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Effect of fungal isolates from different samples upon radionuclide behavior and environmental hazard indices during bioleaching process in Gabal Um Hamd, Um Bogma area, southwestern Sinai, Egypt

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Abstract
Two fungal species were isolated from the studied rock samples and identified morphologically as *Aspergillus hollandicus* and *Penicillium citrinum*. Bioleaching process was applied to W1, W2 and W3. The average concentration activity of ^{238}U, ^{226}Ra, ^{232}Th, and ^{40}K are 5134.03, 5708.64, 189.51, and 1456.8 $BqKg^{-1}$, respectively. Radionuclide's distribution in leach liquor, residual, and fungal adsorption were followed and environmental hazard indices (Ra_{eq}, I_{γ}, H_{ex}, H_{in}, and ECLR) were calculated. From the observed outcomes, the isolated fungal strains have the potential to reduce the harmful effect up to 50% compared to the original. As a result, application of these fungal strains offers a potential strategy for environmental remediation of radionuclides.

Keywords *Aspergillus hollandicus* and *Penicillium citrinum* · Bioleaching process · Radionuclides distribution · Environmental hazard indices

Introduction

Radionuclide contamination has become a part of the surrounding environment in the form of fallout from nuclear weapons, waste from nuclear energy-generating industries, and from medical uses of radioisotopes. Fungi play a significant role in providing ecosystem services in terrestrial ecosystems [1], the importance of organic soil horizons and their microbial communities (fungi and bacteria) highlighted as potential accumulators of nutrient elements and radionuclides within terrestrial ecosystems [2]. According to Steinera et al. [3], fungi are one of the most crucial elements of forest ecosystems because they greatly influence the transit pathways and fate of radionuclides. The immobilization process of radionuclides achieved by saprotrophic fungi, which present in the surrounding environment depends on its ability to increases enormous surface area of hyphae that fitting them for absorbing a large amount of nutrient elements and radionuclides from the environment [4, 5].

One of the main issues regarding ecotoxicology and human health is radionuclides in the environment. Consequently, the use of fungi in bioremediation of radioactively contaminated sites and cleanup of an industrial effluent is one of the most important requirements [6], Although heavy metals are naturally present in the soil, geologic and anthropogenic activities increase the concentration of these elements to amounts that are harmful to both plants and animals. Among there are the mining and smelting of metals, the burning of fossil fuels, the use of pesticides and fertilizers in agriculture, the manufacturing of batteries and other metal products in the industrial sector, and the disposal of sewage sludge [7].

Bioremediation happens spontaneously and is enhanced by providing live substances and fertilizers where the main principle behind biodegradation technology is bioremediation. It means total elimination of hazardous organic

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pollutants into nontoxic, naturally existing, or innocuous substances like carbon dioxide, water, and inorganic substances that are secure for use by people, animals, plants, and aquatic life [8] as well as the degradation of the contaminants is mediated by physical or enzymatic- regulated processes such as bioaccumulation, biosorption, biomineralization, and biotransformation [9]. Qayyum et al. [10] showed that the industrial soil contaminated with heavy metals may be considered a valuable natural source of resistant fungal strains like *Rhizomucor pusillus, Aspergillus flavus, Aspergillus terreus, Aspergillus tubingensis,* and *Neosartorya hiratsukae,* which can be widely used as a bioleaching or bioremediation tool. There are numerous studies that used microorganism like fungi in bioremediation process such as El Dabour et al. [11] were applied *Aspragillus* species on the waste samples that collected from Allouga locality southwestern Sinai, Egypt and demonstrated that *A. lentulus* had a higher bioleaching efficiency of uranium (68%) than *A.niger* (54%). Attia et al. [12] utilizing a natural fungus strain, *Aspergillus nidulans,* as a green technology, the uranium bioleaching efficiency was established at 80% under the ideal conditions of 3 pH, 3% pulp density, 7 days of incubation, and 30 °C incubation temperatures. On the other hand, the microorganism was utilized in the wastewater treatments as biosorption and/or bioaccumulation of contamination through its mycelium, which applied in different fields as pharmaceutical industry, dyes removal, agriculture as pesticides degradation, and metal removal (Th, Ra, U, Cu, Zn, Cd, Pb, Fe, Ni, and Ag) [13] by the mechanism of producing two main types of extracellular chemicals in response to metal stress known as microbial extracellular polymeric substances (EPS) and soluble microbial products (SMP), which affecting the metal binding properties to their active sites [14] as well as the biodegradation of a wide range of organic molecules has been explained by several mechanisms and pathways; for instance, it can occur both with and without oxygen [15].

The natural decay of uranium (^{238}U and ^{235}U) series, thorium (^{232}Th) series, and singly occurring isotopes like potassium (^{40}K) Khandaker et al. [16] contributed that the most of these invisible radiation sources cause environmental pollution.

The aim of this work is to study the relation between the bioleaching process and radionuclides behavior using two fungal isolates; *A.hollandicus* and *P. citrinum*, from the rock samples that collected from Gabal Um Hamd, Sinai, Egypt in addition to calculate their effects on the health hazard indices.

Geological setting

Gabal Um Hamd in southwest Sinai is where the rock samples were collected. Primary and secondary minerals that are allogenic and authigenic make up the research region in Um Hamd, as seen in Fig. 1 radioactive (U, Th), non-radioactive REEs, and base metal-bearing minerals [17]. A lower siltstone-Fe, Mn ore member, a middle siltstone-mudstone-shale member, and an upper dolostone-dolomitic limestone member might all be classified as parts of the Um Bogma

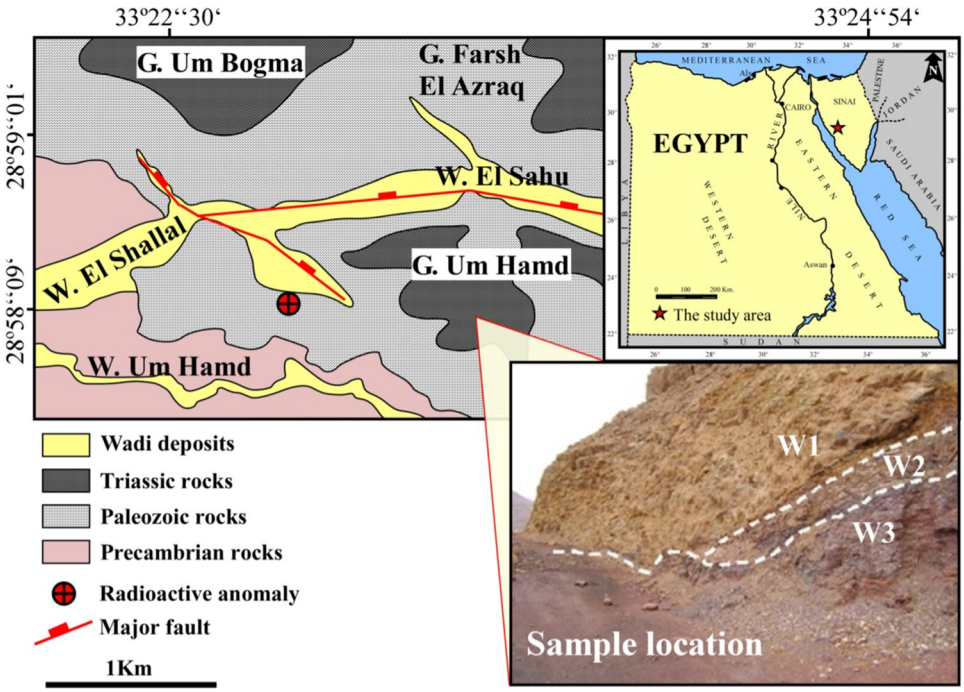

Fig. 1 Geological map showing the location of the studied area and samples location (Modified after Hamza et al., [17])

Formation in Gabal Um Hamd. In the western region of Gabal Um Hamd, where the middle and upper members are the thinnest, otherwise the bottom member, which exhibits an increase in thickness. The three members' rocks vary in their levels of Fe oxide and to a lesser extent of Mn-bearing deposit richness [18]. The Um Bogma Formation's sediments underwent a variety of diagenetic processes, including both iso-chemical and allo-chemical processes. The lower member indicates a maritime habitat that is subtidal to intertidal. The shallow subtidal marine habitat follows the open ocean shelf environment in the middle member. The upper member has shallow subtidal to lower intertidal environments, which are followed by clastic rocks that suggested a regressive sea within a tidal flat [19].

Methodology

Samples preparation

Three geologically gripped radioactive rock samples were collected from Gabal Um Hamd in southwest Sinai. Each sample is a representation of the location where it was gathered. The samples were sealed in sterile polyethylene packets before being crushed, quartered, and ground for analysis. The specification of the studied samples was as follows: i) The first sample (W1) is composed of compact siltstone, which is medium hard and highly feriginous, ii) The second sample (W2) is made up of grey shale, which is fissile ferruginous and gypsiferrous, and iii) The third sample (W3) is made up of sandy dolostone, which is jointed and fractured and is dark grey.

Microbiological studies

The microbial studies upon the collected samples were explained in three steps as follows:

Step1: Fungal isolation

Fine ore powder was spread directly upon the surface of sabouraud dextrose agar plate, this agar media is particularly useful for fungal regrowth [20, 21], in which under septic conditions, sealed and incubated at 30 ºC until the fungal colonies grew [22, 23].

Step2: Purification and identification of isolated fungi

Hyphal tips of each colony were removed and plated upon the surface of sabouraud agar plates. The developed colonies were examined under a microscope to detect contamination. The pure isolated colonies were identified at the regional center for mycology and biotechnology (RCMB) Al-Azhar-University, Cairo, Egypt [23–25]. The microbiological experiments included the fungal strains isolation from the studied rock samples are followed by the bioleaching process.

Step3: Impact of fungi on the test for Bioleaching Ability

Studying the behaviors of isolated fungal strains from studied rock samples (W1, W2 and W3), which contain different grades (high, moderate, and low) of uranium and thorium concentrations**.** Using 100 g of ore from each sample was placed in a1000 mL Erlenmeyer flask then, adding three hundred ml of sabouraud dextrose 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. bioleaching factors investigated included: ore concentration 3%, 1/3 solid liquid ratio, incubation period 7 days at temperature between 30 and 35 °C and pH value equals 3, which applied to the determined optimum conditions.

The radionuclides activity concentrations for the original samples ($BqKg^{-1}$), bioleach liquor (BqL^{-1}), fungal biosorption ($BqKg^{-1}$), and residual ($BqKg^{-1}$) because of bioleaching process phases will be measured using a high-purity germanium (HPGe) detector as a non-destructive examination. The relative efficiency of this detector, which has a resolution of 1.90 keV and a relative efficiency of approximately 50% of the 3″ 3″ NaI (Tl) crystal efficiency, was used to determine the activity concentrations of various radionuclides, including ^{238}U, ^{235}U, ^{234}Th, ^{234} Pa, ^{232}Th, ^{226}Ra, and ^{40}K. The peak/Compton ratio is 69.9 at 1.33 MeV based on ^{60}Co is changing [26].

Radiation hazard indices factors

Absorbed dose rate (DR)

The absorbed dose rate (nGy/h) for the current study has been caculated using Eq. (1)

$$D_R(nGyh) = 0.462 \times A_U + 0.604 \times A_{Th} + 0.0417 \times A_K \quad (1)$$

where A_U, A_{Th}, and A_K are the average specific activities of ^{226}Ra, ^{232}Th, and ^{40}K respectively in Bq kg^{-1}, respectively [27, 28].

Annual effective dose equivalent (AEDE)

Annual effective dose due to radionuclides activities for different studied samples are calculated for outdoor or indoor using Eq. 2, the conversion factor from absorbed dose in air to effective dose must be taken in unit of mSv per year, is calculated from the following Eq. (2) [27, 28].

$$AEDE_{Outdoor}\left(mSvy^{-1}\right) = D_R\left(nGyh^{-1}\right) \times 24h \times 365.24d \times 0.2 \times 0.7\left(SvGy^{-1}\right) \times 10^{-6} \quad (2)$$

where $mSvy^{-1}$ is the effective dose rate, (n Gy h^{-1}) is the dose rate and $SvGy^{-1}$ is the conversion coefficient.

Excess lifetime cancer risk (ELCR)

Excess lifetime cancer risk gives the probability of developing cancer over a lifetime at a given exposure level, considering 70 years as the average duration of life for human beings, as displayed in Eq. (3) [29].

$$ELCR = AEDE * DL * RF \tag{3}$$

where AEDE is the Annual Effective Dose Equivalent, DL is the average Duration of Life (estimated to be 70 years) and RF is the Risk Factor (Sv) represents fatal cancer risk per Sievert. For stochastic effects, ICRP uses RF as 0.05 for the public.

Radium equivalent activity (Ra_{eq})

The combined specific activity of ^{226}Ra, ^{232}Th, and ^{40}K develop a numerical indicator of an external dose to public [23, 30]. Suggested Eq. (4), to calculate radium equivalent and stated the value of 370 Bq kg^{-1} as the maximum allowed dose public.

$$Ra_{eq} = A_{R_a} + 1.43A_{Th} + 0.077A_k \leq 370 \tag{4}$$

where A_{Ra}, A_{Th} and A_K are the specific activities of ^{226}Ra, ^{232}Th and ^{40}K, in Bq Kg^{-1}, respectively.

External hazard (H_{ex})

External hazard index (H_{ex}) are used to measure the external hazard due to the emitted gamma radiation, which has been calculated for the present study using Eq. (5) [27].

$$H_{ex} = \frac{A_{Ra}}{370} + \frac{A_{Th}}{259} + \frac{A_k}{4810} \leq 1 \tag{5}$$

where H_{ex} is the external hazrd index and A_{Ra}, A_{Th} and A_K are the specific activity of ^{226}Ra, ^{232}Th and ^{40}K in Bq Kg^{-1}, respectively.

Internal hazard (H_{in})

The internal radon and its daughter products are quantified by the internal hazard index H_{in}, which is caculated for the studied samples in the present study using Eq. (6):

$$H_{in} = \frac{A_{Ra}}{185} + \frac{A_{Th}}{259} + \frac{A_k}{4810} \leq 1 \tag{6}$$

I-gamma (Level Index)

It is also possible to use an activity utilization index proposed by EC [31] and UNSCEAR [27] due to the raw materials used in building and covering materials, that facilitates the derivation of dose rates in air from different combinations of these three radionuclides has been caculated for the different studied samples using Eq. (7)

$$I\gamma = \frac{C_K}{3000} + \frac{C_U}{300} + \frac{C_{Th}}{30} \tag{7}$$

where C_K, C_U and C_{Th} are the specific activities of ^{226}Ra, ^{232}Th and ^{40}K respectively in $Bqkg^{-1}$ due to building materials. The safty vlue of this index is ≤ 1 [32].

Results and discussion

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

Microorganisms isolation and identification

Two fungal species were isolated from the tested samples. The most dominant fungal strains belong to two species, known as *Asperigillus* and *Penicillium*, identified according to their morphological features, (RCMB), meanwhile, the first one of *Aspergillus* species was identified as *Aspergillus hollandicus* (*A. hollandicus)* and the second of *Penicillium* species as *Penicillium citrinum (P. citrinum)*. They were grown in the presence of sabouraud agar medium at 30–35°C for 7 days.

Gamma counting

The obtained results for the activity concentrations of ^{238}U, ^{226}Ra, ^{235}U, ^{232}Th, and ^{40}K as well as their averages are reported in Table 1 respectively (Bq Kg^{-1}) for the studied rock samples (W1, W2, And W3) by HPGe detector, the radionuclides distribution for different studied samples shown in (Fig. 2). For the sample W1 the activity concentration of ^{238}U, ^{226}Ra, ^{235}U, ^{232}Th and ^{40}K was 2982, 3011,136, 102.6, and 744 Bq Kg^{-1}, respectively. The activity concentration of W2 was 4765.73, 5358.12, 216.09, 198.1, and 1723 Bq Kg^{-1}, respectively. Finally, for the sample W3 the activity concentration was 7654.3, 8756, 347.91, 267, and 1903 Bq Kg^{-1}, respectively. There are respective world-wide concentration limitations of 33, 32, 45, and 420 Bq Kg^{-1} for ^{238}U, ^{226}Ra, ^{232}Th, and ^{40}K [27]. The obtained data demonstrates that it is obvious that Sandy dolostone, jointed and fractured (W3), have the highest concentration of uranium,

Table 1 Radioelements content in the collected soil samples from Gabal Um Hamd area

Sample No	^{238}U Series				^{235}U	^{232}Th Series			^{40}K
	$^{234\,m}$Pa	^{226}Ra	^{214}Bi	^{214}Pb		^{228}Ac	^{212}Bi	^{208}Tl	
W1	2982 ± 46	3011 ± 34	3496 ± 54	3674 ± 57	136 ± 2	109 ± 2	98 ± 2	101 ± 2	744 ± 12
W2	4766 ± 74	5358 ± 83	5839 ± 90	5713 ± 88	216 ± 3	212 ± 3	190 ± 3	193 ± 3	1723 ± 27
W3	7654 ± 118	8757 ± 135	8001 ± 124	7881 ± 122	348 ± 5	301 ± 5	289 ± 5	214 ± 3	1903 ± 29
Average	5134 ± 79	5709 ± 84	5779 ± 89	5756 ± 89	233 ± 4	207 ± 3	192 ± 3	169 ± 3	1456 ± 23

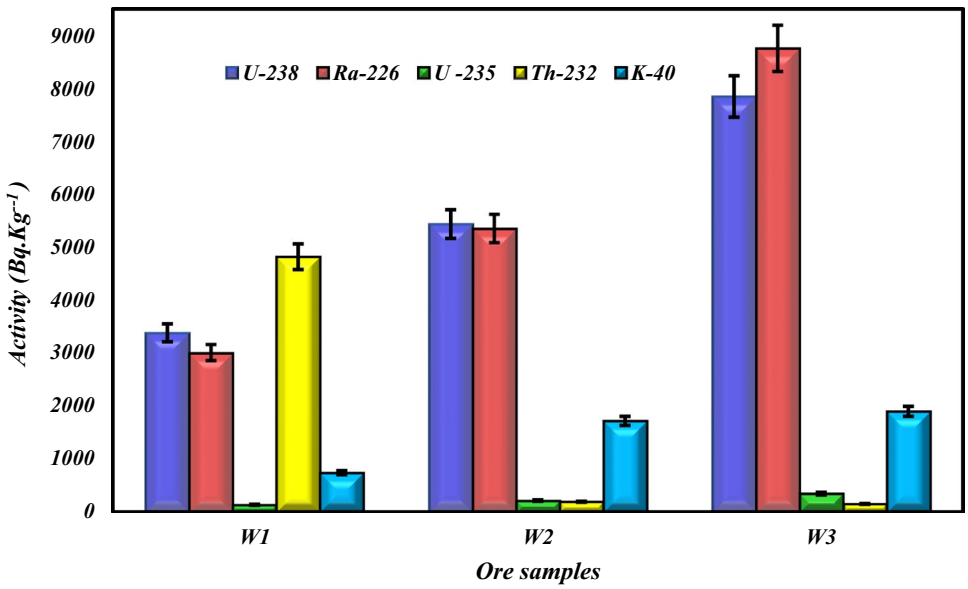

Fig. 2 Radionuclides distribution for different studied samples

thorium, radium, and potassium. The obtained results of ore samples for the activity concentrations showed that all the studied radionuclides in the representative samples have higher values relative to that recorded in the UNSCEAR [27]. As a result, it has higher concentrations of uranium and thorium than sediments described in the IAEA's [33] publication, but lower ^{40}K levels. The Fe oxide cement is also attached to and/or adsorbed with some uranium content.

Fractional dissolution of radionuclides during bioleaching process

Results of specific activities of various isotopes have been examined for each different phase of the bioleaching process: (a) bioleach liquor phase (Bq L^{-1}), (b) fungal biosorption phase (Bq Kg^{-1}) and (c) residual phase (Bq Kg^{-1}) shown in Fig. 3. Leachability percentage for each radionuclide was adjusted varied using *A. hollandicus*, as listed in a group of Tables 2, 3, 4, the investigated measurements with the following characteristics:

(i) Table 2 for sample W1, which recorded radionuclides leachability percent are 69%, 72%, 46%, and 69% for ^{238}U, ^{226}R, ^{232}Th and ^{235}U, respectively. The calculated activity ratio of ^{238}U/^{235}U exhibited within the natural value between 20.00 and 21.92, which reflects a slight deviation from the natural ratio (21.7). Activity ratio of ^{226}Ra/^{238}U recorded 1.009, 0.92, 1.07, and 1.009 for original sample, residual, bioleach liquor, and fungal biosorption, respectively. It is clear from the activity ratio that the calculated values come close to the unity that attained to secular equilibrium while ratio of ^{238}U/^{232}Th ranged from 16.01 to 60.55 showed a deviation from the natural (magmatic) ratio 1:3 in the direction of U-migration-in [11].

(ii) The radionuclides leachability percent for sample W2 are recorded 49%, 53%, 28%, and 48% for ^{238}U, ^{226}R, ^{232}Th and ^{235}U, respectively are shown in Table 3, as well as the calculated ratio of ^{238}U /^{235}U exhibited slightly higher values than the natural value (21.7) between 22.05 and 24.01. the ^{226}Ra/^{238}U ratio recorded 1.12, 1.05, 1.18, and 1.51 for original sample, residual, bioleach liquor, and fungal biosorption, respectively, while the ratio of ^{238}U /^{232}Th ranged from 15.26 to 51.58.

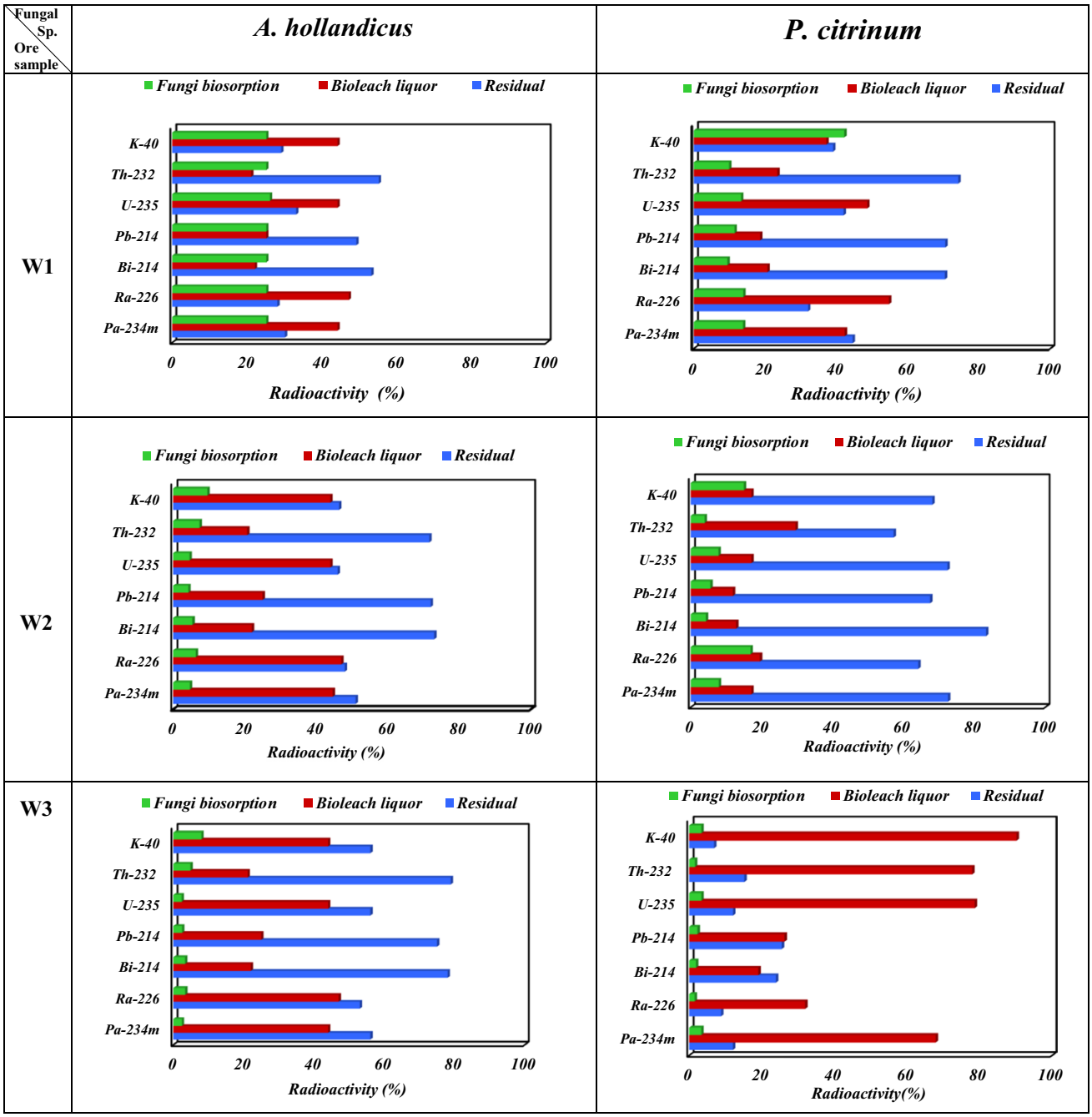

Fig. 3 Radionuclides percentage of bioleaching process in different phases by *A. hollandicus* and *P. citrinum* upon studied samples (W1, W2, and W3)

(iii) The sample W3 recorded radionuclides leachability percent are 46%, 50%, 25% and 46% for ^{238}U, ^{226}R, ^{232}Th and ^{235}U respectively as in Table 4. The calculated ratio of ^{238}U/^{235}U exhibited within the natural value (21.7) between 22.00 and 22.59. The ^{226}Ra/^{238}U ratio recorded 1.14, 1.08, 1.22, and 1.59 for original sample, residual, bioleach liquor, and fungal biosorption, respectively, while the ratio of ^{238}U /^{232}Th ranged from 14.21 to 59.60.

On the other hand, the calculated leachability is varied for each radionuclide using *P. citrinum* as follows:

Table 2 Radionuclides distribution of original and bioleaching different phases by *A. hollandicus* upon sample W1 including leachability percent

		Original (Bq Kg^{-1})	Residual (Bq Kg^{-1})	Bioleach liquor (Bq L^{-1})	Fungi biosorption (Bq Kg^{-1})	Total except original	*(%)
^{238}U Series	$^{234\ mPa}$	2982 ± 46	918 ± 14	1312 ± 20	758 ± 12	2988	69
	^{226}Ra	3011 ± 34	845 ± 13	1415 ± 22	765 ± 12	3025	72
	^{214}Bi	3496 ± 54	1855 ± 29	769 ± 12	888 ± 14	3512	47
	^{214}Pb	3674 ± 57	1822 ± 28	919 ± 14	934 ± 14	3675	50
^{235}U		136 ± 2	46 ± 1	60 ± 1	35 ± 0.5	141	69
^{232}Th Series	^{228}Ac	109 ± 2	61 ± 1	24 ± 0.4	28 ± 0.4	113	47
	^{212}Bi	98 ± 2	56 ± 1	21 ± 0.3	25 ± 0.4	102	46
	^{208}Tl	101 ± 2	55 ± 1	20 ± 0.3	26 ± 0.4	101	45
Average		103 ± 2	57 ± 1	22 ± 0.3	26 ± 0.4	105	46
^{40}K		744 ± 12	218 ± 3	327 ± 5	189 ± 3	734	69
$^{226}_{Ra/}{}^{238}_{U}$		1.009 ± 0.026	0.92 ± 0.330	1.07 ± 0.028	1.009 ± 0.031		
$^{238}_{U/}{}^{235}_{U}$		21.92 ± 0.001	20 .00 ± 0.018	21.86 ± 0.001	21.65 ± 0.001		
$^{238}_{U/}{}^{232}_{Th}$		28.95 ± 0.001	16.01 ± 0.002	60.55 ± 0.0003	28.78 ± 0.001		

*(%): Leachability at optimum conditions (30 °C, 7 days, 1/3 and pH3)

Table 3 Radionuclides distribution of original and bioleaching different phases by *A. hollandicus* upon sample W2 including leachability percent

		Specific activity					
		Original (Bq Kg^{-1})	Residual (Bq Kg^{-1})	Bioleach liquor (Bq L^{-1})	Fungi biosorption (Bq Kg^{-1})	Total except original	*(%)
^{238}U Series	$^{234\ mPa}$	4766 ± 74	2426 ± 37	2126 ± 87	222 ± 9	4774	49
	^{226}Ra	5358 ± 83	2552 ± 39	2518 ± 103	337 ± 14	5407	53
	^{214}Bi	5839 ± 90	4300 ± 66	1284 ± 52	312 ± 13	5896	27
	^{214}Pb	5713 ± 88	4143 ± 64	1428 ± 58	241 ± 10	5812	29
^{235}U		216 ± 3	101 ± 2	95 ± 4	10 ± 0.4	206	48
^{232}Th Series	^{228}Ac	212 ± 3	153 ± 2	46 ± 2	16 ± 0.6	215	29
	^{212}Bi	199 ± 3	142 ± 2	39 ± 2	14 ± 0.6	195	27
	^{208}Tl	193 ± 3	147 ± 2	38 ± 2	14 ± 0.6	199	26
Average		198 ± 3	142 ± 2	41 ± 2	15 ± 0.6	198	28
^{40}K		1723 ± 27	799 ± 12	757 ± 31	165 ± 7	1721	53
$^{226}_{Ra/}{}^{238}_{U}$		1.12 ± 0.027	1.05 ± 0.029	1.18 ± 0.069	1.51 ± 0.054		
$^{238}_{U/}{}^{235}_{U}$		22.05 ± 0.001	24.01 ± 0.001	22.37 ± 0.004	22.5 ± 0.004		
$^{238}_{U/}{}^{232}_{Th}$		24.05 ± 0.001	17.09 ± 0.002	51.85 ± 0.002	15.26 ± 0.005		

*(%): Leachability at optimum conditions (30 °C, 7 days, 1/3 and pH3)

(i) Table 5 shown the sample W1, which recorded radionuclides leachability percent are 56%, 68%, 33%, and 55% for ^{238}U, ^{226}R, ^{232}Th, and ^{235}U, respectively. The calculated ratio of ^{238}U /^{235}U exhibited within the natural value (21.7) between 20.07 and 22.83. The ^{226}Ra/^{238}U ratio recorded 1.01, 0.72, 1.30, and 1.01 for original sample, residual, bioleach liquor, and fungal biosorption, respectively. The ratio of ^{238}U/^{232}Th ranged from 17.43 to 52.48.

(ii) The sample W2 recorded that the radionuclides leachability percentage are 24%, 36%, 33%, and 24% for ^{238}U, ^{226}R, ^{232}Th, and ^{235}U, respectively (Table 6). As well as the calculated ratio of ^{238}U /^{235}U exhibited between 22.05 and 22.33. The ^{226}Ra/^{238}U ratio recorded 1.12, 0.99, 1.28, and 2.39 for original sample, residual, bioleach liquor, and fungal biosorption, respectively. the ratio of ^{238}U/^{232}Th ranged from 13.90 to 51.01. Finally,

Table 4 Radionuclides distribution of original and bioleaching different phases by *A. hollandicus* upon sample W3 including leachability percentage

		Specific activity					
		Original (Bq Kg^{-1})	Residual (Bq Kg^{-1})	Bioleach liquor (Bq L^{-1})	Fungi biosorption (Bq Kg^{-1})	Total except original	*(%)
^{238}U series	$^{234\ mPa}$	118 ± 7654	66 ± 4287	3368 ± 52	185 ± 3	7840	46
	^{226}Ra	135 ± 8757	72 ± 4641	4116 ± 64	295 ± 5	9052	50
	^{214}Bi	124 ± 8001	96 ± 6241	1760 ± 27	267 ± 4	8268	25
	^{214}Pb	122 ± 7881	91 ± 5911	1970 ± 30	19 7 ± 3	8078	27
^{235}U		348 ± 5	3 ± 195	153 ± 2	8 ± 0.1	356	46
^{232}Th series	^{228}Ac	5 ± 301	4 ± 235	66 ± 1	14 ± 0.2	315	26
	^{212}Bi	5 ± 28	4 ± 228	61 ± 1	13 ± 0.2	301	25
	^{208}Tl	3 ± 214	3 ± 171	43 ± 1	12 ± 0.2	226	25
Average		268 ± 4	4 ± 211	57 ± 1	13 ± 2	281	25
^{40}K		1903 ± 29	16 ± 1066	838 ± 13	152 ± 2	2055	51
$^{226}_{Ra/}{}^{238}_{U}$		1.14 ± 0.026	1.08 ± 0.028	1.22 ± 0.025	1.59 ± 0.02		
$^{238}_{U/}{}^{235}_{U}$		22 ± 0.0013	22.01 ± 0.001	22 ± 0.0012	22.59 ± 0.001		
$^{238}_{U/}{}^{232}_{Th}$		28.59 ± 0.001	20.29 ± 0.002	59.6 ± 0.001	14.21 ± 0.002		

*(%): Leachability at optimum conditions (30 °C, 7 days, 1/3 and pH3)

Table 5 Radionuclides distribution of original and bioleaching different phases by *P. citrinum* upon sample W1 including leachability percent

		Specific activity					
		Original (Bq Kg^{-1})	Residual (Bq Kg^{-1})	Bioleach liquor (Bq L^{-1})	Fungi biosorption (Bq Kg^{-1})	Total except original	*(%)
^{238}U Series	$^{234\ m}$ Pa	2982 ± 46	1325 ± 20	1260 ± 19	411 ± 19	2995	56
	^{226}Ra	3011 ± 34	963 ± 15	1644 ± 25	417 ± 6	3024	68
	^{214}Bi	3496 ± 54	2456 ± 38	720 ± 11	329 ± 5	3505	30
	^{214}Pb	3674 ± 57	2585 ± 40	682 ± 11	419 ± 15	3685	29
^{235}U		136 ± 2	57 ± 1	66 ± 1	18 ± 0.3	141	55
^{232}Th Series	^{228}Ac	109 ± 2	83 ± 1	25 ± 0.4	12.38 ± 0.2	120	34
	^{212}Bi	98 ± 2	70 ± 1	23 ± 0.3	11 ± 0.2	103	34
	^{208}Tl	101 ± 2	75 ± 1	24 ± 1	7 ± 0.1	106	30
Average		103 ± 2	76 ± 1	24 ± 0.4	10.15 ± 0.1	24	33
^{40}K		744 ± 12	290 ± 5	276 ± 14	313 ± 5	879.38	79
$^{226}_{Ra/}{}^{238}_{U}$		1.009 ± 0.026	0.72 ± 0.04	1.30 ± 0.023	1.01 ± 0.06		
$^{238}_{U/}{}^{235}_{U}$		21.92 ± 0.001	20.07 ± 0.001	21.99 ± 0.002	22.83 ± 0.003		
$^{238}_{U/}{}^{232}_{Th}$		28.95 ± 0.001	17.43 ± 0.002	52.48 ± 0.001	40.49 ± 0.001		

*(%): Leachability at optimum conditions (30 °C, 7 days, 1/3 and pH3)

(iii) Table 7 demonstrated a sample W3 with the recorded radionuclides leachability percent that achieved 71%, 72%, 16%, and 71% for ^{238}U, ^{226}R, ^{232}Th and ^{235}U respectively. The calculated ratio of ^{238}U/^{235}U exhibited between 21.91 and 22.00. The ^{226}Ra/^{238}U ratio recorded 1.14, 1.79, 1.15, and1.08 for original sample, residual, bioleach liquor, and fungal biosorption, respectively. The ratio of ^{238}U/^{232}Th ranged from 22.59 to 60.58.

From the obtained results of the radionuclides activity concentrations of the studied samples and different bioleaching phases, numerous useful ratios as ^{238}U/^{235}U, ^{226}Ra/^{238}U, and ^{238}U/^{232}Th were calculated. ^{238}U/^{235}U ratios demonstrated that the predominant normality was achieved for each phase around the natural value (21.7) deducing the same behavior of the two radioactive elements [34–36]. Also, the ^{226}Ra/^{238}U ratio represents a meaningful ratio in the geological systems whereas its normal value equals unity, if any

Table 6 Radionuclides distribution of original and bioleaching different phases by *P. citrinum* upon sample W2 including leachability percent

		Specific activity					
		Original (Bq Kg^{-1})	Residual (Bq Kg^{-1})	Bioleach liquor (Bq L^{-1})	Fungi biosorption (Bq Kg^{-1})	Total except original	*(%)
^{238}U Series	$^{234\ mPa}$	4766 ± 74	3449 ± 53	812 ± 16	376 ± 6	4637	24
	^{226}Ra	5358 ± 83	3426 ± 53	1042 ± 12	899 ± 14	5367	36
	^{214}Bi	5839 ± 90	4856 ± 75	745 ± 10	249 ± 4	5849	17
	^{214}Pb	5713 ± 88	3843 ± 59	676 ± 1	314 ± 5	4833	17
^{235}U		216 ± 3	156 ± 2	37 ± 1	17 ± 0.3	210	24
^{232}Th Series	^{228}Ac	212 ± 3	1274 ± 2	66 ± 1	9 ± 0.1	202	35
	^{212}Bi	190 ± 3	108 ± 2	65 ± 1	7 ± 0.1	180	37
	^{208}Tl	193 ± 3	139 ± 2	45 ± 1	7 ± 0.1	191	26
Average		198 ± 3	113 ± 2	58 ± 5	8 ± 0.1	179	33
^{40}K		1723 ± 27	1170 ± 18	293 ± 5	257 ± 4	1722	31
^{226}Ra/^{238}U		1.12 ± 0.027	0.99 ± 0.03	1.28 ± 0.024	2.39 ± 0.013		
^{238}U/^{235}U		22.05 ± 0.001	22.10 ± 0.001	22.05 ± 0.021	22.33 ± 0.002		
^{238}U/^{232}Th		24.05 ± 0.001	30.52 ± 0.001	13.90 ± 0.008	51.01 ± 0.002		

*(%): Leachability at optimum conditions (30 °C, 7 days, 1/3 and pH3)

Table 7 Radionuclides distribution of original and bioleaching different phases by *P. citrinum* upon sample W3 including leachability percent

		Specific activity					
		Original (Bq Kg^{-1})	Residual (Bq Kg^{-1})	Bioleach liquor (Bq L^{-1})	Fungi biosorption (Bq Kg^{-1})	Total except original	*(%)
^{238}U Series	$^{234\ m}$ Pa	7654 ± 118	919 ± 38	5196 ± 80	254 ± 4	6368	71
	^{226}Ra	8757 ± 135	1646 ± 67	5986 ± 92	274 ± 4	7907	71
	^{214}Bi	8001 ± 124	1912 ± 78	1523 ± 24	1489 ± 2	3584	20
	^{214}Pb	7881 ± 122	2009 ± 82	2067 ± 32	178 ± 3	4254	28
^{235}U		348 ± 5	42 ± 2	237 ± 3.65	12 ± 2	327	71
^{232}Th Series	^{228}Ac	301 ± 5	47 ± 2	240 ± 3.70	5 ± 0.1	292	81
	^{212}Bi	289 ± 5	39 ± 2	226 ± 3.48	4 ± 0.1	269	79
	^{208}Tl	214 ± 3	36 ± 2	160 ± 2.46	4 ± 0.1	200	76
Average		268 ± 4	41 ± 2	209 ± 3	4 ± 0.1	254	79
^{40}K		1903 ± 29	130 ± 5	1717 ± 26.49	63 ± 27	1910	93
^{226}Ra/^{238}U		1.14 ± 0.026	1.79 ± 0.045	1.15 ± 0.026	1.08 ± 0.028		
^{238}U/^{235}U		22.00 ± 0.001	21.92 ± 0.004	21.92 ± 0.001	21.91 ± 0.008		
^{238}U/^{232}Th		28.59 ± 0.001	22.59 ± 0.004	24.90 ± 0.001	60.58 ± 0.001		

*(%): Leachability at optimum conditions (30 °C, 7 days, 1/3 and pH3)

deviation is recorded whether greater or lesser pointing to deviation from the secular equilibrium, may be that due to ^{226}Ra in excess of equilibrium with ^{238}U enhanced leaching of ^{224}U according to the occurring of alpha decay- recoil phenomenon causing nucleus damage to the crystal structure of the ore [37]. Finally, the obtained ratio ^{238}U/^{232}Th are compared with the ratio in the upper crust is typically 0.8 [38] indicating a relatively enhancement of ^{238}U to ^{232}Th. Therefore, the slight enhancement of uranium relative to thorium in some samples may be due to dissolution and re-adsorption of uranium.

Radiological hazards (EhzIs)

From Table 8 The absorbed dose rate was ranged from (2666.83–4286 n Gy/h), and the annual effective dose equivalent calculated for outdoor from 1.82 to 5.25 mSv/y. Since the results of absorbed dose rate (D_R) and the annual effective dose rate (AEDE) are higher than the international

Table 8 Radiological hazard indices (EHzIs) of the original samples

Sample No	Dose rate (nGy/h)	AEDE (mSv/y)	Iγ	H_{in}	H_{ex}	Ra_{eq}	ELCR × 10^{-3}
W1	1484	2	22	17	9	3728	6
W2	2667	3	39	30	16	6966	11
W3	4286	5	62	49	25	10,603	18

recommended values, which are 60 n Gy/h and 1 mSv/y, respectively [27, 28].

The Level index I-gamma calculated values were ranged from 21.59 to 62.3, which found to be higher than the world level index I-gamma ($I_\gamma \leq 6$) [27]. The obtained values for calculated H_{ex} ranges from 8.96 to 25.09 are higher than the world external hazard (unity) [27], likewise, the values of internal hazard index (H_{in}) ranges from 16.44 to 25.09 are higher than the world internal hazard unity, as well as the radium equivalent activity (Ra_{eq}) ranged from (3728.86 Bq Kg^{-1}) to (10,603.12 Bq Kg^{-1}), which is higher than the recommended maximum value (370 Bq Kg^{-1}) [26]. The value of the excess life cancer risk (ELCR) varied from (6.37×10^{-3} to 18.39×10^{-3}), which is greater than the standard ELCR that is given as ($0.29 \times 10^{-3)}$ [29]. From the previous results can be concluded that *A. hollandicus* decreased the AEDE value in the three studied samples as in Table 9. For sample W2 and W3 the value of D_R, AEDE, I_γ, H_{ex}, H_{in}, Ra_{eq}, and ECLR in residual phase was higher than that in the leach liquor represented low leachability percentage. On the other hand, the calculated hazard indices using *P. citrinum*, exhibited an effective role in decreasing the D_R, AEDE, Iγ, H_{ex}, H_{in}, Ra_{eq} in the residual phase R1 as (497.84 n Gy/h), (0.61 mSv/y), (7.28), (5.49), (2.92), (1278.9BqKg^{-1}), and (2.13×10^{-3}), respectively. and R3 as (957.76 n Gy/h), (1.17 mSv/y), (14.19), (9.95), (5.60), (3265.53 BqKg^{-1}), and (4.11×10^{-3}). As well as in fungal biosorption phase whereas it has high values of D_R, AEDE, H_{ex}, H_{in}, Ra_{eq}, also in B2 as (461.11nGy/h), (0.56 mSv/y), (5.11), (2.71), and (1179.83BqKg^{-1}), respectively. These results agreed with Maity et al. [39], showed that high doses of gamma radiation cause dose-dependent inhibitory effects in the fungi that make soil fungal community structure appeared to shift toward species that may be more radiation resistant, and melanin-containing fungi ascended to dominate the soil fungal communities with increase in radionuclide pollution [40].

Furthermore, the obtained results of the bioleaching process, the effect of both two studied fungi upon the environmental hazard indices (EhzIs) were observed (Fig. 4). Firstly, the effect of *A. hollandicus* was achieved a significantly decreasing of the EhzIs percentage in different bioleaching phases. (i) The leach liquor (L1, L2, and L3) approached to 46% as a reduced value comparing with the original samples hazard indices and (ii) the fungal phase similarly recorded decreasing of their percentage as 25% (B1), 6% (B2), and 4% (B3). Consequently, concluded that the bioleaching process can clean up (remediate) the original samples by 70%, 51%, and 49% for W1, W2, and W3, respectively. Likewise, the remaining percentage values of EHzIs in the residual achieved 29%, 49%, and 54% for W1, W2, and W3, respectively. Secondly, in the same manner, the effect of *P. citrinum,* was significantly decreasing the environmental hazard indices (EhzIs) percentage in different bioleaching phases. The leach liquor (L1, L2, and L3) recorded 52%, 19%, and 65% as a reduced value comparing with ore hazard indices and the fungal phase recorded decreasing percentage as 14% (B1), 17% (B2), and 3% (B3) comparing with the original samples. As result, bioleaching process can clean up (remediate) the original samples by 83%, 50%, and 72% for W1, W2, and W3, respectively. Therefore, the remaining percentage values of EhzIs in the residual achieved 33, 63, 22% for W1, W2, andW3, respectively.

Conclusion

Bioremediation will be required as a highly effective way of pollutant removal and nutrient recycling in an ecosystem. Fungi, as decomposers have the unusual capacity to metabolize various organic and inorganic contaminants, which utilizing them as energy and carbon sources as well as reducing them to non-hazardous concentrations. Radiometric studies clearly show high values of uranium contents in the rock samples and bioleaching process phases (leach liquor, residual, and fungal adsorption) relative to those international recorded values. The EHzIs were calculated for the original samples cleared that (D_R), (AEDE) and (Ra_{eq}) were more than the international recommended limits. The effect of the two fungal isolates upon radionuclides were followed in the three phases of bioleaching process showed that *A. hollandicus* exhibited a high leaching efficiency with low uranium

Table 9 Specific activity concentration and radiological hazard indices of different bioleaching phases by *A. hollandicus* and *P. citrinum*

Fungi sp.	Sample No	^{226}Ra (BqKg^{-1})	U (BqKg^{-1})	Th (BqKg^{-1})	K (BqKg^{-1})	Dose rate (nGy/h)	AEDE (mSv/y)	Iγ	H_{in}	H_{ex}	Ra_{eq}	ELCR $10^{-3}\times$
A. hollandicus	L1	1415	1312	21	327	680.05	0.83	9.86	7.78	3.97	1696.82	2.91
	L2	2518	2126	41	757	1219.66	1.49	17.70	13.90	7.12	3159.52	5.24
	L3	4115	3367	56	837	1969.87	2.42	28.55	22.61	11.51	4839.57	8.46
	R1	845	918	57	218	433.93	0.53	6.35	4.80	2.54	1094.37	1.86
	R2	2552	2426	141	799	1297.56	1.59	18.96	14.43	7.61	3368.86	5.57
	R3	4641	4286	211	1065	2316.08	2.84	33.76	26.02	13.58	5762.78	9.94
	B1	756	758	26	218	374.08	0.45	–	4.21	2.18	961.04	–
	B2	337	222	14	165	171.04	0.21	–	1.90	0.99	484.07	–
	B3	294	185	13	152	150.02	0.18	–	1.67	0.88	429.63	–
P. citrinum	L1	1644	1259	10	313	778.62	0.95	11.26	8.98	4.55	1899.31	3.34
	L2	1041	812	8	293	497.99	0.61	7.22	5.72	2.91	1278.05	2.13
	L3	5986	5195	41	130	2795.73	3.43	40.40	32.52	16.36	6144.73	12.00
	R1	963	1325	68	284	497.84	0.61	7.28	5.49	2.92	1278.92	2.13
	R2	3426	3448	113	1169	1699.86	2.08	24.75	19.14	9.93	4487.72	7.29
	R3	1646	918	208	1717	957.76	1.17	14.19	9.95	5.60	3265.53	4.11
	B1	417	411	24	145	213.21	0.26	–	2.36	1.25	562.97	–
	B2	899	375	58	257	461.11	0.56	–	5.11	2.71	1179.83	–
	B3	274	254	4	63	131.63	0.16	–	1.51	0.76	328.23	–

*L1, L2, L3 for Bioleach liquor (Bq. L^{-1}) of sample W1, W2, W3 respectively

*R1, R2, R3 for Residual (Bq. Kg-1) of sample W1, W2, W3 respectively

*B1, B2, B3 for Fungi biosorption (Bq. Kg^{-1}) of sample W1, W2, W3 respectively

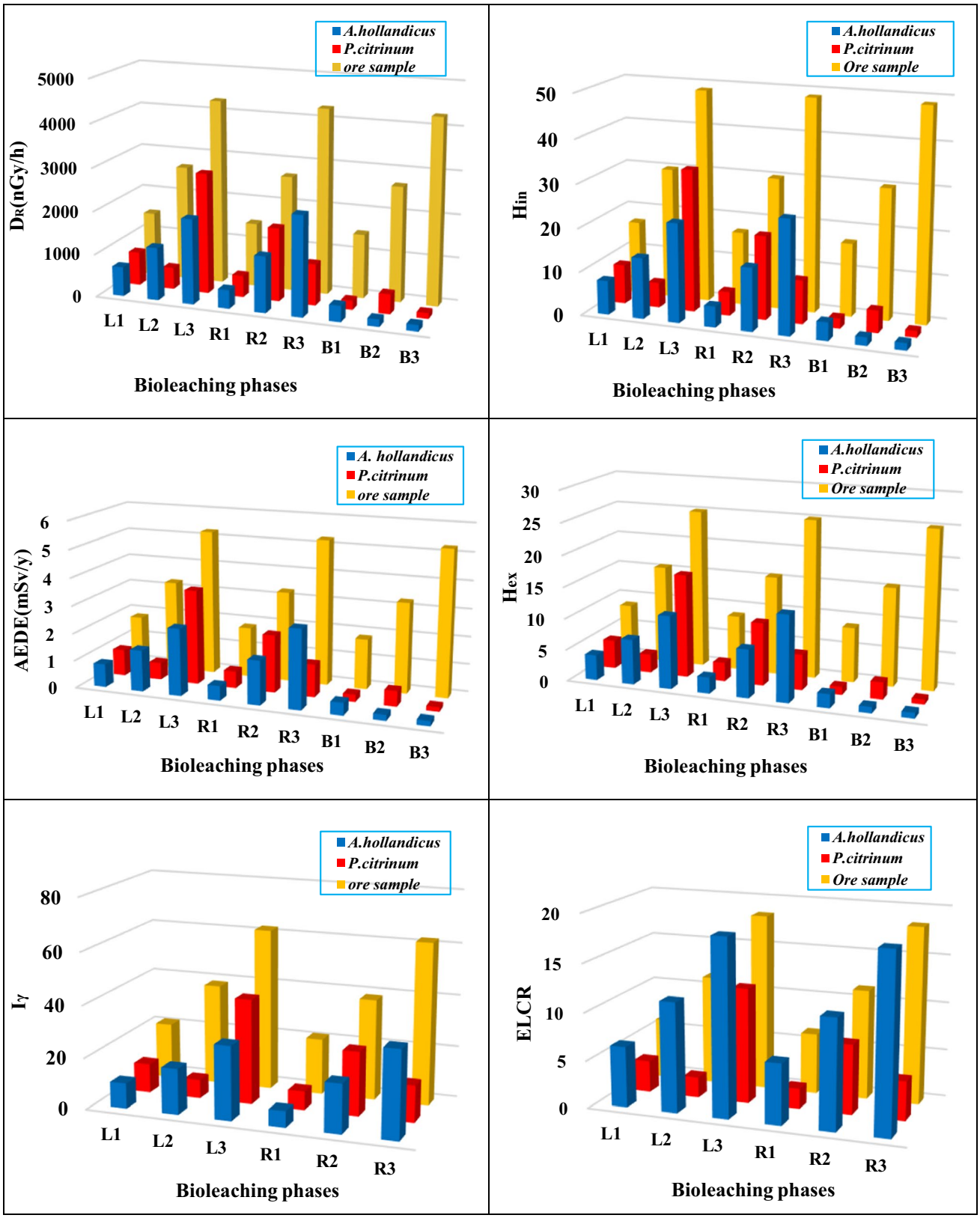

Fig. 4 Environmental hazard indices (EHzIs) values in different bioleaching phases by *A. hollandicus* and *P. Citrinum* of the studied samples (W1, W2, W3)

contents samples as well as achieved a low leaching efficiency with high uranium content samples. Since *P. citrinum* can withstand the high uranium content samples, also it exhibited a high leachability of uranium whereas displays a better result to decrease the EHzIs in the studied samples. Consequently, can utilize the *P. citrinum* to remediate the environment by applying bioleaching process several times to reach the safe international limit values of EHzIs. The hazard indices in leach liquor phase are directly proportional to the leachability percentage otherwise in the case of residual phase are inversible. Therefore, it is recommended to use a combined leaching experiment and hazard index calculation as an indicator of fungal leaching efficiency.

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