COMPARISON OF FUNGAL SPECIES FOR LEAD TOLERANCE AND PLANT GROWTH PROMOTION IN CONTAMINATED SOIL

Maria Manzoor[a], Iram Gul[a], Jean Kallerhoff[b], Muhammad Arshad[a]*
[a] *Institute of Environmental Sciences & Engineering, School of Civil & Environmental Engineering, National University of Sciences & Technology, H-12 sector, Islamabad, 44000, Pakistan*
[b]*Ecolab, ENSAT, Auzeville-Tolosane, Av. Agrobiopoˆle, Castanet-Tolosan cedex, 31326, France*

**Muhammad Arshad: marshad@iese.nust.edu.pk*

***Abstract*—The following study have been designed to investigate the beneficial role of lead tolerant fungal strains for plant growth promotion in lead contaminated soil. Five fungal strains including *Trichoderma harzianum*, *Penicillium simplicissimum*, *Aspergillus flavous*, *Aspergillus niger* and *Mucor* spp. were examined for tolerance against lead. Multiple heavy metal tolerance of tested fungi was also recorded. Afterwards the effect of lead tolerant fungi upon plant growth was monitored under different soil Pb concentrations (0, 1000 & 1500 mg kg^{-1}). Parameters including, germination percentage, root shoot length, dry biomass, number of leaves were monitored. The results indicated that application of heavy metal tolerant strains significantly improved the growth of plants even under high metal stress condition. Among tested strains *Aspergillus niger* significantly improved plant growth as compared to other strains and control. The root shoot length was increased by 1.175 and 1.26 -folds as compared to control. Root shoot biomass was increased by 1.45 and 1.2 -folds. No significant difference was observed in number of leaves when compared to plants treated with other fungal strains however significant difference was observed when compared to control. Results suggested that *Aspergillus niger* has shown a high level of tolerance to all metals tested and promoted plant growth under elevated concentrations of Pb in soil which makes it attractive potential candidate for further investigation regarding plant growth promoting characteristics under elevated Pb concentrations.**

KEYWORDS: lead, tolerant fungi, chlorophyll, growth promotion

1. INTRODUCTION

Soil has become contaminated with multiple heavy metals and metalloids as a result of ever increasing anthropogenic activities including heavy metals containing improper waste disposal, pesticide, petrochemicals, paints, coal and fuel combustion, batteries manufacturing and recycling [1]. The most commonly found heavy metals in soil is lead (Pb). The presence of Pb in soil seriously effect plant growth and development and including reduced vigor, low biomass production, decline in chlorophyll content and buildup of metal concentrations in crop plants posing serious threat to animals and human health [2].

Soil microorganisms particularly fungi can play important role to sustain crop growth and production in soil contaminated with Pb. There are many versatile groups of fungi that can survive in wide range of pH, temperature, nutrient availability, moisture conditions and tolerate high levels of heavy metals [3-4]. In our previous studies we have worked on *Pelargonium hortorum* for Pb phytoextraction [5-6-7]. But decrease in biomass at elevated Pb concentration is hindering the phytoremediation efficiency of plant. Keeping in mind that these fungal strains can ameliorate the negative effects of lead in soil plant system, the objectives of current study are to find out the multiple heavy metal tolerant fungal strains and their effect on plant growth under elevated Pb contaminated soil.

2. MATERIAL AND METHODS

Heavy metal tolerant fungal strains: Five fungal strains were obtained from fungal culture bank, university of Punjab, Lahore. These strains were previously isolated from industrial area soil. To check the extent of tolerance, fungal strains were grown in potato dextrose agar (PDA) media

containing different concentrations of Pb [8] and Tolerance Index (TI) was recorded using equation 1 [9]. Further the MIC was recorded for different heavy metals in order to check the multiple heavy metal resistance of the tested fungi.

TI= fungal growth in the presence of metals/ fungal growth in the control plate---------- (i)

The results were verified by growing cultures in potato dextrose broth (PDB) amended with metal concentrations and measuring the optical density (OD) at 570 nm [10].

Pot experiment: Soil was collected from botanical nursery National University of Science and Technology (NUST), Islamabad Pakistan. The soil properties are listed in Table 1.

Table 1: Soil physioco- chemical characteristics used in pot experiment

Sr.No.	Parameters	Values
1	Soil pH	7.32
2	EC (dS/m)	0.45
3	Texture	Clay loam
4	Organic matter content (carbon (%))	0.47
5	Nitrogen	0.033
6	Phosphorus	6.48
7	Potassium (mg/Kg)	721
8	Magnesium (mg/Kg)	344
9	Iron (mg/Kg)	3000.25

Lead concentration found in contaminated soil around battery recycling units severed as reference for selection of treatment levels. The soil was artificially contaminated with Pb @ 0, 1000 and 1500 mg kg^{-1}. After one month of metal stabilization, soil was pot filled and seeds of *Pelargonium hortorum* were sterilized with 5% Calcium hypochlorite prior to sowing in order to remove any bacterial or fungal contamination. After 3 weeks of germination fungal spore suspensions (1.5 OD at 580 nm) of selected heavy metal resistant strains were applied [8] @ 10mL pot^{-1}. Un-inoculated plants were taken as control. The experimental design was a randomized complete block design with five replicates for each treatment. The plants were allowed to grow for two months under typical greenhouse conditions [16h light at 28°C; 8h dark at 20°C]. Plants were daily watered with distilled water to maintain 60% moisture content. Chlorophyll was measured on regular intervals (chlorophyll meter) during experiment. After harvesting physiological growth parameters including root shoot length, biomass and number of leaves were recorded

Germination and root elongation assay: The effect of fungal strains to promote seed germination and root growth was determined using assay described by [11] with some modifications. Fungal inoculums were prepared as described above. Glass plates with filter paper were sterilized by autoclaving (120°C @ 15 Pa for 1 h) and seeds were sterilized as above to remove any contamination. Ten seeds were place in each petri plate and ten ml of spore suspensions supplemented with different levels of heavy metals were added to moisten the filter paper. Plates were then incubated for 15 days at 28°C in dark. The assay was performed with three replicates for each treatment.

3. RESULTS AND DISCUSSION

Heavy Metal Tolerance

The extent of heavy metal tolerance and TI of tested fungal strains have been presented in table 2 and 3 respectively. Results suggested that *Aspergillus niger* showed highest tolerance against all heavy metals except Zn where *Penicillium simplicissimum* could tolerate up to 1200 mgL^{-1}. These results are parallel to those reported in literature. In a study conducted by Iram et al. [12] *Aspergillus niger* and *Aspergillus* sp. exhibited more tolerance against heavy metals in order of Zn > Ni > Pb >Cd as compared to *Penicillium* sp., and *Fusarium* sp. In another study *Penicillium simplicissimum* showed exceptional adaptive behavior for Zn while tolerating up to 8000 ppm of Zn [13].

Table 2: The final concentration (ppm) up to which each fungus could tolerate.

Fungal strains	Pb	Cd	Cu	Zn	Cr
T.harzianum	1000	200	600	1000	700
P.simplicissimum	700	150	400	1200	500
A.flavous	1000	200	600	800	700
A.niger	1000	300	1500	1000	1100
Mucor spp.	700	150	700	500	600

Table 3: Tolerance index of tested fungal strains for different heavy metals

Fungal strains	Pb	Cd	Cu	Zn	Cr
T.harzianum	0.35	0.30	0.35	0.33	0.33
P.simplicissimum	0.26	0.29	0.26	0.59	0.41
A.flavous	0.29	0.32	0.37	0.34	0.34
A.niger	0.45	0.42	0.37	0.39	0.50
Mucor spp.	0.33	0.26	0.31	0.33	0.31

Results of the present investigations shows that multiple heavy metal tolerance in fungi follows the pattern *Aspergillus niger* > *Trichoderma harzianum* > *Aspergillus flavous* > *Mucor* spp. > *Penicillium simplicissimum*.

Effect on Plant Growth in Heavy Metal Contaminated Soil

Fungal strains positively affected plant growth parameters. The results for biomass and root shoot length are presented in figure 1 and 2 respectively. From results it is obvious that by increasing metal concentration the plant biomass and root shoot length tends to decrease. Similar results of decrease in biomass and growth of *Pelargonium hortorum* at elevated concentration of Pb (1500 mg kg^{-1}) have been reported by Gul et al. [14].

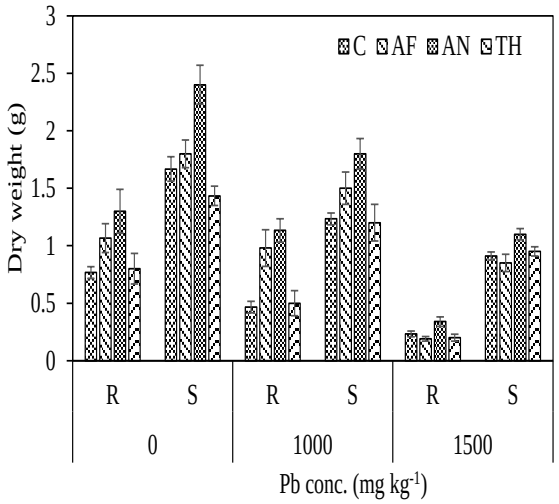

Figure 1: Effect of fungal treatments on root shoot (R& S) biomass of plants in Pb contaminated soil

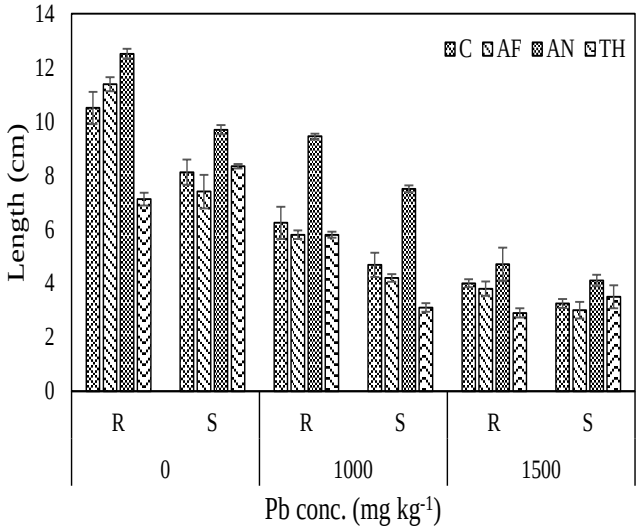

Figure 2: Effect of fungal treatments on root shoot (R& S) length of plants in Pb contaminated soil

Among fungal treatments *Aspergillus niger* significantly improved plant dry biomass and root shoot length at increasing soil metal concentrations when compared to plant treated with other fungi and control (no fungi added). This may be attributed to high Pb tolerance of this strains and sustaining plant growth by production of important plant growth promoting enzymes and hormones like Oxalic acid, Citric acid indole and acetic acid (IAA) [15].

Chlorophyll content was measured with chlorophyll meter and results are presented in figure 3. At increasing metal contamination i.e. from control to 1500 mg kg^{-1}, significant ($p<0.05$) decline in chlorophyll content was observed when compared to control (no Pb and no fungi added). The addition of fungi particularly *Aspergillus niger* slightly improved the chlorophyll content however the change was not significant.

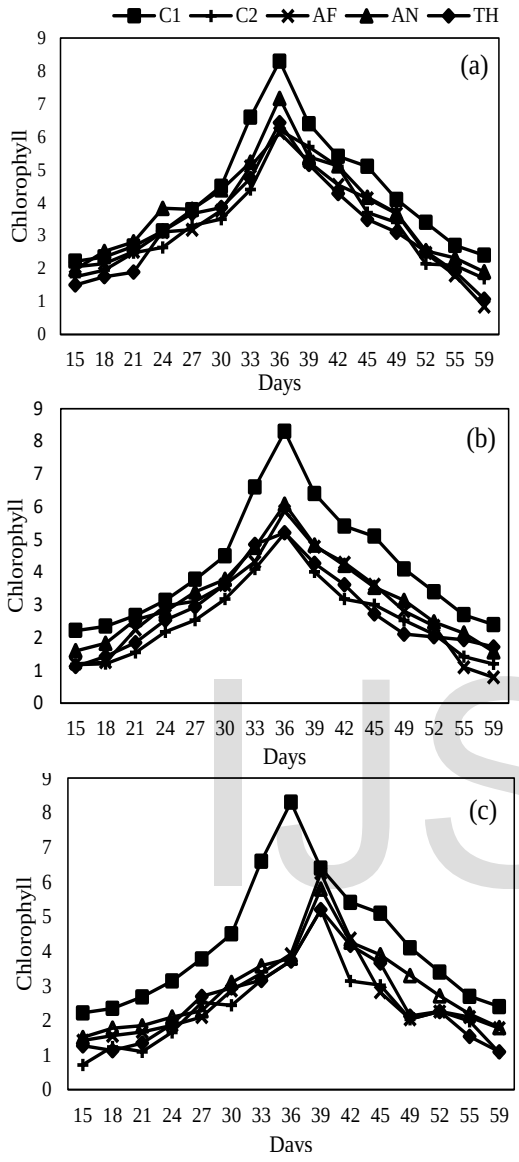

Figure 2: Effect of fungal treatments on chlorophyll contentment at different intervals of time in control (a); 1000 mg kg^{-1} (b); and 1500 mg kg^{-1} (c) Pb contaminated soil.

The results of germination percentage and no of leaves are presented in table 2. The results confirmed the positive plant growth promoting characteristics of *Aspergillus niger*. At heavy metal concentrations of control and 1000 mg kg^{-1} *Aspergillus niger* significantly improved the germination percentage by 8.3 and 18.33 % however at highest soil Pb concentrations (1500 mg kg^{-1}) the effect was not significant. Contrary *Trichoderma harzianum* and *Aspergillus flavous* did not improve the germination percentage instead a slight decline was observed.

Table 4 Effect of Fungi on plant physiological growth parameters under heavy metal stress

Treatments		% Germination	No. of leaves
Control		^{a}93.25±3.5	^{a}4.33±0.33
H1	NF	^{c}74.33±2.5	^{b}3.25±0.21
	Aspergillus flavous	^{c}68.25±2.5	^{a}4.50±0.27
	Aspergillus niger	^{b}80.5±1.25	^{a}4.25±0.35
	Trichoderma harzianum	^{c}67.25±3.5	^{b}3.50±0.19
H2	NF	^{d}60.5±4.33	^{b}3.75±0.46
	Aspergillus flavous	^{cd}63.75±5.5	^{b}3.33±0.33
	Aspergillus niger	^{c}71.5±2.17	^{b}3.25±0.26
	Trichoderma harzianum	^{de}55.5±4.28	^{a}4.50±0.31
H3	NF	^{e}51.25±2.5	^{c}2.25±0.29
	Aspergillus flavous	^{e}48.33±3.5	^{c}2.75±0.11
	Aspergillus niger	^{e}52.5±3.75	^{b}3.50±0.33
	Trichoderma harzianum	^{de}56.0±2.33	^{c}2.75±0.15

For number of leaves up to 1000 mg kg^{-1} level of soil heavy metal concentration plants treated with *Aspergillus niger and Trichoderma harzianum* produced good number of leaves. However at highest soil Pb contamination level (1500 mg kg^{-1}) *Aspergillus niger* was only able to sustain leave production.

4. CONCLUSION

From obtained results under laboratory conditions it is concluded that *Aspergillus niger* was the most tolerant against all the tested heavy metals considering tolerance index values followed by *Trichoderma harzianum* and *Aspergillus flavous*. Pot experiment conducted revealed that at elevated concentrations of Pb in soil, growth and physiological processes were affected in *Pelargonium hortorum*. Application of heavy metal tolerant fungal strains particularly *Aspergillus niger* significantly enhanced plant growth by improving the processes of germination, root shoot elongation, plant biomass and chlorophyll content.

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