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# EFFECT OF INCUBATION TIME ON COPPER UPTAKE AND BIOMASS OF ASPERGILLUS TERREUS

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## ABSTRACT:

**Fifty pure isolates were obtained from *Aspergillus terreus* which isolated from rivers at Basrah/Iraq to study the ability of fungus growth at different concentration of copper at different incubation periods. The capacity of the biological removal of copper was calculated by the fungus. The results showed that the rate of accumulation of the copper element in the fungus cells increases with the incubation period of the fungus with the element and the removal capacity of *Aspergillus terreus* depend on the copper concentration and the time of incubation.**

**Key words:** *biological removal, accumulation, pollution, fungi, heavy metals.*

## INTRODUCTION:

**Heavy metals pollution is becoming more serious problem for the environment and public health. Worldwide, heavy metals are transported via aerial, terrestrial, and aquatic systems [1]. Because of their persistence in the environment, heavy metals can accumulate in organismal tissues through the food chain. When heavy metals are not removed by an organism, especially toxic metals, they become toxic to that organism [2,3]. As a result, heavy metal pollution is a significant global problem. The input of heavy metals into aquatic ecosystems is affected by natural processes and anthropogenic activities, including geological weathering, mining effluents, industrial effluents, domestic effluents, rural nonpoint source pollution, and atmospheric deposition [1,4]. Association between different microbes has been used for wastewater treatment as bioremediations, as it was considered to have greater capability over pure and single isolates. The axenic cultures were devised to be mineralized rapidly and completely by the metabolic activity of different microorganisms [5]. From microorganisms fungi have diverse mechanisms for bioremediation of heavy metals, including metal uptake by cell-wall components, accumulation inside the cell or extracellular chelation by phytochelatins and metal lothioneins [6].**

**The objective of the present study is to investigate the use of fungal biomass as the biosorbent for the removal of copper from an aqueous solution that contains copper sulfide nanoparticles.**

## MATERIAL AND METHODS:

### • Sample collection and fungus isolation:

**For the present study, water samples were collected from three river places at Basrah/Iraq.**

**The samples were cultured in a pure plate method and after examining the dishes and identified the fungus by usual methods. Fifty pure fungal isolates of *Aspergillus terreus* were used in the present study.**

- ## Preparation of adsorbent:

***Aspergillus terreus* biomass was prepared in potato dextrose broth (PD broth) medium. For preparation of 100 ml of the PD broth, 30ml of potato broth and 2 gm of glucose was added in conical flask and completed to 100ml by distilled water. Flask was tightly closed with a cotton plug and aluminum foil and autoclaved at 121$^0$C and 15 Pascal for 20 minutes. Later, flask was opened under laminar flow and fungus was inoculated into each flask. The flasks were agitated on a rotatory shaker for 3-4 days at 150 rpm and at 30ºC temperature. After 3-4 days thick bed of fungal biomass developed was further used for biosorption experiment.**

- ## Metal biosorption:

**For investigation the biosorption capacity of *Aspergillus terreus* isolates towards the copper with various initial heavy metal concentrations and optimal cultural conditions. Copper solutions pH 5.0 of 11.07, 22.155 and 44.31mg/l were prepared using copper sulfate ($CuSo_4.7H_2O$). Then 1 ml of fungal biomass was suspended in 100 ml of copper solution in 250ml conical flask. The flasks were agitated on a rotatory shaker at 150rpm and at 30ºC with contact time of 24 hrs, 48 hrs and 72 hrs. The initial pH and biosorption contact time was chosen based on previous reported studies [7, 8].**

- ## Equation calculating the proportion of compounds removed [9]

$$R\% = ((C0 - C1) / C) *100$$

**Where: R = the percentage of removal.**

**C0 = the concentration of compounds in the primary solution.**

**C1 = the concentration of compounds in the final solution.**

# RESULTS AND DISCUSSION:

**Pollution by heavy metals affects the diversity of soil biota, their abundance and activity [10, 11]. When metals are discharged with effluent, they may result in severe contamination of downstream ecosystems [12]. In an aquatic environment, metals occur both in the dissolved or soluble fraction and in particulate matter. Elevated levels of metal ions are generally toxic and cause major damage to cells [13]. Interactions between different heavy metals and microorganisms might be antagonistic, additive or synergic. These interactions might be multifaceted and distinguished which depends on the type of heavy metal ion and microbial species consortium. The collective effect of different heavy metals may be toxic or growth enhancement in the same microbial consortium assorted from the additive effect of the single metal ions [14]. Biosorption technique employing microbial biomass has been illustrated. In this process both alive and heat killed dead biomass of several filamentous fungi (*Mucor spp.*, *Aspergillus spp.*, *Penicillium spp.*, *Rhizopus spp.*) have been employed [15].**

**The results of the current study showed that the rate of accumulation of the copper element in the fungus cells was decreased with the incubation period of the fungus with the element at lower concentration as shown in Table 1.**

**Table 1: Average of bioaccumulation of the copper element in *Aspergillus terreus***

| Copper concentrations (mg/l) | one day | two days | three days |
|---|---|---|---|
| 11.070 | 11.030 | 9.946 | 6.400 |
| 22.155 | 21.210 | 18.841 | 17.120 |
| 44.310 | 43.780 | 43.620 | 40.530 |

**As the percentage of the biological removal of the copper component was calculated by the fungus, the results showed that the percentage of biological removal increases with the incubation period of the fungus with the polluted element as shown in Table 2**

**Table 2: Biomass removal rate of copper element by *Aspergillus terreus***

| Copper Concentrations (mg/l) | one day | two days | three days |
|---|---|---|---|
| 11.07 | 0.36 | 10.1 | 42.18 |
| 22.155 | 4.26 | 14.9 | 22.72 |
| 44.31 | 1.19 | 1.55 | 8.53 |

**Capacity of fungi in biosorption of hazardous metals due to contain of fungi on chitin and lignin, exudation of organic acids by fungi or adsorption of metal ions to fungal cell wall [16,17]. This shows that the biosorption mechanism on fungal biomass surface is caused by interaction between heavy metals and different fungal species. The current results are similar to earlier studies which indicated the role of functional groups on fungal cell surface and the formation of complex bond among metal ions and various functional binding group [18,19].**

**Copper is rarely found in natural water, but is found in man polluted environments. Any copper present normally originates from industrial effluents, seepage, water from refuse dumps, pesticides or corrosive water that has come into contact with fitting and pipes containing copper [20,21]. Trace amounts of copper are essential for life, but it also catalyzes the synthesis of reactive oxygen species leading to severe damage to cytoplasmic constituents through oxidation of proteins, fragmentation of DNA and RNA, and lipid peroxidation [22].**

**The results of biosorption vary from species to species because the process is dependent on factors including: fungal species, biosorbent size, metal solution concentration, solution pH, shaking time and ionic composition. Fungi constitute a high proportion of the microbial biomass in soil. Being widespread in soil their large surface to volume ratio and high metabolic activity and can contribute significantly to heavy metal dynamics in soil [23].**

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# تأثير وقت الحضانة على امتصاص النحاس والكتلة الحيوية لفطر الاسبريجلس تيروس

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**الملخص العربي:**

**أدى زيادة النشاط الصناعي والطلب على المعادن الثقيلة مثل النحاس والرصاص والزئبق والكادميوم وغيرها الكثير إلى زيادة الكمية العالمية لمياه الصرف الملوثة بالمعادن الثقيلة. غالبًا ما تكون التقنيات الحالية المستخدمة لإزالة المعادن الموجودة بتركيز منخفض من مياه الصرف من خلال عملية مثل الترسيب والتبادل الأيوني باهظة الثمن وغير فعالة. لذلك تم البحث عن آلية غير مكلفة وفعالة مثل عمليات الامتصاص الحيوي والتراكم البيولوجي في الفطريات كنظم ممكنة لإزالة المعادن. في هذا البحث تم الحصول على خمسون عزلة نقية من فطر *Aspergillus terreus* من ثلاثة مواقع نهرية بمدينة البصرة /العراق لدراسة قدرتها علي النمو وإزالة عنصر النحاس تحت تركيزات مختلقة وخلال مدد إحتضان مختلفة. أظهرت الدراسة أن معدل تراكم العنصر النحاسي في خلايا الفطريات يقل مع زيادة فترة حضانة الفطريات مع العنصر وخاصة مع الجرعات المنخفضة نظرًا لأن النسبة المئوية للإزالة البيولوجية لعنصر النحاس التي تم حسابها بواسطة الفطريات أظهرت أن النسبة المئوية للإزالة البيولوجية تزداد مع فترة حضانة الفطريات باستخدام العنصر الملوث . خلصت الدراسة إلي أن قدرة الفطر علي إزالة عنصر النحاس تعتمد علي تركيزة وفترة التعرض له.**