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Effect of Saharan Dust on Biodegradation of Phenol by White Rot Fungi

S. Sanin,[1] M. Arısoy,[2] R. Tıpırdamaz,[3] C. Saydam[1]

[1] Hacettepe University, Department of Environmental Engineering, 06532 Beytepe, Ankara, Turkey
[2] Ankara University, Faculty of Health Education, Department of Basic Health Science and Institute of Biotechnology, 06690, Ankara, Turkey
[3] Hacettepe University, Department of Biology, 06532 Beytepe, Ankara, Turkey

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Dust originating and transported from Africa affects Southern and Eastern parts of Europe. Deposition of Saharan dust on Mediterranean region is a known and documented concept (Chester et al. 1984; Gullu et al, 1996). Major dust transport patterns travel to Europe through Western and Eastern Mediterranean and atmospheric deposition events originating from Africa are reported in England, Spain and Turkey. Turkey is in the Eastern transport pattern of Saharan dust plumes and wet and dry Saharan dust depositions are reported especially during spring and autumn (Gullu et al. 2004). A typical dust composition (Table 1) indicates that Saharan dust is low in organic content but contain chemicals which can be considered as nutrients (Avila 1998). Saharan dust samples collected at different locations may have different compositions (Ryall et al. 2000, Glavas and Moschonas 2002, Griffin et al. 2001).

In recent years, it was shown that photochemical reactions are also possible during atmospheric transport of the dust (Sulzberger and Laubscher 1995). The latest addition to the fast developing knowledge on Saharan dust is the production of oxalate and Fe(II), a biologically significant chemical, in the presence of UV light by using Saharan dust in laboratory (Saydam and Senyuva 2002). There is evidence to support the idea that Saharan dust can be one of the reasons behind the algal blooms (Ridame et al. 2003).

Available literature may lead to the conclusion that Saharan dust composition has the potential to support microbial activity in different ways. Several parameters, (i) composition of Saharan dust, (ii) presence of microorganisms in the dust and (iii) photochemical reactions, may be responsible for the production of biologically significant chemicals during long range atmospheric dust transport.

Fungi, which are among the natives of soil systems, adapt easily to different environmental conditions and are able to biodegrade complex organic compounds such as phenols. Among the very large variety of fungal species, *Pleurotus sajor-caju* and *Trametes versicolor* are the two members of white rot fungi family, which have found use in environmental control and biodegradation applications. White rot fungi that cause white-rot of wood have recently become

Correspondence to: S. Sanin

Table 1. Physicochemical composition of Northern Africa soil.

Source Area	Composition (%)					
	Na	K	Ca	Mg	S	P
Western Sahara	0.45	2.31	1.36	1.69	0.0068	0.0767
Moroccan Atlas	0.42	2.02	6.70	1.71	0.0050	0.0624
Central Algeria	0.11	0.58	0.44	1.36	0.0014	0.0222

the object of increasing attention of researchers in hazardous waste treatment field. These fungi normally grow on decaying wood and forest litter, and appear to be unique among microorganisms that can rapidly depolimerize and mineralize lignin to carbon dioxide (Hammel 1989).

White rot fungi are also able to degrade a wide variety of environmental pollutants to carbon dioxide, including the difficult to biodegrade chemicals, such as chlorinated and phenolic pollutants (Aust 1995). Biodegradation ability of white rot fungi is a result of lignin degrading enzyme system that they posses (Shah et al. 1992). Fungi's ability to degrade such a diverse group of compounds has been shown to depend on the nonspecific and nonstereoselective lignin degrading system expressed by these microorganisms, under nutrient (nitrogen, carbon or sulfur) limiting conditions (Bumpus et al. 1988).

In this research we hypothesize that Saharan dust can be used as a nutrient media. To investigate its use as a nutrient media we selected two types of fungi, which are capable of biodegrading phenol. The purpose of the study was to show that selected fungi can grow and biodegrade phenol in a suspension prepared by using Saharan dust.

MATERIALS AND METHODS

Pleurotus sajor-caju and *Trametes versicolor* were used in the experiment. Each fungi was grown in Saharan dust and nutrient media reactors to be able to identify the differences. *Pleurotus sajor-caju* was obtained from Dr. Zadrazil (Institut fur Bodenbiologie, Bundalsalle 50 D-33 Braunsweigh Germany). *Trametes versicolor* ATTC 200801 was obtained from Dr. Yeşilada (University of Malatya, Faculty of Science, Turkey). The stock cultures of organisms were grown and maintained on malt agar slants until inoculation. Nutrient growth media was prepared according to the modification given by Eaton (1985). Nutrient medium contained (mM) 27.7 glucose, 2.24 NH_4Cl, 14.9 K_2HPO_4, 2.03 $MgSO_4$, 0.9 $CaCl_2$, 0.003 thiamin. The pH value of the medium was 4.5. Other culture medium was prepared with Saharan dust (SD media) which did not contain additional nutrients. Saharan dust used in experiments, a sub-sample of 40 tons, was obtained from Tozeur region in Tunisia. Saharan dust solution was prepared by suspending 100 g of Saharan dust in 1 liter distilled water. The suspension was filtered through a 0.45 μm membrane filter to remove suspended particles from the solution. Final pH in reactors were adjusted to 4.5 using 0.1 N HCl and 0.1 N NaOH. Biochemical

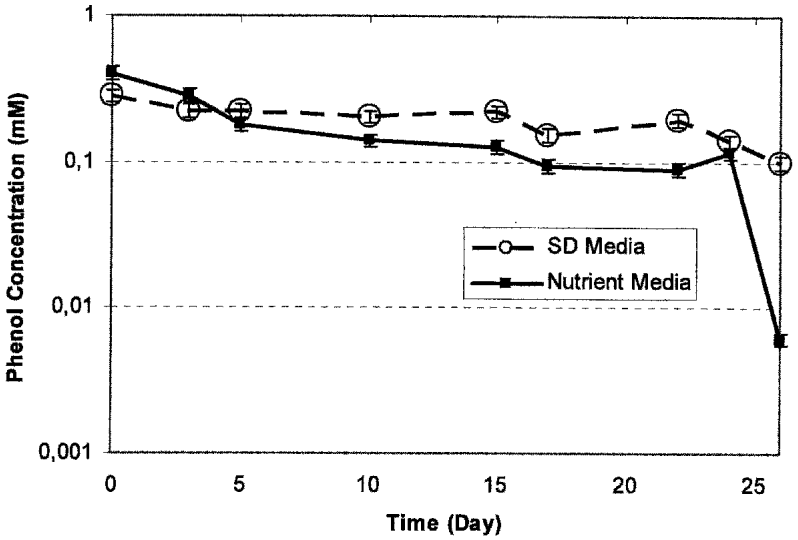

Figure 1. Biodegradation of phenol in SD and Nutrient media reactors by *Trametes versicolor*

Oxygen Demand (BOD) of Saharan dust, an indicator of biologically available carbon in solution, was 10 ppm.

After sterilization, solution was cooled down to room temperature and phenol was added via syringe connected to a sterile 0.22μm filter, to provide a final concentration of 0.38 and 0.29 mM in reactors. The experiments were repeated in two separate experimental sets. The analytical results reported in the article are the averages of three replicates.

The difference in start-up concentrations in reactors is due to manual injection of phenol into the system. The reactors used in the experiments were incubated at 30 °C for 26 days. Growth of white rot fungi cultures in reactors was determined gravimetrically at the end of incubation period, using dry weight of the mycelia, after filtration.

For control studies, phenol in distilled water was incubated in the batch reactors during the experiments. The fungi species used in the studies were also incubated in phenol containing distilled water without nutrients to observe any growth under starvation conditions. Samples collected from reactors at certain times during incubation period were centrifuged and analyzed for phenol spectroscopically using Standard Methods (APHA, 1989). All the data reported are the corrected results with respect to the control reactors.

RESULTS AND DISCUSSION

Both species, *Trametes versicolor* and *Pleurotus sajor-caju* were able to biodegrade phenol under all experimental conditions. *Trametes versicolor* had

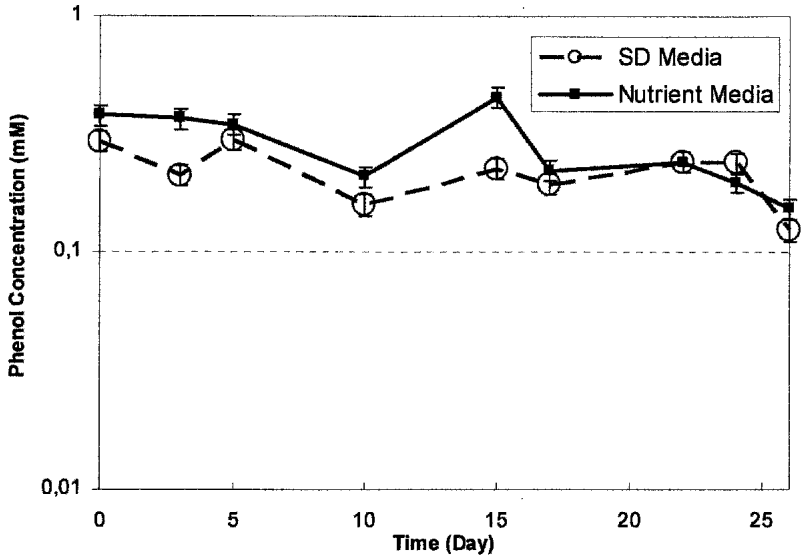

Figure 2. Biodegradation of phenol in SD and nutrient media reactors by *Pleurotus sajor-caju.*

better bioremoval performance then *Pleurotus sajor-caju.* Biodegradation of phenol in the SD reactors indicate that Saharan dust composition contains nutrients necessary for the growth of fungal population. Biodegradation performances of *Trametes versicolor* and *Pleurotus sajor-caju* under experimental conditions are given in Figure 1 and Figure 2, respectively. According to these results, both species showed similar removal performances in SD media reactors whereas in nutrient media rectors *Trametes versicolor* performed better then *Pleurotus sajor-caju.*

When biodegradation performance of *Trametes versicolor* in SD and nutrient media reactors were analyzed, different phenol removal efficiencies with similar biodegradation patterns was observed (Figure 1). Our results indicate that phenol concentration in nutrient media reactors were decreased from 0.4 mM to ~0.05 mM. At the end of the incubation period, *Trametes versicolor* have biodegraded 98% of the phenol in nutrient media reactors. Whereas in Saharan Dust reactors, initial concentration of 0.28 mM phenol was reduced to 0.1 mM, which corresponds to 65% removal (Figure 3).

Phenol removal in SD and nutrient media reactors at the end of the incubation period indicate that *Pleurotus sajor-caju* is capable of biodegrading phenol. According to these results phenol concentration in nutrient media reactors were decreased from 0.38 mM to 0.15 mM at the end of 26 days, which corresponds to 60 % removal efficiency for *Pleurotus sajor-caju* (Figure 2).

In SD reactors, which contained a mixture of phenol with Saharan dust, the initial concentration of 0.29 mM phenol concentration was reduced to 0.13 mM at the

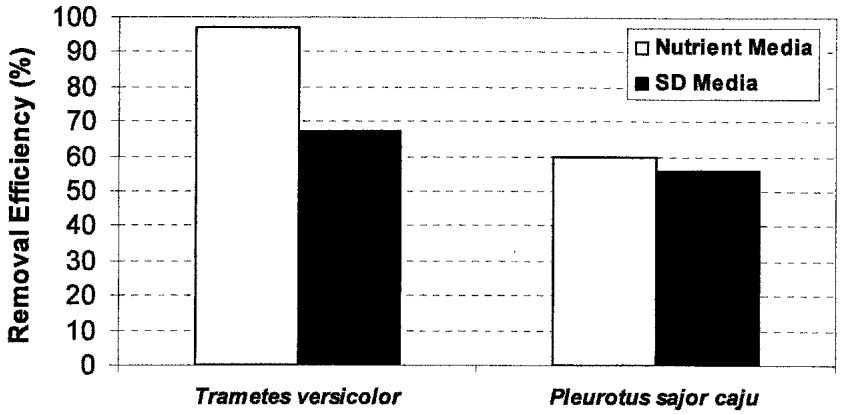

Figure 3. Phenol removal efficiencies of *Trametes versicolor* and *Pleurotus sajor-caju*

end of incubation period, which corresponds to 58% removal efficiency for *Pleurotus sajor-caju* (Figure 3).

Time dependent concentration changes observed in the reactors indicate shifting order reactions, which is the characteristics of enzymatic activity for both microorganisms. At the beginning, the reactions were independent of substrate concentration, as the time passed and the fungal concentration in the reactors were increased, a dependency to the phenol concentration was observed. Overall reaction rates of *Pleurotus-sajor caju* and *Trametes versicolor* were best represented with a zero-order reaction plot and calculated rate constants are given in Table 2. Calculated fungal doubling times based on the rate constants are also given in Table 2. As the rate constants and fungal doubling times indicate microorganisms incubated in nutrient media biodegraded phenol and grew faster than Saharan Dust inoculums. For *Pleurotus sajor-caju* doubling time in nutrient media was one third of the doubling time in Saharan Dust reactors. For *Trametes versicolor* doubling time of the fungi in nutrient media reactors was half the Saharan Dust reactors. These results indicate the difference among the species as well as their response to different environmental conditions.

Table 2. Overall phenol removal rate constants and doubling time of fungal species

White Rod Fungi	Saharan Dust		Nutrient Media	
	Removal rate (day^{-1})	Doubling time (days)	Removal rate (day^{-1})	Doubling time (days)
Trametes versicolor	0.02	35	0.04	17
Pleurotus sajor-caju	0.01	69	0.03	23

When the overall removal efficiency of *Pleurotus sajor-caju* in SD and in nutrient media are examined, no statistically significant difference was observed (around

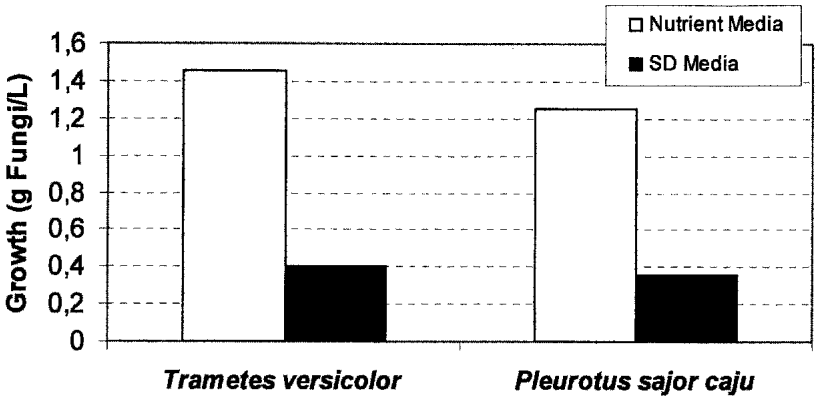

Figure 4. Growth of *Trametes versicolor* and *Pleurotus sajor-caju*

%60). Removal efficiency of *Trametes versicolor* in nutrient media (%98) is found to be significantly higher than *Trametes versicolor* in SD (%67). *Trametes versicolor* in nutrient media had the highest removal efficiency, 98%, and *Pleurotus sajor caju* in Saharan dust solution had the lowest (%58), when all reactors are compared (Figure 3). Removal efficiencies of *Trametes versicolor* (%67) and *Pleurotus sajor-caju* (%58) in the Saharan dust reactors were different.

Trametes versicolor had better phenol degradation performance in nutrient media then SD media (Figure 3). Total phenol removal at the end of the experimental study was similar for *Pleurotus sajor-caju*, in both reactors. This may mean addition of external nutrients is not needed to improve the biodegradation performance of *Pleurotus sajor-caju.*

Fungal masses of *Pleurotus sajor-caju* and *Trametes versicolor* obtained in reactors at the end of the incubation period were given in figure 4. Fungal mass obtained in Saharan Dust reactors were about 70% less then the fungal mass in nutrient media, for both species. When overall growth of the species in SD and nutrient media was compared, no significant differences in total mass was observed for either fungi in SD reactors.

Although the mass of *Pleurotus sajor-caju* accumulated in SD and in nutrient media reactors were different at the end of incubation period, 0.38 g-Fungi/l and 1.3 g-Fungi/l (Figure 4), observed removal efficiencies were similar, %58 and %60, respectively (Figure 3). On the other hand, the amount of *Trametes versicolor* in SD and nutrient media reactors and observed phenol removals were related linearly.

Two different control sets were used during the experiments. The first control reactor included distilled water without microorganisms to observe the loss of phenol from the system. No significant phenol loss was observed in this reactor. In the second control reactor setup, fungal species were incubated in distilled water in the absence of nutrients to see if there is any growth. In this reactor, fungal species were monitored for 10 days and at the end of the incubation period

microorganisms were filtered, dried and weighed, no fungal mass increase was observed in these reactors, either.

In conclusion, we showed that Saharan dust is capable of supporting the growth of the fungal species *Trametes versicolor* and *Pleurotus sajor-caju* in controlled environments. This finding supports our hypothesis that Saharan dust transported continuously through Eastern Mediterranean region, may provide nutrients for microorganisms. These findings also indicate that Saharan dust has a potential as a natural growth media in biodegradation processes of hazardous organics.

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