RESEARCH ARTICLE Agricultural Science Digest, Volume 44 Issue 3: 565-570 (June 2024)

Effect of pH on Trace Elements Removal from Batteries and Compact Disks by *Aspergillus terreus* Pellets

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ABSTRACT

Background: The aim of this study is the removal of three trace elements (Nickle Ni, Cadmium Cd and Lead Pb) by an environmentally friendly method from Batteries and compact discs. This study aimed to rapidly remove trace elements from E-waste (batteries) by filamentous fungi pellets.

Methods: *Aspergillus* was the best heavy metal tolerated isolate under the following incubation parameters: pH (5), temperature (25°C), ground electronic waste powder doses (0.5, 1 and 1.5 g/L) absorbed by fungal pellets biomass 10 gm/L of wet weight.

Result: The results showed the best removal percentage R% of Ni, Cd and Pb removal were (89, 91 and 96) observed at pH 5. Images from SEM showed accumulations of metal particles on the surface of the fungal hyphae and Fourier Transform Infra-Red Spectroscopy (FT-IR) was used to determine the effective groups in the fungal cell wall responsible of biosorption of trace elements and this study indicates that an application may be possible *Aspergillus terreus* as a promising candidate for biological sorption and recycling of trace elements and as an ecologically friendly and effective process in terms of time and cost.

Key words: *Aspergillus*, Batteries, CD, pH, Trace elements.

INTRODUCTION

Most e-waste consists of various metals (Fe, Ni, Cd, Cu, Al, Au, Ag, Pd, Li and to a lesser extent Hg, Pb, Cd and rare earths as well as plastics and ceramic Islam and Huda (2019). Metals it is a global and growing problem due to factory operations and is considered a serious threat to the environment and public health, even at low concentrations and is toxic to organisms (Ayangbenro and Babalola, 2017; Kalia *et al*., 2021). To reduce pollution and meet the constant demand for trace elements, it is vital to recover and recycle E-waste. Different methods are commonly used for recycling and recovery of metal elements from electronic waste, like chemical and physical (Kaya, 2016; Shahabuddin *et al.,* 2022). Nevertheless, these energy-intensive methods have very high energy consumption, which makes them unsuitable for separating trace elements from E-waste. Therefore, there is a need to find alternative, innovative and practical technology to these traditional methods, such as Biosorption, which utilizes the natural capability of microorganisms to recycle metals (Narayanasamy *et al*., 2020; Narayanasamy *et al.,* 2018; Al-Shammari *et al.,* 2023). As organic acids are produced by microorganisms, metals that are insoluble are oxidized to become soluble (Karaffa *et al*., 2021). In the bioabsorption process, metals are bound to the surface of microorganisms in the environment (Kumar *et al.,* 2018). Several microorganisms are currently used for efficient bioabsorption, such as *Micrococcus luteus* and *Pseudomonas balearica* (Kumar *et al.,* 2018; Pham *et al*., 2022). Usually, a fungus is considered to be the best fungus to leach and bioremediate trace elements from soil polluted with electronic waste Yaashikaa *et al*., 2022; Trivedi and Hait, 2020), which have chitosan in their cell walls and play an active role in the surface adsorption of pollutants. In addition, the lack of production of secondary compounds was potentially toxic. Filamentous Fungi are more Suitable for e-waste bioleaching and bioremediation (Patel and Lakshmi, 2021). This study aims to remove trace elements by biosorption from E-waste using filamentous fungi pellets as an eco-friendly and economical method.

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How to cite this article: Makhrib, M.A., Al-Shammari, R.H.H. and Alshammary, A.G. (2024). Effect of pH on Trace Elements Removal from Batteries and Compact Disks by *Aspergillus terreus* Pellets. Agricultural Science Digest. 44(3): 565-570. doi: 10.18805/ag.DF-594.

Submitted: 08-01-2024 **Accepted:** 15-02-2024 **Online:** 09-03-2024

MATERIALS AND METHODS

Samples gathering

Collection of electronic waste

Preparation of E-waste samples of Compact Disks and Batteries from shops in Baghdad 2022. Whole e-waste samples are hammer-milled into spheres approximately 2 cm in diameter. The samples were ground using a modified method (Patel and Lakshmi, 2021) using a grinder for 20 min, turned into powder. Through a nationwide standard test sieve with a pore size of 80 to 155 microns and particles with a diameter of 80 to 155 microns were collected.

Isolation identification of fungal genera and its ability to pellet formation

Filamentous fungi *were* isolated from 10 polluted soil samples near industrial factories in Baghdad from September to November 2022 isolation and purification experiments were achieved in solid sterile potato-dextrose agar at 25±2°C, fungal isolate was grown for seven days and then purified based on its morphological structure. Identifying fungi using a key after purifying and diagnosing with an optical microscope (Barnett and Hunter, 1972).

A pure fungal colony of *A. terreus* was used to collect the conidia, which were then prepared by dilution with normal saline to achieve the necessary spore suspension concentration of 1×10^6 spores/10 ml. A drop of spore suspension was added to 500 mL of liquid culture (grams/liter) which consisted of glucose 100, $NaNO_3$ 1.5, KH_2PO_4 0.5, $MgSO_4$ $7H_2O$ 0.025, KCl 0.025 and yeast extract 1.6. The liquid medium was autoclaved at 121°C for 18 minutes and then incubated at 25°C. A pure fungal colony of *A. terreus* was used to collect the conidia, which were then prepared by dilution with normal saline to achieve the necessary spore suspension concentration of 1×10^6 spores/ml in 10 ml. with rotating shaky at 170 rpm for seven days following the method of Makhrib and Al-Shammari (2023); Noor *et al*. (2020).

Biosorption experiments by fungal pellets

The E-waste powder was incubated for two weeks with *A. terreus* pellets at 25°C and 150 rpm with 0.1, 0.5 and 1.0 grams of powder added daily. PDA plates with *A. terreus* attended as the positive control (+ve control). Medium with E-waste powder attended as negative control (Al-Shammari *et al.,* 2023). To determine pH during biosorption experiments, 5 mL of culture media was tested once a day. The pH was monitored using a portable pH meter and adjusted by adding 0.1 M of Hcl or 0.1 M of NaOH to reach the desired pH value (Trivedi and Hait, 2020).

Atomic absorption spectrometry of E-waste and bio leached residue

The acidic dissolution of the metals was carried out using the method described by Patel and Lakshmi (2021) for determining metals in electronic waste. One gram was placed in a 250 mL flask and dissolved in 40 mL of a 1:3 mixture of concentrated nitric acid (70% w/v) and HCL (40% w/v). The mixture was left for 24 hours before being centrifuged at 8000 rpm for 15 minutes and the supernatant was kept at 4°C. The metal concentrations in the acidified samples were then analyzed using atomic absorption spectroscopy. 10 ml of the bio sorbent solution from the bioleaching experiment was filtered through Whatman #. 1 Filter paper to remove particles and centrifuge at 12,000 rpm for 15 minutes to remove fungal deposits. The supernatants were then analyzed for mean metal content (mg/l).

Determination of trace elements uptake by fungal pellets

Fungal biomass was filtered through a funnel with 0.1 mm porous, filtrates washed five times with deionized distilled water to remove soluble compounds and dried at 90°C overnight. the percentage of elements absorbed by fungal pellets was calculated by the following equation:

$$R = \frac{Po - Pe}{Po} * 100$$

R = Metals removal by fungal pellets.
Po= Preliminary concentration of trace elements.
Pe= The last concentration of elements mg/l.

FT-IR spectra of bleach residues

FT-IR analysis was achieved by removing solid particles from the biofilter waste using Whatman #1 filter paper. The detergent waste was filtered before being analyzed by FT-IR. To remove fungal pellets, centrifuge at 11,000 rpm for 15 min, then rinse with physiological saline and aerate at 85°C for one day. Finally, fungal pellets were crushed to a fine powder and hard-pressed into disks and analyzed on a Perkin-Elmer Spectrum instrument. The instrument was used to identify the chemical structure and pulp compounds before and after bioleaching by scintillation in the spectral range 400-4000 cm^{-1} at room temperature (Acosta-Rodríguez *et al*., 2018). All the above tests and characterization achieved in Ministry of Science and Technology, Baghdad, Iraq.

Statistic evaluation

To ascertain whether group variance was significant (p 0.05), a two-way ANOVA of variance, least significant difference (LSD) and correlation was conducted. Statistical significance was evaluated using the SPSS program.

RESULTS AND DISCUSSION

Analysis of E-waste atomic absorption spectroscopy

The trace elements present in the compact discs and batteries in Table (1) showed that the most metals presented in (1 gram) were Cu, Cr, Sr, Br, Ti, Zn and the highest concentrations were Pb, Cd and Ni the last three metals recognized as significant environmental pollutants (Barnett and Hunter, 1972).

Identification of isolated fungal genera and their ability to pellet formation

Identification of selected isolates fungal colony morphologic investigations of cotton-shaped colonies in PDA plates prepared from selected fungi, Five fungal isolates (*Aspergillus niger*, *Aspergillus terreus*, *Aspergillus fumigatus*, *Penicillium* sp. and *Alternaria alternate*) were tested to form pellets (Table 2) shows the first and second genera were able to form pellets and after 7 days only *A. terreus* pellets were stay stable after 14 days as showed in (Fig 1). Trace elements biosorption was studded by wet biomass of *Aspergillus terreus* pellets, the uptake of trace elements after 48 hrs. of incubation is shown in Fig 2, Electron microscope image (SEM) of *A. terreus* Before preparation. same fungal genera isolated from soil (Kichu *et al*., 2020; Temjen *et al*., 2022).

Effect of pH value on metal biosorption by fungi

In this study, metal ion recovery is strongly influenced by pH. Its changes in the culture media and metal ion adsorption sites have a high effect on cell surface and chemical structure of the metal in water affected by the initial pH on absorption of metal ions, The effect of pH changes on metal ions showed in Fig 3. This study evaluates effect of pH on absorption of metal ions with different values (Shahabuddin *et al.,* 2022; Narayanasamy *et al*., 2018). In the experiments, the maximum removal percentage R% of all metals was (89, 91 and 96) observed at pH 5 and R% is significantly ($p \leq 0.05$) less than the other pH values.

Effect of different amounts of E-waste powder in solution on the recovery rate in Cadmium, Nickel and lead metals

The effect of different amounts of E-waste powder (0.5,1 and 1.5) g/L on the removal of metals was performed in Fig 4. In all amounts, the effect of E-waste powder in the solution no significant differences ($p \leq 0.05$) on the removal rate of Cadmium, nickel and lead metals, the effect of metal concentration on the removal of metals by *A. terreus*, the absorption capacity of Cd was about 78-80% and for Ni 969-75% for Pb 90-94%.

Table 1: Trace elements composition of CD and battery analysis by AAS.

Metals	Concentration (mg/l)
Pb	6929 mg/l
Cd	1596.45 mg/l
Ni	2647.299 mg/l
Zn	496.562 mg/l
Al	574.103 mg/l
Br	850.85 mg/l
Sr	677.678 mg/l
Ti	907.284 mg/l
Au	945.202 mg/l
Ba	954.642 mg/l

FT- IR spectra of biosorption by fungal pellets

The bioaccumulation of trace elements is a function of the primary functional groups during the biosorption process by FT-IR spectral analysis. Fig 5 shows the transmittance bands at 646.49,699.90 cm^{-1}. In biosorption *A. terreus*, transfer bands based on electronic-waste powder density were attained, 3913.22, 2620.04, 1770.37 and 1630.15 cm^{-1}, which are correlated to C-H-C-H, hydroxylic group and RCO-OH, correspondingly. FT-IR analysis presented that the four main functional groups, which contain amines, hydroxyls, alcohols and amides, play an important role in the bioaccumulation of trace elements during the biosorption experimentations. FT-IR peaks obtained from mycelium fungal biomass isolates were compared with standard interpretation charts for FT-IR peaks.

Isolation identification of fungal genera and its ability to pellet formation

Heavy metal pollution in affluent nations makes them heavily reliant on collecting electronic debris, a conceivable solution is bioremediation and recycling are both possible with microorganisms because of their environmental friendliness and cost-effectiveness. Study results show that filamentous fungi have an excellent ability for heavy metal biosorption under the same conditions (Parveen *et al*., 2023; Bahafid *et al*., 2017). Fungi isolated from polluted soil as previous studies have confirmed that microbial populations can adapt to high levels of heavy metal contamination in

Table 2: Fungal pellets forming of isolated fungi strains derived from polluted soil.

Fungi Isolates	Pellet formation capability	After 3 days	After 7 days	After 14 days
Aspergillus niger	+	+	-	-
Aspergillus terreus	+	+	+	+
Aspergillus fumigatus	-	-	-	-
Penicillium sp1.	-	-	-	-
Alternaria sp.	-	-	-	-

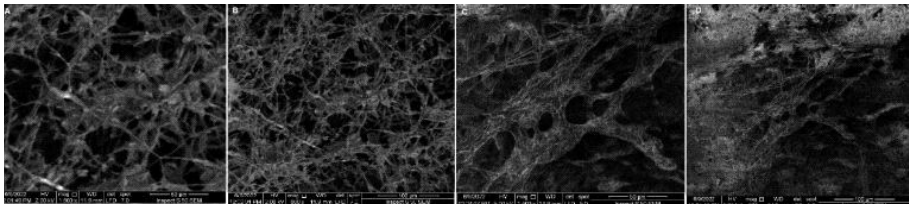
\+ Represent fungal pellet formation.

\- Represent no pellets formation.



Fig 1: Fungal pellets diameter ranged (1-5) cm in diameter.

heavy metal-polluted environments (Al-Shammari *et al*., 2022; Fan *et al*., 2018). Because the long-term toxic effects of heavy metal pollution tend to increase the number of native microbial diversity, fungi with a high potential for tolerance to trace elements have been found in these areas, particularly due to their increased adaptability to



A and B (fungal hyphae without treatment 50 µm, 100 µm), C and D fungal hyphae after biosoprtion of trace elements 50 µm,100 µm).

Fig 2: *A. terreus* fungus with electron microscope scanning (SEM).

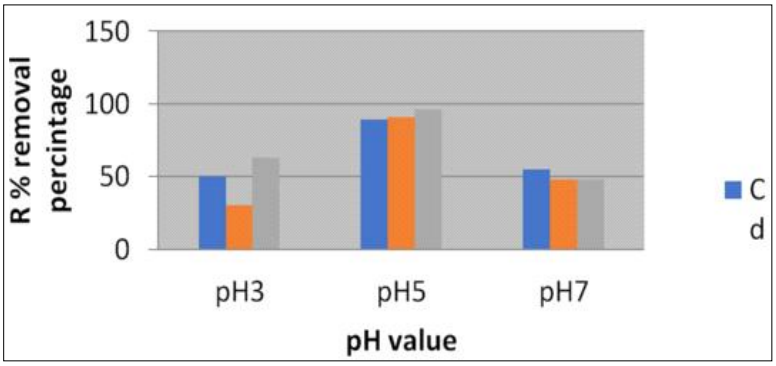


Fig 3: The maximum capacity in experiments (c) and lead (b) nickel, (a) in solution on the recovery rate of cadmium ions pH.

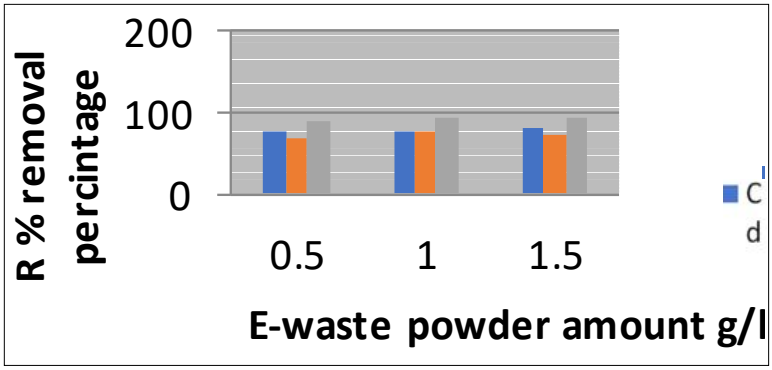


Fig 4: Effect of changes in the amount of E-waste powder g/l on the recovery rate of Ni, Cd and Pb ions.

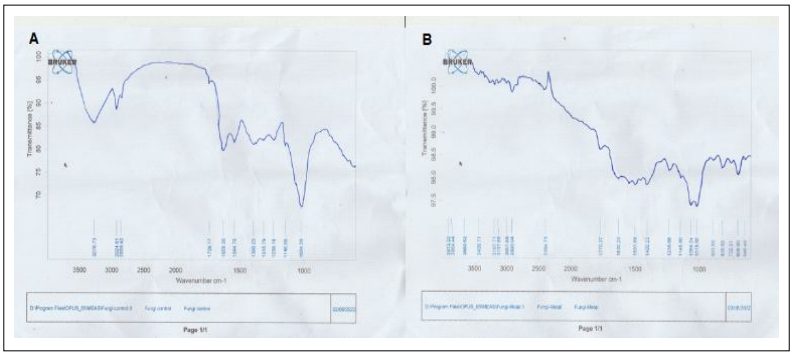


Fig 5: FT IR spectra of biosorption by fungal pellets (A) before treatment, (B) after 48 hrs. of treatment.

long-term exposure to pollutants. Some fungi thrive in high (toxic) concentrations of metal ions (Cárdenas González *et al*., 2019).

Acidic pH = 5 was the optimum for removal percent, Therefore, under acidic conditions the cause of competition of H+ ions with heavy metal cations for placement on the surface of fungal hyphae mass, sorption occurs previous studies recorded pH values effect on biosorption of Co (II), 250 mg l^{-1} to maximize biosorption, the dehydrated mycelium was incubated at pH 5.5 for one day. *Aspergillus niger*, *Penicillium* spp. absorbed 93, 77.5 and 70.4% of Co (II), (Cárdenas González *et al*., 2019). Another study found that with *Paecilomyces* absorption of cobalt increased with the changing of pH from acidic to neutral. The highest biosorption was verified at pH 7.0 according to the cobalt precipitation (Li *et al.,* 2014). These findings can be clarified by the fact that there was less competition between hydrogen atoms with positive charge and Co^{2+} functional groups.

According to (FT-IR) analysis *A. terreus* used for the bioremediation of trace elements from electronic-waste FT-IR conducted on the trace elements resistant *A. terreus* exposed that 4 main functional groups were noticed on the surface of the fungal hyphae which are amines, hydroxyl, alcohols and amides groups. Bioremediation involves the interaction of trace elements with functional groups on the surface of the fungal cell wall (amines, hydroxyls, alcohols and amides), causing slight shifts or changes in peaks which agreed with previous studies (Gazem and Nazareth, 2013). FT-IR analysis of the mycelium biomass of fungal species showed that the changes in peak location and concentration were caused by the complexation of functional groups on the surface of the mycelium biomass caused by the hydrolysis of some polysaccharides on the fungal cell wall to shorter saccharides, such as oligosaccharides. A similar finding was reported during the biosorption process of these functional groups with trace elements (Temjen *et al*., 2022; Ozsoy *et al*., 2008), that hydroxyl groups in conjunction with carbonyl groups in mycelial biomass were indicative of carboxylic acid groups. There are amino acids present in the cell wall of the mycelial biomass, which can explain the presence of an amino group and a hydroxyl group with a carbonyl group. The result of this study agreed with results from Ozsoy *et al.* (2008), Al-Shammari *et al*. (2023) All reported that the process of bioaccumulation of trace elements at the fusional groups proceeded by creating a negatively charged surface on the cell wall of fungal cell walls and chitin through the ionization of functional groups, thereby enhancing the binding of heavy metal ions.

Conclusion

A significant source of trace elements that are inappropriately collected, recycled and reused is electronic waste, which poses a considerable environmental risk. Recycling provides a substitute for disposal. Recycling e-waste is primarily done to enhance metal recovery and lessen its hazardous impact on the environment. *A. terreus* fungal pellets were utilized to accomplish this purpose and to get the best removal of metals (Ni, Cd and Pb) from e-waste dust at an acidic pH. As a result, the biosorption technique is seen as a rising option for recycling e-waste. Only if this strategy is used in the industrial sector (middle and big firms) and its efficacy is thoroughly examined based on the operational and economic possibilities, does it make sense to do so.

Conflict of interest

All authors declare that they have no conflicts of interest.

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