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Investigation of the Capability of Some Fungal Species to Immobilize and Concentrate NORM

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Abstract: Microbiota are extremely likely to play a key role in in-situ recovery (ISR) mining at all stages. They can have a beneficial or negative impact on uranium recovery efficiencies, and they play a critical role in mine site remediation. It has been established that fungi can convert uranium solids into secondary mycogenic uranium minerals. Fungal interactions with uranium species have received little attention compared to bacterial subsurface U transformations. In the current study, we investigated the ability of seven locally isolated fungal species which are *Aspergillus oryza*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Ulocladiumatrum*, *Cladosporium* sp, and *Fusarium oxyporiumto* immobilize naturally occurring radioactive materials (NORM). *Aspergillus oryza* and *Aspergillus niger* were shown to be possible good candidates for immobilizing NORM with removal percentages of 97.1% and 98.55% in 48 hours, respectively. *Ulocladiumatrum* showed a possible capability of specifically immobilizing uranium species with removal percentages of ~100% in 48 hours. *Cladosporium* sp species showed specificity for uranium species as species *Ulocladiumatrum* but its rate was 58.39% in 48 hours and required a much longer time to completely immobilize all the activity in the media..

Keywords: Fungi, NORM, Immobilization, Aspergillus, Ulocladium, Cladosporium, Fusarium.

1 Introduction

Uranium (U) use has been rapidly increased, driven by the world's pace with the fast need for nuclear power. As per conservative estimates, yearly demand for U in 2030 would range between 80,000 and 148,500 t [1], up 50 to 179 percent from the 58,000 t produced in 2012. Kazakhstan (36.5 percent), Canada (15 percent), and Australia (12 percent) presently produce over 63.5 percent of the world's U. Underground mining, open-pit mining, and in situ recovery (ISR) technologies have all been used to extract uranium from a wide range of deposits [2].

The usage of ISR has steadily increased over the last two decades, and it now contributes to 45 percent of global U production [3].

In 907 A.D., the Chinese appear to have been the first to use ISR for copper extraction, with references to solution mining reaching as far back as 177 B.C. [4, 5]. This was followed by the French ISR of elemental sulfur and the Russians' ISR of gold [4, 5].

The ISR of U was developed by the United States and the Soviet Union in the 1960s [6]. ISR accounted for 95 percent of U mined in the United States by the 1990s, and the technique is now being used more widely around the world [3, 7].

The in-situ recovery of U involves drilling boreholes into the ore deposit [8], pumping a leaching solution down injection boreholes, flowing the solution through the mineralized horizon to dissolve the ore, retrieving the solution from production boreholes, and extracting U from the solution in a surface plant. Natural rock porosity, mineral dissolution (acid leach), or engineered fragmentation can all allow the solution to pass through the ore (hydraulics or explosives).

The leaching solution might be alkaline or acidic, depending on the deposit's mineralogical and geochemical characteristics. Careful monitoring and management of leach solutions are essential to prevent the spread of U, other radionuclides, and metals into previously uncontaminated ground water. Microorganisms play a significant role in the mobility of a wide range of metals, including iron, manganese, gold, copper, and uranium, and are well-known actors in Earth's elemental cycles such as the carbon, and nitrogen, sulfur, and phosphorous cycles [9].

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Numerous studies have shown that microbiota has a substantial impact on U mobility [10-17].

Microorganisms convert U from U(VI) to U(IV), which has an impact on the status and distribution of U in the environment [12].

Microorganisms can use U as an energy source, and a large variety of microorganisms can convert U: Aerobic metal-oxidizing microbes catalyze the oxidation of reduced metals, thus solubilizing and mobilizing U; anaerobic bacteria catalyze metal reduction, thus immobilizing U. [18, 19].

Some bacteria, for example, promote U mobility by producing acid during iron and sulfur oxidation (i.e., bioleaching) [20-23]; others, on the other hand, limit U mobility by reducing uranyl and generating very insoluble nano-particles of secondary U(IV)minerals such uraninite [12] and coffinite [24].

As a result, microbiota are extremely likely to play a key role in ISR at all stages: They can have a beneficial or negative impact on U recovery rates and play a critical role in mine site remediation. Fungi can dissolve metal-containing minerals and re-precipitate mobilized metal or metal radionuclide species within the mycelium or microenvironment through acidity and chelation [25-28].

Organic and inorganic metal precipitation as secondary mineral phases might result in metal and radionuclide immobilization [25-36].

It has been established that fungi can convert uranium solids into secondary mycogenic uranium minerals [33-36].

Even though fungi are an essential component of the soil microbiota, playing a key role in a variety of important metal, radionuclide, and mineral transformations [26, 27], Because fungi are generally overlooked in geomicrobiology, fungal interactions with uranium species have received little attention compared to bacterial subsurface U transformations [28, 37].

This study aims to investigate the ability of several fungal isolates to immobilize and concentrate uranium and other naturally occurring radioactive materials (NORM).

2 Experimental Section

2.1 Fungal Specimens

Fungal specimens were isolated from soil samples collected from several sites at the Nuclear Research Center, Egyptian Atomic Energy Authority in Inshas. The Fungal Isolates were identified morphologically on genus and famous species levels only to be continued in later studies. The fungal candidates used in this study were, *Aspergillus oryza*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillusterreus*, *Fusarium oxyporium*, *Cladosporium sp.*, and *Ulocladiumatrum*.

2.2 Media Preparation

Fungal growth media was prepared by dissolving 20g of sucrose, 2g NaNO3, 1g K2HPO4, 0.5g KCl, 0.5g MgSO4.5H2O, 0.003g FeCl3, 0.01g Mgcl3, and 0.03 MnSO4 in one liter. To study the effect of pH on NORM immobilization efficiency, pH was adjusted for media solutions of the same components at 4, 5, 6, 7, 8, and 9 using diluted HCl and NaOH solutions. The media was solidified by adding 20 g of purified Agar as support. The media was autoclaved before isolation, purification, and cultivation steps which were performed in Petri dishes and slants. Submerged cultures were then transferred to 100 ml of sterilized liquid media with the same above-mentioned composition and mixed with monazite waste containing U-238, Th-232, and U-235 that had activity concentrations of 620± 46, 50 ± 2,and 20 ± 1 Bq/kg respectively. One hundred grams of growth media were used for each culture jar which had a total calculated activity of 62±4.6 Bq U-238, 5 ±0. 2Bq Th-232, and 2±0.1Bq U-235. Fungal specimens were inoculated in growth media jar replicates as 5 replicates for each specimen. Two growth stages were conducted. Fungal specimens were grown on media containing dissolved NORM with measured activity concentrations for 48 hours in the first stage. In the second stage, the grown fungal spores, and hyphae were from the growth media and new inoculums from the same species were inoculated to grow for 14 more days. To study the temperature effect, fungal cultures were incubated at 25and 45 ^{o}C. All grown fungal samples were harvested from culture jars and dried at 105 ^{o}C for 1 hour.

2.3 Gamma Spectroscopy

Individual dried fungal samples were analyzed for gamma-ray radioactivity using High Purity Germanium detector (HPGe). The efficiency of the germanium detector was calibrated using decay gammas from standard sources of Ba-133[38], Cs-137 [39], andEu-152 and ^{154}Eu [40, 41].

The immobilization percentage of fungal cultures understudy was calculated according to the following equation:

$$(C_o\text{-}C)/C_oX100$$

Where;

C_o is the initial activity of an isotope in growth media

C is the final activity of an isotope in growth media

3 Results and Discussion

3.1 ability of growth:

The ability of seven fungi strains *Aspergillus oryza*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Ulocladiumatrum*, *Cladosporium sp*, and *Fusarium*

oxyporium to extract, and immobilize NORM was evaluated in this study. The ability of the studied strains to grow varied in the used growth media, as shown in Table 1, where *Aspergillus niger* showed the highest ability to grow, as the dry weight its at the end of the experiment reached 2.79 g, with a slight difference from *Aspergillus oryza* whose dry weight reached 2.72 g. As for the rest of the strains, the dry weights of *Ulocladiumatrum*, *Aspergillus flavus*, *Aspergillus terreus*, *Cladosporium sp*, and *Fusarium oxyporium* reached 0.848, 0.838, 0.465, 0.25, and 0.13 g , respectively.

3.2 Extraction of NORM

As shown in table 1, Only four of the studied strains were able to extract and immobilize NORM, which are *Aspergillus oryza*, *Aspergillus niger*,*Ulocladiumatrum*, and *Cladosporium sp*, while the strains *Aspergillus flavus*, *Aspergillus terreus*, and *Fusarium oxyporium* did not have the ability to immobilize NORM. The strains that immobilized NORM varied in the speed of extraction, and the specificity. Table 1 shows *Aspergillus oryza* and *Aspergillus niger* had the ability to extract and immobilize almost all of the NORM within 48 hours of cultivation. On the other hand, *Ulocladiumatrum* and *Cladosporium sp* showed specialization in extracting uranium only, noting that strain *Ulocladiumatrum* was able to extract and immobilize almost all uranium within 48 hours of cultivation.

3.3 Immobilization Capacity

By comparing the immobilization capacity expressed in Bq/g, it was found that although *Cladosporium sp* was the slowest of the strains in NORM immobilization, it showed the largest capacity (256 Bq/g). There is an additional observation shown in Table 1, which is that the concentration of NORM has an effect on the growth of the fungal strains, as it was found that the weight of the NORM immobilizing strains after 48 hours was large, given that the growth media had the highest concentration of NORM, while, their growth was weak in the 14 days period of incubation, as there was very little left of the NORM in the growth media.

3.4 Effect of pH

A study was carried out on the effect of change in pH on fungal growth, the ability to immobilize NORM, and the capacity of immobilization of the NORM immobilizing fungal strains. The fungal strains were grown in media with pH 4,5,6,8, and 9. The effect of the change in pH appeared clearly on *Aspergillus oryza*, as shown in Table 2 and figure 1, where it did not succeed in growing at a pH less than 6 or higher than 8, and its speed in fixing theNORM slowed down, as it needed a period longer than 48 hours to immobilize NORM, which is its speed at neutral pH.

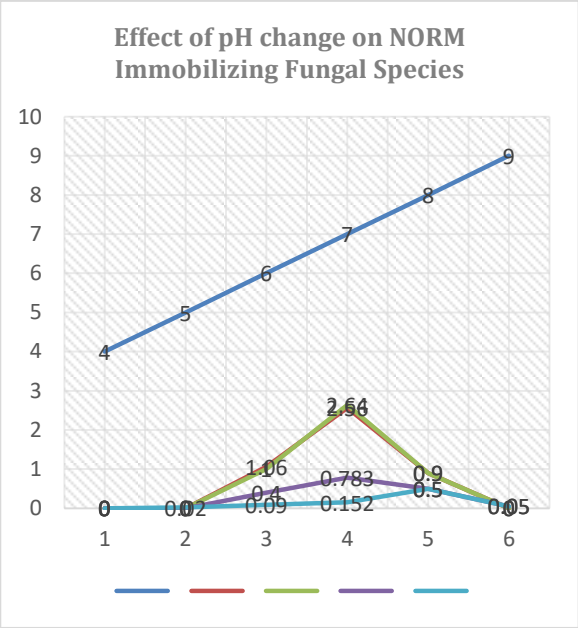

Fig. 1: Effect of pH change on NORMImmobilizing Fungal Species.

When comparing the capacity of NORM immobilization under the influence of the change in pH, as shown in table 2 and figure2, it turned out that the change in pH had a positive effect on the immobilization capacity of *Aspergillus oryza*. Both cases were higher than the capacity of *Aspergillus oryza* to immobilize the NORM at neutral pH, which was 25.37 Bq/g.

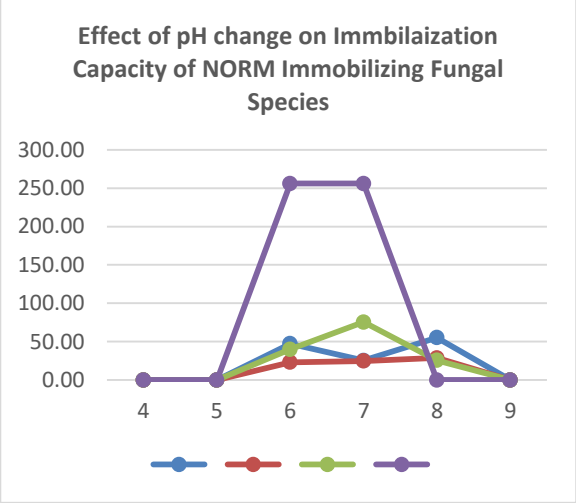

Fig. 2: Effect of pH change on Immbilaization Capacity of NORM Immobilizing Fungal Species.

The effect of the change in pH on the growth of fungus *Aspergillus niger* was the same as in the case of fungus *Aspergillus oryza* as shown in Table 2 and Figure 1, but it differed in its effect on the capacity of NORM immobilization as shown in Table 2 and Figure 2, where the effect of the base pH was positive on The

Table 1: Measured activities of Th-232, U-235, and U-238 in Fungal cultures harvested after 48 hours and 14 days

Fungal Sp.	Mass(g)		Th-232		U-235		U-238		Total Mass(g)	Total Activity (Bq)		Total activity immobilized %	Capacity for immobilization Bq/g
			Bq/unit		Bq/unit		Bq/unit						
	48 hrs	14days	48 hrs	14days	48 hrs	14days	48 hrs	14days		48 hrs	14days		
Aspergillus oryza	2.56	0.16	5±0.5	N.D.	2±0.04	N.D.	60±3	2±0.05	2.72	67	2	100	25.37
Aspergillus flavus	0.811	0.02	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.831	B.G.	B.G.	0	0.00
Aspergillus niger	2.64	0.15	5±2	N.D.	2±0.06	N.D.	61±4	1±0.1	2.79	68	1	100	24.73
Aspergillus terreus	0.46	0.005	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.465	B.G.	B.G.	0	0.00
Ulocladiumatrum	0.783	0.065	N.D.	N.D.	2±0.03	N.D.	60±4	2±0.06	0.848	62	2	92.75	75.47
Cladosporium sp	0.152	0.098	N.D.	N.D.	0.8±0.2	0.5 ±0.01	25±3	16±2	0.25	25.8	16.5	61.3	169.20
Fusarium oxyporium	0.095	0.035	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.13	B.G.	B.G.	0	0.00

Table 2: Measured activities of Th-232, U-235, and U-238 in fungal cultures harvested after 48 hours and 14 days at different Temperatures at pH7.

pH		Mass(g)		Th-232		U-235		U-238		Total Mass(g)	Total Activity (Bq)		Total activity immobilized %	Capacity for immobilization Bq/g
				Bq/unit		Bq/unit		Bq/unit						
		48 hrs	14days	48 hrs	14days	48 hrs	14days	48 hrs	14days		48 hrs	14days		
4	*Aspergillus oryza*	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Aspergillus niger	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Ulocladiumatrum	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Cladosporium sp	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
5	*Aspergillus oryza*	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Aspergillus niger	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Ulocladiumatrum	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Cladosporium sp	0.02	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02	N.D.	N.D.	N.D.	0.00
6	*Aspergillus oryza*	1.06	0.4	2±0.5	3±0.09	0.9±0.08	1.1 ±0.07	30 ±4	32 ±3	1.46	32.9	36.1	100	47.26
	Aspergillus niger	1	2	2.25 ±0.5	2.75± 0.08	0.8±0.08	1.2±0 .07	28 ±4	34±3	3	31.05	38	100	23.02
	Ulocladiumatrum	0.4	1.2	N.D.	N.D.	0.9±0.08	1.1±0 .09	28 ±4	34.1±3	1.6	28.8	35.2	92.75	40.00
	Cladosporium sp	0.09	0.16	N.D.	N.D.	0.3±0.08	1.7±0 .05	9.5 ±2	52.5±4	0.25	10	54	92.75	256.00

7	*Aspergillus oryza*	2.56	0.16	5±0.5	N.D.	2±0.04	N.D.	60±3	2±0.05	2.72	67	2	100	25.37
	Aspergillus niger	2.64	0.15	5±2	N.D	2±0.06	N.D.	61±4	1±0.1	2.79	68	1	100	24.73
	Ulocladiumatrum	0.783	0.065	N.D.	N.D.	2±0.03	N.D.	60±4	2±0.06	0.848	62	2	92.75	75.47
	Cladosporium sp	0.152	0.098	N.D.	N.D.	0.8±0.2	1.2 ±0.01	25±3	37±2	0.25	25.8	38.2	92.75	256.00
8	*Aspergillus oryza*	0.9	0.35	1.8±0.5	3.2±0.04	0.6±0.1	1.4±0.05	28±4	34±3	1.25	30.4	38.6	100	55.20
	Aspergillus niger	0.9	1.5	2±0.5	3±0.05	1±0.1	1±0.04	31±4	31±2	2.4	34	35	100	28.75
	Ulocladiumatrum	0.5	2	N.D.	N.D.	1±0.1	1±0.04	31±4	31±2	2.5	32	32	92.75	25.60
	Cladosporium sp	0.5	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.5	N.D.	N.D.	N.D.	0.00
9	*Aspergillus oryza*	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Aspergillus niger	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	N.D.	0.00
	Ulocladiumatrum	0.05	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.05	N.D.	N.D.	N.D.	0.00
	Cladosporium sp	0.05	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.05	N.D.	N.D.	N.D.	0.00

immobilization capacity of *Aspergillus niger*, reached 28.75 Bq/g, while the effect of the acidic pH was negative, as the immobilization capacity of *Aspergillus niger* decreased to 23.02 Bq/g compared with the immobilization capacity of *Aspergillus niger* at neutral pH, which was 24.73 Bq/g. The effect of the change in pH was negative on the immobilization speed as well as the immobilization capacity of *Ulocladiumatrum*, as shown in Table 2 and Figures 2 and 3, as it needed more than 48 to fix uranium, and its immobilization capacity decreased to 40 Bq/g in the acidic pH media and to less than This is when cultivated in a media with an alkaline pH of 25.6 Bq/g, compared to its immobilization capacity in an environment with a neutral pH of 75 Bq/g. *Cladosporium sp* did not succeed in growing in an alkaline media environment, nor did it succeed in growing in an environment with an acidic pH of less than 6, but this did not affect its speed or capacity to immobilize uranium, as shown in Table 2 and Figures 2 and 3, where it was able to immobilize uranium in the incubation period was 14 days, and its immobilization capacity was 256 Bq/g.

3.5 Effect of Temperature

The effect of temperature change was also evident on the characteristics of NORM immobilizing fungi in terms of growth, immobilization speed, and immobilization capacity, as *Cladosporium sp* did not succeed in growing at temperatures other than 37 °C. The effect of temperature also varied on the rest of the NORM immobilizing fungal strains, as shown in Table3 and Figures 3 and 4. *Aspergillus oryza* did not grow at a temperature of 45 °C, while its growth accelerated when it was incubated at a temperature of 25 °C, as shown in Table 3 and Figure 3, but despite the acceleration of its growth at 25 °C, its immobilization capacity was negatively affected. It reached 23 Bq/g compared to its immobilization capacity at 37 °C, which was 25 as shown in Table 3 and Figure 4.The effect of temperature on *Aspergillus niger* was similar as it failed to grow when incubated at 45 °C and its growth accelerated when incubated at 25 °C, but it was negatively affected by the temperature as its immobilization capacity decreased to 23 Bq/g compared to its immobilization capacity at 37 °C which was 24.73 Bq/g as shown in Table 3 and Figures 3 and 4 .As in the case of other NORM immobilizing fungal strains, *Ulocladiumatrum* also failed to grow when it was incubated at 45 ° C. It succeeded in growing at 25 ° C. But unlike the others, its growth rate or its fixative capacity was not affected by the change in temperatures at which it succeeded in growing as shown in Table 3 and Figures 3 and 4.

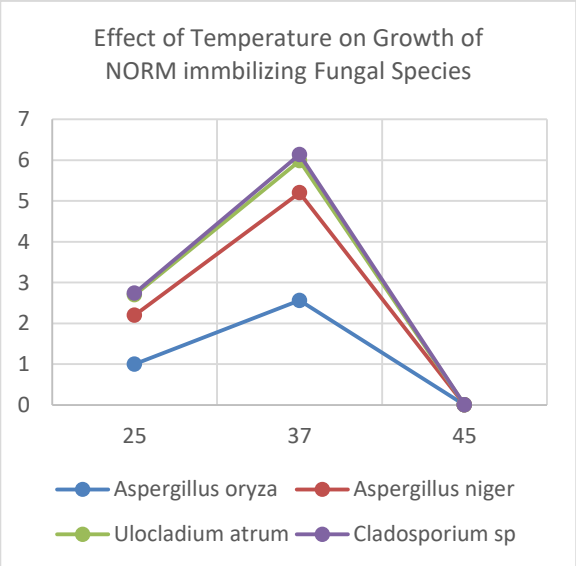

Fig. 3: Effect of Temperature on Growth of NORM immbilizing Fungal Species.

Table 3: Measured activities of Th-232, U-235, and U-238 in fungal cultures harvested after 48 hours and 14 days at different Temperatures at pH7.

T	Fungal Species	Mass(g)		Th-232 Bq/unit		U-235 Bq/unit		U-238 Bq/unit		Total Mass(g)	Total Activity (Bq)		Total activity immobilized %	Capacity for immobilization Bq/g
		48 hrs	14days	48 hrs	14days	48 hrs	14days	48 hrs	14days		48 hrs	14days		
25	*Aspergillus oryza*	1	2	1.5±0.4	3.5 ±0.05	0.8±0.08	1.2±0.07	27.9±3	34.1±3	3	30.2	38.8	100	23.00
	Aspergillus niger	1.2	1.8	3 ±0.5	2±0.05	1.2±0.08	0.8±0.06	41.2±4	20.8±3	3	45.4	23.6	100	23.00
	Ulocladiumatrum	0.5	0.5	N.D.	N.D.	1.4±0.06	0.6±0.09	43.4±4	18.6±3	1	44.8	19.2	92.75	64.00
	Cladosporium sp	0.04	0.14	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.18	N.D.	N.D.	0	0.00
37	*Aspergillus oryza*	2.56	0.16	5±0.5	N.D.	2±0.04	N.D.	60±3	2±0.05	2.72	67	2	100	25.37
	Aspergillus niger	2.64	0.15	5±2	N.D.	2±0.06	N.D.	61±4	1±0.1	2.79	68	1	100	24.73
	Ulocladiumatrum	0.783	0.065	N.D.	N.D.	2±0.03	N.D.	60±4	2±0.06	0.848	62	0	92.75	73.11
	Cladosporium sp	0.152	0.098	N.D.	N.D.	0.8±0.2	1.2 ±0.01	25±3	37±2	0.25	25.8	38.2	92.75	256.00
45	*Aspergillus oryza*	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	0	0.00
	Aspergillus niger	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	0	0.00
	Ulocladiumatrum	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	0	0.00
	Cladosporium sp	0	0	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0	N.D.	N.D.	0	0.00

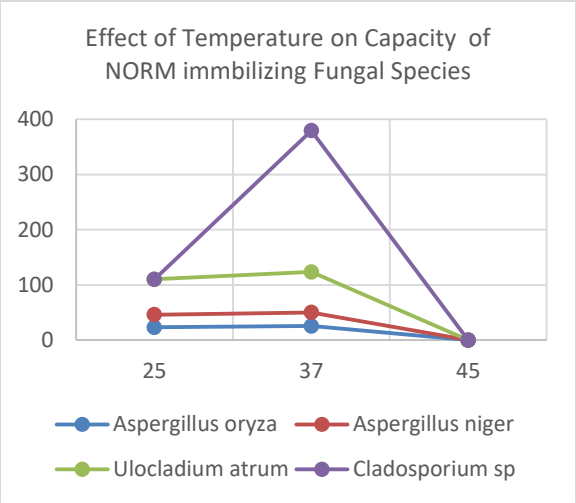

Fig. 4: Effect of Temperature on Capacity of NORM immbilizing Fungal Species.

4 Conclusions

In the current study, we investigated the ability of some locally isolated fungal species to utilize and immobilize NORM. *Aspergillus oryza* and *Aspergillus niger*were were shown to be possible good candidates for utilizing and immobilizing NORM. *Ulocladiumatrum* showed a possible capability of specifically utilizing and immobilizing Uranium species. *Cladosporium sp* species showed specificity for Uranium species as species *Ulocladiumatrum* but required a much longer time to completely immobilize all the activity in the media. Regarding NORM Immbilization capacity, *Cladosporium sp*showed the highest capacity in Uranium species immobilization. The uranium and Thorium immobilization fungal strains *Aspergillus oryza*and *Aspergillus* niger had nearly the same capacity in immobilizing NORM. The optimum pH and temperature for successive NORM fixation in studied fungal species are pH=7 and 37 ^{o}C, respectively. It can be concluded that the changes in NORM uptake may be controlled in response to changes in pH and temperature.

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