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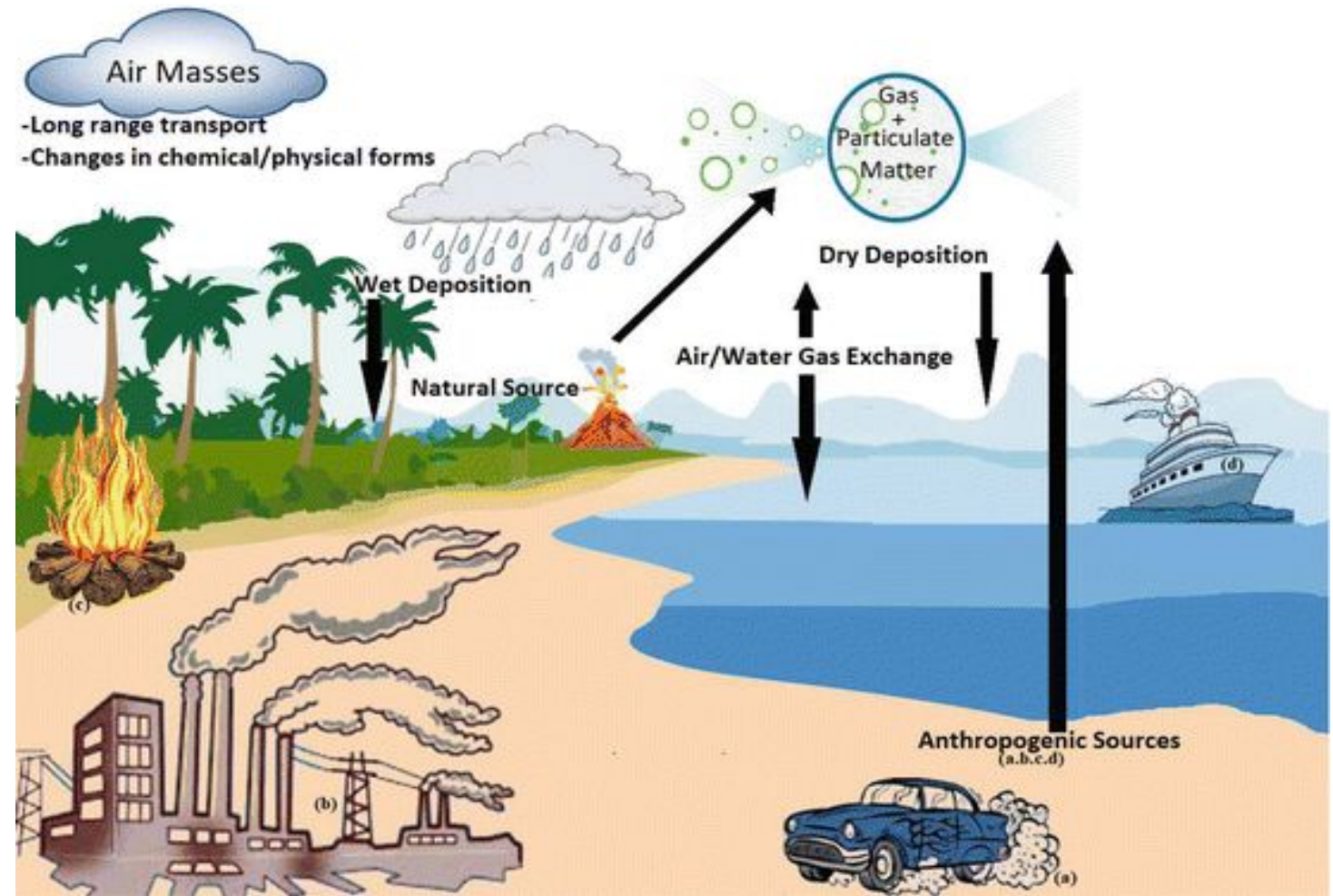
# Enhancing laccase activity towards Phenanthrene degradation: a systematic analysis using *Phellinus noxius* BRB 11 laccase with three synthetic mediators

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Location & Date of Presentation:  
Corfu, Greece (Friday, 17/June/2022)

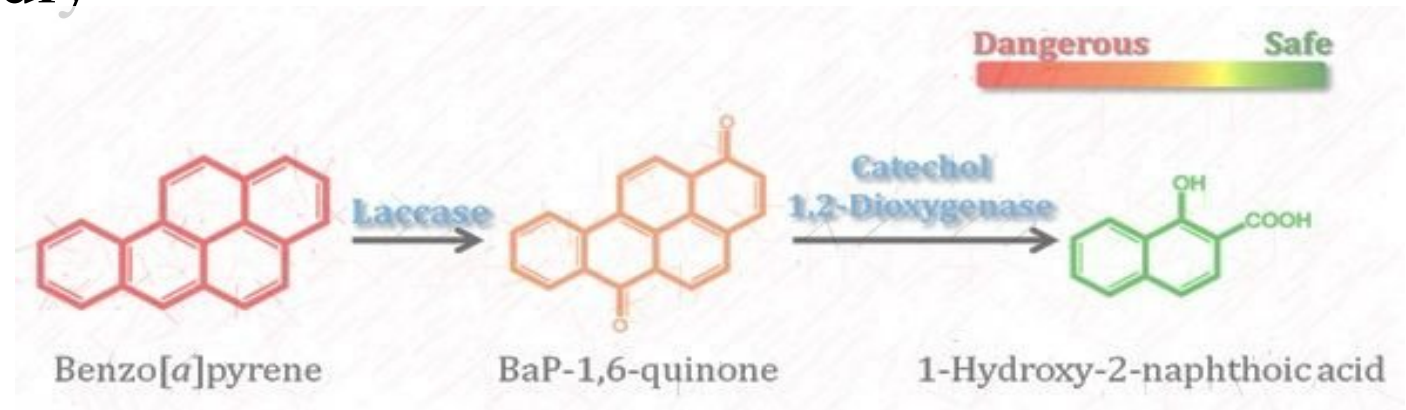
# Background

- Polycyclic aromatic hydrocarbons (PAHs) are life-threatening environmental pollutants
- Composed of 2-3 or more aromatic rings
- Highly recalcitrant
- US-EPA enlisted 16-PAH compounds for Degradation



# Objectives

- Biodegradation of PAH through different Eco-friendly practices
- Bio-waste utilization for laccase production



- Optimization of mediators 1-Hydroxybenzotriazole (1-HBT), 2,2,6,6-Tetramethylpiperidinyloxy (TEMPO), Violuric acid (VA) to enhance degradation



# Oil Palm Empty Fruit Bunch for Enzyme Production

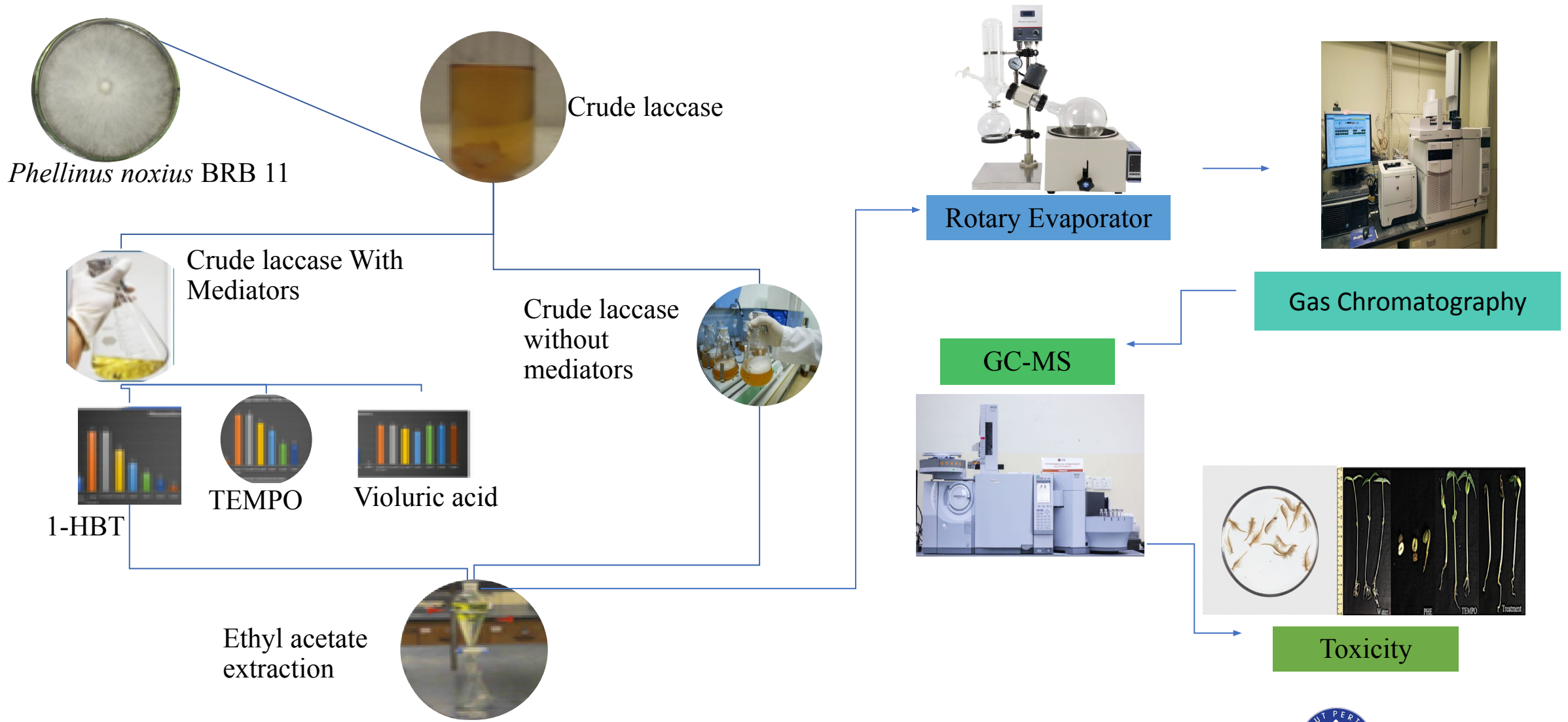


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# Experimental set-up



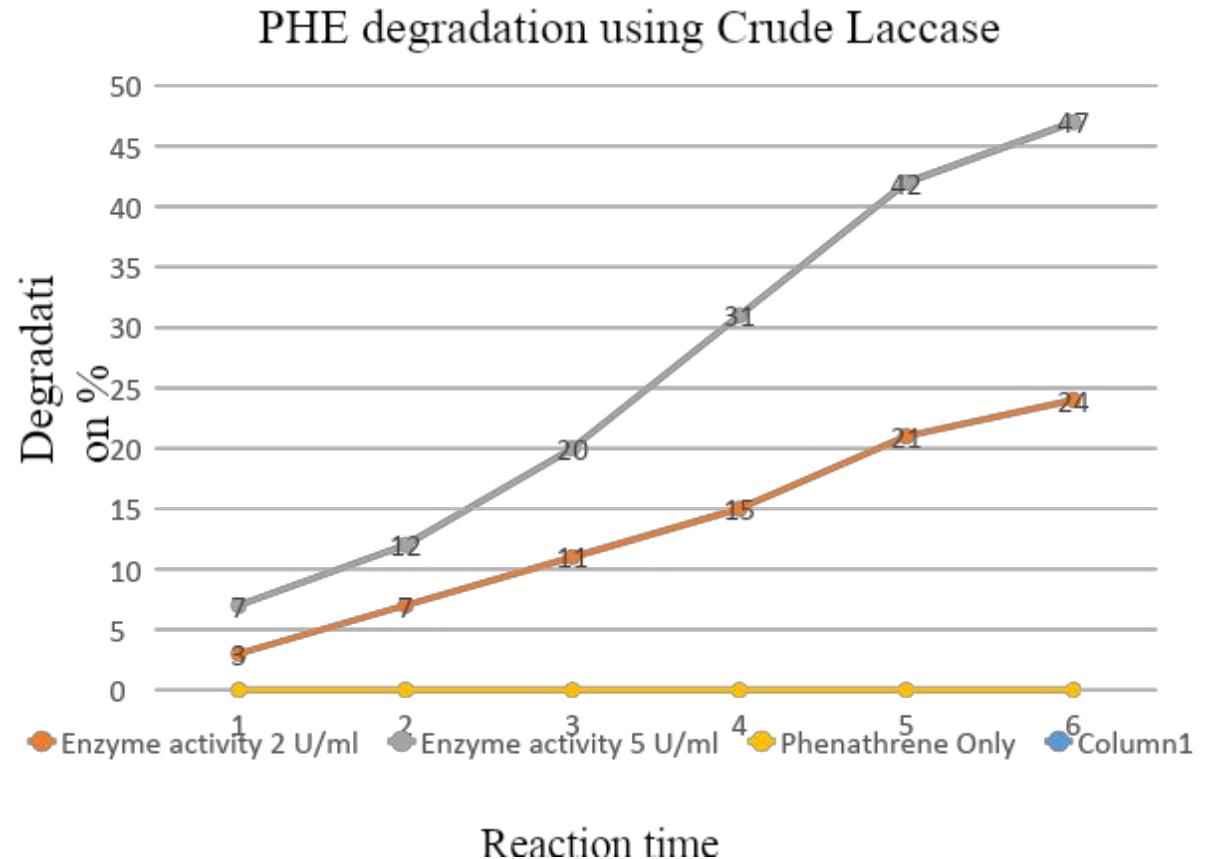
# Important Formulas

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- *Laccase activity*  $\left(\frac{U}{mL}\right) = \frac{Abs}{\varepsilon} \times V_{solution} \frac{\mu L}{10^6} \times \frac{60/t}{V_{enzyme} (\mu l) 10^3}$
- *Biodegradation efficiency (%)* =  $\frac{C_c - Ct}{C_c} \times 100$
- *Mortality (%)* =  $\frac{Control - Test}{Control} \times 100$

# Results

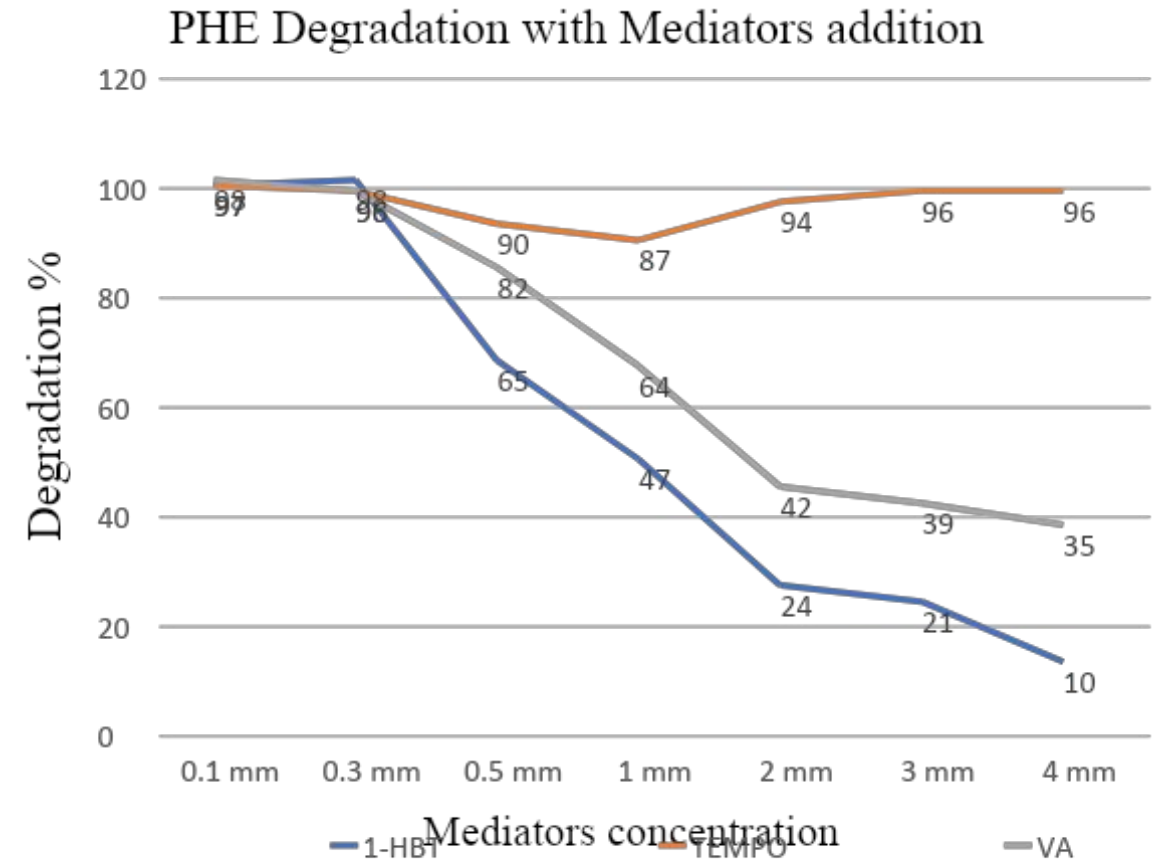
- Phenanthrene (100 ppm) without laccase showed no degradation.
- Degradation was observed 47% with laccase 2 U/ml activity while 24% with 0.5 U/ml activity.





# Mediators added degradation GC analysis

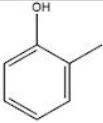
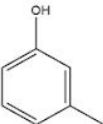
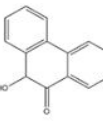
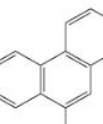
- All mediators; 1-HBT, TEMPO, and Violuric acid showed 99% degradation with 0.1 and 0.3 mM concentration
- TEMPO as mediator showed low laccase inactivation

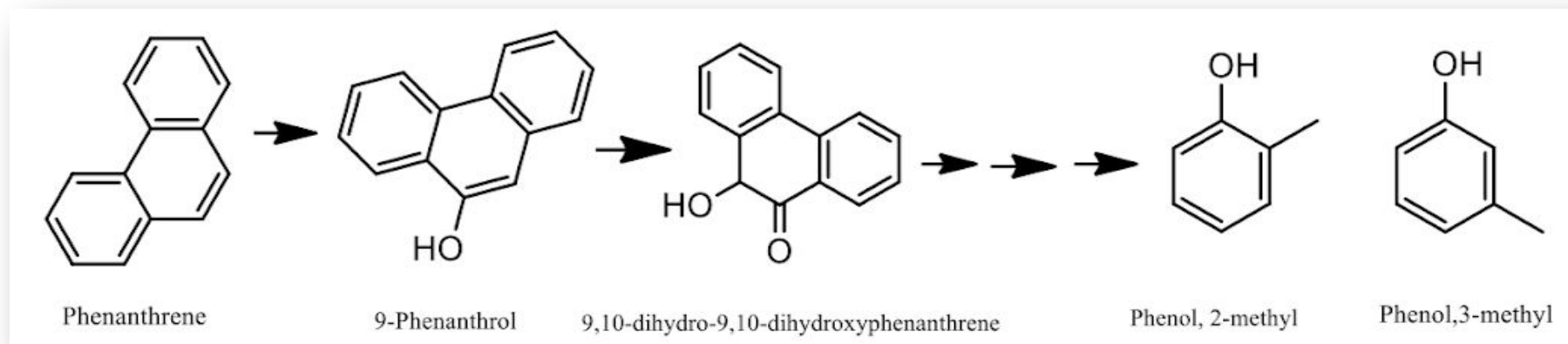




# GC-MS metabolites Analysis

- GC-MS and 4 metabolites were detected in the degraded samples
- Fungal strain initially transformed PHE to 9-Phenanthrol followed by 9,10-dihydro-9,10-dihydroxyphenanthrene

Retention time	Molecular weight	Name	Structure
4.00	108	Phenol, 2-methyl	
4.32	108	Phenol, 3-methyl	
16.82	212	9,10-Dihydro-9,10-dihydroxyphenanthrene	
17.27	194	9-Phenanthrol	



# Cytotoxicity Analysis



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- Control of Phenanthrene without any mediator was observed highly toxic
- The degraded metabolites were observed less toxic compared to pure Phenanthrene

Control	Samples	LC50 Values
Positive Control	Water	135
Negative Control-1	PHE	4
Negative Control-2	TEMPO	10
Treatment	Degraded metabolites	8

# Phytotoxic analysis



- Phenanthrene samples showed no germination using 5% of the PHE concentration

Sample	Stem length	Root length	Leaf length		Leaf width	
			Right	Left	Right	Left
Water	11.9 ± 0.2	3.5 ± 0.7	2.6 ± 0.1	2.9 ± 0.2	1 ± 0.4	1.1 ± 0.4
PHE	0.3 ± 0.6	0.6 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
TEMPO	5.8 ± 1.2	2.5 ± 0.4	1.7 ± 0.3	1.9 ± 0.2	0.7 ± 0.0	0.1 ± 0.1
Treatment	4.6 ± 0.4	3,2 ± 0.2	1.1 ± 0.1	1.3 ± 0.1	0.4 ± 0.2	0.3 ± 0.2

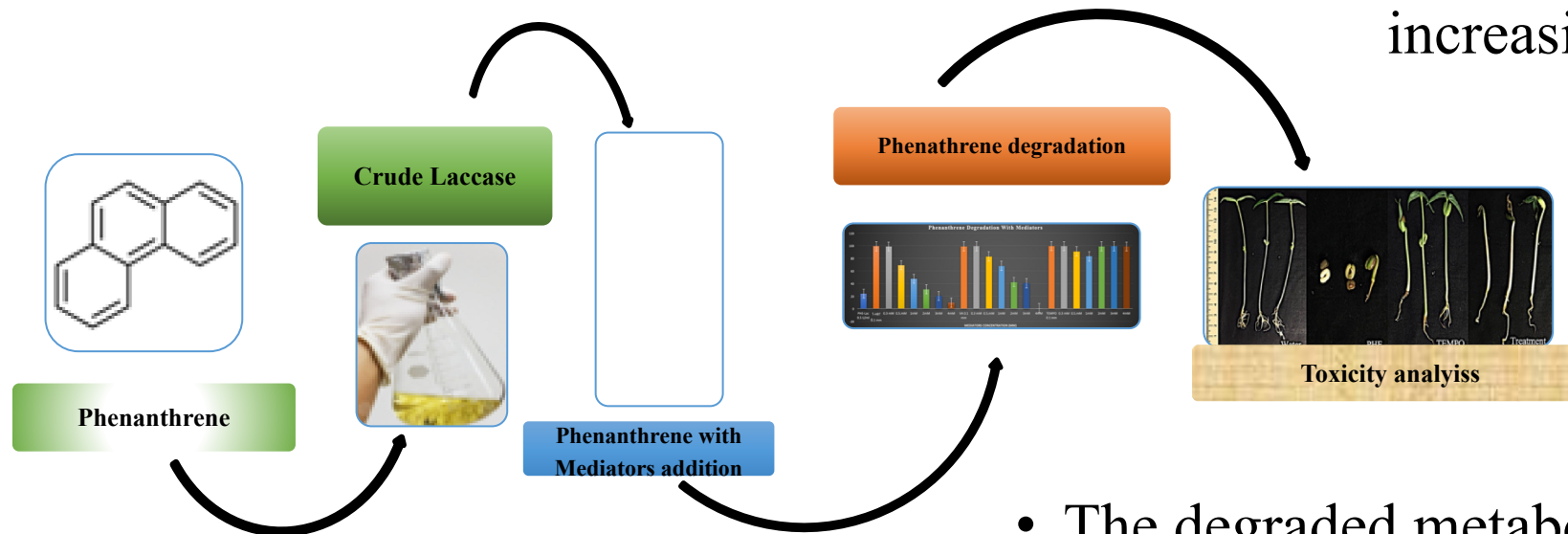
- Negative control (TEMPO) showed low toxic effect



# Conclusion

- Crude laccase was produced using OPEFB by *Phellinus noxius* BRB 11 species

- TEMPO shows low effect of laccase inactivation with increasing the concentration



- TEMPO showed high degradation

- The degraded metabolites showed low cytotoxic and phytotoxic effects on *A. salina* and *V. radiata* used as indicator organisms



**Thank you  
very much for  
your kind  
Attention**



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