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Phytoremediation of tannery effluent polluted soils of Dindigul, Tamil Nadu, using Arbuscular mycorrhizal fungi inoculated *Azadirachta indica*

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ABSTRACT

A survey was undertaken from three different sites of tannery effluent polluted soils of Dindigul and their colonized arbuscularmycorrhizal fungi in the roots of the plants commonly grown on three sites, site 1, 2 and 3were observed. The root-zone soils of the common plants were collected and observed for the occurrence of mycorrhizal fungal spores. There was 21 AM fungi were isolated from the root-zone soils and Glomusgeosporum was observed as the dominant AM fungi in the tannery effluent polluted soil sites. The percentage of arbuscularmycorrhizal fungi colonized in the roots of the 18 angiosperm plants collected from the polluted area also analyzed. The tannery effluents were collected from the three different sites, and were analyzed for their physico-chemical parameters and heavy metal composition. The tannery effluents of the 3 sites were mixed and treated to the experimental plants with half strength tannery effluent, mixed with water. For the selection of a suitable experimental plant, 4 plants grown commonly in the tannery effluent polluted sites such as Azadirachta indica, Eucalyptus sp., Tamarindusindica, and Pongamiaglabra were treated half strength tannery effluent and measured for growth parameters. Among the 4 plants, Azadirachtaindica was selected as the experimental plant, as it showed the best performance in growth parameters. The experimental plant was inoculated with dominant mycorrhizal fungi, G. geosporumfor the effluent treatment. There were 4 treatments given, namely, Control, Effluent, AM fungi, and Effluent + AM fungi. The experimental plants were harvested on 45, 90 day intervals after the inoculation of AM fungi and were analyzed for heavy metals such as Cd, Cr, Cu Pb and Zn. Among the effluent treated plants, the plants inoculated with AM fungi G. geosporum showed a great influence on restriction of heavy metals, especially Cr, from the root to shoot tissues of the experimental plant was recorded. This study helped to find a suitable host plant for phytoremediation of tannery effluent polluted soil and we suggest the native common host plant A. indica inoculated with G. geosporum, a native dominant AM fungi as a good option for phytoremediation of tannery effluent polluted soils in Dindigul, India.

Key words: AM fungi, Heavy metals, Phytoremediation, Tannery effluent, Inoculation.

INTRODUCTION

Industrialization caused a serious pollution problem due to the disposal of sewage and industrial effluents to water bodies and agriculture lands. Tanning is one of the industry causing major sources of pollution in Tamil Nadu, India, releases large quantities of effluents and sludge chromium (Cr) and the other metal salts in the surrounding soil and water environment [1]. The effluents coming out of these tanning industries carry several heavy metals, like Cr, Cd, Cu, Pb, Zn, Ni and Hg and deposited in the soils of the surrounding area and even it passes to the agricultural lands. When accumulated in soils, water bodies and aquatic life, they cause toxic effects, although each heavy metal has unique bio functions or bio toxicities [2]. In Tamil Nadu state of India, there are about 60% of tanning industries located in Vellore, Dindigul, and Erode districts. In Dindigul, about 84 tanning industries available causing toxic effects to the living organisms, including plants in and around, due to the release of effluents. In these tanning industrial areas, in the past few decades, disposal of tanning effluents to water bodies has caused a serious pollution problem in the ecosystem, including human, because unlike many other pollutants, heavy metals are difficult to remove from the environment [2].

In tanning industries, a variety of chemicals used, including chromium sulphate, magnesium oxide, sodium formate, sodium sulphide, lime, sodium chloride, sodium carbonate, ammonium chloride, sulphuric acid, formic acid, tannins, dyes, etc. A large quantity of water is needed for processing leather. It depends on ground water sources for their daily requirements. The dischargedeffluents from the processing units are storedin large lagoons and pollution occurs as thedissolved salts percolate into the surroundingsoil, and even the ground water sources to a great extent, when tanneries are in close location to each other and simply, it is evident that the tannery effluents causing a significant pollution effect on the soil and affecting the plants and lives in several ways has been evidenced with severely affected survival problems [3]. In this connection, a remedy is an expected need to save the livestock in and around the tannery effluent polluted area of Dindigul, Tamil Nadu state, India.

In addition, interactions among several heavy metals also create different toxic effects on ecosystems, compared with the effects of single pollutants [4]. So, converting tannery effluent polluted soils into usable land form would be beneficial to the vegetation, animals and human. In converting the heavy metal polluted soils into normal soil, free from pollution, several techniques are in application. Washing and disposal of heavy metals [5], is one of the remediation strategy for heavy metal polluted soils. But, this remediation technology is very expensive and gives rise to a considerable amount of chemical wastes.

But, phytoremediation, a process of remedying soil, using plants and microorganism is an effective, economic, environmentally-sustainable solution in the stabilization and potential recovery of tannery effluent polluted lands. Among the microorganisms, arbuscularmycorrhizal fungi (AM fungi) are playing significant roles in protecting plants from heavy metal pollution [6]. Earlier researchers [7,8] proved the role of AM fungi help in alleviating metal toxicity to plants by reducing metal translocation.

It was recorded that AM fungi contribute as a functional component of the soil, plant system that is critical for sustainable productivity in stressed soils and promote plant growth to reduce or eliminate the bioavilibility of plants [9]. Also, there are variations in metal accumulation between plant species, and that depends on different factors like host-plant, root density, soil characteristics, metals and their availability. Metal tolerant AM fungi can decrease metal absorption capacity of these fungi, which could filter metal ions during uptake as reported earlier [10]. The role of AM fungi in increasing its host's uptake of nutrients and improving growth and resistance to environmental stresses also reported earlier [11,12].

Hence, AM fungi could prove beneficial in the phytoremediation system as they can increase the rate of plant survival and establishment, reduce plant stress and increase plant nutrient acquisition, increase carbon and nitrogen deposition into soil, thereby contributing to bacterial growth and increase the volume of soil being remediated [13]. As it could be a good phytoremediation system, the present work was designed to use a dominant AM fungi isolated from tannery effluent polluted soils of Dindigul, Tamil Nadu state, India, as an inoculant to a host plant, commonly available in the polluted tannery effluent ecosystem to study the influence of AM fungi on phytoremediation against tannery effluent treatment. The aim of the present study is to use the phytoremediation strategies of AM fungi along with a host plant with a possible aim of remedying the tannery effluent polluted soil of Dindigul, Tamil Nadu state, India.

EXPERIMENTAL SECTION

Collection of plant root, soil and effluent samples

The sampling area is located in Dindigul, Tamil Nadu state, India. It is located at 10.35°N 77.95°E and has an average elevation of 265 m (869 ft). The town is in Dindigul district of the South Indian state, Tamil Nadu, 420 km (260 mi) from Chennai and100 km (62 mi) south-west of Tiruchirappalli. Dindigul is located in the foothills of Sirumalai hills. The topography is plain and hilly, with the variation resulting in climatic changes. There are about 84 tannery industries available in and around Dindigul and the release of effluents from these industries are health hazards cause severe pollution to the lives of the soil and the land.

Three sampling sites were chosen along the tannery effluent channels running along the town for the present study. The Site 1 is a tannery effluent channel located on Madurai road; Site 2 is a tannery effluent channel in Vattalagundu road and the Site 3 is located in a tannery effluent channel on Ponmandurai road. For sampling, these three sites were observed for harbouring of plant species and from each site, few plant species were selected and the root-zone soils of the selected samples, along with the fine root samples were collected and transported to the laboratory for further analysis. The effluent samples also collected in containers and transported to the laboratory for further analysis. The collection was made during October 2010.

Physico-Chemical and Heavy metal Analysis of Tannery effluents

Triplicate effluent samples for each site were collected from the tannery effluent channel and analyzed for physical property such as pH, and chemical properties, like bicarbonate, chloride, calcium, magnesium, sodium ions. Heavy metals like chromium, cadmium, copper, lead, and zinc were also estimated. Standard methods were used for collection and analysis of effluents [14,15]. Effluent samples were collected and stored in a clean polythene bottle that had been pre-washed with 10% nitric acid and thoroughly rinsed with de-ionized water. Soil samples were collected in fresh polythene bags.

Assessment of arbuscularmycorrhizal colonization

To determine root percentage colonization, the roots were washed in tap water and boiled in 10% KOH, treated in and 10% H_2O_2, and stained with pen blue ink and acetic acid [16]. The presence of fungal structures within the roots was verified by a method that used root segments mounted on slides and observed under the microscope [17]. Ten slides were mounted for each sampled point, containing ten 1 cm-long root segments each. Percent colonization was calculated as the number of colonized root segments divided by the total number of root segments observed, using the following formula:

$$\text{Percentage of colonization} = \frac{\text{Number of root segments with colonization}}{\text{Total number of root segments observed}} \times 100$$

Isolation and identification of arbuscularmycorrhizal fungi spores

Root-zone soils collected from 30 cm depth from the root-zone regions of different angiosperm plant species available in three tannery effluent polluted sites of Dindigul were taken into the laboratory and analyzed for AM fungal spores. About 1 kg of soil sample was collected from each site and labeled. The soil samples were analyzed for the presence of spores of AM fungi. The spores present in the soil samples were isolated by wet-siewing and decanting method [18]. For the isolation, 100 g soil from each sample was used and the spores were observed in a Trinocular Microscope and identified using synoptic keys of Trappe et al. [19,20,21]. The spores were morphologically grouped under the microscope and the dominant spores were identified. After identification of dominant spores, groups of 10 ± 20 apparently similar spores were employed as starting inoculum for multiplication, using *Allium cepa* L. as a trap plant as described in Miyasaka et al. [22]. For inoculation, 1 g soil inoculum with approximately 500 spores was used.

Pot-experiments

Seeds of *Azadirachtaindica, Eucalyptus* sp., *Pongamiaglabra* and *Tamarindusindica* were purchased from the Government Fruit Garden, Kanyakumari and were surface sterilized by washing with dilute sulfuric acid followed by soaking in sterile distilled water. For germination, soaked seeds were kept in the dark on the sterilized fine sand moistened with sterile distilled water. To carry out pot-experiments, red soil and sand (1:3 ratios) and the mixture was autoclaved to remove indigenous microbes and AM propagules.

For the selection of a suitable host plant for tannery effluent treatment, seedlings were raised from the above 4 plants. After one month growth, the seedlings were transplanted into pots filled with sterilized soil as mentioned above and treated with half-strength tannery effluents, and tested for their survival against tannery effluent treatment for two months. The tannery effluent treated plants were analyzed for growth parameters suchas shoot length, root length and total dry weight. To measure dry biomass, fresh plant from each treatment was washed in distilled water and kept in oven at 60°C for drying and finally dry weight was recorded. The best performed plant was selected for further studies to carry out the inoculation experiment with AM fungi.

Pot-experiment for AM fungi inoculation along with tannery effluent treatment

Seedlings were prepared for the best plant selected for inoculation study, and the inoculation of AM fungi was done with the following 4 treatments. 1. Control, the pots filled with sterilized soil and irrigated with water, without inoculation and effluent treatment. 2. Effluent treatment, the pots filled with sterilized soil, and plants treated with half-strength tannery effluent. 3. AM fungi, the pots filled with sterilized soil, inoculated with dominant AM fungi, *G. geosporum*. 4. AM fungi + Effluent, as shown for 3 and treated with half-strength tannery effluent. The plants were harvested on 45, 90 days after inoculation and analyzed for heavy metals such as Cd, Cr, Cu, Pb and Zn from the root and shoot tissues of the experimental plants and the data were recorded.

Analysis of Heavy metals from plant tissues

Samples, approximately 0.2 g for plants were weighed for digestion and subsequently a mixture of HCl and HNO3 in a 3:1 ratio (v/v) was added. Samples were left for 1 week to soak in the acid after which they were digested in an open heat block (Environmental express 54 Hot block SC154) for 2 h. After cooling, the samples were diluted to 100 ml with HCl 0.1 M and stored until metal analyses.

Prior to measurements the solutions were filtered through filter paper. The concentrations of heavy metals in digesting solutions were analyzed immediately using a flame atomic absorption spectrophotometer. The wavelength (nm), precision (as relative standard deviation, %) and detection limit (mg/kg) of elements were: 283.31, 1.0 and 0.05 for Pb; 213.86, 2.31 and 0.005 for Zn; 324.75, 1.2 and 0.010 for Cu; 228.80, 1.7 and 0.002 for Cd; 309.27, 1.0 and 0.7 for Al; 253.65, 0.2 and 0.7 for Hg; 357.87, 1.7 and 0.078 for Cr; 242.80, 1.7 and 0.33 for Au. The reproducibility of the method used for digesting the plant samples was checked by triplicate analyses [23]. Two blank were also run simultaneously to estimate background metal contamination from the digestion procedure. Analytical grade chemical reagents and highly purified, deionized water were used in the chemical analysis.

RESULTS

The physico-chemical characteristics of the tannery effluents collected from the three sites showed a highly acidic pH ranges from 4.5 to 5.2; the bicarbonate ion level was 10.2 to 12.1 g/L; Cl was 9.6 to 11.2; Ca was 7.4 to 8.2; Mg was 8.3 to 10.6; and Na was 2.4 to 3.2 g/L. The data for these ions were showing a very low level of nutrients. The level of heavy metals such as Cr (3340 to 3600 ppm), Cu (193 to 235 ppm), Pb (86 to 98 ppm), and Zn (785 to 815 ppm) showed very high concentrations of these heavy metals, especially Cr [24,25] were recorded (Table 1).

From the three tannery effluent polluted sites, there was 18 plant species, belonged to 10 different families were selected (Table 2). Among them, 5 (*Acacia planifrons*, *Melettiapinnata*, *Pithecellobumdulce*, *Prosopisjuliflora* and *Tamarindusindica*) were belonged to Fabaceae; 3 (*Eclyptaprostrata*, *Physalis minima* and *Tridaxprocumbens*) belonged to Asteraceae, 2 (*Amaranthusspinosus* and *Gomphrenaglobosa*) belonged to Amaranthaceae, 2 (*Acalyphaindica* and *Euphorbia rosea*) belonged to Euphorbiaceae, 1 (*Commelinabengalensis*) of Commelinaceae,1 plant from Meliaceae (*Azadirachtaindica*), 1 (*Eucalyptus globulus*) of Myrtaceae, 1 (*Cyanodondactylon*) Poaceae and 1 (*Phyllanthusniruri*) Phyllanthaceae were recorded. Among these, 4 plants such as*Acalyphaindica*, *Azadirachtaindica*, *C. dactylon* and *P.juliflora*were common to the 3 polluted sites. There were 6 plants such as *P. minima*, *E. rosea*, *T. indica*, *P. dulce*, *A. planifrons* and *Eucalyptus globules* found only in one site and the other 8 plants such as *A. spinosus*, *G. globosa*, *P. oleracea*, *E. prostrata*, *C. bengalensis*, *M. pinnata*, *T. procumbens* and *P. niruri* were recorded in two among the three sites. The percentage of AM fungi colonization was recorded highest (95%) in *Azadirachta indica*. The lowest record in colonization was in *Euphorbia rosea* (35%); whereas the other plants showed an incidence of mycorrhizal colonization from 40 (*A. spinosus*, *Acalyphaindica*) to 90% (*P. juliflora*) ranges. The number of spores counted as the highest (521 spores/100 g soil) in *Azadirachta indica*. The least spore number was recorded in *E. rosea* (75 spores/100 g soil). The other plant species showed a range of spore numbers

from 89 (*Acalypha indica*) to 470 spores/100 g soil (*P. juliflora*). All the 18 plants showed mycorrhizal colonization also reported (Table 2).

Table 1.Physico-Chemical characteristics and heavy metal levels in tannery effluents from three sites of Dindigul

Studysite*	pH	Ions (g/L)					Heavy metals (ppm)			
		HCO^-_3	Cl^-	Ca^{2+}	Mg^{2+}	Na^+	Cr	Cu	Pb	Zn
Site 1	4.7 ± 1.1	12.1 ± 0.9	11.2 ± 1.1	8.2 ± 0.5	10.6 ± 0.5	2.7 ± 0.2	3450 ± 23.2	193 ± 14.3	95 ± 6.4	815 ± 16.3
Site 2	5.2 ± 0.8	10.5 ± 0.8	10.3 ± 0.6	7.4 ± 0.3	8.3 ± 0.4	2.4 ± 0.3	3340 ± 20.4	218 ± 12.5	86 ± 8.7	785 ± 12.7
Site 3	4.5 ± 0.9	10.2 ± 0.6	9.6 ± 0.5	7.6 ± 0.6	8.7 ± 0.7	3.2 ± 0.2	3600 ± 22.1	235 ± 15.4	98 ± 4.9	790 ± 11.8

**Abbreviations as in Table 1*

Table 2. Percentage of root colonization, number of AMF spores in the root-zone soil, and name of AMF associated with the roots of plant species collected from three tannery effluent polluted sites of Dindigul, Tamil Nadu state.

Sl. No.	Name of Plant species	Family	Sites*	Percentage of root colonization	Number of AMF spores / 100 g soil	Name of AM Fungi associated
1	*Amaranthusspinosus*L.	Amaranthaceae	1, 3	40	95	LGSP, LMCC, LFLV, SPCC,
2	*Gomphrenaglobosa*L.	Amaranthaceae	1, 2	65	149	LGSP, SCVS, SPCC
3	*Portulacaoleracea* L.	Portulacaceae	2, 3	55	123	AELG, LGSP, LMCL, SSNS
4	*Ecliptaprostrata*L.	Astraceae	1, 3	65	155	GABD, LAGR, LGSP, LMCC
5	*Physalis minima* L.	Asteraceae	3	60	120	LGSP, LMCC, LFLV, SSNS
6	*Euphorbia rosea* Retz.	Euphorbiaceae	3	35	75	LFSC, CCRL, LGSP, SNSS
7	*Acalyphaindica* L.	Euphorbiaceae	1, 2, 3	40	89	ABRT, LGSP, LINR, GMRG, CCRL
8	*Commelinabengalensis* L.	Commelinaceae	1, 3	70	266	GMRG, LFSC, LGSP, SSNS
9	*Prosopisjuliflora*(Sw.) DC.	Fabaceae	1, 2, 3	90	470	GABD, LGSP, CCRL
10	*Azadirachtaindica* L.	Meliaceae	1, 2, 3	95	521	LGSP, SCVS, SPCC
11	*Tamarindusindica*L.	Fabaceae	2	75	372	LFSC, LGSP, LMSS, SRBF
12	*Pithecellobiumdulce*(Roxb.) Benth.	Fabaceae	3	70	390	LCND, LFSC, LGSP, SMCC
13	*Melettiapinnata*(L.) Panigrahi	Fabaceae	1, 2	85	295	GDCP, LGSP, LMCL, SSNS
14	*Eucalyptus globulus*Labill.	Myrtaceae	1	70	347	LFSC, CCRL, SCVS, SPCC
15	*Cyanodondactylon* Pers.	Poaceae	1, 2, 3	50	120	AELG, GMRG, LGSP
16	*Tridaxprocumbens*L.	Asteraceae	2, 3	75	250	LGSP, LMSS, SCVS
17	*Acacia planifrons*Wight &Arn.	Fabaceae	1	80	328	GMRG, LFSC, LGSP, LVSF
18	*Phyllanthusniruri*L.	Phyllanthaceae	1, 2	60	146	GDCP, LMCL, LMSS, SSNS

**Site 1: Tannery effluent channel in Madurai road, Site 2: Tannery effluent channel in Vattalagunduroad; Site 3: Tannery effluent channel in Ponmandurairoad*

There were 21 AM fungal spores recorded from the three tannery effluent polluted sites. Among them, 11 species such as *Acaulospora bireticulata*, *A. elegans*, *Gigaspora albida*, *G. fasciculatum*, *G. geosporum*, *G. intraradices*, *G. maculosum*, *G. mosseae*, *Sclerocystis pachycaulis*, *Scl. Sinuosa*, and *Scutellospora corralloides* were observed in all the 3 sites; 6 species such as *Gi. decipiens*, *Gi. margarita*, *G. aggregatum*, *G. fulvum*, *G. macrocarpum*, and *Scl. Clavispora* were observed in 2 sites; and 4 species such as *G. canadense*, *G. versiforme*, *Scl. microcarpus*, *Scl. Rubiformis* were observed in only one site. Among the spores listed in only one sites, *G. canadense* and *Scl. microcarpus* were recorded only in site 3, whereas, *G. versiforme* was recorded only in site 1 and *Scl. rubiformis* was recorded only in site 2 (Table 2, 3, 4). Among all the AM fungi, *G. geosporum* was recorded in 16 plants andit followed by *G. fasciculatum* recorded in 6 plants, and *G. canadense*, *G. versiforme*, *Scl. microcarpus* and *S. rubiformis* recorded as the rare AM fungi in the soils of the tannery effluent polluted sites (Table 2, 3, 4).

Among the one month grown nurseries treated with tannery effluent and measured for shoot length, root length and total dry weight. The result showed that the shoot length and root length and total dry weight were highest in *A.*

indica (25.61, 16.12 cm; 5.21 g/plant), it was followed by *T. indica* (17.56, 14.61 cm; 4.96 g/plant), *E. globulus*(15.43, 10.46 cm; 4.48 g/plant) and the least in *P. glabra* (12.37, 9.64 cm; 3.72 g/plant).

Table 3. List of AM fungi isolated from Dindigul tannery effluent sites and their abbreviations

Sl. No.	Abbreviations used	Full name of AM fungi
1	ABRT	*Acaulosporabireticulata*
2	AELG	*A. elegans*
3	GABD	*Gi. albida*
4	GMRG	*Gi. margarita*
5	GDCP	*Gi. decipiens*
6	LAGR	*Glomusaggregatum*
7	LCND	*G. canadense*
8	LFLV	*G. fulvum*
9	LFSC	*G. fasciculatum*
10	LGSP	*G. geosporum*
11	LINR	*G. intraradices*
12	LMCC	*G. macrocarpum*
13	LMCL	*G. maculosum*
14	LMSS	*G. mosseae*
15	LVSF	*G. versiforme*
16	CCRL	*Scutellosporacorralloides*
17	SCVS	*Sclerocystisclavispora*
18	SMCC	*Scl. microcarpus*
19	SPCC	*Scl. pachycaulis*
20	SRBF	*Scl. rubiformis*
21	SSNS	*Scl. sinuosa*

Table 4. Distribution of AM fungi in the root-zone soils of plant species collected from three different tannery effluent polluted sites of Dindigul

Sl. No.	Name of AMF species*	Name of sites		
		Site 1	Site 2	Site 3
1	ABRT	+	+	+
2	AELG	+	+	+
3	GABD	+	+	+
4	GMRG	+	-	+
5	GDCP	+	+	-
6	LAGR	+	-	+
7	LCND	-	-	+
8	LFLV	+	-	+
9	LFSC	+	+	+
10	LGSP	+	+	+
11	LINR	+	+	+
12	LMCC	+	-	+
13	LMCL	+	+	+
14	LMSS	+	+	+
15	LVSF	+	-	-
16	CCRL	+	+	+
17	SCVS	-	+	+
18	SMCC	-	-	+
19	SPCC	+	+	+
20	SRBF	-	+	-
21	SSNS	+	+	+

**Abbreviations as in Table 2*

Table 5. Growth measurement and dry weight of one month grown nurseries, treated with tannery effluent collected from Dindigul

Name of plantspecies	Shootlength (cm)	Rootlength (cm)	Total Dry weight (g/plant)
Azadirachta indica	25.61 ± 2.43	16.12 ± 2.14	5.21 ± 0.82
Eucalyptusglobulus.	15.43 ± 1.54	10.46 ± 0.92	4.48 ± 0.39
Pongamia glabra	12.37 ± 1.12	9.64 ± 0.72	3.72 ± 0.28
Tamarindus indica	17.58 ± 1.65	14.61 ± 1.35	4.96 ± 0.36

Table 6. Concentration of heavy metals analyzed from experimental plants on 45 days after inoculation of Arbuscularmycorrhizal fungi.

Analyzed plant part	Name of Treatment	Concentration of metals in plant tissues (ppm)				
		Cd	Cr	Cu	Pb	Zn
Shoot	Control	1.93± 0.15	2.43 ±1.95	2.43 ±0.15	1.91 ±0.15	3.28 ±0.18
	Effluent	9.37 ± 1.12	174.72 ± 15.34	59.31 ± 5.22	237.22 ± 19.74	348.67 ± 32.12
	AMF	0..97 ±0.03	1.12 ±0.08	2.27 ±0.27	0.96 ± 0.09	1.24 ±0.11
	Effluent + AMF	4.75 ± 0.31	51.18 ±4.82	30.18 ± 1.88	17.66 ± 1.48	16.44 ±1.72
Root	Control	2.34 ±0.19	3.47 ±0.21	5.83 ± 0.36	1.52 ±0.34	4.6 ±0.77
	Effluent	20.55 ± 2.13	782.85 ± 72.45	198.69 ±17.65	578.05 ± 51.55	655.38 ±63.24
	AMF	1.68 ±0.08	1.32 ±0.25	1.22 ±0.09	1.18 ±0.07	10.84 ± 1.12
	Effluent + AMF	12.12 ±1.66	235.51 ±20.41	32.59 ±3.11	54.13 ±4.99	82.52 ± 7.91

Table 7. Concentration of heavy metals analyzed from *Azadirachtaindica* on 90 days after inoculation of *Glomusgeosporum*

Analyzed plant part	Name of Treatment	Concentration of metals in plant tissues (ppm)				
		Cd	Cr	Cu	Pb	Zn
Shoot	Control	2.22 ± 0.19	3.88 ± 0.24	2.65 ± 0.18	2.04 ± 0.16	3.64 ± 0.21
	Effluent	12.16 ± 2.54	208.14 ± 18.68	73.07 ± 6.54	273.46 ± 25.33.	382.15 ± 29.76
	AMF	1.06 ± 0.08	1.77 ± 0.09	2.39 ± 0.12	1.37 ± 0.11	1.33 ± 0.15
	Effluent + AMF	5.50 ± 0.43	63.35 ± 7.73	37.43 ± 2.88	25.71 ± 1.97	21.89 ± 2.19
Root	Control	3.15 ± 0.22	5.81 ± 0.39	6.95 ± 0.72	1.94 ± 0.46	5.75 ± 0.86
	Effluent	27.80 ± 3.65	925.12 ± 55.47	247.44 ± 19.66	630.23 ± 54.99	764.69 ± 81.95
	AMF	1.85 ± 0.06	2.17 ± 0.15	1.51 ± 0.08	1.27 ± 0.09	14.65 ± 1.38
	Effluent + AMF	15.90 ± 2.14	292.88 ± 25.42	49.83 ± 3.55	96.85 ± 8.61	149.21 ± 15.27

The experimental plant harvested on 90 days after inoculation showed a highest level of Cd in the root and shoot tissues (27.8, 12.16 ppm) of effluent alone treated plants, whereas in *G. geosporum* inoculated plants treated with effluents showed 15.9, 5.5 ppm level of Cd, and the level of this heavy metal in *G. geosporum* alone treated plants, the level of Cd was the least (1.85, 1.06 ppm) in the root and shoot than the control (3.15, 2.22 ppm). The result was same in the plants harvested on 45 days after inoculation of the AM fungi also, except a little lower level of Cd in the plant tissue recorded (Table 6, 7).

The level of Cr also was the highestin the root and shoot tissues (925.12, 208.14 ppm) of effluent alone treated plants, whereas in *G. geosporum* inoculated plants treated with effluent showed 292.88, 63.35 ppm level of Cr, and the level of Cr in *G. geosporum* alone treated plants it showed the least concentration (2.17, 1.77 ppm) in the root and shoot and even lower thanthe control (5.81, 3.88 ppm). The result was same in the plants harvested on 45 days after inoculation of the AM fungi also, except a little lower level of Cr in the plant tissue recorded (Table 6, 7).

The level of Cu, Pb and Zn also were the highest in the root and shoot tissues (247.44, 630.23, 764.69 ppm in root tissues; 73.07, 273.46, 382.15ppm in shoot tissues) of effluent alone treated plants, whereas in *G. geosporum* inoculated plants treated with effluent showed a record of 49.83, 96.85, 149.21 ppm in root tissues, and 37.43, 25.71, 21.89 ppm in shoot tissues and the level of these heavy meals in *G. geosporum* alone treated plants showed the least concentration in root (2.17, 1.51, 1.27 ppm) and shoot (37.43, 1.37, 1.33ppm in) tissues, and even lower than the control in root (6.95, 1.94, 5.75 ppm) and shoot (2.65, 2.04 and 3.64 ppm) tissues of the experimental *A. indica* plants. The result was same in the plants harvested on 45 days after inoculation of the AM fungi also, except minor changes with a little slightly lower concentration of Cu, Pb and Zn was recorded in the plant tissues (Table 6, 7).

DISCUSSION

The data recorded from tannery effluents of Dindigul tannery effluent polluted sites for level of micronutrients, nutrient ions were very low in concentration; whereas heavy metals showed a very high concentration as shown in published standards [24,25]. The level of heavy metals such as Cr (3340 to 3600 ppm), Cu (193 to 235 ppm), Pb (86 to 98 ppm), and Zn (785 to 815 ppm) showed higher concentrations than normal ranges. The pH of the tannery effluent samples showed acidic. The acidic nature of tannery effluent, and low level of nutrient ions such as HCO_3, Ca, Mg, Na, and higher level of heavy metals are common phenomenon in tannery effluents and it has been reported earlier in industrial effluents [26,27]. The higher level of heavy metals, highly acidic pH and low level of nutrients are biologically non-essential systems and they can cause toxicity to the vegetations grown in the soil [28]. The

presence of acidic pH, low level nutrients and higher level of heavy metals could be toxic and this effect of the tannery effluents could be unsuitable for plant growth [28].

The presence of 18 plants of angiosperm member belonged to 10 different families, and their presence as common species in some sites could be due to the widespread occurrence of such plants in tannery effluent polluted sites and rare presence or showing site specificity of some plants could be due to the differences in the edaphic, climatic or biological factors of the sites [29,30].

All the 18 plants collected from the tannery effluent sites showing colonization of arbuscularmycorrhizal fungi, showed that mycorrhizal condition is widespread as reported earlier [27,31,32], in the natural ecosystem, including tannery effluent polluted sites. Also, few plants from families reported as non-mycorrhizal also reported as mycorrhizal in the current work. *A. spinosus*, *G. globosa* of Amaranthaceae, *E. rosea*, *A. indica* of Euphorbiaceae, and *P. niruri* of Phyllanthaceae shown to be a non-mycorrhizal plant species were reported as mycorrhizalas previously reported [29,30]. The presence of 100% mycorrhizal colonization in plants colonized in tannery effluent polluted soil as recorded in this present work would be a rare phenomenon; however, it showed the importance of mycorrhizal colonization in helping harboured vegetations to survive against heavy metal pollution. All the plant species recorded in the three polluted sites occurred with the association of more than two AM fungal species; and 16 plant species showed more than 3 species of AM fungi, and this showed the absence of host-preferred association of the AM fungi colonized with these plant species [33].

There was common, dominant and rare occurrence of spores of AM fungi noticed among some species of AM fungi. The common occurrence of 11 AM fungi species in three sites could be attributed to their nature of colonization with any plant species showing no preference in host-specific association, and with a broad host range and that may be a reason for those spores to present in all the three sites, and association with different plants. The spore of AM fungi, *G. geosporum* was recorded as the dominant, and *G. fasciculatum* was recorded as the subdominant spore, among the soils of the three tannery effluent polluted sites. The other reason for dominant occurrence of *G. geosporum* could be due to the association of broad host ranges and also due to its virulent nature to survive and colonize with the different plant species grown on the tannery effluent polluted sites [34]. Whereas, *G. canadense*, *G. versiforme*, *Scl. microcarpus* and *S. rubiformis* were colonized only in selective plant species, suchas *M. pinnata*, *C. dactylon*, *M. pinnata*, and *P. dulce* showing a host specific association; among them, *S. rubiformis* showing both host and site specific association, as the host plants are recorded only in site 3. The host specific and site specific association of AM fungi have also been reported earlier [35]. However, the reason for such host specific associations are still poorly understood as reported earlier [36].

The maximum number of AM fungi spores and maximum percentage of colonization were reported from the plant species common to all the three sites; whereas the least number of spores and percentage of colonization reported from plants belonged only to a specific site, as seen in *E. rosea* recorded only from site 3. There was a correlation between the number of spores and percentage of root colonization recorded as highest number of spores and percentage of colonization in *A. indica* (521, 95%) and the lowest number of spores and percentage of colonization as recorded in *E. rosea* (75, 35%). The reason for failure of some plants to form a maximum percentage of colonization and the number of spores could be due to release of volatile substances from the root tissues and due to the presence of heavy metals in the root-zone soil not favoring the colonization and spore production, as exemplified earlier [37]. It could be stated that the occurrence of AM fungi colonization in all the plants observed from the tannery effluent polluted sites could be helpful for those plants for their survival against the heavy metal pollution in those sites. The reports of earlier works also support this view [29,30].

Prior to the inoculation experiment, selection of a suitable host plant was undertaken to choose a host plant showing suitability to face the toxic effect of the tannery effluent and to study the inoculation experiment with tannery effluent treatment.For selection of suitable host plant, 4 plants of angiosperms commonly grown in the tannery effluent polluted sites were tested with tannery effluent treatment and as the result showed *A. indica* the best plant by showing comparatively higher shoot, root length and total dry weight, selected to test the influence of AM fungi in green house condition. Earlier studies of Abdullateef et al. [38,39] also reported *A. indica* as a plant for indicating environmental pollution and as a suitable plant for phytoremediation.

The toxic levels of heavy metals like Cr, Cd, Cu, Pb and Zn from plant root and shoot were reported. The standard of Rudnick and Gao [40], showed the level of heavy metals estimated from the effluent alone treated plant of the

present study was higher than the normal range.The pot-experiment studied in the experimental plant *A. indica* inoculated with *G. geosporum* and treated tannery effluent showed that there was a great influence of AM fungi on reducing the uptake and transport of heavy metals, especially Cr, and also Cd, Cu, Pb, and Zn, especially to the aerial part of the experimental plant. The results for all these above heavy metals showed in the experimental plants harvested on 45, 90 days after inoculation of the AM fungi, *G. geosporum* along with tannery effluent treatment showed a higher restriction of heavy metals to the shoot region, than the effluent alone treatment. It was reported by several researchers that arbuscularmycorrhizal (AM) fungi inoculations reduced adverse stress effect on plants by the role of AM fungi on binding of heavy metals on its extra-radical hyphae [41,42,43,8]. It is hypothesized that metal transporters and plant-encoded transporters involved in uptake, binding and tolerance of heavy metal are the mechanisms involved and that may be reasons for the prevention of heavy metal movement to the upper part of the experimental plant and that helped in better survival of the AM fungi, *G. geosporum* inoculated experimental plant against tannery effluent treatment [44,45].

Also, the occurrence of AM fungi in the tannery effluent polluted soils of Dindigul could be helpful for the plants of *A. indica* for its easy survival against the tannery effluent polluted soil environment. As the heavy metals including Cr trapped from the soil is mostly prevented to move to the aerial part of the AM fungi, *G. geosporum* helped the plants to escape from severe heavy metal toxicity. It indirectly helped the heavy metals concentration to decrease in the soil and that gradually make the soil free from heavy metal pollution, including chromium. This proved that AM fungi *G. geosporum* strain isolated from the tannery effluent site could be a highly heavy metal tolerant strain. The result of the current work proved the statement of Joner and Leyval [9], stated AM fungi contributes as a functional component of the soil plant system that is critical for sustainable productivity in stressed soils and promote plant growth to reduce or eliminate the heavy metals translocation to support plants. The metal tolerant strain of AM fungi, *G. geosporum* helped to decrease of metal absorption, and filter metal ions during uptake. This record is highly co-inside with the earlier reports from other AM fungi species also [10,44,41,42].

CONCLUSION

A remedy is an expected need to save the livestock in and around the tannery effluent polluted area of Dindigul, Tamil Nadu state, India. This study helps us to suggest the application of *A. indica*, inoculated with AM fungi *G. geosporum* in the tannery effluent polluted soils of Dindigul, for phytoremediation of the tannery effluent polluted soils in and around Dindigul, Tamil Nadu state, India.

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