Radiometric and microbiological investigations of ore and waste samples with especial emphasis on suitable fungus for remediation processes

El Dabour, S.E[a]., Harpy N.M.[a], Nada, A.A.[b], Sallam, A.M[c]., El Aassy, I.E[a]. and El Feky, M.G[a].

a; Nuclear Materials Authority, Cairo, Egypt.

b; Faculty of Women for Art, Science, and Education – Ain Shams University, Cairo, Egypt.

c; Physics Department, Faculty of Science Ain Shams University, Cairo, Egypt.

Abstract:

The capacity of the micro-organisms in ore transformations makes bioleaching technique a very interesting alternative to conventional processes. Radiometric studies indicate high uranium contents in ore and waste samples. This paper describes the suitable fungus species for mobilization of U from low-grade U ores and their resulting wastes. The high bioleaching efficiency in waste samples is mainly attributed to the presence of high sulfate contents in these samples which converted by the aiding of microorganisms into sulphuric acids and hence, increase the bioleaching efficiency. Fungal leaching technique demonstrated an adequate recovery of uranium, with an efficient and cost-effective means from waste samples and respect to the reuse of waste for economic and environmental purposes

Keywords: Uranium ore, Bioleaching, Aspergillus, Remediation.

1. Introduction:

Uranium tailings are a waste by-product of uranium milling. In mining, raw uranium ore is brought to the surface and crushed into fine sand. The valuable uranium-bearing minerals are then removed via heap leaching with the use of acids or bases, and the remaining radioactive sludge, called "uranium tailings", is stored in huge impoundments. Uranium tailings can retain up to 85% of the ore's original radioactivity **(Robinson *et al*. 1979).**

If uranium tailings are stored above ground and allowed to dry out, the radioactive sand can be carried for great distances by the wind, entering the food chain and water resources. The danger posed by such sand dispersal is uncertain at best given the dilution effect of dispersal. The majority of tailing mass will be exposed to atmospheric oxygen, which can substantially alter their chemical behavior.

1.1 Role of fungi in pollution abatement

Use of microbiological methods for environmental remediation has come up as an appreciable concept, which often acts as an eco-friendly approach (**Prasenjit and Sumathi, 2005).** Regarding sustainability, biological processes can contribute to a large extent to future technologies, including also waste treatment **(Ghazala *et al., 2019)***.

In general, bioleaching is a microbiological process described as being "the dissolution of metals from their mineral source by certain naturally occurring microorganisms.

Bioleaching is mainly used in recovering copper, uranium, and gold from ores **(Narayan and Sahana 2009).** There have also been many studies related to bioleaching of

the waste from Cu mining (**Dopson *et al.*, 2009**) in different mining areas from Europe, Africa and Chile (**Andraš *et al.*, 2008, Trois *et al.*, 2007**, **Escobar *et al.*, 2009**).

Several mechanisms are involved in bioleaching, including (i) acidolysis; (ii) complexolysis; (iii) redoxolysis; and (iv) bioaccumulation (**Simate *et al.*, 2010).**

The current study focusses on the investigation of the ability of isolated fungi in bioleaching of uranium from the ore and its resulting residual.

2. Geological aspects of the study area:

Sinai is considered as one of the promising, valuable and strategic areas in Egypt; if it is not the most important at all. It is characterized by great biodiversity in different environs as well as richness in natural and mineral resources. Field studies and surveys have been applied in some regions with a high concentration of radioactive ores (especially uranium mineralization), from these regions the study area, which is known as Allouga locality southwestern Sinai, Egypt. It lies between longitudes 33˚ 20́ and 33˚ 26́ E and latitudes 29˚ 06́ and 29˚ 12́ N. as shown in Fig. (1). The uraniferous ore sample was collected from Um Bogma Formation at Allouga locality.

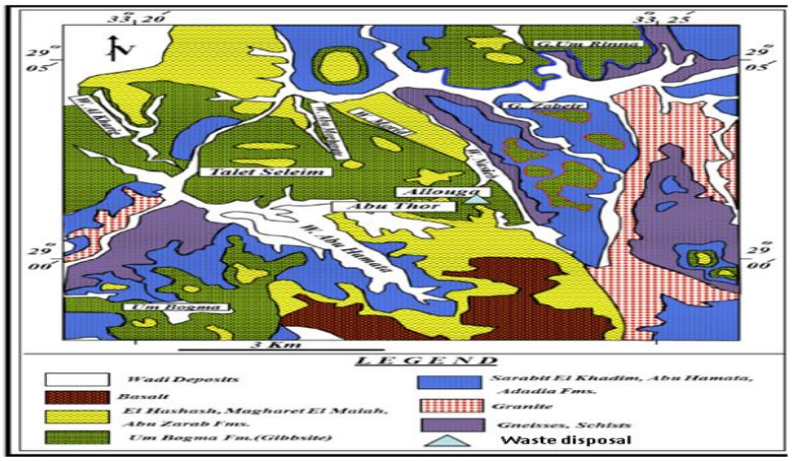

Figure (1): Geological map showing the studied locality and location of tailing wastes (**After Al Shami 2003**)

3. Materials and methods

3.1 Sampling

Two samples were collected; one ore sample (Q8) collected from Allouga locality representing the ore used for uranium extraction while the other sample represents the resulting solid waste after processing (W6) and collected from the sites of waste accumulation. Waste materials were stored after acidic heap leaching and agitated tank leaching of sedimentary rock ores. The resultant wastes were grouped in piles and generally open-air stacked. The studied waste piles were stacked in a rectangular shape with defected sides (Fig. 2). The studied waste sample was collected from the surface of their waste pile.

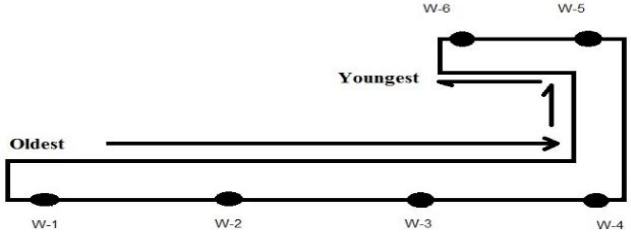

Figure (2): Sketch showing the waste piles distribution in the studied area.

3.2 Chemical analysis

A representative portion of the collected technological sample was properly prepared through crushing and grinding to size minus 200 mesh. The major oxides were examined by the rapid silicate analytical procedure **(Shapiro and Brannock, 1962).** This procedure involves preparation of two main solutions; namely, an alkaline one for SiO_2 and $A1_2O_3$ and an acidic one solution for the other oxides determination of (CaO, MgO, total iron, Na_2O, K_2O, etc.) Special sample portions were used for the determination of the weight loss at various temperatures to estimate the loss on ignition (LOI).

3.2.1 Chemical determination of uranium

The uranium content of the samples was determined according to the method described by **(Davies and Gray 1964),** This solution was titrated against standard NH_4VO_3. the endpoint will be obtained at the appearance of a purplish color.
The uranium concentration will be calculated according to the following equation:

$$\textbf{U (mg/L)} = x = \frac{\mathrm{T} \times \boldsymbol{V_1}\, x10^3}{\mathrm{V}} \text{ ppm} \qquad \textbf{(1)}$$

Where: T is the titration intensity of NH_4VO_3 solution, V_1 is the volume of NH_4VO_3 solution consumed and V is the volume of the measured sample.

3.3. Radiometric analysis

Radiometric analysis of radionuclides was done using γ-ray High Purity Germanium HP-Ge Detector, in Nuclear Materials Authority (NMA), Qattameya, Egypt. The system used for the analysis consisted mainly of High Purity Germanium Detector Model GMX60P4 with its electronic circuits. The crystal has a diameter of 69.5mm and a length of 86.7 mm. Its relative efficiency is 60% and the Peak-to-Compton ratio is 56:1. The FMWM at 1.3 MeV of ^{60}Co is 2.3 keV. The efficiency calibration was performed by using three reference materials obtained from IAEA for U, Th, and K which are named RGU.1, RGTh.1 and RGK.1 **(Aghighi, *et al*., 2004, Atlas, 2006)**.

The collected samples were measured for their U, Th, Ra and K γ-activities. About 300 g from each sample was packed in a plastic container, sealed well and stored for about 30 days before analysis to allow the in-growth of uranium and thorium decay products, prevent the escape of radiogenic gases ^{222}Rn and ^{220}Rn and allow secular equilibrium between ^{238}U, ^{232}Th, and their daughter products. After attainment of secular equilibrium, each sample was counted for at least 24 hours **(Tsuruta, 2005, Yang and Volesky 1999).**

3.4. Microbiological techniques

3.4.1. Media preparation

Growing media composed of;1: $NaNo_3$, 2: KH_2PO_4, 1: $MgSO_4.7H_2O$, 0.5: KC1, 0.5; $FeSO_4$. $5H_2O$ traces; sucrose, 30: Agar, 15 and 5g. Yeast extract. The pH value of the medium was adjusted to a value of 6.5 as described by **(Raper and Thom 1954).**

3.4.2 Fungal isolation

Two techniques were used for the fungal strain's isolation from the ore samples, the first one was the direct plating technique, in which fine ore powder was spread directly upon

the surface of Dox agar plates under septic conditions, sealed and incubated at 30^{o}C until the fungal colonies grew. The second one was the dilution plate technique in which 1 g of ore powder 0.2 mm mesh size was taken under the septic condition and mixed well with 9 m1 of sterile distilled water, then a serial dilution was prepared, and 0.1 m1 of this mixture was spread under a septic plate. The plates were incubated at 28^{o}C± 2 until colony development was obtained **(Gilman, 1957 and Pitt, 1979).**

3.4.3 Purification and identification of isolated fungi

Hyphal tips of each colony were removed and plated upon the surface of Dox agar plates. The developed colonies were examined under a microscope to detect contamination. The pure isolated colonies were identified according to **(Gilman, 1957, Pitt, 1979, Moubasher, 1993 and Mostafa, 2006**). The fungal isolates were maintained on Czapek's-Dox agar slants and sub-cultured every two weeks.

The tested fungi were identified based on macroscopic and microscopic characteristics according to **(Kilch 2002**, **Watanabe 2002 and Humber 1997)** up to species level.

3.4.٤ Fungal activity determination

Direct method

One hundred ml of Dox liquid medium was placed in 250 ml Erlenmeyer flasks. The flasks were supplemented with 1% of ore sample concentration. Triplicate sets of flasks were prepared for each organism and ore. The flasks were autoclaved at 1.5 atm for 20 minutes, after cooling the flasks were inoculated with 1 ml spore suspension and finally incubated at 30^{o}C for 7 days in an orbital shaker at 100 rpm. At the end of the incubation period, the culture medium was filtered and centrifuged at 4000 rpm to precipitate more particles. This filtrate was kept for uranium determination.

3.4.5 Analysis of leach liquor

Estimation of uranium in leach liquor was done according to **(Davies and Gray 1964) and (Farag *et al.* 2015)** by titration against 0.1% ammonium metavanadate.

4. Results

4.1 Major oxides

The obtained results showed in table (1) indicate that the ore and waste samples are mainly composed of SiO_2, CaO, MgO, Fe_2O_3, and Al_2O_3. Some oxides of major elements such as SiO_2, Fe_2O_3, MgO, SO_3^{--} and Al_2O_3 display enrichment in the ore and waste samples. The waste sample has lower CaO and L.O.I content suggesting lower carbonate and so organic carbon contents.

The waste sample has nearly two folder SiO_2 of the ore sample due to the presence of the remained gangue minerals fig (3). On the other hand, MnO, K_2O, Na_2O TiO_2, and P_2O_5 show low contents in the ore and waste samples. Uranium was analyzed radiometrically and chemically. Chemically measured uranium of samples was higher than the radiometrically measured uranium indicating the presence of recent uranium deposits resulting from later

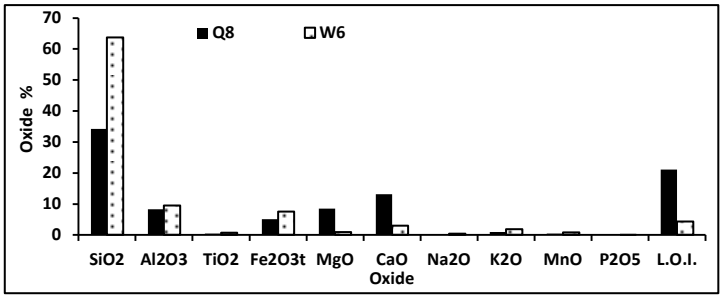

Figure (3): Major oxides percent in ore sample and waste sample.

processes affecting waste and surrounding rocks suggesting the necessary need for waste management. Uranium contents reach up to 1650 and 302 ppm in the ore and waste samples, respectively.

Table (1): Concentrations of major oxides in (wt%) for ore and waste sample.

Oxide	Original sample	Waste sample
%	Q8	W6
SiO_2	34.25	63.61
Al_2O_3	8.29	9.41
TiO_2	0.48	0.62
$Fe_2O_3^t$	5.08	7.52
MgO	8.49	0.9
CaO	13.19	2.92
Na_2O	0.04	0.31
K_2O	0.99	1.81
MnO	0.49	0.78
P_2O_5	0.03	0.08
$SO3^{--}$	7.57	7.72
S	3.03	3.08
L.O.I.	21.14	4.32
Total	100.04	100
U* (ppm)	1506	240
U** ppm	1650	302

$Fe_2O_3^t$: total iron L.O.I.: Loss of ignition
U* Uranium concentration using HP-Ge detector.
U**: Uranium concentration by chemical analysis.

4.2 Radiometric investigations:

In this work, Hyper-pure germanium (HP-Ge) based high-resolution gamma spectrometry is applied for detection and measurement of natural radioactivity. The activity concentrations are expressed in $Bq.Kg^{-1}$ for the measured radionuclides ^{238}U and ^{232}Th series indicate the radioactive equilibrium/disequilibrium as also noticed by **Walley El Dine (2007)**. Equilibrium achieved if the activity between parent/ daughter or between daughter/ parent is equal to unity.

4.2.1 Distribution of radionuclides in study samples (Allouga locality)

The obtained results for the activity concentrations of ^{238}U, ^{235}U, ^{234}U, ^{230}Th, ^{226}Ra, ^{40}K as well as their ratios are reported in Table (2) in (Bq/kg) for the studied Allouga representative sample (Q8) by HP-Ge detector. The activity concentrations of ^{238}U, ^{232}Th, ^{226}Ra, and ^{40}K are 21208.8±311.13, 52.07±14.13, 12573.24±75.25 and 235.69±24.71 $Bq.Kg^{-1}$, respectively. The world concentration limits of ^{238}U, ^{232}Th, ^{226}Ra, and ^{40}K are equal to 33, 45, 32 and 412 $Bq.Kg^{-1}$, respectively **(UNSCEAR, 2008)**. The activity concentrations of all

the studied radionuclides in the representative sample have higher values relative to that recorded in the **(UNSCEAR, 2008)**.

$^{234}U/^{238}U$, $^{230}Th/^{238}U$, and $^{226}Ra/^{238}U$ ratios are 0.72±0.14, 0.62±0.05, 0.59±0.02 Bq/kg which show disequilibrium in the direction of uranium concentrations and indicate U-migration-in for these sedimentary rocks. The $^{238}U/^{232}Th$ ratios support the previous observation as it is 407.31 which deviate from the natural (magmatic) ratio 1:3 in the direction of U-migration-in.

$^{238}U/^{235}U$ ratio was 21.78±1.25 which is within the natural ratio (21.7). $^{234}U/^{235}U$ ratio 15.70±3.31 is diverted from the normal ratio. It has been known for decades that there is a decreased amount of ^{234}U in soil due to that the ^{234}U is more leachable than ^{238}U as a result of the alpha-recoil phenomenon **(Gregory, *et al.*, 2010 and Kumar, *et al.*, 2016)**. The ratio of ^{226}Ra /^{230}Th was 0.96±0.06 show equilibrium between ^{230}Th and his daughter ^{226}Ra. Similarly, the ratio of $^{214}Pb/^{226}Ra$ and ^{214} Bi/^{226}Ra were 0.90±0.01 and 0.95±0.01 which also show equilibrium between ^{226}Ra and their daughters ^{214}Pb and ^{214} Bi which could be due to radon accumulation and aided by poor mobility of ^{214}Pb and ^{214} Bi. So, from the obtained results of these ratios indicate that U-migration-in these sedimentary rocks could be the main cause for the disequilibrium in these samples.

Table (2) also represent the activity concentration of mill tailing sample. It was found that the activity concentration of ^{238}U 2350.96±113.11 Bq/Kg, and that ^{232}Th of 64.74±6.58 Bq/Kg, for ^{226}Ra 8215.78±69.87 Bq/Kg , while ^{40}K has 462±10.1Bq/Kg. The studied tailing also has higher values relative to that recorded in the **(UNSCEAR, 2008)**.

In several uranium mines around the world, the presence of oxidizing sulfides in the tailings and waste rock is the main reason for the most important potential environmental problem **(Fernandes *et al.*, 1998)**. $SO3^{--}$ content in the studied waste reach up to 7.72 wt.% (Table-1) which has a plausible effect in radionuclides translocation if reaches to the water table.

The $^{234}U/^{238}U$ (2.6 ± 0.24) >1 in tailing waste sample which may result from α- recoil and re-adsorption or co-precipitation of ^{234}U by amorphous materials. It has been demonstrated experimentally that some minerals such as clays or iron/manganese oxide phases are efficient in removing radionuclides from the soluble phase **(Duff *et al.*, 2002)**.

The organic matter content may also contribute significantly to chemical fractionation between uranium and its long-lived daughters **(Dosseto *et al.*, 2006)**. Tailings from waste sample show a clear disequilibrium ($^{230}Th/^{238}U$ >1), this probably results from the leaching of U that would be fixed again to the solid phase. Preferential complexation of thorium with dissolved organic matter, which enhances its mobility, and hence leads to depletion in residual compared to uranium, However, $^{230}Th/^{234}U$ (0.79± 0.4) < 1in waste sample due to the large existence of the ^{234}U in these wastes. The radioactive disequilibria of $^{230}Th/^{234}U$ are mainly produced by α-decay enhanced dissolution, re-adsorption and re-precipitation of ^{234}U. Thorium isotopes are significantly associated with colloids **(Short 1988)**. Mobility of ^{226}Ra is suppressed by high concentrations of sulfate ions leading to the formation of stable (precipitated) $RaSO_4$, and thus reducing the mobility of radium, leading to $^{226}Ra/^{238}U$ and $^{226}Ra/^{230}Th$>1, in addition to $^{214}Bi/^{226}Ra$ and $^{214}Pb/^{226}Ra$<1 for tailing waste sample. ^{226}Ra shows equilibrium with their daughters ^{214}Pb and ^{214} Bi which may be due radon accumulation and aided by poor leaching of ^{214}Pb and ^{214} Bi as ^{226}Ra. It was found that $^{238}U/^{235}U$ ratios are within the natural ratio (21.7). So, leaching of the two elements has the

same behavior and the same leaching efficiency. Vice versa the $^{234}U/^{235}U$ ratio which out of the natural ratio which could be resulted from α- recoil and re-adsorption or co-precipitation of ^{234}U by amorphous materials.

Table (2): Measured HP-Ge-specific activity concentrations in Bq/kg for radionuclides of ^{238}U-, ^{235}U-, ^{232}Th-series and ^{40}K with their total uncertainty values of ore and waste sample.

Nuclide	Ore sample Q8	Waste sample W6
Uranium-series(^{238}U)		
^{234}Th	21023.58±154.93	2337.78±50.04
^{234m}Pa	21394.01±467.32	2364.14±176.18
Av.	21208.8±311.13	2350.96±113.11
^{234}U	15287.89±2681.59	6119.28±273.01
^{230}Th	13078.71±716.95	4858.53±368.62
^{226}Ra	12573.24±75.25	8215.78±69.87
^{214}Pb	11310.10±41.72	6466.41±22.34
^{214}Bi	11418.43±34.65	6464.19±18.18
^{210}Pb	10621.33±107.26	5510.52±47.79
Uranium-series (^{235}U)		
^{235}U	973.62±34.35	107.72±13.28
Thorium-series (^{232}Th)		
^{228}Ac	51.71±7.91	64.53±8.22
^{208}Tl	52.44±4.23	65.95±3.3
Av.	52.07±14.13	64.74±6.58
^{40}K	235.69±24.71	462±10.1
$^{238}U/^{235}U$	**21.78±1.25**	**21.82±3.74**
$^{234}U/^{235}U$	**15.70±3.31**	**56.81±9.54**
$^{234}U/^{238}U$	**0.72±0.14**	**2.60±0.24**
$^{226}Ra/^{238}U$	**0.59±0.02**	**3.49±0.2**
$^{230}Th/^{238}U$	**0.62±0.05**	**2.07±0.26**
$^{230}Th/^{234}U$	**0.86±0.11**	**0.79±0.4**
$^{226}Ra/^{230}Th$	**0.96±0.06**	**1.69±0.14**
$^{214}Pb/^{226}Ra$	**0.90±0.01**	**0.79±0.01**
$^{214}Bi/^{226}Ra$	**0.95±0.01**	**0.79±0.01**
$^{238}U/^{232}Th$	**407.31±1.3**	

These hazard wastes need remediation and management, so we must isolate the suitable microorganism for remediation of these wastes.

4.3 Microbiological investigations

The microbiological assays comprise microorganisms isolation of the most growing strains from two samples Q8 as original sample (ore sample) and W6 as tailing waste sample.

4.3.1 Microorganisms isolation and identification

Five fungal species were successfully isolated from the tested samples. Examination of the isolated fungi revealed that they belong to the genus *Aspergillus*. They have septate mycelium with a vesicle at the end of sporophore bearing catenate conidiospores and also form ascospores. The cultures on Czapek's agar plates are shown in Fig. 4. The tested isolates belong to 5 species (*Asp. Lentulus*, *Asp. flavus*, *Asp. Niger*, *Asp. Felis*, and *Asp. fumigatus*).

4.3.2 The direct microbial leaching studies on Q8 and W6

Indigenous fungal isolates showed variable U bioleaching abilities using direct techniques. The direct bioleaching efficiencies were different for the tested isolates (Fig. 5).

All of the tested fungi could grow in the presence of 1% ore concentration of the studied waste sample (W6) and their leaching efficiencies were tabulated in Table (3) and Fig. (5), which reveals that *A. lentulus* was superior and followed by *Asp. Niger (*68 and 54%) respectively bioleaching efficiency of uranium. Since the bioleaching efficiency of uranium obtained with *A. lentulus* was the highest relative to the other fungi.

The high bioleaching efficiencies for those isolates using direct method may be due to the increased interaction between the hyphae and the rock sample and in the same time withstanding the toxicity of radionuclides and/or heavy metals leached from the sample into the culture filtrate due to having certain features that enables them to resist heavy metals and/or radionuclides' action. It may also be because the genes are responsible for the production of secondary metabolites and enzymes that aid in the bioleaching process was upregulated in the presence of the sample. Since the bioleaching efficiency of uranium obtained with *A. lentulus* was the highest relative to the other fungi. **Sharma *et al*. (2009)** studied the effect of *Aspergillus lentulusAMLO5* species on the treatment of Cr (VI) from industrial zone effluents at Wazirpur, India. They found that the prevailing factor helps the removal of Cr (VI) with 99.8% under pH=6.5 for 120 hours as an optimum condition.

Kaushik and Malik (2011) applied three variables of dye concentration, urea, and nutrient glucose to control dye remediation by *Aspergillus lentulus FJ172995*. they reached to dye treatment/ removal efficiency up to 99.7% by optimizing the mentioned controlling conditions.

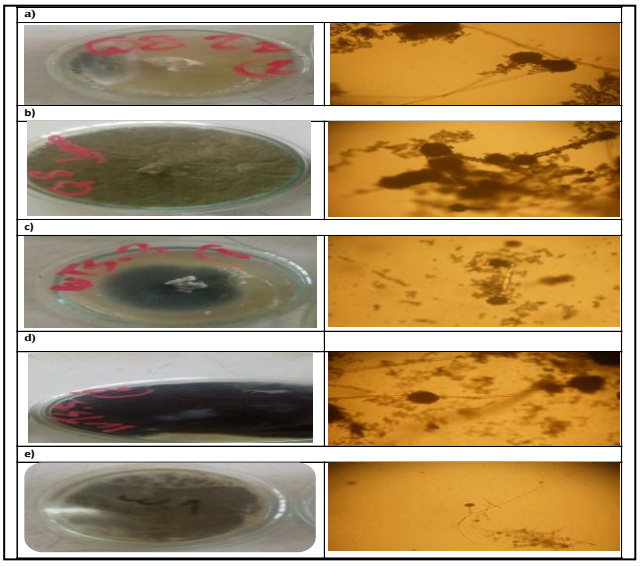

Figure (4): Microscopic and culture pictures of isolated fungus from the studied samples by a binocular microscope (X400). **a:** *Asp. Flavus*, **b:** *Asp. Lentulus*

c: *Asp. Fumigatus* **d:** *Asp. Niger* **e:** *Asp. Felis*

Table (3): Leaching efficiencies of the isolated species for uranium

Species	Uranium Bioleaching efficiency %
Aspergillus Lentulus	68
Aspergillus Niger	54
Aspergillus Flavus	42
Aspergillus Fumigatus	37
Aspergillus Felis	26

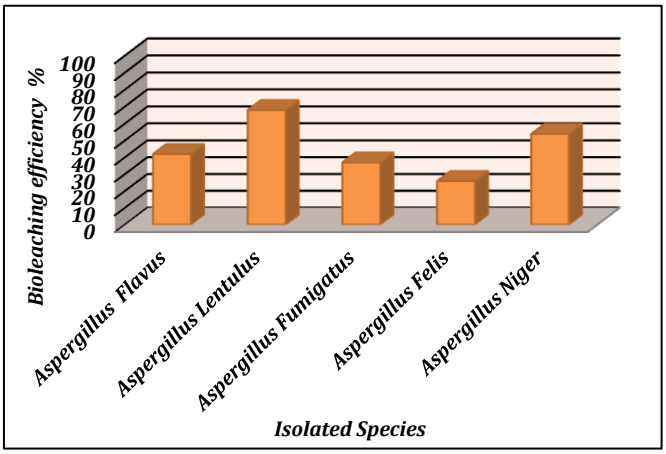

Figure (5): Leaching efficiencies for the isolated species from the studied sample

5. Conclusion and recommendation:

Bioleaching has been proved to be an alternative and economically friendly technique that should be passed to a larger scale to maximize its efficient role. Radiometric studies clearly show high uranium contents in the ore and waste samples. The noticeable difference between radiometrically and chemically measured uranium, in addition to a high sulfur concentration in the ore and waste samples, confirms that uranium and other radio-and toxic elements were mobilized to the surrounding environment and indicate the vital need for waste management especially with the annually occurring of flash floods. Microbiological studies show that *Aspergillus species* represent the main isolates. Bioleaching experiments confirm that *A. lentulus* is the most efficient species in uranium bioleaching and so in bioremediation processes. More studies are necessary for bioremediation processes concerning other radio-and toxic elements rather than uranium.

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الملخص باللغة العربية

تحقيقات إشعاعية وميكروبيولوجية لعينات الخام والنفايات مع التركيز بشكل خاص على الفطريات المناسبة لعمليات المعالجة

سامح السيد محمد* و د. ناريمان مصلح حربى* و ا. د/ عفاف عبد اللطيف ندا و ا.د/ عبد الستار محمد سلام *** و ا.د/ ابراهيم القطانى العاصى * و ا.د/ محمد جلال حسين الفقى ***

** هيئة المواد النووية - القطامية - مصر*
***قسم الفيزياء - كلية البنات للأداب والعلوم والتربية - جامعة عين شمس*
****قسم الفيزياء - كلية العلوم - جامعة عين شمس*

إن قدرة الكائنات الحية الدقيقة على معالجة الخامات تجعل تقنية الإذابة الحيوية بديلاً مثيراً للاهتمام للغاية عن العمليات التقليدية. تشير الدراسات الإشعاعية إلى وجود نسبة عالية من اليورانيوم في عينات الخامات والنفايات. الهدف من البحث هو دراسة أنواع الفطريات المناسبة لإذابة اليورانيوم من خاماته منخفضة التركيز والنفايات الناتجة عنها. تُعزى كفاءة الإذابة الحيوية العالية في عينات النفايات بشكل أساسي إلى وجود محتويات عالية من الكبريتات في هذه العينات والتي تم تحويلها بمساعدة الكائنات الحية الدقيقة إلى أحماض الكبريتيك ، وبالتالي زيادة كفاءة الإذابة الحيوية. أظهرت تقنية الإذابة الحيوية استردادًا مناسبًا لليورانيوم ، مع إثبات أنها وسيلة ذات كفاءة عالية وفعالة من حيث التكلفة ومعدل الإستخلاص لبعض العناصر بالنسبة لعينات النفايات مما يؤكد على إعادة استخدام النفايات للأغراض الاقتصادية والبيئية للإستفادة من محتواها.