

Effect of Lead and Cadmium on the Fungal Population in Rhizosphere and Non-Rhizosphere Soils of *Eucalyptus* species

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Received 12 January 2017; Accepted 14 February 2017; Published online 4 March 2017

Abstract High concentrations of heavy metals in soil have an adverse effect on micro-organisms and microbial processes. Among different types of microorganisms of soil fungi are the only ones providing a direct link between soil and roots and can therefore be of great importance in heavy metal availability and toxicity to plants. In paper discusses the interactions between heavy metals and fungi. Effects of heavy metals on the occurrence of fungi, heavy metal tolerance in these microorganisms and their effect on different *Eucalyptus* species. Toxic effect of heavy metals on the population of fungi from rhizospheric and non-rhizospheric soils of four *Eucalyptus* species are discussed. The possible use of mycorrhizal fungi as bioremediation agents in polluted soils or as bioindicators of pollution is also discussed.

Keywords Fungal population, Heavy metals, Rhizosphere, Non-rhizosphere.

Introduction

Pollutants permitted to the biosphere including heavy metals due to industrial and agricultural and domestic activities has created a serious problem for the safe utilization of sod soils [1]. Toxic effect of heavy metals on fungal population is already known by world [2]. The influence of heavy metals on fungal activities was studied at 30 DAS and 180 DAS in microbiology laboratory. Pot culture experiment was conducted in two types of forest soil clay loam and sandy loam. Three different concentrations of metals lead and cadmium were imposed in both soils. The fungal activity decreased with increased metal concentrations. Fungal activity initially increased with the level of metal contamination, being up to 3 and 7 times higher than that in the control samples during the first week at the highest levels of Zn and Cu addition, respectively. This is the first direct evidence that activities in soil are differently affected by heavy metals. The different responses of fungi to metals were reflected in an increase in the relative fungal.

Materials and Methods

The experiment was conducted at the nursery of Forest College and Research Institute, Mettupalayam. Air dried red sandy loam and black clayey loam soils were spiked with respective metal levels up to saturated condition and about 6 kg of spiked soil is into plastic pots of (17 cm in diameter and 15 cm in length) dimensions. The good quality seeds of *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* were directly sown (10 seeds pot⁻¹) on

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plastic pots. The seedlings were thinned after 30 days by keeping uniform sized seedling (3 seedlings pot⁻¹). In the pot culture experiment no recommended fertilizers were applied for the plants. Three replications were fixed for each treatment in a factorial completely randomized design (FCRD). Soil samples rhizosphere and non-rhizosphere soil were recorded in two intervals 30 and 180 DAS.

The microbial load were analyzed in rhizosphere and non-rhizosphere soils. The enumeration of microbes was carried out on solid media using the serial dilution and plating technique, one gram of soil samples was taken and serial dilutions were carried out in sterile water. seven dilutions viz 10⁻², 10⁻³ 10⁻⁴, 10⁻⁵ 10⁻⁶ and 10⁻⁷ were prepared and used for the isolation of microorganisms 10⁻⁶ for fungi [3].

Rose bengal agar (RBA) medium were used to isolate fungi. Three replications were maintained for each isolation. The petri plates were incubated at room temperature and colonies of different microorganisms enumerated after appropriate time interval 2-3 days for fungi.

Factor	Particulars	Details
Factor 1	Soil types	2 S ₁ -Sandy loam S ₂ -Clay loam E ₁ - <i>Eucalyptus tereticornis</i> E ₂ - <i>E. camaldulensis</i> E ₃ - <i>E. globulus</i> E ₄ - <i>E. citriodora</i>
Factor 2	Tree species	4 T ₀ -Control M ₁ T ₁ -Cadmium 25 ppm M ₁ T ₂ -Cadmium 50 ppm M ₁ T ₃ -Cadmium 100 ppm
Factor 3	Treatments	7 M ₂ T ₁ -Lead 100 ppm M ₂ T ₂ -Lead 125 ppm M ₂ T ₃ -Lead 250 ppm
	Replications design	3 FCRD

Results and Discussion

The data on the heavy metals viz., cadmium and lead in the fungal population of rhizosphere and non-rhizosphere sandy and clay loam soil of four *Eucalyptus* species at 30 DAS was given in Table 1. The results showed that the fungal population in the four

Eucalyptus species was significantly affected due to the application of heavy metals in different concentrations in the rhizosphere and non-rhizosphere soils when compared to control. The fungal population significantly reduced due to the toxicity of different metal concentrations. The rhizosphere is considered a major niche for microbial activity, where a substantial number of microorganisms may exert a beneficial effect on plant fitness and development, particularly phosphate solubilisation, which is considered to be one of the most important features associated with plant nutrition.

The fungal population in the rhizosphere soil of *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* decreased under cadmium treated sandy loam soil and it was 8.22 CFU g⁻¹, 6.22 CFU g⁻¹, 7.56 CFU g⁻¹ and 9.89 CFU g⁻¹ respectively. In lead treated sandy loam soil, it was 10.55 CFU g⁻¹, 6.67 CFU g⁻¹, 8.00 CFU g⁻¹ and 10.67 CFU g⁻¹ respectively. In cadmium treated clay loam soil, the fungal population in the non-rhizosphere soil *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* was 7.00 CFU g⁻¹, 5.33 CFU g⁻¹, 6.33 CFU g⁻¹ and 9.00 CFU g⁻¹ respectively and it was 9.00 CFU g⁻¹, 5.89 CFU g⁻¹, 7.22 CFU g⁻¹ and 9.44 CFU g⁻¹ respectively in lead treated clay loam soil.

The fungal population in the non-rhizosphere soil of *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* decreased under cadmium treated sandy loam soil and it was 4.22 CFU g⁻¹, 3.44 CFU g⁻¹, 3.89 CFU g⁻¹ and 7.00 CFU g⁻¹ respectively. In lead treated sandy loam soil, it was 5.89 CFU g⁻¹, 4.33 CFU g⁻¹, 5.11 CFU g⁻¹ and 7.67 g⁻¹ respectively. In cadmium treated clay loam soil, the fungal population in non rhizosphere soil of *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* was 4.00 CFU g⁻¹, 2.55 CFU g⁻¹, 3.33 CFU g⁻¹ and 6.33 CFU g⁻¹. respectively and it was 5.11 CFU g⁻¹, 3.56 CFU g⁻¹, 4.89 CFU g⁻¹ and 6.89 CFU g⁻¹ respectively in lead treated clay loam soil. The fungi population was significantly varied with soil types, toxic metals level and species. The fungi were showed sensitivity towards toxic metals. The toxic effect of metals reduced the activity of the fungi. It showed decreasing trend with increasing metal concentration [4]. The sandy loam soil was showed higher fungal

Table 1. Total counts of fungi as influenced by cadmium and lead at 30 DAS (CFU g⁻¹).

Soil type	Treatments	Fungi 30 DAS (CFU g ⁻¹)					Non-rhizosphere				
		Rhizosphere					Non-rhizosphere				
		E ₁	E ₂	E ₃	E ₄	Mean	E ₁	E ₂	E ₃	E ₄	Mean
S ₁	To	12.33	11.67	11.33	11.00	11.58	7.67	8.00	8.33	8.00	8.00
	M ₁ T ₁	10.33	7.67	9.33	11.00	9.58	5.67	5.00	5.33	8.00	6.00
	M ₁ T ₂	8.33	6.00	7.67	10.00	8.00	3.67	3.00	3.33	7.00	4.25
	M ₁ T ₃	6.00	5.00	5.67	8.67	6.34	3.33	2.33	3.00	6.00	3.67
	Mean	8.22	6.22	7.56	9.89	7.97	4.22	3.44	3.89	7.00	4.64
	M ₂ T ₁	11.33	8.00	9.33	11.67	10.08	6.67	5.67	6.00	8.33	6.67
	M ₂ T ₂	11.00	6.67	8.00	10.67	9.09	6.00	4.00	5.00	7.67	5.67
	M ₂ T ₃	9.33	5.33	6.67	9.67	7.75	5.00	3.33	4.33	7.00	4.92
	Mean	10.55	6.67	8.00	10.67	8.97	5.89	4.33	5.11	7.67	5.75
	To	10.33	10.33	10.00	9.67	10.08	7.00	7.00	7.33	7.33	7.17
S ₂	M ₁ T ₁	9.00	6.33	8.00	10.00	8.33	5.00	4.00	4.67	7.33	5.25
	M ₁ T ₂	7.00	5.33	6.00	9.00	6.83	4.00	2.33	3.00	6.33	3.92
	M ₁ T ₃	5.00	4.33	5.00	8.00	5.58	3.00	1.33	2.33	5.33	3.00
	Mean	7.00	5.33	6.33	9.00	6.92	4.00	2.55	3.33	6.33	4.05
	M ₂ T ₁	9.67	7.00	8.67	10.33	8.92	6.00	5.00	5.67	7.67	6.09
	M ₂ T ₂	9.00	6.00	7.00	9.33	7.83	5.00	3.00	5.00	7.00	5.00
	M ₂ T ₃	8.33	4.67	6.00	8.67	6.92	4.33	2.67	4.00	6.00	4.25
	Mean	9.00	5.89	7.22	9.44	7.89	5.11	3.56	4.89	6.89	5.11

Table 1. Continued.

	Rhizosphere	
	SEd	CD (0.05)
S	1.27	2.51
E	1.79	3.55
M	1.27	2.51
T	1.54	3.08
S × E	2.53	5.02
S × M	1.79	3.55
S × T	2.19	4.35
E × M	2.53	5.02
E × T	3.09	6.15
M × T	2.19	4.35
S × E × M	3.58	7.10
S × E × T	4.38	8.70
S × M × T	3.09	6.15
E × M × T	4.38	8.70
S × E × M × T	6.19	12.30

Table 1. Continued.

	Rhizosphere				
	E ₁	E ₂	E ₃	E ₄	Mean
S ₁	9.39	6.45	7.78	10.28	8.47
S ₂	8.00	5.61	6.78	9.22	7.41
Mean	8.70	6.03	7.28	9.75	7.94
M ₁	7.61	5.78	6.95	9.45	7.45
M ₂	9.78	6.28	7.61	10.06	8.43

Table 1. Continued.

	Non-rhizosphere	
	SEd	CD (0.05)
S	0.07	0.14
E	0.09	0.19
M	0.07	0.14
T	0.08	0.17
S × E	0.14	0.27
S × M	0.09	0.19
S × T	0.11	0.24
E × M	0.14	0.27
E × T	0.17	0.33
M × T	0.12	0.24
S × E × M	0.19	0.39
S × E × T	0.24	0.47
S × M × T	0.17	0.33
E × M × T	0.24	0.47
S × E × M × T	0.34	0.67

Table 1. Continued.

	Non-rhizosphere				
	E ₁	E ₂	E ₃	E ₄	Mean
S ₁	5.06	3.89	4.50	7.34	5.20
S ₂	4.56	3.06	4.11	6.61	4.58
Mean	5.06	3.89	4.50	7.34	5.20
M ₁	4.11	3.00	3.61	6.67	4.35
M ₂	5.50	3.95	5.00	7.28	5.43

growth (8.47 CFU g⁻¹) as compared to clay loam soil (7.41 CFU g⁻¹) in rhizosphere soil. In case of non-rhizosphere soil similar results was found. The fungal growth was counted more in rhizosphere soil as compared to non-rhizosphere soil at the early stage (30 DAS). Some ions of heavy elements necessary for the growth of fungi, when added low concentrations fall within the limits of carrying fungus, but increased concentrations of ions limits of carrying fungus adversely affect the growth and reproduction, have been observed this phenomenon when exposing fungi under study to different concentration of heavy metals [5]. Among all species the fungi colonies were higher in *E. citriodora* seedling (9.75 CFU g⁻¹) over the other species in rhizosphere soil at 30 DAS.

The data on the heavy metals viz., cadmium and lead in the fungal population of the rhizosphere and non-rhizosphere sandy and clay loam soil of four *Eucalyptus* species at 180 DAS was given in Table 2. The results showed that the fungal population in the four *Eucalyptus* species was significantly affected due to the application of heavy metals in dif-

ferent concentrations in the rhizosphere and non-rhizosphere soils when compared to control. The fungal population significantly reduced due to the toxicity of different metal concentrations.

The fungal population in the rhizosphere soil of *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* decreased under cadmium treated sandy loam soil and it was 15.22 CFU g⁻¹, 9.22CFU g⁻¹, 14.33 CFU g⁻¹ and 17.56 CFU g⁻¹ respectively. in lead treated sandy loam soil, it was 17.78 CFU g⁻¹, 12.00 CFU g⁻¹, 16.44 CFU g⁻¹ and 18.44 CFU g⁻¹ respectively. In cadmium treated clay loam soil, the fungal population in the rhizosphere soil of *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* was 13.33 CFU g⁻¹, 8.67 CFU g⁻¹, 11.78 CFU g⁻¹ and 14.67 CFU g⁻¹ respectively and it was 14.22 CFU g⁻¹, 9.56 CFU g⁻¹, 123.44 CFU g⁻¹ and 15.56 g⁻¹ respectively in leadtreated clay loam soil.

The fungal population in the non-rhizosphere soil of *E. tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* decreased under cadmium treated sandy loam soil it was 10.44 CFU g⁻¹, 6.56 CFU g⁻¹,

Table 2. Total count of fungi as influenced by cadmium and lead at 180 DAS (CFU g⁻¹).

		Fungi 180 DAS (CFU g ⁻¹)									
		Rhizosphere					Non-rhizosphere				
Soil type	Treatments	E ₁	E ₂	E ₃	E ₄	Mean	E ₁	E ₂	E ₃	E ₄	Mean
S ₁	To	19.33	18.67	21.00	19.33	19.58	13.67	12.67	13.67	12.67	13.17
	M ₁ T ₁	18.67	12.33	17.00	19.67	16.92	12.33	7.67	11.00	12.67	10.92
	M ₁ T ₂	15.33	8.33	14.67	17.67	14.00	10.33	7.00	9.00	10.67	9.25
	M ₁ T ₃	11.67	7.00	11.33	15.33	11.33	8.67	5.00	7.67	9.00	7.59
	Mean	15.22	9.22	14.33	17.56	14.08	10.44	6.56	9.22	10.78	9.25
	M ₂ T ₁	19.00	13.67	17.67	20.00	17.59	13.00	11.33	11.67	13.00	12.25
	M ₂ T ₂	17.67	11.67	16.33	18.33	16.00	11.33	9.67	10.00	13.00	11.00
	M ₂ T ₃	16.67	10.67	15.33	17.00	14.92	10.67	8.33	9.00	11.00	9.75
	Mean	17.78	12.00	16.44	18.44	16.17	11.67	9.78	10.22	12.33	11.00
	To	16.00	16.33	16.00	16.00	16.08	11.00	10.33	10.67	11.33	10.83
S ₂	M ₁ T ₁	15.33	11.00	14.33	16.33	14.25	10.67	7.00	9.33	11.33	9.58
	M ₁ T ₂	14.00	9.00	12.00	15.00	12.50	9.33	6.00	8.33	10.00	8.42
	M ₁ T ₃	10.67	6.00	9.00	12.67	9.59	7.33	4.00	6.67	8.00	6.50
	Mean	13.33	8.67	11.78	14.67	12.11	9.11	5.67	8.11	9.78	8.17
	M ₂ T ₁	15.33	11.67	15.00	17.00	14.75	11.00	8.00	10.00	11.67	10.17
	M ₂ T ₂	14.33	10.00	13.33	15.67	13.33	10.00	6.67	9.00	11.00	9.17
	M ₂ T ₃	13.00	7.00	12.00	14.00	11.50	9.00	5.33	8.00	10.00	8.08
	Mean	14.22	9.56	13.44	15.56	13.19	10.00	6.67	9.00	10.89	9.14

Table 2. Continued.

Rhizosphere		
	SEd	CD (0.05)
S	0.09	0.19
E	0.14	0.28
M	0.09	0.19
T	0.12	0.24
S × E	0.19	0.39
S × M	0.14	0.27
S × T	0.17	0.34
E × M	0.19	0.39
E × T	0.24	0.48
M × T	0.17	0.34
S × E × M	0.28	0.55
S × E × T	0.34	0.68
S × M × T	0.24	0.48
E × M × T	0.34	0.68
S × E × M × T	0.48	0.96

Table 2. Continued.

Rhizosphere					
	E ₁	E ₂	E ₃	E ₄	Mean
S ₁	16.50	10.61	15.39	18.00	15.13
S ₂	13.78	9.12	12.61	15.12	12.65
Mean	15.14	9.87	14.00	16.56	13.89
M ₁	14.28	8.95	13.06	16.12	13.10
M ₂	16.00	10.78	14.94	17.00	14.68

9.22 CFU g⁻¹, and 10.78 CFU g⁻¹ respectively. In lead treated sandy loam soil, it was 11.67 CFU g⁻¹, 9.78 CFU g⁻¹, 10.22 CFU g⁻¹ and 12.33 CFU g⁻¹ respectively. In cadmium treated clay loam soil, the fungal population in the non-rhizosphere soil of *E.tereticornis*, *E. camaldulensis*, *E. globulus* and *E. citriodora* was 9.11 CFU g⁻¹, 5.67 CFU g⁻¹, 8.11 CFU g⁻¹ and 9.78 CFU g⁻¹ respectively and it was 10.00 CFU g⁻¹, 6.67 CFU g⁻¹, 9.00 CFU g⁻¹ and 10.89 CFU g⁻¹ respectively in lead treated clay loam soil.

Similarly at 180 DAS, the fungal growth was found to be higher in sandy loam soil (15.13 CFU g⁻¹) compared to clay loam (12.65 CFU g⁻¹) rhizospheric soil. In non-rhizosphere soil, the fungal colonies were less as compare to rhizosphere (10.13 CFU g⁻¹) in sandy loam soil and (8.66 CFU g⁻¹) in clay loam soil. The fungi must be able to sequester essential trace metal ions from various

Table 2. Continued.

Non-rhizosphere		
	SEd	CD (0.05)
S	0.09	0.18
E	0.13	0.26
M	0.09	0.18
T	0.11	0.23
S × E	0.18	0.37
S × M	0.13	0.26
S × T	0.16	0.32
E × M	0.19	0.37
E × T	0.23	0.45
M × T	0.16	0.32
S × E × M	0.26	0.52
S × E × T	0.32	0.64
S × M × T	0.23	0.45
E × M × T	0.32	0.64
S × E × M × T	0.46	0.9

Table 2. Continued.

Non-rhizosphere					
	E ₁	E ₂	E ₃	E ₄	Mean
S ₁	11.06	8.17	9.72	11.56	10.13
S ₂	9.56	6.17	8.56	10.34	8.66
Mean	10.31	7.17	9.14	10.95	9.40
M ₁	9.78	6.12	8.67	10.28	8.71
M ₂	10.84	8.23	9.61	11.61	10.07

sources, where the metals can be present in concentrations ranging from trace to toxic levels. The concentration of heavy metal ions in their main source, wood, is usually low. In beech (*Fagus sylvatica*), Cd and Pb concentrations are usually below 1 ppm, Zn concentration can reach tens of parts per million[6]. At 180 DAS, the fungal population was increased in *E. citriodora* (16.56 CFU g⁻¹) over the other *Eucalyptus* spp.

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