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Effect of Mycorrhiza on the Growth of *Paraserianthes falcataria* (L.) I.C Nielson under Hg-contamination

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Abstract

Mercury (Hg) at 50 mg kg^{-1} is toxic to microorganisms, but its toxicity to Arbuscular Mycorrhizae Fungi (AMF) associated with *Paraserianthes falcataria* (L.) I.C. Nielsen has not been widely studied. Effect of Hg on seed germination was evaluated to measure the rate of seed deterioration. The toxic concentration obtained from the germination study was used to adjust the Hg concentration of media for the seedling growth study. The aim of this study was to evaluate the effect of Hg on mycorrhiza. The research was conducted in two consecutive experiments. Rate of germination of seed under mercury condition, soil physicochemical properties, the profile of symbiont (AMF), and soil microbial activities were measured. Microbial activities were assayed using Fluorescein diacetate (FDA) hydrolysis and phosphatase activities. Seed germination was inhibited to 21.67–81.85% by Hg in a range of 30-100 mg L^{-1} Hg. The medium dose was chosen to evaluate the behavior of the symbiont (AMF) and its effect on soil physicochemical properties. Mercury inhibits microbial activity. Combination of compost and AMF treatment reduced toxicity effect while increasing soil microbial activity.

Key words – FDA – Germination – Hg-toxication – Mycorrhiza – *Paraserianthes falcataria* – Phosphatase

Introduction

Several technologies have been developed to find out the most cost-effective strategy to remove mercury (Hg) contaminants in soil. The Hg that occurs naturally in the environment and anthropogenic activities (i.e., mining, coal burning, and smelting of metal) have increased the widespread occurrence of Hg to reach levels of pollution (Beckers & Rinklebe 2017). The concentration of Hg in the environment varies greatly from 230–630 ng g^{-1} (Li et al. 2016). In soil, Hg is mostly associated with humic acids forming strong complexes with sulfur-containing functional groups. Hg in the soil can be found in many forms, such as elemental mercury, ionic mercury, MeHg, mercury hydroxide ($Hg(OH)_2$) and mercury sulfide (HgS). Hg^{2+} is the predominant toxic form of mercury. Hg can be transported and accumulated in the plant. Thorough mixing remains to be very difficult during *in situ* stabilization (Mulligan et al. 2001, Wang et al. 2020).

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The form of Hg commonly found in the environment is Hg^{2+}. It can be taken up directly by plants leading to Hg-toxicity in plants. Understanding the behavior of Hg in plants is crucial to optimize Hg-phytoremediation strategies. The most notorious behavior of Hg is their bioaccumulation potential in plant needles that reported a low uptake capacity of total Hg levels (0.03–6.68 mg kg^{-1}). Therefore many remediation technologies have been explored to reduce the environmental and biological hazard of Hg (Barquero et al. 2019). Various studies indicated that Hg is not taken into roots in significant amounts compared to the total amount of available Hg in the rhizosphere (Marrugo-Negrete et al. 2016). Their strong complex forms with soil chemicals such as humic acids, and sulfur-containing substances makes Hg-removal from the soil very complicated.

The most environmental friendly technology for Hg removal could be Hg-phytostabilization using plants such as *Paraserianthes falcataria*. *Paraserianthes falcataria* is a fast-growing tree and is a good candidate for removing Hg in contaminated sites. Naturally, *P. falcataria* can grow in a wide range of soil types, including nutrient-deficient soils or marginal lands. Their growth in marginal lands will be much slower and N-P-fertilization is necessary to increase growth. Symbionts, such as AMF plays a key important role in promoting the growth of *P. falcataria* under critical conditions (Wulandari et al. 2016). However, the effectiveness of this protection depends on the type of the fungus, a particular heavy metal involved, heavy metal concentration in the soil, soil properties, plant species and growing conditions, etc. Yu et al. (2010) reported that plant inoculation with AMF significantly increased immobilization of Hg in soil and reduced Hg root uptake to protect the plant from Hg toxicity. Tulod et al. (2012) reported that plants inoculated with AMF increased Hg uptake. Hg uptake is affected by mycorrhiza symbiont and plant species. AMF may influence the speciation, mobility and bioavailability of Hg in soil, consequently modulating its behaviour in soil-plant systems. Only a few research reported about the behaviour of microorganisms under high Hg concentrations, particularly by the plant symbionts. The effect of Hg on microorganisms and soil microbial activity in the presence of *P. falcataria* seedling remains unclear. Since its growth is affected by their associated microbial symbiont, AMF is crucial to evaluate the effect of Hg toxicity on the AMF population and its influence on *P. falcataria* growth.

Materials & Methods

Soil source

The study was conducted in a glasshouse at LIPI Cibinong, Bogor from October 2019 to February 2020. Soil samples for the pot trials were collected in LIPI Cibinong, Bogor (6°29’34” S 106°50’58” E). The soil was collected in October (dry season) from the near-surface at a depth of 10-20 cm. The physicochemical properties of this soil are shown in Table 1.

Table 1 Soil physicochemical and biological properties

No.	Parameter	Value	Status
1.	pH	6.71	Neutral*
2.	CEC	10.94 cmol kg^{-1}	Low*
3.	N total	143 kg ha^{-1}	Good*
4.	P total	46.1 kg ha^{-1}	Medium*
5.	Available P	19.4 kg ha^{-1}	Less*
6.	Organic Carbon	2.37%	More than sufficient*
7.	AMF Population**	170-210 $100g^{-1}$	
8.	Total Hg	Not detected	Very low

Criteria (*) Iram & Khan (2018)
** 90 % live spores

Experimental design

The research was performed in two experiments. First experiment was conducted to investigate the toxic concentration of Hg for the seed germination of *P. falcataria*. This experiment was set up in a completely randomized design with one factor, Hg concentration level. After deciding on the toxic concentration, it was continued to the second experiment to evaluate the effect of AMF on the growth of *P. falcataria* under Hg-contamination as well as its effect on soil physicochemical (total phosphate, available phosphate, and organic carbon), biological (spore population and root colonization, total microbial activity and soil enzyme) properties. The second experiment was set up in a Factorial Completely Randomized Design with two factors, namely AMF and compost treatments. A total of eight treatments were arranged with three replicates for each. Same arrangement was used without Hg contamination as controls.

Hg-toxicity on seed germination

The Hg solution was prepared, ranging from 0–100 mg L^{-1} Hg (Malar et al. 2015). Seeds of *P. falcataria* were obtained from the local market in Bogor. Seeds were surface-sterilized to avoid contamination, soaked with warm water to break the dormancy (Lindsey et al. 2017), and then soaked in the Hg solution for 24 hours before subjected to germination test. Sterile distilled water was used as a control (0 mg L^{-1} Hg). The germination test used ten seeds per treatment with three replications and was conducted after it dried in a sterile petri dish with a Whatman paper mat. The test was monitored after 24 hours of incubation in the dark at room temperature (Lin & Xing 2007).

***Paraserianthes falcataria* seedling preparation**

Surface-sterilized seeds were sown into the sterile compost media in the seedling tray. Two weeks after sowing, AMF-inoculated seedlings were prepared by inoculating the seedlings with spores of AMF. AMF spores were extracted from commercial mycorrhiza in the vermiculite propagule by choosing uniform spores. The wet sieving method was used for spore isolation (picking), and its identification was carried out based on the shape, colour, and size of the spores under the microscope (Brundrett et al. 1996). Seedling roots were inoculated with ten spores of mycorrhiza each by individually picking and attaching with tweezers. The inoculated seedlings were grown in a tray consisting of sterilized compost media. They were placed in the growth chamber for three weeks and irrigated with sterile water daily. The un-inoculated seedlings were also prepared in a similar manner.

Hg-toxicity on *P. falcataria* seedling growth and its symbiont

Hg-concentration used in this second experiment was the minimal concentration that reduced the seed germination rate by 50%. For the calculation and preparation of Hg doses, the method used by Ambarsari & Qisthi (2017) was followed. Briefly, 50 ml of 200 mg L^{-1} $HgCl_2$ stock solution was mixed with 200 g dried soil to get a final concentration of 50 mg kg^{-1} Hg in soil. The inoculated and uninoculated seedlings were transplanted into the soil in the glass pot following the combination rule in Table 1. The seedlings were placed in the growth chamber for two weeks. After two weeks, the seedlings were transferred to the greenhouse and monitored for eight weeks. The seedlings were irrigated daily with sterile water. The response of *Paraserianthes falcataria* under Hg-toxicity was observed by monitoring soil nutrient content, particularly soil organic carbon, total and available phosphate, biological and biochemical properties of soil.

Soil Physicochemical properties

Physicochemical properties such as soil organic carbon, total and available phosphate content were analysed to determine the effect of Hg toxicity on AMF and soil mineralization. The analyses followed the standard method of the Soil Research Agency (2009).

Soil Biological properties

Spore number in the soil was determined using the wet sieving method (Brundrett et al.

1996). Root colonization was observed using the staining method (Phillips & Hayman 1970). Soil microbial activity was determined using Fluorescein Diacetate (FDA) hydrolysis method (Adam & Duncan 2001), and phosphatase (acid and alkaline) activities were determined following the methods as described by Tabatabai & Bremner (1969).

Data analyses

Data presented are mean values ± standard error (SE). All data gathered were subjected to analyses of variance (ANOVA). The significance of the difference between exposed and control plants was tested by Duncan's Multiple Range Test ($P<0.01$).

Results

Hg-toxicity on seed *germination*

Hg significantly affected seed germination after 24 hours of incubation (Fig. 1). Hg concentration of 10 and 20 mg L^{-1} increased seed germination by 8.37% and 15.42%, respectively, compared to 0 mg L^{-1}. Hg-toxicity that significantly inhibited seed germination started at 30 mg L^{-1}, in which seed germination decreased to 21.67% compared to 0 mg L^{-1}. The percentage of seed germination decreased with the increase of Hg concentration. The highest toxicity effect resulted at 100 mg L^{-1} of Hg that reduced seed germination by 81.85% compared to 0 mg L^{-1}. These data were used to adjust the Hg concentration in the phytoremediation experiment.

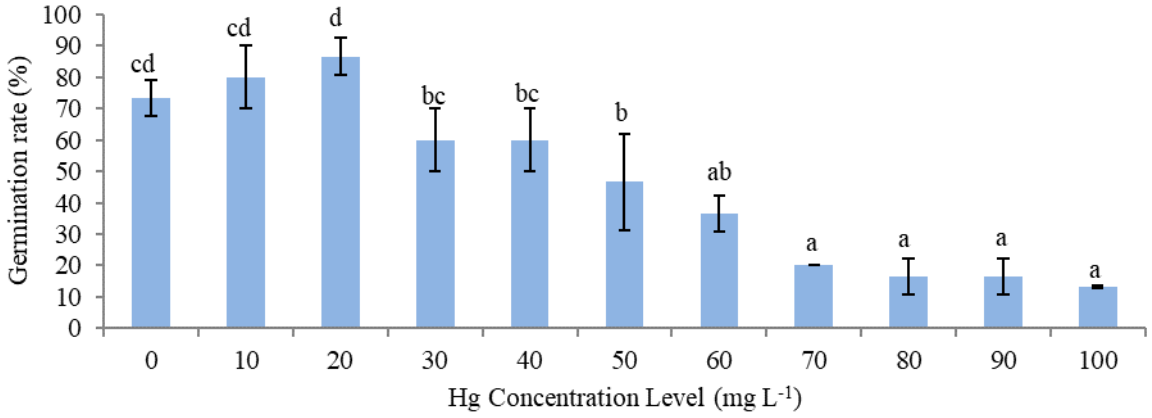

Fig. 1 – Hg-Toxicity effect on *Paraserianthes falcataria* seed germination. Values presented are means of three replicates with standard deviations. Bars with different characters indicate significant differences relative to Hg-concentration ($P<0.01$, ANOVA).

Effect of Hg on *P. falcataria*

Hg treatment on *P. falcataria* exhibited the potency of AMF to increase plant biomass under metal stress. Plant growth in Hg-treatment showed clear symptoms of toxicity (Fig. 2). Data showed that the plant height increment was twice higher than the control (Table 2).

Table 2 Agronomy Characters of *Paraserianthes falcataria* on Hg-condition

Treatment	Plant Height Rate (cm week^{-1})		Number of Leaves		Diameter (mm)	
	Mercury	Control	Mercury	Control	Mercury	Control
Mver	4.33±0.11 a*	2.16±0.35 d	6.67±0.57 ab*	8.00±0.00 ab	1.36±0.23 a	1.36±0.15 a
Mind	4.70±1.47 a *	2.33±0.57 ab	7.67±0.57 abc	6.67±2.08 a	1.56±0.55 ab*	1.76±0.25 ab
Mver+Mind	6.03±0.32 ab*	3.00±0.00 b	8.33±0.57 abc	8.33±1.52 ab	2.06±0.05 ab*	1.70±0.36 ab
M0	4.26±0.75 a*	2.00±0.00 a	6.33±1.52 a	7.67±0.57 ab	1.23±0.20 a	1.36±0.05 a

Table 2 Continued.

Treatment	Plant Height Rate (cm week^{-1})		Number of Leaves		Diameter (mm)	
	Mercury	Control	Mercury	Control	Mercury	Control
Mver+K	6.67±0.75 ab*	3.33±0.57 b	10.00±1.00 bc	9.33±1.52 ab	1.93±0.28 ab	1.86±0.15 ab
Mind+K	7.06±2.08 ab*	2.33±0.57 ab	10.33±2.08 c	9.00±1.00 ab	1.96±0.15 ab	2.00±0.10 b
Mver+Mind +K	8.83±1.40 b*	2.66±0.57 ab	9.00±2.00 abc	10.00±0.00 b	2.33±0.55 b*	2.63±0.32 c
M0+K	4.56±0.47 a*	2.00±0.00 a	8.33±1.15 abc	8.33±0.57 ab	1.23±0.35 a*	1.40±0.10 a

Mver = AMF inoculated seedling growth in sterile soil; Mind = Un-inoculated seedling growth in non-sterile soil; M0 = Un-inoculated seedling growth in sterile soil; MVer+Mind = AMF inoculated seedling growth in non-sterile soil; MVer+K = AMF inoculated seedling growth in sterile soil + compost; MInd+K = Un-inoculated seedling growth in non-sterile soil + compost; M0+K = Un-inoculated seedling growth in sterile soil+ compost; MVer+MInd+K = AMF inoculated seedling growth in non-sterile + compost. Values presented are means of three replicates with standard deviation. Values with different characters indicate significant differences between each treatment and control ($P<0.01$, ANOVA). * indicates significant difference ($P<0.01$, ANOVA) between each treatment and the control

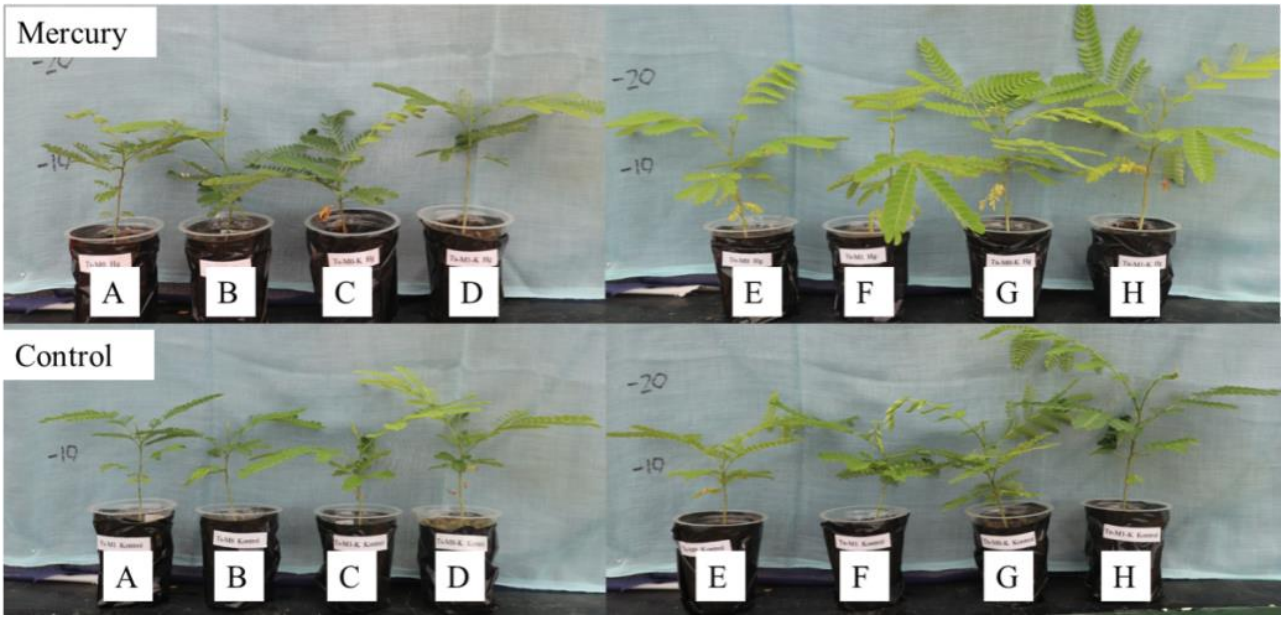

Fig. 2 – Agronomic characters of plants in pot trial. A Mver = AMF inoculated seedling growth in sterile soil. B M0 = Un-inoculated seedling growth in sterile soil. C MVer+K = AMF inoculated seedling growth in sterile soil + compost. D M0+K = Un-inoculated seedling growth in sterile soil+ compost. E MVer+Mind = AMF inoculated seedling growth in non-sterile soil. F Mind = Un-inoculated seedling growth in non-sterile soil. G MVer+MInd+K = AMF inoculated seedling growth in non-sterile + compost. H MInd+K = Un-inoculated seedling growth in non-sterile soil + compost.

Effect of Hg on soil nutrient behavior

Plants subjected to Hg contamination showed a significant effect on soil nutrient levels (Figs 3–4). Compost and Hg treatment affected soil physicochemical properties, including total phosphate, available phosphate, and organic carbon.

The amount of phosphate that remained in the soil was shown by the total phosphate in the soil (Fig. 3A). A high level of total phosphate found in the M0 treatment and a low value in the MVer+MInd treatment. This result shows that Hg contamination in the soil can reduce phosphate uptake by the plant. However, when compost and AMF were applied, nutrient uptake significantly

increased under Hg-condition, compared to treatment without compost and AMF. Hg in soil inhibits the absorption of phosphorus by plants, but AMF and compost treatments reduce the inhibition effect significantly. The availability of phosphate in the soil is also reduced by Hg contamination (Fig. 3B). A higher available phosphate was found in the MVer+MInd+K treatment, and the lower value was found in the M0 treatment. A higher amount of available phosphate with mycorrhiza addition indicated the AMF's ability to provide phosphate for plants even under toxic conditions. Compost treatment also increased the available phosphate in the soil. Hg treatment significantly affected organic carbon residue (Fig. 4). The higher amount of organic carbon was found in the MInd+K and a lower amount in the MVer+MInd treatment. Organic carbon level before trial was 2.37%, and compost addition increased organic carbon in the soil. The absorption of organic carbon under Hg-contaminated conditions was twice as higher as controls. These results imply that carbon was much more needed by plants and microorganisms for their metabolism under Hg-stressed condition.

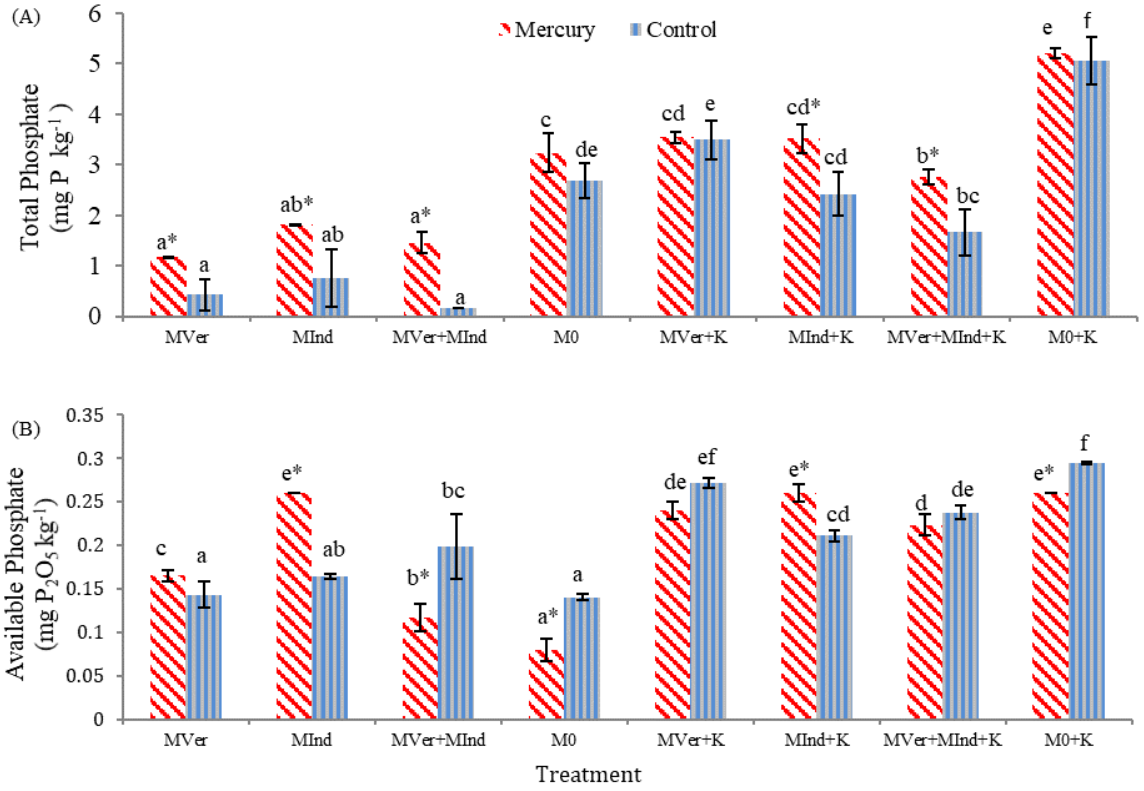

Fig. 3 – Phosphate Analysis. A Total phosphate. B Available phosphate. Values presented are means of three replicates with standard deviations. Bars with different characters indicate significant differences among respective Hg or control treatments within the column ($P<0.01$, ANOVA). * indicates a significant difference ($P<0.01$, ANOVA) between each individual treatment and the control. Mver = AMF inoculated seedling growth in sterile soil; Mind = Un-inoculated seedling growth in non-sterile soil; M0 = Un-inoculated seedling growth in sterile soil; MVer+Mind = AMF inoculated seedling growth in non-sterile soil; MVer+K = AMF inoculated seedling growth in sterile soil + compost; MInd+K = Un-inoculated seedling growth in non-sterile soil + compost; M0+K = Un-inoculated seedling growth in sterile soil+ compost; MVer+MInd+K = AMF inoculated seedling growth in non-sterile + compost.

AMF and microbial activity on Hg-condition

Inoculation of AMF has succeeded in increasing spore population and colonization (Fig. 5A, B). The highest spore concentration was found in MVer + K treatment, while the highest colonization found in MVer + MInd treatment. Hg contamination in soil has significantly

influenced spore population and colonization. The spore population of AMF was increased, while the colonization was reduced under Hg contamination. Similarly, compost addition gave a significant effect by increasing the spore number but not on root colonization.

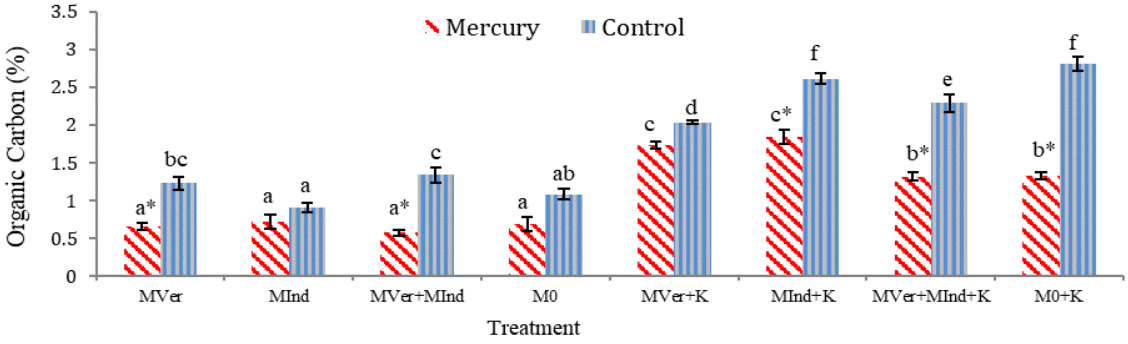

Fig. 4 – Soil Organic Carbon. Values presented are means of three replicates with standard deviations. Bars with different characters indicate significant differences among respective Hg- and control treatments within the column (P<0.01, ANOVA). * indicates a significant difference (P<0.01, ANOVA) between each individual treatment and the control. Mver = AMF inoculated seedling growth in sterile soil; Mind = Un-inoculated seedling growth in non-sterile soil; M0 = Un-inoculated seedling growth in sterile soil; MVer+Mind = AMF inoculated seedling growth in non-sterile soil; MVer+K = AMF inoculated seedling growth in sterile soil + compost; MInd+K = Un-inoculated seedling growth in non-sterile soil + compost; M0+K = Un-inoculated seedling growth in sterile soil+ compost; MVer+MInd+K = AMF inoculated seedling growth in non-sterile + compost.

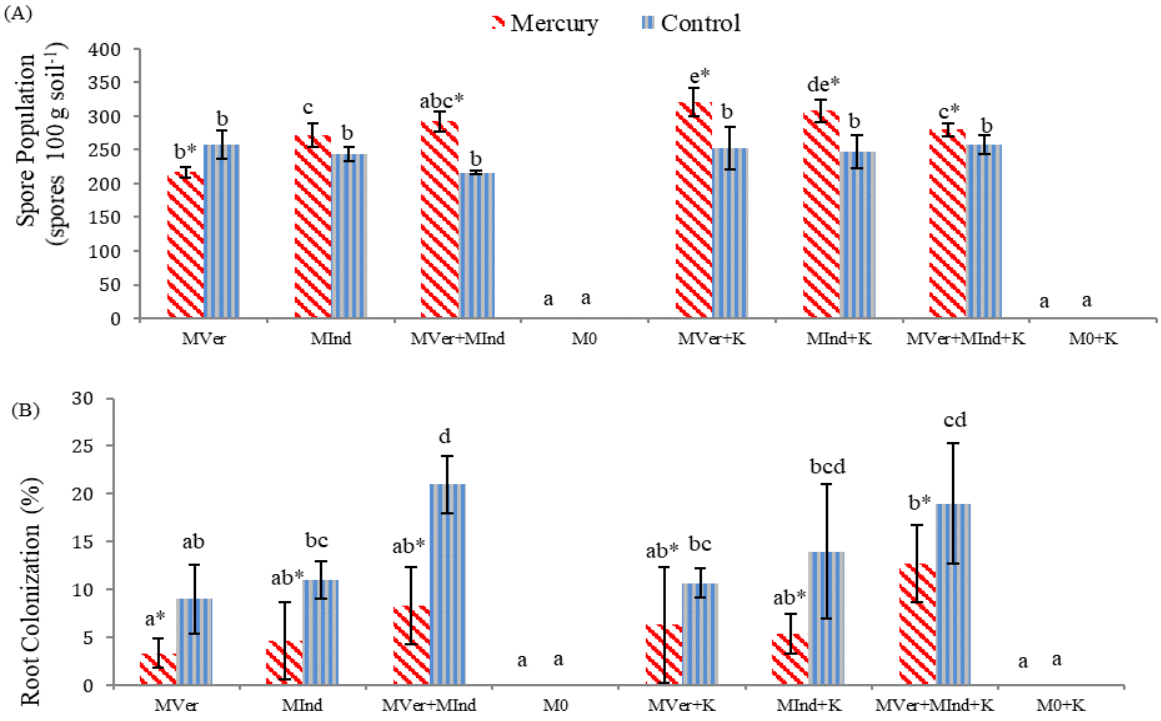

Fig. 5 – AMF. A = Spore population. B = Root colonization. Values presented are means of three replicates with standard deviations. Bars with different characters indicate significant differences among respective Hg or control treatments within the column (P<0.01, ANOVA). * indicates a significant difference (P<0.01, ANOVA) between each individual treatment and the control. Mver = AMF inoculated seedling growth in sterile soil; Mind = Un-inoculated seedling growth in non-sterile soil; M0 = Un-inoculated seedling growth in sterile soil; MVer+Mind = AMF inoculated

seedling growth in non-sterile soil; MVer+K = AMF inoculated seedling growth in sterile soil + compost; MInd+K = Un-inoculated seedling growth in non-sterile soil + compost; M0+K = Un-inoculated seedling growth in sterile soil+ compost; MVer+MInd+K = AMF inoculated seedling growth in non-sterile + compost.

Hg-toxicity affected FDA (Fluorescein Diacetate) and phosphatase activity (Fig. 6A, B , C). A higher FDA and acid-phosphatase levels were found in the controls than in Hg treatments (Fig. 6A, B). Meanwhile, Hg treatments recorded levels higher than the control in the alkali-phosphatase. The results showed that Hg treatment only affects soil microorganisms, especially in their metabolism. Based on FDA activity, phosphatase activity was also increased by the compost and AMF treatments.

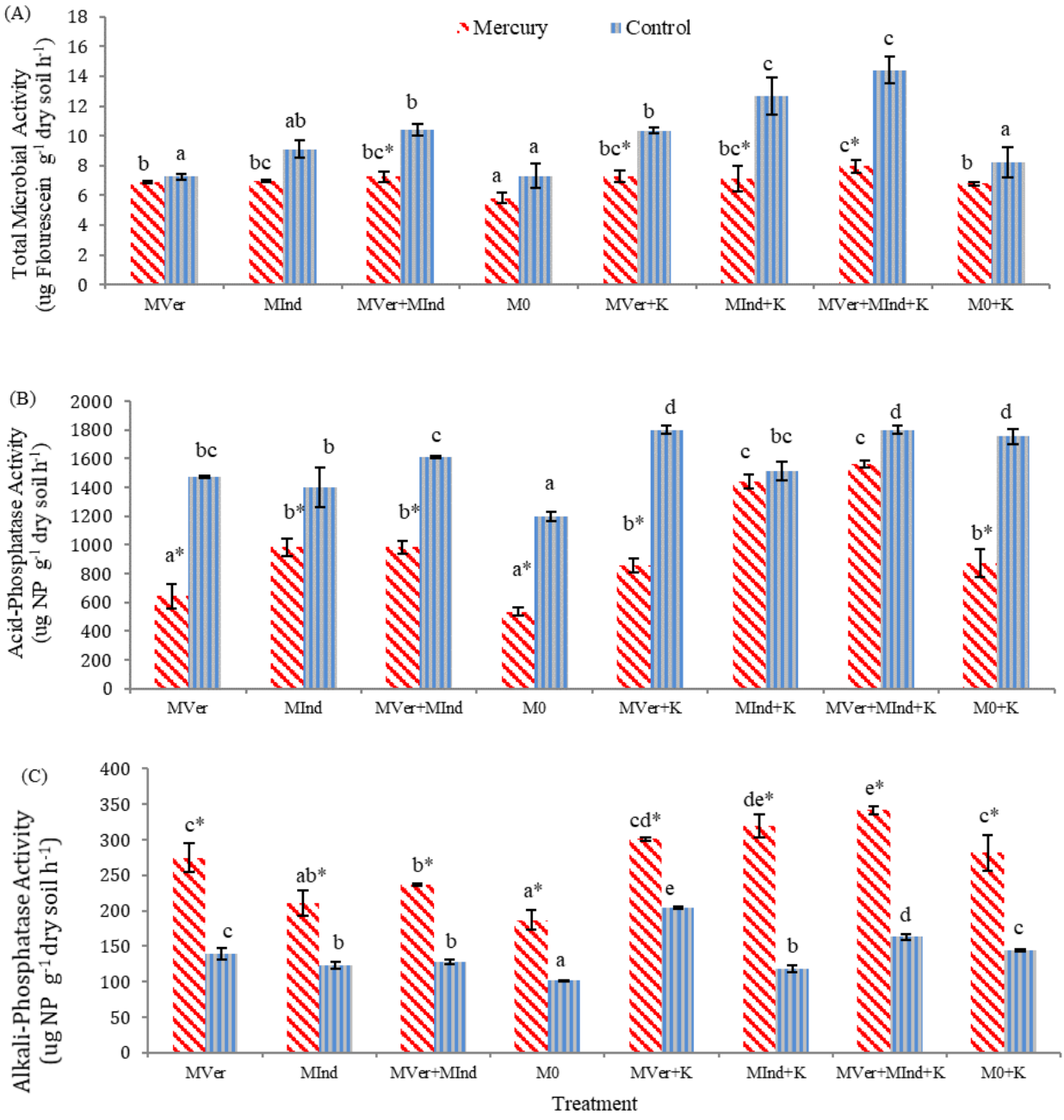

Fig. 6 – AMF Activity. A FDA. B Acid phosphatase. C Alkaline phosphatase. Values presented are means of three replicates with standard deviations. Bars with different characters indicate significant differences among respective Hg or control treatments within the column (P<0.01, ANOVA). *indicate significant differences (P<0.01, ANOVA) between each individual treatment

and the control. Mver = AMF inoculated seedling growth in sterile soil; Mind = Un-inoculated seedling growth in non-sterile soil; M0 = Un-inoculated seedling growth in sterile soil; MVer+Mind = AMF inoculated seedling growth in non-sterile soil; MVer+K = AMF inoculated seedling growth in sterile soil + compost; MInd+K = Un-inoculated seedling growth in non-sterile soil + compost; M0+K= Un-inoculated seedling growth in sterile soil+ compost; MVer+MInd+K = AMF inoculated seedling growth in non-sterile + compost

Discussion

Several studies have reported that various plants can grow in Hg contaminated environments (Lin & Xing 2007). *Paraserianthes falcataria* grows in Hg contaminated soils (Indraningisih et al. 2016). The experiment on *P. falcataria* seed germination under Hg contamination showed that seed germination has increased at low Hg concentrations (10-20 mg L^{-1}) and significantly decreased at medium to high concentrations (50-100 mg L^{-1}) (Fig. 1). Thus, a middle dose (50 mg L^{-1}) was chosen for further study to evaluate the behavior of symbiont (AMF) and its effect on soil nutrient behavior. The fact that the Hg at lower concentrations (10-20 mg L^{-1}) has increased seedling growth is quite intriguing, which needs further plant physiological explanation. This phenomenon was suggested as hormesis. The hormesis growth stimulation has frequently been observed in plants exposed to potentially toxic metals (e.g. Cd, Cr, Al, Pb) in low concentrations. Under abiotic stress, metals can act as elicitors of defense response and express phytohormones in plants (Emamverdian et al. 2020). Various effects of Hg on plants have been identified and observed. However, the mechanism through which Hg was toxic to plant and microorganism was not well understood. It was reported that Hg is able to block the important macromolecules, affecting the antioxidant defense system, and reducing photosynthesis (Azevedo & Rodriguez 2012). According to Majid et al. (2017), Hg in plant tissues binds to glutathione and phytochelatin. Hg has a strong affinity to sulfhydryl (S-H), which disrupts the protein-membrane and affects seed germination. But, the toxicity effect and Hg bond forms depend on organic matter, chloride ions, pH, and biological processes (Xu et al. 2015).

Our results showed that Hg reduced AMF activity to de-composite phosphate, so that the available phosphate is low than under uncontaminated condition (Fig. 3). These results were in line with those published by Debeljak et al. (2018) that Hg decreased the phosphate uptake by *Zea mays*. Hg can bind with phosphate groups and form insoluble complexes, hence plants cannot absorb these nutrients (Tangahu et al. 2011). Hg was reported as having the ability to alter the translocation of nutrient uptake (i.e., phosphate, nitrogen) (Azevedo & Rodriguez 2012), replacement of essential ions (i.e., Mg^{2+}) and disrupting functional mechanisms in plants (Küpper et al. 2007). However, the existence of AMF in plant roots can reduce the Hg blocking effect on phosphate uptake. It was well known that AMF assisted plants to acquire nutrients, particularly the phosphates through hyphal expansion and release of various organic acids to solubilize insoluble forms of phosphate and through the increased activity of phosphatase. The level of carbon in soil has increased under Hg-contaminant. This proves that more carbon produced by plants and microorganisms through their metabolism under stress due to Hg-condition. Wang et al. (2015) reported that protein (Acyl-CoA) made by photosynthesis used to detoxify Hg. This finding supports the hormesis condition proposed previously. Plants will grow faster as a defense response to the toxic metal, hence it needs more energy sources. Organic carbon is the main source of energy for microorganisms and plant growth (Huang et al. 2016), therefore increased the growth of plant at initial low Hg rates.

Studies on the evaluation of the toxic effect of Hg on soil microorganisms have revealed that Hg contamination in soil has a negative effect on soil microorganisms particularly on plant symbionts (AMF). Hg contamination in soil has reduced root infection by AMF and increased sporulation. Spore formation is normally induced under critical conditions (Radha et al. 2015), and on the nutrient levels (Zhou et al. 2017). As proven by spore population levels and colonization, *P. falcataria* has successfully formed a symbiosis with AMF. Symbiosis among *Rhizobium*, AMF, and plants involved sophisticated and complex molecular communication (Chang et al. 2017). Hg

contaminations in soil are expected to disrupt this molecular communication so as to inhibit their growth. The results imply that AMF was not tolerant of Hg. Understanding the symbiotic association between symbionts and their host is critical for the success of Hg-phytoremediation. Symbiosis among *Rhizobium*, AMF and the plants are called "*tripartite symbiosis*" which can further enhance growth even under more critical conditions (Chang et al. 2017).

Determining FDA activity in soil has been widely used to estimate soil microbial activities and soil respiration (Adam & Duncan 2001). Hg contaminations in soil reduced the overall microbial activities. Our finding was in line with the study by Nie et al. (2018), who demonstrated that FDA activity was increased under higher amounts of the substrate (organic matter) when the plants were not under abiotic or biotic stress. The increase in FDA activity was in line with the abundance of biodiversity of soil biota. The change of these AMF activities in soil due to the effect of Hg toxicity can be used as a bioindicator for measuring and assessing soil and environmental health (Tazisong et al. 2012). Results showed that Hg was toxic to soil microbes, but the addition of compost and the presence of plant symbiont (AMF) could maintain soil microorganisms sufficiently in the Hg-contamination environment. Soil enzyme analysis showed the abnormal activity of microorganisms due to Hg-contamination (Fig. 6). Acid phosphatases decreased by the Hg contamination, and these results are in line with the study reported by Xu et al. (2007). Hg in soil could inhibit acid phosphatase activity along with the increase of its concentration. However, AMF and compost treatment can reduce such inhibition caused by Hg contamination. Besides, organic matter in compost can bind Hg and reduce the toxicity of Hg in soil (Różański et al. 2016). On other hand, the alkaline-phosphatase was stimulated by Hg-contaminated soil. Alkaline-phosphatases are mostly produced by bacteria and the increase of alkaline activities might be due to the increase of heavy metal resistant bacteria in the soils contaminated by Hg. There is no straightforward explanation for this physiological behavior, and need further studies in AMF. It is well known that soil enzyme activities are influenced by the soil type, topography, climate, vegetation, and time. Soil enzyme activity might be suggested as a bioindicator for soil pollution since its activities are sensitive to the toxic compounds, caused particularly by heavy metals (Casucci et al. 2003).

Conclusions

This study was to evaluate the effect of Hg toxicity on seed germination and growth of *Paraserianthes falcataria* as well as their associated microbial symbiont, AMF. Hg toxicity on seed germination is concentration-dependent. At lower concentrations (10-20 mg L^{-1}), it increases seed germination by becoming toxic at higher concentrations (>30 mg L^{-1}). At lower concentrations (10-20 mg L^{-1}), seed germination increases by 8.37%, while at high concentrations (>30 mg L^{-1}), it inhibits the germination up to 21.67-81.85%. It is crucial to determine the Hg-toxic threshold to seed germination prior to any Hg-contaminant experiment, i.e. 30 mg L^{-1}. It will affect the level of allowable Hg-condition to get good seedling growth. At 50 mg kg^{-1}, Hg did not inhibit seedling growth in pot experiments as found in previous seed germination experiments. Hg contaminant altered physicochemical properties of soil including available phosphates and organic carbon. These results proved that carbon was needed more by plants and microorganisms in their metabolism under stressed Hg-condition. This finding supports the hormesis condition that was proposed previously. Plants will grow faster as a defense response to the toxic metal, requiring more energy sources. AMF is crucial in stimulating *P. falcataria* growth under a high Hg-stressed environment. Determining FDA and phosphatase activities of Hg-contaminated soil can be used as an indicator of pollution status. The behavior of Hg is quite unique as it stimulates *P. falcataria* growth when the Hg-contamination is low. This is quite advantageous for phytoremediation of low Hg-contaminated areas. The mechanism behind this phenomenon is open for further study.

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Accessibility of data

Provide identifiers of material and data deposited in (preferably publicly accessible) public herbaria, collections, databases and data repositories.

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