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HPTLC ANALYSIS OF STRESS INDUCED METABOLITES IN *A. TERREUS* FROM POLLUTED SITES

Vijayalakshmi. S., Riyaz Ahmad Rather & Kathiravan. G.
Department of Biotechnology, VELS University, India.

ABSTRACT

In recent years, pollution in large areas of land by heavy metals, plastics and chemicals has become a major concern. Accumulations of solid waste impacts a serious threat posing ecological problems and health hazard risks that even alters the soil edaphic characters. The present study is focused on the occurrence and distribution of mycoflora in the sites polluted by heavy metals, plastics and chemicals, their growth strategies, chemical profiling and the associated parameters. Physico chemical analysis of the soil samples markened their notorious "polluted" environment. *Aspergillus* was found to be the dominant genus occurring amongst the diversified mycoflora that have been identified includes*: Mortierella sp*., *Acremonium sp., Penicilllium sp.,* Chemical profiling of methanolic extract of *A. terreus* isolate from distinguishing polluted sites and HPTLC fingerprinting elucidated variations, that in the HPTLC chromatograms of *A.terreus* from plastic dumpsite, the production of a metabolite with Rf value of 0.51 (2.75%) markens a variation being absent in other two extracts tested. These results markens various stress adaptive mechanisms whose semiquantitaive production of varied analytes was observed in HPTLC profiling.

KEYWORDS: Plastic dumpsite, HPTLC, *A. terreus, Mortierella sp.*

INTRODUCTION

In recent years, pollution in large areas of land by heavy metals, plastics and chemicals has become a major concern. The discharge of these effluents would also change the physico – chemical characteristics of the soil in turn results in decreased soil microbial activity and soil fertility, and yield losses. The presence of distinguishing pollutants not only affects the soil edaphic characters but also creates a selection pressure on occurrence and distribution of fungi that could have serious direct and indirect effects on the ecosystem (Ruhling *et al.*, 1984).

Also, the quality of chemical compounds (secondary metabolites) produced may also vary dramatically among the isolates from these distinguishingly polluted soils (Frisvad *et al.,* 2007). The chemical diversity can be a chemotaxonomic characteristic of a particular species that the persistence of pollutants induces the production of certain stress metabolites which might be of evolutionary significance. This necessitates us to study presently on the occurrence and distribution of mycoflora in the polluted site, their growth strategies, chemical profiling and associated parameters that would aid in prospects of relying upon their heavy metal tolerance and in identification of stress metabolites in future.

MATERIALS AND METHODS

Sampling was done from the well-defined depth of the selected sites, that three polluted soil samples were collected: the tannery affected site in Ranipet near Vellore, chemical industrial effluent polluted site in Mathuranthakam and plastic dumpyard site in Pallikaranai, Chennai. The samples were stored in thin- walled polythene bags in a refrigerator and analyzed with in 48 hours of collection for various physico – chemical parameters including heavy metals and chemicals. Similarly, garden soil samples were obtained and also subjected for analysis that pertained as control.

Soil samples were then subjected to serial dilution (soil dilution) and warcup methods for the isolation of fungi (Warcup, 1950). Czapek Dox Agar amended with chloramphenicol (12mg/100ml) was employed (Raju *et al*., 2007). The inoculated plates were kept for incubation at room temperature 25^{o}C, for 5-7 days and observed for growth of fungal colonies and identified (Barnett and Hunter, 1972). The density and percentage of frequency of the fungal isolates was also calculated (Aneja, 2005).

Among the fungi, *Aspergillus terreus* that was screened among the polluted soil samples was selected for broth culture in Czapek dox broth (incubated for 2 weeks at 26^{0}C at 150 rpm). The culture filtrate was then extracted with two equal volumes of methanol and the crude extract was then subjected for chemical analysis (Frisvad *et al.,* 2007). The fungal matt obtained was then dried in oven at 56^{0}C and powdered, extracted using methanol and analysed using HPTLC. Thin layer chromatography (TLC) for the fungal samples was carried out on 0.25 mm (10 cm × 20 cm) aluminum precoated silica gel plates (Merck, Germany). Mobile phase was ethylacetate: hexane: acetic acid (4:6:2drops) and chromatograms were monitored at 254 nm (Srinivas Reddy et al., 2008).

RESULTS AND DISCUSSION

Soil samples being analysed for its physico – chemical parameters yielded the following data (Table. 1). The presence of chromium in the tannery polluted soil with higher electrical Conductivity can alters the chelating properties of receiving water systems, which create conditions for free metal availability to flora and fauna.

Further, the higher level of organic matter can also act upon the bio - availability of heavy metals. Higher level of Zinc and Potassium can result in their hyperaccumalation in the biota. And, variations could well be revealed from the data between the different soil samples comparative to the garden soil, markens the notorious "polluted" environment of the soil samples.

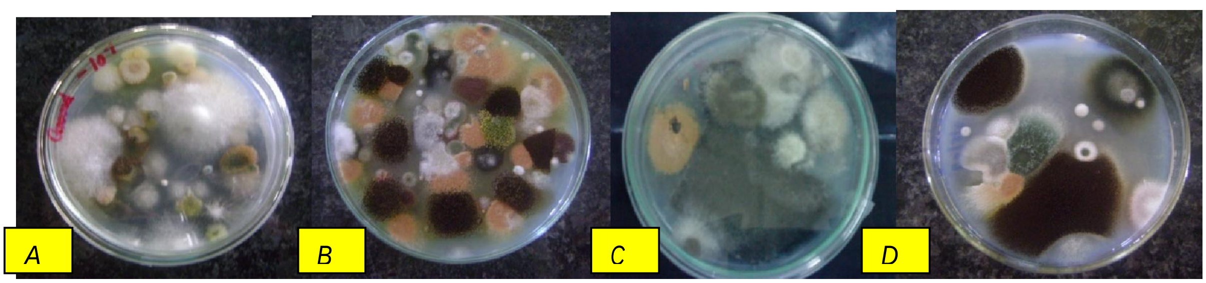
TABLE 1. Physico chemical parameters of soil samples

S. No	Parameters	LEVEL IN POLLUTED SOILS			Level in garden soil
		Tannery soil	Chemical soil	Plastic dump site	
1.	pH	6.6	8.38	7.72	6.7
2.	Electrical conductance	354 µmhos/cm	940µmhos/cm	263µmhos/cm	230µmhos/cm
3.	Nitrogen	0.01% (Available N_2)	2.11% (TKN)	2.24% (TKN)	0.04% (Available N_2)
4.	Total Phosphorous	200mg/kg	131 mg/kg	527 mg/kg	138 mg/kg
5.	Total Potassium	293 mg/kg	337 mg/kg	2482 mg/kg	135 mg/kg
6.	Organic Carbon	3.2%	2.66%	1.75 %	0.40%
7.	Iron	0.42%	0.55%	0.95%	0.32%
8.	Zinc	233 mg/kg	61.8 mg/kg	2067 mg/kg	142 mg/kg
9.	Lead	144 mg/kg	Nil	216 mg/kg	Nil
10.	Nickel	Nil	Nil	32 mg/kg	Nil
11.	Chromium	<0.02mg	Nil	Nil	Nil
12.	Cobalt	Nil	Nil	Nil	Nil
13.	Arsenic, Cyanide & Phenolics	-	BDL	Nil	Nil

TKN: Total Kjeldhal Nitrogen; BDL: Below Detection Limit.

Concerning fungal isolation *Aspergillus* was found to be the dominant genus occurring amongst the diversified mycoflora that have been identified includes*: Mortierella* sp., *Acremonium sp.*, *Penicilllium sp.*, (Fig. 1).

FIGURE 1. Soil dilution plates of fungal isolates: A. Garden soil, B. Tannery soil, C. Plastic site, D. Chemical soil



The distribution of fungal isolates is well elucidated (Table. 2) revealing the prevalent occurrence and wide distribution of *Aspergillus niger* > *Aspergillus terreus*> *Aspergillus sp.* & other fungal isolates in the polluted soil.

TABLE 2. Occurrence of fungi in soil samples

S.No	Name of fungus	Tannery soil		Chemical soil		Plastic dump site		Garden soil	
		%	Density	%	Density	%	Density	%	Density
1.	*A. fumigatus*	83	0.8	-	-	100	3	100	3.67
2.	*A. niger*	100	4.6	100	2	80	4	67	2
3.	*A. terreus*	83	4.5	83	1.5	83	3.5	65	1.2
4.	*A. flavus*	50	0.8	50	1	-	-	-	-
5.	*Mortierella sp.,*	67	1.6	-	-	-	-	-	-
6.	*Penicillium sp.,*	-	-	-	-	65	2.1	100	1.67
7.	*Acremonium sp.,*	-	-	-	-	-	-	50	1.3
8.	*Trichoderma sp.,*	50	1	-	-	50	1	65	2
9.	*Curvularia sp.,*	-	-	-	-	-	-	50	1
10.	*Mucor sp.,*	-	-	-	-	50	0.8	-	-

Chemical profiling of *A. terreus* isolates from three distinguishing polluted environments (Fig. 2) suggests the qualitative presence of different metabolites (Tab. 3).

FIGURE 2. *Aspergillus terreus* isolates.

TABLE 3. Chemical profiling of A. terreus from different soil samples

S.No.	Chemical group & test	Tannery soil	Chemical soil	Plastic dump site	Garden soil
1.	Alkaloids (Dragendorff's test)	+	+	+	+
2.	Carbohydrates (Molisch's test)	+	+	+	+
3.	Steroids (Lieberman Burchand test)	-	+	+	-
4.	Terpenoids (Lieberman Burchand test)	+	-	+	-
5.	Tannins (Ferric chloride test)	+	+	+	+
6.	Proteins (Ninhydrin test)	+	+	+	+
7.	Phenolic compounds (Ferric chloride test)	+	+	+	+

TABLE 4. HPTLC profiling of A. terreus from polluted soil samples

S.No.	Tannery soil Isolate Rf value	Area [%]	Chemical soil Isolate Rf value	Area [%]	Plastic dump Site isolate – Rf value	Area [%]
1.	0.04	13.29	0.03	2.68	0.04	9.11
2.	0.08	1.52	0.04	7.30	0.06	2.90
3.	0.22	2.81	0.09	2.55	0.21	9.27
4.	0.26	1.96	0.22	5.90	0.25	2.17
5.	0.36	2.53	0.39	3.58	0.35	4.27
6.	0.65	48.75	0.63	72.25	0.51	2.59
7.	0.80	17.62	0.92	5.74	0.65	50.11
8.	0.92	8.20	-	-	0.77	11.21
9.	0.95	3.31	-	-	0.92	6.88
10.	-	-	-	-	0.95	1.47

FIGURE. 3 HPTLC profiling of *A. terreus* isolate from chemical soil

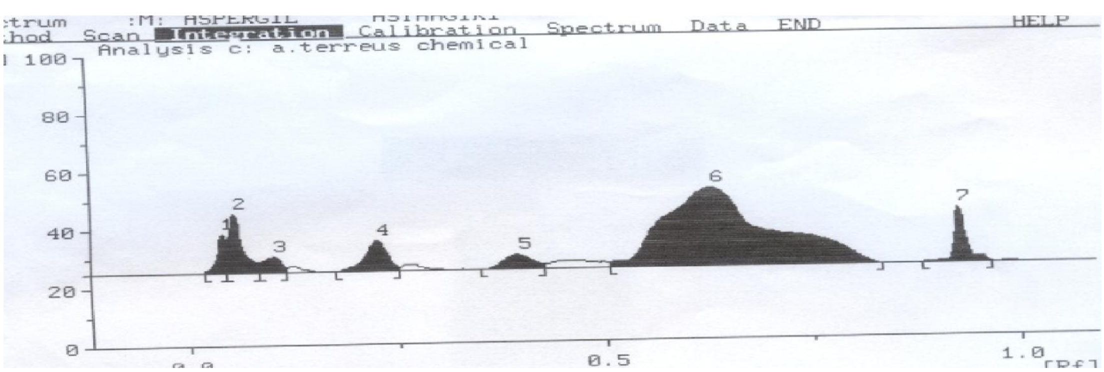


FIGURE. 4 HPTLC profiling of *A. terreus* isolate from plastic dumpsite

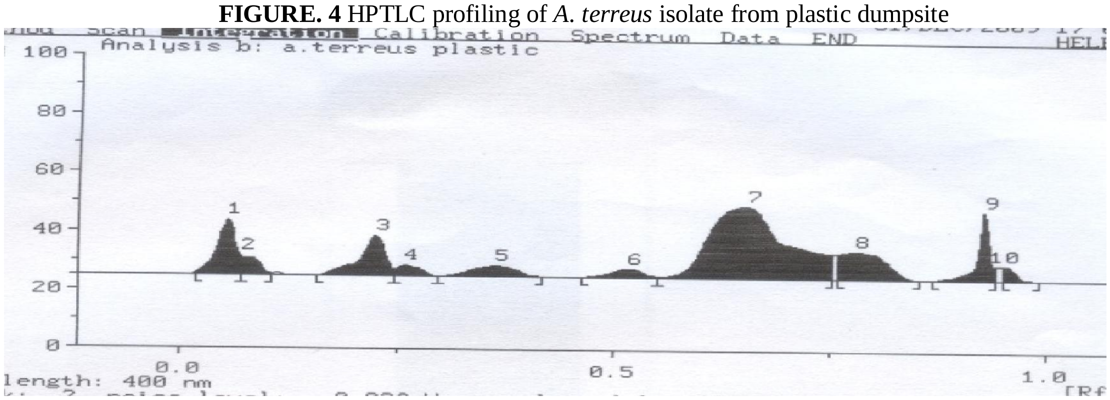
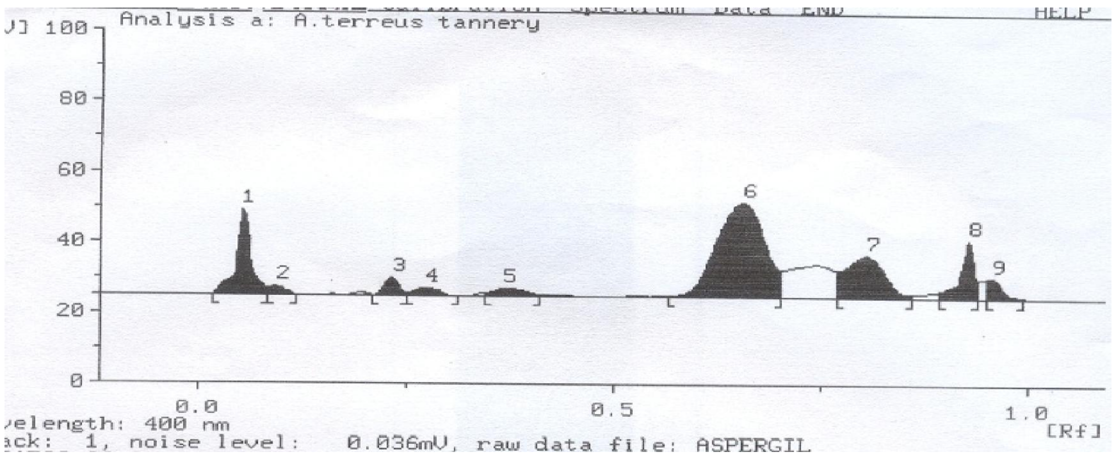


FIGURE. 5 HPTLC profiling of *A. terreus* isolate from tannery soil



These results markens various stress adaptive mechanisms whose semiquantitaive production of varied analytes was observed in HPTLC profiling. Future prospective by inferring these results along with molecular characteristics could insight into the details of stress tolerance genes and metabolites and their individual mechanisms in counteracting the impact of distinguishing pollutants.

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