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Biouptake of Copper and Their Impact on Fungal Fatty Acids

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Abstract: *Aspergillus terreus* and *Alternaria alternata* were able to tolerate copper metal ion in the growth medium up to 1000 ppm and 800 ppm respectively, but *A. alternata* failed completely to grow at 1000 ppm. Their growth increased at 100 ppm but then markedly decreased with increasing metal concentrations in the medium. Fifty % growth inhibition of *A. terreus* and *A. alternata* occurred approximately at 400 ppm . On the other hand, the rate of sporulation (conidiospoes production) decreased with increasing copper metal concentration. Metal content of copper in the mycelium of *A. terreus* was subsequently increased with increasing metal concentrations in the growth medium and the maximum accumulated amount of copper was detected at 400 ppm , then reached to minimum concentration at 800 ppm. Copper had the highest influence on the fatty acids content of *A. terreus* at low copper (100 ppm) and high concentrations (800 ppm) ,The ratio of saturated to unsaturated fatty acids in the fungus treated with low and high concentration was determined to be 6.89 and 2.36 respectively . Most of the detected fatty acids at low concentration (100 ppm) was high in the concentration compared with their concentrations at high metal concentration (800 ppm) and with the control. Among the detected fatty acids, two unsaturated fatty acids linoleic acid (18:2) and oleic acid (18:1) were markedly increased at high copper concentration particularly linoleic acid.

Key words: Biouptake, Heavy metals, Fatty Acids

INTRODUCTION

The uptake and accumulation of heavy metals by fungal biomass is receiving increasing attention in a biotechnological context since microbe based technologies may provide an alternative or adjunct to conventional techniques of metal removal from polluted effluents and waste waters (Brierley et al. 1985; Gadd 1986; Ksheminska *et al*. 2003). Therefore, a better understanding of the mechanisms allowing fungi to survive in high concentrations of essential or non-essential metal ions is of both environmental and economic significance (Ledin, 2000; Gadd, 2004; S1aba *et al*., 2005; Paraszkiewicz *et al.*, 2009; Shugaba *et al*. 2010, Krzysztof *et al*. 2011). Fungi, due to their mycelial nature and well documented ability to accumulate metals of all families (Gadd, 1993), are good candidates for bioremediation of metals from wastes. de Rome and Gadd (1987) studied the ability of *Rhizopus arrhizus*, *Cladosporium resinae*, and *Penicillium italicum* to remove copper from aqueous solution. Heavy metal uptake by fungi is of fundamental importance to organisms growing in polluted habitats since tolerance may be determined by the ability to prevent cellular entry of a potentially toxic metal or the ability to compartmentalize or detoxify it within the cell. Heavy metals accumulated mainly in the fungal cell wall and in the vacuoles of *Glomus intraradices*, while minor changes in metal concentrations were detected in the cytoplasm (Gonzalez *et al.* 2008; Elisa *et al*. 2009). The cell walls of some microbes appear to have a greater and more selective ability to accumulate some metals. A large portion of the Cu^{2+} taken up by *Penicillium ochro-chloron* was accumulated in the cell walls (Motohiro *et al*. 1983).

Copper and cobalt are toxic to *Chaetomium globosum* and *Stachybotrys chartarum* where their growth markedly decreased with increasing Cu and Co concentrations in the growth medium. However, both fungi were able to tolerate Cu and Co up to a concentration of 800 mg/L (Hefnawy, *et al*.2009) . In other work, it was found that *Penicillium citrinium* was able to tolerate copper in the growth medium up to 400 mg/L respectively (Azab & Hefnawy, 1999). A strain of *Fusarium oxysporum* was found to tolerate Cu in the growth medium up to 600 mg/L (Hefnawy & Razak, 1998). Similar results were also observed where fungi isolated from metal contaminated agricultural soil belonged to genera *Aspergillus*, *Penicillium*, *Alternaria*, *Geotrichum*, *Fusarium* and *Trichoderma* showed a significantly tolerance to heavy metals. The minimum inhibitory concentration ranged from 0.6 to 9 mg/mL for Cu and 0.1 to 5 mg/mL for Co depending on the isolate (Zafar

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et al. 2007). Metal absorption and resistance to toxicity are related phenomenon. This point was made in 1982, when it was shown that multiple copies of copper metallothionein in *S. cerevisiae* enhanced its resistance to copper (Fogel and Welch, 1982). Petr (2010) stated that saprotrophic fungi are especially sensitive to heavy metals since they rely heavily on extracellular enzymes for nutrient acquisition (ligninolytic, cellulolytic, and hemicellulolytic enzymes, as well as several others), and these enzymes are often a target of heavy metal toxicity. To cope with heavy metal toxicity, fungi have evolved a set of response mechanisms that limit the toxicity of the metal to their cells. In addition, saprotrophic and mycorrhizal fungi are also the biogenic agents of metal mobilization from minerals and immobilization into novel, mycogenic minerals, such as metal oxalates. Copper is considered to be accumulated passively by the spores by unspecific reaction with cell constituents (Somers, 2008).

Studies by Kazy *et al*. (2002) concerning extracellular polysaccharides of copper-sensitive and copper-resistant *Pseudomonas aeruginosa* strains revealed that both polymers were acidic in nature and contained alginate as a major component along with various neutral- and amino-sugars**.** Studied by Doss *et al*. (2003) the extracellular matrix of the Botrytis cinerea strain was found to be a mixture of carbohydrates, simple lipids, proteins, and a dark pigment that was identified as melanin. One of the suggested roles for extracellular mucilaginous material is the involvement in cell protection against heavy metal toxicity (Paraszkiewicz *et al*., 2007; Vesentini *et al*., 2007). Heavy metal toxicity can be manifested by altering or blocking enzyme activity, inhibiting macromolecule synthesis as well asinducing disruption of cellular and organellar membranes (Denkhaus and Salnikow, 2002). A reduction in polyunsaturated fatty acid content is also a frequently reported effect caused by some heavy metals (Garcia *et al*., 2005). The present study is aimed at bioaccumulation of copper metal and their impact on fungal fatty acids

MATERIALS AND METHODS

Used Fungi and Their Culture Condition:

Two fungal species *Aspergillus terreus* and *Alternaria alternate* were obtained from fungal laboratory, Faculty of Sience, AL-Azhar University., cultivated at different concentrations of copper acetate ranged from 0 to 1000 ppm on Dox medium containing (per liter): $NaNO_3$, 2 g; KH_2PO_4, 1 g; KCl, 0.5 g; $MgSO_4$ $7H_2O$, 0.5 g; $FeSO_4$ $7H_2O$, 0.001 g; and agar, 20 g. Then incubated at 28 ºC for 6 days, after incubation periods the fungal growth and sporulation percentage were estimated with assay colony radius and heamocytometer.

Gas Chromatography/mass Spectrometry (GC/MS) Analysis of Fatty Acids:

Fungal mycelium was grinded in 10 ml chloroform: Methanol (2:1) and then filtrated and concentrated into 1 ml. The concentrated extract was placed in GC auto-sampler vials until they were analyzed. A Varian Star 3400 Cx Ion Trap GC/MS Shimadzu GCMS-QP 5050 A. software class 5000. Searched library: Wiley 229 LIB. Column: DBI, 30m, 053 mm ID; 1.5 um film. Carrier gas: Helium (flow rate 1 ml/min.).Ionization mode: El (70 ev). Temperature program: 70 (static for 2 min) then gradually increasing (at a rate of 2 /min) up to 220 (static for 5 min).Detector temperature 250 injector temperature 250 The chromatographs were compared and individual peaks were identified by comparing mass spectra to the library references. At the Regional Center for Mycology and Biotechnology AL-Azhar University.

Elemental Analysis:

Fungal mycelium was collected from broth medium after incubation period and dried for obtaining 0.25 mg dry weight, then grinded and inoculated on Scaning electron microscope grads and the percentage of metals content was measured with x ray.(Scaning electron microscope JSM-500 LV, with coated in Spi- MoDule. SPUTTER Coated) At the Regional Center for Mycology and Biotechnology AL-Azhar University.

Results:

Aspergillus terreus and *Alternaria alternata* were able to tolerate copper metal ion in the growth medium up to 1000 ppm and 800 ppm respectively, but *A. alternata* failed completely to grow at 1000 ppm. Their growth increased at 100 ppm but then markedly decreased with increasing metal concentrations in the medium (Table 1). Fifty % growth inhibition of *A. terreus* and *A. alternata* occurred approximately at 400 ppm copper . From the result (Table 1) *A. terreus* was more resistant to copper metal than *A. alternata* . On the other hand, the rate of sporulation (conidiospoes production) decreased with increasing copper metal concentration (Table 2) , Although the growth of *A. alternata* and *A. terreus* was observed and measured at 800 ppm and 1000 ppm but failed completely to sporulate at this concentrations respectively.

Table 1: Growth of *Aspergillus terreus* and *Alternaria alternate* at different concentrations of copper metal

Metal concentration (ppm)	Colony radius (cm)	
	Aspergillus terreus	*Alternaria alternata*
0	6.56 ±0.069	5.36±0.027
100	7.03±0.070	5.60±0.082
200	5.50±0.074	3.40±0.047
400	3.30±0.074	2.66±0.029
800	1.76±0.054	1.30±0.047
1000	1.43± 0.027	0.0±0.00

Table 2: Sporulation % of *Aspergillus terrius* and *Alternaria alternate* at different concentrations of copper metal

Copper metal concentration (ppm)	Conidiospores production %	
	Aspergillus terreus	*Alternaria alternata*
0	100 ± 0.00	100 ± 0.00
100	93.0 ± 0.94	86.3 ±1.36
200	63.0 ± 1.41	41.66 ±0.72
400	34.3 ± 0.54	21.66 ±0.72
800	14.0 ± 0.47	0.00 ± 0.00
1000	0.0 ± 0.00	0.00 ± 0.00

Sporulation % regarded to control (0 ppm) as 100 %

Metal content of copper in the mycelium of *A. terreus* was subsequently increased with increasing metal concentrations in the growth medium and represented high affinity to absorb copper. The maximum accumulated amount of copper was detected at 400 ppm , where their percentage was 7.67% compared to % at 100 ppm (5.47 %) , then reached to minimum concentration at 800 ppm. From the result in table (3) high concentration of copper metal in the growth medium inhibit their uptake by the fungus and enhance the fungus to absorb other elements.

Table 3: Percentage % Copper accumulated in *Aspergillus terreus* at different concentration of copper metal

Copper concentration (ppm)	Percentage % Copper and other elements in fungal cells	
	Copper	Other elements
0.0	0.86	99.14
100	5.47	94.53
200	6.53	93.47
400	7.67	92.33
800	4.50	95.50

Table 4: Fatty acids of *Aspergillus terreus* at different concentrations of copper

Fatty acids	Different concentrations of copper metal (ppm)		
	0.0	100	800
Capric (C10)	1.72	0.00	0.57
Lauric (C12)	0.29	2.22	2.00
Tridecanoic (C13)	0.21	0.00	0.50
Myristic (C14)	1.23	8.59	4.09
Pentadecanoic (C15)	0.26	0.00	0.20
Palmitic (C16)	49.03	66.12	53.27
Palmitoleic (C16:1)	0.00	0.00	0.00
Heptadecanoic (C17)	0.37	1.73	1.28
Stearic (C18)	30.89	5.38	6.29
Oleic (C18:1)	14.29	11.32	18.04
Linoleic (C18:2)	1.46	1.35	11.68
Arachidic (C20)	0.25	3.29	2.08
Behenic (C22)	0.00	0.00	0.00
Total Saturated	84.25	87.33	70.28
Total Unsaturated	15.75	12.67	29.72

Copper had the highest influence on the fatty acid of *A. terreus* at low copper (100 ppm) and high concentrations (800 ppm) ,The ratio of saturated to unsaturated fatty acids in the fungus treated with low and high concentration was determined to be 6.89 and 2.36 respectively (Table 4 & Fig.2). Most of the detected fatty acids at low concentration (100 ppm) was high in the concentration compared with their concentrations at high metal concentration (800 ppm) and with the control. Among the detected fatty acids, two unsaturated

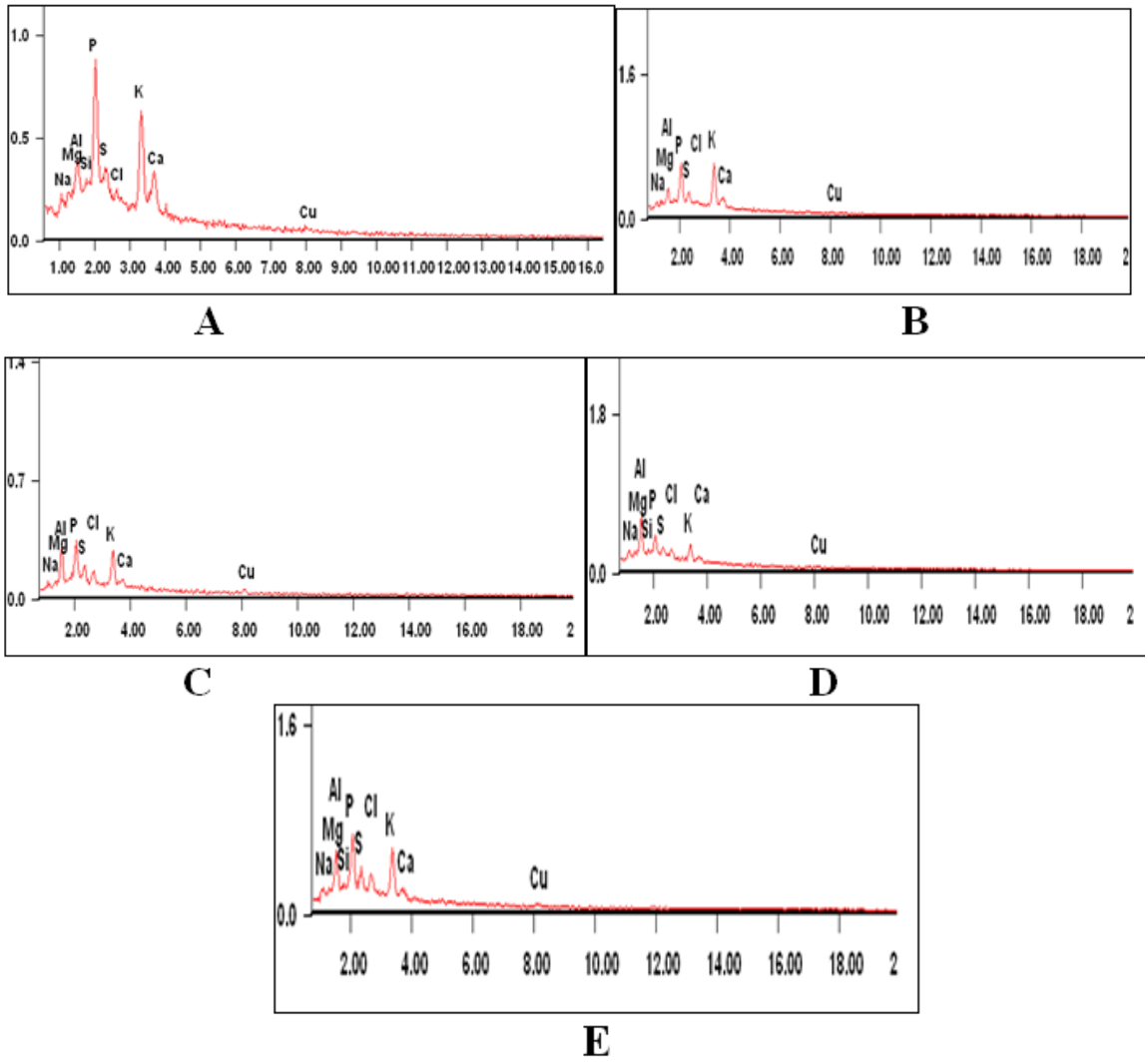
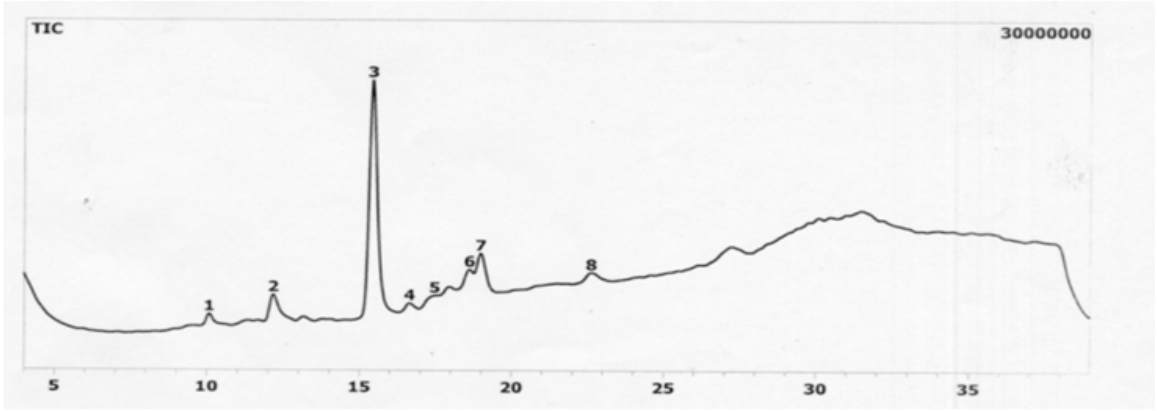


Fig. 1: Copper Metal chromatogram of *A. terreus* at different concentrations of copper (A, 0; B,100; C,200; D,400; D,800 ppm)

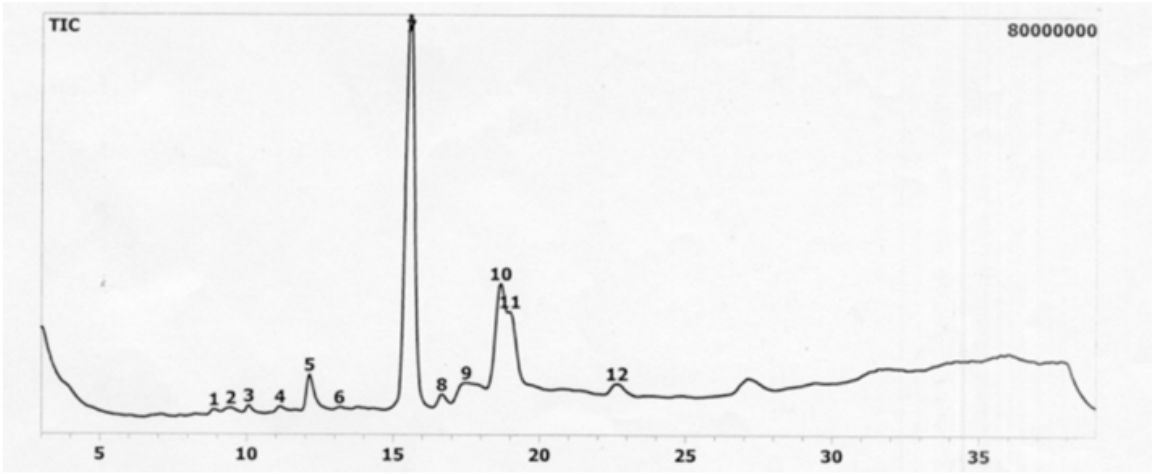
fatty acids linoleic acid (18:2) and oleic acid (18:1) were markedly increased at high copper concentration particularly linoleic acid. On the other hand myristic acid (C14) as saturated fatty acid decreased at high copper concentration compared at low concentration with approximately 52 %. At low copper concentration (100 ppm) Capric acid (C10) was not detected but detected at control and at high copper concentration.

Discussion:

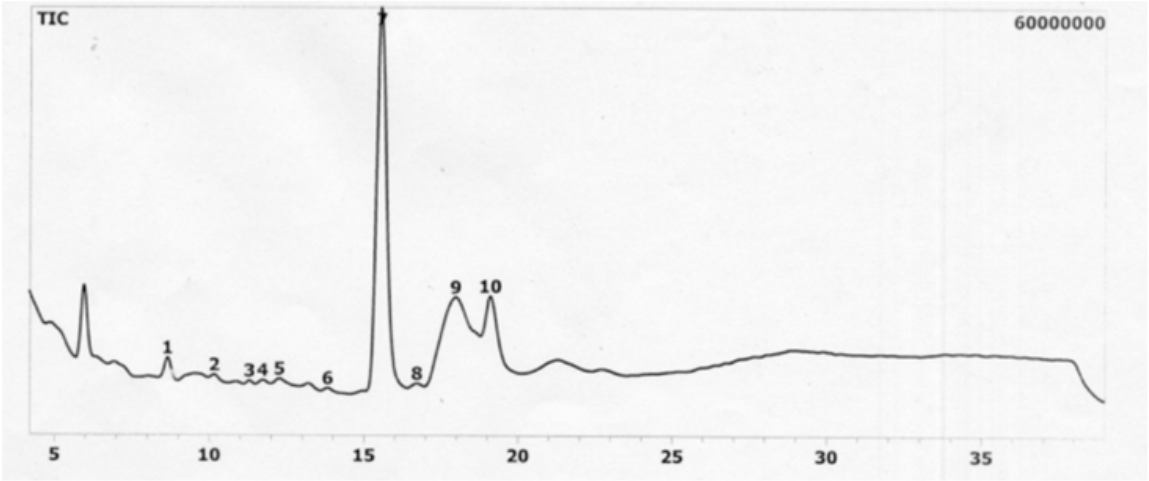
Copper metal at high concentration is toxic to *A. terreus* and *A. alternata* where their growth markedly decreased with increasing copper concentrations in the growth medium. However, both fungi were able to tolerate Cu up to a concentration 1000 ppm and 800 ppm respectively. In other work, it was found that *Streptomyces anulatus* and *Penicillium citrinium* were able to tolerate copper in the growth medium up to 1000 and 400 mg/L respectively (Azab and Hefnawy, 1999). A strain of *Fusarium oxysporum* was found to tolerate Cu in the growth medium up to 600 mg/L (Hefnawy and Razak, 1998; Mehta *et al* 2010). Similar results were also observed where fungi isolated from metal contaminated agricultural soil belonged to genera *Aspergillus, Penicillium, Alternaria, Geotrichum, Fusarium* and *Trichoderma* showed a significantly tolerance to heavy metals. The minimum inhibitory concentration (MIC) ranged from 0.6 to 9 mg/ml for Cu depending on the isolate (Zafar *et al.* 2007). From our results copper at low concentration (100 ppm) increased fungal growth,



A



B



C

Fig. 2: Fatty acids chromatogram of *A. terreus* at different concentrations of copper (A, 0; B,100; C, 800 ppm).

this may be due to copper is trace element needed for fungal metabolism. Nevertheless, like other above certain concentrations they become toxic. Heavy metal toxicity can be manifested by altering or blocking enzyme activity, inhibiting macromolecule synthesis as well as inducing disruption of cellular and organellar membranes (Denkhaus and Salnikow, 2002; Gasic *et al.* 2006). A decrease in microbial enzymes activity was also noted when the assay was performed in the presence of heavy metals (Ni^2 and Cu^2) (Poli *et al.* 2009; Wang *et al.* 2010). From the obtained result sporulation rate of fungi under study was more affected by the growth on high concentration of copper . The perithecia and spores of fungi were markedly decreased in their number with damage of seta, conidiophores and phialides (Hefnawy *et al.* 2009).

Copper metal uptake by the *A. terreus* mycelium was affected by their concentration , where Cu content in the mycelium was increased with elevated concentrations of metal in the growth medium. Similar results were also observed with *Schizosaccharomyces pombe* and *Candida glabrata* grown in the medium containing 100 mg Cd/L could incorporate 20 and 8 mg Cd/g dry mass respectively (Krumov, *et al.* 2007),also is similar with result obtained with (Hefnawy *et al*. 2009). Previous studies reported that high metal concentrations may limit the bioaccumulation in the fungi (Moore *et al*.2008).

Copper had the highest influence on the fatty acid of *A. terreus* at low copper (100 ppm) and high concentrations (800 ppm), where the concentrations of the detected fatty acids were less at high concentration than lower concentration of copper metal. This observation is further supported by the progressive decrease in the growth of *A. terreus* at high metal concentration. Hefnawy *et al*. (2009) noted that, fungal total lipid showed a slight increase in the mycelium with elevated copper concentration up to 400 mg/L and decreased above this concentration. In our studies two unsaturated fatty acids linoleic acid (18:2) and oleic acid (18:1) were markedly increased at high copper concentration particularly linoleic acid ,this acids may play an important role in fungus tolerance to copper metal stress. A reduction in unsaturated fatty acid content is also a frequently reported effect caused by some heavy metals and the ability of metals to generate oxidative stress (Howlett and Avery, 1997; Garcia *et al*., 2005). In other study (Frostegard 1993), many branched Phospholipid fatty acids, of microorganisms increased in the high metal-content soils (Pennanen *et al*.,1996)**.** Most of cell membrane structure is fatty acids and therefore may affected with stress of metals. Stohs and Bagchi (1995) stated that high concentrations of heavy metals like Cu (II) and Zn have been reported to cause a rapid decline in membrane integrity, which is generally manifested by leakage of mobile cellular solutes and cell death. Therefore, the increased in polyunsaturated (18:2) fatty acid concentration observed in high copper treated mycelia of *A. terreus* allows us to suggest that retardation or inhibition of lipid peroxidation may be involved in copper ion toxicity towards the studied fungus.

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REFERENCES

Azab, A.A., M.A. Hefnawy, 1999. Copper metal-protein in *Streptomyces anulatus* and *Penicillium citrenium*. J. Union Arab Biol, Cairo (8) B Microbiol. And Viruses, pp: 403-415.

Brierley, C.L., D.P. Kelly, K.J. Seal, D.J. Best, 1985. Materials and biotechnology. In: Higgins I J, Best DJ, Jones J (eds), Biotechnology. Blackwell, Oxford, pp: 163-212.

Chudzynski, K., J. Grazyna, S. Aleksandra, F. Jerzy, 2011. Mercury content and bio-concentration potential of Slippery Jack, *Suillus luteus*, mushroom. Food Chemistry, 25: pp: 986-990.

de Rome, L. and G.M. Gadd, 1987. Copper adsorption by *Rhizopus arrhizus*, *Cladosporium resinae* and *Penicillium italicum*. Applied Microbiology and Biotechnology, 26: 84-90.

Denkhaus, E. and K. Salnikow, 2002. Nickel essentiality, toxicity, and carcinogenicity. Critical Reviews in Oncology/Hematology, 42: 35-56.

Doss, R.P., J. Deisenhofer, H.A. Krug von Nidda, A.H. Soeldner, R.P. McGuire, 2003. Melanin in the extracellular matrix of germlings of *Botrytis cinerea*. Phytochemistry, 63: 687-691.

Elisa, G., L. Guido, B. Graziella and R.G. Bernard, 2009. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can. J. Microbiol., 55(5): 501-514.

Fogel, S. and J.W. Welch, 1982. Tandem gene amplification mediates copper resistance in yeast. Proceedings of the National Academy of Sciences USA, 79: 5342-5346.

Frostegard, A., A. Tunlid, E. Baath, 1993. Phospholipid fatty acid composition, biomass, and activity of microbial communities from two soil types experimentally exposed to different heavy metals. Appl. Environ. Microbiol., 59: 3605-3617.

Gadd, G.M., 1986 .The uptake of heavy metals by fungi and yeasts: the chemistry and physiology of the process and applications for biotechnology. In: Eccles H, Hunt S (eds) Immobilisation of ions by bio-sorption. Ellis Horwood Ltd, Chichester, pp: 135-147.

Gadd, G.M., 1993. Tansky Review No. 47 - Interactions of fungi with toxicmetals. The New Phytologist, 124: 25-60.

Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma, 122: 109-119.

Garcia, J.J., E. Martinez-Ballarin, S. Millan-Plano, J.L. Allue, C. Albendea, L. Fuentes, J.F. Escanero, 2005. Effects of trace elements on membrane fluidity. Journal of Trace Elements in Medicine and Biology, 19: 19-22.

Gasic, K., S.S. Korban, 2006. Heavy metal stress, in: K.V.M. Rao, A.S. Raghavendra, K.J. Reddy (Eds.), Physiology and Molecular Biology of Stress Tolerance in Plants, pp: 219-254.

Gonzalez, G.M., L.H. Melville, N. Ferrol, J.N. Lott, A.C. Azcon, R.L. Peterson, 2008. Ultrastructural localization of heavy metals in the extraradical mycelium and spores of the arbuscular mycorrhizal fungus Glomus intraradices. Can J. Microbiol., 54(2): 103-110.

Hefnawy, M.A., M.I. Ali and S.A. Abdul-Ghanay, 2009. Influence of Copper and Cobalt Stress on Morphology and Ultra-structure of *Chaetomium Globosum* and *Stachybotrys Chartarum*. Australian Journal of Basic and Applied Sciences, 3(4): 3158-3165.

Hefnawy, M.A. and A.A. Razak, 1998. Alteration of cell wall composition of *Fusarium oxysporum* by copper stress. Folia Microbiol., 43(5): 453-458.

Howlett, N.G. and S.V. Avery, 1997. Induction of lipid peroxidation during heavy metal stress in *Saccharomyces cerevisiae* and influence of plasma membrane fatty acid unsaturation. Applied and Environmental Microbiology, 63: 2971-2976.

Kazy, S.K., P. Sar, S.P. Singh, A.K. Sen, S.F. D'Souza, 2002. Extracellular polysaccharides of a copper-sensitive and a copper-resistant *Pseudomonas aeruginosa* strain: synthesis, chemical nature and copper binding. World Journal of Microbiology and Biotechnology, 18: 583-588.

Krumov, N., S. Oder, I. Perner- Nochta, A. Angelov, C. Posten, 2007. Accumulation of Cd nanoparticles by yeasts in a fed-batch bioprocess. J. Biotechnol., 132(4): 481-486.

Ksheminska, H., A. Jaglarz, D. Fedorovych, L. Babyak, D. Yanovych, P. Kaszycki and H. Koloczeke, 2003. Bioremediation of chromium by the yeast *Pichia guilliermondii:* toxicity and accumulation of Cr (III) and Cr (VI) and the influence of riboflavin on Cr tolerance. Microbiol. Res., 158: 59-67.

Ledin, M., 2000. Accumulation of metals by microorganisms -processes and importance for soil systems. Earth-Science Revi., 51: 1-31.

Mehta, K.D., D. Chitrangada, K. Rakesh, B.D. Pandey, S.P. Mehrotra, 2010. Effect of mechano-chemical activation on bioleaching of Indian Ocean nodules by a fungus Minerals Engineering, 23: 1207-1212.

Moore, B.A., J.R. Duncan, J.E. Burgess, 2008. Fungal bioaccu-mulation of copper, nickel, gold and platinum, Miner. Eng., 21: 55-60.

Motohiro, F., Y. Sunao, T. Shozo, 1983. Distribution of copper in the cells of heavy metal tolerant fungus, *Penicillium ochrochloron*, cultured in concentrated copper medium. Agric. Biol. Chem., 47: 1367-1369.

Paraszkiewicz, K., A. Frycie, M. Slaba, J. D1ugon Ski, 2007. Enhancement of emulsifier production by Curvularia lunata in cadmium, zinc and lead presence. Biometals, 20: 797-805.

Paraszkiewicz, K., B. Przemys1aw, D. Jerzy, 2009. Effect of nickel, copper, and zinc on emulsifier production and saturation of cellular fatty acids in the filamentous fungus *Curvularia lunata*. International Biodeterioration & Biodegradation, 63: 100-105.

Pennanen,T., A. Frostegard, H. Fritze, E. Baath, 1996. Phospholipid Fatty Acid Composition and Heavy Metal Tolerance of Soil Microbial Communities alongTwo Heavy Metal-Polluted Gradientsin Coniferous Forests. Applied and Environmental Microbiology, 62(2): 420-428.

Petr, B., 2010. Effect of Heavy Metals on Saprotrophic Soil Fungi. Soil Biology, 19: 263-279.

Poli, A., A. Salerno, G. Laezza, P. di Donato, S. Dumontet, B. Nicolaus, 2009. Heavy metal resistance of some thermophiles: potential use of a-amylase from Anoxybacillus amylolyticus as a microbial enzymatic bioassay. Research in Microbiology, 160: 99-106.

Slaba, M., M. Bizukojc, B. Pa1ecz, J. D1ugonski, 2005. Kinetic study of the toxicity of zinc and lead ions to the heavy metals accumulating fungus *Paecilomyces marquandii*. Bioprocess and Biosystems Engineering, 28: 185-197.

Shugaba, A., A.J. Nok, D.A. Ameh and J.A. Lori, 2010. Studies on the Growth of Some Filamentous Fungi in Culture Solutions Containing Hexavalent Chromium. International Journal of Biotechnology and Biochemistry, 6(5): 715-722.

Somers, E., 2008.The uptake of copper by fungal cells. Annals of Applied Biology, 51(3): 425-437.

Stohs, S.J. and D. Bagchi, 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radical Biol. Med., 18: 321-336.

Vesentini, D., D.J. Dickinson, R.J. Murphy, 2007. The protective role of the extracellular mucilaginous material (ECMM) from two wood-rotting basidiomycetes against copper toxicity. International Biodeterioration and Biodegradation, 60: 1-7.

Wang, F., J. Yao, Y. Si, H. Chena, M. Russel, K. Chen, Y. Qian, G. Zaray, E. Bramanti, 2010. Short-time effect of heavy metals upon microbial community activity. Journal of Hazardous Materials, 173: 510-516.

Zafar, S., F. Aqil, I. Ahmad, 2007. Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour. Technol., 98(13): 2557-2561.