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Mini-Review

Potential fungi to remediate polycyclic aromatic hydrocarbons in contaminated soil: A mini-review

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are often present in man-made pollutants such as diesel engine oil, pesticide and dye. PAHs are often deposited in soil and absorbed by plants without any further degradation. Therefore, PAHs are considered dangerous in terms of food safety and human health as they are often mutagenic and carcinogenic. This mini-review is to identify potential fungi candidates by evaluating their degradation percentage of PAHs and the types of PAHs effectively degraded by fungi. Based on a reported study, *Ganoderma lucidum* and *Aspergillus flavus* achieved the highest degradation percentage whereby nearly all PAH pollutants in the soil were completely degraded, that, thereby making both of them the best potential candidates for the degradation of PAHs. In contrast, *Anthracophyllum discolor*, *Pleurotus chrysosporium Peniophora incarnata*, *Pleurotus ostreatus*, *Trametes versicolor*, *Aspergillus niger*, *Trichoderma asperellum*, *Fusarium solani*, *Penicillium simplicissimum* and *Scopulariopsis brevicaulis* were relatively less effective in the degradation of PAHs since only moderate degradation percentage of PAHs was achieved, despite being capable of degrading at least two types of PAHs. On the other hand, *Lasiodiplodia theobromae* and *Irpex lacteus* were not good candidates since they were only able to degrade one type of PAH with low degradation percentage. In conclusion, *G. lucidum* and *A. flavus* are the fungi with the greatest potential for mycoremediating PAHs as shown by their degradation percentage. PAH remediation through the use of fungi holds great potential, offering affordable, effective and environmentally friendly alternatives for removing a wide array of PAHs from polluted environments.

KEYWORDS: review, polycyclic aromatic hydrocarbons, mycoremediation, fungi.

INTRODUCTION

Industrial productions were found often to contaminate the environment through the release of hydrocarbon content especially polycyclic aromatic hydrocarbons (PAHs) [1]. PAHs have been shown to have negative environmental and ecological impacts that affect the human health [2]. More adversely, PAHs are a type of recalcitrant xenobiotic pollutants, and these contaminants are durable and difficult to be degraded. As a result, they remain for long periods in the environment which causes a significant threat as many of the PAHs possess mutagenic and carcinogenic characteristics [3]. Hence, PAH pollutant has acquired significant public attention. PAHs are natural components that exist in petroleum, coal and fossil fuels [4]. Burning of these fuels, especially incomplete burning, leads to the release of PAHs into the environment. PAHs are able to adsorb onto dust or soot particles and enter the atmosphere and are eventually deposited into the soil and plants [5]. As a consequence, PAHs are widely distributed to different parts of the environment. Thus, they are categorized as one of the prioritized pollutants that need to be removed from the environment.

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Currently, chemical and physical approaches such as combustion, landfill, photolysis, and ultrasonic decomposition are used to remediate soil contaminated by PAHs. These approaches may remove PAHs with high efficiency and reduce the cost of remediation [6]. However, the use of these approaches could cause negative effects to the environment which in turn could lead to secondary pollutions, especially the use of chemical techniques wherein PAH remediation might result in the formation of toxic compounds [7]. Additionally, PAHs do not degrade physically at ambient temperature and hence might require more carbon to generate more heat energy for degradation. This could result in greenhouse gas emission in the surrounding environment [2]. In order to overcome this issue, mycoremediation has been shown as a better option in remediating PAHs from contaminated soil [8]. The catabolism of certain species of fungi that is native to the soil environment could be used as a potential tool for PAH remediation [9]. Compared with the conventional approaches, mycoremediation of PAHs has more key advantages, including ease of implementation, environmental friendliness, cost effectiveness and suitability for use in large area [10].

In Peninsular Malaysia, rapid development of industrial urban areas has a significant impact on the quality of the environment. Hence, the issue of PAH pollution has become a major concern of the society. In Malaysia, the total PAH content accumulated in the sediments increased significantly from 20 - 112 ng/g in 2013 [11] to 481.3 - 976.6 ng/g in 2015 [12], a drastic increment within a short two-year period. It is projected the total PAHs would continuously accumulate in the sediment of Peninsular Malaysia, and consequently, increasing the risk of PAHs entering the food chain with negative effects on the ecosystem [13]. As such, the increasing environmental pollution stresses the need for environmentally friendly remediation technologies especially mycoremediation. Therefore, the objective of this mini-review is to detect potential fungi candidates towards mycoremediation of PAHs by assessing their PAHs degradation percentage and the types of PAHs that can be degraded.

Mycoremediation of PAHs

Mycoremediation is a form of bioremediation that uses fungi to degrade or convert pollutants into harmless or less harmful compounds. The advantage of mycoremediation is the ability of fungi to adapt to the complex conditions of a polluted environment. Generally, studies on mycoremediation can be classified based on types of fungi used, and the different types of metabolism expression (ligninolytic or non-ligninolytic). Ligninolytic refers to fungi that produce and secrete extracellular ligninolytic enzymes including lignin peroxidases, manganese peroxidases and laccases. These enzymes are known to degrade a broad range of organic compounds [14]. Fungi that are unable to produce ligninolytic enzymes are known as non-ligninolytic fungi [15]. Non-ligninolytic fungi usually oxidize PAHs through the use of cytochrome P450 monoxygenase enzymes and convert PAHs into oxidized products and trans-dihydrodiol [16]. Pozdnyakova *et al*. [17] observed that PAHs can be converted into trans-dihydrodiol and phenol, and subsequently be further metabolized to glucoside, glucuronide, sulfate, or xyloside by non-ligninolytic fungi.

Ligninolytic fungi

Results in Table 1 show *Ganoderma lucidum* has the highest potential to degrade PAHs. This fungus can degrade four different PAHs namely, naphthalene, fluorene, phenanthrene and pyrene, and achieves 99% degradation with the last two PAHs [3], most probably due to the presence of laccase, manganese peroxidase and lignin peroxidase, which were secreted in high amounts [3, 18, 19]. A recent study by Torres-Farrada *et al*. [18] showed that laccase secretion alone by *G. lucidum* can achieve up to 64% of fluorene and 73% of naphthalene degradation. Apart from laccase, Agrawal *et al*. [3] reported that *G. lucidum* degraded 99.65% and 99.58% of recalcitrant phenanthrene and pyrene, respectively, and the authors attributed the PAHs' degradation efficiency to the large amount of manganese peroxidase and lignin peroxidase secreted.

Although *Anthracophyllum discolor* can degrade five types of PAHs which is more than *G. lucidum*, the degradation percentage is only moderately high [20]. This fungus is also reported to secrete manganese peroxidase [20], and is responsible for the degradation ofbenzo[a]pyrene (75%), anthracene (73%), phenanthrene (62%), pyrene (60%), and fluoranthene (54%). *A. discolor* isolated from the

Table 1. The efficiency of potential ligninolytic fungi to remediate various PAHs.

Species	PAHs	Degradation %	Incubation	References
Anthracophyllum discolor	Benzo[*a*]pyrene	75%	60 days	[20]
	Anthracene	73%	60 days	
	Phenanthrene	62%	60 days	
	Pyrene	60%	60 days	
	Fluoranthene	54%	60 days	
Ganoderma lucidum	Phenanthrene	99.65%	30 days	[3, 18]
	Pyrene	99.58%	30 days	
	Naphthalene	73%	7 days	
	Fluorene	64%	7 days	
Irpex lacteus	Anthracene	38%	61 days	[30]
Phanerochaete chrysosporium	Pyrene	85.87%	40 days	[28]
	Phenanthrene	60.62%	40 days	
Peniophora incarnata	Phenanthrene	86.5%	40 days	[29, 30]
	Fluoranthene	82.6%	40 days	
	Pyrene	77.4%	40 days	
	Anthracene	< 40%	40 days	
Pleurotus ostreatus	Phenanthrene	> 90%	14 days	[31, 17]
	Fluorene	> 90%	14 days	
	Naphthalene	86.47%	11 days	
	Anthracene	27.87%	11 days	
Trametes versicolor	Phenanthrene	> 90%	14 days	[17]
	Fluorene	> 90%	14 days	

forest of Chile was also able to degrade PAHs in the three to four aromatic rings range. Despite the fact that *A. discolor* have the ability to degrade recalcitrant PAHs which comprise of 3-4 aromatic rings, this fungus received relatively little interest from researchers in relation to PAH mycoremediation. The reason probably could be higher removal percentage of PAHs, which is not outstanding enough to be notable, by *A. discolor* can only be achieved after a relatively longer incubation period of 60 days. Another possible reason could be that *A. discolor* might only be able to grow in selected locations such as in the forests of Chile [21].

Phanerochaete chrysosporium is reported to degrade various types of pollutants including pesticides, dyes and PAHs [22]. Bumpus *et al*. [23] and Song [24] reported that *P. chrysosporium* can degrade PAHs into more soluble compounds. This is because *P. chrysosporium* secretes lignin peroxidases that can depolymerize lignin by cleaving the alpha and beta bonds and convert the polyaromatic structure into monoaromatic structures. It is suggested that PAHs can be similarly metabolized using the lignin peroxidases, as reported by Pozdnyakova [25] wherein lignin peroxidases were observed as the primary enzyme to degrade phenanthrene, pyrene and benzo[a]pyrene in contaminated soil. In addition, secretion of manganese peroxidases together with hydrogen peroxide oxidizes Mn^{2+} in soil or wood to generate Mn^{3+} ions, to better support growth of *P. chrysosporium* [26]. However, in a recent study by Cao *et al*. [27], *P. chrysosporium* was found to be unable to utilize benzo[a]pyrene as the sole carbon source, and it was speculated that low secretion of lignin peroxidase was responsible for the failure. Lee *et al*. [28] reported that the removal of PAHs by *P. chrysosporium* demonstrated that

initial degradation percentage of phenanthrene was 19.71% and pyrene was 52.21% after three days of incubation. However, with the increase in incubation time (until 40 days), the degradation percentage of phenanthrene gradually increased from 19.71% to 60.62% while the degradation percentage of pyrene gradually increased from 52.21% to 85.87%. Results in Table 1 also show that *P. chrysosporium* was only able to degrade pyrene efficiently (85.87%), with similar extended incubation period of 40 days. This indicated that the ability of *P. chrysosporium* to degrade PAHs is highly dependent on incubation time.

Peniophora incarnata is reported to be a good candidate to degrade PAHs in soil [29]. Lee *et al*. [29] demonstrated that *P. incarnata* was able to degrade 86.5% of phenanthrene, 82.6% of fluoranthene, and 77.4% of pyrene, with the detection of laccase and manganese peroxidase. In a recent report, Lee *et al*. [28] further showed that *P. incarnata* was able to degrade more than 70% phenanthrene, fluoranthene and pyrene, in water, but less than 40% of anthracene was degraded (< 40%). This may be due to the low solubility of anthracene (0.015 mg/L) in water compared to phenanthrene (1.8 mg/L), fluoranthene (0.25 mg/L), and pyrene (0.18 mg/L) [17, 30, 31].

Pleurotus ostreatus is the best candidate if the target is to achieve >90% degradation of phenanthrene and fluorene [17]. *P. ostreatus* also can secrete laccase and manganese [17, 30]. Pozdnyakova *et al.* [17] showed that *P. ostreatus* has the ability to mineralize >90% phenanthrene and fluorene by laccase and manganese peroxidase in a basidiomycete-rich medium incubated at 26 °C for 14 days. Elhusseiny *et al*. [31] showed *P. ostreatus* was able to degrade 86.47% of naphthalene and to a lesser extent 27.87% of anthracene in high concentration of nitrogen after three days of incubation. A further incubation showed that naphthalene was completely degraded within five days. Thus, both *P. incarnata* and *P. ostreatus* similarly were relatively inefficient in degrading anthracene with the degradation percentage reported being as low as 27.87% [30]. One of the possible reasons could be that *P. ostreatus* was unable to produce biosurfactants to increase the bioavailability of anthracene, the least water-soluble PAH tested [30, 31].

Young *et al*. [32] investigated the degradation of Bunker C fuel oil collected from Blackstone Canal, United States of America (USA) using several types of white-rot fungi including *T. versicolor* grown on malt yeast agar (MYA) at 27 °C and incubated for 180 days. From the results, it was reported that *T. versicolor* could produce laccase and manganese peroxidase, achieving >70% degradation of phenanthrene. A study by Pozdnyakova *et al*. [17] on *T. versicolor* grown in basidiomycete-rich medium at 26 °C for 14 days also showed mineralization of phenanthrene and fluorene exceeding 90%. *T. versicolor* showing a higher bioavailability towards phenanthrene (1.8 mg/L) and fluorene (1.89 mg/L) is due to the fact that both have relatively higher water solubility [16, 17].

Lastly, *Irpex lacteus* seem to be the least efficient ligninolytic fungi to be used to degrade PAHs as the fungus can only degrade one type of PAH with a low degradation percentage of 38%, despite a very long 61 days' incubation period [30]. *I. lacteus* have a relatively slow growth rate [30, 33]. Baborova *et al.* (2006) [34] reported that the only major ligninolytic enzyme produced by *I. lacteus* is manganese peroxide and that it is secreted in low amounts although studies have shown that manganese peroxide cleaves PAHs that contain three to four aromatic rings. Jove *et al*. [30] reported that *I. lacteus* can only metabolize 4-ring anthracene with 38% degradation. A study conducted by Byss *et al*. [33] similarly reported that *I. lacteus* was only able to achieve a degradation percentage ranging from 32% to 49%, for PAHs containing three aromatic rings.

Non-ligninolytic fungi

Data in Table 2 suggests that *Aspergillus flavus* is the best non-ligninolytic fungi to degrade PAHs, since it can degrade at least six types of PAHs within a short incubation period and achieved 100% degradation [35]. A study by Haritash & Kaushik [36], showed that *A. flavus* achieved the highest PAH degradation with naphthalene (68.8%), followed by fluoranthene (62.2%), phenanthrene (61.6%). and pyrene (59.8%) after 12 days incubation with low-molecular weight PAHs. Al-Dossary *et al*. [35] also reported that *A. flavus* was able to degrade 16 types of PAHs, and achieved 100% degradation on six of the PAHs including naphthalene, acenaphthylene, fluoranthene, pyrene, chrysene, and benzo[b]fluoranthene, within 15 days.

The rest of the PAHs were completely mineralized when growth of *A. flavus* was stimulated using nutrient amendments within another 15 days. The efficiency of *A. flavus* to degrade PAHs was attributed to the secretion of enzymes in large amount including epoxide hydrolase, endoglucanase, and beta-glucosidase [37]. Therefore *A. flavus* has the highest potential to be used in bioremediation of PAH pollutants.

According to Table 2, *Aspergillus niger* is the best to be used to specifically degrade phenanthrene to achieve a high 97% degradation in only 5 days [38]. In another study, Chukwura *et al.* [39] pointed out that *A. niger* was able degrade 79% of benzo[a]pyrene, 99% of pyrene and 99% of phenanthrene in soil samples after 28 days of incubation. Hamzah *et al.* [38] further highlighted (that?) *A. niger* (fungi) was relatively more than *Pseudomonas putida* (bacteria) in removing phenantherene from contaminated soil. The result reported was that *A. niger* was able to achieve 97% phenanthrene degradation, which was almost five times higher than that of *P. putida* (20%) after 5 days. The author suggested that the uptake mechanisms of phenanthrene by *A. niger* could be passive transport mechanisms, which thus increases the metabolizing rate of PAHs by intracellular enzymes including cytochrome P-45 enzyme [40]. Therefore, *A. niger* has greater potential than bacterial in removing PAHs in the environment.

Both of the *Aspergillus* species performed well in degrading PAHs and this was probably due to the high surface area of the fungal mycelia [41], allowing for a larger surface area to come in contact with

Table 2. The efficiency of potential non ligninolytic fungi to remediate various PAHs.

Species	PAHs	Degradation %	Incubation	References
Lasiodiplodia theobromae	Benzo[*a*]pyrene	32%	10 days	[27]
Aspergillus niger	Pyrene	99%	28 days	[38, 40]
	Phenanthrene	97%	5 days	
	Benzo[a]pyrene	76%	28 days	
Aspergillus flavus	Naphthalene	100%	15 days	[35, 36]
	Acenaphthylene	100%	15 days	
	Pyrene	100%	15 days	
	Fluoranthene	100%	15 days	
	Chrysene	100%	15 days	
	Benzo[a]pyrene	100%	15 days	
	Phenanthrene	61.6%	12 days	
Fusarium solani	Naphthalene	84.82%	10 days	[51]
	Chrysene	57.84%	10 days	
	Phenanthrene	40.09%	10 days	
	Benzo[a]pyrene	35.06%	10 days	
Trichoderma asperellum	Benzo[a]pyrene	81%	14 days	[46]
	Phenanthrene	74%	14 days	
	Pyrene	63%	14 days	
Penicillium simplicissium	Anthracene	86%	61 days	[30, 43]
	Pyrene	60%	28 days	
Scopulariopsis brevicaulis	Benzo[*a*]pyrene	82%	30 days	[47]
	Phenanthrene	75%	30 days	
	Pyrene	64%	30 days	
	Fluoranthene	62%	30 days	

PAHs, whereby degradation could be achieved within a shorter period of time compared to others genus. Additionally, the *Aspergillus* species produce more than one type of degradative enzyme that enhances the potential to degrade PAH compounds [37]. Therefore, the *Aspergillus* genus has better PAH degradation ability than other genus in general [9, 42].

Penicillium simplicissimum is the fungi that can specifically degrade anthracene efficiently (86%), despite needing a long incubation period of 2 months [30]. Saraswathy and Hallberg [43] reported that *P. simplicissimum* can also use pyrene (around 60%) as carbon food source in liquid cultures. In a recent study, *P. simplicissimum* was also used to remediate a mixed hydrocarbon pollutant oil [44]. Ravelet *et al*. [45] in his report showed that *P. simplicissimum* secretes laccases to degrade PAHs.

Both *Trichoderma asperellum* and *Scopulariopsis brevicaulis* are shown to achieve moderately high degradation percentage against benzo[a]pyrene, phenanthrene and pyrene (Table 2); *T. asperellum* a comparatively better candidate as it required only half of the incubation period of *S. brevicaulis* to degrade the three PAHs. The reason could be that *T. asperellum* is capable of secreting laccase, peroxidase and catechol dioxygenase to degrade PAHs efficiently to obtain a carbon source to aid its growth but *S. brevicaulis* could not secrete those enzymes [46, 47].

Zafra *et al*. [46] discovered that after 14 days of incubation *T. asperellum* was able to degrade 81% of benzo[a]pyrene, 74% of phenanthrene, and 63% of pyrene by using enzyme laccase, peroxidase and catechol dioxygenase. Based on a previous study conducted by Zafra and Cortés-Espinosa [48], *T. asperellum* was found to have the highest potential to degrade PAHs among the other seven *Trichoderma. sp* because *T. asperellum* has excellent ability to degrade a wide range of low and high-molecular weight PAHs, and high tolerance towards a variety of PAHs. However, more information on using *T. asperellum* to remediate PAHs are limited, since studies on the potential of *T. asperellum* to bioremediate PAH pollutants was only first reported in 2015.

Mao & Guan [47] isolated *S. brevicaulis* from contaminated soil and identified the potential of using this fungus to remove PAHs. In their report, *S. brevicaulis* was able to achieve 82% degradation of benzo[a]pyrene, 75% degradation of phenanthrene, 64% degradation of pyrene and finally 62% degradation of fluoranthene within 30 days of incubation. Godoy *et al*. [49] reported that *S. brevicaulis* degrades PAH pollutants through co-metabolic processes, using a variety of enzymatic pathways. The ability of *S. brevicaulis* to co-metabolize PAHs allows the fungus to be used for bioremediating other xenobiotics with similar structures in the environment [50].

Based on Table 2, *Fusarium solani* can efficiently degrade naphthalene (84.82%), but is less efficient against phenanthrene, chrysene and benzo[*a*]pyrene with moderate to low degradation percentages [51]. Thion *et al*. [52] reported secretion of laccase by *F. solani* degrades PAHs with three to five aromatic rings, which include phenanthrene (3 rings), pyrene (4 rings), and dibenz[a,h]anthracene (5 rings), present in contaminated soil, with degradation percentage reported at 46%, 51% and 30%, respectively. In another study using agar plates coated with PAHs, *F. solani* isolated from oil-contaminated soil in Egypt was reported to achieve 35.06% degradation of benzo[a]pyrene (5 ring), 57.84% of chrysene (4 ring), 40.09% of phenanthrene (3 ring) and 84.82% of naphthalene (2 ring) after ten days of incubation [51]. The author demonstrated that increasing number of aromatic rings contributed to higher difficulty for *F. solani* to degrade the PAHs. A recent study by Delsarte *et al*. [53] also showed poor degradation percentage (37%) towards 5-ring benzo[*a*]pyrene by *F. solani*. However, when *F. solani* was mixed with the catalase (3-Amino-1,2,4-trizole), the degradation percentage of benzo[*a*]pyrene increased to approximately 40% to 76%, demonstrating that addition of catalase could greatly improve the degradation of PAHs with higher number of aromatic rings.

The potential of *Lasiodiplodia theobromae* in remediating PAH-contaminated soil was first reported by Balaji *et al*. [54], showed a similar degradation percentage (30%) as *F. solani* against benzo[a]pyrene. However, *L. theobromae* was reported to produce a lesser quantity of lipase and laccase during the degradation process. Table 2 shows that *L. theobromae* can only degrade a single type of PAH making it the least-useful mycoremediation agent to degrade PAHs [27]. However, there is a

report demonstrating that *L. theobromae* is able to degrade pristine (contain 17 carbon) and pyrene (contain 18 carbon) in the presence of lipase [54]. Cao *et al.* [27] observed that *L. theobromae* isolated from contaminated soil in Beijing, China can degrade 32% of benzo[a]pyrene in 10 days. The degradation percentage can be increased to up to 90% when benzo[a]pyrene is mixed with an emulsifying agent (Tween 80). This shows that the lipase and laccase enzymatic activities in fungi have lower bioavailability towards high-molecular weight PAHs as they are less water soluble [55].

CONCLUSION

Based on the current mini-review, numerous studies greatly demonstrated the feasibility of using fungi to remediate PAH pollutants. However, the efficiency of PAH degradation by fungi was found to be highly dependent on the fungi species, types of PAHs and also incubation parameters, particularly, length of incubation. *G. lucidum* has the highest efficiency to degrade four different types of PAHs with 99% degradation achieved, in the category of ligninolytic fungi. In contrast, *A. discolor, P. chrysosporium, P. incarnate, P. ostreatus*, and *T. versicolor* were relatively less effective and were able to degrade only two types of PAHs. *I. lacteus* does not seem to be a good candidate amongst the ligninolytic fungi as *I. lacteus* was only able to degrade one type of PAH, with a low degradation percentage. Among non-ligninolytic fungi, *A. flavus* has the highest efficiency as it can degrade more than six types of PAHs, while *A. niger, T. asperellum, S. brevicaulis, P. simplicissium* and *F. solani* achieved moderate degradation percentage towards at least two types of PAHs. *L. theobromae* did not seem to be a good candidate for mycoremediation as it can only degrade one type of PAH making it the least useful as a mycoremediation candidate.

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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