Uranium adsorption by *Articulospora tetracladia*: can aquatic hyphomycetes be natural bioremediators of uranium contaminated streams?

V. Ferreira[*,1], A. L. Gonçalves[1], J. Pratas[2] and C. Canhoto[1]

[1] IMAR-Institute of Marine Research & Dept. Zoologia, Universidade de Coimbra, 30004-517 Largo Marquês de Pombal, Coimbra, Portugal

[2] Dept. Ciências da Terra, Universidade de Coimbra, 3000-272 Largo Marquês de Pombal, Coimbra, Portugal

Uranium concentration in the streams around abandoned uranium mines in central Portugal can be as high as 1.8 mg/L. Herein we assessed the kinetics of uranium adsorption by *Articulospora tetracladia* mycelium at 200 and 2000 μgU/L over 6h. Uranium adsoption was relatively fast with 18–50% uranium remaining in solution after 15 minutes and maximum adsorption of ~140 mgU/gDM at 2000 μgU/L after 6h. The fitting of the uranium uptake data to the Freundlich isotherm indicates monolayer uranium adsorption at the surface of the mycelium. The stability of the uranium monolayer is high (n<1), as well as the adsorption capacity (at 1 μgU/L the uranium uptake is 1.73 mgU/gDM). Since the uranium uptake was not significantly different between live and dead mycelium, the uranium adsorption over the 6h study period was probably a physico-chemical process, independent of biological activity. The applicability of the Michaelis-Menten-type model indicates that adsorption at the mycelium surface progresses towards saturation, indicating that the limiting factor for uranium binding is the number of surface sites; maximum uranium uptake rate was 182 mgU/gDM, and 196 μgU/L was the half saturating uranium concentration. The high extraction factors (EF=28–41) and distribution coefficients (Kd>48203 mL/g) found for *A. tetracladia* indicate that this species can be considered a good biosorbent and has the ability to retrieve uranium from very dilute solutions (stream water). Aquatic hyphomycetes seem to have the potential to act as natural bioremediators of streams running through uranium contaminated areas.

Keywords adsorption; aquatic hyphomycete; *Articulospora tetracladia*; uranium contamination

1. Introduction

Uranium is an important environmental contaminant in some areas of the world [1], such as in central Portugal, where there are several abandoned uranium mines (e.g. Urgeiriça and Cunha Baixa mines, Viseu; [2,3]). Here, the uranium concentration in water can be as high as 1.8 mg/L [3], which potentially affects the aquatic biota (e.g. fish, invertebrates, algae; [4]). Several studies have evaluated the adsorption and accumulation of uranium by microorganisms [5, 6, 8], on the perspective of using them as bioremediators. The reported tolerance of microorganisms to uranium is in part due to their ability to adsorb, bioaccumulate and/or transform uranium [9, 10]. Several studies have shown that the ability of microorganisms to adsorb/accumulate uranium results mainly from physico-chemical processes at the cells surface and is not dependent on metabolism [5, 9, 11, 12]. To our knowledge, there is no information regarding the capacity on aquatic hyphomycetes (anamorphs of ascomycete and basidiomycete fungi) to adsorb uranium, although this would be of great interest given their pivotal role on litter decomposition, which fuels aquatic food webs in small shaded streams [13].

In this study we assessed the kinetics of uranium adsorption by *Articulospora tetracladia* as a first attempt to address the potential for aquatic hyphomycetes to act as natural bioremediators of uranium contaminated streams.

2. Methods

2.1 Kinetics of uranium biosorption

Erlenmeyer flasks (100 mL) were filled with 25 mL a 200 or 2000 μgU/L sterile solution, which mimic the U concentration found at a U contaminated stream and at the mine residual waters, respectively. Each flask was inoculated with ~0.0192 g of mycelium, which was produced as described by Gessner and Chauvet [14]. Flasks were incubated on a shaker (100 rpm) at 20ºC for 6 h. At each of 16 sampling times the mycelium suspensions (n=3) were filtered through a pre-weighed ignited glass fiber filter, the mycelium was oven dried (105ºC, 24 h)

[*] Corresponding author: e-mail: veronica@ci.uc.pt, Phone: +351 239 855 760, Fax: +351 239 855 789

and weighed (±1 mg), while the filtered solution was poured into a scintillation vial and acidified with 65% HNO3 to pH=2 and stored until analyzed for U concentration [15].

The U adsorption (q) to the mycelium was calculated as q (mgU/gDM)=[Ui]-[Uf]/DM, where [Ui] and [Uf] are the initial and final U concentrations in solution, respectively, and DM is the dry mass. Uranium adsorption (log transformed) over time was compared between 200 and 2000 μU/L by 2-way ANOVA, followed by Tukey's test (Statistica 6), and fitted to linear or non linear regression models. The rate (k, /min) was calculated as the slope of the linearized plot of the Lagergren equation: ln(qe-qt) vs. t, where qe and qt are the amount of U adsorbed by the mycelium at equilibrium and at time t, respectively [16].

2.2 Adsorption isotherms

Erlenmeyer flasks (n=5) were filled with 25 mL of sterile uranium solution (100, 200, 400, 800 and 2000 μgU/L), inoculated with ~0.0136 g of mycelium, and incubated on a shaker for 6h, after which mycelium and solution were sampled, as above.

The U adsorption at equilibrium (qe) was calculated as above. The ratio [Ue] on mycelium/[Ue] on solution, where [Ue] is the U concentration at equilibrium, gives the adsorption distribution coefficient (Kd; ml/g) [5, 9]. The extraction factor (EF) was calculated as [Ui]/[Uf], where [Ui] and [Uf] are the initial and final U concentrations [17]. Uranium concentration and adsorption at equilibrium (log transformed), Kd and EF at equilibrium were compared among U concentrations by 1-way ANOVA, followed by Tukey's test (Statistica 6).

Uranium adsorption data were fit to the Freundlich isotherm [5, 11, 16, 18]. Log(qe) *vs*. log[Ue] gives the linearized plot of the Freundlich equation, with the slope (n) indicating the intensity of adsorption (n>1 and n<1, indicate repulsive and attractive forces, respectively, between the surface layer and the sorbent) and the intercept (k, mgU/gDM) giving the adsorption capacity at 1 μgU/L [10]. The relationship between U adsorption at equilibrium (log transformed) and initial U concentration was also assessed via Michaelis-Menten-type saturation model, which allows the determination of the maximum U adsorption rate (Vmax) and the U concentration at Vmax/2 (Km).

2.3 Adsorption by live and dead mycelium

In order to evaluate if U adsorption by mycelium is a metabolic or a physico-chemical process we assessed the U adsorption by live and dead mycelium at 200 and 2000 μgU/L. Erlenmeyer flasks were filled with 25 mL of sterile U solutions, inoculated with ~0.0146 g of live or dead (120ºC, 15min) mycelium (n=5), and incubated on a shaker for 6h, after which mycelium and solution were sampled, as above. Uranium adsorption was compared among mycelium types and U concentrations by 2-way ANOVA, followed by Tukey's test (Statistica 6).

3. Results

3.1 Kinetics of uranium biosorption

Uranium adsorption by *A. tetracladia* mycelium over the 6h period was dependent on the initial U concentration ($p<0.001$), being 8–17 times higher at 2000 μgU/L than at 200 μgU/L (e.g. 146.3 *vs*. 8.6 mgU/gDM at equilibrium; **Fig. 1a, b**). Uranium adsorption was very fast during the first 15min, mostly at 2000 μgU/L (linear regressions, $R^2=0.92$ and $p<0.00001$; slopes: 0.22 (at 200 μgU/L) and 2.47 (at 2000 μgU/L); **Fig. 1a, b**), although it resulted in lower percentage of U remaining in solution at 200 μgU/L (18%) than at 2000 μgU/L (50%). After the first 15min, U adsorption roughly stabilized at 200 μgU/L (8.6 mgU/gDM; **Fig. 1a**); after 6h there were only 1.5% U remaining in solution. At 2000 μgU/L, U adsorption still increased from 65.1 mgU/gDM at 15min to 146.3 mgU/gDM at 6h (logarithmic regression, $R^2=0.81$; **Fig. 1b**), resulting in only 2.8% U remaining in solution. The rate constants (k) given by the slope of the linearized plots of the Lagergren equation were 0.0078/min at 200 μgU/L ($R^2=0.60$, $p<0.00001$) and 0.0296/min at 2000 μgU/L ($R^2=0.90$, $p<0.00001$) (**Fig. 1c, d**).

3.2 Adsorption isotherms

The U concentration at equilibrium increased linearly ($R^2=0.98$ and $p<0.00001$) with increasing initial U concentration (**Table 1**). The U adsorption at equilibrium was dependent on the initial U concentration, increasing linearly ($R^2=0.96$ and $p<0.00001$) from 6.5 mgU/gDM at 100 μgU/L to 135.9 mgU/gDM at 2000 μgU/L ($p<0.001$; **Table 1**). The adsorption distribution coefficients were high across all U concentrations (Kd>48203 mL/g), although higher at 800 μgU/L than at 100 and 200 μgU/L ($p=0.009$), and the extraction

factors varied between 28 and 41, and were significantly higher at 800 μgU/L than at 200 μgU/L (p=0.018) (**Table 1**).

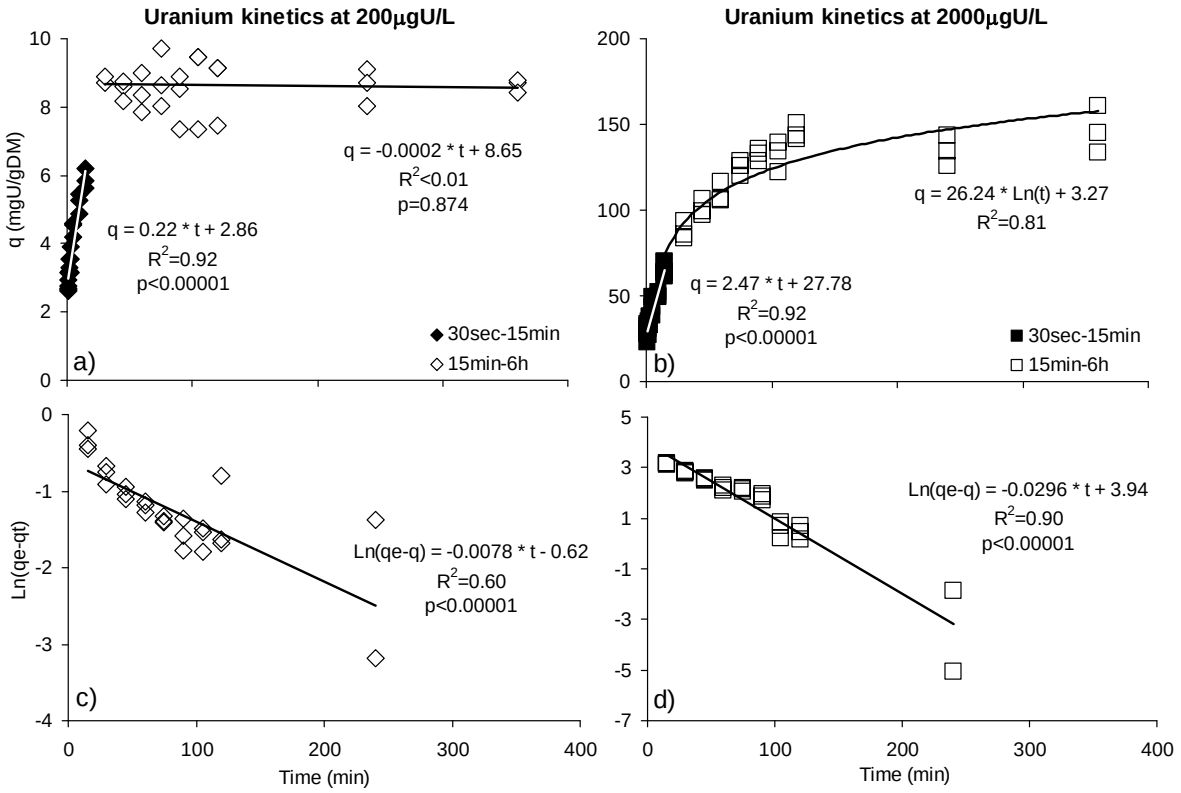

Fig. 1 Uranium adsorption (q) by *A. tetracladia* live mycelium (a, b), and linear plot of the Lagergren equation for U adsorption Ln(qe-qt)=-k*t+ln(qe), where qe and qt are the U adsorption (mgU/gDM) at equilibrium and at time t, respectively, and k (/min) is the rate constant (c, d), at 2 U concentrations over 6h.

Table 1 Initial ([Ui]) and equilibrium ([Ue]) U concentrations, U adsorption (qe), adsorption distribution coefficients (Kd) and extraction factors (EF) of A. tetracladia exposed for 6h at 5 U concentrations. Values are averages±1SE. Comparisons were made among U concentrations by 1-way ANOVA; different letters indicate significant differences (Tukey's test).

[Ui] (μg/L)	[Ue] (μg/L)	qe (mgU/gDM)	Kd (mL/g)	EF
100	3.7^{a} ± 0.5	6.5^{a} ± 0.3	48203^{a} ± 6302	30^{ab} ± 5
200	7.3^{b} ± 0.3	14.6^{b} ± 1.2	49416^{a} ± 2400	28^{a} ± 1
400	11.2^{c} ± 0.3	29.6^{c} ± 1.9	66037^{ab} ± 3268	36^{ab} ± 1
800	19.8^{d} ± 0.7	58.7^{d} ± 3.7	74826^{b} ± 6670	41^{b} ± 1
2000	52.8^{e} ± 2.9	135.9^{e} ± 11.4	65314^{ab} ± 7624	38^{ab} ± 2
1-way ANOVA (p)	<0.001	<0.001	0.009	0.018

The U adsorption data fit the Freundlich isotherm model (linearized plot, R^2=0.95 and p<0.00001), with n=0.89 and k=1.73 mgU/gDM (**Fig. 2a**). The Michaelis-Menten-type model also gave a good fit (R^2=0.99 and p<0.00001), with maximum U adsorption rate of 182 mgU/gDM, and 196 μgU/L as the half saturating U concentration (**Fig. 2b**).

3.3 Adsorption by live and dead mycelium

Uranium adsorption was not significantly different between live and dead mycelium at both U concentrations (200 μgU/L: 11.9 *vs*. 13.8 mgU/gDM; 2000 μgU/L: 142.3 *vs*. 150.1 mgU/gDM, for live and dead mycelium, respectively; p=0.655), although it was higher at 2000 μgU/L than at 200 μgU/L for both mycelium types (p<0.001).

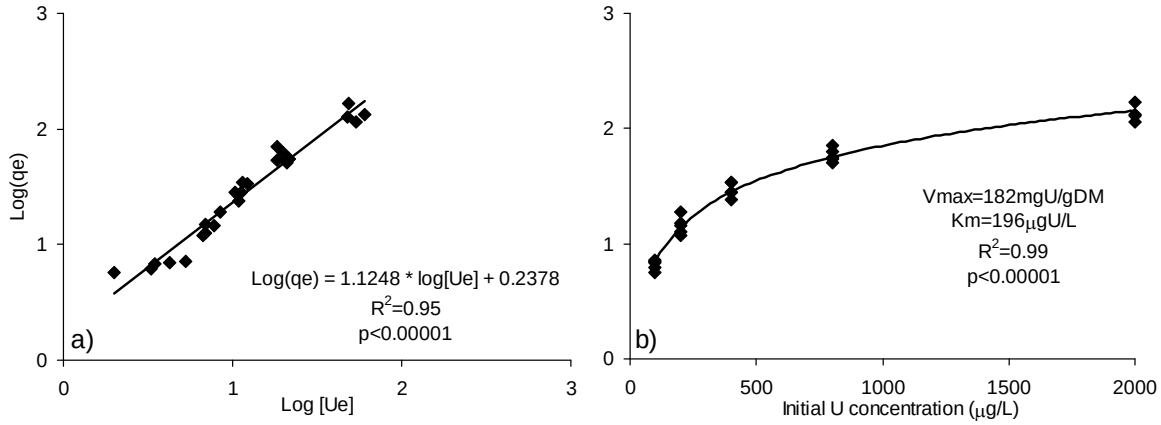

Fig. 2 Linear plot of the Freundlich U adsorption isotherm Log(qe)=1/n*log[Ue]+logk, being qe the adsorption rate at equilibrium (mgU/gDM), [Ue] the U concentration at equilibrium (mg/L), n the intensity of adsorption and k the adsorption capacity at 1μgU/L (a), and relationship between log U adsorption at equilibrium (qe, mgU/gDM) and initial U concentration ([Ui], μg/L). Data are fit into Michaelis-Menten-type model Log(qe)=(Vmax*[Ui])/(Km+[Ui]), being Vmax the maximum adsorption rate and Km the U concentration at half maximum adsorption rate (b).

4. Discussion

We assessed the kinetics of U biosorption by *A. tetracladia* mycelium in two U concentrations (200 μgU/L and 2000 μgU/L), which are 1–3 orders of magnitude lower than the concentrations used in previous studies [5, 12, 19]. However, most of these studies addressed the use of microorganisms as bioremediators retrieving U from concentrated waste waters [7, 12, 19]. Here, we were interested in assessing if aquatic hyphomycetes, a group of organisms naturally occurring in headwater streams, have the ability to adsorb U at concentrations equivalent to those found in streams running through contaminated areas. Although in these streams the U concentration is far lower than in waste waters [2], it still remains a threat to the aquatic life [4].

Uranium adsorption by *A. tetracladia* mycelium was relatively fast with 18% (200 μgU/L) – 50% (2000 μgU/L) U remaining in solution after 15 minutes, and less than 3% remaining after 6h. Rapid and efficient bioadsorption of U by live microbial biomass has been reported previously; *Aspergillus fumigatus* removed 75% U from solution within 2 min of contact [9], *Pseudomonas* removed >90% U from solution within 10 min of contact [12], and 21 basidiomycetes species removed >80% U from solution within 1h of contact [7].

The U loading capacity attained at equilibrium increased with increasing initial U concentration, as expected for low U concentrations [5]. At very high U concentrations, Horikoshi *et al*. ([5]; >8–12 mgU/L) reported stabilization and Bhainsa and D'Souza ([9]; >200 mgU/L) and Tawfik *et al*. ([20]; >100 mgU/L) observed a decrease in U uptake. This might suggest a unimodal relationship between U loading capacity and U concentration over a wide concentration range. The maximum U adsorption observed in this study (~140 mgU/gDM at 2000 μgU/L) was higher than those found for most species previously assessed [5, 8, 21], but was lower than that reported for *Talaromyces emersonii* (q=280 mgU/gDM, [19]).

The fitting of the U adsorption data to the Freundlich isotherm indicates monolayer U adsorption to the surface of the mycelium, which was also suggested in previous studies [11, 12, 18]. The stability of the U monolayer is high as a result of the attractive forces between the surface layer and the mycelium (n<1) [10]. The U adsorption capacity is also relatively high given that at 1 μgU/L the U adsorption is 1.73 mgU/gDM. Uranium adsorption was not significantly different between live and dead mycelium which further indicates that the U adsorption over the 6h period was a physico-chemical process, and independent of biological activity. This was already observed in previous studies where metabolic inhibitors, dead biomass and different temperatures were used [5, 9, 11, 12]. Our experiment does not allow to clarify the mechanism for U binding at the mycelium surface, however, some authors have suggested that it involves the amino group of polysaccharides (e.g. chitin and chitosan) from the cell walls [22, 23].

The fitting of the adsorption data to the Michaelis-Menten-type model indicates that U adsorption at the mycelium surface progresses towards saturation, indicating that the limiting factor is the surface area. The maximum possible U loading was calculated as 182 mgU/gDM, which is higher than most uptake rates reported, although lower than that found for *T. emersonii* [19].

The distribution coefficients for *A. tetracladia* (Kd>48000 mL/g for residual concentrations <53 μgU/L) were in the upper range reported in the literature [5, 9], and indicate *A. tetracladia* ability to retrieve U from very dilute solutions such as stream water. The high extraction factor corroborates the indication that the mycelium of *A. tetracladia* can be considered a good biosorbent.

Aquatic hyphomycetes seem therefore to have the potential to act as natural bioremediators of streams running through U contaminated areas. In these systems, U sequestration has been attempted with the use of macrophytes [2], but small shaded streams are light limited which might compromise the success of this approach. Aquatic fungi, on the other hand, are heterotrophic organisms, which depend on terrestrially derived organic matter as a source of energy and carbon, and an increase in litter availability will result in an increase of the associated fungal biomass [24]. In fact, the accumulation of U by submerged organic matter was already observed [2] and might in part be due to its colonization by aquatic fungi. This might suggest another approach to decrease U transportation to downstream reaches: the reforestation of riparian areas, usually highly degraded in mining areas, with deciduous tree species.

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References

[1] Winde F, Sangham LA. Uranium pollution of South African streams – an overview of the situation in gold mining areas of the Witwatersrand. GeoJournal. 2004;61:131-149.
[2] Merkel J, Hasche-Berger A, eds. *Uranium in the environment*. Berlin Heidelberg: Springer-Verlag Publ; 2006.
[3] Antunes SC, Figueiredo DR, Marques SM, Castro BB, Pereira R, Gonçalves F. Evaluation of water column and sediment toxicity from an abandoned uranium mine using a battery of bioassays. *Science of the Total Environment*. 2007;374: 252-259.
[4] Sheppard SC, Sheppard MI, Gallerand MO, Sanipelli B. Derivation of ecotoxicity thresholds for uranium. *Journal of Environmental Radioactivity*. 2005;79:55-83.
[5] Horikoshi T, Nakajima A, Sakaguchi T. Studies on the accumulation of heavy metal elements in biological systems. *Applied Microbiology and Biotechnology*. 1981;12: 90-96.
[6] Nakajima A, Sakaguchi T. Selective accumulation of heavy metals by microorganisms. *Applied Microbiology and Biotechnology*. 1986;24:59-64.
[7] Nakajima A, Sakaguchi T. Accumulation of uranium by basidiomycetes. *Applied Microbiology and Biotechnology*. 1993;38:574-578.
[8] Nakajima A, Tsuruta T. Competitive biosorption of thorium and uranium by *Micrococcus luteus*. *Journal of Radioanalytical and Nuclear Chemistry*. 2004;260:13-18.
[9] Bhainsa KC, D'Souza SF. Biosorption of uranium(VI) by *Aspergillus fumigatus*. *Biotechnology Techniques*. 1999;13:695-699.
[10] Kalin M, Wheeler WN, Meinrath G. The removal of uranium from mining waste water using algal/microbial biomass. *Journal of Environmental Radioactivity*. 2005;78: 151-177.
[11] Pons MP, Fusté MC. Uranium uptake by immobilized cells of *Pseudomonas* strain EPS 5028. *Applied Microbiology and Biotechnology*. 1993;39:661-665.
[12] Sar P, D'Souza SF. Biosorptive uranium uptake by a *Pseudomonas* strain: characterization and equilibrium studies. *Journal of Chemical Technology and Biotechnology*. 2001;76:1286-1294.
[13] Hieber M, Gessner MO. Contribution of stream detritivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*. 2002;83:1026-1038.
[14] Gessner MO, Chauvet E. Ergosterol-to-biomass conversion factors for aquatic hyphomycetes. *Applied Environmental Microbiology*. 1993;59:502-507.
[15] Paulo C, Pratas J, Rodrigues N. Rhizofiltration of uranium from contaminated mine water. *Metal Ions in Biology and Medicine*. 2006;9:187-192.
[16] Mahramanlioglu M, Bicer IO, Misirli T, Kilislioglu A. Removal of uranium by the adsorbents produced from coffee residues. *Journal of Radioanalytical and Nuclear Chemistry*. 2007;273:621-624.
[17] Merten D, Kothe E, Büchel G. Studies on microbial heavy metal retention from uranium mine drainage water with special emphasis on rare earth elements. *Mine Water and the Environment*. 2004 ;23:34-43.
[18] Zhang X, Luo S, Iang Q, Zhang H, Li J. Accumulation of uranium at low concentration by the green alga *Scenedesmus obliquus* 34. *Journal of Applied Phycology*. 1997;9:65-71.
[19] Bengtsson L, Johansson B, Hackett TJ, MaHale L, MaHale AP. Studies on the biosorption of uranium by *Talaromyces emersonii CBS 814.70* biomass. *Applied and Microbiological Biotechnology*. 1995;42: 807-811.
[20] Tawfik Z, Abu-Shady M, Haythan M. Uranium uptake by some locally isolated and some reference bacterial species. *Acta Pharmaceutica*. 2005;55:93-105.
[21] Tsuruta T. Cell-associated adsorption of thorium or uranium from aqueous system using various microorganisms. *Water, Air Soil Pollution*. 2004;159:35-47.
[22] Tsezos M. The role of chitin in uranium adsorption by *R. arrhizus*. *Biotechnology and Bioengineering*. 1983;25:2025-2040.
[23] Guibal E, Roussy J, Le Cloirec P. Photochemical reaction of uranium with glucosamine, acetylglucosamine and related polymers: chitin and chitosan. *Water SA*. 1996;22:19-26.
[24] Laitung B, Pretty JL, Chauvet E, Dobson M. Response of aquatic hyphomycete communities to enhanced stream retention in areas impacted by commercial forestry. *Freshwater Biology*. 2002;47:313-323.