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Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by *Phanerochete Chrysosporium* and the Effect of Gamma Radiation

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Abstract

Phanerochete Chrysosporium is a strain of White Rot Fungi (WRF) in the present study was screened for its ability to degrade Polycyclic Aromatic Hydrocarbons (PAHs) with low- (naphthene, acenaphthene, anthracene, and phenanthrene) and high- (Pyrene, fluoranthene) molecular weight and used those as a sole carbon and energy source. The abilities of the fungal strain to grow on Basal Salt Media (BSM) amended with 5, 10, 50, 100, 150 mg/L of six examined PAHs have been determined by measuring their growth and secretion of extracellular protein after zero, 3, 7, 14, 21, and 28 days spectrophotometrically. The degradation of six examined PAHs, have been determined quantitatively by High Performance Liquid Chromatography (HPLC).

Phanerochete Chrysosporium was able to grow and utilize all the tested PAHs as sole carbon and energy source. *P. Chrysosporium* degraded 87.77%, 61.27%, 29.23%, 57.17%, 86.38% and 51.90% of 150 mg/L of fluoranthene, phenanthrene, acenaphthen, naphthene, anthracene and Pyrene respectively. Exposure to elevated doses of gamma radiation reduced the viable count of *P. Chrysosporium* gradually. Doses 6.0 and 7.0 KGY reduced the viable count by 7.70 and 10.44 Log cycles respectively. Exposure of *P. Chrysosporium* to low doses (0. 5 and 1.0 KGY) induced *P. Chrysosporium* growth on Flu., Phen., Acen., Naph., Anth. And Pyr. more than parent strain by (2.4, 2.8) , (2.6 ,2.0) , (3.8 ,2.9), (1.5 ,1.5), (2.8 , 3.6) and (2.2 , 1.2) times respectively, while dose 1.5 KGY induced protein secretion by *P. Chrysosporium* than its parent strain (Non- irradiated).

Keywords: Degradation percentage; Gamma irradiation; Growth; Polycyclic aromatic hydrocarbons (PAHs); White-rot fungi (WRF)

Introduction

A group of several hundred of organic compound that are chemically related and environmentally persistent and known as Polycyclic Aromatic Hydrocarbons (PAHs) [1-4].PAHs pass varied toxicity, genotoxic, mutagenic and/or carcinogenic properties[5,6]. PAHs enter the environment via varied ways, which include natural, human activity sources and found as mixture involving two or more of these chemical compounds. The disposal of petroleum products and forest fires represent the natural sources. While thermal decomposition and industrial processing represent the human activity sources [7]. The thermal decomposition occurs via pyrolysis and pyrosynthesis of organic molecules. Otherwise, industrial processing caused by incineration or combustion such as wood combustion, fuel combustion (gas, oil, cool) and waste incineration resulted in PAHs release [8-12].

PAHs found in polluted atmosphere transferred to human and plant. However, it can be filtered from soil into water. Accidental spills and industrial effluent pollute the water (Sihage et al., 2014) [13] PAHs can be found in meats, fishes, vegetables and fruits [14]. Exposure to high levels of PAHs cause diarrhea, nausea, vomiting and eye irritation [15]. Chronic Human health effects include kidney and liver damage, lung function abnormalities, decrease immune function, asthma, skin inflammation, cataracts and breathing problems could be from long exposure to PAHs [16,17].

Ability of microorganisms to transform, degrade or detoxify organic compounds by converting harmful compounds to non - toxic compound is called biodegrading. Degradation of Permanent Organic Pollutants (POPs) like PAHs, PCBs, PPCBs and Pesticides is an important research activity [18-22]. Biodegradation, biotransformation, biostimulation and bio augmentation process have been carried out via various microorganisms by breaking down the complex compounds via potential enzymes [23,24].

Fungi can degrade PAHs[25-28].White -Rot Fungi (WRF) can degrade Low Molecular Weight (LMW) as well as High Molecular Weight (HMW) PAHs with four or more fused benzene rings. This issue represent a great interest for the research groups. WRF are the most important fungi that can eliminate lignin, cellulose and hemicellulose at nearly equal rates [29-33].

WRF produce Laccase Enzyme (Lac) (Benzene diol: oxygen oxidoreductase), a multi-copper; Lignin-Peroxidase (Lip) and Manganese Peroxidase (MnP) which are lignin degrading enzymes. The ionization potential is the main aspect for removal of PAHs by those low substrate specify extracellular enzymes [5,30,34-39].

Phanerochaete chrysosporium; *Ganoderma* strains; *Pleurotus ostratus*; *Pleurotus sajor-caju*; *Pleurotus plumonarius*, *Pseudotrametes gibbosa; Bjerkamdera adusta* and *Pycnoporus* sp. are the most commonly species used for degradation of PAHs as a sole carbon and energy source [33,40-44].

The aim of the present study is to determine the ability of *Phanerochaete chrysosporium* as a White Rot Fungus (WRF) to degrade six different PAHs with low and High Molecular Weight (LMW-PAHs and HMW-PAHs) as a soul carbon and energy source. Also studying the role of gamma radiation in inducing mutants with hyper-activities.

Materials and Methods

Microorganism

The White-Rot Fungus (WRF) used in the present study was *Phanerocheate chrysosporium* ATCC 32629 purchased from Microbiological Resources Center (MIRCEN), Faculty of Agriculture, Ain-Shams University, Cairo, Egypt.

Maintenance of WRF

Phanerocheate chrysosporium ATCC 32629 was maintained on 2% (w/v) malt agar (Oxoid, 1982) [45] slants and kept at 4°C until need. *P. chrysosporium* can be activated by sub- culturing on Malt Extract (ME) agar plates and Potato Dextrose Agar (PDA) plates (Oxoid, 1982) [45] for 14 day at 28±2°C.

Chemicals used

Six Polycyclic Aromatic Hydrocarbons (PAHs) with a low and high molecular weight were used in the present study. Naphthene (99٪ purity), was purchased from El- Gomhoria Company, Cairo, Egypt. While Pyrene, phenantherene, fluoranthene, Anthracene and acenaphthene (all 99٪ purity), purchased from Sigma-Aldrich, USA. All PAHs were dissolved in chloroform (Stock solution).

Degradation Culture media

The liquid Basal Salt Medium (BSM) [41] with modification [18-21,46] was used for biodegradation of PAHs by *Phanerocheate chrysosporium*. The BSM was amended by five concentrations (5, 10, 50, 100, 150 mgL-1) of each PAHs as sole carbon and energy source.

Preparation of inoculum

Five agar plugs (6 mm diameter) were cut out from the margin of a 14 days actively growing fungal culture of *Phanerocheate chrysosporium* ATCC 32629 on Malt Extract (ME) agar plates. These plugs were used as inoculum.

Biodegradation of PAHs by *Phanerocheate Chrysosporium*

The biodegradation experiment was performed in 500ml conical flasks containing 150ml of BSM amended by five concentrations (5, 10, 50, 100, 150 mgL^{-1}) of six examined PAHs (naphthene, anthracene, phenantherene, Pyrene, fluoranthene and acenaphthen). Every flask was inoculated by 5 agar plugs (6mm) of the actively growing fungal strain as inoculum. Three replicates were used for each concentration of each PAH compound. The inoculated BSM as mention above were incubated at 28 ±2°C with continuous shaking at 150 rpm for zero, 3, 7, 14, 21 and 28 days.

Growth and protein determination

After each incubation period, mycelia of *Phanerocheate chrysosporium*were removed by filtration from the cultures through Whitman filter paper No.1. The fungal mycelia (biomass) were used for the quantitative determination of the fungal growth. The fungal filtrates were used for protein determination according to Lowery et al., (1951) [47] as well as for determination of degradation rates by HPLC.

HPLC Analysis

Liquid / Liquid (1:1 v/v) extraction; of *P. Chrysosporium* BSM / Chloroform were used for quantitative analysis of residual PAHs. The extracted sample (Chloroform layer) was analyzed by High Performance Liquid Chromatography (HPLC) at Micro Analytical Center, Faculty of Science, Cairo University, Giza, Egypt. PAHs were quantified by YL 9100 HPLC system made in South Korea (Quaternary pump No. YL9110, UV/Vis detector No. YL 9120 and Column compartment No. YL 9131) with the 150 mm reversed phase column hypersil ODS-C18, 5µm. The mobile phase consisted of acetonitrite and deionized water. The acetonitrite / deionized water ratio is 85:15 by manual injection with 1 ml/min flow rate at 254nm UV and 40°C.

All the experiments and measurements were done in duplicates and arithmetic averages were used throughout the data analysis and calculations. Degradation percentages were analysis and calculated for two concentrations (5, 150 mgL^{-1}) of each PAHs examined.

Gamma radiation

Preparation of spore suspension

Five Erlenmeyer flasks (250 ml) containing 100 ml [48,49] of Malt Extract (ME) agar medium were sterilized by autoclaving. The flasks after sterilization were left to cool on a horizontal surface. After cooling and solidification of ME agar medium, the flasks were inoculated by *P. Chrysosporium* disc (6 mm) on the center of ME agar medium. After that, the inoculated flasks were incubated stagnant for 7 days at 28°C.

Well-grown *P. Chrysosporium* spores were harvested by sterile saline (30 ml) containing tween-80 (0.1 % v/v). Flasks were shacked gently and spore suspension were collected from each flask into new sterile one to form a pool. This pool represent stock spores suspension to be used for inoculation. Five ml spore suspension (~ 7 × 107 CFU/ml) was distributed in sterile screw-capped tubes. These tubes were exposed to different doses (0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 KGy) of gamma radiation from Indian Chamber of Cobalet-60 (60CO) at National Center for Radiation Research And Technology (NCRRT), Egyptian Atomic Energy Authority (EAEA), and have been conducted at dose rate 1 KGy / 12.5 min. at ambient temperature at the time of experiment [50].

Determination of dose-response curve

Surface plate technique have been followed by inoculating serially diluted control (non-irradiated and irradiated suspension on the surface of ME agar plates (0.1 ml). After that, inoculated plates were incubated for 7 days at 28°C to determine their count (dose-response curve). The count of *P. Chrysosporium* (CFU/ml) was determined and log number of survivors have been plotted against absorbed gamma radiation doses [50,51].

Induction of mutants

From the edge of 14 days actively growing *P. Chrysosporium* exposed to different doses of gamma radiation and having a morphological changes, five plugs (6 mm) were cut out from each colony of gamma irradiated and non-irradiated (parent strain). These *p. chrysosporium* discs were used to inoculate 150 ml of BSM supplemented with 100 mg/L of the six examined PAHs. The inoculated BSM was incubated at 28 ±2°C with continuous shaking at 150 rpm for zero and 14d. The growth (dry weight) and enzyme activity (extracellular protein secretion) have been determined.

Results and Discussion

Growth and extracellular protein of *Phanerochete chrysosporium* on different concentrations of different PAHs

The growth of *P. chrysosporium* on the five concentrations of fluoranthene reached the maximum growth at day 21 for the first four concentrations except for the higher concentration 150 mg/L which reached its maximum growth after 7 days incubation period as indicated in Figure 1. An extracellular protein secreted reached their maximum productivity at 3rd day of incubation for the first four concentrations of fluoranthene except for the highest concentration 150 mg/L which reached the highest protein at day 7 as indicated in Figure 2.

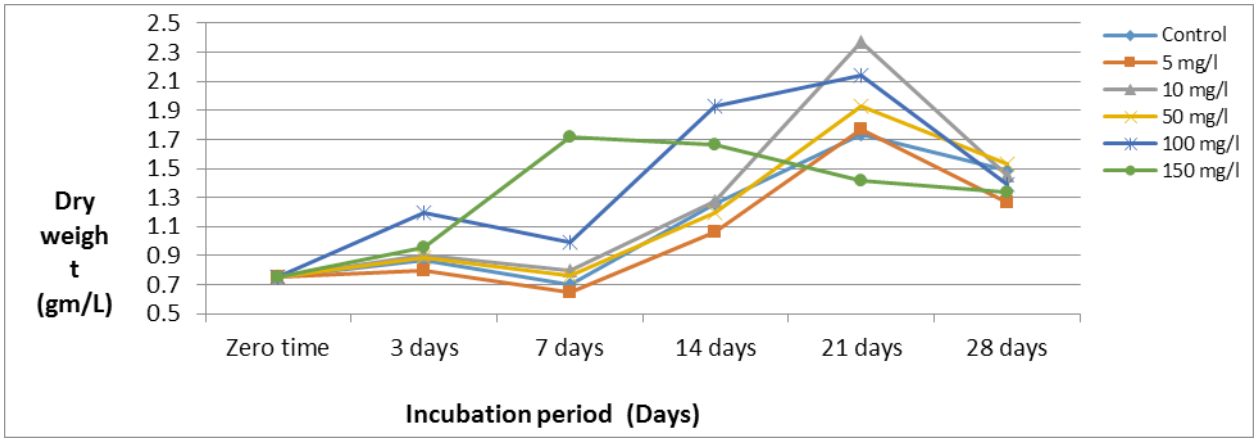

Figure 1: Growth of *Phanerocheate chrysosporium* on different concentrations of Fluoranthene.

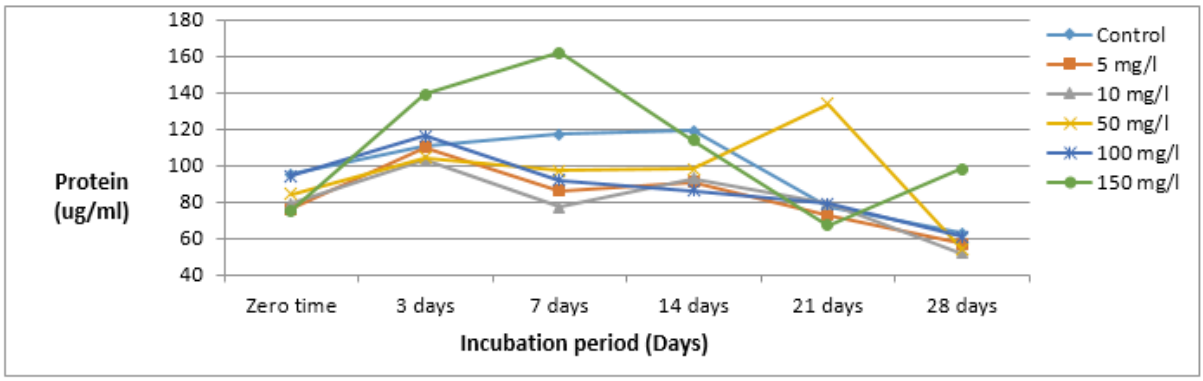

Figure 2: Extracellular protein of *Phanerocheate chrysosporium* on different concentrations of Fluoranthene.

The maximum growth of 100 mg/L of phenanthrene has been reached after 21 days while the higher concentration of 150 mg/L reached its maximum growth at 14 days. The lowest concentrations (5 and 10 mg/L) need more time to reach their maximum productivity (28 days) as shown in Figure 3.

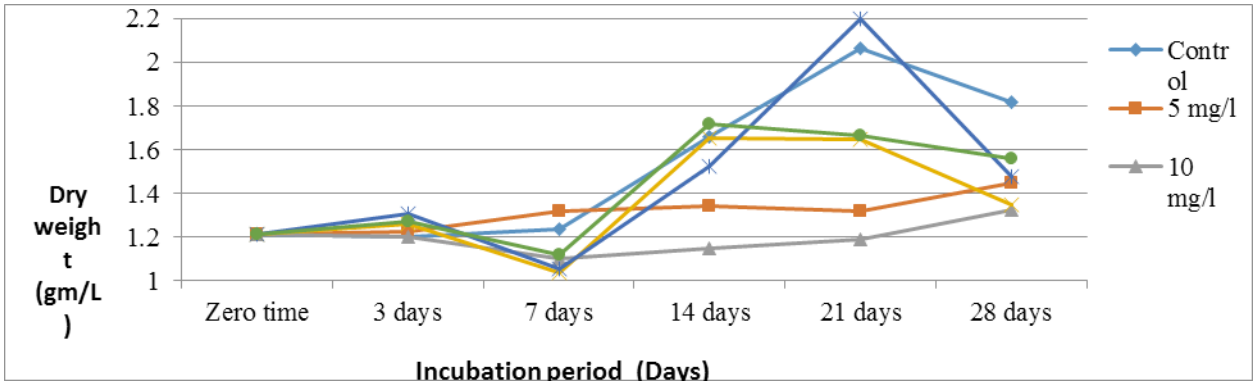

Figure 3: Growth of *Phanerocheate chrysosporium* on different concentrations of Phenantherene.

However, protein secretion has been recorded after 3rd day for the lowest concentrations (5 and 50 mg/L) phenanthrene as indicated in Figure 4.

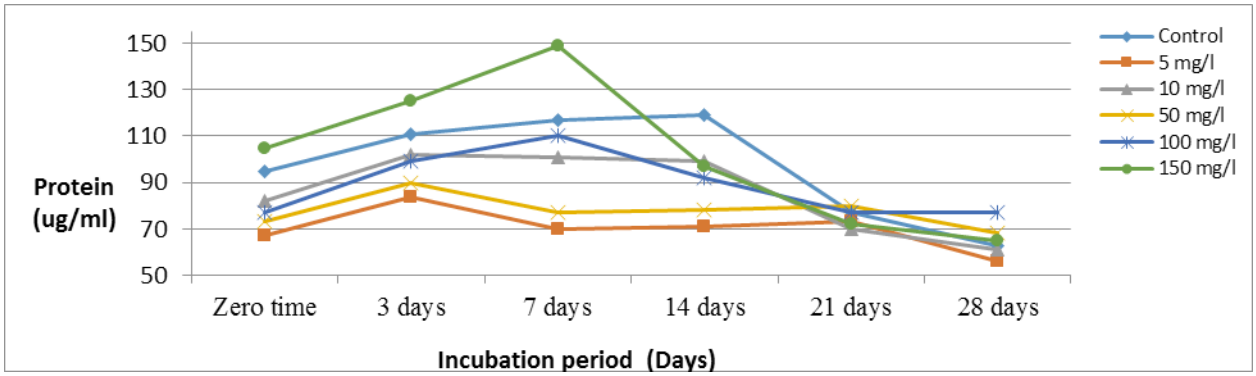

Figure 4: Extracellular protein of *Phanerocheate chrysosporium* on different concentrations of Phenantherene.

The maximum growth of *P. chrysosporium* on different concentrations of acenaphthene was recorded after 21 days of incubation and also the maximum protein secretion as shown in Figure 5 and Figure 6respectively.

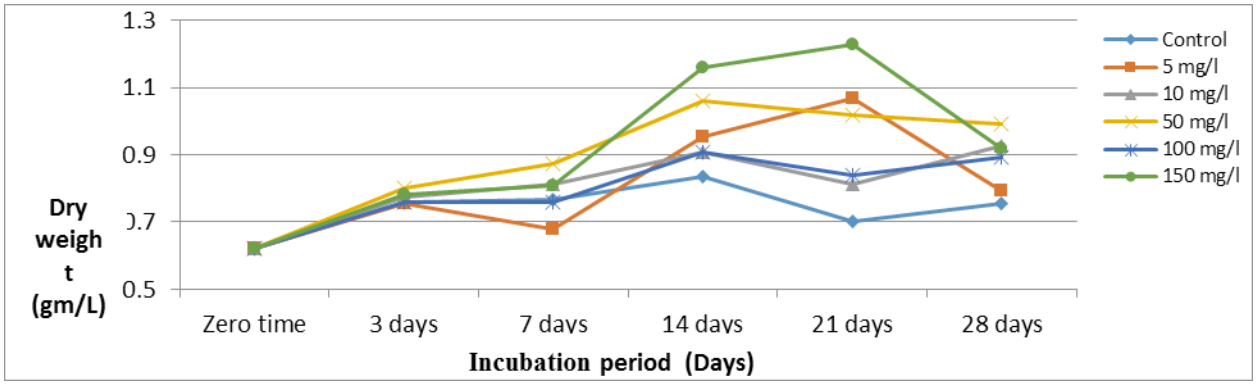

Figure 5: Growth of *Phanerocheate chrysosporium* on different concentrations of Acenaphthene.

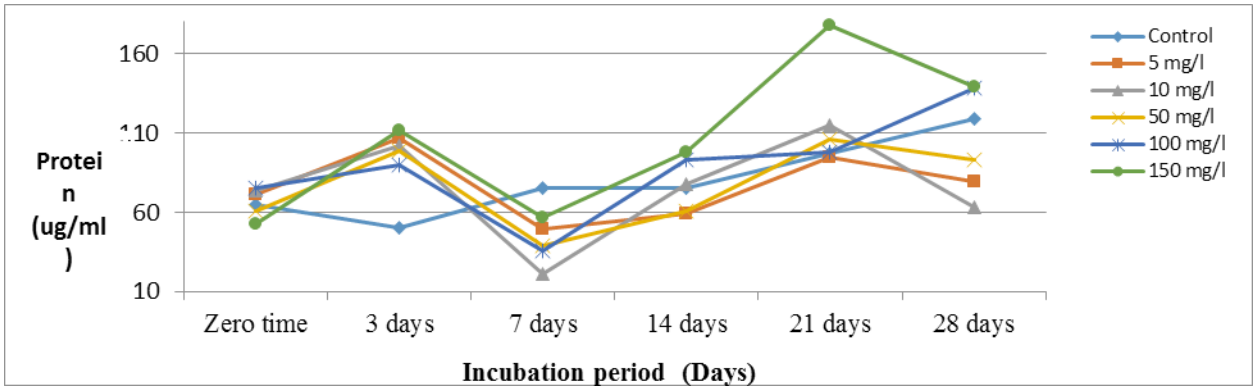

Figure 6: Extracellular protein of *Phanerocheate chrysosporium* on different concentrations of Acenaphthene.

Results of the growth of *P. chrysosporium* on naphthene revealed that the maximum growth was recorded at the end of the incubation period (28 days) as indicated in Figure 7. While the maximum protein secretion reached maximum production after 14 days Figure 8.

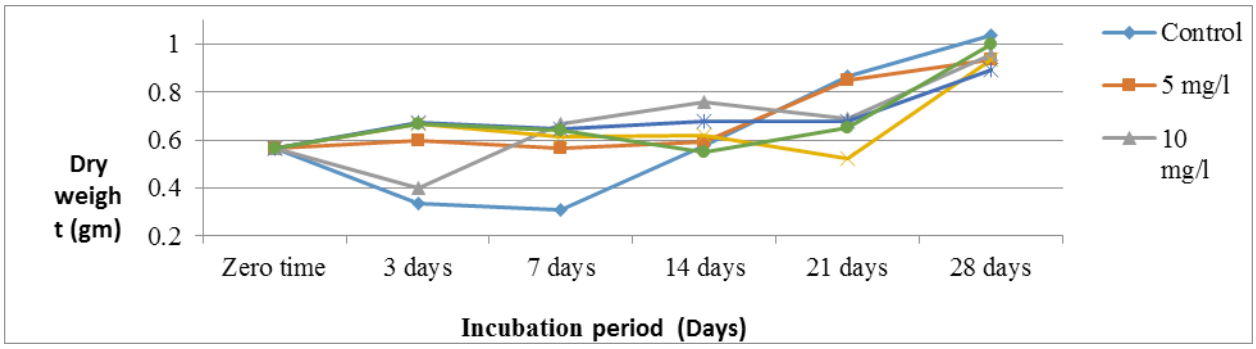

Figure 7: Growth of *Phanerocheate chrysosporium* on different concentrations of Naphthene.

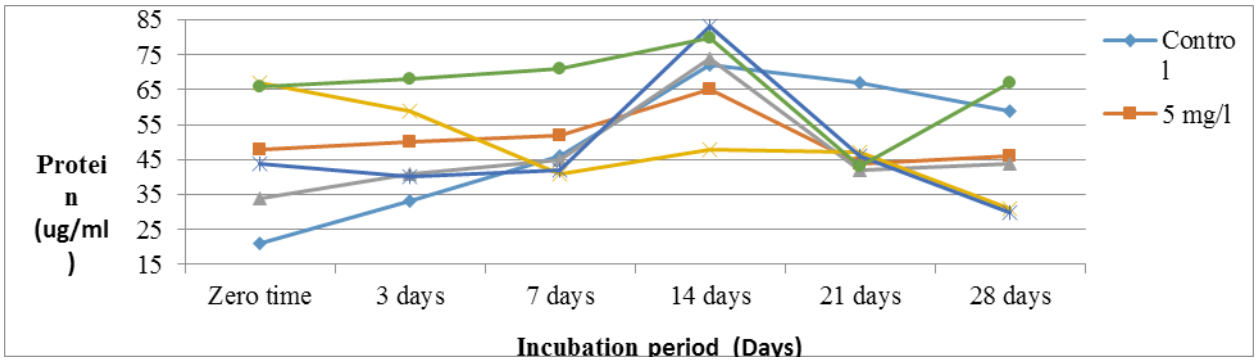

Figure 8: Extracellular protein of *Phanerocheate chrysosporium* on different concentrations of Naphthene.

The results revealed that growth of *P. chrysosporium* on the first four concentrations on anthracene reached their maximum growth at the end of incubation (28 days) except for the higher concentration (150 mg/L) which reached its maximum growth earlier (14 days) as shown in Figure 9. Secreted protein by *P. chrysosporium* reached their maximum productivity at 7th day while at the highest concentration of anthracene (150 mg/L) the maximum productivity has been recorded after 21 days Figure 10.

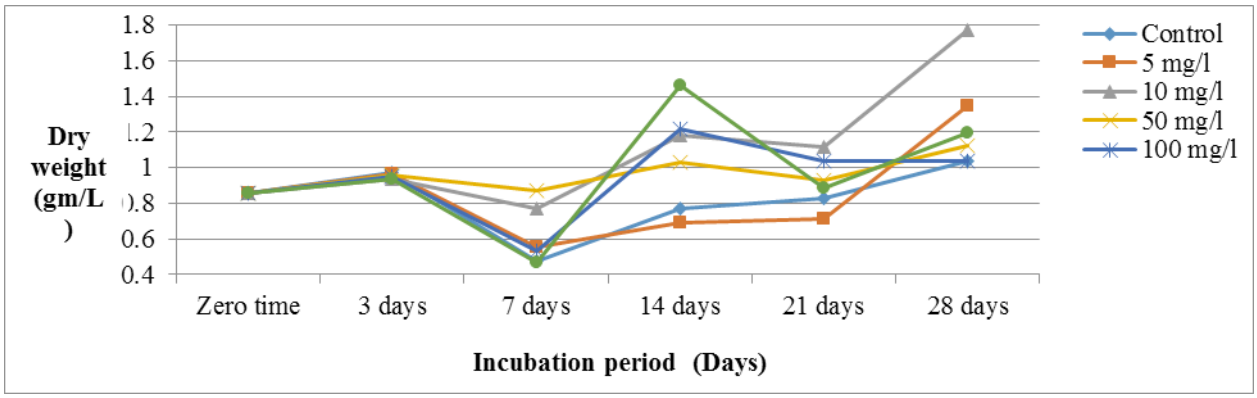

Figure 9: Growth of *Phanerocheate chrysosporium* on different concentrations of Anthracene.

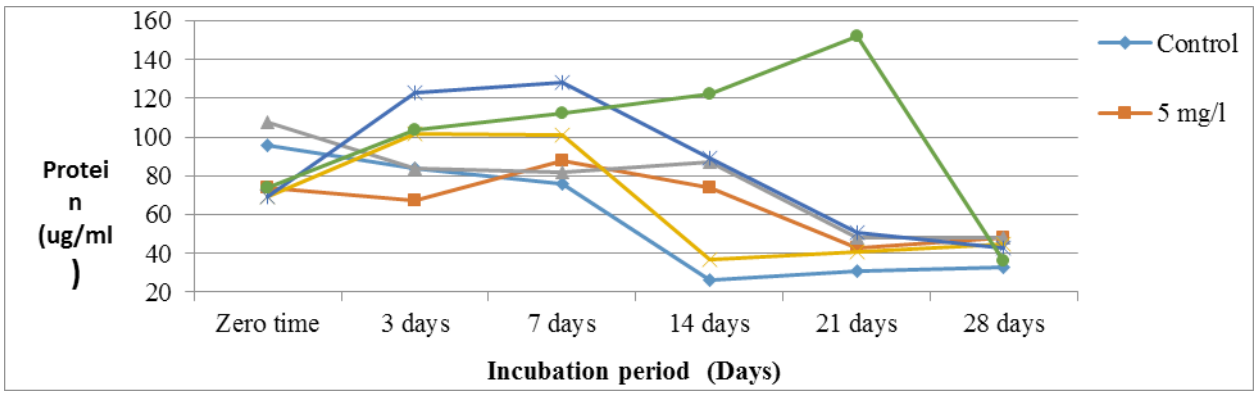

Figure 10: Extracellular protein of *Phanerocheate chrysosporium* on different concentrations of Anthracene.

The maximum growth of *P. chrysosporium* on pyrene has been reached at the end of the incubation period (28 days) for all the five concentrations Figure 11. However, the maximum protein secretion was recorded at 3rd and 7th days except for concentration (10 mg/L) which reached maximum protein secretion at 21 days as indicated in Figure 12.

The results of the present study, showed that growth and extracellular protein secreted by *P. chrysosporium* reached their maximum activities at concentrations (5, 50, 100 and 150 mgL^{-1}) of the six examined PAH compounds i.e at low and high concentration of PAHs. While most studies are based on low concentrations only [26, 67].

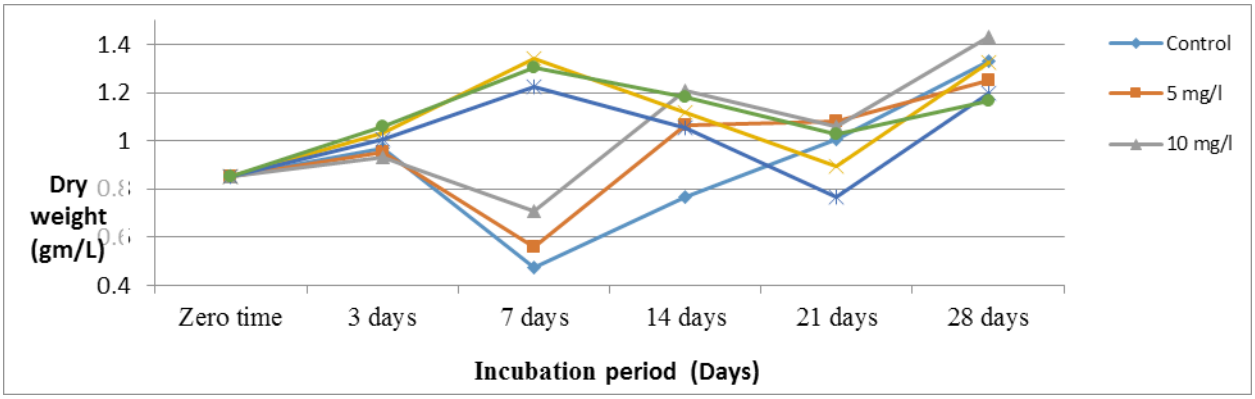

Figure 11: Growth of *Phanerocheate chrysosporium* on different concentrations of Pyrene.

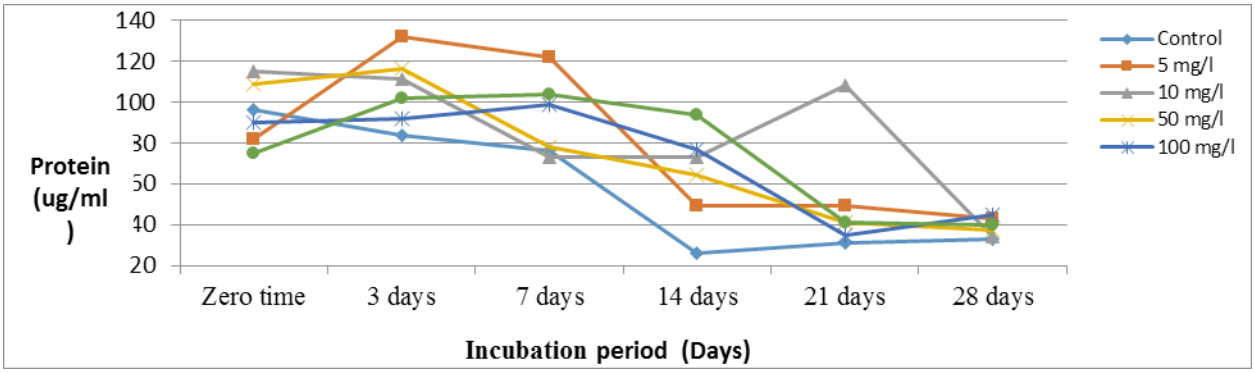

Figure 12: Extracellular protein of *Phanerocheate chrysosporium* on different concentrations of Pyrene.

Degradation of different PAHs

Degradation of six different PAHs by *Phanerochete chrysosporium* has been determined after 28 days of the incubation period (the end of incubation). The results as indicated in Table 1 revealed that *Phanerochete chrysosporium* degraded 87.77 % and 86.38 %, 61,27 % , 57.17 % , 51.90% and 29.23% of 150 mg/L of fluoranthene (Flu.), anthracene (Anth.), phenantherene (Phen.), naphthene (Naph.), pyrene (Pyr.) and acenaphthen (Acen.) respectively. The results also of the higher concentration of Flu.,Anth., Phen. , Naph. And Pyr. were degraded by *P. chrysosporium* more than the lower concentration. However *Phanerochete chrysosporium* degraded the lower concentration (5 mg/L) of Acen. more than the higher concentrations (150 mg/L). The degradation of Acen. Was 64.80 %. The results of Bishnoi et al., (2008) and Wang et al., (2009) [52,53] proved that *Phanerochete chrysosporium* having the ability to best degradation of PAHs than other WRF such as *Trichoderma* (Yao, 2015)[54], *Pycropous* (Li et al., 2018) [33]or other fungi such as *Aspergillus* sp.[26] (Lahkar and Deka, 2016).

Polycyclic aromatic hydrocarbons compounds (PAHs)	Concentration	Degradation percentage %
Fluoranthene	5 mg/L	78.92%
	150 mg/L	87.77%
Phenantherene	5 mg/L	58.31%
	150 mg/L	61.27%

Acenaphthene	5 mg/L	64.84%
	150 mg/L	29.23%
Naphthene	5 mg/L	27.90%
	150 mg/L	57.17%
Anthracene	5 mg/L	32 .50 %
	150 mg/L	86.38%
pyrene	5 mg/L	25.50%
	150 mg/L	51.9 0%

Table 1: Degradation rate of PAHs by *Phanerocheate Chrysosporium*.

The results of the present study were confirmed by the results of other investigators. Oxidoreductase enzyme-based bio-catalytic processes for the biodegradation and biotransformation of a wide array of harmful environmental contaminants have been studied [55]. Immobilized white-rot fungus *P. chrysosporium* removed greater than 90 % after 60 h. of phenantherene, pyrene, and benzo[a] pyrene in Rotating Biological Contactor (RBC) reactor [56]. The same authors (Zheng and obbard, 2002 b),[57] found that the key factors affecting the oxidation of PAH *P. chrysosporium* inclusive Mn2+ concentrations on the extracellular enzyme production.

*P. chrysosporium*is known to produce the MnP enzyme[35]. *P. chrysosporium* act synergistically with soil indigenous microorganisms in the oxidation of low molecular weight PAHs (LMW-PAHs) e.g Acenaphthene, fluorine, Phenantherene, Fluoranthene, and Pyrene, where oxidation was enhanced by up to 43 % in the presence of fungus. [57]. Meanwhile, the white-rot fungus Trametestrogii degraded more than 90 % of 250 - 500 ppm of nitrobenzene and anthracene within 12 - 24 days [58]. *P. chrysosporium* was able to degrade the highly persistent irgarol compound also. Via MnP enzyme [59].

Immobilized MnP enzyme was able to degrade Pyrene (> 86 %), anthracene (>65%) alone or in a mixture, and to less extent fluoranthene (< 15.2 %) and phenantherene (< 8.6 %) by white-rot fungi, *Anthracophyllum discolor*.[60,61]

A defined mixture of fluoranthene, phenantherene, anthracene, pyrene, and benzo [a] anthracene have been degraded by + 60 % fluoaranthene + 40 % phenantherene, and benzo [a] anthracene was 15.2 % and 15.8 % being achieved with *Phanerochete chrysosporium*[62].

MnP enzyme was able to degrade the three PAHs anthracene, dibenzothiophene, and pyrene[63]. In sterile soil, degradation in 42 days incubation of five PAHs by *P. chrysosporium* was as follows: Phen. 98.96 %; anth. 92.6 %; Pyrene 92.2 %; acenaphthene 83.8 % and fluoranthene 79.8 % [52].While, *P. chrysosporium* degraded phenantherene; pyrene and benzo [a] pyrene with rang 72.77 % to 25.50 % (Wang et al., 2009)[53].When a consortium of WRF (CW-1) was used for PAHs degradation, the removal of Phen. (70 - 80 %) and Pyr. (90 %) by live fungi [64].

The oxidation of Anth.,Pyr. andFluo. Detected within 24 h. were 59.7 % , 50.7 % and 49.8 % respectively, whereas only 25.3 % of benzo [a] pyrene and 32.4 % of Phen. were oxidized by white rot fungus *Pycnoporus coccineus* enzyme actively [65].Another WRF, *Pseudotrametes gibbosa* was proved to be able to utilize pyrene as the sole carbon and energy source [41].

The results of metabolic peculiarities of Anth. andPyr. by WRF *Pycnoporus sanguineu*s H1 showed that both extracellular laccase and intracellular catabolic enzymes may perform a significant function in the initial oxidation of Anth. Whereas Pyr. could be only oxidized by catabolic enzymes [66].Another WRF, *Armillaria* sp. F022 is capable to degrade a wide range of four-ring aromatic hydrocarbon like Fluoranthene. *Armillaria* sp. was able to degrade 10 mg/L of Fluo. by 100 % within 30 days [67].

Effect of Gamma radiation on the viable count of *Phanerochete chrysosporium* (dose - response Curve)

Results of exposing of *P. chrysosporium* to different doses of gamma radiation revealed that as the dose of gamma radiation increased, the viable count of spore suspension decreased gradually. The results also showed that 7.0 KGy reduced the viability of *P. chrysosporium* completely as indicated in Table 2 and Figure 13. Dose 6.0 KGy reduced the viable count by 7.70 log cycles.

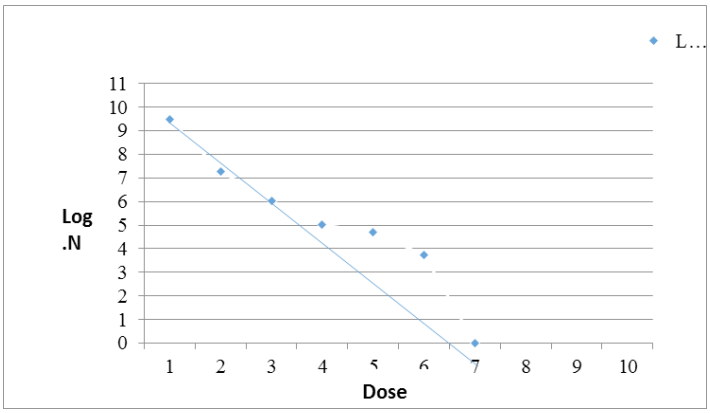

Figure 13: Effect of Gamma radiation on *Phanerocheate chrysosporium* (Dose response curve).

Dose (KGy)	Count (cfu/ml)	Log N
Cont.	2.8×10^{10}	10.44
0.5	2.0×10^{10}	10.3
1	3.2×10^{9}	9.5
1.5	1.5×10^{8}	8.17
2	1.9×10^{7}	7.27
3	1.1×10^{6}	6.04
4	1.1×10^{5}	5.04
5	0.5×10^{4}	4.69
6	5.5×10^{2}	2.74
7	-ve	0

Table 2: Effect of Gamma radiation on *Phanerocheate chrysosporium* (Dose - response curve).

Effect of Gamma radiation on growth and extracellular protein non-irradiated (control) and irradiated *Phanerochete Chrysosporium*

Table 3 indicated the effect of gamma radiation on growth and extracellular protein secreted by *P. chrysosporium* on different PAHs. The low dose of gamma radiation - induced the growth of *P. chrysosporium* (i.e 0.5 and 1.0 KGy) and utilized PAHs as a sole carbon and energy source. The growth of P. *chrysosporium* was more than its dry weight at the beginning by 6.0, 6.6, 6.6, 3.8, 4.3 and 6 times on 100 mg/L of Flu.,Phen., Acen., Naph. , Anth. andPyr. expose to 0.5 KGy respectively. Also, exposing to 1.0 KGy induced the growth of *P. chrysosporium* than their beginning by 7.1, 5.0, 5.0, 3.8, 5.5, and 3.3 times for Flu.,Phen., Acen., Naph., Anth., and Pyr. respectively.

PAHs compods / Gamma Dose		Fluoranthene		Phenantherene		Acenaphthene		Naphthene		Ancerathene		Pyrene	
		Dry Weight (gm/L)	Protein (ug/ml)	Dry Weight (gm/L)	Protein (ug/ml)	Dry Weight (gm/L)	Protein (ug/ml)	Dry Weight (gm/L)	Protein (ug/ml)	Dry Weight (gm/L)	Protein (ug/ml)	Dry Weight (gm/L)	Protein (ug/ml)
Control	Zero time	0.130	14	0.130	20	0.130	33	0.130	28	0.13	16	0.130	18
	14 days	0.330	26	0.330	47	0.230	51	1.300	36	0.200	31	0.360	28
0.5 KGy	Zero time	0.060	19	0.060	25	0.060	30	0.060	15	0.060	17	0.060	30
	14 days	0.360	36	0.400	49	0.400	43	0.230	28	0.260	29	0.360	45
1.0 KGy	Zero time	0.060	18	0.060	23	0.060	30	0.060	22	0.060	35	0.060	27
	14 days	0.430	32	0.300	48	0.300	47	0.230	37	0.330	60	0.200	49

1.5 KGy	**Zero time**	0.200	18	0.200	23	0.200	30	0.200	22	0.200	35	0.200	27
	14 days	0.250	67	0.270	87	0.270	70	0.270	68	0.280	60	0.430	65
2.0 KGy	**Zero time**	0.330	36	0.330	25	0.330	35	0.330	31	0.330	30	0.330	30
	14 days	0.400	45	0.410	48	0.380	62	0.370	57	0.410	51	0.430	34
3.0 KGy	**Zero time**	0.260	36	0.260	38	0.260	40	0.260	35	0.260	24	0.260	27
	14 days	0.350	45	0.280	66	0.290	76	0.290	54	0.310	42	0.340	58

Table 3: The effect of different Gamma radiation Doses on Growth and Extracellular protein excreted by *Phanerocheate chrysosporium* non - irradiated (control) and irradiated ones on different PAHs.

Exposure of *P. chrysosporium* to 0.5 and 1.0 kGy gamma radiation induced the growth on Flu. , Phen., Acen.,Naph., Anth. And Pyr. More than parent strain (non- irradiated control) by 2.4,2.8; 2.6,2.0 ; 3.8,2.9 ; 1.5,1.5 ; 2.8,3.6 and 2.2,1.2 times respectively. The highest growth rate, in general, was recorded at 0.5 and 1.0 KGy doses of gamma - irradiated *P. chrysosporium*. Also, in general, the highest growth rate has been recorded by *P. chrysosporium* exposed to 1.0 KGy on 100 mg/L Flu. After 14 days incubation period by 7.1 times.

The results of protein secreted by *P. chrysosporium* exposed to 1.5 KGy revealed that protein was more than protein determined at their beginning on different PAHs by 3.7, 3.7, 2.3, 3.1, 1.7, and 2.4 times for Flu., Phen., Acen., Naph., Anth., and Pyr. Respectively.

Generally, gamma radiation at low doses induced growth of *P. chrysosporium* than its parent strain (non-irradiated) P. *chrysosporium* and 1.5 KGy induced protein secretion more than non-irradiated *P. chrysosporium* showed their maximum at 14 days of incubation with 100 mgL^{-1}concentrations of six examined PAHs. Gradually decrease (dose - response curve) of the present studytill the lethal dose may be explained on the basis that gamma radiation causes a variety of damage to DNA in cells [68]. Survival curves of *Phanerocheate chrysosporium* spores showed a rise in viability due to radiation - induced germination before a logarithmic decline [69].

Several studies show that gamma radiation can change the genomic structure and induce mutants [70-73]. Also, Abo-State (2003), [48] indicated that *Fusarium neoceras* mutant number 1 and number 6 were exposed to 1 min. UV- radiation recorded the highest CM Case activity while the CM Case activity from *Fusarium oxysporum* was mutant number 4 which was exposed to 4 min. UV - radiation. El-Batal and Abo-State (2006) [74], found that enhanced production in Avicelase CM Case, Pictinase, FPase, and xylanase and proteinase by gamma irradiation at dose 1.0 KGy with increased percentage 10 %; 8 %; 31 %; 20 %; 4 % and 34 % respectively. Rajoka (2006) [75] reported enhanced production (1.6 fold) of extracellular endoglucanase enzyme activity over the parent strain.

Abo-State et al., (2010) [49], proved that gamma radiation reduced the viability of *Aspergillus* MAM-F23 and MAM-F35 gradually. Mutant number 4, which exposed to 0.5 KGy produced higher cellulases than the parent strain.

A gradual decrease in the growth of *P. sajor-caju* was observed as a gamma - ray dose increased. However, irradiated by gamma - ray, enhanced MnP production at low doses by 4.5 times that of the parent strain [36].

The dose - response curve of two fungal isolates MAM-F15 and MAM-F48 isolated from antibiotics revealed that 6.0 KGy and 5 KGy reduced the viability completely. These doses reduced the viable count of MAM-15 by 4.48 log cycles[76].

In another study, Abo-State et al., (2019)[77]. Found that three fungi isolated from third and fourth - generation of cephalosporins when exposed to gamma radiation doses the viable count reduced gradually as the doses increased. Doses 6.0 and 7.0 KGy reduced the viable count completely.

Conclusion

*Phanerochete chrysosporium*was able to utilize PAHs as a sole carbon and energy source.

P. chrysosporium was able to grow on the six tested PAHs (Flu.,Phen., Anth., Naph., Acen., and Pyr.).

P. chrysosporium degraded 87.77 %; 86.38 %; 61.27 %, 29.23%, 51.90% and 57.17 %, of 150 mg/L Flu., Anth. , Phen., Acen. , Pyr. And Naph.respectively.

As the dose of gamma radiation increased, the viable count of *P. chrysosporium* decreased gradually.

Dose 6.0 KGy reduced the viable count by 7.7 KGy, while 7.0 KGy reduced the viability of *P. chrysosporium* completely.

Low doses of gamma radiation (0.5 and 1.0 KGy) induced the growth of *Phanerochete chrysosporium* than the non-irradiated ones (parent strain).

Dose (1.5 KGy) induced protein secretion by *P. chrysosporium* than the non -irradiated ones.

P. chrysosporium can be used as a good candidate for the biodegradation of PAHs (biotechnological treatment).

References

1. Gurjeet P, Kothiyal NC,Kumar V (2014) Hydrocarbons (PAH) from soil using *Sphingobium indicum*, *Sphingobium japonicum* and *Stenotrophomonas maltophilia* bacterial strains under aerobic conditions. J Environ Res Develop 8: 395-405.
2. Ghosal D, Ghosh S, Tapan K, Dutta K, Ahn Y (2016) Current State of Knowledge in Microbial Degradation of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. Frontiers Microbiol 7:1369.
3. Abdulazeez TL (2017) Environmental chemistry, pollution & waste management-Polycyclic aromatic hydrocarbons. A review. Cogent Environmental Science 3: 1-89.
4. Kong W, Lars H, Carlos FJ, Lutz AO (2019) Rhoda electrocatalysis for Annulative C-H Activation: Polycyclic Aromatic Hydrocarbons by Versatile Double Electrocatalysis. Angewandte Chemie 10: 1433-7851.
5. Torres-Farrada G, Manzano-Leon AM, Rineau F, Leal MR, Thijs S, et al. (2019) Biodegradation of polycyclic aromatic hydrocarbons by native Ganoderma sp. strains: identification of metabolites and proposed degradation pathways. Applied Microbiology and Biotechnology 103: 7203-7215.
6. Zhang J, Rujing HH, Sun WR (2019) Historical Pollution and Source Contributions of PAHs in Sediment Cores from the Middle Reach of Huai River, China. Bull Environ ContaminToxic 102: 531-537.
7. Lee H, Jang Y, Choi Y, Kim M, Lee J,et al. (2014): Biotechnological procedures to select white rot fungi for the degradation of PAHs. Journal of Microbiological Methods 97: 56-62.
8. Rey-Salgueiro L, García-Falcón MS, Martínez-Carballo E, Simal-Gándara J (2008) Effects of a chemical company fire on the occurrence of polycyclic aromatic hydrocarbons in plant foods. Food Chemistry108: 347-353.
9. Rey-Salgueiro L, García-Falcón MS, Martínez-Carballo E, Simal-Gándara J (2008) Effects of toasting procedures on the levels of polycyclic aromatic hydrocarbons in toasted bread. Food Chemistry 108: 607-615.
10. Lee BK, Vu VT (2010) Sources, distribution and toxicity of polyaromatic hydrocarbons (PAHs) in particulate matter. In: Villanyi V [ed]. Air pollution. Rijeka: Sciyo; InTech, ISBN: 978-953-307-143-145.
11. Inomata Y, Kajino M, Sato K, Ohara T, Kurokawa JI, et al. (2012) Emission and atmospheric transport of particulate PAHs in Northeast Asia. Environ Sci Technol 46: 4941-4949.
12. Seidel T, Orasche J, Ruppert H, Schnelle-Kreis J, Hartmann H (2013) Emissions of organic and inorganic pollutants during the combustion of wood, straw and biogas. In: Ruppert H, Kappas M, Ibendorf J, editors. Sustainable bioenergy production-an integrated approach. Berlin: Springer 387-422.
13. Sihag S, Pathak H, Jaroli DP (2014) Factors affecting the rate of biodegradation of polyaromatic hydrocarbons. Int J Pure Appl Bio Sci 2: 185-202.
14. Yebra-Pimentel I, Fernandez-Gonzalez R, Martínez-Carballo E,Simal-Gandara J (2015)A critical review about the health risk assessment of PAHs and their metabolites in foods. Crit Rev Food Sci Nutr55: 1383-1405.
15. Bolling AK, Pagels J, Yttri KE, Barregard L, Sallsten G,et al. (2009) Health effects of residential wood smoke particles: the importance of combustion conditions and physicochemical particle properties. Part Fibre Toxicol 6: 29.
16. Khairy MA, Kolb M, Mostafa AR, El-Fiky A,Bahadir M (2009)Risk assessment of polycyclic aromatic hydrocarbons in a Mediterranean semi-enclosed basin affected by human activities (Abu Qir Bay, Egypt). J Hazard Mater 170: 389-397.
17. Kim KH, Jahan SA, Kabir E,Brown RJ (2013) A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ Int 60: 71-80.
18. Abo-State MA, Saleh Y, Aziz N,Partila AM (2013a) Isolation of polycyclic aromatic hydrocarbon degrading bacterial strains isolated from indigenous microbial communities of petroleum contaminated soils. World Applied Sciences Journal 23: 554-564.
19. Abo-State MA, Saleh Y,Partila AM (2013b) Identification of polycyclic aromatic hydrocarbon degrading bacterial strain and its ability to degrade pyrene. World Applied Sciences Journal 23: 515-525.
20. Abo-StateMAM, EL- Dars FMS,Abdin BA (2018 a) Isolation and identification of Pyrene degrading bacterial and its pathway from Suez Oil Processing Company, Suez , Egypt. J Ecol Health Environ In 6: 63-76.
21. Abo-State MAM, Riad BY,Abdel Aziz MF (2018 b) Biodegradation of naphthalene by *Bordetella avium* isolated from Petroleum refinery wastewater in Egypt and its pathway. J RadRes Appl Sci 11: 1-9.
22. Abo-State MAM,El-Kelani TA (2020) Bacterial Biodegradation pathways of polycyclic aromatic hydrocarbons (PAHs). Arch Pet Environ Biotechnol 5: 158
23. Integra Environmental (2011)Bioremediation overview. Integra Environmental, Houston, TX, USA.
24. Gaur N, Narasimhulu K,PydiSetty Y (2018) Recent advances in the bio-remediation of persistent organic pollutants and its effect on environment. Journal of Cleaner Production 198: 1602-1631.
25. Chen Y, Peng Y, Dai C,Ju Q (2011)Biodegradation of 4-hydroxybenzoic acid by *Phomopsisliquid ambari*. Applied Soil Ecology 51: 102-110.

26. Lahkar J,Deka H (2016) Isolation of Polycyclic Aromatic Hydrocarbons (PAHs) Degrading Fungal Candidate from Oil-Contaminated Soil and Degradation Potentiality Study on Anthracene. Polycyclic Aromatic Compounds Taylor and Francis 37: 141-147.

27. Mao J,Guan W (2016) Fungal degradation of polycyclic aromatic hydrocarbons (PAHs) by *Scopulariopsis brevicaulis* and its application in bioremediation of PAH-contaminated soil. Journal of Environmental Science and Health, Part B Pesticides. Food Contaminants and Agricultural Wastes. 52: 399-405.

28. Umar ZD, Abd Aziz NA, Zulkifli SZ, Mustafa M (2016) Rapid biodegradation of polycyclic aromatic hydrocarbons (PAHs) using effective *Cronobacter sakazakii* MM045 (KT933253). Polycyclic Aromatic Compounds MethodsX 4: 104-117.

29. Alexopolous CJ, Mims CW,Blackwell M (1996) Introductory mycology. 4thed, New York.

30. Kadri T, Rouissi T,Brar SK, Cledon M, Sarma S,et al. (2017) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. Industrial Crops and Products J Environ Sci51: 52-74.

31. Treu R,Falandysz J (2017) Mycoremediation of hydrocarbons with basidiomycetes-a review. Journal of Environmental Science and Health, Part B Pesticides. Food Contaminants and Agricultural Wastes 52: 148-155.

32. Kresinova Z, Linhartova L, Filipova A, Ezechias M, Masin P,et al. (2018) Biodegradation of endocrine disruptors in urban wastewater using *Pleurotus ostreatus* bioreactor. New Biotechnology 43: 53-61.

33. Li Z, Yanshuo P, Hu S, Sheng Y, Cheng Y, et al. (2018a) Diversity of phenanthrene and Benz[a]anthracene metabolic pathways in white rot fungus *Pycnoporus sanguineu*s 14. J Sci Total Environ 134: 25-30.

34. Mester T, Tien M (2000)Oxidation mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants. Biochem Mol Biol 46: 51-59.

35. Abo-State MAM, Othman M, Khatab O,Abd-Elfattah EA (2011a) Enhancement production of Mn P enzyme produced by *Pleurotus sajor-caju* exposed to Gamma Radiation. World Appl Sci J 14: 1457-1468.

36. Abo-State MAM, Khatab O, Abo- El Nasr A,Mahmoud B (2011b) Factors affecting laccase production by *Pleurotus ostreatus* and *Pleurotus sajor-caju*. World Appl Sci J 14: 1607-1619.

37. Abo-State MAM, Reyad B, Gomaa O, Youssif EA (2011 C) Comparing decolorization of dye by white rot fungi , free enzyme and immobilized enzyme. World Appl Sci J 14: 1469-1486.

38. Li pX, Chen AY, Wu Y, Wu L, Xiang L,et al. (2018b) Applying β-cyclodextrin to amaranth inoculated with white-rot fungus for more efficient remediation of soil co-contaminated with Cd and BDE-209. New Biotechnology 634: 417-426.

39. Dao ATN, Vonck J, JanssensTKS, Dang HTC, Brouwer A,et al. (2019) Screening white-rot fungi for bioremediation potential of 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin. IndCrops Prod Animal Ecol 128: 153-161.

40. Li X, Lin X, Yiu R, Wu Y, Chu H,et al. (2010) Optimization of Laccase-mediated Benzo[a]pyrene Oxidation and Bioremedial Application. Polycyclic Aromatic Hydrocarbons contaminated soil J Health Sci 56: 534-540.

41. Wen J, Gao D, Zhang B, Liang H (2011) Co-metabolic degradation of pyrene by indigenous white-rot fungus Pseudotrametesgibbosa from the northeast China. International Biodeterioration& Biodegradation 65: 600-604.

42. Li X, Wang Y, Wu S, Qiu L, Gu L, et al. (2014) Peculiarities of metabolism of anthracene and pyrene by laccase-producing fungus Pycnoporussanguineus H. Biotechnology and Applied Biochemistry 61: 549-554.

43. Luis FB, Gabriel M, Raquel S (2015) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by laccase from Trametes versicolor covalently immobilized on amino-functionalized SBA-15. Chemosphere 136: 273-280.

44. Vasiliadou IA, Molina R, Pariente MI, Christoforidis KC, Martinez F, et al. (2019) Understanding the role of mediators in the efficiency of advanced oxidation processes using white-rot fungi. Chemical Engineering Journal 359: 1427-1435.

45. Oxoid (1982) TheOxoid Manual of Culture Media Ingredients and other Laboratories Services (5th ed.), Oxoid Limited, England.

46. Abo-State MA, Abdallah N, Nader B (2017a) Biodegradation of high molecular weight polycyclic aromatic hydrocarbons (HMW-PAHs) mixtures by bacteria isolated from soils polluted with petroleum oil. First Int Conf Biol Environm Sci Appl (ICBESA) 23-25.

47. Lowery OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 139: 265-275.

48. Abo-State MAM (2003) Production of CarboxymethylCellulase by *Fusarium oxysporium* and *Fusarium neoceros* from Gamma-Pretreated Lignocellulosic Wastes. J Biotechnol 15: 151-168.

49. Abo-State MAM, Hammad MAM, Swelim M, Gannam RB (2010) Enhanced production of cellulose (s) by *Aspergillus* spp. isolated from agriculture wastes by solid state fermentation. American-Eurasian J Agric Environ Sci 8: 402-410.

50. Abo-State MAM, Husseiny SHM, Helimish FA, Zickry ARA (2012) Contamination of Eye Drops with *Bacillus* species and Evaluation of their Virulence Factors. World Appl Sci J 19: 8470-855.

51. Abdallah AHA (2014) Inhibitory Effect of Gamma Radiation in Degrading and Preventing Fungal Toxins. J Food Agri Environ 12: 77-81.

52. Bishnoi K, Kumar R, Bishnoi RN (2008) Biodegradation of polycyclic hydrocarbons by white rot fungi *Phanerocheate chrysosporium* in sterile and unsterile soil. J Sci Ind Res 67: 538-542.

53. Wang C, Sun H, Li J, Li Y, Zhang Q (2009) Enzyme activities during degradation of polycyclic aromatic hydrocarbons by white rot fungus *Phanerochete chrysosporium* in soils. J Chemosphere 77: 733-738.

54. Yao L, Teng Y, Luo Y, Christie P, Ma W, et al. (2015) Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Trichodermareesei FS10-C and Effect of Bioaugmentation on an Aged PAH-Contaminated Soil. Bioremediation Journal 19: 9-17.

55. Bilal M, Adeel M, Rasheed T, Zhao Y, Iqbal HMN (2019) Emerging contaminants of high concern and their enzyme-assisted biodegradation-A review. Environ Int 124: 336-353.

56. Zheng Z, Obbard JP (2002) Removal of polycyclic aromatic hydrocarbon from soil using surfactant and white rot fungi *Phanerochaete chrysosporium* in a rotating biological contractor. J Biotechnol 96: 241 -249.

57. Zheng Z, Obbard JP (2002) Polycyclic aromatic hydrocarbon removal from soil by surfactant solubilization and *Phanerochaete chrysosporium* oxidation. J Environ Qual 31: 1842-1847.

58. Levin L, Viale A, Forchiassin A (2003) Degradation of organic pollutants by the white rot basidiomycete Trametestrogii. Int BiodeteriorBiodegra 52: 1-5.

59. Ogawa N, Okamura H, Hirai H, Nishida T (2004) Degradation of the antifouling compound Irgarol 1051 by manganese peroxidase from the white rot fungus *Phanerochaete chrysosporium*. Chemosphere 55: 487-491.

60. Acevedo F, Pizzul L, Castillo MD, González ME, Cea M, et al. (2010) Degradation of polycyclic aromatic hydrocarbons by free and nanoclay-immobilized manganese peroxidase from *Anthracophyllum discolor*. Chemosphere 80: 271-278.

61. Acevedo F, Pizzul L, Castillo MD, González ME, Cea M, et al. (2011) A Practical Culture Technique for Enhanced Production of Manganese Peroxidase by *Anthracophyllum discolor* Sp4. Brazil Arch Biol Technol Biol Appl Sci 54: 1175-1186.

62. Tekere M, Read JS, Mattiasson B (2005) Polycyclic aromatic hydrocarbons biodegradation in extracellular fluids and static batch cultures of selected sub-tropical white rot fungi. J Biotech 115: 367-377.

63. Eibes G, Chau LUT, Feijoo G, Moreira MT, Lema JM (2005) Complete degradation of anthracene by manganese peroxidase in organic solvent mixtures Enzyme Microbe. Technol 37: 356-372.

64. Chen B, Wang Y, Hu D (2010) Biosorption and biodegradation of polycyclic aromatic hydrocarbons in aqueous solutions by a consortium of white rot fungi. J Hazardous materials 179: 845-851.

65. Thongkred P, Lotrakul P, Prasongsuk S, Imai T (2011) Oxidation of polycyclic aromatic hydrocarbons by a tropical isolate of *Pycnoporus coccineus* and its laccase. J Science Asia 37: 225-233.

66. Wang F, Yu S, Liu K, Chen FE, Song Z, et al. (2013) Acute Intraocular inflammation caused by Endotoxin fter intravitreal injection of counterfeit Bevacizumab in Shanghai China. J Ophthalmology 120: 355-361.

67. Hadibarata T, Kristanti RA (2015) Biotransformation Studies on Fluoranthene, a Four-ring Polycylic Aromatic Hydrocarbon, by White-Rot Fungus Armillaria sp. F022. AgriAgricult Sci Proc 3: 45-50.

68. Hutchinson F (1985) Chemical changes induced in DNA by ionized radiation produced free radicals. Rad Res 86: 185-195.

69. Liwicki RA, Paterson MJ, Macdonald, Broda P (1985) Phenotypic classes of Phenoloxidases- negative mutants of the lignin- degrading fungus *Phanerochete chrysosporium*. J Bacteriol 162: 641-644.

70. Zolan ME, Termel CJ, Pukkila PJ (1988) Production and characterization of radiation-sensitive meiotic mutants of *Coprinus cinereus*. Genetics 120: 379-387.

71. Boominathan KS, Dass B, Randall TA, Reddy CA (1990) Nitrogen-deregulated mutants of *Phanerochete chrysosporium*-a lignin degrading basidiomycetes. Arch Microbiol 153: 521-527.

72. Fukuoka H, Kageyama Y, Yamamoto K, Takeda G (1994) Rapid conversion of r DNA intergenic spacer if diploid mutants of rice derived from gamma ray irradiated tetraploids. Mol Gen Genet 243: 166-172.

73. Nam Ks (2002) Low dose gamma irradiation as means of isolating carotenoid-hyper producing yeast mutant. J Microbial Biotechnol 12: 1010-1012.

74. El-Batal AI, Abo-State MA (2006) Production of cellulose, xylanase, pectinase, amylase and protease enzymes cocktail by Bacillus spp. and their mixed culture with *Candida tropicalis and Rhodotorula glutinis* under solid state fermentation. Egypt Rad Sci Appli 19: 139-156.

75. Rajoka MI (2006) Double mutants of *cellulomonas biazotea* for production of cellulose and hemicellulases following growth on straw of a perennial grass. World J Microbiol Biotechnol 21: 1063-1066.

76. Abo-State MAM, Husseiny SH ,Ahmed HA, Moawad A (2017b) The effect of gamma radiation on cephalosporin and aminoglycosides antibiotics. J Rad Nucl Appl 2(3): 75-85.

77. Abo-State MAM, Yassin MH, El-Shora MM (2019) Influence of gamma radiation on third and fourth genration cephalosporin antibiotics. World J Pharm Pharmaceut Sci 8: 321-347.