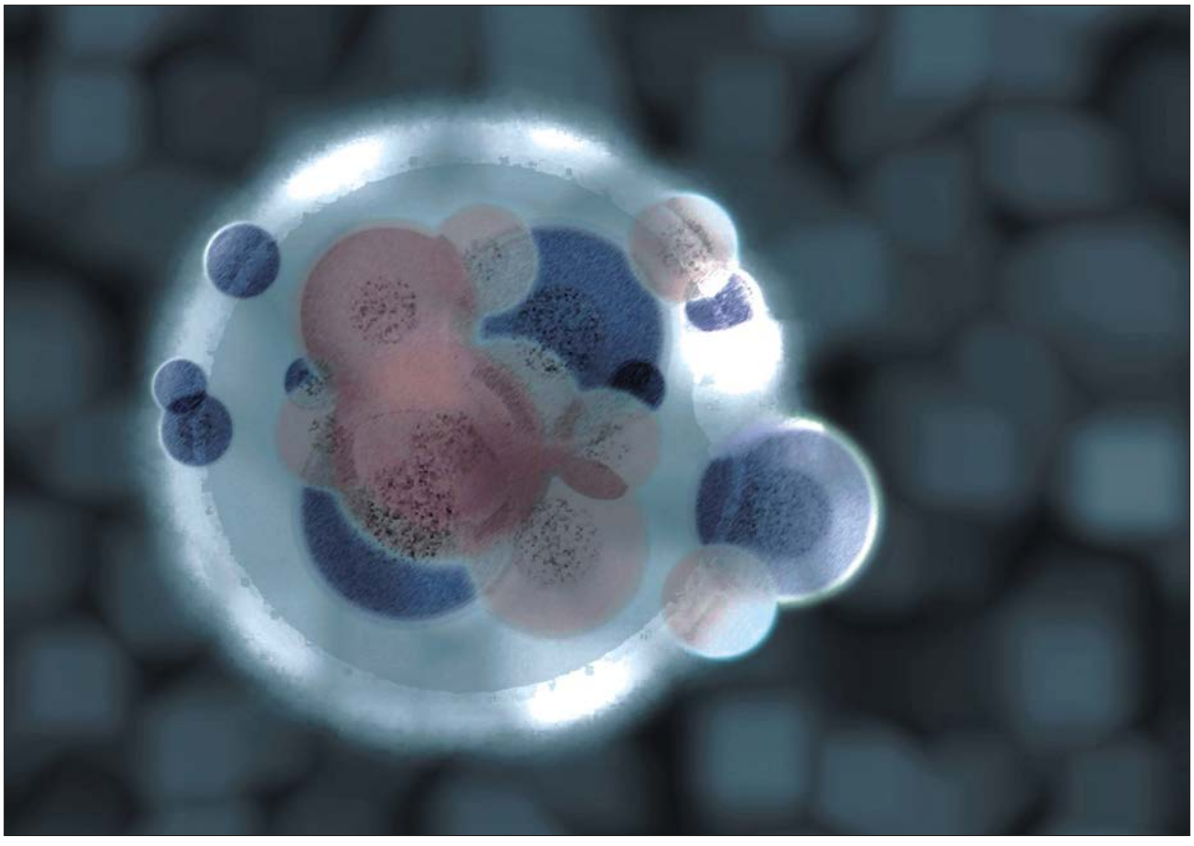

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Soil Analysis of Contaminated Soil from Riyadh City, Saudi Arabia and Influence of Aluminium and Cobalt Ions on the Growth of Fungi Isolated

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Abstract: Twenty soil samples from Eastern, Western, Northern an Southern directions of Gold and Silver manufactory in second industrial city, Riyadh, Saudi Arabia were collected and analyzed chemically for heavy metal concentrations, total soluble salts and pH and mechanically for composition of soils. Saturation percent were determined also. From soil samples collected thirteen fungal isolates were able to grew in media containing 10 mM of either aluminium or cobalt ions. *Aspergillus* were predominant and represented by four species. *Fusarium* and *Penicillium* were represented by 3 species. While *Auerobasidium*, *Mucor* and *Monascus* were represented by one species each. Intraspecific variability in growth response to Al^{3+} and Co^{2+} on agar media was studied among isolated fungi.

Key words: Soil analysis, heavy metals, fungal growth, Saudi Arabia

INTRODUCTION

Human industrialization increase release of heavy metals into the environment. Since, heavy metals readily accumulate in soil, the amount in soil is likely to continue to increase. Excessive heavy metal concentration in the soils was reported to cause decrease in microbial population (Mc Grath *et al*., 1995), change in the population structure (Bardgett *et al*., 1994) and physiological activity (Contrufo *et al*., 1995). Thus making heavy metals an important class of environmental pollutants.

In Saudi Arabia several investigation have been made on heavy metals contaminated sites to study the soil composition and mycoflora of these sites, another investigation take care to study the effect of heavy metals on the growth of fungi isolated from the soil (Hashem, 1990, 1992, 1993a-c, 1994; Hashem and Al-Johany 1994; Hashem and Bahkali, 1994; Hashem and Parvez, 1994; Hashem, 1995a,b, 1996, 1997; Barakah *et al*., 2001; Hashem and Kuchari, 2001; Abed and Alwakel, 2002).

Hashem (1993b) collected soils and water samples from different places in Saudi Arabia (Abha, Arar, Dammam, Gizan, Hail, Madinab, Riyadh, Tabouk, Taif and Yanbu) and analyzed chemically for heavy metal concentrations. Soil samples from these places differed greatly in concentration of aluminium, bromide, cadmium, cobalt, copper, iron, lead and zinc, while water samples differed only slightly.

Hashem (1993c) isolated twenty four isolates species belonging to eleven fungal genera from soil samples of ten localities from industrial Yanbu city, Saudi Arabia. *Aspergillus* were predominant and represented by six species. Numbers of *Aspergillus flavus* and *A. niger* colonies were predominant as compared to other detected *Aspergillus* sp. *Alternaria* and *Penicillium* were represented by 3 species each. While, two species of *Curvularia*, *Fusarium*, *Mucor* and *Ulocladium* were isolated. *Cladosporium*, *Drechslera*, *Neurospora* and *Rhizopus* were represented by one species each. Soil type is sandy, alkaline and differs in the organic matter content, total soluble salts, moisture content and total metal content.

Hashem and Al-Johany (1994) selected ten soil sampling in the Al-Madinah area. Five individual locations were selected within each sampling site. Each sample processed and passed through a 2 mm sieve. A 0.5 g sub-sample was digested in concentrated analar HNO_3 analyzed for B, Co, Cu, Fe, Mn, Pb and Zn and for Ca and Mg. Organic matter contents, total soluble salts and pH were determined. Five replicate water samples were collected from five locations. The organic matter contents of soil samples vary between 0.02 and 0.86%, the total soluble salts fluctuate between 0.03 and 0.66% and soil pH vary between 7.01 and 7.55. The concentration of Ca are the highest followed by the concentrations of Mg and Fe.

Abed and Alwakel (2002) collected soil samples from four localities from Sabic industrial area in Riyadh city, Saudi Arabia and analysis for their heavy metals content and fungal flora were studied. Soil samples differed greatly in the concentrations of Fe, As, Cd, Co, Cr, Pb and Zn. Five isolates of fungal species were isolated from soil samples of the four localities *Fusarium* sp., *Helminthosporium* sp., *Fusarium solani*, *Mucor flavus* and *Ulocladium curbitea*. *Mucor flavus* was isolated from Sabic industrial in Riyadh city soil. Its tolerances to zinc as tested on liquid media containing different concentrations of this metal (100, 200, 300, 400 and 500 ppm). The concentrations of Zn inhibited growth of the fungus.

The present study aimed to isolated fungi flora in contaminated soils in Riyadh, Saudi Arabia with soils analysis for these sites and to study the effect of aluminium and cobalt ions on mycelial growth of these fungi to determined fungal isolated from contaminated sites resistance to heavy metals exposure.

MATERIALS AND METHODS

Characteristics of soil: Method describe by Piper (1955) was used for determination the soils type. Chemical analysis of the soil samples were done by methods used by (Chapman and Pratt, 1961). Heavy metals (Al, Co, Cu, Pb) in soil content were measured by an atomic absorption spectrophotometer after digestion soil samples with a mixture of HNO_3-HCl (Soon and Abboud, 1993). Soil analysis was done with help of Soil Science Department, College of Food and Agriculture Science, King Saud University.

Collection and isolation of fungi: Soil samples from Eastern, Western, Northern an Southern directions of Gold and Silver manufactory in second industrial city, Riyadh, Saudi Arabia were collected according to the method described by Johnson *et al.* (1960) at a depth of 1-10 cm during the month of February (2006) in which the temperature was 30°C and the percentage humidity was 24% (five individual locations were selected within each sampling site). The samples were stored in sterile plastic bags and transported to the laboratory. Five collections of a total weight of 4000 g from each direction were mixed throughly. Approximately half of the mixed samples were used for soil analysis, the rest were sieved through screens with a 0.5 and 0.1 mm diameter opening to remove stones and other debris and used for isolation of fungal content. Soil plates method of Warcup (1957) was used for isolation fungal flora.

Aliquots of soil samples were dispatched into Petri dishes (90 mm diameter) and covered with steril medium, Pepton-dextrose agar containing rose bengal and antibiotic (Martin, 1950) with 2 mmole of either $Al(NO_3)_3 . 9 H_2O$ or $CoCl_2 6H_2O$.

Dishes were incubated at 25±2°C for one week and examined daily. Further inspection of the dishes were made two weeks after plating to record slow growing fungi. Fungal growing on these dishes were regarded as having at least low level of Al and Co tolerance and were retreated on modified Dox agar (Naguib, 1967) plates for isolation of singal colonies.

Screening for high aluminium and cobalt tolerance: The isolated fungi from the previous experiment were, therefore, screened. For their abilities to tolerate the level of 10 mM of aluminium and cobalt ions. All isolates were separately inoculated onto modified Dox agar plates with 10 mM aluminium or cobalt ions There were 3 replicates per isolate. All plates were incubated at 25±2°C for one week. Isolates that did not grow were discarded whereas ones that grew were regarded as being tolerant to a high level of aluminium and cobalt ions concentration and were used for further testing.

Identification of fungal isolate: Slides of hyphay, conidiophores and conidia were prepared by mounting with lacto-fuchsine and examined by viewing at 1600X magnification using a compound microscope. Size and color of fungal colonies on media also were also recorded. All fungal isolates were identified according to Samson *et al.* (1996) and Ellis (1971 and 1976).

Effect of aluminium and cobalt ions concentration on fungal growth: Inoculum (8 mm disk) from 7 day old culture of isolates were inoculated on the center of modified Dox agar plates (9 cm) diameter containing two concentrations i.e., 0, 5 and 10 mM L^{-1} of $Al(NO_3)_3 9 H_2O$ or $CoCl_2$ 6 H_2O. There were three replicates for each experiment. The media without metal served as control. After 7 days of incubation at 25±2°C the diameters of mycelial growth, in four directions and the radial growth rates (in cm per day) were determined (Babich and Stotzky, 1977).

RESULTS AND DISCUSSION

Characteristics of soil: Table 1 demonstrated that soils type is sandy clay loam, sandy loam or only loam. The pH value of the soil samples tested was neutral alkaline (range 7.5-8.2). The concentration of Ca^{2+} are the highest compared with another cations ranged between 170-1546 meq^{-1}, followed by Mg^{2+} ranged between 118.6-420.0 meq^{-1}, while Na^+ and K^+ concentrations are low ranged between

Table 1: Chemical analysis of soil samples

Sample direction	SP (%)*	pH	EC** (ds m^{-1})	Cations (meq^{-1})				Anions (meq^{-1})				SAR***
				Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	CO_3^{2-}	HCO_3^{-}	Cl^{-}	SO_4^{2-}	
Northern	30	7.7	72.5	210.0	135.2	15.9	20.09	0.00	10.00	1510.00	95.60	1.2
Southern	30	7.5	54.0	250.0	118.6	9.8	9.00	0.00	6.00	620.00	84.30	0.7
Eastern	26	7.5	64.0	170.0	197.6	13.0	15.26	0.00	14.00	828.00	54.80	1.0
Western	27	8.2	133.1	1546.0	420.0	43.7	45.48	0.00	120.00	1940.00	93.40	1.4

*Saturation percent,**Electric conductivity,***Sodium saturation ratio

Table 2: Mechanical analysis as well as heavy metals content of soil samples

Sample direction	Partical size (%)			Texture class	$CaCO_3$ (%)	Available (ppm)			
	Sand	Silt	Clay			Al	Co	Cu	Pb
Northern	59.92	24.00	16.08	Sandy clay loam	34.00	1.162	0.282	22.12	18.0
Southern	65.92	14.00	20.08	Sandy loam	20.42	0.294	0.294	2.18	2.83
Eastern	57.92	29.00	13.08	Sandy loam	39.50	0.556	0.186	4.036	7.244
Western	35.92	46.00	18.08	Loam	40.69	0.348	0.204	5.070	8.656

Table 3: Growth of isolated fungi at 5 and 10 mM concentrations of aluminum and cobalt ions on modified Dox agar plates over 7 day incubation at 25±2°C

Organism	Mean (±SD) colony diameter in (cm)				
	Control	5 mM Al^{3+}	10 mM Al^{3+}	5 mM Co^{2+}	10 mM Co^{2+}
Aspergillus flavus Link	5±0.000	4.5±0.010	4±0.057	5±0.010	4±0.028
Aspergillus niger van Tieghem	5±0.000	4.4±0.060	4.2±0.100	4.8±0.100	4.4±0.010
Aspergillus oryzae (Ahlburg) Cohn	4.4±1.115	4.2±0.023	4±0.052	4.2±0.110	4±0.052
Aspergillus wentii Wehmer	3.5±0.238	3.5±0.011	3.4±0.021	3±0.230	3±0.010
Auerobasidium pullulans (De. Bary) Arnaud	4±0.010	4±0.264	3.8±0.045	4±0.043	3.8±0.050
Fusarium equiseti (Corda) Sacc.	8.8±0.006	8.5±0.021	7.5±0.020	8.5±0.055	8.5±0.000
Fusarium poae (Peck) Wollenw	7±0.0153	6.8±0.0162	6±0.026	6.4±0.016	6.2±0.021
Fusarium subglutinans (Wollenw and Reinking) Nelson, Toussoun and Marasas	5.5±0.016	5±0.000	5±0.024	5.5±0.000	5.5±0.010
Penicillium corylophilum Dierckx	6±0.010	5.8±0.055	5.8±0.044	5.7±0.011	5.6±0.110
Penicillium camemberti Thom	9±0.000	8.2±0.015	7.8±0.000	7.6±0.020	7±0.055
Mucor hiemalis Wehmer f. hiemalis	3.5±0.025	3.4±0.032	3.2±0.029	3±0.00	3±0.015
Monascus ruber v. Tieghem	3±0.100	3±0.052	2.6±0.000	2.8±0.020	2.6±0.010
Penicillium roqueforti Thom	5±0.000	4.4±0.050	4.2±0.020	4.8±0.000	4.4±0.064

9.8-43.7 and 9.0-45.48 meq^{-1}, respectively. However the concentration Ca^{2+}, Mg^{2+} and K^{+} were less than that reported before for Saudi Arabia soils (Hashem, 1993c; Hashem and Al-Johany, 1994). Also Table 1 show that the concentration of Cl^{-} are the highest compared with another anions ranged between 620-1940 meq^{-1}. Table 2 show that the concentrations of Al^{3+} in the tested sample soil was less than reported before for Saudi Arabia soils (Hashem, 1990, 1993 b-c). In the present study also Co concentration of tested sample soil was less than reported before for samples collected from industrial Yanbu city and samples from Al-Madeinah (Hashem, 1993c; Hashem and Al-Johany, 1994).

Lead concentration in the tested soil sample was to be similar to those studies reported before by (Hashem, 1993b-c), but was highest than that study reported by (Hashem and Al-Johany, 1994).

The concentration of copper in this study was less than other studies reported before in some Saudi Arabia soils (Hashem, 1990, 1993 b-c).

Effect of aluminium and cobalt concentration on fungal growth: Table 3 indicated the results of aluminum and cobalt applied as nitrate and chloride salts, respectively at the level 5 and 10 mM to the media. Results clear that the growth of *Aspergillus flavus* not affected with 5 mM concentration of cobalt ions, while 10 mM concentration of cobalt and aluminium ions decreased fungal growth 20%, also 5 mM aluminium ions concentration decreased fungal growth 10% when compared with that of control. Hashem (1997) demonstrated that *Aspergillus flavus* tolerant substantial amount of cobalt, while high concentration of cobalt ions inhibit fungal growth. 5 mM concentration of cobalt ions caused a little inhibition 4% on *Aspergillus niger* growth, while 5 and 10 mM concentration of aluminium and cobalt ions, respectively caused 12% inhibition on fungal growth, also 10 mM concentration of aluminium ions decreased fungal growth 16% when compared with that of control. 5 mM concentration of aluminium and cobalt ions caused a little inhibition 5% on *Aspergillus oryzae* growth, while 10 mM concentration of aluminium and cobalt ions decreased fungal growth 9% when compared with that of control. No stimulation on growth of *Aspergillus wentii* was observed at 5 mM concentration of aluminium ions, while both concentration of cobalt ions 5 and 10 mM inhibited fungal growth 15% when compared with that of control. 5 mM concentration of both metals not stimulated the growth of *Auerobasidium pullulans*, while 10 mM

concentration of both metals inhibited it 5% when compared with that of control. 5 and 10 mM concentration of cobalt ions and 5 mM concentration of aluminium ions inhibited *Fusarium equiseti* growth only 3%, while 10 mM concentration of aluminium ions decreased fungal growth 15% when compared with that of control. Results shown in Table 3 demonstrated that *Fusarium poae*, *Mucor hiemalis* and *Penicillium roqueforti* growth decreased with increase of both metals ions concentrations. There was no influence of cobalt ions on growth of *Fusarium subglutinans*, while aluminium ions inhibited it 10% when compared with that of control. 5 and 10 mM concentration of aluminium ions inhibited *Monascus ruber* growth only 3%, while 5 and 10 mM concentration of cobalt ions decreased fungal growth 5 and 7%, respectively as compared with that of control. *Penicillium camemberti* growth deceased with increased aluminium ions concentration, while both concentrations of cobalt ions inhibited fungal growth 14% when compared with that of controls. *Penicillium corylophilum* growth decreased in 5 mM concentration of cobalt ions 7%, also 10 mM concentration of aluminum and cobalt ions decreased fungal growth 15% when compared with that of control, while 5 mM concentration of aluminum ions not affected fungal growth.

These results clear that the fungal isolates from contaminated soil from Riyadh, city, Saudi Arabia appeared to be more tolerant to heavy metals toxicity and we can use it for further studies in future.

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