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Dioxygenases present in phenanthrene and fluoranthene degradation by bacterial and fungal co-cultures

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PAHs

Polyaromatic Hydrocarbons (PAH) are Ubiquitous (present in air, water and soil)

PAHs in soil (most common)

- **Soils are reservoir of PAHs because PAHs**
  - **readily adsorb to soil particles**
  - **persistently remain in soil due to their hydrophobic**

- **Impacts negatively on ecosystem sustainability**

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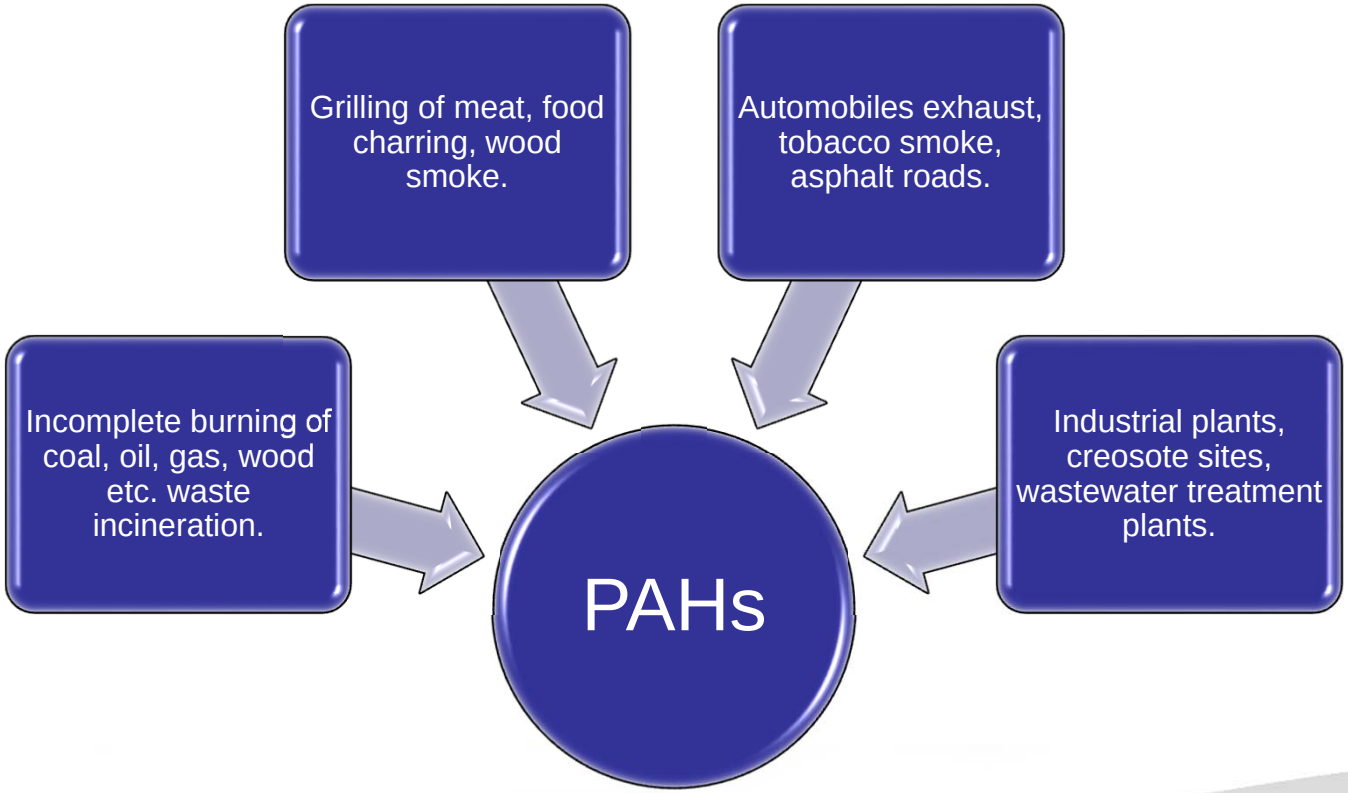
PAHs (Examples)



Phenanthrene and fluoranthene – typical polycyclic aromatic hydrocarbons (PAHs)

- **Phenanthrene are considered prototypic PAHs and serve as signature compounds to detect PAH contamination**

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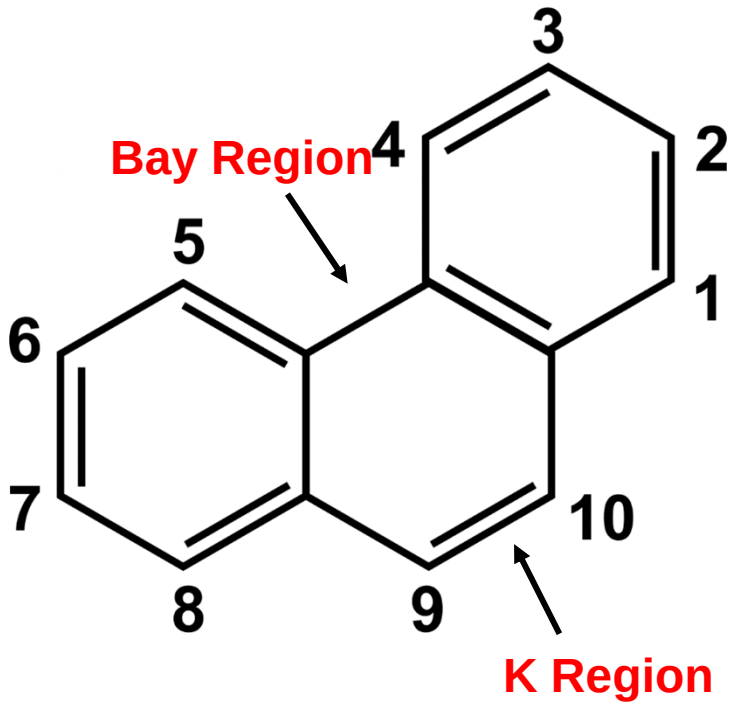
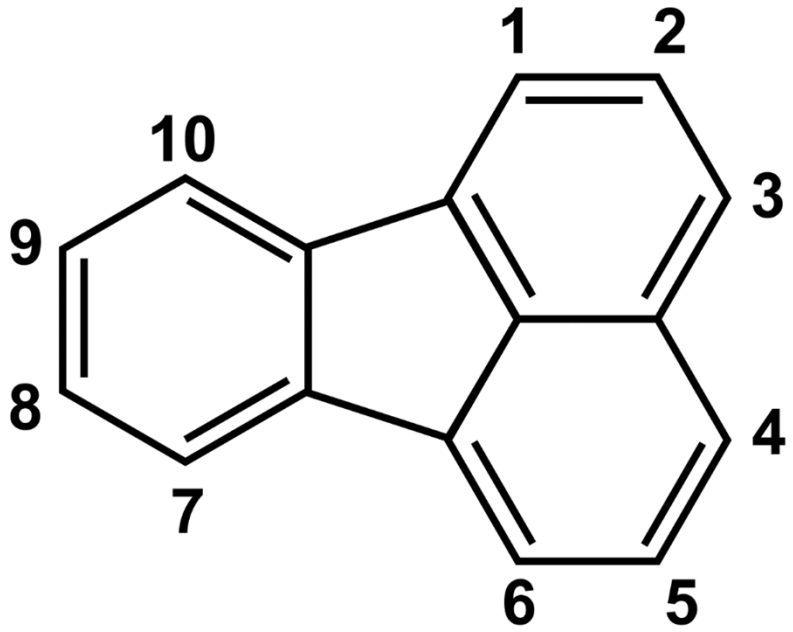
Phenanthrene and fluoranthene – typical polycyclic aromatic hydrocarbons (PAHs)



Phenanthrene



Fluoranthene

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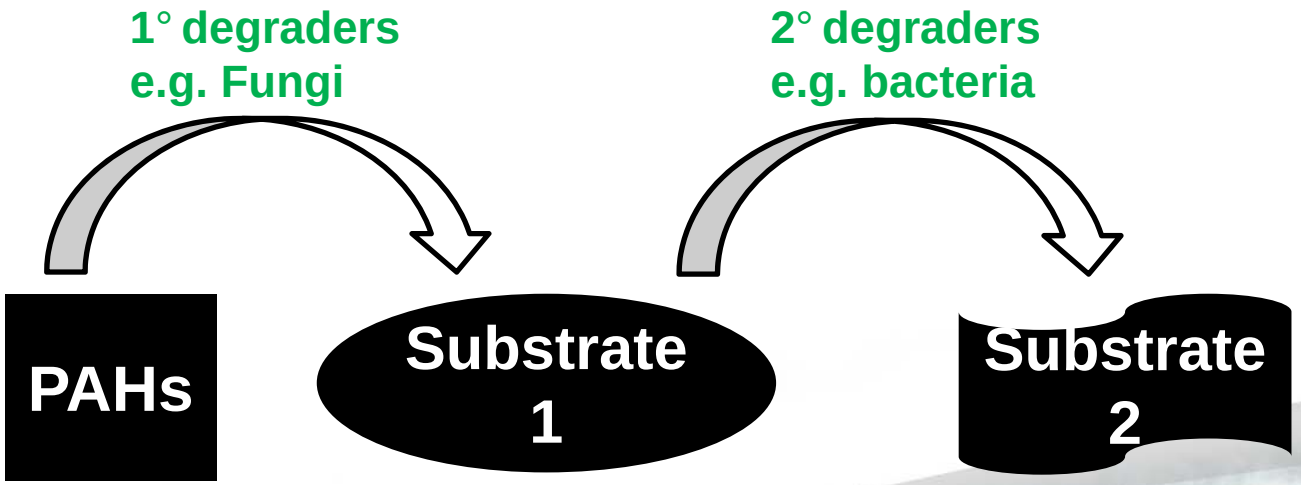
PAH Biodegradation by co-cultures

- **Different organisms (bacteria, fungi, plants, algae, archaea, earthworms etc.) are used**
- **Co-cultures are recommended for enhanced biodegradation**



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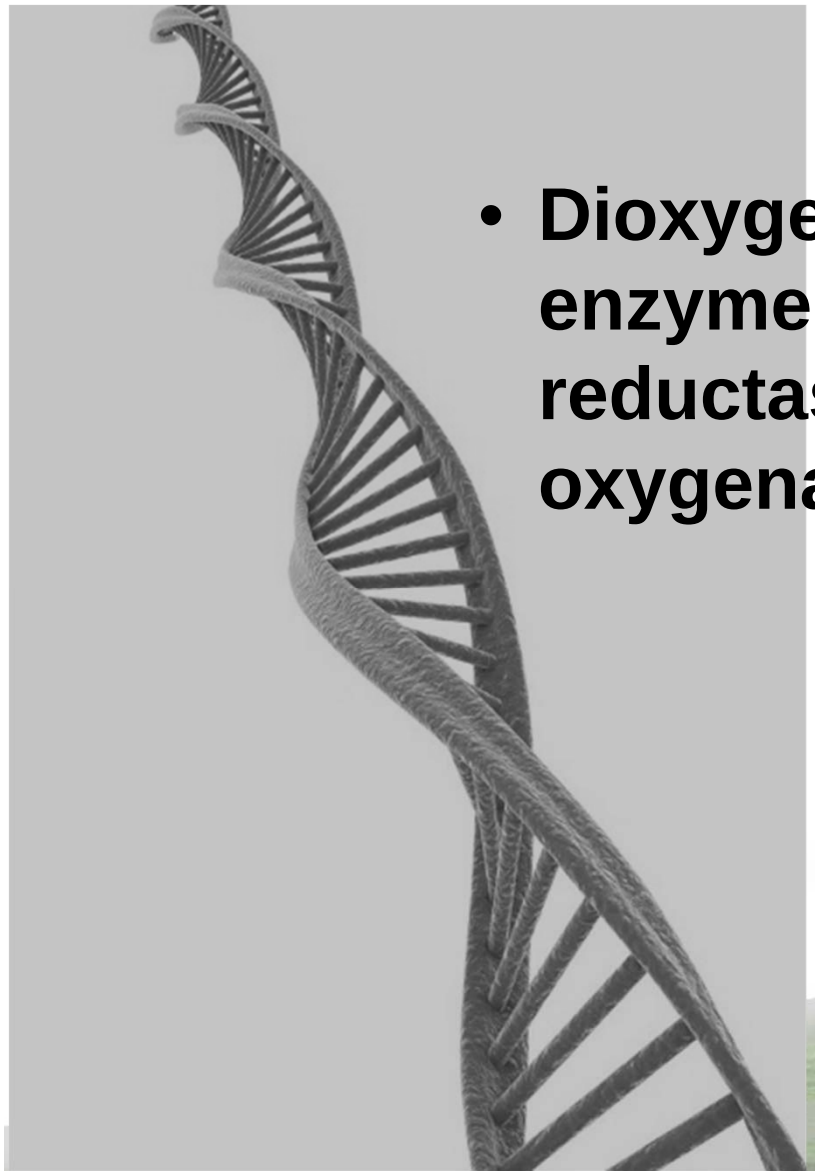
Dioxygenases (PAH-RHD) in PAH biodegradation



- **Dioxygenase is a multicomponent enzyme generally consisting of reductase, ferredoxin, and terminal oxygenase subunits**

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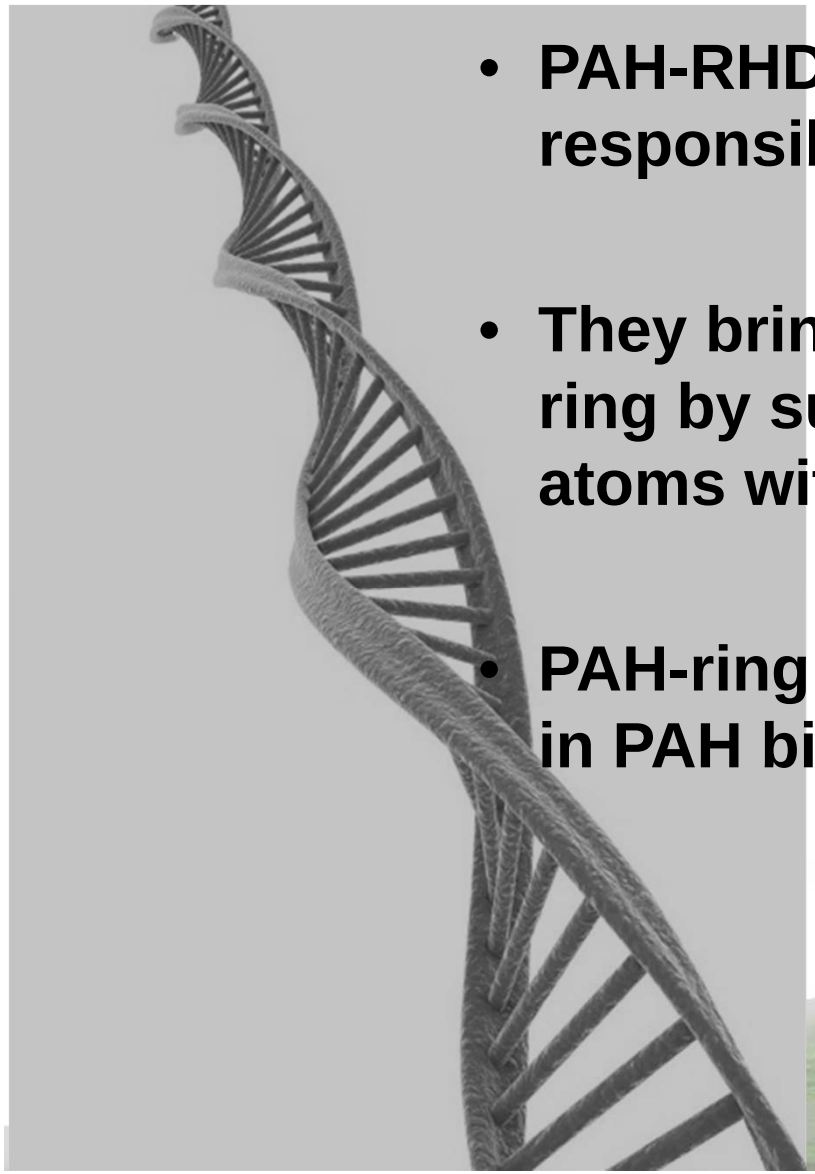
Dioxygenases (PAH-RHD) in PAH biodegradation



- **PAH-RHDs are usually bacterial enzymes responsible for opening the PAH ring**
- **They bring about hydroxylation of aromatic ring by substitution of adjacent hydrogen atoms with hydroxyl groups**
- **PAH-ring hydroxylation is a rate-limiting step in PAH biodegradation**

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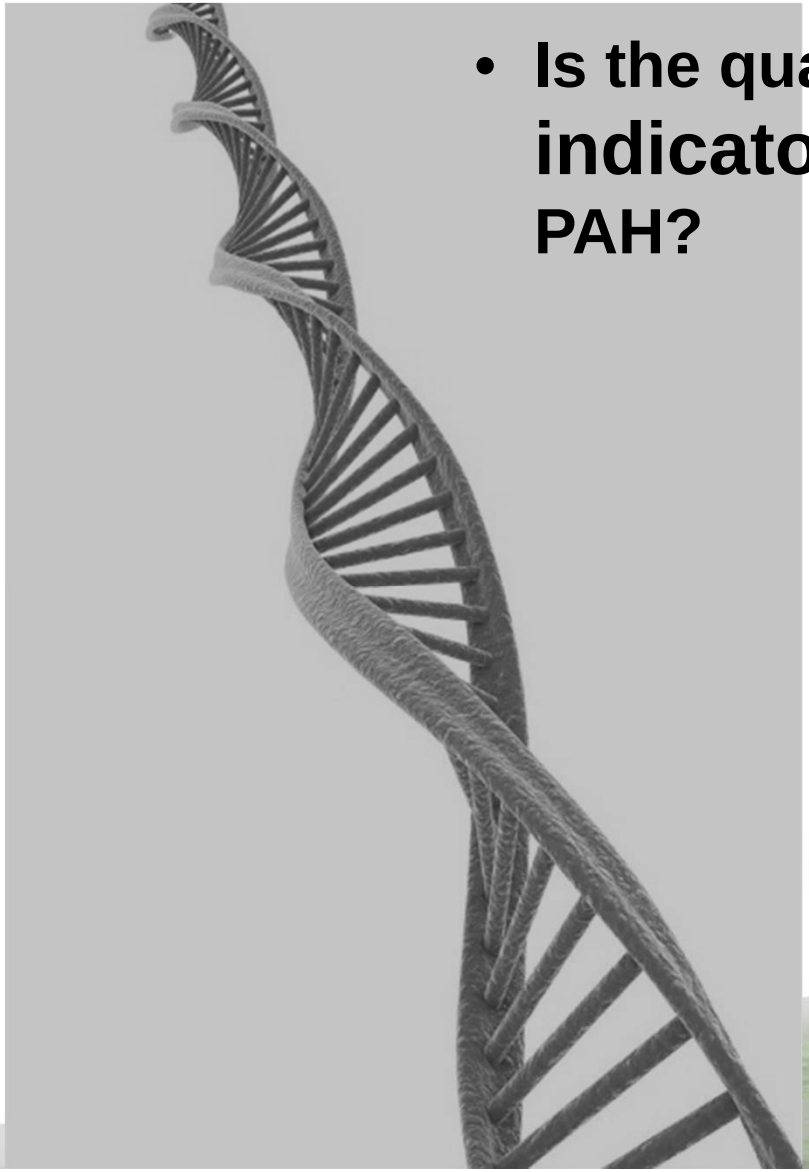
Dioxygenases (PAH-RHD) in PAH biodegradation



- **Is the quantification of PAH-RHD sufficient indicator for aerobic biodegradation of PAH?**

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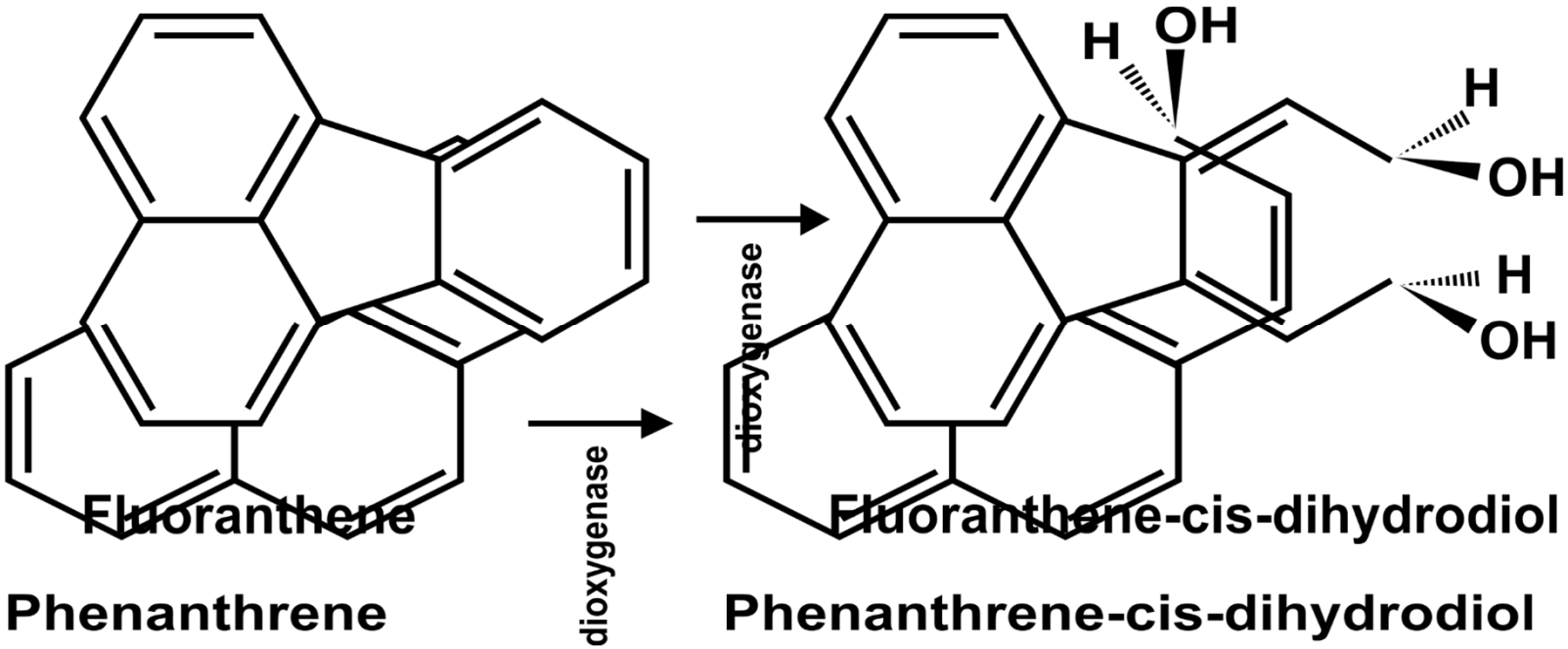
Dioxygenase in action



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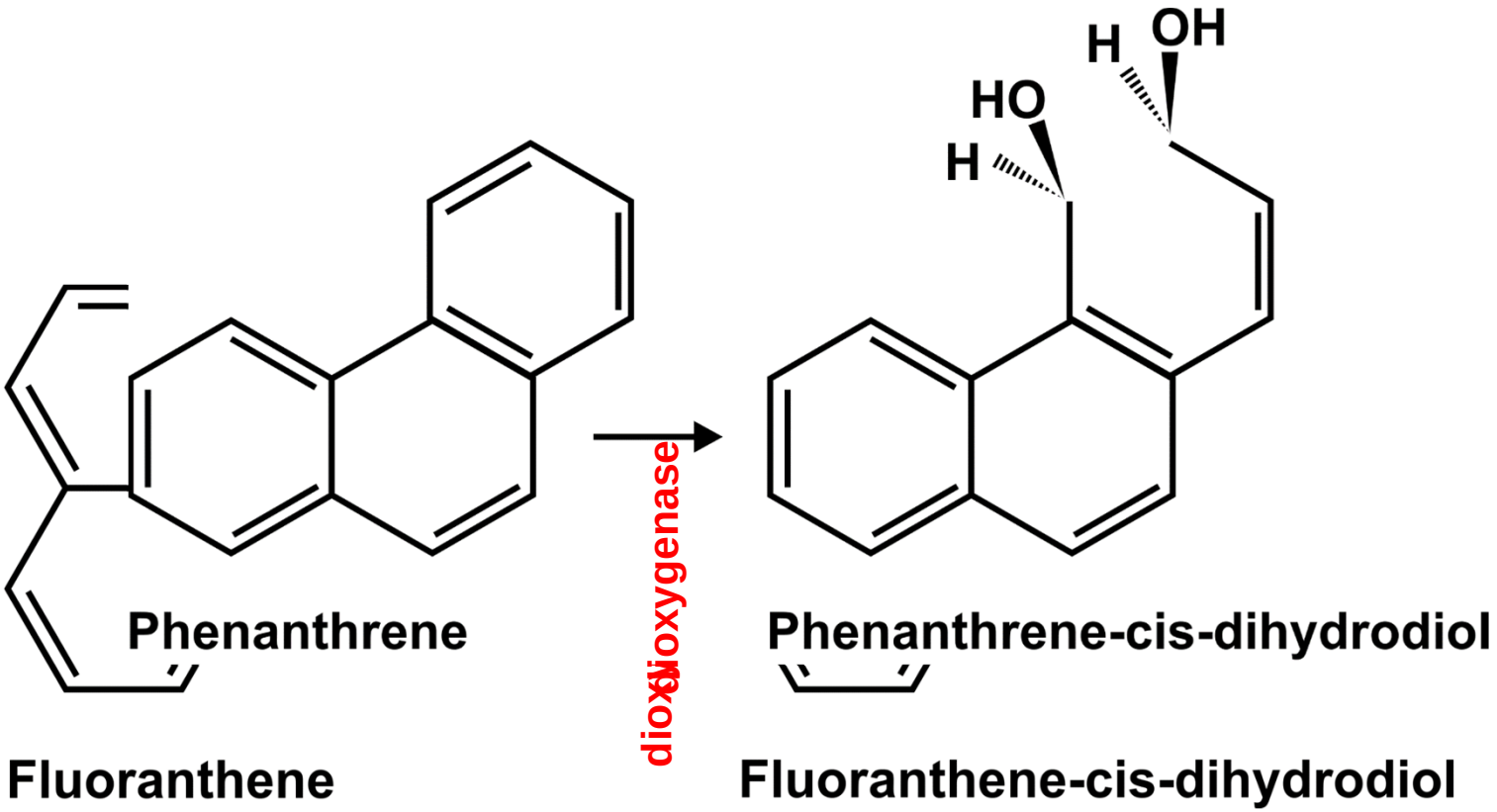
Dioxygenase in action



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Significance/justification

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Aim

Evaluation of specific bacterial degradation genes involved in biodegradation of PAHs by the synergism of mechanism of ectomycorrhizal fungi and rhizosphere bacteria in association with pine plant

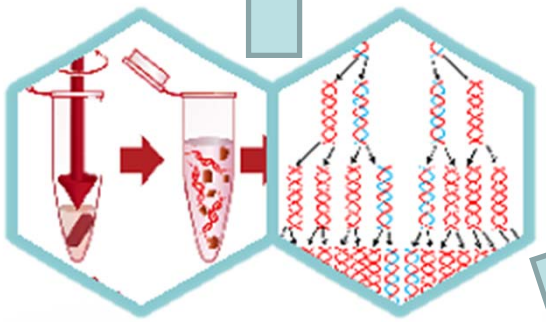
Methods

Experimental set up

ECMF d bacteria introduction

Samples taken for analyses

DNA extraction from soil samples for PCR, qPCR, NGS



GC-MS

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Characterisation of bacterial isolates

- **Bacterial isolates were obtained from artificially polluted agricultural soil**
- **Isolates were screened for their PAH biodegradation capabilities as well as soil fertility attributes such as phosphate solubilization, atmospheric nitrogen fixation, and indoleacetic acid (IAA) production**

  – **To enhance growth of pine plant during the degradation process**

Compatibility study

- **Co-existence of ectomycorrhizal fungi (ECMF) bacterial isolates and *Pinus patula*.**
- **PAH tolerance of each of the ECMF and pine seedlings was evaluated.**

Methods

Organisms and soil obtained and prepared

Phe and Fl introduced into soil at 0.1 g/Kg

Planting and germination of Pine

Bacteria and fungi introduced

Treatment Description	
PAH soil with ECMF, pine plant and bacteria	PEPB
PAH soil with bacteria and pine plant without ECMF	PBP
PAH soil with ECMF and pine plant without bacteria	PEP
PAH soil with ECMF and bacteria without pine plant	PEB
A negative control PAH soil with no organisms	PS
Second control with no PAHs, but just soil with pine plant	C

Analyses

1.Molecular analyses

2.Root staining for mycorrhization

3. PAH extraction, GC-MS

Statistical analysis of results

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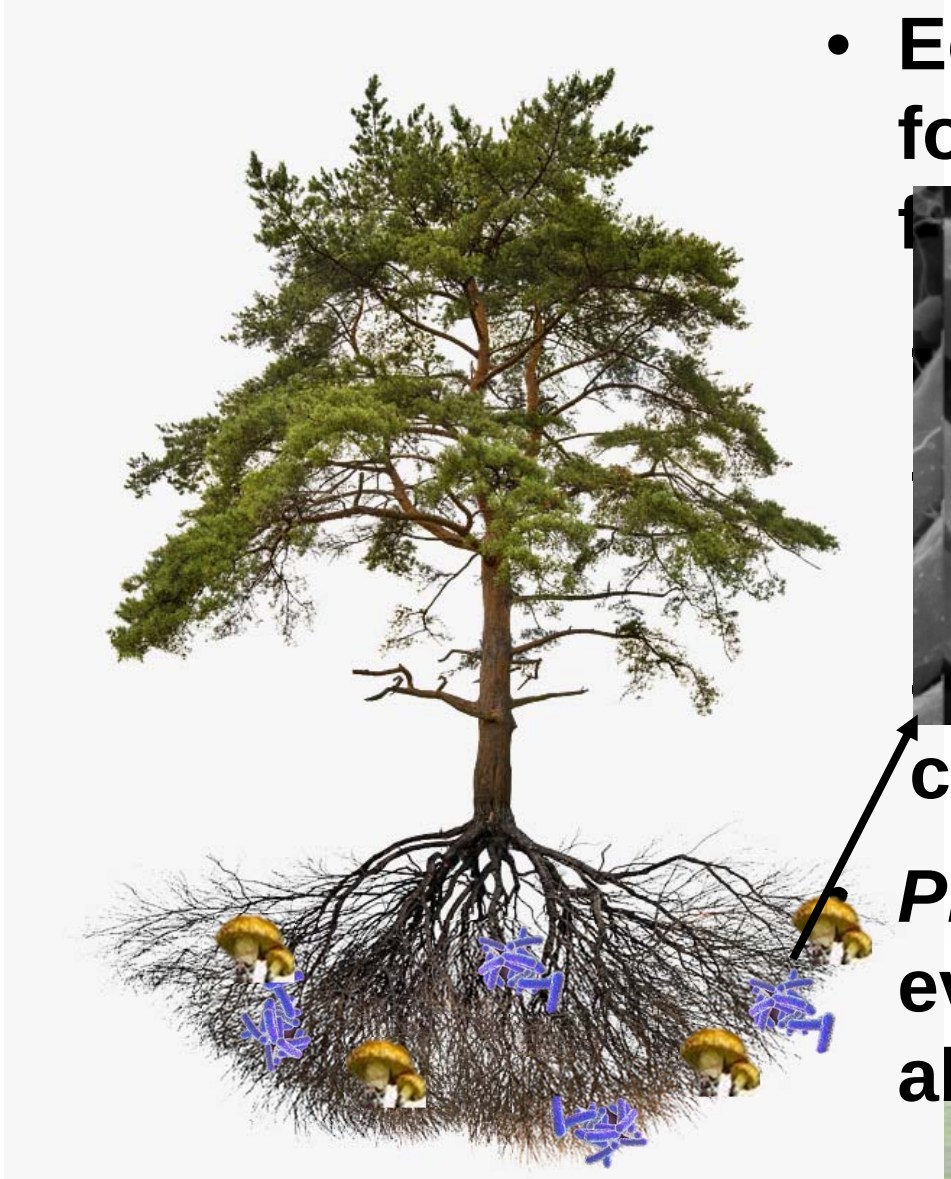
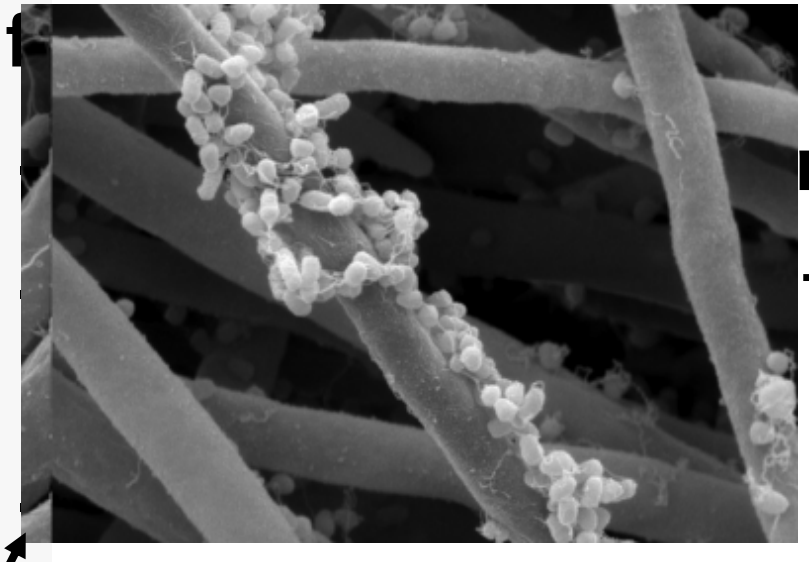
Pinus mycorrhizosphere



- Ectomycorrhizae are known for various ecosystem f ment from contaminants



- *Pinus patula* is an evergreen plant and it's abundant in South Africa

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Results – Bacterial Isolates

- **PAH-degrading bacteria from enriched cultures:**
  - **A total of 44 isolates**
  - ***Acinetobacter, Arthrobacter, Bacillus, Flavobacterium, Microbacterium, Ochrobactrum, Pseudomonas, Pseudoxanthomonas. Rhodococcus* and**

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Research Article

Potential of Polycyclic Aromatic Hydrocarbon-Degrading Bacterial Isolates to Contribute to Soil Fertility

Maryam Bello-Akinosho,[1,2] Rosina Makofane,[2,3] Rasheed Adeleke,[2,4] Mapitsi Thantsha,[1] Michael Pillay,[3] and George Johannes Chirima[2,5]

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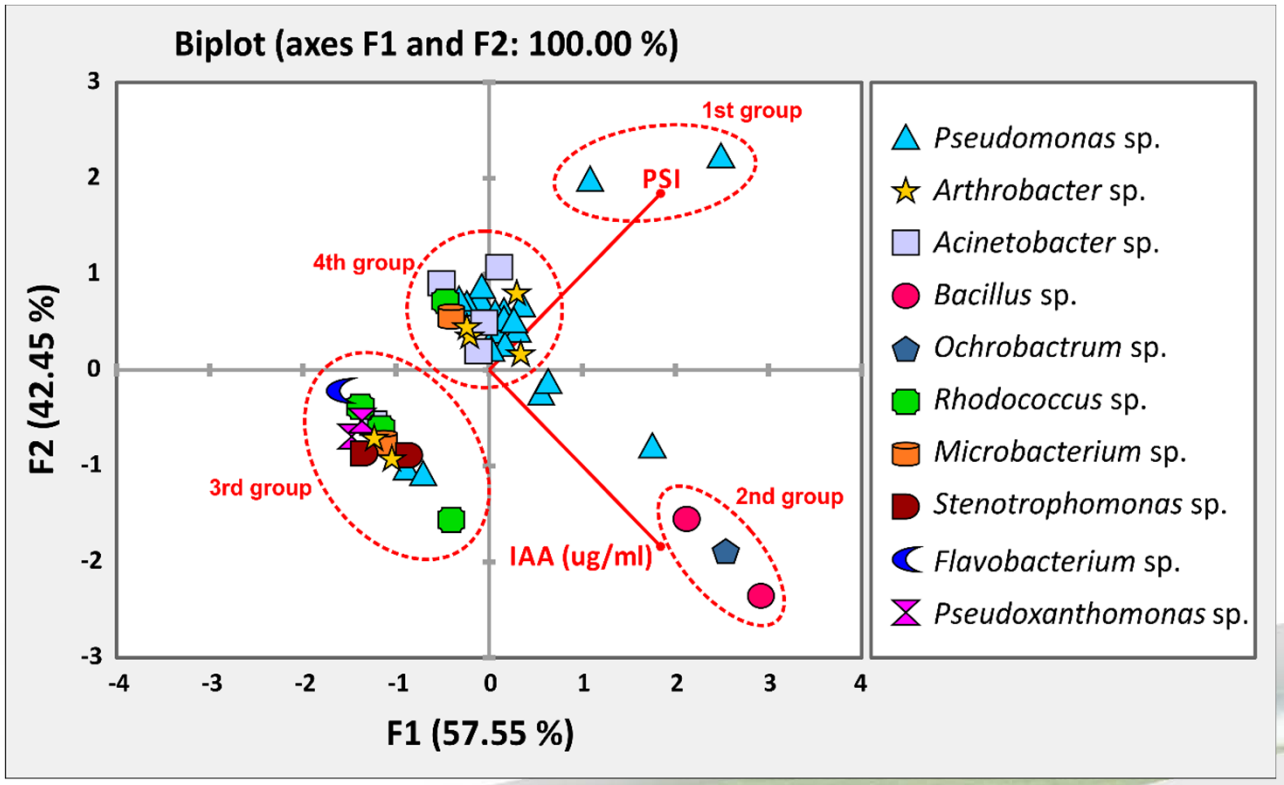
Results – Selection of Bacterial Isolates

- ***Pseudomonas, Acinetobacter, Arthrobacter* and *Rhodococcus* had the best soil fertility attributes**



Principal component analysis (PCA) of the 44 isolates in relation to their abilities to solubilize phosphate (PSI) and produce indoleacetic acid (IAA) as a function of their potential ability to contribute to soil fertility.

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Results – Selection of Bacterial Isolates

- **Only *Acinetobacter, Arthrobacter* and *Rhodococcus* were selected for the co-culture experiment**

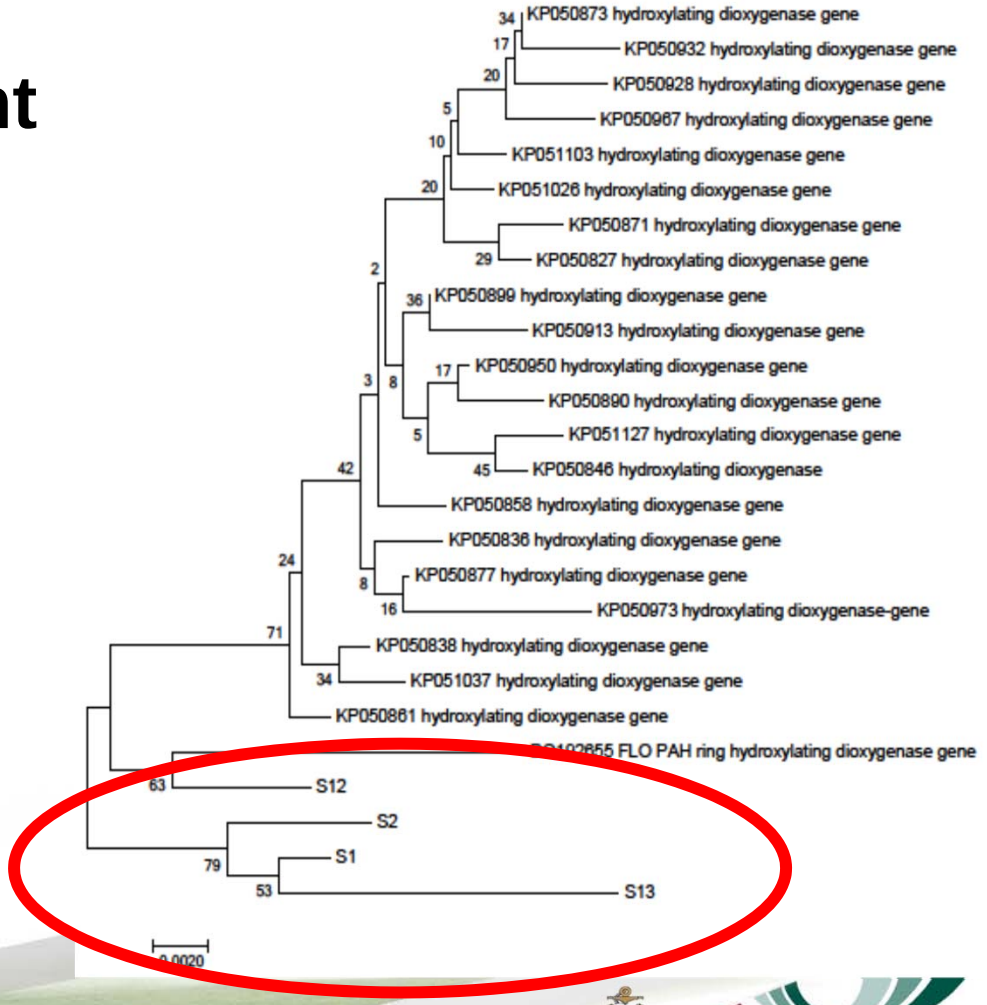
Results – Co-culture experiment

- The ectomycorrhizal culture was a mixture of the 3 ECMF - *Suilus tomentosus*, *Boletus edulis* and *Lacaria bicolor*

- For the bacterial inoculum, a mix of 3 bacteria- *Ochrobactrum*, *Bacillus* and *Rhodococcus*

Results – PAH-RHD genes

- **Abundant in all samples with Phe and Fl irrespective of treatment**
- **These genes formed different clade from existing genes**



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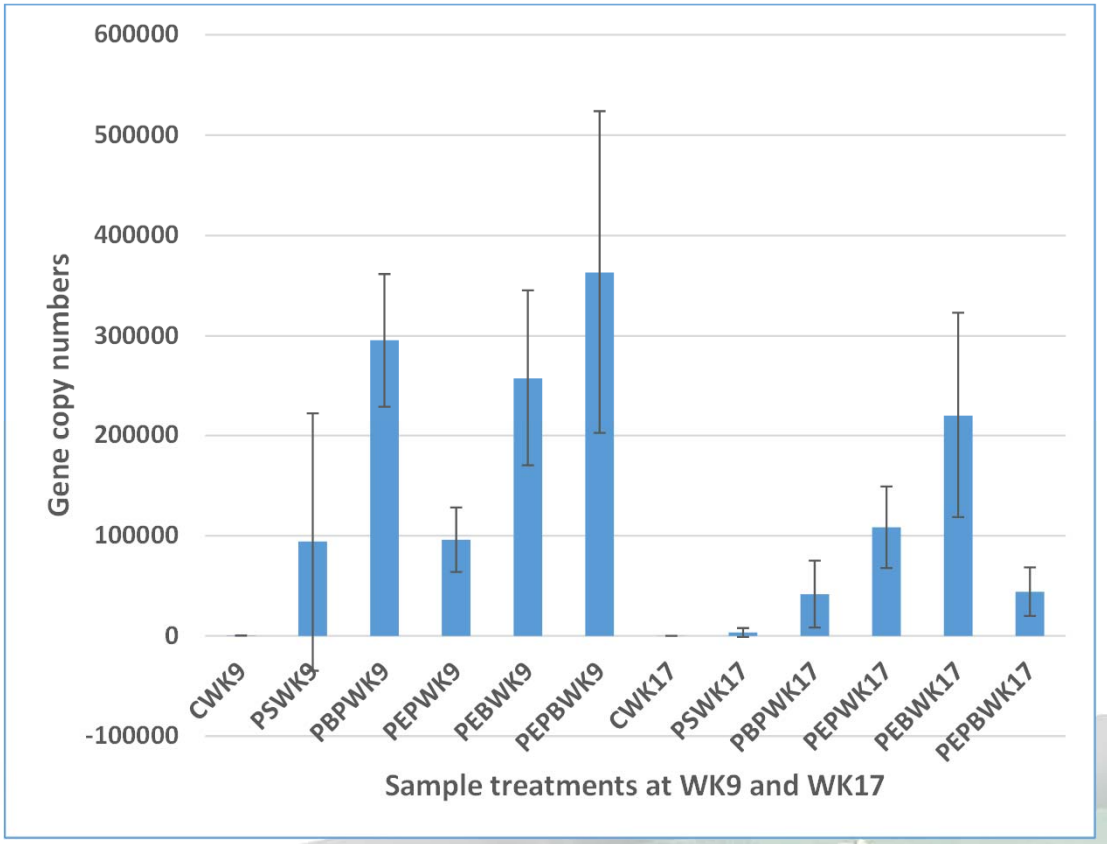
Gene copy numbers

- **PAH-RHD gene copy number in the range 4 to 3.68 X 10^5**



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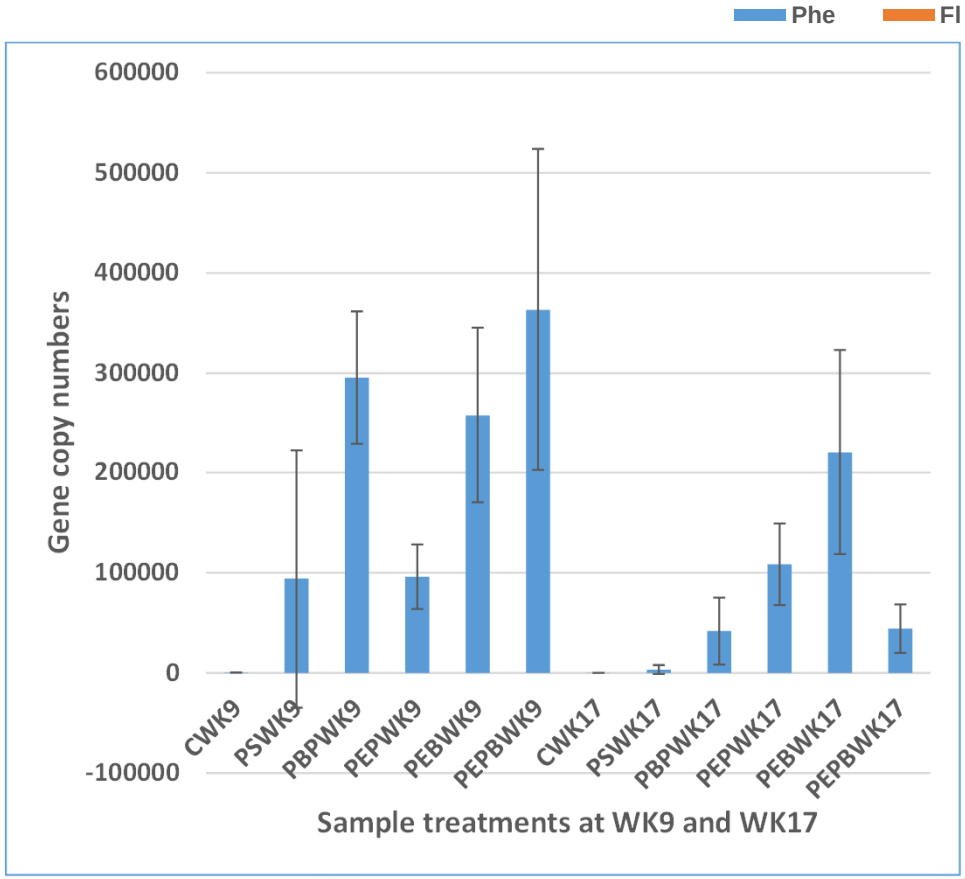
Soil residual PAHs



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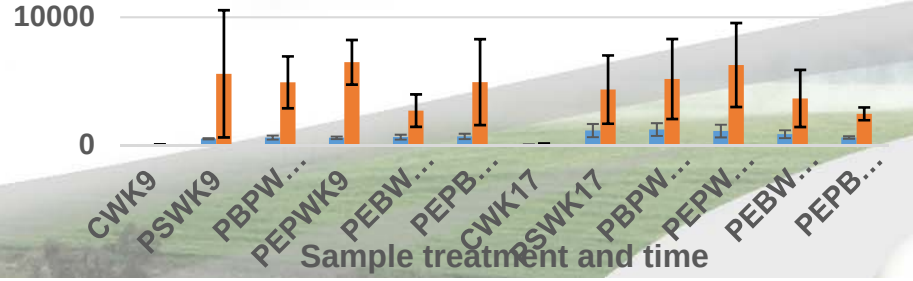

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Discussion and significance of results

- **The degradation of Ph and Fl is a complicated process - different catabolic pathways are involved and many intermediates are produced, accumulated and consumed when the degradation proceeds**
- **1,2-Dioxygenase and 2,3-Dioxygenase are usually responsible for the first step in the aerobic degradation of polyaromatic compounds, catalyzing the hydroxylation of the substrate to the corresponding cis-dihydrodiol**
- **The catalytic meta-cleavage of catechol by 1,2-Dioxygenase and 2,3-Dioxygenase seems to be the most common pathway in the subsequent steps of PAH degradation.**
- **We are now in the process of tracing the activities of these two important enzymes in our research**

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Discussion and significance of results

- **Higher residual Fl content than the Phe in all treatments**
- **Dissipation of Phe higher, a range of 98.92 - 99.92%, than found in Fl, range of 88.35 – 95.14%**
- **The higher dissipation of Phe over Fl could be attributed to volatility as well as mass transfer rate of PAHs to microorganisms being influenced by PAH. A higher PAHs concentration would then influence a higher microbial degradation of the PAH**

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Conclusion and recommendation

- **Co-cultures of bacteria and fungi especially in the presence of plants are self-sustaining, thus promising to be exploited in soil PAH biodegradation**

- **Bacterial dioxygenases, which are biomarkers of PAH-degrading bacteria, were abundantly present, signifying active biodegradation**

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Thank you for listening!

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QUESTIONS?

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