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Research article

Lead tolerant endophyte *Trametes hirsuta* improved the growth and lead accumulation in the vegetative parts of *Triticum aestivum* L.

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ABSTRACT

Rapid industrialization and increasing population are continuously adding contaminants to our environment. Among those, heavy metals are considered to be one of the serious threats to the ecosystem due to their persistent nature. Microbe assisted phytoremediation is an effective tool for metal remediation as microbes enhance the metal availability and uptake to the host plants or reduce it by binding them intracellularly or extracellularly. An endophytic fungus, *Trametes hirsuta*, was isolated from *Chenopodium album* L. plant growing in the lead (Pb) contaminated soil of an industrial area. This is the first study citing *Trametes hirsuta*, as a root endophyte of *Chenopodium album* L. This endophytic fungus was found to be tolerant to high concentration of Pb i.e., 1500 mg L^{-1}, when tested *in-vitro*. Wheat (*Triticum aestivum* L.) seedlings were infected by *Trametes hirsuta* and Pb tolerance was observed. With the fungal inoculation plants cumulative growth and total chlorophyll content increased by 24% and 18%, respectively as compared to their respective non-inoculated controls at 1000 mg kg^{-1} Pb. Similary, 50% more Pb accumulation was measured in the shoots of fungal inoculated plants at 1500 mg kg^{-1} Pb as compared to control. Thus, the results of the present study suggest that mutualism with endophytic fungi can improve the survival of host plants in metal contaminated soils, additionally it can also assist the phytoextraction of heavy metals from polluted sites by increasing their uptake by the host plants.

1. Introduction

Increased worldwide industrialization, modern agriculture and urbanization have hosted a wide variety of chemicals into the environment which may result in serious environmental and human health problems (Rajkumar et al., 2012). Among these pollutants, heavy metals are considered as main concern to health related issues of humans due to their carcinogenicity, cytotoxicity and genotoxicity (Dautovic et al., 2019). Thus, the remediation of heavy metal contaminated environment has considerably gained attention. Also, it is a bit difficult task owing to the fact that metals cannot be degraded like organics, so generally need to be physically removed or immobilized (Aishwarya et al., 2014).

The present study focuses on Pb in terms of its remediation as it ranks fifth in terms of industrial production and comes after Cu, Fe, Al and Zn (Wuana and Okieimen, 2011). It is reported as the second most hazardous substance right after arsenic by the Agency for Toxic Substances and Disease Registry (ATSDR) due to its toxicity, frequency of occurrence and potential for human exposure (Titah et al., 2013). Pb induces phytotoxicity by changing cell membrane permeability, by combining with either ADP or ATPs; exclusively with their phosphate groups; by reacting with active groups of various metabolic enzymes and by provision of substitutes of vital ions (Kumar et al., 2017). Likewise, Pb inhibits plants normal functioning; root elongation, seed germination, chlorophyll

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production, and plant growth; transpiration and water/protein content and seedling development (Maestri et al., 2010).

To avoid the short comings of phytoremediation i.e., phytotoxicity, the microbe-plant partnership is a best-established approach as it enhances the nutrients availability and mitigate the harmful effects of heavy metals on plants (Ashraf et al., 2017). Endophytes are organisms which may induce infections asymptomatically within plant tissues with no disease symptoms (Aishwarya et al., 2014). In endophyte-plant symbiosis, the endophytes get carbohydrates from plants and in return help plant's adaptability and resistance to biotic and abiotic stresses (Aslam et al., 2019). Endophytes basically produce a glut of bioactive compounds i.e., plant growth regulators, antifungal, insecticidal, antibacterial, and antiviral compounds to facilitate plant growth (Nisa et al., 2015). Owing to the fact that fungi form extended mycelial networks makes it well suitable for bioremediation purposes and these linear organs of aggregated hyphae facilitate fungal translocation. In addition, fungi possess higher tendency to build a physical and enzymatic contact with its surrounding environment due to its greater cell to surface ratio (Jacob et al., 2018). The appliance of filamentous fungi can be a potential way in situations of bacterial malfunction when contaminants are unreachable physically up to unicellular organisms or pollution is too stern to preserve bacterial survival as bacteria cannot outline its extended mycelial network to respond to contaminants easily (Harms et al., 2011). Fungi also contribute in enhancing the soil nutrient bioavailability such as they promote P and K solubilization through siderophores production and release of CO_2, H^+, and organic acids (Rashid et al., 2016).

Though, potential fungal pathogens can also be isolated as endophytes but literature states that endophytes that are pathogenic to some host plants might be asymptomatic to others (Malcolm et al., 2013). Furthermore, fungal endophytic species may also switch between pathogenic, commensal or mutualistic modes and this depends upon the environmental conditions, host plant and the endophytic species. Like, in harsh environments where survival of both plants and microbe has become hard and they need each other's assistance so they would become symbionts and provide benefit to each other (Schulz and Boyle, 2005).

According to Ripa et al. (2019), endophytic fungal communities can be exploited as bio-fertilizers or bio-agents to create a sustainable crop production system because fungal endophytes of their study exhibited tolerance to abiotic stresses i.e., temperature, salinity, drought and heavy metals. Importance of fungal endophytes colonization for survival in stress conditions can also be evaluated by the findings of Yamaji et al. (2016) as their experimental results showed that *Clethra barbinervis* can scarcely grow under the heavy metals stress without root fungal endophytes while the inoculated plants tolerated high concentrations in the presence of fungal endophytes; *Phialocephala fortinii*, *Rhizoscyphus*, and *Rhizodermea veluwensis* sp. by growth augmentation which increased K uptake and reduced metal uptake. Likewise, fungal endophyte *Alternaria alternata* was assessed to evaluate its role in host survival and stress mitigation of *Solanum nigrum* L. and showed better growth attributes such as shoot, root length, dry biomass, chlorophyll contents and leaf area under Cd stress as compared to controls and it also decreased Cd uptake in plant (Khan et al., 2015). Thus, it is evident that heavy metal tolerant fungal endophytes can help in promoting plant growth and tolerance in the presence of metal stress.

Therefore, present study was designed to isolate a Pb tolerant endophytic fungus from *Chenopodium album* L. roots grown on Pb contaminated soil and to evaluate the affects of that endophytic fungus on *Triticum aestivum* L. growth in the presence of Pb stress as well as to assess its role in *Triticum aestivum* L. tolerance to Pb.

2. Materials and methods

2.1. Collection of plant samples for endophytic fungal isolation

The sampling area is located along a drain of I-9 sector Islamabad. It is an industrial area loaded with pharmaceuticals, steel, polymer and marble industries. Being the dominant plant species, five healthy plants of *Chenopodium album* L. were uprooted randomly. Sampled plants were stored in paper bags and transported to the laboratory immediately and processed for fungal endophyte isolation within 24 h.

2.2. Isolation of endophytic fungus

After washing the roots of five individual plants by running tap water and distilled water, they were surface sterilized with 70% ethanol for 1 min, then 15% hydrogen peroxide solution for 15 min and 70% ethanol for 1 min. Then plant roots were washed with sterilized distilled water to remove reagents, dried on sterile filter paper and cut into 5-mm pieces approximately with a sterilized scalpel (Yamaji et al., 2016). These surface sterilized root pieces were then aseptically placed on potato dextrose agar (PDA) improved with 0.5 g L^{-1} streptomycin sulfate to restrain bacterial growth. Then plates had been incubated at 25 °C for about two weeks and growing fungi out of the plant tissues were shifted to fresh PDA plates. Fungal colonies were purified by hyphal-tipping method (Strobel et al., 1997) and purified colonies were stored at 4 °C. The efficacy of the surface sterilization was established by making marks of surface sterilized root pieces on PDA plates from which no fungal growth was noticed (Li et al., 2016).

2.3. Selection of Pb tolerant endophytic fungi

Two dominant root endophytic fungi which were having different colony color were selected for further testing for heavy metal tolerance on PDA, spiked with increasing concentrations of Pb (Teng et al., 2018). Lead nitrate [$Pb(NO_3)_2$] was used as a source of Pb. Three replicates of each treated PDA plate were inoculated with a 5 mm agar plug from the edge of seven day old culture plates of both isolates. PDA plates without Pb were used as controls. The plates were then incubated at 25 °C for seven days. At 300 mg L^{-1} Pb, the fungus strain # 15 showed better growth than fungus strain # 10 so it was further tested at 600, 1000, 1500, 1600 and 1700 mg L^{-1} concentration of Pb (Khan et al., 2015).

2.4. Molecular identification of Pb tolerant fungus

The endophytic fungus was characterized based on molecular characterization. For this purpose, genomic DNA was extracted from 5 days old pure fungal culture grown on PDA plates by the method of Lee et al. (1988). Polymerase chain reaction (PCR) was then performed to amplify the ITS1-ITS4 region of the internal transcribed spacer (ITS) of extracted gDNA by using the universal primers; ITS1-F (5′-TCCGTAGGTGAACCTGCGG-3′), ITS4-R (5′-TCCTCCGCTTATTGATATGC-3′) (White et al., 1990). PCR was carried out with the following amplification steps: initial denaturation for 3 min at 95 °C followed by 29 cycles of 45 s at 95 °C, 30 s at 55 °C and 40 s at 72 °C; and final extension of 10 min at 72 °C. The PCR products were run on (1%) agarose gel to confirm the amplified product. The amplified product was sent to Macrogen, Korea for sequencing. The nucleotide sequence results were blasted on NCBI to identify the fungus.

2.5. Pot experiment of Triticum aestivum L. with root fungal endophyte under Pb stress

Sand was filtered via 2 mm sieve and acid rinsed to eradicate the adsorbed nutrients by soaking overnight in 0.1 N HCl. Sand was then washed several times with distilled water until pH 6.0 (Idayu et al., 2017). The acid washed sand was air dried and sterilized twice by autoclaving at 15 psi at 121 °C for 2 h. Then it was spiked with Pb solutions according to Li et al. (2011) to establish the Pb concentration of 0, 500, 1000 and 1500 mg kg^{-1}. Then the spiked sand was left for one month for metal stabilization. Uniform *Triticum aestivum* L. seeds (Pakistan 2013; the characteristics of this variety can be found on http://www.parc.gov.pk/index.php/en/faq-s/60-faqs/270-faqs-wheat) were surface sterilized first by the method of Shen et al. (2013) then inoculated

by fungal mycelial suspension (0.1 g fresh mycelia ml^{-1}) for 1 h and seeds for control treatment were soaked in sterile water (Deng et al., 2014). The sterilized seeds were aseptically grown in plastic pots lined with polythene bags and filled with 400 g of spiked sand. Six seeds were planted in every pot with three replicates per treatment. Subsequent to the appearance of the first pair of true leaves, three uniform seedlings were kept per pot. Pots were daily irrigated as per requirement with sterilized distilled water and nutrients were supplied by the application of half strength Hoagland's nutrient solution on a weekly basis. Plants were harvested after 45 days of growth.

After plant harvesting, fresh plant samples were weighed to obtain fresh biomass and then air dried at 65 ± 5 °C for 48 h in drying oven until constant weight is attained. The photosynthetic pigments were measured in terms of chlorophyll a, carotenoids and chlorophyll b in fresh plant samples in accordance with the procedure proposed by Arnon (1949) while using UV-spectrophotometer (LRI Germany, 5405007) at 470, 645 and 663 nm wavelengths, respectively and quantified by using the equations proposed by Lichtenthaler (1987). Plant samples were prepared for metal analysis by acid digestion method; 0.1 g of dried plant sample was first grinded, then 3 ml of concentrated nitric acid (HNO_3) was added followed by the addition of 3 ml of H_2O_2 and 0.5 ml of concentrated HCl (Estefan et al., 2013). The concentration of Pb in the *Triticum aestivum* L. roots and shoots was measured by atomic absorption spectrophotometer (AAS) (Perkin Elmer, AAS-700).

2.6. Microscopic observation of endophyte infected Triticum aestivum L. roots

Plant fresh roots were taken from the inoculated treatments for observing the fungal root colonization. Roots were washed thoroughly and preserved informol acetic alcohol (FAA) solution having 70% ethanol, 35% formaldehyde and glacial acetic acid in 90:5:5 ratios and stored at 4 °C (An et al., 2015). At the time of observation, these fixed roots were washed carefully and cut into 2 cm pieces. Then they were cleared with 10% KOH at 90 °C for 15 min in water bath and stained with 0.05% (*v/v*) trypan blue in lactophenol for 5 min (Shahabivand et al., 2012). The presence of fungal infection in roots was observed under a compound light microscope (Olympus CX41 equipped with Tucsen ISH500 camera).

2.7. Statistical analysis

Pot experiment was done with three replicates per treatment. All of the values stated in present study are mean of three values ±standard error (SE). Data of all parameters was statistically analyzed with the help of SPSS 20 and Microsoft Excel 2010. Two-way ANOVA was performed to compare the means of treatments by using Duncan's multiple range test at significance level of $p < 0.05$ and significantly different values were shown with different letters.

3. Results

3.1. Morphological characteristics of endophytic fungus and its molecular identification

Pb tolerance testing for fungus strain # 15 showed that it can grow up to 1500 mg L^{-1} of Pb concentration (Figure 1). The endophyte possesses white colony mycelia on PDA plates. It has thread-like mycelia with septate hyphae (Figure 2). Molecular characterization was done on the basis of internal transcribed spacer (ITS) region and blast results revealed that the fungal DNA sequences possess 100% homology with the corresponding gene sequences of *Trametes hirsuta*. The partial sequence is provided as supplementary sequence 1 in supplementary material.

3.2. Microscopic observation of root colonization by endophytic fungus

Root pieces of *Triticum aestivum* L. inoculated by *Trametes hirsuta* were randomly collected at the completion of pot experiment and were stained with trypan blue. The dark blue color appeared which represents the presence of fungal hyphae in the root tissues of *Triticum aestivum* L. (Figure 3).

3.3. Effect of endophytic Trametes hirsuta inoculation on growth of Triticum aestivum L.

Trametes hirsuta inoculation helped the plants to tolerate high Pb concentrations. With increasing concentration of Pb, the shoot and root fresh and dry weights decreased, but plants with *Trametes hirsuta* showed better growth and higher biomass as compared to their respective non inoculated controls. At 1000 and 1500 mg kg^{-1} Pb stress, the shoot fresh weight significantly decreased in control and observed to be 0.53 g and 0.41 g, respectively, but *Trametes hirsuta* inoculated plants showed significantly higher fresh biomass by having 0.66 g and 0.53 g, respectively (Figure 4a). Similarly, at 500 mg kg^{-1} Pb, significantly higher root biomass when inoculated by *Trametes hirsuta* was obtained as compared to its control, by having 0.95 g fresh and 0.32 g dry mass. Pb stress higher than 1000 mg kg^{-1} significantly decreased the plant root fresh weight but *Trametes hirsuta* inoculated plants had significantly higher biomasses (Figure 5a).

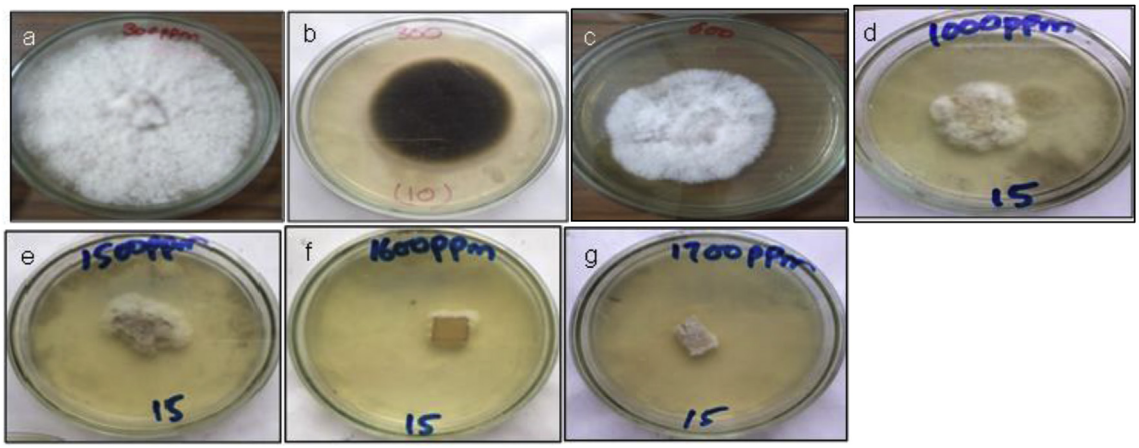

Figure 1. Endophytic fungal strains growth after seven days of incubation at 300 mg L^{-1} Pb, strain # 15 (a) and #10 (b). Growth of strain # 15 (*Trametes hirsuta*) at 600 (c), 1000 (d), 1500 (e), 1600 (f) and 1700 (g) mg L^{-1} Pb stress.

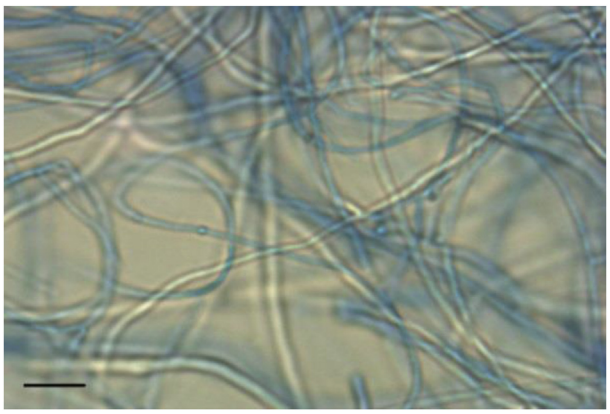

Figure 2. Microscopic view of the *Trametes hirsuta* growing on PDA. The picture is taken by using compound light microscope after isolation. Scale bar = 12.5 μm.

3.4. Effect of Trametes hirsuta inoculation on chlorophyll content

Trametes hirsuta inoculation improved the plant metal stress tolerance as plants with fungal inoculation showed higher chlorophyll content as compared to their respective non-inoculated plants (control). At 1000 mg kg^{-1} and 1500 mg kg^{-1}, *Trametes hirsuta* inoculated plants had 1.5 mg g^{-1} and 1.13 mg g^{-1} FW total chlorophyll, respectively, while it was 1.27 mg g^{-1} and 0.97 mg g^{-1} FW in their respective controls (Figure 6).

3.5. Effect of Trametes hirsuta inoculation on plant Pb uptake

Pb accumulation in roots and shoots increased with increasing external metal concentration. In terms of shoot accumulation, fungal inoculation significantly promoted the Pb uptake on each concentration as compared to their respective control. The highest shoot accumulation observed was 410.75 mg kg^{-1} DW in *Trametes hirsuta* inoculated treatment at 1500 mg kg^{-1} Pb stress while it was 273.75 mg kg^{-1} DW in non-inoculated treatment (Figure 7a). Roots accumulated more metal as compared to the plant shoots and *Trametes hirsuta* significantly promoted the metal uptake in roots at higher Pb stress, i.e., 1000 and 1500 mg kg^{-1} as compared to their respective non-inoculated treatments (Figure 7b).

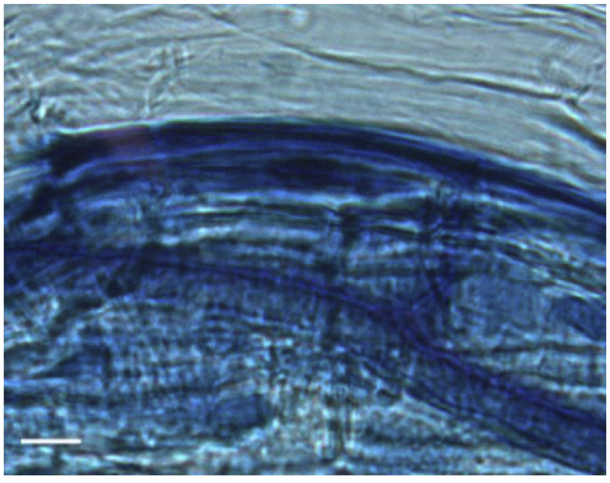

Figure 3. Fungal colonization in roots of inoculated wheat after 45 days, confirmed by trypan blue staining. The picture is taken by using compound light microscope. Scale bar = 12.5 μm.

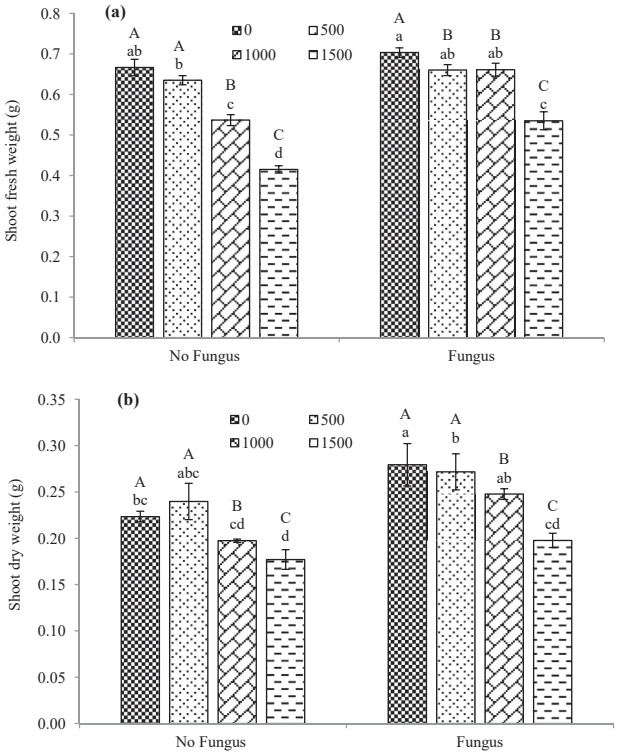

Figure 4. Shoot fresh (a) and dry weight (b) of *Triticum aestivum* after 45 days of Pb exposure (0, 500, 1000 and 1500 mg kg^{-1} of sand). Each bar represents values of 3 replicates and letters represents significant difference at $p < 0.05$ according to Duncan's test. Small letters show the difference in growth between Fungus and No Fungus group while capital letters show the difference in growth within a group. Fungus is for fungal inoculated pots, while No Fungus is for non-inoculated pots.

4. Discussion

Trametes hirsuta has also been identified as endophyte by different studies conducted on diverse plant species. Puri et al. (2006) isolated *Trametes hirsuta* as an endophyte from rhizomes of *Podophyllum hexandrum* (Himalayan mayapple). *Trametes hirsuta* along with *Trametes gibbosa* and *Trametes versicolor* have also been recovered from *Triticum aestivum* L. plants by Comby et al. (2016). However, this is the first study citing *Trametes hirsuta*, as an endophyte of *Chenopodium album* L. and this endophyte showed high Pb tolerance. The metal tolerance in endophytic fungi is attributed to cell-wall binding, the extracellular precipitation, efflux of metals ions from cell, intracellular chelation, cellular compartmentalization and antioxidant systems (Jacob et al., 2017).

Results of pot experiment showed that with increasing concentration of Pb, plant fresh and dry weights decreased. These effects on growth are generally linked to metal toxicity which may arise due to the metal binding to sulfhydryl groups in proteins which may inhibit their activity or affect their structure. Metals may also cause the displacement of essential elements, which results in deficiency effects (Jayasri and Suthindhiran, 2017).

In the present study, a gradual decrease in plant chlorophyll content in response to increased concentrations of Pb was observed. However, it was observed in the current study that *Trametes hirsuta* infected plants possessed better growth, which suggests that this endophyte helped the plants in mitigating the Pb toxicity effects and they had better growth environments. This effect of *Trametes hirsuta* inoculation on plant growth is comparable with the findings of Taghinasab et al. (2018) who observed the effect of *Trametes versicolor*, a fungal endophyte isolated from the root of *Galium album*, inoculation on *Triticum aestivum* L. and revealed that colonized plants exhibited higher grain yield (37%), straw yield (8.5%)

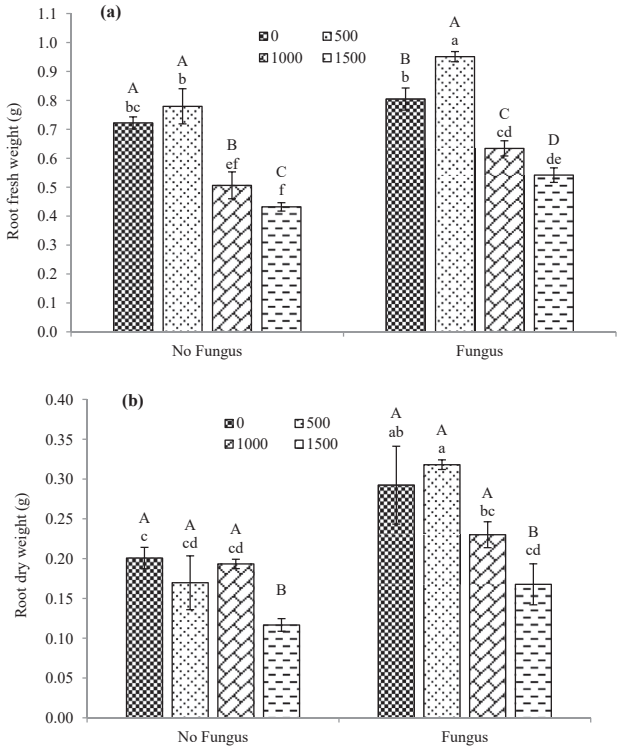

Figure 5. Root fresh (a) and dry weight (b) of *Triticum aestivum* after 45 days of Pb exposure (0, 500, 1000 and 1500 mg kg^{-1} of sand). Each bar represents values of 3 replicates and letters represents significant difference at $p < 0.05$ according to Duncan's test. Small letters show the difference in growth between Fungus and No Fungus group while capital letters show the difference in growth within a group. Fungus is for fungal inoculated pots, while No Fungus is for non-inoculated pots.

and P content as compared to non-colonized plants. A synergistic action of arbuscular mycorrhizal (AM) fungi and *Coriolopsis rigida* (a member of *Polyporaceae* family similar to *Trametes hirsuta*) on plant growth has been observed by Arriagada et al. (2009) as their dual inoculation to *Eucalyptus globulus* in sewage sludge amended soil had shown improved plant growth and nutrient acquisition evident by high K, Ca, Mg and Fe concentrations in plants.

This plant growth promoting characteristic of endophytic fungi is generally attributed to the production of plant growth regulators and protectants in stressful environments which include the production of plant growth promoting phyto-hormones like indole-3-acetic acid (Sukumar et al., 2013), gibberellins (Leitao and Enguita, 2016), auxins (Waqas et al., 2012) and cytokinins along with the fact that endophytes also help the plant in nutrient absorption (Shahabivand et al., 2012).

Fugal inoculation may increase or decrease the metal availability to the host plants and this trend varies according to the fungal species, type and concentration of metals (Shahabivand et al., 2012). However, it was seen in the present study that treatments with *Trametes hirsuta* inoculation had shown higher metal uptake as compared to non-inoculated treatments when exposed to high concentration of Pb stress. Similar results were also obtained by Shen et al. (2013) where fungal inoculation by *Peyronellaea* sp. significantly enhanced metal availability to the maize plants in Cd stress. A possible reason to that behavior might be attributed to the fact that fungal mycelium extended through the plants tissues can bind more heavy metals with it. Results of the current study also coincide with the findings of Shi et al. (2017) which showed that *Penicillium* sp. and *Fusarium* sp. inoculation significantly enhanced Pb availability in soil and more Pb concentration was observed in shoots of *Brassica napus* as compared to control treatments. *Trametes hirsuta* can also be helpful in enhancing the phytoremediation potential of Pb hyperaccumulators as *Aspergillus flavus* treated *Pelargonium hortorum* L. (a Pb hyperaccumulator) showed significant Pb accumulation at 2000 mg kg^{-1} while accumulation was significantly reduced in control plants due to high toxicity induced by increased concentrations of Pb (Manzoor et al., 2019).

Roots being the first part to be exposed to toxic metals, accumulated more metal as compared to the plant shoots as in case of pine (*Pinus sylvestris*) when inoculed with fungal endophyte *Trichoderma* sp. significantly enhanced the metal uptake in roots of plants by having 30% more Cd, 62% more Ni, 25% more Cu, 33% more Pb, 28% more As and 26% more Zn, as compared to the control seedlings (Babu et al., 2014). Thus, fungi can play an important role in metal translocation from root to the shoot tissues.

The high metal availability to plants is directly associated with the activity of microbes present in the vicinity of plant roots. Microbes enhance metal solubility and change the metal speciation via production of organic ligands and exudating metabolites i.e., siderophores, organic acids and various degrading enzymes (Rajkumar et al., 2012) which may lead to high metal uptake in plants. The mechanism of microbial metal mobilization by organic acids involves the replacement of metal cations

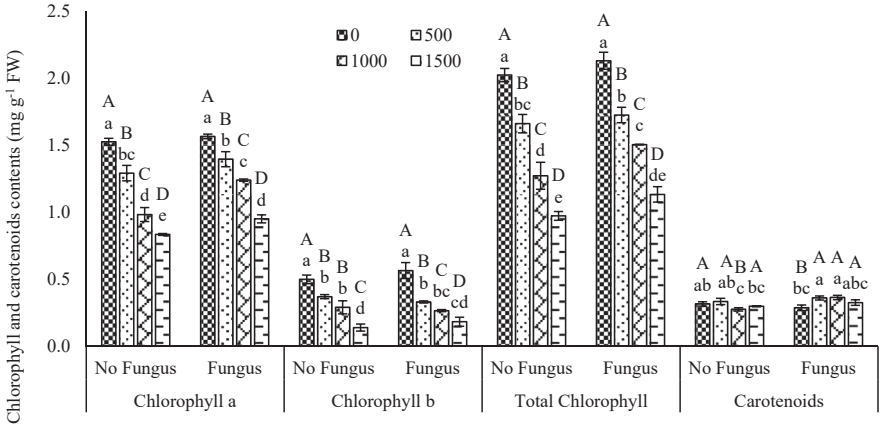

Figure 6. Chlorophyll and carotenoids contents of *Triticum aestivum* after 45 days of Pb exposure (0, 500, 1000 and 1500 mg kg^{-1} of sand). Each bar represents values of 3 replicates and letters represents significant difference at $p < 0.05$ according to Duncan's test. Small letters show the difference in growth between Fungus and No Fungus group while capital letters show the difference in growth within a group. Fungus is for fungal inoculated pots, while No Fungus is for non-inoculated pots.

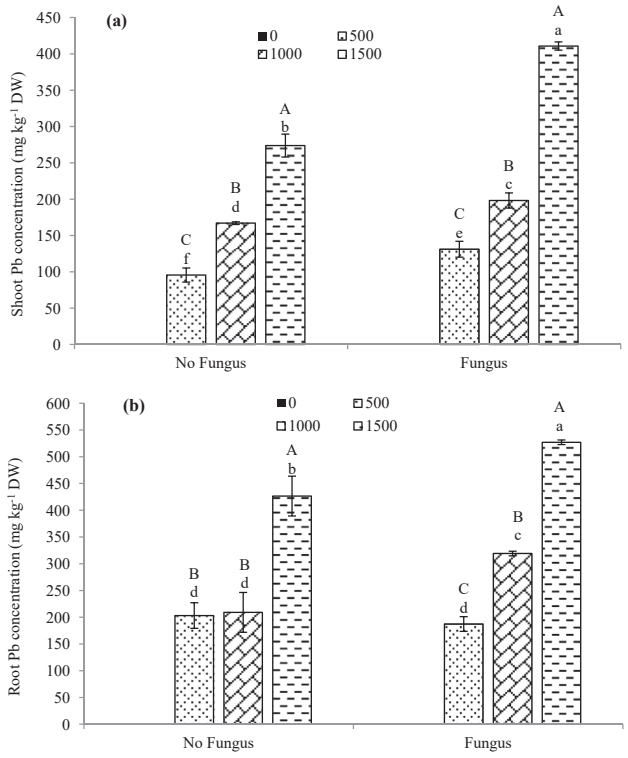

Figure 7. Lead (Pb) accumulation in shoots (a) and roots (b) of *Triticum aestivum* after 45 days of Pb exposure (0, 500, 1000 and 1500 mg kg^{-1} of sand). Each bar represents values of 3 replicates and letters represents significant difference at $p < 0.05$ according to Duncan's test. Small letters show the difference in growth between Fungus and No Fungus group while capital letters show the difference in growth within a group. Fungus is for fungal inoculated pots, while No Fungus is for non-inoculated pots.

with protons at sorption sites thus dissolve the metal containing minerals (Gadd, 2010). The role of organic acid as observed by Fomina et al. (2005) revealed that soil fungi *Beauveria caledonica* accelerated the solubilization of $Zn_3(PO_4)_2$ and pyromorphite through acidolysis (protonation) reaction. Likewise, Aslam et al. (2019) found that *Piriformospora indica* releases acid phosphatases which mobilize inaccessible organic P from its immobilized sources, thus facilitating host plants to absorb sufficient P and support plant growth. Metal tolerance behavior of *Trametes hirsuta* in the present study can also be endorsed by these mechanisms as it increased Pb uptake in *Triticum aestivum* L. plants. However, further experimentation is needed to examine the exact reasons and mechanisms involved in *Trametes hirsuta* induced growth promotion and metal tolerance.

5. Conclusion

Present study revealed that endophyte *Trametes hirsuta* is able to tolerate high Pb concentration and its endophytic infection made host plant more tolerant to Pb as compared to their respective non inoculated controls. Inoculation also facilitated the high metal uptake in roots and shoots of *Triticum aestivum* L. plants, suggesting the beneficial role of *Trametes hirsuta* in restoration of heavy metal polluted soils via promoting phytoextraction. Thus, it can be concluded that heavy metal tolerant fungal endophytes which are capable of promoting plant growth and heavy metal extraction, can have high potential for eco-friendly and cost-effective alternative for the remediation of contaminated soils along with some potential plant species.

Declarations

Author contribution statement

Mazhar Iqbal, Ismat Nawaz: Conceived and designed the experiments; Wrote the paper.

Amna Malik: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Tayyab Ashfaq Butt, Syed Tatheer Alam Naqvi, Ghazanfar Farooq: Analyzed and interpreted the data.

Sohail Yousaf, Muhammad Kamran Qureshi, Mazhar Iqbal Zafar: Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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