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Research Article

Study the Fungal Leaching Activity in Relation to the Extracellular Protein Secretion on Different Samples from Um Bogma Formation in Gabal Um Hamd, Southwestern Sinai, Egypt

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Abstract: Bioleaching is a mining and biohydrometallurgical process used to extract valuable metals from different ores with the help of microorganisms such as fungi. Five fungal isolates were isolated from seven soil samples collected from Um Bogma Formation in Gabal Um Hamd, southwestern Sinai, Egypt, and were coded from W1 to W7, which were classified geologically into three categories as siltstone (W1, W2, W4), dolostone (W5, W6) and grey shale (W3, W7). The isolated fungi were morphologically identified as *Aspergillus hollandicus* (*A. hollandicus*), *Penicillium citrinum* (*P. citrinum*), *Trichoderma harzianum* (*T. harzianum*), *Fusarium roseum* (*F. roseum*), and *Aspergillus fumigatus* (*A. fumigatus*). The chemical characterization of the studied samples for uranium revealed a wide variation, with values ranging from 20 ppm in W4 to 9,706 ppm in W7. Additionally, the thorium values obtained ranged from 23 ppm in W2 to 122 ppm in W3. The maximum values of Rare Earth Elements (REEs) were recorded as 153 and 33 ppm in W1 and W6, respectively. The bioleaching activity of uranium, thorium, and REEs exhibited wide variation when applied to the studied samples as follows: 53% (W5), 74% (W3), and 55% (W7) (*F. roseum*), 58% (W3), 49% (W3), and 41% (W3) (*A. fumigatus*), 60% (W5), 65% (W1), and 76% (W5) (*T. harzianum*), 64% (W1), 77% (W5), and 89% (W5) (*A. hollandicus*), and 70% (W1), 88% (W4), and 33% (W1) (*P. citrinum*), respectively. Furthermore, the extracellular protein secretion played a major role in bioleaching activity, showing a significant increase at the end of each experiment as the maximum level was recorded as 20.1 and 13.8 mg/L for W6 and 11.7, 10.7, and 5.8 mg/L for W5. This may be considered as a defense mechanism of fungi to generate more energy to face the overdose of metals or radiation toxicity and the increase in fungal digestion enzymes may contribute to enhanced bioleaching activity. Consequently, proteins act as a co-factor in the bioleaching process and a protective agent for fungi to survive against exposure to different environmental stresses such as irradiation or radioactive metals.

***Keywords*:** fungi, bioleaching, uranium, thorium, Rare Earth Elements (REEs), protein

1. Introduction

Gabal Um Hamd is an area located in southwestern Sinai and is considered one of the most important areas for uranium occurrence in Egypt. The Lower Carboniferous Um Bogma Formation in G. Um Hamd consists of argillaceous

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and carbonate rocks, and it can be subdivided into a lower siltstone-Fe, Mn ore member, a middle siltstone-mudstone-shale member, and an upper dolostone-dolomitic limestone member formed of carbonate rocks such as calcite, dolomite, quartz, hematite, ilmenite, malachite, chalcopyrite, galena, U_3O_8, kasolite, uranothorite and corondite.[1] Um Bogma rocks consist of a large assemblage of primary and secondary minerals that are allogenic and/or authigenic. They include non-radioactive (Rare Earth Elements (REEs)-and base metals-bearing minerals) and radioactive (uranium and thorium) minerals. Abdel-Azeem[2] and Sallam[3] identified three different uranium minerals in the Um Bogma formation, which are more concentrated in its lower part.[4]

Naturally, the occurrence of radioactive materials such as potassium ^{40}K, uranium ^{238}U and ^{235}U, and thorium ^{232}Th causes more than 80% of the human exposure to ionizing radiation.[5] Several types of pollutants and toxins that come from human and natural activities lead to an increase in metals that do not degrade naturally and make their removal from the soil more difficult, and are considered the major cause of soil pollution.[6] The exposure to uranium, thorium radionuclides, and rare earth elements has a hazardous effect on the biological system.[7] Silva et al.[8] showed the cadmium and zinc effects on *Oreochromisniloticus*, Abdel-Rahman et al.[9,10] and Abdel Kader et al.[11] studied the uranium hazards on the brain of rats. Although toxic properties of REEs are still not completely known, they affect metabolism or enzyme activities.[12,13]

The efficiency of Uranium (U) and Thorium (Th) as natural radionuclides in soil refers to their use as fuel.[7] Besides the presence of U and Th, the presence of REEs are found naturally in geosphere in trace amounts.[6]

Microbial applications have an effective role in solving many environmental issues by bioleaching, biosorption or bioremediation activity. Compared to chemical leaching, fungal bioleaching was found to be a better method to recover valuable metals from low-concentration, for which extraction was not economically feasible with the former.[14,15]

The proteins, which are secreted in a broad spectrum of fungi, have a significant role in nutrition, carbon release, and energy production. They also contribute to the generation of new enzymes (protein production) that have a key role in providing a protective mechanism (as bioleaching or biosorption) against toxic damage and during detoxification.[16] One of the most important fungi that are used in metal bioleaching is the *Aspergillus* and *Penicillium* genus.[17] Amin et al.[18] showed that *A. niger* and *A. terreus* from uraniferous sedimentary rocks exhibited the highest leaching efficiencies in the bio-dissolution experiments. Rezk and Morse[19] demonstrated the high tolerance that associated with a high biosorption capacity of *A. niger* to lanthanum in a concentrated sample.

The bioleaching of uranium, thorium, and rare earth elements from the Um Bogma formation in Gabal Um Hamd is very demandable in the detoxification process of contaminants in the environment using bioremediation techniques that involve the use of fungal enzymes. The aims of the present study are to evaluate the uranium, thorium, and rare earth elements concentrations in seven samples collected from different layers in Um Bogma, in addition to demonstrating and identifying the several types of isolated fungi from these samples, as well as to study the bioleaching activity of the recorded fungi to U, Th, and REEs in relation to the extracellular protein secretion.

2. Materials and methods

2.1 *Sampling*

From Um Bogma Formation in Gabal Um Hamd, southwestern Sinai, seven samples were collected from different layers coded from W1 to W7.

2.2 *Chemical analysis of samples*

2.2.1 *Sample digestion*

After the samples were crushed and ground, each one was digested by an acid attack mechanism. In a Teflon beaker, a mixture of 10 ml of conc. Hydrofluoric acid (HF), 5 ml of conc. Nitric acid (HNO_3) and 5 ml of perchloric acid was added to 0.5 g of the sample, then heated at 220 °C till complete dryness, followed by adding 5 ml of 1 : 1 diluted Hydrochloric acid (HCl), and then up to a volume of 50 ml by adding distilled water.[20] All the chemical analyses were conducted in the laboratories of the Nuclear Materials Authority.

2.2.2 *Sample analysis*

The major elements, in the oxide form, were identified by the wet analysis technique, in which the SiO_2, Al_2O_3, TiO_2, and P_2O_5 were determined by a colorimetric spectrophotometric method (LABOMED: model-SPECTRO UVD, USA), while the total oxides such as Fe_2O_3, MgO, and CaO were evaluated by traditional titration techniques. Also, the contents of Na and K were determined using a flame photometric technique (JENWAY: model-PEP7, UK). The Loss on Ignition (L.O.I) was calculated gravimetrically. The estimated error for the major constituents is less than ± 1%. The identity and concentration of the trace elements were determined using X-Ray Fluorescence (XRF) spectrometry under the conditions of a W-target tube, Lithium Fluoride (LIF)-220 crystal, gas flow proportional counter, and scintillation counter, at 70 kV and 15 mA with a detection limit of 2 ppm. The analytical precision was ± 3%. On the other hand, the Uranium (U) content of the samples studied was measured by a titration method.[21] This method was utilized for both ore samples and bioleach liquors against standard ammonium metavanadate, where the endpoint was obtained at the appearance of a purplish color, after which the uranium concentration was calculated according to the following equation (1):

$$\text{U (mg/L)} = \frac{T \times V_1 \times 10^3}{V} \text{ ppm} \quad (1)$$

where, T is the titration intensity of the NH_4VO_3 solution, V_1 is the volume of NH_4VO_3 solution consumed, and V is the volume of the measured sample. Meanwhile, thorium concentration was determined using the thoron method, in a 25 ml volumetric flask containing: 1 ml of the studied sample solution, 1 ml of 5% (w/v) tartaric acid (dissolved 5 g of tartaric acid in bi-distilled water and then the volume was completed to 100 ml), 1 ml of 1% (w/v) ascorbic acid (dissolved 1 g of ascorbic acid in bi-distilled water and the volume was completed to 100 ml), 2 ml of 0.1% (w/v) thoron solution (prepared by dissolving 50 mg of thoron in bi-distilled water and diluting the solution to 50 mL in a volumetric flask and then transferred to a dark bottle, where it is stable for at least seven days) were added, and sufficient hydrochloric acid to make its concentration 0.25 M after dilution to the mark with water. The absorbance of the solution was measured at 540 nm, using a reagent blank solution as the reference.[22] The total rare earth elements were measured by Arsenazo-I solution (0.05%: dissolve 0.05 g of Arsenazo-I in 100 ml of water), and Buffer solution (weight boric acid 10.53 gm with borax 2.8 gm and complete with water to 1,000 ml: pH 7-7.5). In a 25 ml volumetric flask containing adequate volume of the sample solution, 1.5 ml of Arsenazo-I solution was added, then completed to 2.5 ml with the buffer solution. Finally, measure the absorbance at 580 nm using a reagent blank solution as the reference.[22]

2.3 *Microbiological studies*

2.3.1 *Media preparation*

Sabouraud dextrose was the medium selected for fungi cultivation. This medium was prepared by dissolving 10 g of peptone, 40 g of dextrose, and 20 g of agar in 1,000 ml of bi-distilled water, then was sterilized by autoclaving at 15 lbs, pressure (120 °C) for 15 minutes. Mycological Peptone provides nitrogenous compounds, dextrose provides an energy source, and the low pH favors fungal growth and inhibits contaminating bacteria.[21]

2.3.2 *Fungal growth, isolation, and identification*

The direct plating technique was used by spreading a fine powder of each sample on Sabouraud agar plates and incubating them for 7 days for fungal growth.[21] Then, from each separate colony, hyphal tips were removed and plated on the surface of another Sabouraud agar plate to get a pure fungal isolate. Each pure isolated colony was morphologically identified according to Amin et al.[23] at the Regional Center for Mycology and Biotechnology (RCMB) Al-Azhar-University, Cairo, Egypt.

2.4 *Protein assay*

To detect the peptide bonds for the total protein determination, the biuret experiment was used according to

Mahesha,[24] where a violet-colored compound was formed when the atoms of nitrogen in the peptide bonds of a protein reacted with the copper ions. The formed color intensity is directly proportional to the total protein concentration, according to the Beer-Lambert law, which relates to the physical material optical attenuation containing a single attenuating species of uniform concentration, the optical path length through the sample, and absorptivity of the species, and was calculated from: the Absorbance (Ab), the absorbing capacity (E) of the attenuating species, the optical path Length (L), and the Concentration (C) of the attenuating species (equation (2)).[25]

$$\text{Ab} = ELC \tag{2}$$

The used apparatus for the colorimetric studies is Ultraviolet (UV)-Visible spectrophotometer (Metertech Ino: model Sp-5001).

2.5 *Fungal leaching activity*

One gram of ore from each sample was placed in a 250 ml Erlenmeyer flask, then, adding one hundred ml of sabouraud media to be autoclaved. Inoculation of 10% (v/v) of 1×10^8 Spore/ml from each fungus separately with each sample in a separate flask. Finally, the flasks were incubated at 30 °C for 7 days with pH (5.6 ± 0.2). At the end of the incubation period, the mycellial mats were collected and washed with distilled water several times, dried for 24 hours at 85 ℃ and the dry weight was determined (mg) to calculate the Growth Inhibition (GI) efficiency percentage, estimated by equation (3):

$$\text{GI}\ (\%) = \frac{(C_m - T_m)}{C_m} \times 100 \tag{3}$$

where C_m is the mycelium growth without ore sample, T_m is the test species growth of mycelium with ore sample, and GI is growth inhibition efficiency.[26]

Then the culture medium was filtered and centrifuged at 4,000 rpm to precipitate more particles. This filtrate was kept for uranium, thorium and the total REEs bioleaching efficiency percentage determination by equation (4),[27] all steps were triplicated.

$$\text{Bioleaching efficiency}\ (\%) = \frac{C_o - C_f}{C_o} \times 100 \tag{4}$$

where C_o is the concentration of the element in the ore sample before bioleaching and C_f is the concentration of the element after bioleaching.

The results are presented as mean ± stander error, using one-way analysis of variance with Statistical Package for the Social Science (SPSS) version 20.

3. Results

3.1 *Sample characterization*

The mineralogical classification of the samples showed three samples as siltstone (W1, W2, W4), dolostone (W5, W6) and two samples as grey shale (W3, W7) as shown in Table 1.

The major oxide elements were chemically measured in the seven samples as tabulated in Table 2, as well as the uranium concentration in the studied samples showed a wide variation value starting from 23 ppm in W4 and increasing to reach its maximum value in W7 of 9,706 ppm. Whereas, the thorium concentrations attained a low value in all samples as compared to the uranium concentration. Moreover, the maximum concentration value of REEs was recorded as 153 ppm in W1 and decreased to 33 ppm in W6.

Table 1. Geological descriptions of the studied samples

Sample no.	Description
W1	Compact siltstone, medium hard highly ferruginous
W2	Siltstone compact of hard high ferruginous
W3	Shale grey, fissile ferruginous and gypsiferrous
W4	Siltstone, brown and medium hard
W5	Sandy dolostone, jointed and fractured, dark
W6	Sandy dolostone, grey to dark grey, medium hard to hard, fractured
W7	Shale, grey to dark grey, soft with yellow secondary uranium mineralization

Table 2. Chemical characterization of the samples studied (major oxides: wt. % and U, Th, REEs: ppm)

	W1	W2	W3	W4	W5	W6	W7
SiO_2	44.33	47.4	50.45	32.11	28.8	27.1	62
TiO_2	0.75	0.86	0.95	0.12	0.13	0.05	0.67
Al_2O_3	13.9	12.4	12.71	12.8	15.9	15.7	14.7
Fe_2O_3	23.9	18.7	20.4	22.2	4.8	3.2	9
CaO	3.8	3.4	2.18	3.6	20.02	26.3	3.8
MgO	0.25	0.21	0.32	0.65	13.7	14.5	0.7
P_2O_5	0.3	0.15	0.24	0.31	0.17	0.22	0.15
Na_2O	1.99	1.71	2.12	1.34	0.76	0.45	2.16
K_2O	0.98	0.88	1.06	0.69	0.12	0.09	1.33
L.O.I	9.8	13.87	8.17	24.15	10.9	10.5	1.5
U	250	333	391	20	1,161	400	9,706
Th	119.3	23	122.2	12	44.75	46.50	76
REEs	153	56	91.25	43	60.3	33	40

Silica oxides were analyzed and recorded as 50.45% as the maximum value in W3, whereas in W6, it was 27.1% as the minimum value. The ferric oxide percentage reached its maximum value in W4 at 22.2% while, calcium oxide was recorded as 26.3% in W6 with a minimum value of 3.4% in W2. Loss of ignition for the samples displayed a wide variation, starting from 1.5% to 24.15% for W7 and W4, respectively. Furthermore, the trace element's identity and concentration were determined using X-Ray Fluorescence (XRF) spectrometry as demonstrated in Figure 1. With corresponding concentrations of Cr and Sr, the analyzed sample W1 showed a higher content of 4,026 ppm (65%) and 377 ppm (6%), respectively, than the other samples, as well as the Rb and V content of W2 being higher than that of W1, W3, W4, W5, W6, and W7, recording 2,601 ppm (29%) and 968 ppm (11%), respectively. However, the sample W3 examined had higher concentrations, 1,244 ppm (19%) and 434 ppm (7%) of Zn and Pb, respectively. In

comparison to other samples, the sample W4 exhibited higher contents of Ni, Sr, and Ba at 702 (20%), 259 (8%), and 699 ppm (20%), respectively. On the other hand, the obtained results showed that the samples W5 and W6 contain a greater concentration of Cu and Ga: 3,646 ppm (77%) and 603 ppm (14%), respectively. Finally, a higher content of Zr and Nb: 1,426 ppm (19%) and 55 ppm (0.71%), respectively, was found in the sample W7.

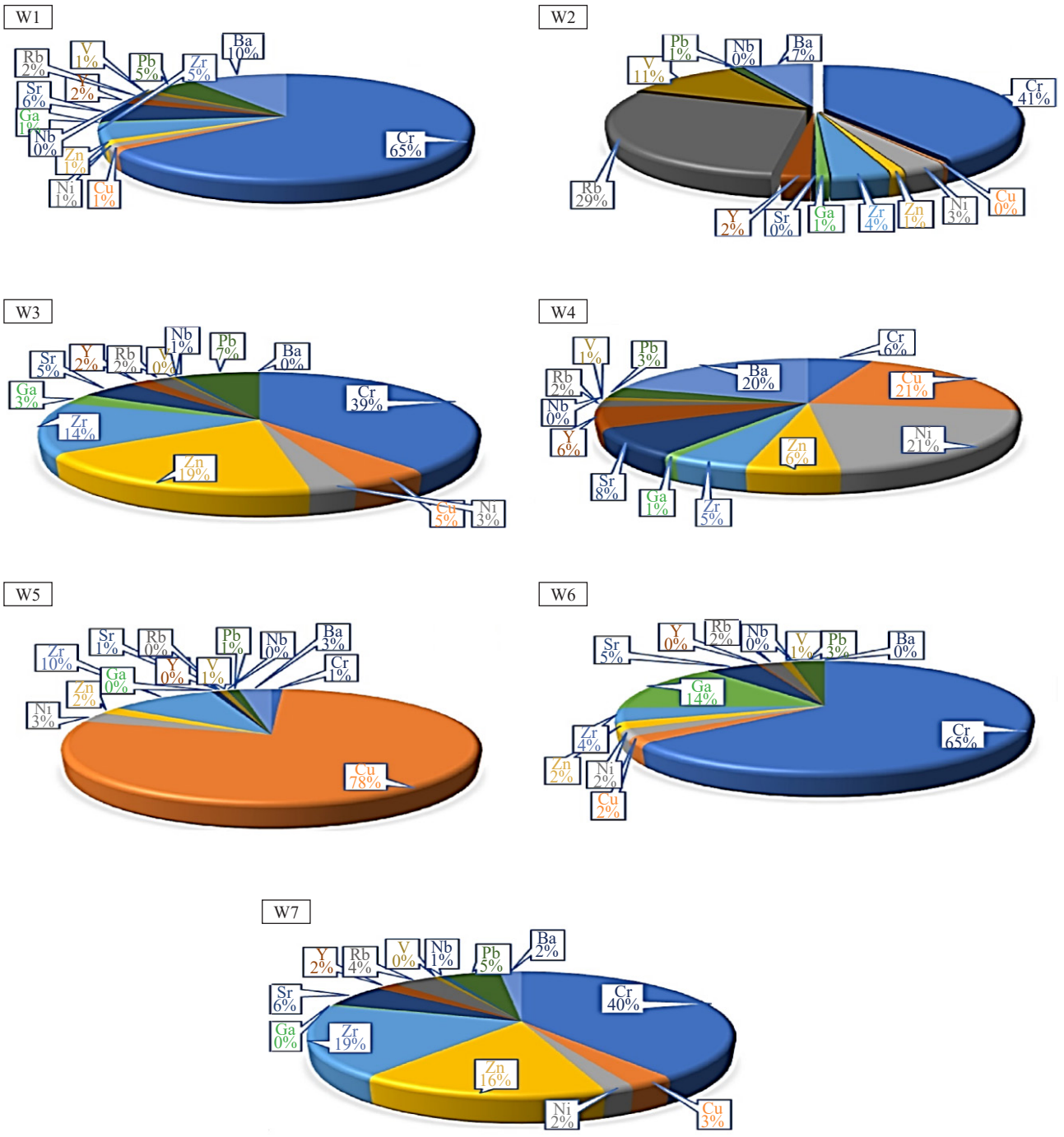


Figure 1. Chemical distribution of trace elements % in the studied samples

3.2 *Microbiological studies*

The microbiological experiments included the fungal strain isolation from the studied rock samples are followed by the bioleaching ability assay.

3.2.1 *Microorganisms isolation and identification*

As shown in Table 3 and Figure 2, five fungal species were isolated from the tested samples. The fungal strains were identified according to their morphological features, belonging to four species, known as *Aspergillus*, *Penicillium*, *Trichoderma*, and *Fusarium*.

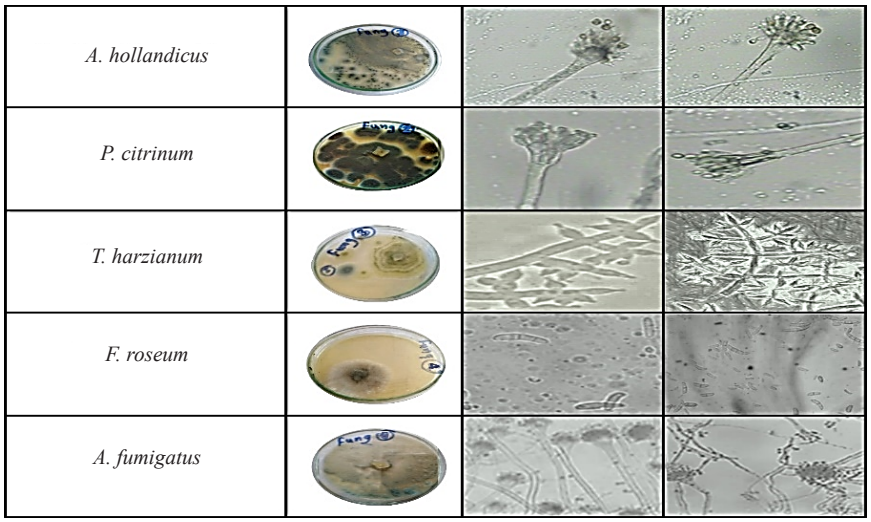


Figure 2. Image analysis and culture pictures of isolated fungi from the studied samples: *A. hollandicus*, *P. citrinum*, *T. harzianum*, *F. roseum*, and *A. fumigatus*

Table 3. Morphological features of isolated stains

Parameters	RCMB 1	RCMB 2	RCMB 3	RCMB 4	RCMB 5
Colony color	Velvety, white, buff to brown colonies, reverse yellow to brown	White, grayish, mycelium to deep green. Reverse pale yellow	Greyish green colored, broad concentric rings; aggregated with pustules	A white mycelium that becomes grey violet	Greyish turquoise or dark turquoise to dark green to dull green
Conidial shape	Radiate too loosely columnar	Mono and bi-verticillate are present	Pyramidal with opposing branches	Macroconidia are slender with tapering apical, and microconidia are oval, or club shaped with a flattened base	Conidial head columnar
Vesicle shape	Globose, 22.0 µm	Septate	-	-	Rarely globose, 5-24 µm
Sterigmata number and position	8.0 × 4.0 µm	Conidiophore diameter 2.5 µm	Many entire surfaces	Branched	One layer
Conidiospore color/ morphology	Brown	Pale yellow	Pale green colored	Magenta	Velutinous
Identification of fungal strains	*A. hollandicus*	*P. citrinum*	*T. harzianum*	*F. roseum*	*A. fumigatus*

3.3 *Effect of fungal activity on uranium, thorium, and total REEs leaching*

By applying the identified five fungal strains upon the seven studied samples to show the bioleaching ability of uranium from ore samples (W1, W2, W3, W4, W5, W6, and W7), which contain different grades of uranium concentrations (high, intermediate, and low). Meanwhile, the thorium and total REEs concentrations were measured during the bioleaching process.

Table 4. The fungi bioleaching activity to U, Th, and REEs in the seven samples studied

		W1	W2	W3	W4	W5	W6	W7
A. hollandicus	U (%)	64 ± 0.88	26 ± 0.4	32 ± 0.49	25 ± 0.38	57 ± 0.87	51 ± 0.78	6 ± 0.09
	Th (%)	73 ± 1.12	21 ± 0.32	85 ± 1.31	35 ± 0.54	77 ± 1.18	24 ± 0.22	41 ± 0.45
	REEs (%)	71 ± 1.09	54 ± 0.83	33 ± 0.50	51 ± 0.81	89 ± 1.37	80 ± 1.23	66 ± 1.01
P. citrinum	U (%)	70 ± 1.08	15 ± 0.23	28 ± 0.43	20 ± 0.30	59 ± 0.91	62 ± 0.95	15 ± 0.23
	Th (%)	69 ± 1.06	43 ± 0.66	88 ± 1.35	21 ± 0.32	77 ± 1.18	27 ± 0.41	21 ± 0.32
	REEs (%)	33 ± 0.50	23 ± 0.35	20 ± 0.32	18 ± 0.24	39 ± 0.53	28 ± 0.41	26 ± 0.39
T. harzianum	U (%)	42 ± 0.64	35 ± 0.54	29 ± 0.44	35 ± 0.54	60 ± 0.92	27 ± 0.41	8 ± 0.12
	Th (%)	65 ± 1.00	47 ± 0.72	48 ± 0.74	57 ± 0.87	34 ± 0.52	17 ± 0.26	26 ± 0.40
	REEs (%)	19 ± 0.29	39 ± 0.54	48 ± 0.74	41 ± 0.63	76 ± 1.17	75 ± 1.15	60 ± 0.92
F. roseum	U (%)	49 ± 0.75	16 ± 0.24	52 ± 0.80	25 ± 0.38	53 ± 0.81	51 ± 0.78	6 ± 0.09
	Th (%)	31 ± 0.47	47 ± 0.72	74 ± 1.14	21 ± 0.32	20 ± 0.30	25 ± 0.35	17 ± 0.26
	REEs (%)	17 ± 0.26	50 ± 0.77	30 ± 0.46	25 ± 0.38	30 ± 0.46	28 ± 0.43	55 ± 0.84
A. fumigatus	U (%)	41 ± 0.63	30 ± 0.46	58 ± 0.89	15 ± 0.23	37 ± 0.57	30 ± 0.46	9 ± 0.13
	Th (%)	18 ± 0.27	34 ± 0.52	49 ± 0.75	42 ± 0.64	47 ± 0.72	27 ± 0.41	21 ± 0.32
	REEs (%)	22 ± 0.33	21 ± 0.32	41 ± 0.63	27 ± 0.41	19 ± 0.29	39 ± 0.60	35 ± 0.54

It clearly appears from Table 4 that all the tested fungi had different U, Th, and REEs bioleaching activities. Especially, *P. citrinum* and *A. hollandicus* were able to leach the highest amount of uranium when applied to W1, recording 70% and 64%, respectively. Furthermore, the larger leachability percentage of thorium concentration was established in W3 by *A. hollandicus*, *P. citrinum*, and *F. roseum*, recording 85%, 88%, and 74%, respectively. The obtained bioleaching percentage of the total REEs in W5 and W6 was recorded as 89% and 75% in the most effective fungi *A. hollandicus* and *T. harzianum*, respectively. Also, the *A. fumigatus* achieved the lowest effectiveness of bioleaching activity with all studied samples.

3.4 *The fungi extracellular total protein secretion after its exposure to different samples*

The results concerning the protein concentration in the fungal extracts are presented in Table 5.

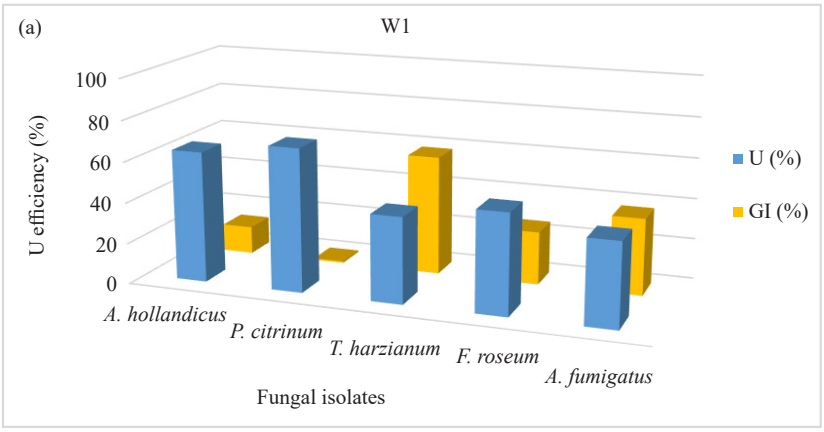
The data analysis revealed a clear difference in the capability of the five fungal strains to produce Total Protein (T.P) after exposure to the different studied samples. The T.P control values showed a variable change between the fungi ranging from 3.4 to 6.5 mg/L. As each fungus was exposed to a sample type, the value of T.P secretion varied. In *A. hollandicus*, the maximum significant level of T.P was recorded as 20.1 mg/L when exposed to W6 while the minimum

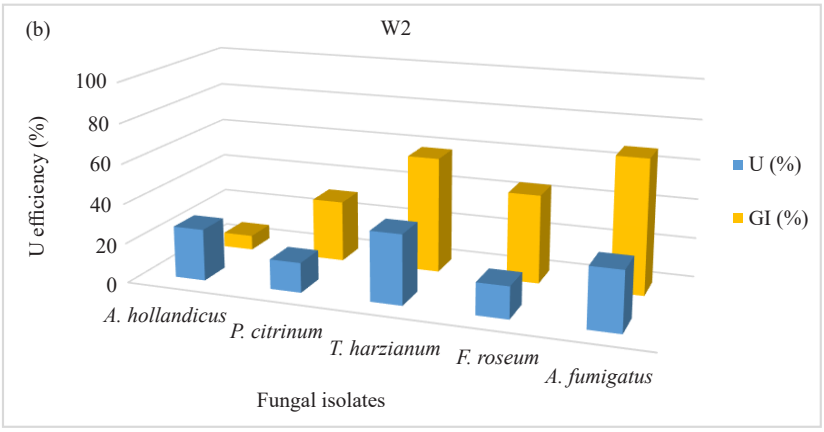
level was 2.7 mg/L in W4 as compared to its control value. The determined T.P of *P. citrinum* after its exposure to W5 was 10.7 mg/L as the maximum value between all samples versus control, and 3.1 mg/L as the lowest one. However, in the case of *T. harzianum*, the T.P reached the highest value of 13.8 mg/L in W6, and 5.2 mg/L as the minimum one. Likewise, the T.P in *F. roseum* was 11.7 mg/L as maximum value (W5) and 3.2 mg/L as the smallest value (W4) against control. Finally, *A. fumigatus* expressed 5.8 mg/L as the largest value (W5), and 3.7 mg/L as the lowest value (W3) in comparison with the control value.

Table 5. The level of extracellular protein (mg/L) after fungi exposure to the different samples

Fungi sp.	W1	W2	W3	W4	W5	W6	W7	Control
A. hollandicus	6.3 ± 0.07	7.5 ± 0.11	6.5 ± 0.19	2.7 ± 0.31	12.7 ± 0.13	20.1 ± 0.08	8.8 ± 0.07	4.7 ± 0.07
P. citrinum	6.2 ± 0.09	6.4 ± 0.03	6.3 ± 0.09	3.1 ± 0.01	10.7 ± 0.13	8.8 ± 0.08	4.8 ± 0.07	5.1 ± 0.07
T. harzianum	7.3 ± 0.1	7.8 ± 0.12	7.7 ± 0.11	5.2 ± 0.04	6.3 ± 0.21	13.8 ± 0.08	5.3 ± 0.10	6.5 ± 0.07
F. roseum	5.5 ± 0.08	6.1 ± 0.07	6.1 ± 0.09	3.2 ± 0.16	11.7 ± 0.06	10.8 ± 0.08	4.3 ± 0.06	5.1 ± 0.09
A. fumigatus	5.2 ± 0.07	5.1 ± 0.07	4.9 ± 0.07	3.7 ± 0.07	5.8 ± 0.07	3.7 ± 0.07	5.3 ± 0.07	3.4 ± 0.07

3.5 *Effect of the fungal GI factor by different mineralogical samples*





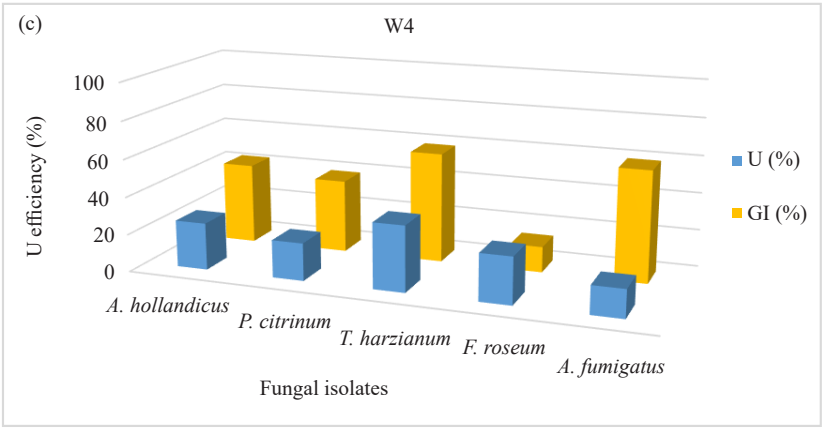
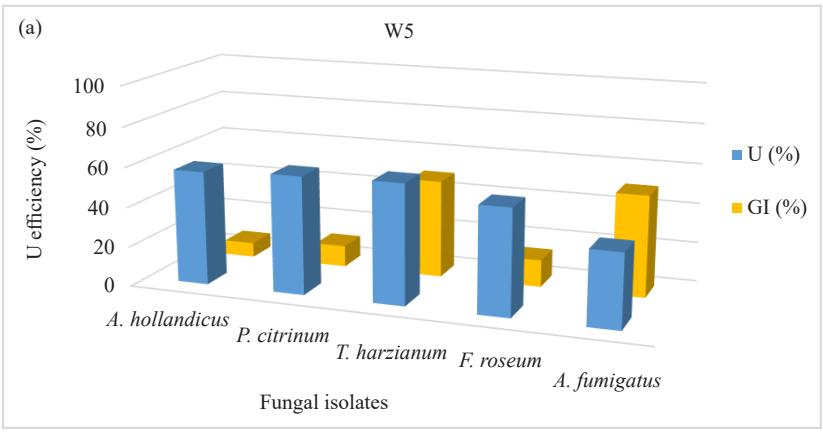


Figure 3. Fungal Growth Inhibition efficiency (GI (%)) vs. Uranium efficiency (U (%)) in the siltstone samples: (a) W1, (b) W2, and (c) W4



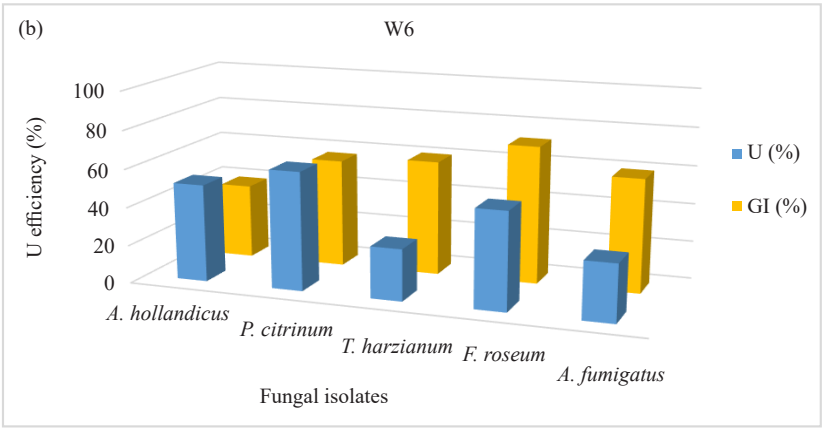


Figure 4. Fungal Growth Inhibition efficiency (GI (%)) vs. Uranium efficiency (U (%)) in the dolostone samples: (a) W5 and (b) W6

The relationship between sample mineralogical constituents and growth inhibition efficiency of all isolated fungi changed in an unsteady manner as shown in Figure 3, 4, and 5: (i) In the siltstone samples (W1, W2, and W4), *P. citrinum* achieved a low percentage of GI (1%) that tolerate the sample toxicity due to high uranium leachability (70%), otherwise in W2 and W4, the GI factor was exhibited as 7% and 14% by *A. hollandicus* and *F. roseum*, respectively.

(ii) In the grey shale samples (W3 and W7), the GI factor was 6% by *P. citrinum* in sample W3, but all isolated fungal species could not tolerate the toxicity of the sample W7, which may be due to its high radioactivity concentration. (iii) In the dolostone samples (W5 and W6), *A. hollandicus* (7%) and *P. citrinum* (11%) implemented approximate values of GI factor (7%), (11%) respectively. Conversely, for W6, GI percentages of 39% and 56% were achieved by *A. hollandicus* and *P. citrinum*, respectively.

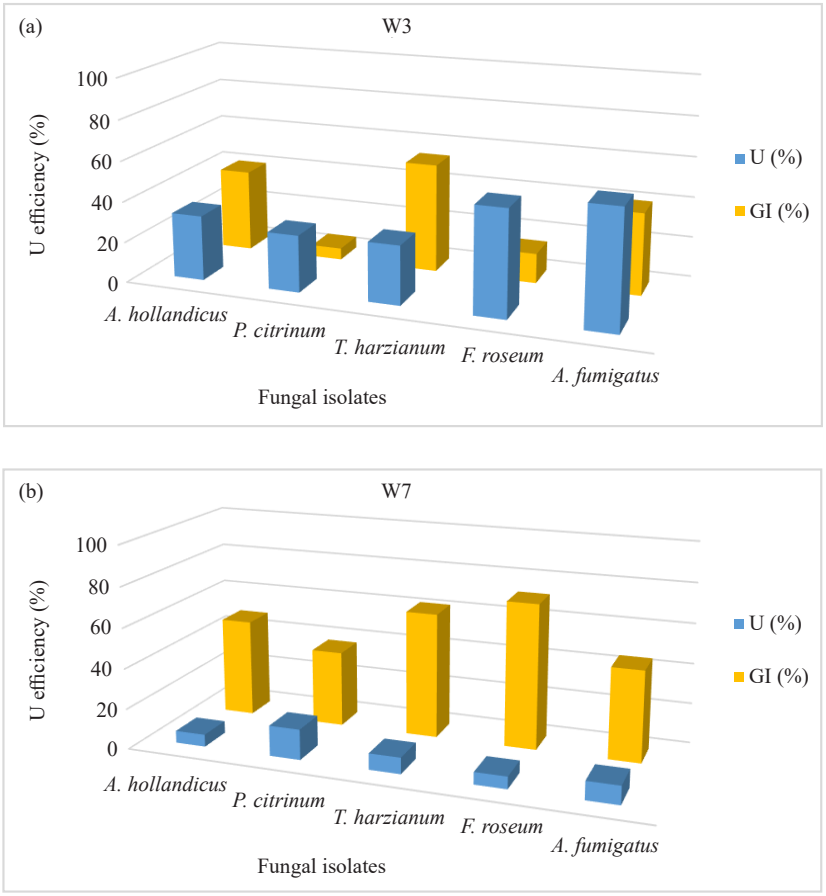


Figure 5. Fungal Growth Inhibition efficiency (GI (%)) vs. uranium efficiency in the grey shale samples: (a) W3 and (b) W7

4. Discussion

The presence of a small amount of pollutants can act as a stimulant by turning on the operation of bioremediation enzymes.[28,29] Microorganisms' enzymatic pathways act as biocatalysts, facilitating the progress of biochemical reactions to degrade the desired pollutant or helping them generate energy and nutrients to build more cells.[30,31]

Myco-remediation is a type of bioremediation that uses fungi to consume and break down environmental pollutants.[32,33] *Pinus tabulaeformis* fungi's bioleaching capacity for cadmium may be due to their exogenous protein secretion, which improves the fixation capacity of heavy metals.[34]

It is known that proteins secreted as enzymes or amino acids can cross the cell wall into the external media, where they may also be involved in recognition processes. Additionally, the phenomenon of protein aggregation within the fungal cell depends on the uptake or export of toxic elements in and out of the cell. Some proteins (e.g., hydrophobins) may have a distinguished role in the aggregation of fungal hypha, making a multicellular tissue that may have a major role in the interaction of the fungi with the external metal exposure.[35]

In general, the protein typically provides three to seven coordinating ligands in natural metalloproteins that

precisely fit the metal's stereochemical needs. However, as few as two ligands, if positioned correctly, can form chelating sites that are useful for protein and peptide crosslinking and stabilization.[36]

The first site of the direct contact between fungus and metal is the fungal cell wall; it contains small amounts of proteins as well as glucan, chitin, and galactosamine-containing polymers. The cell wall and its composition exhibit a vast number of potential binding sites, such as the presence of unbound carboxyl, amino, hydroxyl, phosphate, and mercapto groups.[37-39] It has also been reported that several filamentous fungi, regardless of their ability to produce organic acids, possess primary resistance to metals due to their cell wall and membrane characteristics.[40] Others have indicated the ability of exogenous polysaccharides to play a role in the bioleaching process.[41] In the present study, fungi showed good bioleaching activity, which may be due to the activation of Krebs cycle processes to increase the extracellular secretion of protein and organic acids, leading to changes in the overall system pH value. So, the combination of all these factors has a significant role in the bioleaching process.

Fungi perform many bioleaching processes as a defense mechanism to avoid exposure to toxicants or to use some metals in their vitality. These defense mechanisms may be carried out by the secretion of protein (amino acids, enzymes) or by the bioleaching processes, which proceed through a lot of steps such as acidolysis, complexolysis, redoxolysis, bioaccumulation, biosorption, and alkaloysis.[40]

One of the advantages reported for fungi is their strong adaptability and high tolerance capacity to the environmental stress of metal pollution, which improves mineral bioleaching,[41] due to the great ability of fungi to secrete biodegradative extracellular enzymes and their hyphal growth, which increase their bioleaching capacity.[42]

In the present study, the significant increase in fungi protein secretion may be a defense mechanism when they are exposed to uranium, thorium, and rare earth elements in the different samples of Um Bogma. This result is in agreement with Mohamed et al.,[43] and Geweely et al.,[44] who recorded an increment in total proteins and some amino acids of gamma irradiated *Paecilomyces violacea* and *Fusarium solani*, highlighting the protective role of protein against harmful environmental exposure. Additionally, a new protein may be formed to protect cells against many harmful environmental stresses, such as metal exposure.[44]

Furthermore, fungi showed a resistance effect against many types of radiation by secreting specific types of amino acids such as cystine, methionine and histidine, which contain sulphur bonds and those having double bonds (histidine) are highly resistant to gamma radiation.[45] *Aspergillus niger* exposed to different doses of gamma radiation shows changes in the amino acid profile between irradiated and unirradiated.[46] Dawoud and Taleb[47] recorded an increment in β-glucans in *Pleurotus ostreatus* after its exposure to a low gamma radiation dose. All these findings could explain the resulting increment of total protein secretion in the present study, making protein a protective factor for fungi against exposure to different environmental stresses such as irradiation or radioactive metal toxicity.

From the obtained results and the strategy of mineral-fungi interactions, it could be concluded that there are many factors that can affect the bioleaching activity of fungi. These factors are dependent on the sample's major oxide components, trace elements and uranium content, as well as on the percentage of fungal protein secretion. All these factors work together to provide the fungi with energy, nutrients, and support for fungal growth functions, leading to an increase in their bioleaching capacity.[48]

It is known that the relation between the microorganisms and chemical elements stems from the essentiality of these elements. Essential elements play a crucial role in biology and are integral to all activities, including metabolism and its regulation. Toxic concentrations of non-essential metals, or even of essential ones, cause an increase in reactive oxygen species in fungal cells, leading to an increase in oxidative stress, which is one of the most influential reasons for growth inhibition, ultimately causing fungal death.[44,49]

As the sample constitution differs, so does the efficiency of fungal growth inhibition and bioleaching activity. This observation is clearly apparent when the fungi are exposed to the different samples, where the siltstone samples, which are enriched with major oxides: SiO_2, Fe_2O_3, and Al_2O_3, as well as varying concentration distributions of Cr, Ba, Rb, V, Cu, Ni, Zn, Sr as trace elements, show a slight decrease in the growth inhibition efficiency of *A. hollandicus* and *P. citrinum*, leading to an increase in the uranium bioleaching activity. Meanwhile, in ferruginous siltstone samples, the decrease in Fe_2O_3 level and increase in SiO_2 has a significant effect upon many fungal species like *A. terreus* and *P. spinulosum* by increasing their uranium leaching ability.[50] Conversely, in the grey shale samples the uranium bioleaching activity was slightly decreased, although its enrichment with the essential metals as Zn, Zr, Fe, Cu, and Mn.[51-53] On the other hand, the dolostone samples were characterized by their mineralogical constituents that are rich in Ca, Mg,

and Cu, all of which have a key role as bio essential components highly needed to maintain the fungal metabolism and growth.[54] Also, Cu is an integral trace metal (metal co-factor) required by the enzymes to work properly as biological catalysts.[55]

5. Conclusion

From the present study and the previous one it could be concluded that:

(1) There is a relation between the geological composition of the studied samples and the activity of the bioleaching process by the five adapted potent fungal strains (*A. hollandicus*, *P. citrinum*, *T. harzianum*, *F. roseum*, and *A. fumigatus*).

(2) Fungi showed high bioleaching activity toward different economic elements as U, Th, and REEs.

(3) Proteins have a significant role in the fungal bioleaching process, being considered as a protective factor for fungi to survive exposure to different environmental stress, by the strategy of metal removal (metal sequestration), which can be caused by binding to proteins/ligands, thus enabling growth under stress.

(4) *A. hollandicus* and *P. citrinum* exhibited a great ability to grow under the stress of bio-toxic substances and oxidative pressure from reactive oxygen species.

(5) All these findings confirmed the fact that fungi are a promising bioleaching factor that can be applied.

Conflict of interest

The authors declare no conflict of interest.

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