Identification of contaminated soil from isolated fungi in Riyadh province

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Abstract: The chemical and microbiological (on the basis of fungi) analysis of the soils was carried out in the Riyadh region with thirteen mycobiota samples, which were surveyed in Explosive Institute in Riyadh. This study aims to explore does isolated fungi in contaminated soils can be used as bioremediation agents for explosive materials. High significant differences in fungal frequency between presented in five regions. Fungal distribution analysis was performed by dilution plating of the explosive-contaminated soil. The fungal genera found in explosive-contaminated soil in the state in order of decreasing predominance were *Fusarium sambucinum*, *Chaetomium globosum, Nigrospora sacchari,* and *Ulocladium chartarum*. From the present study we conclude that some of the isolated fungi of contaminated soils can be used as bioremediation agents for explosive materials.
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Introduction

The major component of earth's ecosystem is soil, comprises the organic matter, minerals, gasses and large numbers of macro and microorganisms (Chandrasekhar *et al.,* 2014). The major role of fungi in soil is enormously complex one and is fundamental to the soil ecosystem. The important fungal component of the soil microbiota typically constituting more soil biomass than bacteria, depending on soil depth and nutrient conditions (Soni *et al.,* 2014). In the agricultural soils and near surroundings, the isolation of fungi belongs to *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* genera species (Pit *et al.,* 2009). Fungi are eukaryotic, filamentous, most commonly spore-producing organisms, saprophyte or parasite in animals and humans, may be found everywhere. The intensity of fungi spores increases depending on air pollution (Suerdem *et al.,* 2009). The *Aspergillus* section *Flavi* strains are also commonly isolated worldwide from agricultural soils (Saito *et al.,* 1986; Klich *et al.,* 2002; Horn *et al.,* 2003; Barros *et al.,* 2003). Peanuts, corn, and soybeans are crop ecosystems often invaded before harvest by these *Aspergillus* species both in tropical and subtropical regions (Nesci *et al.,* 2006). Long-term contaminated soil (aged contaminated soil) longer periods of contact of Persistent organic pollutants (POPS) with soil constituents allow more time for sorption reactions to occur and subsequent slow migration and/or diffusion of POPS into soil micro-pores renders pollutants unavailable for microbial transformation even by extracellular enzymes. General microbial activity is known to be affected by pore size; for example, carbon turnover rates were lower when organic substrates were located in smaller soil pores at low soil metric potential (Killham *et al.,* 1997) and nitrifying bacteria were found to be restricted to soil pores of 136 to 214 mm in size (Fair *et al.,* 1994). Due to the limited research and knowledge in the Saudi soil and importance of fungi, we aimed to explore this research to regulate does the fungal isolation in contaminated soil can be used as bioremediation agents for explosive materials.

Materials And Methods

Soil Samples. Soil samples were collected from 5 different regions in Explosive Institute (EI) in Riyadh, Saudi Arabia. 13 samples of mycobacteria were collected from different points as core sampler technique. The samples were sieved at the site with 10 mm sieve, stored in glass jars and frozen until analysis. The dry weights, ignitions losses and mineral soil concentrations were determined (Bjorklof *et al.,* 2009). The fungal community in soil was more diverse.

Isolation and characterization of fungi. Serial dilution agar plating, Warcup soil plate, and Waksman Direct inoculation methods were employed for the isolation of microbes from soil; the suspension was diluted up to 10-5. The aliquots were cultured for fungus on Czapek Dox Agar (NaNO3 2.0 g, KCl 0.5 g, K2HPO4 1.0 g MgSO4.7H2O 0.5 g, FeSO4. 7H2O 0.01 g,); and Potato Dextrose Agar (Peeled potato200.0 g, Dextrose 20.0g) media. For primary isolation Rose Bengal (30mg/L) was also added to the medium. Three plates from each soil samples were incubated for 24-96 h at 25+2 °C, and each morphologically unique fungal colony was sub-cultured and purified using standard techniques. The fungal species were identified and characterize based

on their morphological characters and microscopic analysis was carried out with taxonomic guides and standard procedures. The following morphological characteristics were evaluated: colony growth (length and width), presence or absence of aerial mycelium, colony color, the presence of wrinkles and furrows, pigment production etc. (Rohilla *et al.,* 2012).

Results

Thirteen isolates of fungi have been isolated from explosive-contaminated soil samples collected from EI. The fungal community in soil was more diverse. The most frequent *Fusarium* and *Drechslera* fungi were two species of the genus. High significant differences in fungal frequencies was presented in five regions and documented as tables (1-5) and showed in the occurrence of fungal species isolated from explosive soil. The mycological analysis was performed by dilution of plating of the explosive-contaminated soil. Table 1 showed the lists and occurrence of fungi isolated from explosive contaminated soil. The fungal genera found in explosive-contaminated soil in the state in order of decreasing predominance were *Fusarium sambucinum, Papulaspora irregularis, Aspergillus fumigates,* and *Drechslerabiseptata*. While, the low fungal species contaminated-soil with explosive material were *Ulocladium consortiale* and *Fusarium equiseti*. Other isolated fungal genera including *Macrophomina phaseolina* and *Coleophoma empetri* had a very low percentage of occurrences (Table 1).

Mycological analysis was performed by dilution plating of the explosive-contaminated soil and showed the occurrence of each fungal species isolated from multiple sites. Table 2 showed the lists and occurrence of fungi isolated from explosive contaminated soil. The fungal genera found in explosive-contaminated soil in the state in order of decreasing predominance were *Fusarium sambucinum, Phoma exigua, Ulocladium chartarum*, and *Papulaspora irregularis*. While, the fungal species contaminated-soil with explosive material were *Drechslera sp*. and *Fusarium equiseti*. Other isolated fungal genera including *Drechslerabiseptata* and *C. cladosporioides* had a very low percentage of occurrences (Table 2).

Fungal distribution analysis was performed by dilution plating and extracting the fungal species of the explosive-contaminated soil. Table 3 showed the lists and occurrence of fungi isolated from explosive contaminated soil. The fungal genera found in explosive-contaminated soil in the state in order of decreasing predominance were Fusarium sambucinum, *Chaetomium globosum, Nigrospora sacchari,* and *Ulocladium chartarum*. While, the low fungal species contaminated-soil with explosive material were *Ulocladium consortiale* and *Embellisia allii*. Other isolated fungal genera including *Fusarium equiseti* and *Fusarium solani* had a very low percentage of occurrences. Mycological analysis was performed by dilution plating of the explosive-contaminated soil. The isolation of fungal species were separated from five sites. Table 4 showed the lists and occurrence of fungi isolated from explosive contaminated soil. The fungal genera found in explosive-contaminated soil in the state in order of decreasing predominance were *Mucor circinelloides, Fusarium sambucinum, Macrophomina phaseolina, Ulocladium consortiale*, and *Drechslerabiseptata*. While, the low fungal species contaminated-soil with explosive material were *Chaetomium globosum sp*. and *Embellisia allii*. Other isolated fungal genera including *Phoma herbarumand Nigrospora sacchari* had a very low percentage of occurrences.

Fungal distribution analysis was performed by dilution plating of the explosive-contaminated soil and fungal species were separated. Table 5 showed the lists and occurrence of fungi isolated from explosive contaminated soil. The fungal genera found in explosive-contaminated soil in the state in order of decreasing predominance were *Aspergillus fumigates, Fusarium sambucinum, Ulocladium sp., Mucor circinelloides*, and *Fusarium equiseti*. While, the fungal species contaminated-soil with explosive material were *Ulocladium consortiale* and *Drechslerabiseptata*. Other isolated fungal genera including *Phoma astragali* and *Embellisia alii* had a very low percentage of occurrences (Table 5).

ANOVA (Table 6) showed very highly significant (P = 0.0000) effects of site x region interaction on all the tested parameters.

Discussion

Soil microbial communities are among the most complex and diverse assemblages in the biosphere and they have important role in all of the ecosystem services provided by soils (Bjorklof *et al.,* 2009). Soil is the habitat for many organisms including bacteria, fungi, algae, viruses and protozoa. It supports the growth of a variety of unstressed plants, animals and soil microorganisms usually by providing a diverse physical, chemical and biological habitat. Microorganisms are found in large numbers in soil usually between one and ten million microorganisms are present per gram of soil with bacteria and fungi being the most prevalent. The different microorganisms like fungi, bacteria are known to tolerate and accumulate heavy metals and findings of the fungal activity was effected minorly which was in accordance with earlier studies indicating that fungi were less sensitive to heavy metals than bacteria. The short-term effects on microbial activity clearly indicate that bacteria and fungi were affected

differently by the addition of the metals (Iram *et al.,* 2011; Muller *et al.,* 2001; Khan *et al.,* 2002; Yoder *et al.,* 1937). Fungi play a central role in biodegradation/decomposition, produces an arrays of extracellular enzymes. Filamentous fungi has been linked up with biodegradation, wide range of Nitromatic and nitroamines explosives which could contribute significantly to bioremediation efforts (Hughes *et al.,* 2012; Ferrari *et al.,* 2011). The ligninolytic fungi can secrete extracellular oxidative enzymes, which enable lignin degradation (Liers *et al.,* 2011). Lignin is an amorphous and complex biopolymer whose aromatic structure is similar to the aromatic molecular structure of some environmental pollutants, such as cyclic nitramines, pesticides, polychlorinated biphenyls and polycyclic aromatic hydrocarbons (Nagendran *et al.,* 2014). *Phanerochaete chrysosporium* is a white rot fungus capable of mineralizing RDX and HMX. The fungus mineralized 52.9% of an initial RDX concentration in 60 days in liquid culture to mainly CO_2 and N_2O and 20% of an initial HMX concentration in 58 days when added to soil slurries of ammunition contaminated soil, to yield the same end products. Historically, TNT is one of the most widely used military explosives in the world (Sheramata *et al.,* 2000; Fournier *et al.,* 2004; Rosenblatt *et al.,* 1991; Eaton *et al.,* 2011). Harter *et al.,* (1985) estimated the worldwide production of TNT to be at 106 kg per year. The persistence of TNT in soils contaminated during World War II and the Korean conflict reveals its relative resistance to degradation by indigenous micro-organisms, which is a result, in part, of its toxicity to biological systems (Esteve-Nunez *et al.,* 2001). The electrophilic nature of the nitro group causes TNT readily to oxidize biological reductants, causing toxicity directly or by formation of other reactive products such as nitroarene radicals (Manson *et al.,* 1985). In addition, the nitro groups draw electrons from the aromatic bonds, effectively reducing the electron density of the conjugated aromatic system. As a result, TNT is resistant to degradation via electrophilic attack by oxygenases (Vorbeck *et al.,* 1998; Rieger *et al.,* 1995). In order for the aromatic ring to be cleaved, organisms must first remove or transform the nitro groups. The abundance, persistence and resistance of TNT make it one of the most intensely studied hazardous organonitro compounds with respect to bioremediation. Consequently, the largest body of work and the bulk of this literature focus on the fungal degradation of TNT, especially by *P. chrysosporium*. Some of the earliest published work on fungal degradation of TNT was that of by Klausmeier *et al.,* (1974).

Fungal growths were observed, identified and reported in colony forming units per gram of dust (cfu/g of dust). The appearance and the color were recorded macroscopically. The microscopic investigation was conceded and identified at the required site. The bulk of obtained culture was placed and teased out into a clean glass slide upon a drop of lacto phenol cotton blue using sterile inoculating needles and covered with clean coverslip and then observed under the microscope using x4, x10 and x40 objectives. Identification was based on characteristic morphology and compared to the mycological atlas for confirmatory identification (Buhari *et al.,* 2012).

Table 1. Mean of occurrence for fungal genera and species isolated from contaminated soil samples with explosive material collected from Explosive Institute (EI) in Riyadh (Region1).

Fungal species	Mean
Aspergillus fumigatus	66.67 B-E
Cochliobolus lunatus	23.33 I-P
Coleophoma empetri	17.77 K-P
Drechslera biseptata	47.70 C-L
Embellisia allii	42.57 C-O
Fusarium equiseti	28.23 H- P
Fusarium sambucinum	70.87 A-D
Fusarium solani	24.07 I-P
Macrophomina phaseolina	18.53 J-P
Papulaspora irregularis	58.87 C-D
Ulocladium consortiale	38.87 D-O
white sterile mycelium	29.10 H-P

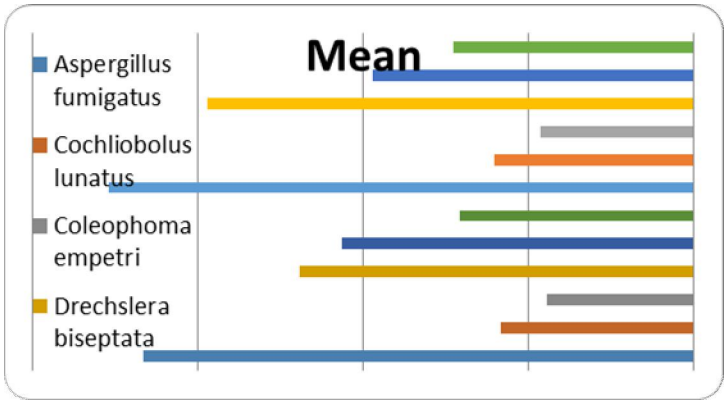

Table 2. Mean of occurrence for fungal genera and species isolated from contaminated soil samples with explosive material collected from Explosive Institute (EI) in Riyadh (Region2).

Fungal species	Mean
Aspergillus fumigatus	44.33 C-N
C. cladosporioides	4.667 P
Coleophoma empetri	29.00 H-P
Drechslera biseptata	15.50 K-P
Drechslera sp.	43.60 C-N
Fusarium equiseti	48.60 C-K
Fusarium sambucinum	100.0 A
Fusarium subglutinans	71.00 A-C
Macrophomina phaseolina	51.37 C-J
Papulaspora irregularis	56.37 C-I
Phoma exigua	95.33 AB
Ulocladium chartarum	63.33 C-F
Ulocladium consortiale	40.17 D-O
White sterile mycelium	36.67 E-P

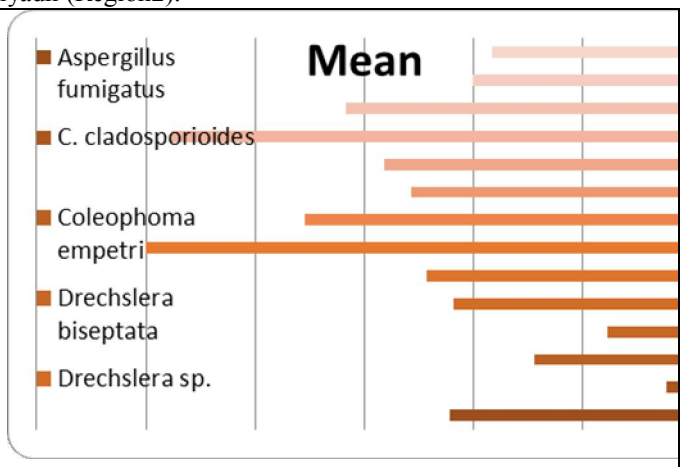

Table 3. Mean of occurrence for fungal genera and species isolated from contaminated soil samples with explosive material collected from Explosive Institute (EI) in Riyadh (Region 3).

Fungal species	Mean
Alternaria alternata	31.33 F-P
Chaetomium globosum	48.00 C-L
Coleophoma empetri	29.63 H-P
Embellisia allii	28.43 H-P
Fusarium equiseti	17.83 K-P
Fusarium solani	11.67 NOP
Fusarium sambucinum	48.83 C-K
Nigrospora sacchari	33.33 F-P
Phoma astragali	20.67 J-P
Phoma herbarum	25.93 J-P
Ulocladium chartarum	31.43 F-P
Ulocladium consortiale	28.43 H-P

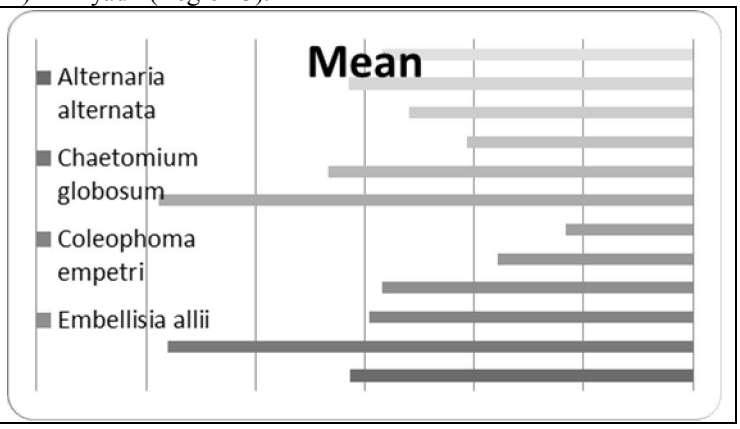

Table 4. Mean of occurrence for fungal genera and species isolated from contaminated soil samples with explosive material collected from Explosive Ins6tute (EI) in Riyadh (Region 4).

Fungal species	Mean
Chaetomium globosum	26.67 H-P
Drechslera biseptata	33.30 F-P
Drechslera biseptata	36.50 E-P
Embellisia allii	25.00 I-P
Fusarium equiseti	66.40 B-E
Fusarium sambucinum	25.00 H-P
Macrophomina phaseolina	73.33 ABC
Mucor circinelloides	100.0 A
Mucor sp.	9.667 OP
Nigrospora sacchari	22.50 J-P
Phoma herbarum	22.67 J-P
Ulocladium consortiale	38.50 E-O

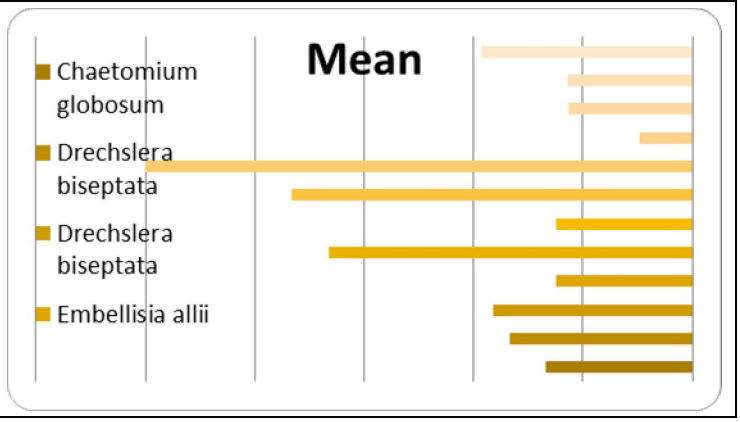

Table 5. Mean of occurrence for fungal genera and species isolated from contaminated soil samples with explosive material collected from Explosive Ins6tute (EI) in Riyadh (Region 5).

Fungal species	Mean
Aspergillus fumigatus	100.0 A
Drechslera biseptata	30.33 G-P
Embellisia allii	11.50 NOP
Fusarium equiseti	39.17 D-O
Fusarium sambucinum	47.50 C-P
Macrophomina phaseolina	15.00 L-P
Mucor circinelloides	62.67 C-G
Nigrospora sacchari	21.00 J-P
P. aurantiogriseum	22.17 J-P
Phoma astragali	12.67 M-P
Phoma herbarum	19.33 J-P
Ulocladium chartarum	16.00 K-P
Ulocladium consortiale	37.00 E-P
Ulocladium sp.	45.83 C-M

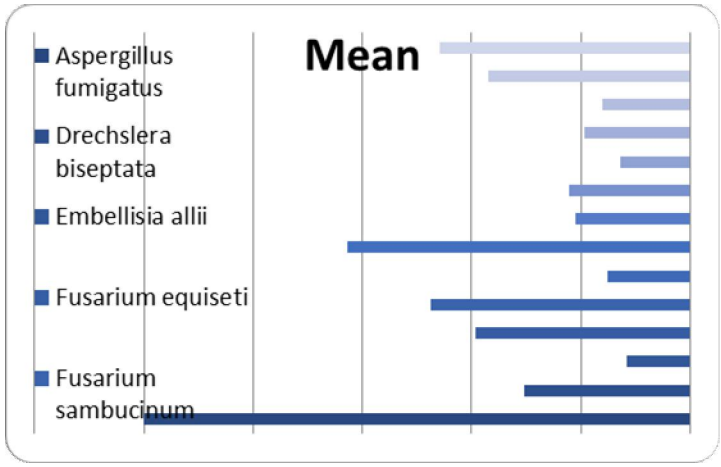

Table 6: Analysis of variance (ANOVA)

Source	Degrees of freedom	Sum of Squares	Mean Squares	F-Value
Replication Factor A Error	2 63 126	52.733 98829.406 32755.387	26.366 1568.721 259.963	0.1014 6.0344

Conclusion

The results of our study conclude that 13 fungal species were isolated and some of the isolated fungi of contaminated soils can be used as bioremediation agents for explosive materials.

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