3 (Heavy metal without AMF) in heavy metal cadmium and mercury has the highest heavy metal uptake. The study further recommend that A. pintoi, a widely available and abundant plant species with mycorrhyzal fungi interaction will be a beneficial procedure in reducing heavy metal pollution in soil.

Keywords: *Arachis pintoi*, Arbuscular Mycorrhizal Fungi, Bioremediation, Heavy metal, Pollution

Heavy metals and metalloids, such as cadmium (Cd) and mercury (Hg) which are released into the environment by mining, agriculture and other industries that utilizes heavy metal material later poses a threat to the environment and human health (Lombi et al., 2001). Heavy-metal toxicity can cause a variety of adaptive responses in plants and can exert an adverse effect on microorganisms and microbial processes on the soil (McGrath et al., 1995; Sanita & Gabbrielli, 1999). Among the various toxic metals, cadmium and mercury are one of the most common soil pollutants. Cadmium enters agricultural soils through aerial deposition, phosphate fertilizers, and land application of sewage sludge (Kabata-Pendias and Pendias, 1992) and mercury in soil can enter and accumulate in the food chain through plant uptake, posing a potential danger to human health and welfare (Gnamuš et al., 2000).

One way of removing the toxicity of the soil is by the use of plants inoculated with arbuscular mycorrhizal fungi which aids the facilitation of the plant absorption of the heavy metals from the soil. Arbuscular mycorrhizal fungi have proven to improve plant tolerance to heavy metal stress in polluted soils (Leyval et al., 1997). These fungi, which forms symbioses with two out of three of all plant species, are believed to be obligate biotrophs that are wholly dependent on the plant partner for their carbon supply (Trappe, 1987; Smith & Read, 1996). Among soil microorganisms, mycorrhizal fungi are the only ones providing a direct link between soil and roots, and are renowned for their ability to improve plant mineral nutrients, including heavy metals (Tonin et al., 2001). Arbuscular mycorrhizal fungi may be potential biotechnological tools for enhancing phytoremediation of heavy metal-contaminated soils (Gaur & Adholeya, 2004).

One of the key factors in heavy metal remediation is plants. Pinto peanut (*Arachis pintoi*) is considered as multiple use legume because it is grown for forage, forest and orchard ground covers, in low tillage system, soil erosion control, and ornamental purposes (Carvalho et al., 2010). A recent study by Bangoy et al., (2011) have shown that *A. pintoi* with mycorrhizal fungi can absorb Lead (Pb) contamination in soil. In addition to their work, a study using another set of heavy metals such as cadmium and mercury was used to investigate the bio-remediating capacity of *A. pintoi* and arbuscular mycorrhizal fungi. Thus, the success of this study will add present information related to the efficiency of pinto peanut inoculated with arbuscular mycorrhizal fungi in removing cadmium and mercury of the soil. This study will lead to a more efficient, less costly and effective alternative method of bioremediation.

The main objective of this study was to investigate the heavy metal (Cd and Hg) absorption of *A. pintoi* associated with arbuscular mycorrhizal fungi (AMF). Specifically, this study aimed to: 1.) evaluate the growth of *Arachis pintoi* propogated in sterile soil, 2.) evaluate the growth of *Arachis pintoi* applied with cadmium and mercury inoculated with arbuscular mycorrhizal fungi and; 3.) determine and compare the cadmium and mercury uptake of *Arachis pintoi* inoculated with arbuscular mycorrhizal fungi in sterile soil.

Materials and Methods

Preparation and Sterilization of Soil

A mixture of loam and sandy soil was used as planting media of the test plants. Soil was transferred in aluminum plates and was sterilized using an oven at 60-180°C for 2 hours. This should be done to assure killing of all soil-borne disease producing organism that might possibly affect plant growth and development (Miller, 1950). Three kilograms of this sterilized soil was placed in rectangular trays without holes to avoid leakage.

Soil Analysis

The initial cadmium and mercury concentration in soil was determined and was added to the amount of cadmium and mercury that was applied on the soil analysis of the soil which includes the organic matter; it was done using the standard procedure used at the University of Southern Mindanao Agricultural Research Center (USMARC), University of Southern Mindanao, Kabacan, Cotabato. While the initial cadmium and mercury content of the soil was analyzed using the standard procedures used at Davao Analytical Laboratories Inc., Davao City and F.A.S.T Laboratories, Cagayan de Oro City.

Treatments and Experimental Design

Randomized Complete Block Design was used as the experimental design. Sterile soil was used; there were four treatments were established. Each treatment has the following treatments: (1) Cadmium, Treatment one was applied with Cd $(NO_3)_2$ and inoculated with AMF, treatment two was applied with AMF but without Cd $(NO_3)_2$, treatment three was applied with Cd $(NO_3)_2$ but without AMF and treatment four was the control set-up; (2) Mercury, For mercury uptake, it was treated by the following treatments: Treatment one was applied with $HgCl_2$ and inoculated with AMF, treatment two was applied with AMF but without $HgCl_2$, treatment three was applied with $HgCl_2$ but without AMF and treatment four was the control set-up.

Propagation of Plant Samples and Inoculation of Arbuscular Mycorrhizal Fungi

Unrooted stolons of *Arachis pintoi* were used as the source of cuttings. Two cuttings about 8 to 10 cm long with 2 to 3 nodes were planted each tray. Twenty five grams of mycorrhizal fungi was inoculated into the soil a week after the cuttings were planted. For non-AMF plant set-ups, plant cuttings were propagated on separate trays with soils without AMF. After a month, plants were transferred on respective soil treatments for heavy metal uptake and growth analysis.

Application of Heavy Metals into the Soil

Two grams of Cd $(NO_3)_2$ and three grams of $HgCl_2$ were applied in each tray containing three kilograms of soil. Prior to application, Cadmium Nitrate (Cd $(NO_3)_2$ and Mercury Chloride ($HgCl_2$) were dissolved in distilled water to ensure that it will easily mix with the soil. After mixing the solutions in the soil, it was allowed to stabilize for one week.

Determination of Plant Growth, and Heavy Metal Uptake (Cd & Hg)

The growth of *A. pintoi* was determined by counting the number of leaves and measuring the length of the lateral stem a week after transplantation to different soil treatments. The plant growth will be evaluated every week for two months. Fresh weight and dry weight of the plant was measured to get the biomass at the end of the planting period. The measuring of the number of flower emergence of *A. pintoi* was evaluated weekly for 2 months.

Cadmium and Mercury uptake were determined in accordance to the laboratory protocol for Dry Ashing Atomic Spectrophotometry at Davao Analytical Laboratories Inc., Davao city and Cold Vapor Atomic Spectrometry F.A.S.T. Laboratories, Cagayan de Oro City respectively.

Statistical Analysis

The data on the growth of *A. pintoi* was measured in terms of the length of the shoot and the number of the leaves, flower emergence, fresh weight and dry weight was subjected to Two- way Analysis of Variance (Two-way ANOVA). The data on Cadmium and Mercury uptake of the plant were presented as percentage.

Results and Discussion

Growth Performance of *Arachis pintoi* with AMF Association in Response to Heavy metal Concentration

The number of leaves and stem length were used as parameters of plant growth. Figure 1 shows the growth of *Arachis pintoi* in sterile soil in terms of number of leaves for eight weeks of observation in Cd and Hg set-ups. Treatment 2 in both set ups showed the highest number of leaves, followed by treatment 1. As observed in both treatments, it shows the beneficial effects of arbuscular maycorrhizal fungi in the growth performance of *Arachis pintoi* even in the presence of heavy metals such as cadmium and mercury. The inoculation of arbuscular mycorrhizal fungi increased the number of leaves of *Arachis pintoi* than in plants treated with cadmium and mercury and plants in control set-up. According to Heijden et al. (1998), Arbuscular mycorrhizal fungi improve plant nutrition and promote plant diversity. AMF have been shown to differentially colonize plant roots, causing a variety of effects on plant growth. Thus, the extent of plant growth promotion by AM fungi can depend upon the specific plant and fungal combination (Heijden et al., 1998).

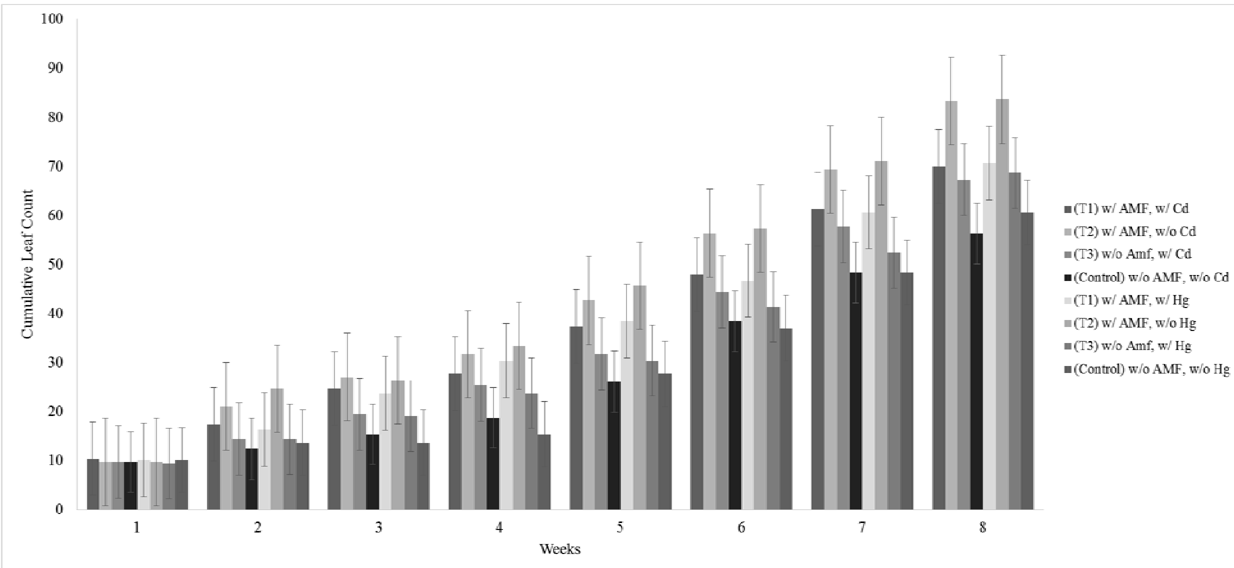

Figure 1. Cumulative leaf count after 8 weeks of AMF inoculation under heavy metal stress

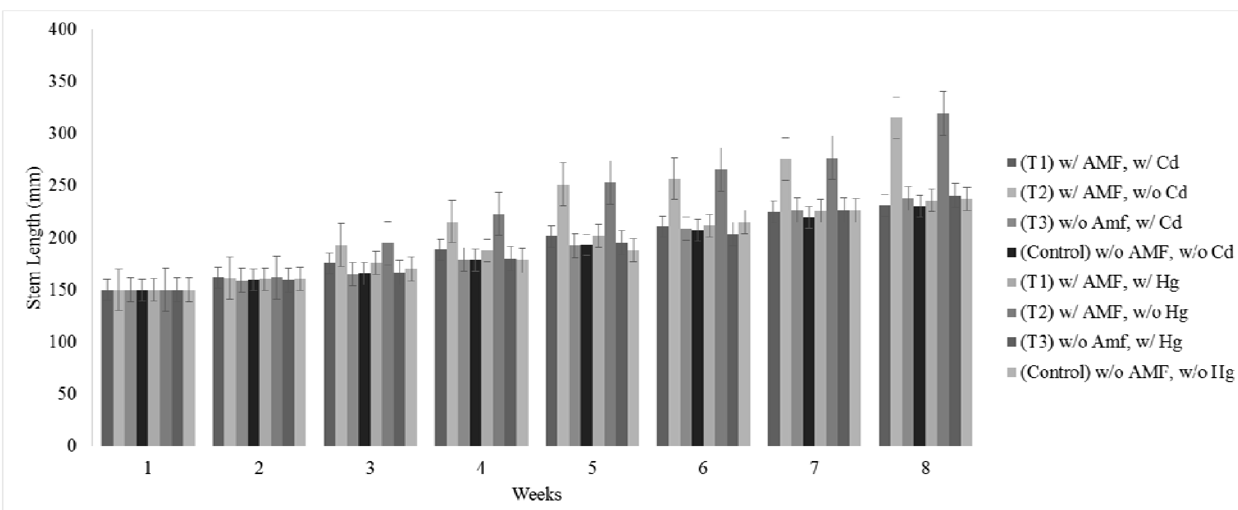

Figure 2. Stem length after 8 weeks of AMF inoculation under heavy metal stress

Analysis of variance on the mean number of leaves of *Arachis pintoi* in cadmium and mercury set-up in terms of treatments, weeks and their interaction. The computed results of analysis indicate that the main effect of treatment is highly significant in cadmium and mercury set-up (F = 556.277, df = 3, p-value = .000; F = 620.593, df = 3, p-value = .000, respectively), the main effect of weeks is highly significant (F = 3.381E3, df = 7, p-value

= .000; F = 3.114E3, df = 7, p-value = .000, respectively) and their interaction is highly significant (F = 22.465, df = 21, p-value .000; F = 22.897, df = 21, p-value .000, respectively). This was done to determine if the observed difference on the mean number of leaves of *Arachis pintoi* in cadmium and mercury set-up among the four treatments, weeks and their interaction were statistically significant. This shows that the four treatments are significantly different regardless of weeks applied towards the *Arachis pintoi* and the weeks are significantly different regardless of the treatments applied towards the *Arachis pintoi*.

Since there is an evidence of significant differences among the four treatments applied, a multiple comparisons among the mean number of leaves of *Arachis pintoi* in cadmium and mercury set-ups were done to determine the most effective treatment. Treatment 2 in cadmium and mercury set-up (42.625^{ab}, 43.958^{ab}, respectively) are comparable with treatment 1 (37.08333^{a}, 37.083^{a}, respectively) but differs significantly from treatment 3 and control set-up. It can be concluded that treatment 2 in cadmium and mercury set-up was the most effective treatment that could significantly increase the mean number of leaves of *Arachis pintoi*. Arbuscular Mycorrhizal fungi have an extraordinary importance since they increase nutrient acquisition by the plant as well as resistance to biotic and abiotic stress (Barea et al., 2002). The fungal symbiont increases its host's uptake of nutrients and can improve its growth and resistance to environmental stresses (Smith & Read, 2008).

Length of stem in Cadmium set-up was measured after eight weeks of observation (Figure 2). It was found out on the observation that treatment 2 in both set-up has the longest length of stem, followed by treatment 1, treatment 3, and the shortest length of stem was observed in control set-up. Populations of AMF are the key factor in soil development and successful plant establishment. According to Sylvia & Williams (1992), their presence may reduce stress caused by lack of nutrients or organic matter. Rivera-Becerril et al. (2002) stated that AM fungi not only provide nutrient to the plant but also play an important role in plant tolerance to heavy metals.

The analysis of variance on the mean length of stem of *Arachis pintoi* in cadmium and mercury set-up in terms of treatments, weeks and their interaction was done to determine if the observed difference on the mean length of stem of *Arachis pintoi* in cadmium and mercury set-up among the four treatments, weeks and their interaction were statistically significant. The computed results of analysis indicate that the main effect of treatment is highly significant in cadmium and mercury set-up (F = 1.042E3, df = 3, p-value = .000; F = 1.623E3, df = 3, p-value = .000, respectively), the main effect of weeks is highly significant (F = 2.040E3, df = 7, p-value = .000; F = 3.090E3, df = 7, p-value = .000, respectively) and their interaction is highly significant (F = 76.701, df = 21, p-value .000; F = 113.865, df = 21, p-value .000, respectively).

Since there is an evidence of significant difference among the four treatments applied, the comparison among the mean length of stem in cadmium and mercury set-up of *Arachis pintoi*. Treatment 1 in cadmium set-up is comparable with treatment 2 but differs differently in treatment 3 and control. It can be concluded that treatment 2 was the most effective treatment that could significantly increase the length of stem in cadmium set-up of pinto peanut. For mercury set-up, treatment 2 is comparable with treatment 1 but differs differently in control and treatment 3. The most effective treatment could significantly increase the length of stem is the treatment 2. According to Wang et al., (2007), the mechanisms exerted by AMF to alleviate heavy metals stress in plants may include the immobilization of heavy metals in the mycelium, heavy metals adsorption to chitin in the cell walls, the improvement of plant mineral nutrition, changes in rhizosphere pH, and the regulation of gene expression under stress conditions.

Cadmium and Mercury uptake of *Arachis pintoi*

Cadmium is an important environmental contaminant released into the environment as a by-product of various industrial processes (Kurek & Bollag, 2004). It is primarily used in plastics, ceramics, and paints, plating iron, television tubes and manufacturing of nickel-cadmium batteries (Al-Khedhairy et al., 2001). This heavy metal has been ranked number seven among the top ten of the 1999 priority hazardous substances (Kamnev & Lelie, 2000). The fate of cadmium in the environment is of particular concern. According to Uminska (1993), plants accumulate cadmium especially in conditions of great acidification of the soil. Yang (2004) stated that cadmium contamination is a great threat to human health as cadmium is easily transferred to human food chain from the soils. This metal has been determined to be mutagenic, exhibiting toxic effects on animals and humans, as well as on plants (Newhook et al., 1994). According to Bernard & Lauwerys (1986), in industry, cadmium enters the organism mainly by inhalation.

Mercury is a worldwide hazard released to the environment by anthropogenic activities such as coal burning and mining (Han et al., 2002). Among the top ten of the 1999 priority hazardous substances, mercury has been ranked number three (Kamnev & Lelie, 2000). It was formerly included in some pharmaceutical products, agricultural chemicals, dry cell batteries, and paints. Other uses of this metal include chloralkali production, switches and electrical apparatus, fluorescent light bulbs, and dental amalgam (Brooks, 2006). Chien et al., (2010) stated that mercury pollution is a widespread environmental problem due to atmospheric transport and deposition and also to point sources of pollution. According to Nies (1999), mercury is a toxic metal that accumulates through the food web. Mercury pollution has been a global public health problem of concern. The effect mercury is to cause damage to the brain and the central nervous system.

Figure 3 shows the cadmium and mercury uptake of pinto peanut with the initial concentrations of heavy metals in soil. Treatment 3 in both set up has the highest uptake of cadmium and mercury (24.5, 19.7 respectively), followed by the treatment 1 (19.1, 18.5 respectively). The result have found out that treatment 3 has the highest heavy metal uptake, however it is also observed that treatment 1 with AM fungi inoculation is also observed to have heavy metal uptake capacity.

However it was observed that treatment without AM has higher compared to that of with AM fungi, according to Iram et al. (2009) the heavy metal capacity of plant inoculated with AM fungi may depend on the extent of toxicity and nature of the metals and soil characteristics, and the complex interactions between metals and the environment. It is also supported by the report of Donnelly and Fletcher (1994) that mycorrhizal fungi appear to partially protect plants against the toxicity of heavy metals this may be the reason of the low metal uptake of the plant set-up. This result may be due to the beneficial role of ectomycorrhizal fungi has been reported earlier by many investigators (Galli et al. 1994) contributing to the heavy metal uptake.

The mechanism suggested for the protective effect of the fungus is the prevention of translocation of heavy metals into the host. Protection of plants against heavy metals is by the physical barrier or mantle (Brundrett,

2002) and may include metabolic processes such as intracellular metal accumulation and the extracellular precipitation of metals by metabolites in exudates. A key point for understanding the interactions between heavy metals and mycorrhizae is to take into account the functional diversity of mycorrhizal fungi and their ability to accumulate heavy metals.

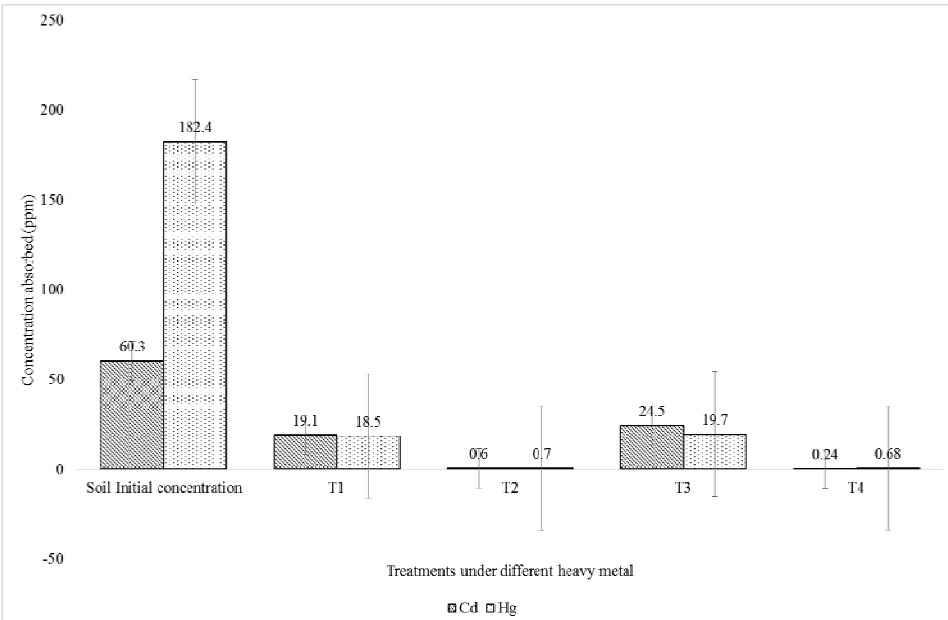

Figure 3. Amount of heavy metal absorbed in different treatments

There are a number of different types of fungi that form associations with plants, but for agriculture it is the arbuscular mycorrhizal fungi (AMF) of the Phylum Glomeromycota (Schussler et al., 2001) that are most important. These fungi comprise the most common mycorrhizal association and form mutualistic relationships with over 80% of all vascular plants (Brundrett, 2002).

Conclusion

The study was to investigate the heavy metal (Cd & Hg) absorption of *A. pintoi* associated with arbuscular mycorrhizal fungi (AMF). Specifically, evaluates the growth of *Arachis pintoi* applied with cadmium and mercury inoculated with arbuscular mycorrhizal fungi and determine and compare the cadmium and mercury uptake of *Arachis pintoi* inoculated with arbuscular mycorrhizal fungi in sterile soil. Considering the results of this study, the following conclusions were drawn:

1. The inoculation of arbuscular mycorrhizal fungi increased the number of leaves, length of stem, flower emergence of *Arachis pintoi* as well as of fresh weight and dry weight of the plant than to that of non-mycorrhizal fungi treatments.
2. The result have found out that treatment 3 in heavy metal cadmium and mercury has the highest heavy metal uptake, however it is also observed that treatment 1 with AM fungi inoculation is also observed to have heavy metal uptake capacity.

3. With the result on hand, it further suggest that *Arachis pintoi,* a widespread, easy to grow, and abundant plant species with AMF association could pave way in a low-cost and effective procedure in detoxifying soil heavy metal pollution and protecting plants against heavy metal contamination.
4. It is further recommended to conduct a test on the heavy metal accumulation in different plant parts (i.e. roots, leaf, and stem) investigate heavy metal uptake capacity using different metal to determine the total capacity of the plant in bioremediation techniques.

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