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# Phylloremediation of pyrene and anthracene by endophytic fungi inhabiting tea leaves (*Camellia sinensis* (L.) Kuntze) in Sri Lanka

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**ABSTRACT**

Polyaromatic hydrocarbons (PAHs) released from vehicular emissions and oil refineries deposit on the phyllosphere, compromising the quality of leaf-based food products by posing many health issues. Nevertheless, the tea phyllosphere harbours a variety of endophytes that are highly effective at degrading polyaromatic hydrocarbons, anthracene, and pyrene. The present study attempts to analyse the pyrene and anthracene degrading capability of phyllosphere endophytic fungi that inhabit *Camellia sinensis* (L.) Kuntze leaves. The frequency of occurrence of endophytic fungi in different leaf tissue layers was examined using light and scanning electron microscopy (SEM). The best pyrene and anthracene degrading strains were selected based on the High-Performance Liquid Chromatography (HPLC) results, and further kinetic assays. Light microscopy and SEM observations highlighted a heterogeneous endophytic fungal distribution among leaf tissue layers; the upper epidermis had the highest fungal distribution compared to other leaf layers. HPLC results revealed that *Phyllosticta capitalensis, Colletotrichum gloeosporioides, Colletotrichum siamense, Pseudopestalotiopsis chinensis,* and *Daldinia eschscholtzii,* have higher pyrene and anthracene degradation respectively and their PAH degradation kinetics follow the first-order kinetic model. The best anthracene and pyrene degrader, *P. capitalensis* showed the lowest half-life. The present investigation highlights the potential of *P. capitalensis*, the best pyrene and anthracene degrader that can remediate PAHs deposited on the phyllosphere of tea leaves.

## Introduction

Polyaromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants derived from the combustion of gasoline, diesel-like carbonaceous fuel materials in vehicles and oil refineries (Kavouras et al. 2001; Liu et al. 2001). Current PAH concentration

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in urban air is significantly high due to many anthropogenic activities: daily increasing vehicular emissions and oil refinery combustion (Fang et al. 2004; Lammel et al. 2015). Polyaromatic hydrocarbons with a molecular weight range between 128 and 276 Da were identified as one of the priority pollutants by the US Environmental Protection Agency. Of them, pyrene and anthracene are considered hazardous compounds due to their high carcinogenicity and genotoxicity to all living beings (Ren et al. 2017a).

PAH molecules are deposited in a variety of ground-level open spaces and in the phyllosphere (Venkataraman et al. 1994). PAH pollutants remain on the phyllosphere due to the leaf structure's thick waxy epicuticle and inner cuticle layer (Wagrowski and Hites 1998; Forster et al. 2004; Sharma et al. 2016). PAH deposition on the phyllosphere of tea leaves generates health issues since tea is an infusion of its leaves (Ishizaki et al. 2010; Drabova et al. 2012; Ciemniak et al. 2019). Since tea cultivation sites do not have any cover crops or leaf cover above bush level, tea leaves are directly exposed to air pollutant depositions (Kuroda and Hara 1999; Ziegenhals et al. 2008). These contaminants may affect tea yield and quality and pose a health threat to tea consumers (Zachara et al. 2018). Further, the presence of PAHs was detected in some processed teas and fresh tea leaves worldwide, indicating the accumulation of PAHs in the environment (Lin et al. 2006; Vinas et al. 2007). It has also been assessed whether the tea infusion prepared from tea leaves contains any PAHs (Pincemaille et al. 2014). Black tea delivered from China had PAH values of 9650, 8800, and 1162 g/Kg (Fiedler et al. 2002; Lin and Zhu 2004; Lin et al. 2005), whereas dried black tea delivered from German supermarkets had PAHs content ranging between 14 and 89 g/Kg. The phyllosphere of tea leaves is an excellent niche for diverse bacterial and fungal species that stabilise the chemical composition of tea leaves (Mei et al. 2009). Furthermore, the tea phyllosphere is a collection of endophytic and epiphytic microorganisms that make symbiotic relations to maintain a favourable chemical environment in the phyllosphere (Thambugala et al. 2021). These microorganisms can resist the stress conditions created by PAH contaminants in the phyllosphere and show great potential for biocontrol against some plant pathogenic fungi (Thambugala et al. 2022). As an adaptation, they can degrade PAH compounds by utilising them as their sole carbon source. For instance, the fungal species isolated from the phyllosphere of ornamental plants collected from the urban areas in Sri Lanka showed significant degradation abilities in phenanthrene and naphthalene (Undugoda et al. 2016a). Even though much research was conducted on phylloremediation of PAH compounds, no such research was carried out to investigate the PAH degrading capabilities of phyllosphere endophytic fungal species inhabiting the leaves of Camellia sinensis (L.) Kuntze.

The present research attempt was to analyse the pyrene and anthracene degradation abilities of tea phyllosphere endophytic fungi in Sri Lanka. Moreover, the distribution of endophytic fungi in the different tissue layers of the tea leaves was examined. Furthermore, a kinetic assay was conducted to evaluate the degradation pattern of each fungus and to analyse their first-order kinetic models.

## Materials and methodology

### Fungal samples

*Colletotrichum gloeosporioides* (MZ160522), *Colletotrichum siamense* (MZ160504), *Daldinia eschscholtzii* (MZ160510), *Phyllosticta capitalensis* (MZ160517) and

*Pseudopestalotiopsis chinensis* (MZ160509) which were isolated from the leaf tissues of tea plants in three regions (Kandy, Kegalle, and Nuwara Eliya) of Sri Lanka (Thambugala et al. 2021) were taken to investigate their capabilities in degrading pyrene and anthracene.

### Determination of the distribution pattern of the endophytes in tea leaf tissues

Freehand sections of the leaves, which were used to isolate phyllosphere fungal consortium were taken to assess the presence of fungal endophytes in the internal leaf tissue layers. Thin leaf cross sections were stained using a drop of cotton blue, and a drop of glycerol (50%) was used to mount the specimens. The leaf specimens were then observed under mid-power (x10) and high-power (x40) of a bright field compound microscope (Carl Zeiss, Germany). The percentage distribution of endophytes in each of the particular leaf tissue layers were calculated using the below formula.

$$\text{Percentage distribution of fungal endophytes in a particular leaf tissue}$$
$$= \frac{\text{Number of particular leaf tissue with fungal endophytes}}{\text{Total number of tissues observed}}$$

### Endophytic fungal observations through SEM

A critical point dryer was used to dry the leaves after they had been surface disinfected (Pamphile et al. 2008). The samples were coated with a thin layer of gold (at 27 °C, 50 mA, 260 seg) and placed vertically in stubs using the adhesive tape conductors of the SEM. The fragments were first seen at low magnification using an SEM, and then the magnification was gradually increased to 50x and 100x using an emission field of 5 kV and at a distance of 7 mm.

### Determination of pyrene and anthracene degradation ability of phyllosphere fungi

#### Screening of pyrene and anthracene degradation ability of fungi using the plate assay

Each fungus underwent three days of starvation after being placed on Bacto Bushnell Hass (BBH) agar medium. A BBH agar plate was divided into 25 squares and inoculated with an isolated fungal strain after the starvation period, followed by the addition of pyrene and anthracene at a concentration of 100 ppm. The control samples were prepared without adding anthracene and pyrene to the BBH medium. Finally, the number of squares out of 25 that had colony growth was counted after seven days of incubation at 28 °C.

#### Confirmation of pyrene and anthracene degradation ability of fungi using HPLC analysis

Fifty millilitres of BBH broth medium, including 100 ppm of pyrene and anthracene, was added to one millilitre of starved fungal broth, and the mixture was cultured for six days at 28 °C. After rotary evaporation to concentrate the sample, the culture solution was

filtered through a 0.2 m nylon syringe filter. The filtrate was then extracted using an acetone: hexane (1:1) solvent combination. A UV detector with an HPLC was used to evaluate the rotary evaporated residue after it had been reconstituted in 1 mL of acetonitrile (HPLC grade, assay 99.8%). At a flow rate of 1.0 mL/min, acetonitrile: water (90:10) combination was used as the mobile phase for pyrene and anthracene, and the UV detector was set at 254 nm for compound identification.

## Determination of pyrene and anthracene degradation kinetics of the best degraders

### Determination of degradation rate according to the different PAH substrate concentrations

Fifty millilitres of mineral salt medium containing pyrene and anthracene as the only carbon sources were inoculated with 1 millilitre of the fungal suspension (Undugoda et al. 2016b). The well-developed fungal cultures were used to create samples with pyrene and anthracene concentrations of 100, 150, 200, 250, 300, 350, and 400 mg/L. After that, samples were stored for seven days at 28 °C and 200 rpm in a shaking incubator. Then, each fungal strain's rate of degradation was examined.

### Analysis of substrate consumption

Daily, an HPLC analysis was used to evaluate the remaining PAH concentration following the fungal breakdown of various PAH concentrations. Using an HPLC (Waters 600, Waters, Milford, MA, USA) fitted with a 4.6 150-mm reverse-phase C18 column and methanol/water (90:10) as the mobile phase at a flow rate of 0.8 mL/min, the residual PAH were extracted using acetonitrile and then measured. Chromatography was carried out at 40 °C with a 245 nm detection wavelength.

### Degradation kinetic assay

To analyse PAH degradation kinetics, first-order equations; Alexander (1999), Kuo and Lotse (1973), and Lafleur (1980), were used.

First-order equation,

$$C_t = C_o \ e^{-kt}$$

$C_t$ – PAH concentration at time (days) $t$
$C_0$ – initial PAH concentration
$k$ – reaction rate constant (1/day)
$t$ – time (days)
Half-life calculations were done using the below equation,

$$T_{\frac{1}{2}} = \frac{\ln 2}{k}$$

The parameters governing PAH degradation model were identified using single linear correlations and equations of regression. The following three indications were used to

find the equation suitable to describe PAH degradation: (i) the coefficient ($R^2$), (ii) the standard error, and (iii) the residuals.

## Statistical analysis

Following one-way ANOVA for HPLC analyses, Tukey's pairwise comparison test was used to evaluate the mean values. The SPSS statistics software was used to conduct the statistical analysis and $P < 0.05$ was used to define statistical significance.

## Results and discussion

### Phyllosphere endophytic fungal distribution in leaf tissue layers

In agreement with Garcia et al. (2012), who studied heterogeneous endophytic fungal colonisation in the leaf tissue layers of Sapindus Saponaria, light microscopy and SEM observations of the present study also highlighted a heterogeneous endophytic fungal distribution among tea leaf tissue layers, upper epidermis, lower epidermis, palisade intracellular, palisade intercellular, spongy intercellular, and spongy intracellular layers (Figure. 1). Further, the light microscopic and SEM observations revealed that the highest percentage of endophytic fungal distribution is in the upper epidermis (85%) compared to the other tissue layers (Figure 2 and 3). Simultaneously, SEM observations also confirmed that the presence of the highest endophytic fungal mycelia in the upper epidermis, which is next to the phylloplane. Ren et al. (2017b) found that most phyllosphere fungi are leaf endophytes mostly colonised in the upper epidermis. The fungal distribution in the lower epidermis was significantly lower than in the upper epidermis.
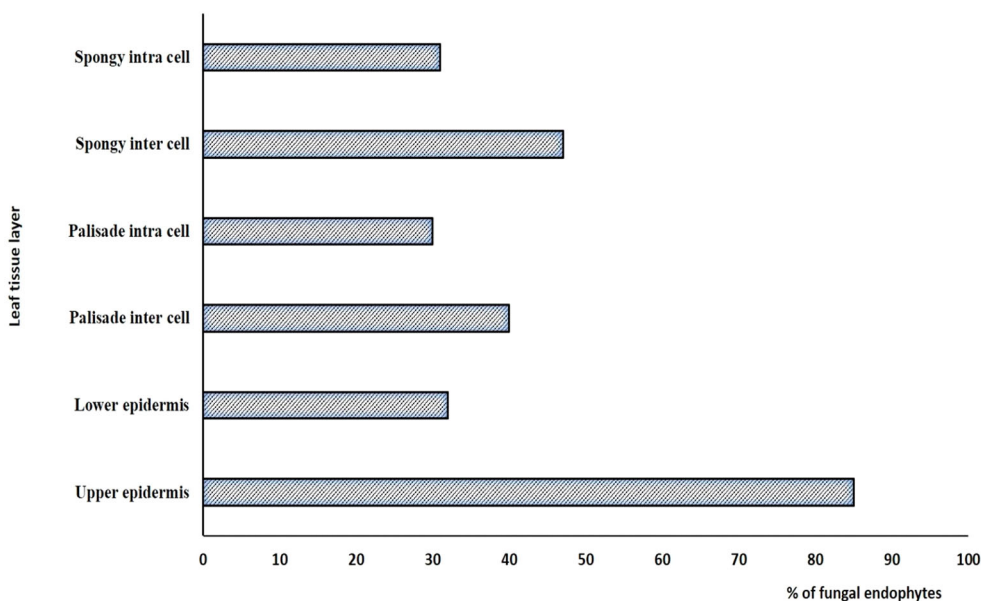


**Figure 1.** Percentage distribution of endophytic fungi in *Camellia sinensis* leaves collected from upcountry areas.

Meanwhile, the second highest distribution was observed in the intercellular spaces of the spongy mesophyll layer, followed by the intercellular spaces of the palisade layer. However, the SEM observations of Duran et al. (2005) indicated that most of the endophytic fungal hyphae were abundant on the palisade parenchyma's surface cells in the Citrus limon leaves. Compared to the intracellular levels, the intercellular spaces of the spongy and palisade layers showed higher fungal distributions than the intracellular levels. Similarly, Gomez-Vidal et al. (2006) found that the endophytic fungi in palm leaves (*Phoenix dactylifera* L.) showed higher fungal colonisation in the intercellular spaces of different tissue layers. Furthermore, they have observed vast disseminated colonisation of the endophytic fungi throughout the leaves in the palisade parenchyma, sclerenchyma, adaxial epidermis, and vascular bundle. Moreover, the finding of Kuldau and Bacon (2008) delineated a higher endophytic fungal colonisation in the intercellular spaces of leaf tissue layers of the family Poaceae due to the higher organic and inorganic nutrient availability.

The mean of five replicates is represented for each data point. By Tukey's multiple comparison test, means in the same row that shares the same letter (s) do not differ substantially ($p > 0.05$).

## Pyrene and anthracene degradation capability of endophytic fungi

Regulation 835/2011 of the European Communities set new limits on foodstuffs containing both benzo[a]pyrene and a total of four PAHs (chrysene, benzo[b]fluoranthene benzo[a]pyrene, and benzo[a]anthracene). Benzo[a]pyrene levels are limited to 10 ng/L under EU Directive (98/83/CE), while benzo[k]fluoranthene, benzo[b]fluoranthene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene levels are limited to 100 ng/L. Tea leaves in the field can be contaminated by pyrene and anthracene in two ways; one is because pollutants in the air can be deposited on the leaves, and
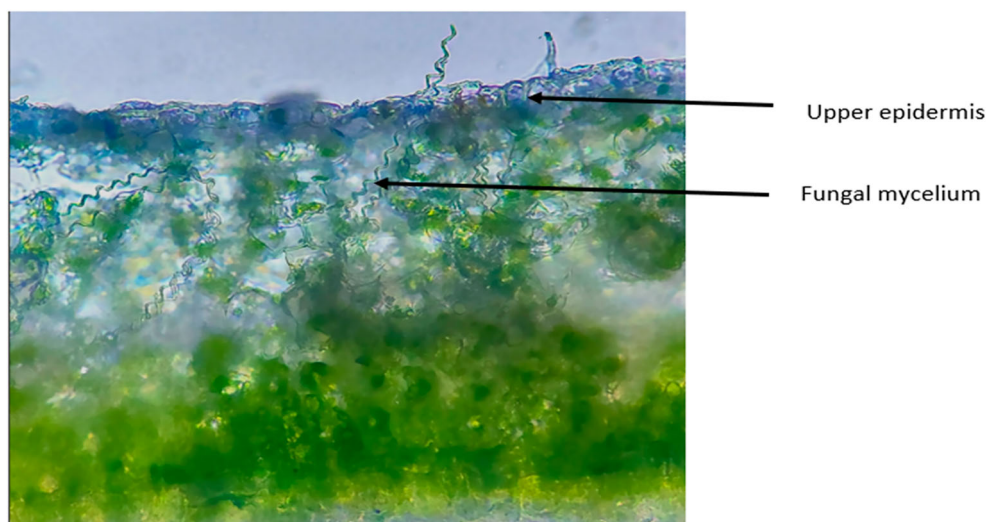


**Figure 2.** Light microscopic view of the distribution of endophytic fungi in different leaf layers of *Camellia sinensis* leaves.
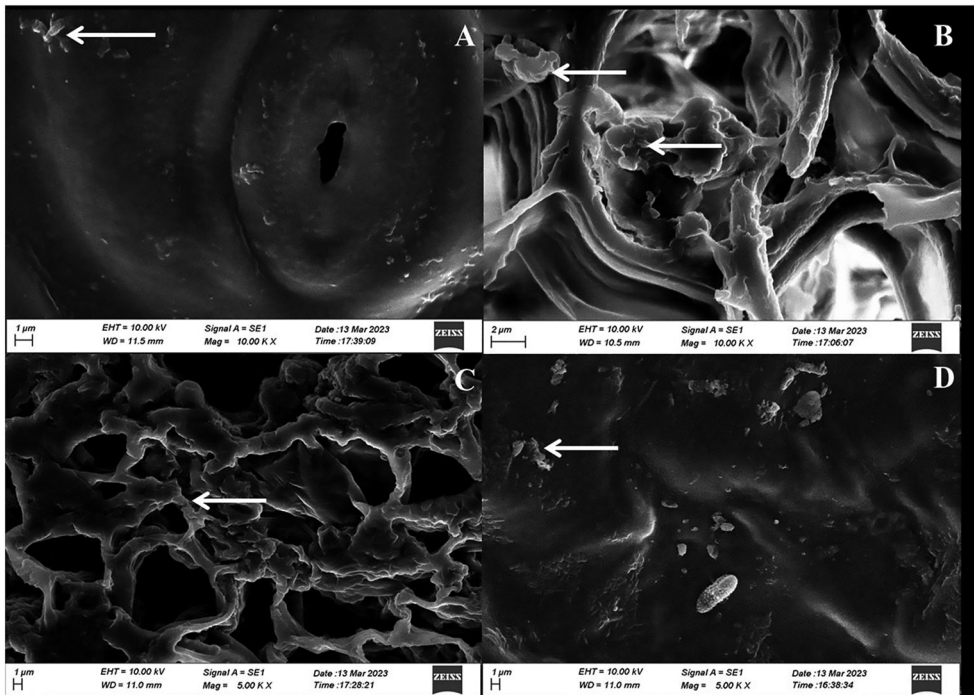
**Figure 3.** SEM view of the distribution of endophytic fungi in different leaf tissue layers of *Camellia sinensis* leaves. Upper epidermis, B – Pallisade layer, C–Spongy layer and D – Lower epidermis.

the second way is during the tea leaves processing PAH pollutants can be mixed due to many combustion steps (Phan Thi et al. 2020). However, according to our experiment results, the tea leaves were analysed just after the plucking and the average value of pyrene concentration is 2 µg/Kg, and anthracene concentration is 1 µg/Kg. Thambugala et al. (2021) revealed that the endophytic fungal diversity of Camellia sinensis inhabiting Kandy, Nuwara Eliya, and Kegalle districts in Sri Lanka is dominated by *Colletotrichum gloeosporioides* (MZ160522), *Colletotrichum siamense* (MZ160504), *Daldinia eschscholtzii* (MZ160510), *Phyllosticta capitalensis* (MZ160517) and *Pseudopestalotiopsis chinensis* (MZ160509). Simultaneously, research findings showed that five fungal strains were the best in vitro pyrene and anthracene degraders. Among them, *P. capitalensis* showed the highest pyrene (97%) and anthracene (89%) degradation capabilities, followed by two *Colletotrichum* spp., which showed more than 80% of pyrene and anthracene degradation (Figure 4). Even though Xie et al. (2020) revealed that *Colletotrichum* spp. were inhabited in the endophytic microbiota in *Camellia sinensis*, no research findings show their pyrene and anthracene degradation. *Phyllosticta capitalensis* exhibited the highest frequency of occurrence (Thambugala et al. 2021) and in this study, it showed the best pyrene and anthracene degradation abilities. The higher occurrence of *Phyllosticta capitalensis* may be due to their excellent adaptation to degrade the PAH depositions on the tea phyllosphere, leading them to survive in the niche.
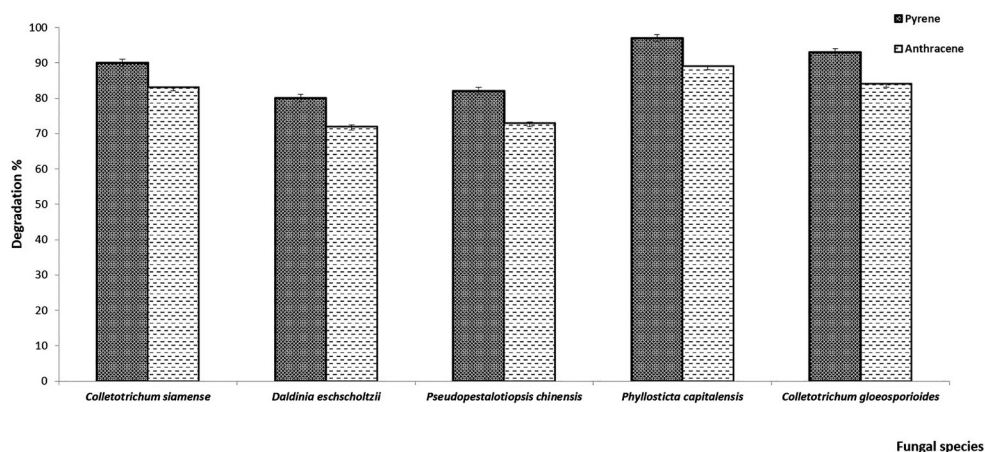
**Figure 4.** Pyrene and anthracene degradation % of endophytic fungi.

## Pyrene degradation kinetics of five endophytic fungal species

The plot of the natural logarithm of pyrene versus time showed a linear correlation coefficient value of more than 0.95 which revealed the degradation pattern fitted to the Monod first order kinetic equation that is $\ln C_t = -kt + A$, where $C_t$ is the pyrene concentration at time $t$, $t$ represents time, $k$ is the first-order rate constant and $A$ is a constant. The lowest half-life was shown by *P. capitalensis* which was 3.02 days compared to the other microorganisms. *Colletotrichum. gloeosporioides* had a half-life which was 4.45 days (Table 1). These findings further revealed when the biodegradation rate constant is getting high, their half-life time is getting reduced. In fact, the highest pyrene degradation rate constant is shown by *P. capitalensis,* with a determination coefficient ($R^2$) of 0.98. Secondly, the highest pyrene degradation kinetics was shown by *C. gloeosporioides* with a degradation rate constant of 0.1559 per day. The other three fungal endophytes isolated from the fresh leaves of *Camellia sinensis* showed a half-life ranging from 9–14 days, and their degradation rate constants are quite similar.

The analysis results of the remaining pyrene concentration in the medium for eight days of incubation by each fungal species are shown in Figure 5A. All five fungal strains degrade pyrene slowly until the second day, and afterward, a rapid reduction of pyrene level could be seen because of the increment of degradation rate. These results revealed that after the second day, fungal strains moved onto exponential growth of their growth curve and utilised pyrene as their sole carbon source. This exponential growth continued until the fifth day of incubation, and after that, we observed less pyrene degradation due to the microbial movement into the stationary phase

**Table 1.** Pyrene degradation kinetic of endophytes in tea phyllosphere.

| Pyrene degrading strain | First-order kinetic equation | Rate constant k (per day) | Half-life (Days) | $R^2$ |
| --- | --- | --- | --- | --- |
| *P. capitalensis* | $c_{pyr} = -0.2296t + 4.984$ | 0.2296 | 3.02 | 0.98 |
| *C. gloeosporioides,* | $c_{pyr} = -0.1559t + 4.875$ | 0.1559 | 4.45 | 0.98 |
| *C. siamense* | $c_{pyr} = -0.0743t + 4.727$ | 0.0743 | 9.33 | 0.97 |
| *P. chinensis* | $c_{pyr} = -0.0664t + 4.715$ | 0.0644 | 10.76 | 0.97 |
| *D. eschscholtzii* | $c_{pyr} = -0.0671t + 4.710$ | 0.0671 | 14.35 | 0.95 |

(Figure 5A). *Phyllosticta capitalensis* had the highest pyrene degradation (80%) due to the lowest half-life time and the highest rate that leads to more than 50% degradation within three days of incubation. However, after the fifth day *P. capitalensis* reached the stationary phase of their growth curve, maintaining an almost constant degradation pattern. All endophytes maintained more than a 10% degradation rate. Additionally, P. capitalensis can degrade high pyrene concentrations, such as 100 mg/L – 450 mg/L. At the highest pyrene concentration (450 mg/L), 42% of pyrene degradation was observed without toxicity. Furthermore, *C. gloeosporioides* showed a second higher degradation rate when exposed to different pyrene levels. Other remaining three fungal strains, *C. siamense, D. eschscholtzii* and *P. chinensis* had low degradation rates even at 100 mg/L of pyrene concentration (Figure 5B). After 350 mg/L concentrations, these three fungal strains showed the lowest degradation rate; reaching 450 mg/L, it was almost below 10%. This may be due to exceeding the minimum tolerating capacity of pyrene to these fungal species. Sack et al. (1997) revealed that wood-decaying fungi *Kuehneromyces mutabilis* and *Trametes versicolor* showed the pyrene degradation ability. Furthermore, the white-rot fungus Pleurotus ostreatus could degrade pyrene and anthracene very well (Bezalel conyr. 1996). The indigenous fungi *Penicillium simplicissimum, P. janthinellum, P. funiculosum,* and *P. terrestre* isolated from the soil samples collected from the gasworks sites showed higher pyrene degradation ability (Saraswathy and Hallberg 2002). Furthermore, the findings of Al-Hawash et al. (2021) revealed that *Ceriporia lacerata* RF-7 isolated from the contaminated soil of the Rumaila oil field had a higher pyrene degradation ability. Although much research was conducted to show endophytic bacteria's pyrene and anthracene-like PAH degradation capability, phyllosphere endophytic fungal-related degradation studies are minimal.

## Anthracene degradation kinetics of phyllosphere fungal endophytes

*Phyllosticta capitalensis* was able to degrade more than 80% of anthracene within eight days of the incubation period. After the third day of degradation, it shows a rapid decline in the anthracene concentration remaining in the medium by revealing the
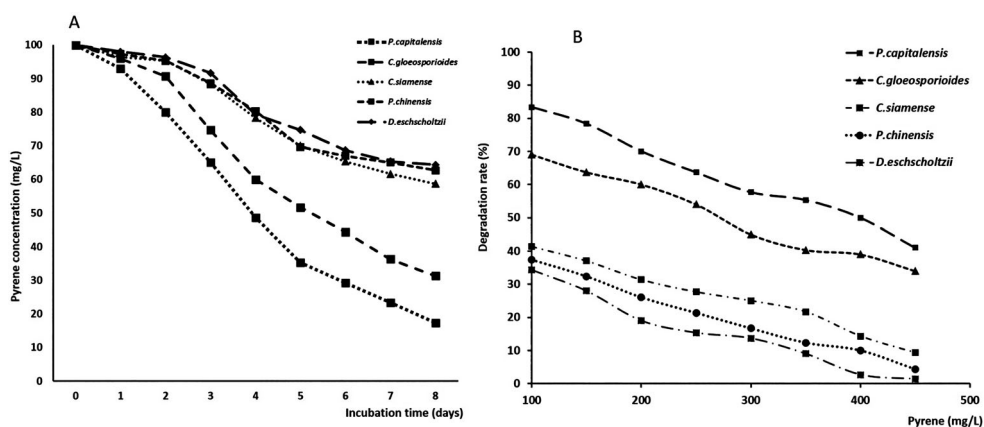


**Figure 5. A.** Different concentrations of pyrene degradation rates of endophytic fungi, **B.**100 mg/L of pyrene degradation with the incubation time.

rapid degradation of anthracene. Most of these endophytic fungi increment in the degradation rate after the third day of degradation (Figure 6A). Until the sixth day, endophytic fungi continued their degradation rate and then came into the stationary phase. The finding of Krivobok et al. (1998) revealed that *Cunninghamella blakesleeana, Bjerkandera adusta, Cryphonectria parasitica, Ceriporiopsis subvermispora, C. elegans*, and *Oxysporus* sp. isolated from soil samples showed higher anthracene degradation ability. Furthermore, the ligninolytic fungi (*Phanerochaete chrysosporium, Irpex lacteus,* and *Pleurotus ostreatus*) and three non-ligninolytic fungi were found in the cork itself. *Aspergillus niger, Mucor racemosus* and *Penicillium simplicissimum* were able to degrade anthracene in the contaminated cork bar (Jove et al. 2016). However, there is no finding to convince the anthracene degradation ability of endophytic fungal species to inhabit the tea phyllosphere. The anthracene degradation rate of *P. capitalensis* is higher than that of other isolated endophytic fungal species from the phyllosphere of fresh tea leaves (Figure 6A). After the 300 mg/L concentration, all fungal species showed a sudden drop in their degradation rate. Furthermore, until 450 mg/L concentration all fungal species showed more than a 20% degradation rate. However, *P. capitalensis* showed more than 30% degradation even at 450 mg/L concentration.

The results in Table 2 revealed that the high coefficient of determination values of each fungal species in their pyrene degradation convinced their anthracene degradation kinetics to fit the first-order kinetic model well. These findings further revealed when the biodegradation rate constant is getting high, their half-life time is getting reduced. In fact, the highest anthracene degradation rate constant is shown by *P. capitalensis,* and it had the highest pyrene degradation rate constant (0.2261) and the lowest half-life, 3.07 days under the coefficient of determination ($R^2$) of 0.97. Secondly, the highest anthracene degradation kinetics was shown by *C. gloeosporioides* with a degradation rate constant of 0.1405 and a half-life of 4.93 days. The other three fungal endophytes isolated from the fresh leaves of *Camellia sinensis* showed a half-life ranging from 7-11, and their degradation rate constants are quite similar.

*Phyllosticta capitalensis* showed the highest degradation rate in pyrene and anthracene, as well as their degradation rates being similar to each other. Moreover, the half-life of anthracene degradation of *P. capitalensis* is also similar to the half-life of pyrene
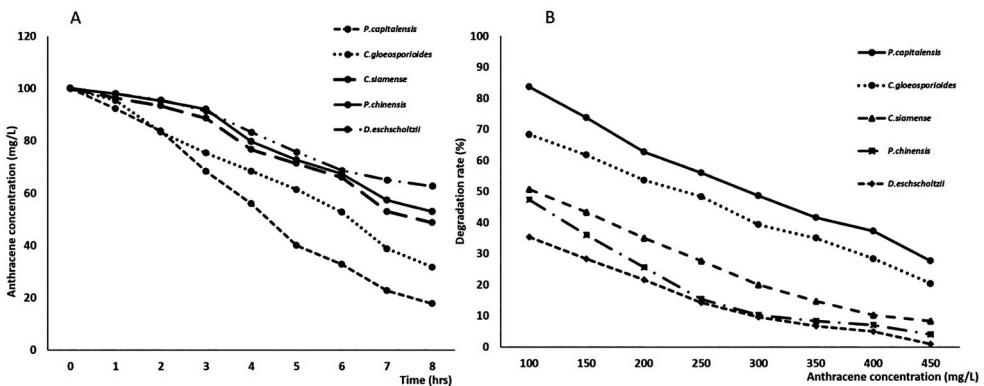


**Figure 6. A.** Different concentrations of anthracene degradation rates of endophytic fungi, **B.** 100 mg/ L of anthracene degradation with the incubation time.

**Table 2.** Anthracene degradation kinetic of endophytes in tea phyllosphere.

| Degrading strain | Degradation kinetic equation | Rate constant k (per day) | Half-life | $R^2$ |
|---|---|---|---|---|
| P. capitalensis | $C_{ant} = -0.2261t + 5.0164$ | 0.2261 | 3.07 | 0.97 |
| C. gloeosporioides, | $C_{ant} = -0.1405t + 4.8494$ | 0.1405 | 4.93 | 0.95 |
| C. siamense | $C_{ant} = -0.0931t + 4.7815$ | 0.0931 | 7.44 | 0.95 |
| P. chinensis | $C_{ant} = -0.0847t + 4.7746$ | 0.0847 | 8.18 | 0.95 |
| D. eschscholtzii | $C_{ant} = -0.0659t + 4.7245$ | 0.0659 | 10.51 | 0.97 |

degradation. The previous finding of Thambugala et al. (2021) showed that *P. capitalensis* had the highest frequency of occurrence in the fresh tea phyllosphere. The higher abundance of these endophytic fungi revealed an excellent adaptation to the phyllosphere niche, and since those tea cultivation sites are situated close to the roads, these plants are exposed well to the depositions of vehicular emissions (Zachara et al. 2018). Since most of these vehicular emissions are PAHs, these endophytic fungal species were able to degrade pyrene and anthracene-like PAHs efficiently.

The best degrader *P. capitalensis* was able to degrade anthracene and pyrene efficiently. Tea is a globally popular beverage that needs to be in good condition. However, daily increasing vehicular emission leads to higher PAH deposition on fresh tea leaves, and even after the tea leaf processing, some PAH amounts remain as it is. Therefore, *P.capitalensis* can be used as an efficient bioremediator to clean the deposited pollutants on fresh tea leaves in the future.

## Conclusion

Light microscopy and SEM observations highlighted a heterogeneous endophytic fungal distribution among leaf tissue layers, upper epidermis, lower epidermis, palisade intracellular, palisade intercellular, spongy intercellular, and spongy intracellular layers. In the present study, out of the isolated fungi, *Colletotrichum gloeosporioides* (MZ160522), *Colletotrichum siamense* (MZ160504), *Daldinia eschscholtzii* (MZ160510), *Phyllosticta capitalensis* (MZ160517) and *Pseudopestalotiopsis chinensis* (MZ160509) showed higher pyrene and anthracene degradation capabilities compared to other isolates. Out of the five fungal isolates, *P.capitalensis* showed the highest pyrene (0.229) and anthracene (0.2261) degradation rates. Furthermore, *P. capitalensis* showed the lowest half-life and highest degradation constant. The anthracene and pyrene degradation abilities of isolated endophytes are best fitted to the first-order kinetic model with more than 0.95 determination coefficient value. Overall, the best pyrene and anthracene degrader *P. capitalensis* can be used to remediate PAH pollutants deposited on the phyllosphere of fresh tea leaves.

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## Disclosure statement

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## References

Alexander M. 1999. Biodegradation and bioremediation. New York: Gulf Professional Publishing.

Al-Hawash AB, Al-Qurnawi WS, Abbood HA, Hillo NA, Ghalib HB, Zhang X, Ma F. 2021. Pyrene-degrading fungus *Ceriporia lacerata* RF-7 from contaminated soil in Iraq. Polycyclic Aromatic Compounds. 42(1):40–48. doi:10.1080/10406638.2020.1713183.

Ciemniak A, Kuźmicz K, Rajkowska-Myśliwiec M, Cadena MF. 2019. Assessing the contamination levels of dried teas and their infusions by polycyclic aromatic hydrocarbons (PAHs). Journal of Consumer Protection and Food Safety. 14:263–274. doi:10.1007/s00003-019-01229-1.

Drabova L, Pulkrabova J, Kalachova K, Tomaniova M, Kocourek V, Hajslova J. 2012. Rapid determination of polycyclic aromatic hydrocarbons (PAHs) in tea using two-dimensional gas chromatography coupled with time of flight mass spectrometry. Talanta. 100:207–216. doi:10.1016/j.talanta.2012.07.081.

Duran EL, Ploper LD, Ramallo JC, Piccolo Grandi RA, Hupper Giancoli ÁC, Azevedo JL. 2005. The foliar fungal endophytes of Citrus limon in Argentina. Canadian Journal of Botany. 83 (4):350–355. doi:10.1139/b05-009.

Fang GC, Wu YS, Chen MH, Ho TT, Huang SH, Rau JY. 2004. Polycyclic aromatic hydrocarbons study in Taichung, Taiwan, during 2002–2003. Atmospheric Environment. 38(21):3385–3391. doi:10.1016/j.atmosenv.2004.03.036.

Fiedler H, Cheung CK, Wong MH. 2002. PCDD/PCDF, chlorinated pesticides and PAH in Chinese teas. Chemosphere. 46(9-10):1429–1433. doi:10.1016/S0045-6535(01)00264-8.

Forster WA, Zabkiewicz JA, Riederer M. 2004. Mechanisms of cuticular uptake of xenobiotics into living plants: 1. Influence of xenobiotic dose on the uptake of three model compounds applied in the absence and presence of surfactants into *Chenopodium album, Hedera helix* and *Stephanotis floribunda* leaves. Pest Management Science: Formerly Pesticide Science. 60 (11):1105–1113. doi:10.1002/ps.918.

Garcia A, Rhoden SA, Rubin Filho CJ, Nakamura CV, Pamphile JA. 2012. Diversity of foliar endophytic fungi from the medicinal plant *sapindus saponaria* L. and their localization by scanning electron microscopy. Biological Research. 45(2):139–148. doi:10.4067/S0716-97602012000200006.

Gomez-Vidal S, Lopez-Llorca LV, Jansson HB, Salinas J. 2006. Endophytic colonization of date palm (*Phoenix dactylifera* L.) leaves by entomopathogenic fungi. Micron. 37(7):624–632. doi:10.1016/j.micron.2006.02.003.

Ishizaki A, Saito K, Hanioka N, Narimatsu S, Kataoka H. 2010. Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection. Journal of Chromatography A. 1217(35):5555–5563. doi:10.1016/j.chroma.2010.06.068.

Jove P, Olivella MÀ, Camarero S, Caixach J, Planas C, Cano L, De Las Heras FX. 2016. Fungal biodegradation of anthracene-polluted cork: a comparative study. Journal of Environmental Science and Health, Part A. 51(1):70–77. doi:10.1080/10934529.2015.1079114.

Kavouras IG, Koutrakis P, Cereceda-Balic F, Oyola P. 2001. Source apportionment of PM10 and PM25 in five Chilean cities using factor analysis. Journal of the Air & Waste Management Association. 51(3):451–464. doi:10.1080/10473289.2001.10464273.

Krivobok S, Miriouchkine E, Seigle-Murandi F, Benoit-Guyod JL. 1998. Biodegradation of anthracene by soil fungi. Chemosphere. 37(3):523–530. doi:10.1016/S0045-6535(98)00067-8.

Kuldau G, Bacon C. 2008. Clavicipitaceous endophytes: their ability to enhance resistance of grasses to multiple stresses. Biological Control. 46(1):57–71. doi:10.1016/j.biocontrol.2008.01.023.

Kuo S, Lotse EG. 1973. Kinetics of phosphate adsorption and desorption by hematite and gibbsite1. Soil Science. 116(6):400–406. doi:10.1097/00010694-197312000-00002.

Kuroda Y, Hara Y. 1999. Antimutagenic and anticarcinogenic activity of tea polyphenols. Mutation Research/Reviews in Mutation Research. 436(1):69–97. doi:10.1016/S1383-5742(98)00019-2.

Lafleur KS. 1980. Loss of pesticides from congaree sandy loam with time: characterization. Soil Science. 130(2):83–87. doi:10.1097/00010694-198008000-00006.

Lammel G, Dvorská A, Klánová J, Kohoutek J, Kukucka P, Prokes R, Sehili AM. 2015. Long-range atmospheric transport of polycyclic aromatic hydrocarbons is worldwide problem-results from measurements at remote sites and modelling. Acta Chimica Slovenica. 62(3):729–735. doi:10.17344/acsi.2015.1387.

Lin D, Tu Y, Zhu L. 2005. Concentrations and health risk of polycyclic aromatic hydrocarbons in tea. Food and Chemical Toxicology. 43(1):41–48. doi:10.1016/j.fct.2004.08.010.

Lin D, Zhu L. 2004. Polycyclic aromatic hydrocarbons: pollution and source analysis of a black tea. Journal of Agricultural and Food Chemistry. 52(26):8268–8271. doi:10.1021/jf048636n.

Lin D, Zhu L, He W, Tu Y. 2006. Tea plant uptake and translocation of polycyclic aromatic hydrocarbons from water and around air. Journal of Agricultural and Food Chemistry. 54(10):3658–3662. doi:10.1021/jf052909c.

Liu K, Han W, Pan WP, Riley JT. 2001. Polycyclic aromatic hydrocarbon (PAH) emissions from a coal-fired pilot FBC system. Journal of Hazardous Materials. 84(2–3):175–188. doi:10.1016/S0304-3894(01)00196-0.

Mei X, Lin DH, Xu Y, Wu YY, Tu YY. 2009. Effects of phenanthrene on chemical composition and enzyme activity in fresh tea leaves. Food Chemistry. 115(2):569–573. doi:10.1016/j.foodchem.2008.12.053.

Pamphile JA, Gai CS, Pileggi M, Rocha CLMSC, Pileggi SAV. 2008. Theory chapters plant-microbe interactions between host and endophytes observed by scanning electron microscopy (SEM). In: Sorvari S, Pirttilä AM, editors. Prospects and applications for plant-associated microbes. A laboratory manual, Part A: Bacteria. Finland: BBI (BioBien Innovations); p. 184–189.

Phan Thi LA, Ngoc NT, Quynh NT, Thanh NV, Kim TT, Anh DH, Viet PH. 2020. Polycyclic aromatic hydrocarbons (PAHs) in dry tea leaves and tea infusions in Vietnam: contamination levels and dietary risk assessment. Environmental Geochemistry and Health. 42(9):2853–2863. doi:10.1007/s10653-020-00524-3.

Pincemaille J, Schummer C, Heinen E, Moris G. 2014. Determination of polycyclic aromatic hydrocarbons in smoked and non-smoked black teas and tea infusions. Food Chemistry. 145:807–813. doi:10.1016/j.foodchem.2013.08.121.

Ren CG, Kong CC, Bian B, Liu W, Li Y, Luo YM, Xie ZH. 2017a. Enhanced phytoremediation of soils contaminated with PAHs by arbuscular mycorrhiza and rhizobium. International Journal of Phytoremediation. 19(9):789–797. doi:10.1080/15226514.2017.1284755.

Ren Y, Zhou B, Tao J, Cao J, Zhang Z, Wu C, Wang J, Li J, Zhang L, Han Y, Liu L. 2017b. Composition and size distribution of airborne particulate PAHs and oxygenated PAHs in two Chinese megacities. Atmospheric Research. 183:322–330. doi:10.1016/j.atmosres.2016.09.015.

Sack U, Heinze TM, Deck J, Cerniglia CE, Martens R, Zadrazil F, Fritsche W. 1997. Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. Applied and Environmental Microbiology. 63(10):3919–3925. doi:10.1128/aem.63.10.3919-3925.1997.

Saraswathy A, Hallberg R. 2002. Degradation of pyrene by indigenous fungi from a former gasworks site. FEMS Microbiology Letters. 210(2):227–232. doi:10.1111/j.1574-6968.2002.tb11185.x.

Sharma S, Kumari A, Sharma M. 2016. Comparative GC-MS analysis of bioactive compounds in methanolic extract of Calotropis gigantea (L) WT Aiton leaf and latex. International Journal of Pharmacognosy and Phytochemical Research. 8(11):1823–1827.

Thambugala K, Daranagama D, Kannangara S. 2022. Biocontrol potential of endophytic fungi in tea (*Camellia sinensis* (L.) Kuntze) leaves against selected fungal phytopathogens. Malaysian Journal of Microbiology. 18(6):665–669.

Thambugala K, Daranagama DA, Kannangara S, Kodituwakku T. 2021. Revealing the endophytic mycoflora in tea (*Camellia sinensis*) leaves in Sri Lanka: the first comprehensive study. Phytotaxa. 514(3):247–260. doi:10.11646/phytotaxa.514.3.5.

Undugoda LJS, Kannangara S, Sirisena DM. 2016a. Aromatic hydrocarbon degrading fungi inhabiting the phyllosphere of ornamental plants on roadsides of urban areas in Sri Lanka. Journal of Bioremediation & Biodegradation. 7(328):2.

Undugoda LJS, Kannangara S, Sirisena DM. 2016b. Genetic basis of naphthalene and phenanthrene degradation by phyllosphere bacterial strains *Alcaligenes faecalis* and *Alcaligenes* sp. 11SO. Journal of Bioremediation & Biodegradation. 7(2):1–5.

Venkataraman C, Lyons JM, Friedlander SK. 1994. Size distributions of polycyclic aromatic hydrocarbons and elemental carbon. 1. Sampling, measurement methods, and source characterization. Environmental Science & Technology. 28(4):555–562. doi:10.1021/es00053a005.

Vinas P, Campillo N, Aguinaga N, Pérez-Cánovas E, Hernández-Córdoba M. 2007. Use of headspace solid-phase microextraction coupled to liquid chromatography for the analysis of polycyclic aromatic hydrocarbons in tea infusions. Journal of Chromatography A. 1164(1–2):10–17. doi:10.1016/j.chroma.2007.06.056.

Wagrowski DM, Hites RA. 1998. Partitioning of polychlorinated dibenzo-p-dioxins and dibenzofurans between the atmosphere and corn. Environmental Science & Technology. 32(16):2389–2393. doi:10.1021/es9706920.

Xie H, Feng X, Wang M, Wang Y, Kumar Awasthi M, Xu P. 2020. Implications of endophytic microbiota in *Camellia sinensis*: a review on current understanding and future insights. Bioengineered. 11(1):1001–1015. doi:10.1080/21655979.2020.1816788.

Zachara A, Gałkowska D, Juszczak L. 2018. Contamination of tea and tea infusion with polycyclic aromatic hydrocarbons. International Journal of Environmental Research and Public Health. 15(1):45. doi:10.3390/ijerph15010045.

Ziegenhals K, Jira W, Speer K. 2008. Polycyclic aromatic hydrocarbons (PAH) in various types of tea. European Food Research and Technology. 228:83–91. doi:10.1007/s00217-008-0909-8.