African Journal of Biotechnology Vol. 10(8), pp. 1384-1392, 21 February, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.1897
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Effect of different concentrations of phenol on growth of some fungi isolated from contaminated soil

Eman H. F. Abd El-Zaher[1]*, Yehia A. G. Mahmoud[1] and Magda M. Aly[2]

[1]Mycology Research Laboratory, Botany Department, Faculty of Science, Tanta University, Egypt.
[2]Botany Department, Faculty of Science, Kafr el-Sheikh University, Egypt.

Accepted 20 January, 2011

Phenol concentration in 25 water samples collected from three Egyptian Governorates (El- Gharbia, Kafer El-Sheik and El-Menofia) was assayed. The wastewater collected from El-Mehalla El-Kobra II (El-Gharbia governorate) was the most polluted sample with phenol and was equal to 0.0 88 mg/L. Czapeks medium was the most suitable among the other tested media for the growth of *Hormodendrum bergeri*, *Fusarium oxysporum* and *Aspergillus flavus var. coulmnaris*. However, where they were able to grow in the media containing 0.1 g/100 ml phenol, they failed to grow in the potato dextrose medium (PDA) with 0.14 g/100 ml phenol. On the other hand, the efficiency of *Aspergillus ochroceus* to grow on phenol was low when compared with *H. bergeri*, *F. oxysporum* and *A. flavus var. coulmnaris*. The growth of *H. bergeri, F. oxysporum* and *A. flavus var. coulmnaris* was optimum on the medium that contained 0.1 g/100 ml phenol after 6 days. The addition of a mixture of vitamins (B1 + B6 + B12) at 0.1% (w/v) to Cazpeks medium enhanced the growth of *H. bergeri*, *F. oxysporum* and *A. flavus var. coulmnaris* in the presence of phenol. Growth in the presence of phenol induced some morphological modification in both *F. oxysporum* and *A. flavus var. coulmnaris*.

Key words: Phenol, growth, fungi, morphological changes.

INTRODUCTION

Wastewaters generated by the chemical, petrochemical and steel industries, frequently contain high concentrations of phenolic compounds, which present a serious ecological problem due to their widespread use, toxicity and occurrence in the environment (Fava et al., 1995). Phenolic compounds are common waste byproducts in the manufacture of industrial and agricultural products. Especially, phenolic compounds are often found in wastewaters from coal gasification, coke-oven batteries, refinery and petrochemical plants and other industries that produce things such as synthetic chemicals, herbicides, pesticides, antioxidants, pulp-and-paper, photo developing chemicals, etc. (Marrot et al., 2006; Bodalo et al., 2008, Jayachandran and Kunhi, 2008). Phenol may persist in air, sea water or surface water, soil or sewage. Now, the associated problem due to phenol is that when it is present in waste water, even in low concentrations, it can be toxic to some aquatic species (Rittmann and McCarty, 2001). Inhalation and dermal contact of phenol causes cardiovascular diseases and severe skin damage, while ingestion can cause serious gastrointestinal damage and death. Even short-term application of phenol to the skin can produce blisters and burns in animals. Therefore, the removal of such chemicals from industrial effluents is of great importance. Phenol readily reacts photochemically and is rapidly biodegraded aerobically or anaerobically to carbon dioxide or methane (HSG, 1994). One of the cheapest possible solutions to resolve phenol contamination problem is by bioremediation using microbial cells. Many studies on biodegradation of phenol using microorganisms have been reported (Shen et al., 2009; Laowansiri et al., 2008; Celik et al., 2008; Santos et al., 2009). A total of 39 phenol- and p-cresol-degraders isolated from the river water continuously polluted with phenolic compounds of oil shale leachate were studied (Heinaru et al., 2000). Species identification by BIOLOG GN analysis revealed 21 strains of *Pseudomonas fluorescens*. The phenolic compounds and polyphenols have high water solubility due to their hydroxyl groups. Planas et al. (1981) showed that *Myriophyllum spicatum*

*Corresponding author. E-mail: eimanabdelzaher@yahoo.com.

contains phenols. Aerobic processes of pollutants biological treatment are generally preferred to degrade these substances, due to the low costs associated with this option, as well as the possibility of a complete mineralization of the xenobiotic (Collins and Daugulis, 1997). It has been demonstrated that various toxic organic compounds are not eliminated by the conventional biological effluent treatment systems, due to the presence of relatively high concentrations of toxic compound. Furthermore, the treatment of small volumes of concentrated toxic compounds at the site of emission, using specific microbial strains and better reactors, is preferable as this procedure allows a higher control over the process and higher removal efficiencies than those obtained in conventional treatment plants (Schröder et al., 1997).

Phenolic compounds degradation may be carried out by eukaryotic and prokaryotic organisms. Aerobic biodegradation of many classes of aromatic compounds is common and proceeds through the key intermediate, catechol. Eukaryotic microorganisms produce catechol from phenol via an epoxide and a transdiol using a monooxygenase. Prokaryotes introduce the entire oxygen molecule by a dioxygenase reaction forming first a cis-diol. Anaerobically, the aromatic ring is not oxidized, but reduced. The key intermediate in this pathway is a cyclohexanone (Bouwer and Zehender, 1993). Biodegradation of phenol and 4-chlorophenol (4-cp) using a pure culture of *Candida tropicalis* was studied. The results showed that *C. tropicalis* could degrade 2,000 mg l^{-1} phenol alone and 350 mg l^{-1} 4-cp alone within 66 and 55 h, respectively. The capacity of the strain to degrade phenol was obviously higher than that to degrade 4-cp (Jiang et al., 2007). Fountoulakis et al. (2002) found that olive mill wastewaters (OMW) represent a major environmental problem. The large amounts generated, combined with the high phenols and chemical oxygen demand concentrations, are the main difficulties in finding a solution for the management of these wastewaters, which are dangerous for the environment. The capability of *Pleurotus ostreatus* to degrade phenols of OMW in different conditions such as in sterilized and thermally processed at 100°C wastewater, is with and without dilution. According to the experimental results, *P. ostreatus* removed phenols from the culture medium, under all the different conditions that were examined. Gerginova et al. (2007) used *Trihosporon cutaneum* R57 strain which has the ability to grow and utilize some organic compounds including phenol and phenol derivatives. It was established that the strain could degrade and assimilate completely up to 1 gl^{-1} phenol for a period of 18 h. Bending and Read (1997) indicated that most mycorrhizal fungi have only low abilities to degrade phenolic compounds relative to the wood decomposing fungi. Dyeing and textile factory in El-Gharbia Governorate especially in El-Mehalla El-Kobera site I and II is the first source of pollution followed by petrochemical factory in Kafr El-Sheikh and El-Menofia. This study was designed to investigate the most tolerant fungi that can grow in phenol containing media following morphological changes during growth.

MATERIALS AND METHODS

Samples collection

Phenol concentration was assayed in the wastewater collected from three Governorates including El Gharbia which represented two regions sites [El-Mehalla El-Kobera (I), El-Mehalla El-Kobera (II)] Kafre El Sheik which represented two sites (Balteem and Kaleen) and El Menofia which represented one site (Shebin El Koom). 10 soil samples were collected from benzene fuel stations from El-Mehalla El-Kobera (II) due to the presence of high number and variety of fungal isolates. Water and soil samples were collected in sterile bottles (100 ml) and in plastic bags, respectively, transferred directly to the laboratory and preserved at 4°C until used.

Fungal isolation and identification

Fungi were isolated from the contaminated soil samples collected from El-Mehalla El-Kobera II using Czapek's dox medium (Difco Laboratories, Detroit, Mich.) with 1 g/l phenol. The most common fungi were recultivated using the same medium until pure colonies were obtained. The isolated fungi were preserved on the same medium without phenol at 4°C until used. These fungi were identified using the methods described by Watanabe (1994). *Aspergillus ochreceous* was obtained from the culture collected from Botany Department, Faculty of Science, Tanta University, and was used as non tolerant species (control).

Effect of different media with different concentration of phenol on fungal growth

Fungi were cultured on three different solid media to select the best medium for growth in the presence of phenol. The media used were Czapek's Dox, Sabouraud agar and Potato Dextrose agar. All the media were supplemented after autoclave sterilization with different concentrations of phenol (0.05, 0.08, 0.1, 0.12 and 0.14 g/100 ml) which were added by microfilter (0.45 µm) after medium sterilization. Fungal disk of 5 days old culture, with 5 mm diameter was used as inoculum on the tested medium. The growth was detected by measuring the diameter of the colony (mm) after 6 days of incubation at 30°C.

Effect of some additives on growth of *Hormodendrum bergeri*, *Aspergillus flavus* var. *coulmnaris* and *Fusarium oxysporum* in the presence of phenol

The effect of the addition of some additives on fungal growth at phenol concentration of 0.1 g/100 ml (w/v) on Czapek's dox medium after 6 days was studied. The studied mate-rials were vitamin B_1, B_6, B_{12}, mixture of B_1 + B_6 + B_{12}, indole acetic acid, nicotinic and tryptophane at concentration of 0.1 g/100 ml, and they were added by microfilter (0.45 µm) after medium sterilization.

Phenol assay

Phenol concentration was determined using 4 amino antipyrine method which was described by Neufeld and Poladino (1985). This method is a colormetric method for determining free monomer

Table 1. Phenol concentrations detected in water samples collected from 5 cities belonging to three different Governorates in Egypt.

Governorate	City	Sample number	Phenol (mg/L)
El Gharbia	El Mehalla El-Kobera (I)	1	0.016 ± 0. 0016*
		2	0.038 ± 0. 0013
		3	0.009 ± 0. 001
		4	0.042 ± 0. 01
	El Mehalla El-Kobera (II)	5	0.033 ± 0. 01
		6	0.088 ± 0. 001
Kafer El Sheik	(Baltem) (I)	7	0.017 ± 0. 001
		8	0.015 ± 0. 01
	Kaleen (II	9	0.008 ± 0. 001
		10	0.022 ± 0. 01
		11	0.036 ± 0. 013
		12	0.017 ± 0. 01
		13	0.011 ± 0. 001
		14	0.011 ± 0.0011
		15	0.017 ± 0. 001
El Menofia	Shebin El Koom	16	0.073 ± 0. 01
		17	0.066 ± 0. 01
		18	0.004 ± 0. 001
		19	0.009 ± 0.001
		20	0.023 ± 0. 01
		21	0.027 ± 0. 017
		22	0.023 ± 0. 01
		23	0.059 ± 0. 01
		24	0.084 ± 0. 001
		25	0.030 ± 0. 01

*Value is the mean ± SD of three replicates.

phenol in aqueous solutions. The blank and samples were treated as follows: to each sample, 2.5 ml of 0.5 N ammonium hydroxide solutions were added and the pH was adjusted to 7.9 with phosphate buffer at pH 6.8. 10 ml of 4 amino antipyrine solution (2 g/100ml) and 1 ml of potassium ferricyannide solution (8 g/100 ml) were added respectively, and mixed well after each addition. 15 min was needed for reproducible color development. After 15 min, the solutions were transferred to cuvettes. The absorbance of the sample was read against the blank in spectrophotometer at 500 nm. The phenol concentration was calculated from a standard curve of phenol (0.05 to 0.5 mg/100 ml).

Scanning electron microscope

F. oxysporum and *A. flavus var. coulmnaris* were chosen to detect the difference between fungal mycelia and spore before and after treatment with phenol at 0.1g/100 ml after six days of growth on Cazpek's dox agar medium. They were examined according to the method described by Hayat (1981) using scanning electron microscope (Model JEOL, JSM-5200LV).

RESULTS

The results indicated that the phenol concentration in the 25 samples collected from the three Governorates (El Gharbia, Kafre El Sheikh and El-Menofia) ranged from 0.008 to 0.088 mg/L (Table 1 and Map 1). Sample No (6) which was collected from El-Mehalla El-Kobera (II) had the highest phenol concentration (0.088 mg/L phenol/ liter) followed by sample No 24 (0.084 mg/L) which was collected from Shebin El Koom and sample No 17 (0.066 mg/L) which was collected from Kaleen city.

The phenol concentration in samples collected from El-Mehalla El-Kobera 1 was in the range of 0.009 to 0.042 with mean value of 0.02625 mg/L but the range was 0.033 to 0.088 mg/ml with mean value of 0.0605 mg/ml for samples collected from El-Mehalla El-Kobera II. The concentration was in the range of 0.008 to 0.036 mg/L with mean value of 0.017 to 0.015 mg/L in samples from Kaleen II but the range was 0.008 to 0.017 mg/L with mean value of 0.016 mg/L in samples from Baltem I. The phenol concentration in samples collected from Shebin El Koom ranged from 0.023 to 0.084 mg/L with mean value of 0.0398 mg/L.

H. bergeri, F. oxysporum and *A. flavus* var*. coulmnaris* were the most dominant fungal species in the collected soils. Therefore, they were isolated, purified, identified

Map 1. Isolation sites in Delta region*.

and chosen for subsequent study. The efficiency of growth of *H. bergeri*, *F. oxysporum* and *A. flavus var. coulmnaris* grown on different culture media containing phenol was evaluated and compared with that of *Aspergillus ochraceous* to determine the optimum concentration of phenol tolerance for the tested fungi.

The growth of *H. bergeri*, *F. oxysporum* and *A. flavus var. coulmmaris* on the three different solid media (Sabouraud, PDA and Czapek's Dox) in the presence of different concentrations of phenol ranging from 0 to 0.14 g/100 ml were determined (Table 2) and compared with the result of *A. ochreceous* as the control. It was found that Czapek's Dox was the most suitable medium for the fungal growth in the presence of phenol up to 0.1 g/100 ml (Table 2). Maximum growth was recorded on Czapek's Dox for *F. oxysporum* and *A. flavus var. coulmnaris* up to 0.1 g/100 ml. In the absence of phenol, the diameter of the growth ranged from 80-86mm, 83-86mm respectively and increased phenol concentration decreased the fungal growth. The lowest growth was found at 0.1 g/100 ml where the colony diameter ranged from 10 to 18 mm. The less tolerant fungus was *A. ochraceous*; no growth was found at 0.12 g/100 ml. On the other hand, *H. bergeri, F. oxysporum* and *A. flavus* var. *coulmnaris* showed mode-rate growth on Czapek's Dox agar medium (growth dia-meter ranged from 8 to 11 mm).

Table 3 shows the different types of vitamin B that were more effective for the fungal growth on 0.1 g/100 ml phenol on Czapek's Dox medium by *H. bergeri* after 6 days. Mixture of vitamins (B1 + B6 + B12) increased colony diameter of *H. bergeri* (86 mm) followed by vitamin B6 alone, vitamine B1 alone, vitamin B12 alone and finally by indole acetic acid at colony diameter 79, 78, 76 and 59 mm, respectively. On the other hand, the mixture of vitamin (B1 + B6 + B12) had more effect on the ability of *F. oxysporum* to grow more than *H. bergeri*, and its growth reach up to 89 mm but *A. flavus var. calumnarius* had 87 mm growth.

Scanning electron microscope

A. flavus var. coulmnaris structure, grown on phenol at 0.1 g/100 ml showed changes in the organism wall and led to smooth membranous wall (Figure 1); when com-

Table 2. Fungal growth (colony diameter, mm) on three different culture media with different phenol concentrations.

Fungal	Media used	Phenol concentration (g/100 ml)					
		0	0.05	0.08	0.1	0.12	0.14
H. bergeri	Sabouraud	75 ± 07*	30 ± 03	18 ± 01	10 ± 01	06 ± 04	05 ± 0
	Czapek's	80 ± 08	59 ± 05	20 ± 02	14 ± 06	08 ± 08	05 ± 0
	PDA	86 ± 08	44 ± 04	24 ± 4	10 ± 01	06 ± 04	05 ± 0
F. oxysporum	Sabouraud	80 ± 11	40± 01	22 ± 01	18 ± 01	06 ± 01	05 ± 0
	Czapek's Dox	87 ± 08	62 ± 01	40 ± 02	22 ± 01	11 ± 02	05 ± 0
	PDA	85 ± 12	45 ± 02	30 ± 03	13 ± 01	07 ± 04	05 ± 0
A. flavus var. columnaris	Sabouraud	83 ± 17	40 ± 01	21 ± 01	11 ± 01	06 ± 04	05 ± 0
	Czapek's Dox	86 ± 11	52 ± 02	42 ± 01	12 ± 01	08 ± 01	06 ± 01
	PDA	86 ± 11	32 ± 01	30 ± 01	10 ± 02	05 ± 0	05± 0
A. ochraceous	Sabouraud	86 ± 17	30 ± 01	21 ± 01	10 ± 01	05 ± 0	05 ± 0
	Czapek's Dox	86 ± 11	32 ± 02	22 ± 01	10 ± 01	05± 0	05 ± 0
	PDA	86 ± 11	35 ± 01	25 ± 01	10 ± 02	05± 0	05± 0

*Value is the mean diameter ± SD. ≤ 10 mm, poor growth, mean diameter; 5 mm, no growth.

Table 3. Effect of some activations on growth of *H. bergeri, A. flavus* var. *columnaris* and *F. oxysporum* on Czapek's Dox medium containing phenol at concentrations of 0.1% (w/v).

Parameter	Growth (colony diameter, mm)		
	A. flavus* var *Columnaris	***F. oxysporum***	***H. bergeri***
Vitamine (B1)	80 ± 11*	82 ± 12	78 ± 6
Vitamine (B6)	77 ± 8	78 ± 5	70 ± 7
Vitamine (B12)	76 ± 8	77 ± 2	70 ± 11
Mix B1 + B6 + B12	87 ± 10	89 ± 4	80 ±10
Indole Acetic Acid	50 ±6	50 ±6	39 ±4
Nicotinic Acid	45 ±4	50 ±6	40±4
Tryptophane	23 ±8	32 ±4	30 ±7
Control	20 ±4	30 ±3	14 ±2

*Value is the mean ± SD of three replicates.

pared to the control, hyphae had spins or hairy like appendages that protruded from the hyphae (Figure 2). *F. oxysporum* grown at phenol of 0.1 g/100 ml, induced a brown pigmentation on the culture medium and the hyphae became thinner and torned (Figure 3) when compared to normal hyhae in the control (Figure 4). In the case of *F. oxysporum*, the spores appeared normal in the control culture (Figure 5). Also, phenol decreased the number of spores of *F. oxysporum* in samples grown on Czapek's medium when compared with the control sample (Figure 6).

DISCUSSION

Widespread contamination of water by phenol has been recognized as an issue of growing importance in recent years. Phenol is a potential or known human carcinogen and is of considerable health concern, even at low concentration. All waste water samples collected from the selected area in Egypt were contaminated with phenol. The level of contamination changed from city to city. Industrial cities like El-Mehalla El-Kobera II which was famous for textile industries had the most contaminated waste water. More quantities of phenol was found in India. The initial concentration of phenol in the coke processing waste water was found to be between 200 and 240 mg L^{-1} (Chakraborty et al., 2010). For instance, Malaysian guidelines limit phenol concentration to 0.001 mg l-1 in wastewater (DOE, 1974). Hence, the treatment of wastewater containing phenol is a necessity. Many technologies have been investigated for removing and degrading phenolic compounds in wastewater. They included, adsorption (Rengaraj et al., 2002), extraction by liquid membrane (Lin et al., 1999), oxidation (Awad and Abuzaid, 2000) and biodegradation (Miland et al., 1996).

It was found that three fungal genera were isolated from the contaminated soil grew in media that contained phenol. Similarly, a total of 97 of the fungal isolates were able to grow in Sabouraud media supplemented with 2

Figure 1. *A. flavus* var. *columnaris* without phenol grown on Czapek's agar medium (control hyphae) (500x).

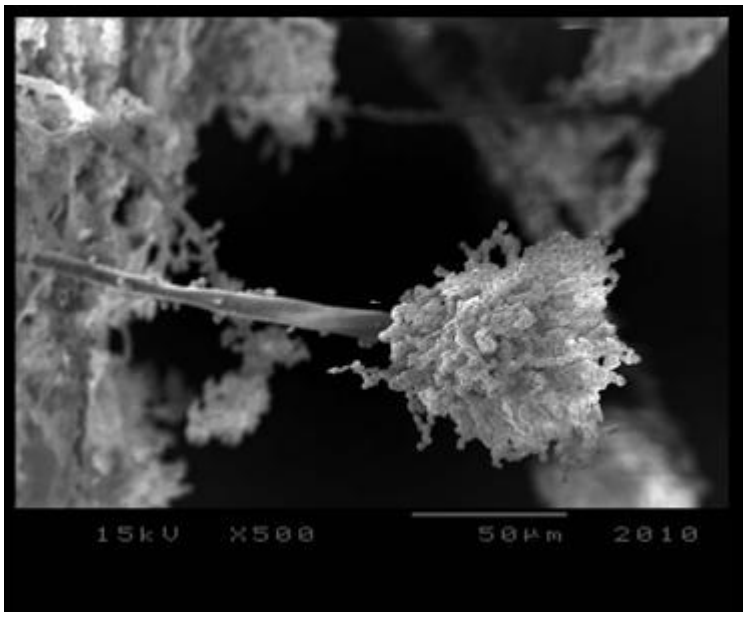

Figure 2. *A. flavus* var. *columnari* without phenol grown on Czapek's agar medium containing phenol (0.1 g/100 ml) treated hyphae (500x).

mM of phenol which were isolated from a stainless steel industry wastewater (Linardi and Santos, 2004). *Graphium* was the most frequent strain (seven) followed by *Penicillium* and *Aspergillus* (three) and *Fusarium* (two). *Graphium, Aspergillus*, *Fusarium* and *Penicillium* can utilize aromatic and aliphatic hydrocarbons (Linardi and Santos, 2004). Studies have shown that *A. fumigatus* is able to grow in cresol, 4 chloro-phenol and phenol as sole carbon sources (Jones et al., 1993). Free or immobilized cells of *Fusarium flocciferum* have been reported to use up to 1 g of phenol/L (10.6 mM) (Anselmo and Novais, 1992). *Graphium* sp. FLB4 degraded 75% of 10 mM of phenol in 168 h (Linardi and Santos, 2004). Alginate immobilized cells of *Aureobasidium pullulans* FE13, degraded 20.45$mgl^{-1}h^{1}$ for 16 mM of phenol but free cell could degrade 18.35 $mgl^{-1}h^{1}$ with the same phe-

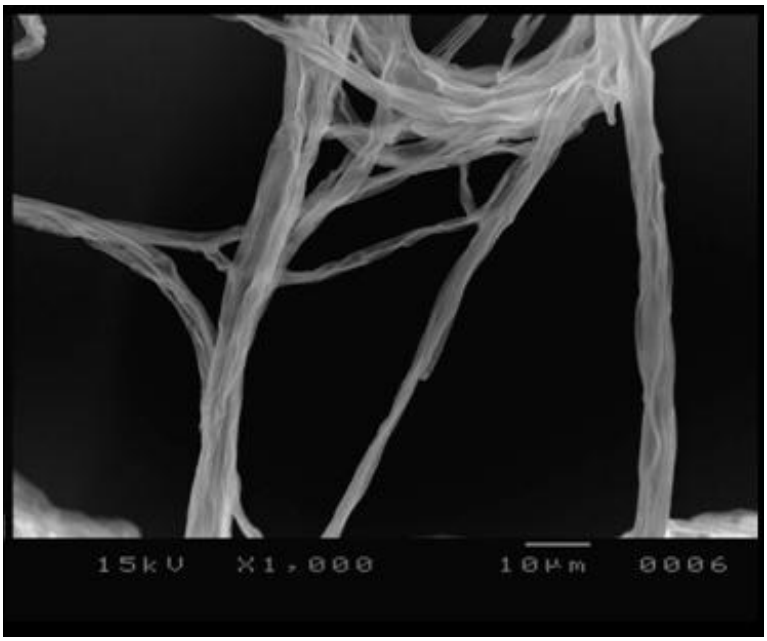

Figure 3. *F. oxysporum* hyphae from culture grown on Czapek's on medium with (0.1 g/100 ml) (1000x).

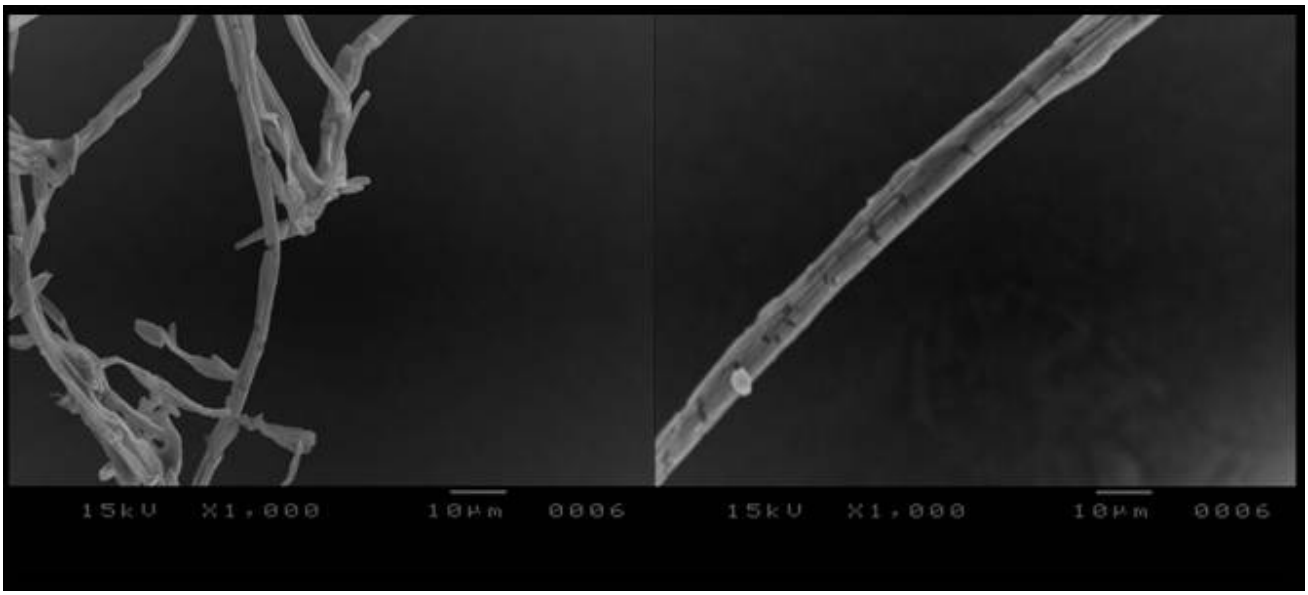

Figure 4. *F. oxysporum* hyphae from culture grown on Czapek's on medium with without phenol at 1000x.

nol concentration (Lúcia Dos Santos et al., 2009).*Candida tropicalis* could degrade 2.000 mgl^{-1} phenol alone and 350 mgl^{-1} 4-chlorophenol alone within 66 and 55 h, respectively (Jiang et al., 2007).

Scanning electron microscope

Morton (1986) found that the mixture of phenol with glycerin mixed with 1:1 (v/v) and 10% polyvinyl alcohol-lactic acid-phenol (56:22:22 v/v/v) caused different swelling of cell wall and laminae in spores of *Acaulospora dilatata* and *Acaulospora rugosa* so that, they resembled adherent unit walls that transformed the beaded wall into a smooth membranous wall. On the other hand, Upadhyay and Hofrichter (1993) found that all cultures of *Pleurotus* grew well up to 4 mM of phenol on Czapek Dox agar except *Agaricus bisporus*, but phenol induced a brown

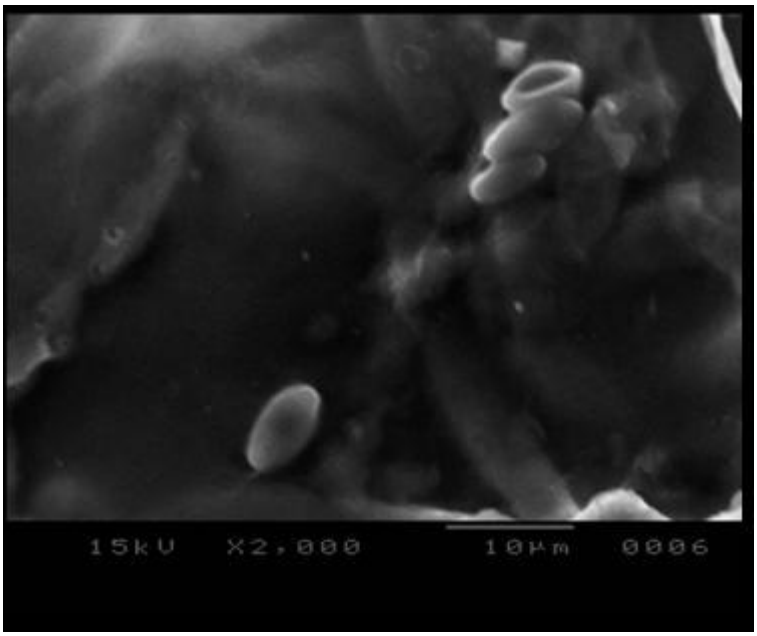

Figure 5. *F. oxysporum* spores from culture grown on Czapek's on medium with 0.1 g/100 ml at 2000x.

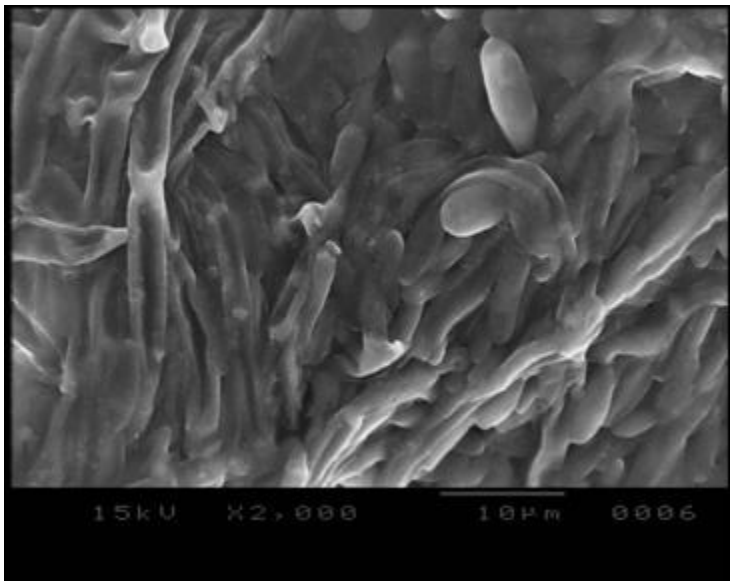

Figure 6. *F. oxysporum* spores from culture grown on Czapek's on medium without phenol at 2000x.

pigmentation of culture medium of *Pleurotus flabellatus* and *Pleurotus pulmonarius* which metabolized 67 and 64 mg/l phenol in 10 days. Similarly, for *F. oxysporum* grown at phenol concentration of 0.1 g/100 ml, a brown pigmentation on the culture medium was gotten and the hyphae became thinner and torned. It was found that the addition of vitamins singly or in combination enhanced fungal growth in the presence of phenol. Kafkewitz et al. (1996) reported that the addition of vitamin solution containing biotin, folic acid, pyridoxine hydrochloride, riboflavin, thiamine hydrochloride, niacin, pantothenic acid, cyanocobalamin, p-aminobenzoic acid and thioctic acid (total final concentration: ≤ 600 ppb) resulted in a 7 to 16% increase in the amount of phenolic compound degraded.

On the basis of these results, *H. bergeri*, *F. oxysporum*

and *A. flavus* var. *coulmnaris* are considered to have good prospects for their application in microbial detoxification of phenol polluted industrial wastewater. A general comparison of the major pathways for the catabolism of aromatic compounds in bacteria and fungi has revealed that the initial conversion steps carried out may be by different enzymes and compounds that are transformed into protocatechuate and catechols as proposed (Van Der Meer et al., 1992).

Conclusion

The contamination of the water by phenol in the selected area was investigated in this work. It was concluded that the most phenol polluted water samples were in El-Mehalla El-Kobera II, Shebin El-Koom, El-Mehalla El-Kobera I, Balteem I and Kaleen II, respectively. *F. oxysporum*, *A. flavus* var. *coulmnaris* and *H. bergeri* had more ability to grow on phenol more than *A. ochraceous*. Also, morphological changes and hairy like appendages in *A. flavus columnaris* and decrease in number of *F. oxysporum* spores were found. So, it could be concluded that tolerant fungi, which have the ability to uptake phenol, had several morphological changes.

REFERENCES

Anselmo AM, Novais JM (1992). Degradation of phenol by immobilized mycelium of *Fusarium flocciferum* in continuous culture. Water Sci .Technol. 25: 161-168.

Awad YM, Abuzaid NS (2000). The influence of residence time on the anodic oxidation of phenol Separation and Purification Technology, 18: 227-236.

Bending GD, Read DJ (1997). Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. Mycol. Res. 101(11): 1348-1354.

Bodalo A, Gomez JL, Gomez M, Leon G, Hidalgo AM, Ruiz MA (2008). Phenol removal from water by hybrid processes: Study of the membrane process step. Desalin. 223: 323-329.

Celik GY, Aslim B, Beyatli Y (2008). Enhanced crude oil biodegradation and rhamnolipid production by *Pseudomonas stutzeri* strain G11 in the presence of Tween-80 and Triton X-100. J. Environ. Biol. 29: 867-870.

Chakraborty S, Bhattacharya T, Patel TN, Tiwari KK (2010). Biodegradation of phenol by native microorganisms isolated from coke processing wastewater. J. Environm. Biol. 31: 293-296.

Collins LD, Daugulis AJ (1997). Biodegradation of phenol at high intial concentrations in two-phase partitioning batch and fed-batch bioreators. Biotechnol. Bioeng. 55: 155-162.

Fava F, Armenante PM, Kafkewitz D (1995). Aerobic degradation and dechlorination of 2-chorophenol, 3-chlorophenol and 4-chlorophenol by a *Pseudomonans picketti* strain. Lett. Appl. Microbiol. 21: 307-312.

Fountoulakis MS, Dokianakis SN, Kornaros ME (2002). Removal of phenolics in olive mill wastewaters using the white-rot fungus *Pleurotus ostreatus*. Water Res. 36(19): 4735-4744.

Gerginova M, Dimova N, Ivanova D, Alexieva Z (2007). Bioremediation of soils contaminated with aromatic compounds. NATO Sci. Series, 76: 67-74.

Hayat MA (1981). Principles and techniques of electron microscopy: Biological applications, Vol. 1. 2nd ed., international pub. house, barkeley heights, New Jersy.

Heinaru E, Truu J, Stottmeister U, Heinaru A (2000). Three types of phenol and p-cresol catabolism in phenol- and p-cresol-degrading bacteria isolated from river water continuously polluted with phenolic compounds. 1 Mar 2000 FEMS Microbiol. Ecol. 31(3): 195-205.

HSG (1999). Phenol Health and Safety guide. Published by the World Health Organization for the International Programme on Chemical Safety. No. 88.

Jayachandran VP, Kunhi AAM (2008). Degradation of 3-chlorobenzoate and phenol singly and in mixture by a mixed culture of two orthopathway- following Pseudomonas strains. J. Ind. Microb. Biotechnol. 5: 120-122.

Jiang Y, Wen J, Lan L, Hu Z (2007). Biodegradation of phenol and 4-chlorophenol by the yeast *Candida tropicalis*. Biodegr. 18(6): 719-729.

Jones KH, Trudgill PW, Hopper DJ. (1993). Metabolism of p-cresol by the fungus *Aspergillus fumigatus*. Appl. Environ. Microbiol. 59: 1125-1130.

Kafkewitz D, Fava F, Armenante PM (1996). Effect of vitamins on the aerobic degradation of 2-chlorophenol, 4-chlorophenol, and 4-chlorobiphenyl. Appl. Microbiol. Biotechnol. 46(4): 414-421.

Laowansiri S, Vinitnantharat S, Chaiprasert P, Ha SR (2008). Anaerobic degradation kinetics of reactive dye with different carbon sources. J. Environ. Biol. 29: 309-314.

Lin SH, Pan CL, Leu HG (1999). Liquid membrane extraction of 2-chlorophenol from aqueous solutions. J. Hazar. Mat. 65: 289-304.

Linardi VR, Santos VL (2004). Biodegradation of phenol by a filamentous fungi isolated from industrial effluents-identification and degradation potential. Process Biochem. 39: 1001-1006.

Lúcia Dos Santos V, de Souza Monteiro A, Braga TD, Santoro MM (2009). Degradation by *Aureobasidium pullulans* FE13 isolated from industrial effluents. J. Hazar. Mat. 161(2-3): 1413-1420.

Marrot B, Barrios-Martinez A, Moulin P, Roche N (2006). Biodegradation of high phenol concentration by activated sludge in an immersed membrane bioreactor. Biochem. Eng. J. 30: 174-183.

Miland E, Smyth MR, Fagain CO (1996). Phenol removal by modified peroxidases, J. Chem. Technol. Biotechnol. 67: 227-236.

Morton J (1986). Effects of mountants and fixatives on wall structure and metzer's reaction in spores of two *Acaulospora* species (endogonaceae). Mycol. 78(5): 787-794.

Neufeld RD, Poladino SB (1985). Comparision of 4-aminoantipyrine and gas chromatography techniques for analysis of phenol compound. J. Water Pollut. Control Fed. 27(10): 1040-1044.

Planas D, Sarhan F, Dube L, Godmaire H, Cadieux C. 1981. Ecological significance of phenolic compounds of *Myriophyllum spicatum*. Verh. Int. Verein. Limnol. 21: 1492-1496.

Rengaraj S, Moon SH, Sivablan R, Arabind B, Murugesan V (2002). Removal of phenol from aqueous solution and resin manufacturing industry wastewater using an agricultural waste: rubber seed coat, J. Hazar. Mat. 89: 185-196.

Rittmann BE, McCarty PL (2001). Environmental Biotechnology: Principles and Applications New York.

Santos VL, Andrea de SM, Braga DT, Santoro MM (2009). Phenol degradation by Aureobasidium pullulans FE13 isolated from industrial effluents. J. Hazar. Mat. 16: 1413-1420.

Schröder M, Müller C, Posten C, Deckwer WD, Hecht V (1997). Inbibition kinetics of phenol degradation from unstable steady-state data. Biotechnol. Bioeng. 54: 567-576.

Shen J, Rui He Yu H, Wang L, Zhang J, Sun X, Li J, Han W, Lu Xu (2009). Biodegradation of 2,4,6-trinitrophenol (picric acid) in a biological aerated filter (BAF). Bioresour. Technol. 11: 1922-1930.

Upadhyay RC, Hofrichter M (1993). Effect of phenol on the mycelial growth and fructification in some of basidiomycetous fungi. J. Basic Microbiol. 33(5): 343-347.

Van Der Meer JR, De Vos WM, Harayama S, Zehnder AJB (1992). Molecular mechanisms of genetrics adaptation to xenobiotic compounds. Microbiol. Rev. 56: 677-694.

Watanabe T (1994). Pictorial Atlas of Soil and Seed Fungi, Morphologies of Cultured Fungi and Key to Species. CRC Press, Lewis Publishers Inc., USA.