Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(2):535-540

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Response of some fungal species to the effect of copper, magnesium and zinc under the laboratory condition

Najwa Mohammed Jameel Ali Abu-Mejdad

Department of Biology, College of Science, University of Basrah, Iraq

---

ABSTRACT

In the present study using Aspergillus niger, Candida albicans and Cryptococcus neoformans for investigating about the effect of (Cu, Mg and Zn) on the growth it under laboratory condition in liquid and solid media. In liquid media Cu, Zn ions were inhibited growth of A. niger where as Mg ion was increase the growth, while lead to added three heavy elements to inhibited of C. albicans and C. neoformans except copper ion in concentration 40 ppm lead to increased growth C. neoformans compared with control. Solid medium was used to study the effect of these ions on the numbers of colonies generally the three fungal species decreased in colony number by effect three heavy metals, except Copper, Magnesium appeared effect positively by increased number of colony for A. niger during 24 hours.

Key words: fungi, heavy metals, growth response

INTRODUCTION

In the atmosphere, most metals are present in particulate form after release by both natural and man-made activities. The emissions of metals from anthropogenic sources occur via diverse pathways including combustion of fossil fuel, waste incineration, industrial processes, roasting and smelting of ores in non-ferrous metal smelters, melting operations in ferrous foundries, and Kiln operations in cement plants [1]

Because the heavy elements of the components of earth's crust natural as well as they exist and concentrations and low in soil and sediment and water and even in living organisms, but the increase in the production and emission of metals which represent persistent inorganic increased danger because they do not break down into water and carbon dioxide Unlike organic pollutants which can be disposed of in this way. [2]

So researchers resorted to the use of methods of treatment vitality using fungi as to get rid of these contaminants and promise a way to combat pollution as Study suggests [3] to the possibility of the use of microbial biomass to remove toxic heavy metals as an alternative method for physiochemical techniques and waste disposal in water microbial biomass non-visible (fungal) show high affinity for heavy metals ions compared with visual biomass.

Fungi play a significant role in human life, besides their utilization in industry, agriculture, medicine, food industry, textiles and bio remediation Mycoremediation[4]

Be adaptation fungi on the toxic effects of heavy metals and their ability to grow their existence and development of resistance towards it make it stand out reviving important in the use of bioremediation methods and in particular fungal Mycoremediation to get rid of these metals in contaminated environments the fungi that favors the bacteria in treatment for a number of reasons: -

1 - usability on the composition of fungal filaments and which enable them to penetrate the contaminated soils to reach the heavy element also provides a greater surface area for adsorption or absorption of the metal in the water
2 - abundantly present in contaminated areas because it is a favorable environment
3 - Some fungi such as *Aspergillus niger* and yeasts, which have the ability to withstand the adverse effects of heavy elements can be used to support the country's economy through the use of the disposal of industrial pollutants where the method is safe and cheap, socially acceptable as vital and the best treatment of chemical or physical methods[5].

As pointed out by several authors to the presence of receptors in natural waters are heavy elements that are generated and flow from industrial sources and then release to the environment without proper treatment, which led to the emergence of real threats to public health because it swells and accumulate vital role in the food chain reasoned negative effects on animals, plants and human health[6].

It studies conducted in this field study[7] which explained that the fungus *Piptoporus betulinus* Ability to respond to cadmium in the solid culture medium as reduced colony growth while in the liquid culture medium fungus was less fluid sensing element as reduced cell growth by a little.
In Pakistan booming industry and the economy, but the presence of heavy metals in soil and water affect agricultural crops causing obstruction of the economy of the country so a study was undertaken by the [8] to see the effect of some inorganic salts of copper on some fungi and fungus *Aspergillus niger* As observed decrease in growth rates due to their toxic effects.

In Malaysia held [9] study of the response of the fungus *Trichoderma atroviride* The copper component and the results showed the ability of this fungus on adsorption element by 85% and absorbed by 47% so researcher pointed to the importance of this fungus in bioremediation.

So the current study aimed to find out the impact on the growth of the isolates *Aspergillus niger Candida albicans* , *Cryptococcus neformans*, Zinc, copper ions and magnesium in the solid and liquid culture media and the extent of the impact of these ions on living cells

MATERIALS AND METHODS

Sample collection

Through The current study 20 samples were collected from the waters of the Shatt al-Arab River and planted directly snapped 0.1 ml of each sample at a rate of three replicates on Sabourauds dextrose agar the (SDA)(Himedia, India) Using sterile publisher L-shape Then incubated at a temperature 27ºC.

Examined the dishes after three days and lasted monitored daily for a week The initial examination of the anatomical occlusion by using a microscope and through developing fungi isolated using sterile needle on petri dishes container Sabourauds Dextrose Agar the purpose of purification and then transferred these isolates transplantation center slant in sterile tubes and kept in the refrigerator after growth.

For the purpose of examining the characteristics and classification of fungi isolated thoroughly examined under an optical microscope Light microscope Prepare slides installed textured Lactophenol Cotton blue containing blue dye doubled and depending on the microscopic characteristics and phenotypic characteristics of the culture media been diagnosed all fungi isolated during the period of study in the laboratory and with the help of the following references[10,11].

Prepare the fungal inoculation

Inoculum was prepared innate user present study the transfer of part of the colony stimulating on Sabourauds agar center SDA For 48 hours of yeasts and 7 days of the fungus *Aspergillus niger* To 5 ml of sterile distilled water suspension strongly commentator and adjust the number of cells 3×10^6 cell according to MacFrland scale [12].

Was added (7) ml of liquid culture media (consisting of 2% glucose and 1% peptone) for each test tube and added ions of heavy metals (zinc, copper and magnesium) salts Cu $(No_3)_2$ And $MgSO_4$ And $ZnCl_2$ To each test tube under sterile conditions, as well as added 1 ml of suspense to free ions pipeline elements (control treatment) was the work of 3 replicates per treatment and incubated transactions at a temperature of 27 m.

I've been up the growth of fungus in the liquid culture media measuring Optical Density (OD) Wavelength (590) nm using a spectrometer optical Helios αv4.60U.V-visible spectrophotometer England. Located in the Department of Physics - Faculty of science - Basra University and for different exposure periods (one week, two weeks).

As for the solid culture media Inoculated culture dishes containing 15 ml of SDA Compatible ions of heavy metals (0.02) ml of suspension cells 3×10^6 cells / ml and then spreading on the dish using L-Shape . Were the work of three replicates per treatment and incubated transactions at a temperature of 27 m [13]

Statistical analysis

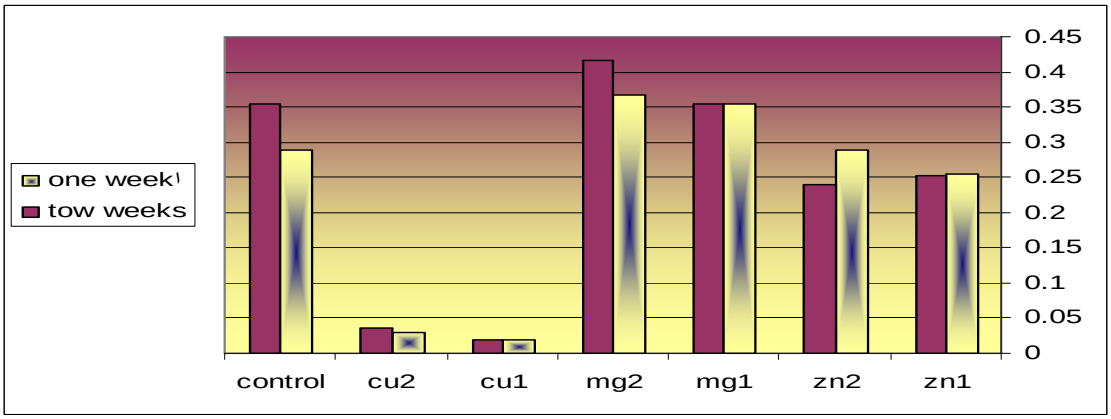
Use statistical analysis software Minitab 87 To analyze the test results of the present study, and has used analysis of variance test ANOVA To see the moral differences between transactions and make test less significant difference in the rate of RLSD To see the moral differences between the averages [14]

RESULTS

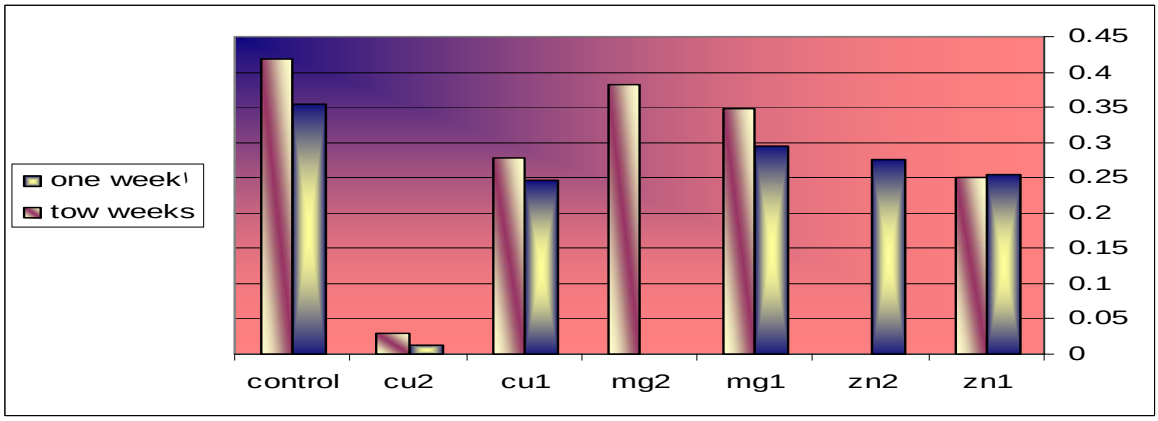
Results of the statistical analysis revealed significant differences below the level of probability P <o.o5 In response to three elements fungi tested in the liquid culture media Figures (1,2,3) depending on the time period and the heavy element concentrations lab as he was less significant difference in the rate as shown below: -

RLSD for fungi = 0.010, RLSD for metals = 0.0103, RLSD for concentrations = 0.0104

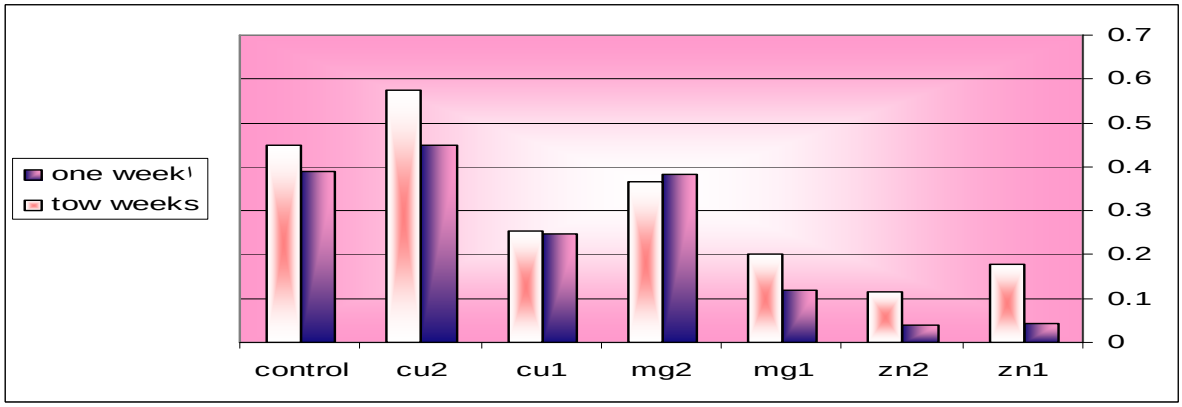
RLSD for time periods = 0.008



(Figure .1): Effect of ions of heavy metals on fungus *Aspergillus niger* In the liquid culture media using concentrations first 20ppm The second 40ppm The two time periods one and tow weeks



(Figure . 2): Effect of ions of heavy metals on yeast *Candida albicans* In the liquid culture media using concentrations first 20ppm The second 40ppm The two time periods one and tow weeks



(Figure.3): Effect of ions of heavy metals on yeast *Cryptococcus neoformans* In the liquid culture media using concentrations first 20ppm The second 40ppm The two time periods one and tow weeks

Results of the statistical analysis revealed significant differences below the level of probability P <o.o5 In the preparation of the solid culture medium three-fungal towards elements tested Table (1,2,3) different concentrations within 24 hours as it was less significant difference in the rate as shown below: -

RLSD for fungi = 11.02 , RLSD for metals = 11.04 , RLSD for concentrations = 11.05

While not show any significant differences in the preparation of the colonies during the period of 48 hours and 72 hours for the three fungi

Table (1) effects of ions of heavy metals on the growth of *Aspergillus niger* on solid culture medium

T.	Elements	The period of time of the exposure to the elements		
		24 hours	48 hours	72 hours
1	Control	200	HG	HG
2	Mg1	250	HG	OG
3	Mg2	300	HG	OG
4	Zn1	150	HG	OG
5	Zn2	200	HG	OG
6	Cu1	250	HG	HG
7	Cu2	300	HG	H. G

Note: 1 = 20 ppm , 2 = 40 ppm , (HG) = Heavy growth , (OG) = Over growth

Table (2) effects of ions of heavy metals on growth *Candida albicans* on solid culture medium

T.	Elements	The period of time of the exposure to the elements		
		24 hours	48 hours	72 hours
1	Control	HG	HG	OG
2	Mg1	300	HG	OG
3	Mg2	300 <	HG	OG
4	Zn1	300	HG	OG
5	Zn2	300 <	HG	OG
6	Cu1	300	HG	OG
7	Cu2	250	HG	OG

Table (3) effects of ions of heavy metals on the growth of *Cryptococcus neoformans* on solid culture medium

T.	Elements	The period of time of the exposure to the elements		
		24 hours	48 hours	72 hours
1	Control	HG	HG	OG
2	Mg 1	300	HG	OG
3	Mg 2	300 <	HG	OG
4	Zn 1	300	HG	OG
5	Zn 2	300 <	HG	OG
6	Cu 1	300	HG	OG
7	Cu 2	300 <	HG	OG

DISCUSSION

Some ions of heavy elements necessary for the growth of fungi, when added low concentrations fall within the limits of carrying fungus, but increased concentrations of ions limits of carrying fungus adversely affect the growth and reproduction, have been observed this phenomenon when exposing fungi under study to different concentration of zinc ions and magnesium and copper.

And globally conducted several studies to identify the extent fungi response to the impact of heavy elements and the factors influencing And study [15] Which showed that some of the factors related to the type of fungal strain and their locations and other factors relating to the metal and specifications, including the type of his cargo, atomic number, mass number is his physical qualities and link sites with metal positive or negative charge on the surface absorption (Fungal cell wall).

During the current study appeared the impact element Zinc and two concentrations (20 ppm And 40ppm) Fungi under study compared with control samples to the decline in growth rates in the liquid culture media, but the yeast *Cryptococcus neoformans* The decline in growth has very high dates because of owning this yeast capsule surrounded it and the impact of this element on the configuration of the capsule and colony viscosity [16] That different concentrations of zinc and magnesium ions have more impact on *Cryptococcus neoformans* Compared with copper as able elements positive charges from the link totals carboxylic existing endings chains sugary multi-constituent Capsules negatively charged and the formation of bonds of ionic inhibit formation capsule and then enter ions inside the cell poignant events vital them, including the process of division, as stated[13] About the role of the heavy elements in the inhibition doubled DNA And then stop the process of division, which led to a decrease in the rate of growth of yeast.

So the current study relied on the use of growth as a guide to fungus response mechanisms under study as stated [17] Measuring growth rates is a good guide to influence the toxic elements in living organisms and reflects metabolism cell as the addition of elements to the culture medium may lead to an increase in growth for fungal resistance, which appear resistant to the toxic effect of the element and repeat development fungus on the center contains the element heavy for more once it adapting for effect it

The fungus-sensitive heavy elements has been observed decline in growth rates due to the toxic effect of the element, which obviously is caused by the presence of two phases of metal adsorption by living organisms

The first is: do not rely on cell metabolism where the metal is linked to the cell surface
The second: It depends on the cell metabolism in principle Aden accumulates inside the cell

As for the element magnesium has been observed that the fungus *Aspergillus niger* Showed an increase in growth compared with fungus the yeasts that showed a decline in growth rates and in general cause ions of heavy metals toxic effects as is the case here, where it may be decreasing in growth rates Of *Candida albicans* Because of the effect on the germination tube configuration to prevent formation and effect on the divisions and growth [13] The decline in the growth rates for *Cryptococcus neoformans* As mentioned above positive element effect similar charge of zinc

The due to the impact of this element in shortening the process of synthesis, which led to an increase Growth rates in the filamentous fungus or because of the different composition of the wall between yeasts and filamentous fungi, which led to the emergence of varied impact Heavy elements As the dominant element heavy cause decreased growth rates, but here the case either because of contradictory
1 - Possession of fungus for resistance against Mechanics Component 2 - Increase colony growth, increased branching fungal filaments so that the fungus growths as sphericules and not fungal filaments i because of severe intensity, increase the production of fungal spores[18]

While all isolates showed a decline in growth rates towards the element copper Except for an increase in the rate of growth of yeast *Cryptococcus neoformans* The focus 40ppm As noted [19] That there are signs of resistance to the presence of a copper caused by the presence of yeast gene responsible for manufacturing protein metallothionein Working, which is the foundation for the copper link fence as the non-yeast protein synthesis in the resistance inhibits the link and encourage yeast to divide normally and increase compared isolates sensitive inhibits growth.

And in general has pointed out [20] That exposing fungi to Salts of inorganic copper is very toxic because effect severe events metabolic fungus as the components the cell wall one thousandth i confidentiality of proteins and fats

and polysaccharides provide convenient locations to link copper and the formation of complexes cause corrosion cell surface fungal integrated and then decompose and death or inhibition.

As for the effect of the time period showed isolates in general and concentrations increase in the rates of growth in the second week, compared with growth rates in the first week in the liquid culture medium while the *Aspergillus niger* The first copper concentration and magnesium focusing first steady increase exposing fungus heavy element may lead to increased likelihood of a link element fungus wall which leads to Increase its toxic effects.

yeasts unicellular less effect because the surface area has little , growth low density and limited extension as well as the hardness of it may not be fungal hyphae either fungus *Aspergillus niger* has shown resistance due to being linear fungus be spinning instinctive saves space Superficial largest so impressed by his biggest either filamentous fungus *A.niger* showed resistance due to being a filamentous fungus be mycelium[3].

either number of the colonies during the period of 24 hours study showed significant differences and different as she was preparing colonies For yeasts Less than control a results were number colonies was close due to being a single-cell fungi small And events metabolic and physiological be close compared with the *Aspergillus niger* As led elemental copper and magnesium to increase the numbers of colonies compared with control was due to The impact of the heavy element could die to increase the growth of mildew because society fungus adaptation on metal While zinc showed a decrease concentration of 20 and equal numbers to control the concentration of zinc 40 reason Sense *Aspergillus niger* For the element.

While the 48 and 72 period did not show the results of statistical analysis, there was no significant difference between the control samples and concentrations of elements toward three isolates because the element often effect in lag phase But after 48 and 72 may be a passing fungus stationary phase So did not show differences.

The solid culture medium also retard the movement heavy element compared liquid culture medium so observed effects of heavy element in liquid culture medium even after two weeks, while in the drive after 48 hours were the results are similar. [15]

CONCLUSION

The results concluded that heavy metals effects on fungi in liquid and solid culture media

REFERENCES

[1]Malekpouri M,. Ehsanpour M. & Afkhami M.*European Journal of Expermental Biology* ,**2012,**2(5):1714-1717.
[2]Jozefczack , M.; Remans, T.; Vangronsveld, J. and Cuypers, A.*Int.J.Mol.Sci*,**2012,** 13 :3145-3175.
[3] Shivakumar, CK and Thippswamy, B, **2012**,*International. Multidisciplinary Research.J*.2 (2) :6-12.
[4] Ranjini R & Padmavathi T. .*European Journal of Expermental Biology* ,**2012,**2(1):75-82.
[5] Dugal, S. and Gangawane, M,**2012**, *J.of chem.& pharm. Res.* 4 (5) :2362-2366.
[6] Chisti, Y., **2004**, *J.Chem. Pharm.Res*.6 :431-432.
[7] Baldrian, P., **2002,***Mycological Society of America.J*.94 (3) :428-436 p.
[8] Nasin, G.; Ilyas, N.; Ali, A. and Munawar, A. **,2008,** *Pak.J.Bo*.40 (1) :427-431.
[9] Yazdani, M.; Yap, CK and Abdullah, F. ,**2010**, *Pertanika.J.Trop.Agric*.33 (1) :71-77.
[10] Ellis, DH,**1994**, *Gillingham. Printers pty. Ltd. Australia.* 166p.
[11] Hoog de, GS & Guarro, J.,**1995,** *center albureau Voor Shimmel-cultures and universitat Rovirai Virgili. Spain b*. 720p.
[12] Collee, J. ; Fraser, A. ; Marmion, B. & Simon, A. 14 th ed, **1996,** *Churchill Liverstone. New York* .978 p.
[13] Pilgrims, M.S.B.**,2004,** *Basra Journal of Veterinary Research*.1(1)::1-14 p.
[14] Al-Rawi, H. M. & Khalaf Allah, A. M ,**2000**, *University of Mosul, Books Publishing House*.488 P.
[15] Manzoor, MT; Shoaib, A. & Bajwa, R.**,2012,***Afric.J.of Microbiol.Res*.6 (2) :236-244.
[16] Nimrichter, L.; Frases, S.; Cinelli, LP; Viana, NB; Nakouzi, A.; Travassos, LR; Casadevall, A. and Rodrigues, ML,**2007,** *Eukaryotic cell.J*.6 (8) :1400-1410.
[17] Sbihi, K.; Cherifi, O.; Elgharmali, A.; Oudra, B. and Aziz, F.**2012,** *J.Mater.Environ.Sci*.3(3) :497-506.
[18] Gabril, J.Kofro, O.; Rychlvsk, P. and Kren, M. ,**1996**,*Bull. Environ.Contam.Toxicol*.57 :383-390.
[19] Jin, YH; Dunlap, PE; McBride, SJ; Al-Refai, H.Bushel, PR and Freedman, JH 2008,**2008**,. *Genet. Pgen.J*.4 (4) :10-17
[20] Alpet, S.; Alpet, K.; Cadirci, BH.; Ozbayrak, O and Yasa, I.**,2010**,.*Electron.J.Biotechnol.13* (5) :45-48.