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Effect of Heavy Metal Ions on the Mycelial Growth of Some Fungi Isolated from the Soil of Al-Jubail Industrial City, Saudi Arabia

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Abstract. The effect of cobalt, copper, lead, molybdenum, manganese, and zinc on the growth of *Alternaria alternata, Aspergillus flavus, Cladosporium herbarum, Curvularia lunata* and *Ulocladium chlamydosporum* isolated from the industrial Al-Jubail city, Saudi Arabia, at concentration of 500, 1000 and 2000 ug/ml were determined. Higher concentrations of cu, and Zn stimulated growth of the tested fungi, while higher concentrations of lead, manganese and molybdenum inhibited growth of some fungi.

Introduction

In recent years, heavy metals have received attention because of their release into the environment from different sources. The tolerance of some fungi to a variety of heavy metals over the world is well documented [1-7]. As no informaiton is available on the resistance of other soil fungi, including some isolated from the industrial Al-Jubail city, Saudi Arabia, the effect of Co, Cu, Pb, Mn, Mo and Zn on the mycelial growth of these fungi, was examined. Jubail is situated on Saudi Arabia's Gulf shore in the heart of the country's oil producing region, about 500 kilometers from Riyadh on the Arabian Gulf [8].

Jubail is currently home to 16 major manufacturing facilities, an oil refinery, steel mill, several petrochemical complexes and methanol and fertilizer factories. This study is a first attempt and part of a more extensive investigation in which the effect of heavy metals on the fungi from different environments of Al-Jubail city, Saudi Arabia, are examined.

The present study was undertaken to determine whether soil fungi growing on

metal contaminated sites develop resistance to heavy metal exposure.

Materials and Methods

Soil samples from different localities of the Jubail industrial city in Saudi Arabia were collected and moisture content, organic matter, total soluble salts and pH value were determined as described earlier by Hashem [8]. Five collections of a total weight of 1000 g from each locality were mixed and used for soil analysis and isolation of fungal flora.

Isolates of *Alternaria alternata, (Fries) keissler, Aspergillus flavus, link Cladosporium herbarum. Linnkex Fries, Curvaluria lunata, (Wakker) Boedijn,* and *Ulocladium chlamydosporum Mouchacca* were obtained from heavy metal contaminated soil of the industrial Al-Jubail city. Cultures were maintained on potato dextrose agar for further studies [8].

Sulphate salts of cobalt, copper, lead, manganese, molybdenum and zinc were individually added to PDA medium to give three concentrations (500, 1000 and 2000 ug/ml) of each compound. The pH of the media was adjusted to 6.0 using NaOH [4]. Five mycelial discs (5 mm diam.) of the tested fungi, from 5-day-old cultures grown on PDA, were inoculated on the metal-amended, sterilized agar media in 9 cm prespex petri dishes. The media without metal served as control. The culture were incubated for ten days at 22°C. The colony diameter were recorded after the 10 days incubation. Five replicates were used for each concentration tested.

Results and Discussion

The effect of heavy metals on the growth of the tested fungi was assessed on the basis of mycelial diameter (Table). *A. alternata, A. flavus.* and *U. chlamydosporum* tolerated substantial amounts of Co, Cu, Pb, Mn, Mo, and Zn. Among the heavy metals screened, Pb and Mo were found very inhibitory to *C. herbarum* and *C. lunata* at higher concentrations. Growth of *C. lunata* was inhibited at 2000 ug/ml Mn.

A. alternata, A. flavus, C. herbarum, C. lunata and *U. chlamydosporum* all showed good colony growth on heavy metal media at 500 ug/ml in comparison to the control. It is clear from Table 1 that the tested fungi grew better on media containing elevated concentrations of Cu and Zn, than on the control medium. The stimulated growth in copper and zinc media may be due to the involvement of Cu and Zn in enzyme synthesis [9].

Higher concentration of Zn and Cu (3000 ug/ml) allowed the growth of *Aspergillus candidus* and *Drechslera rostrata* while higher concentrations of cobalt and lead inhibited growth of *fusarium moniliforme* and *Ulochladium atrum* (3000 ug/ml) [10].

Hashem [3] reported that *Aspergillus niger* and *Penicillium chrysogenum* could survive in a liquid media containing up to 500 ug/ml Cu.

The toxicity of high concentrations of Pb. Mn and Mo to fungi is well known [6, 8, 11, 15]. Cobalt and lead inhibited the growth of *Aspergillus candidus, penicillium notatum* and *Ulocladium atrum* at 3000 ug/ml [11].

Cobalt toxicity to *A. flavus* and *U. chlamydosporum* at 2000 ug/ml may be due to the lack of metabolization of iron by the fungi which in turn inhibits mycelial haem synthesis [12].

Table. Effect of heavy metal ions on the growth of some fungi isolated from the industrial Al-Jubail city. Growth expressed as colony diameter in cm (mean standard error of mean; control = PDA without added heavy metals; – = No growth)

Metal ion	Concentration (ug/ml)	*Alternaria alternata*	*Aspergillus flavus*	*Cladosporium herbarum*	*Curvularia lunata*	*Ulocaldium chlamydosporum*
Cobalt	500	10 ± 0.63	8 ± 0.42	9 ± 0.39	10 ± 0.72	9 ± 0.92
	1000	8 ± 0.31	7 ± 0.23	8 ± 0.30	9 ± 0.61	8 ± 0.71
	2000	7 ± 0.30	–	6 ± 0.26	8 ± 0.49	
Copper	500	10 ± 0.69	11 ± 0.93	10 ± 0.62	9 ± 0.91	9 ± 0.21
	1000	9 ± 0.19	8 ± 0.62	7 ± 0.91	8 ± 0.11	7 ± 0.39
	2000	11 ± 0.11	10 ± 0.92	9 ± 0.11	9 ± 0.18	8 ± 0.92
Lead	500	8 ± 0.11	9 ± 0.12	7 ± 0.29	6 ± 0.11	8 ± 0.29
	1000	6 ± 0.21	7 ± 0.19	5 ± 0.31	5 ± 0.19	6 ± 0.19
	2000	5 ± 0.31	6 ± 0.12	–	–	3 ± 0.11
Manganese	500	6 ± 0.31	5 ± 0.52	5 ± 0.21	4 ± 0.31	5 ± 0.29
	1000	5 ± 0.21	4 ± 0.39	3 ± 0.31	3 ± 0.30	3 ± 0.19
	2000	3 ± 0.11	1 ± 0.11	2 ± 0.12		2 ± 0.21
Molybdenum	500	7 ± 0.91	6 ± 0.31	5 ± 0.21	3 ± 0.11	5 ± 0.11
	1000	6 ± 0.62	4 ± 0.29	3 ± 0.11	2 ± 0.12	4 ± 0.21
	2000	4 ± 0.31	2 ± 0.12	–	–	3 ± 0.13
Zinc	500	11 ± 0.92	10 ± 0.11	9 ± 0.21	10 ± 0.11	8 ± 0.11
	1000	10 ± 0.83	8 ± 0.23	8 ± 0.61	8 ± 0.21	7 ± 0.21
	2000	10 ± 0.72	11 ± 0.31	10 ± 0.31	11 ± 0.31	10 ± 0.31
Control		7 ± 0.32	6 ± 0.32	5 ± 0.39	7 ± 0	6 ± 0.29

Hashem and Al-Sohaibani [13] found that *A. alternata* was able to grow on liquid media containing up to 500 ug/ml Mn and appeared to be more resistance to Mn than to Zn.

Hashem [14] reported that *Ulocladium tuberculatum* isolated from uncontaminated soil from Saudi Arabia was able to grow on liquid media containing up to 300 ug/ml Mn.

Aspergillus species isolated from different soils in Saudi Arabia can grow in media containing higher concentrations of Cd, Co, Pb and Zn [15].

Effects of Cd, Co, Ni, Pb and Zn were tested on the growth of *Aspergillus carneus* and *Aspergillus ellipticus* isolated from medicinal plant *Citrullus colocynthis* from Saudi Arabia, *Aspergillus carneus* was highly resistance to all metals tested [16]. *Curvularia tuberculata* isolated from uncontaminated soil from Saudi Arabia was able to grow on liquid media containing up to 350 ug/ml Cd [17].

The phenomenon of stimulation by low levels of Cd, Co, Pb and zn has been noted in some species of fungi isolated from Saudi Arabian soils [3, 4, 5, 7, 10, 17].

It is clear that comparing the tolerance of a number of different species of the some fungus isolated from different habitats indicated that the same physiological mechanism can detoxify more than one metal. Morcover, the fact that an isolate from a metal-free soil count survive in high concentrations of metals does not mean that same strain content develop genetic tolerance over a number of generation.

Toxic metals affect microorganisms in natural environments by reducing numbers and diversity and selecting a resistant population, and it is commonly assumed that metal exposure leads to the establishment of a resistant or tolerant microbial population [18, 20].

Overall, heavy metals were generally toxic on Al-Jubail industrial city soil fungi, but some fungi appeared to benefit from some concentrations, the degree of toxicity varied among metals studied.

In summary, it appears that the levels of heavy metal resistance achieved by the examined fungi are related to the metal levels which can be predicated to obtain in the natural environment.

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أثر أيونات العناصر المعدنية الثقيلة على نمو بعض الفطريات المعزولة من تربة مدينة الجبيل الصناعية بالمملكة العربية السعودية

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ملخص البحث. تمّ في هذا البحث دراسة أثر العناصر المعدنية السامّة مثل الكوبالت، النحاس، الرصاص، المليبوديتيوم، المنجنيز والخارصين على نمو الفطرة الترناريا الترناتا، اسبرجيللس فلافس، كلادوسبوريوم هرباريوم، كبروفلاريا لاناتا واللوكلاديوم كلامايدوسبورم عند التراكيز ٥٠٠، ١٠٠٠ و ٢٠٠٠ جزء في المليون.

أوضحت النتائج المتحصل عليها أن التراكيز العالية من عنصري النحاس والخارصين تساعد على نمو الفطريات المختبرة بينما التراكيز العالية من العناصر الأخرى تعمل على تثبيط النمو الفطري.