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Zinc Tolerance and Accumulation in *Aspergillus oryzae* *Penicillium citrinum* and *Rhizopus stolonifer* Isolated from Saudi Arabian Soil

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مقاومة وتراكم عنصر الخارصين بواسطة أسبرجيليس أوريزي وبينيسليام سترينيوم وريزوبس ستولونوفر والمعزولة من المملكة العربية السعودية

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تم في هذه الدراسة عزل أسبرجيليس أوريزي وبينيسيليام سترينيوم ورايزوبس ستولونوفر من تربة مدينة الطائف - المملكة العربية السعودية.

وجد أن أسبرجيليس أوريزا وبينيسيليام سترينيوم تستطيعان النمو في البيئات السائلة والمحتوية على (٥٠٠) جزء في المليون وأنهما ذات مقاومة عالية أكثر من رايزوبس ستولونوفر.

تمت مناقشة النتائج المتحصل عليها استنادا إلى مؤشر المقاومة وتراكم العنصر في الغزل الفطري.

Key words : Zinc -Tolerance-Fungi - Soil

ABSTRAC

Aspergillus oryzae, *Penicillium citrinum* and *Rhizopus stolonifer* isolated from Taif soil, Saudi Arabia could survive in a liquid medium containing up to 500 µg/ml Zn. *Aspergillus oryzae* and *Penicillium citrinum* were more tolerant to zinc toxicity than *Rhizopus stolonifer*. The resistance and accumulation of zinc in the tested fungi were discussed according to tolerance index and zinc uptake in the mycellium.

INTRODUCTION

Although there are many studies reporting on the fungal flora of Saudi Arabian soil, little attention has been paid to the effect of heavy metals toxicity on the growth of fungi isolated from Saudi Arabia. Zinc is associated with enzymes, particularly metaloenzymes. It is an essential element for fungal growth [1,2], but toxic at higher concentrations [3]. Excessive amounts of available zinc can influence the uptake and metabolism of other elements [4,5,6].

The resistance of fungi to a variety of heavy metals has been investigated [7,8,9,10]. They have been reported to grow in soils contaminated with higher levels of metals than those tolerated by higher plants [11,12,13].

It is now known that zinc is essential for the activity of more than 25 enzymes, nucleic acid metabolism, the storage or function of certain hormones, vision, wound healing and the synthesis of many industrially and medically significant microbial secondary metabolites [14].

Details of subsequent research concerning the essential and physiological responses of microorganisms to various levels of zinc have been reviewed [15,10,17,18,19,20,21, 22].

Because there is no information available on the effect of zinc on the growth of *A. oryzae*, *P. citrinum and R. stolonifer* isolated from Saudi Arabian soil, this study was designed to determine the toxicity of the above fungi when tested in liquid media containing different concentrations of zinc.

MATERIALS AND METHODS

Soil samples were collected from an exposed soil surface. In each case the soil from Taif, Saudi Arabia was scraped (1-5 cm depth) into a plastic bag using a stainless steel spoon. A total of five pooled samples were collected from five different localities in the city.

On arrival in the laboratory, each sample was passed through a 2.0 mm sieve and was digested in concentrated analar nitric acid to obtain a measure of the total zinc content. The procedure was as follows: 0.5 g of air dried soil from each pooled sample was placed in a 100 ml beaker with 15 ml concentrated nitric acid, covered with a watch-glass and heated at 90°C for 15 min. after digestion. The digest was made up to 50 ml with deionized water analyzed using an atomic absorption spectrophotometer [23]. Five g . of the pooled mixed soil sample were taken at random and suspended in 45 ml of sterile distilled water. Six replicates from each sample were prepared for the isolation of fungi.

The soil dilution plate method was used in the present study. Each soil suspension was cultured on six replicate plates of peptone-dextrose agar containing rose bengal (0.03 g/L) and streptomycin sulphate (0.03 g/L) for the initial isolation of fungi. All plates were incubated at room temperature (22°C) for one week and examined daily. Further inspection of the plates was made two weeks after plating to record slow growing fungi. The recovered fungi were subcultured to agar slants of peptone-dextrose agar (BDH-Chemicals).

The isolated fungi were identified according to Raper and Fennell [24] for *A. oryzae*, Ramirez [25] for *P. citrinum* and Smith [26] for *R. stolonifer*. The pH value, total soluble salts and organic matter content were estimated in the soil sample according to the methods adopted by Black *et al.* [27].

A. oryzae, P. citrinum and *R. stolonifer* were grown on PDA plates, and discs from 5 day-old cultures were inoculated into flasks, using a 4 mm diam. sterile cork borer. Discs of mycelium were cut from the margin of actively growing colonies and transferred to 100 ml conical flasks (1 disc/flask) containing malt extract agar medium (5.0 g malt extract, 1000 ml distilled water) to which zinc as $ZnSO_4$. $7H_2O$ was added to give final concentration of 0, 150, 250, 350 and 500 μg/ml Zn. The pH of the solutions was adjusted to 5.0 before they were sterilized by filtration through millipore filters (0.45 m pore size). All flasks were incubated at room temperature (22°C) in a static condition. Harvests were taken at 10, 20, and 30 days. Mycelia were trnsferred to pre-weighed filter papers, thoroughly washed with deionized water, oven-dried (80°C for 24 h) and weighed. Zinc concentrations in the mycelium were determined by atomic absorption spectrophotometer after nitric acid digestion. The pH values of the residual media were also measured. The results are expressed in terms of tolerance index (T.I.) [2.103].

RESULTS AND DISCUSSION

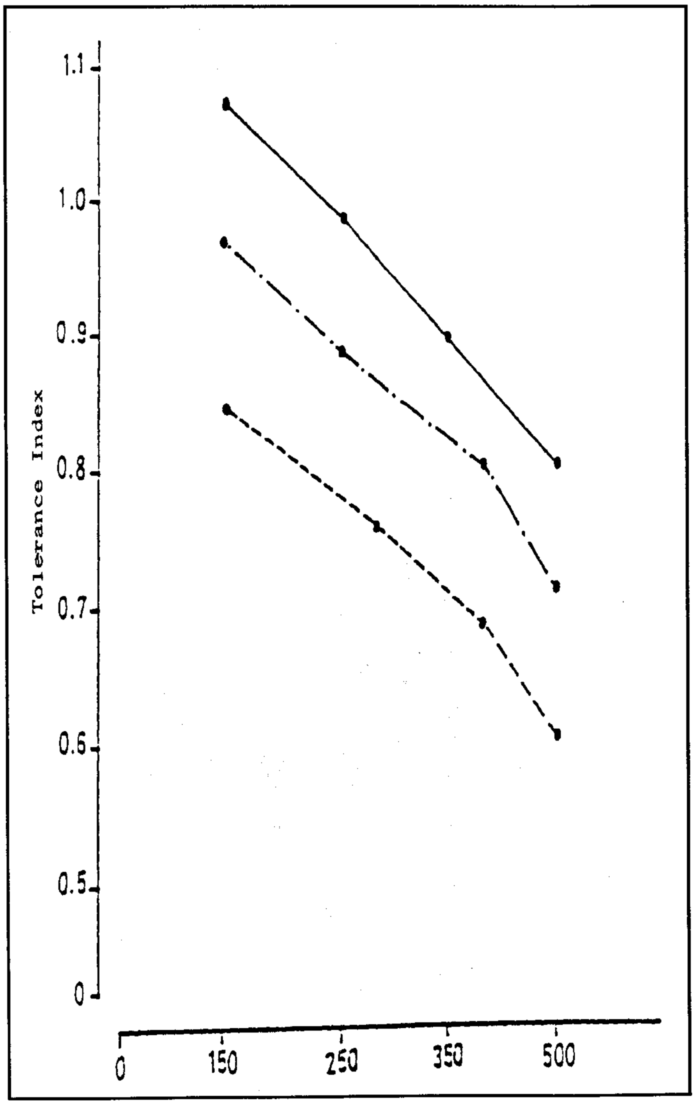
The results obtained in Table (1) show clearly that the organic matter content and total soluble salts of the examined soil were generally low, this is in agreement with findings of Abdel-Hafez [29] and Hashem [30,31]. The pH value was in the alkaline side (7.06).

Table 1: Organic matter content, total soluble salts and pH value of examined soil (n = 5, ± standard deviation).

Total Zn content mg/g	Organic matter content	Total soluble salts %	Ph value
11 ± 0.62	0.69	0.89	7,06

There was a stimulation of growth at 150 and 250 μg/ml Zn for all tested fungi in comparison to the control (Fig. 1). Among the fungi tested, *P. citrinum* was found to be most resistant to zinc, followed by A. oryzae. *R. stolonifer* was by far the most zinc sensitive of the tested fungi.The resistance of the fungi to a variety of heavy metals has been investigated [32,33]. There is little available information on the resistance of fungi to heavy metals toxicity in Saudi Arabia [34,35,36,37].

Many studies have been carried out to determine the nature of the resistance mechanisms possessed by metal tolerant fungi. It is very important to understand the mechanisms of microbial tolerance because of the extensive use of some metals and metal containing compounds as fungicides and disinfectans.



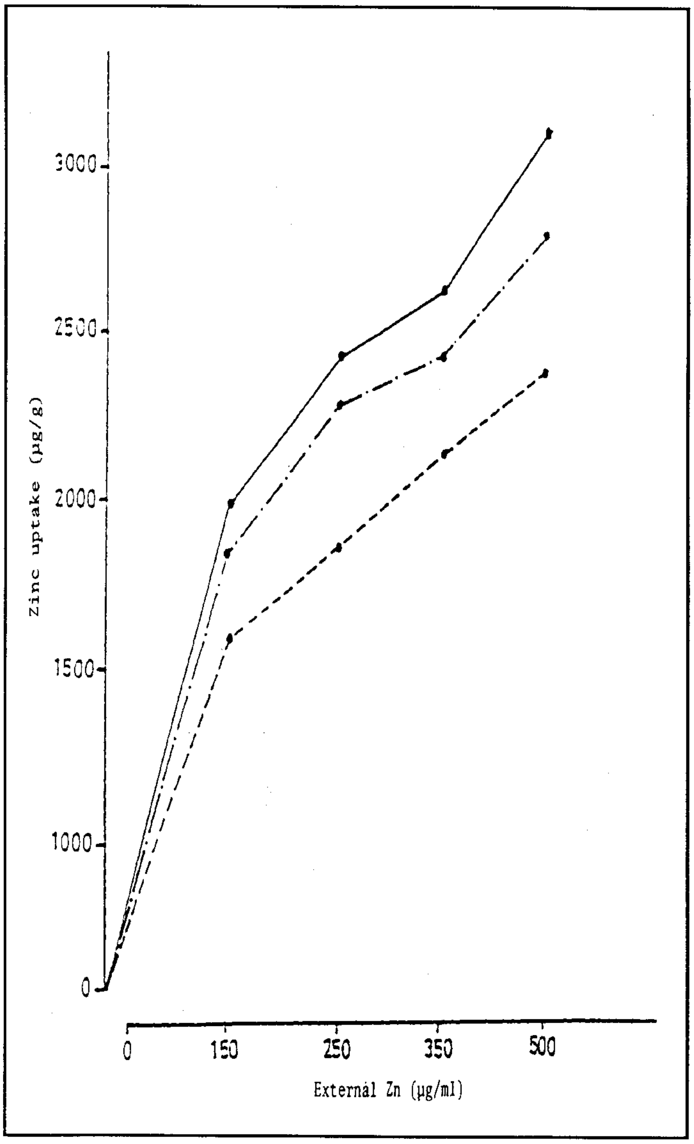
External Zn (μg/ml)

Fig. 1 : Tolerance index (T.I.) from mycelium dry weight (mg) and Zn concenration (μg/ml) after 30 days growth if the tesed fungi. Values given are means of 5 replicates.

—— *P. citrinum*
—●— *A. oryzae*
- - - *R. stolonifer*

Hashem [30] reported that *A. niger* and *P. chrysogenum* could survive in liquid media containing up to 400 μg/ml Zn. There was a stimulation of growth at 100 μg/ml Zn for both tested fungi in comparison to the control.

Mycelium of *P. citrinum* took up zinc to a maximum of 3100 μg/g, in the presence of 500 μg/ml Zn after 30 days, while *A. oryzae* and *R. stolonifer* took up 2600 and 2200 µg/g at 500 µg/ml Zn, respectively after 30 days (Fig. 2). Microorganisms possess mechanisms by which metal can be taken up and accumulated from their environment [38,39,40,41,42].



External Zn (µg/ml)

Fig. 2 : Zinc uptake by the tested after 30 days growth at different concentrations. Values given are means of 5 replicates.

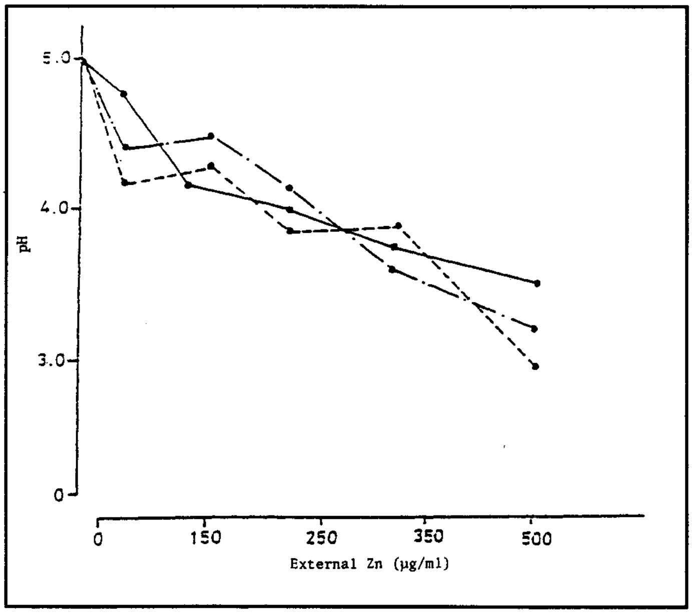
—— *P. citrinum*
—●— *A. oryzae*
- - - *R. stolonifer*

Zi

Zinc appears to be associated with enzymes, particularly metal enzymes [14]. Although zinc has long been known as an essential element for fungal growth, it is also known that it can be toxic at higher concentrations. Excessive amounts of available Zn can influence the uptake and metabolism of other elements [43].

There appear to be two main types of metal uptake by microorganisms. The first involves non-specific binding of the metal to cell surface, extra cellular matrices, etc., where the second involves metabolism-dependent intracellular uptake [33]. In the present study there was a stimulation of growth of the tested fungi at 150 and 250 µg/ml Zn. The phenomenon of stimulation by low levels of a metal has also been noted for root growth in species of higher plants tolerant to the metal [44]. Stimulation is thought to arise because the organisms tend to inactivate the metal as a part of their tolerant mechanisms. Fungi, however, contain much higher concentrations of Cu and Zn than higher plants, and have high requirements for these elements [45,46,47].

The changes in the pH values of filtered medium with the growth of the tested fungi after 30 days are given in Fig. 3. The fall in pH was broadly in proportion to the original zinc levels, the highest metal concentration giving the maximum reduction of pH.



External Zn (µg/ml)

Fig. 3 : The change in the pH of the filtered medium after 30 days growth of the tested fungi. Values are means of 5 replicates.

—— *P. citrinum*
—●— *A. oryzae*
- - - *R. stolonifer*

pH is known to affect Zn toxicity and influence the form and chemical mobility. Thus, a low pH increases the solubility of Zn in soil [48]. It must be noted that in the culture medium, increasing Zn concentrations was associated with increasingly unfavourable pH levels for growth of the tested fungi.

In the present study, zinc was removed from the culture medium to the greatest extent by *P. citrinum,* followed by *A. oryzae* and *R. stolonifer* It appears that this metal must have been transported in high amounts into the cells, because at higher external metal concentrations mycelia growth was severly inhibited.

Finally, much remains to be understood about the genetical, physiological and biochemical bases of fungal esistance to metals.

REFERENCES

[1] **Al-Sharouny, H.M.; Bagy, M. and A.A. El-Shanwany, 1988**. Toxicity of heavy metals to Egyptian soil fungi. International Biodeterioration 24: 49-64.105105

[2] **Ross, I.S. 1994**. Uptake of zinc by fungi. In: Metal ions in fungi. (Eds. Winkelman, G. and Winge, D.), pp. 237-258. Marcel Dekker, Inc., New York.

[3] **Aronson, J.M. 1982**. Cell wall chemistry ultrastructure and metabolism In: Cole. and Hendrick, A. (Eds.) Biology of Conidial Fungi. pp. 345. Sounders Company, London.

[4] **Adriano, D.C.; Poulsen, G.H. and L.S. Murphy 1971**. Phosphorus iron and phosphorus-zinc relationship in corn (Zea mays L.) seedlings as affected by mineral nutrition. Journal of Astonomy 63: 36-39.

[5] **Denny, H.J. 1986**. Zinc tolerance and ectomycorrhizal Betula spp. Ph.D. Thesis, Uiversity of Birmingham, England.

[6] **Starling, A.P. and I.S. Ross, 1991**. Uptake of zinc by *Penicillium notatum.* Microbios 63: 93-98.105105

[7] **Ashida, J. 1965**. Adaptation of fungi to metal *toxicants. Annual Review Phytopathology* 3 : 153-174.

[8] **Ross, I.S. 1975**. Some effects of heavy metals on fungal cells. Transactions of the British Mycological Society 64: 175-193.106

[9] **Stokes, P.M. and J.E. Lindsay, 1979**. Copper tolerance and accumulation in *Penicillium ochro-chloron* isolated from copper plating solution. Mycologia 71 : 796-806.

[10] **Mowell, J. and G. Gadd, 1985**. Effect of vehicular lead pollution on *Phyllophane mycoflora.* Transactions of the Brithish Mycological Society 84: 685-690.

[11] **Harley, J.L. 1969**. The biology of mycorrhizas plant. Sci. Mongr. Leonard Hill Ltd., London, pp. 233.

[12] **Laaksovirta, K. 1978**. Lead, cadmium and zinc content of fungi in the parks of Helsinki. Ann. Bot. Fenn. 15: 253-257.

[13] **Tamamato, H.; Kadzunori, T. and T. Vchiwa, 1985**. Fungal flora of soil polluted with copper. Soil Biology and Biochemistry 17: 785-790.105105

[14] **Valee, B.L. and Wacker, W.E. 1970**. Metalloprotein. In: Neuyath, H. (Ed.). The protein. pp. 192, Academic Press, New York.

[15] **Winder, F.G. and C. O'hara, 1962**. Effect of iron deficiency and of zinc deficiency on the composition of Mycobacterium smegmatis. Journal of Biochemistry 82 : 98-107.

[16] **Windra, A. 1964**. Phosphate directed Y-variation in *Candida ablicans.* Mycopathol. Mycolog. Applicata 23:197-208.

[17] **Uhrmann, G.F. and A. Rothstein, 1968**. Zinc toxicity. Biochem. Biophys. Acta163: 325-3.44

[18] **Harris, A.B. 1969**. Zince toxicity. J. Gen. Microbiol. 56: 27-38.

[19] **Yamaguchi, H. 1975**. Control of dimorphism in *Candida albicans* by zinc: Effect on cell morphology and composition. Journal of General Microbiology 86:370-372.

[20] **Hashem. A.R. 1987**. The role of mycorrhizas in the resistance of plants to metals. Ph.D. Thesis University of Sheffield, England.

[21] **Somashekar, R.K. and Sreenath, K.P. 1988**. Comparative toxicity of heavy metals to some fungi. Geobiosis15, 157-160.

[22] **Ross, I.S. 1993**. Membrane transport processes and response to exposure to heav metals, stress tolerance in fungi. Marcel Dekker, Inc., New York.

[23] **Hashem, A.R. 1990**. Analysis of water and soils from Ashafa, Toroba, Waheet and Wehait. Journal of King Saud University (Sciences) 2: 87-94.

[24] **Raper, K.B. and D.T. Fennell, 1965**. The Genus *Aspergillus*. Williams and Wilkins Co., Baltimore.

[25] **Ramirez, C. 1982**. Manual and Atlas of Penicillia. Elsevier Bromedical Press. Amesterdam.

[26] **Smith, G. 1971**. An Introduction to Industrial Mycology. Edward Arnold Ltd., London.

[27] **Black, C.A. Evans, D.D. Ensmigner, L.E. White, J.L. and F.E. Clark 1965**. Methods of soil analysis. American Society of Agronomy Inc., Madison, Wisconsin.

[28] **Hashem, A.R. and A.A. Al-Homaidan, 1989**. Effect of lead on the growth of *Coprinus micaceus* Transaction of the Mycological Society of Japan 30: 365-371.

[29] **Abdel-Hafez, S.I. 1981**. Halophilic fungi of desert soils in Saudi Arabia. Mycopathologia 75: 75-80.106106

[30] **Hashem, A.R. 1993b**. Fungal flora of soils from Ashafa, Toroba, Waheet and Wehait. Journal King Saud Univ (Science) 5: 47-53.

[31] **Hashem, A.R. 1993c**. Soil analysis and mycoflora of the industrial Yanbu City, Saudi Arabia. Arab Gulf Journal of Scientific Research 11: 91-103.

[32] **Geenway, W. and J. Cowan, 1970**. The stability of mercury resistance in *Pyrenophora avenae* Transactions of the British Mycological 54: 127-138.

[33] **Gadd, G.M. and A.J. Griffiths, 1978**. Microorganisms and heavy metal toxicity. Microbial Ecology 4: 303-317.

[34] **Hashem, A.R. 1989**. Effect of copper on the growth of *Aspergillus niger, Penicillium chrysogenum* and *Rhizopus stolonifer*. Transactions of the Mycological Society of Japan 30: 111-119.

[35] **Hashem, A.R. 1992a**. The role of manganese in the growth of *Fusarium oxysporum and Ulocladium tuberculatum* isolated from Saudi Arabian Soil. Transactions of the Mycological Society of Japan 33: 505-510.

[36] **Hashem, A.R. 1996**. Effect of cobalt on the growth of the pear fruit rot pathogen, *Aspergillus candidus* and *Aspergillus clavatus* isolated from Saudi Arabia. Indian J. Phytopathology 49(1): 72-76.

[37] **Al-Obaid, A.M.; Hashem, A.R. 1996**. Effect of heavy metals on growth of some fungi isolated from industrial Yanbu city, Saudi Arabia. Geobios 23: 107-111.106106

[38] **Somers, E. 1963**. The uptake of copper by fungal cells. Annual Applied Biology 51,106106 425-437.

[39] **Kikuchi, T. 1965**. Studies on the pathway of sulphide production in a copper- adapted yeast. Plant Cell Physiology, Tokyo 6: 195-210.

[40] **Paton, W.H. and K. Budd, 1972**. Zinc uptake in *Neocosmospora vasinfecta.* Journal of General Microbiology 72: 173-184.

[41] **Ross, I.S. 1982**. Effect of copper, cadmium and zinc on germination and mycelial growth in *Candida albicans* Transactions of the Brithish Mycological Society 78: 543-545.

[42] **Hashem, A.R. and M.A. Moslem, 1995**. Boron tolerance and occumulation in Aspergillus flavus and Penicillium citrinum isolated from Saudi Arabian soil. Journal of King Saud University (Science) 7(1): 13-20.

[43] **Erdman, J. Shacklette, H. and J. Keith, 1976**. Elemental composition of selected native plants and associated soils from major vegetation type in Missouri. U.S. Geol. Surv. 954c: 30.

[44] **Antonovics, I.; Bradshaw, A.D. and R.G. Turner, 1971**. Heavy metal tolerance in plants. Advance of Ecological Research 7: 1-85.

[45] **Jacques, L.W.; Brown, E.B.; Barnett, J.M.; Brey, W.S. and Weltner, W. 1977**. A calcium binding carhohydrate. Journal of Biological Chemistry 252: 4533-4538.

[46] **Mutsch, F. Horak, O. and H. Kinzel, 1979**. Zinc and copper in fungi. Z. pflanzen. Physiol. 94: 1-10.

[47] **Hutchinson, T.C. and F.W. Collins, 1978**. Effect of Hion activity and Ca2+ on the toxicity of metals in the environment. Environmental Health Perspect 25: 47-56.

[48] **Budd, K. 1989**. Role of the membrane potential in the transport of zinc by Neocosmo vasinrecta Exp. Mycology 12: 195-203.