KEREGEN GOGEC TEAM 2023

CHAMPIONING PAH SOLUTIONS USING A FIELD ADAPTABLE BIOSENSOR FOR DETECTING TOXIC POLYAROMATIC HYDROCARBONS.

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Background

Rapid industrialization and urbanization have resulted in a plethora of human activities that release a variety of toxic contaminants into the environment, including poly-aromatic hydrocarbons (PAHs) (Mojiri et al., 2019). PAHs, one of the most health-relevant ambient-air pollutants, constitute a broad family of multi-aromatic chemical compounds with two or more fused aromatic rings bound in linear, cluster, or angular configurations (Wang et al., 2021). They are ubiquitous in air, water, soil, and sediments, and have been detected in food, consumer products, and human tissues with a wide range of biological toxicity (Adeniji et al., 2018). Pyrogenic, petrogenic, and biological activities constitute the sources of PAHs, but precisely the point sources of pollution are both natural and anthropogenic emissions. Of which, the latter elicits the main drivers of PAH pollution.

PAH pollution is strongly deteriorating human health, along with other organisms across the planet due to its toxicity, persistence, and bioaccumulation potential (Marris et al., 2020). The disease burden from PAH pollution is becoming more obvious, with a slew of new research finding a strong link between pollution levels and decreased life expectancy(Rengarajan et al., 2015; Tong et al., 2018). The global burden of PAH pollution, which contributes to an estimated 4 million deaths annually attributed to ambient air pollution, is deeply shocking to inter-governmental public health gains (Goshua et al., 2022). Over 90% of the population lives in places where air pollution exceeds World Health Organization limits, and with air pollution levels continually rising in many nations, this problem is only becoming worse (Shaddick et al., 2020).

Developing countries have a higher burden of PAH exposure than their developed counterparts, and within these countries, poorer and marginalized settlements are frequently more exposed (WHO, 2019). Less strict air quality laws, the preponderance of older, more polluting machinery and cars, fossil fuel subsidies, congested urban transportation networks, quickly growing industrial sectors, and cut-and-burn agricultural methods contribute to this heightened trend (Gupte et al., 2 2016; Ravindra et al., 2008). Further exacerbation following population explosion is much expected further constraining the limited available healthcare facilities. This alarming trend and associated factors are synonymous with what is observed in Uganda, one of the countries in SubSaharan Africa. The most current statistics show that Uganda's annual mean Particulate Matter concentration is 50 g/m3, above the advised level of 10 g/m3, which is why the country’s air quality is deemed dangerous by WHO (2019). Consequently, the recently recorded mortality rate due to air pollution is at1east 55.7 deaths per 100,000 people in Uganda (Air Pollution In Uganda: Causes, Effects, And Solutions – N99 and CE Air Pollution Masks | ATC MASK, n.d.).

Despite the high burden of PAH exposure in Uganda and other developing countries, there are significant gaps in the detection and monitoring of PAHs. PAHs are typically measured using expensive and sophisticated equipment, such as gas chromatography-mass spectrometry (GC-MS) and High Performance Liquid Chromatography, which are not widely available in low-resource settings but also requires high expertise to eliminate experimental errors as well is associated with longer Turn Around Time (Kumar et al., 2014). As a result, there is limited monitoring and enforcement of environmental pollution control guidelines. This frailty hampers efforts to understand the extent of the problem, develop effective mitigation strategies, and evaluate the impact of interventions.

Statement of the Problem

The urgency of addressing the problem of PAH exposure in Uganda and other developing countries cannot be overstated. PAHs are known to cause a range of adverse health effects, including cancer, respiratory and cardiovascular diseases, and developmental and reproductive disorders. Children, pregnant women, and vulnerable populations, such as the elderly and people with preexisting health conditions, are particularly at risk (Abdel-Shafy & Mansour, 2016; Liamin et al., 2018; Rengarajan et al., 2015). Moreover, PAHs have a long half-life in the environment and can persist for decades or even centuries, leading to chronic exposure and accumulation in the food chain (Zelinkova & Wenzl., 2015; Li et al., 2008). This means that the impacts of PAHs on human health and the environment are likely to be felt for generations to come. In response, environmental control agencies are reinforcing alleviatory strategies; however, they are broken down into a pothole of scanty understanding of the extent of the problem consequentially hindering appropriate control strategies, yet alone the impact of these 3 interventions can’t be evaluated as expected.

The root cause for this protracted chain of hindrances is attributed to the inefficient and ineffective existing detection framework for poly-aromatic hydrocarbons within the environment. Precisely, PAHs are typically detected using expensive, time-consuming sophisticated equipment, such as gas chromatography-mass spectrometry (GC-MS), which is not widely available in low-resource settings. More so, its dependency on the high expertise and use of highly hazardous reagents preclude its use in low-income countries (Kumar et al., 2014). No wonder, the gap in the prevalence of PAH exposure between rich and poor countries is increasing due to the lack of affordable detection systems. Whereas the hiking trend of PAH exposure leans towards developing countries, their developed counterparts remain equally at risk of PAH pollution regardless of their overarching control strategies. This is because of the long-range transport ability of semi-volatile PAHs via multi-hopping (Jin et al., 2017). Consequently, collective efforts should be geared towards developing a user-friendly that eliminates discrepancies in detection frameworks of PAH detection to eliminate the socio-economic factor as a deterrent from successful global efforts towards containment of toxic PAH Pollution. Just as the study by Li et al. (2021) seeking an appropriate analytical method for detecting PAHs in soil attributes the success of PAH pollution efforts to using reliable detection techniques, the development of a user-friendly biosensor cannot be a missed concept.

Significance

The development of a user-friendly biosensor will provide a proof of concept required for its further optimization for the future development of a tool of desired qualities for improving the detection of Polyaromatic Hydrocarbons. This will enable policymakers to make many better-informed decisions about environmental pollution control measures. Validating the biosensor will earmark its predictive performance to address improved detection of the most abundant toxic polyaromatic hydrocarbon, which will promote an environmentally alert population.

Justification

The Stockholm Convention on Persistent Organic Pollutants (POPs), a global treaty emphasizes the aim to protect human health and the environment from POPs, including Poly Aromatic Hydrocarbons, by eliminating or restricting their production, use, and release into the environment. The Convention requires parties to develop and implement monitoring plans to detect and quantify the presence of POPs including PAHs in the environment (Overview, n.d.). However, the current

4 monitoring methods for PAHs are sophisticated, time-consuming, and costly, limiting their use for real-time monitoring and detection of PAH pollution in under resourced settings. This gap has further constrained global efforts to tackle PAH pollution in the era of globalization.

Developing this biosensor for PAH detection could help fill this gap in the Stockholm Convention by providing a more cost-effective and timely method for monitoring PAHs in the environment (Justino et al., 2017). By integrating biosensors into monitoring plans, policymakers can make more informed decisions on how to manage and reduce PAH pollution, ultimately leading to a cleaner and healthier environment, in line not only with the goals of the Stockholm Convention on POP but also Uganda's National Environment Act of 2019 which aims to protect Uganda's environment for future generations (THE NATIONAL ENVIRONMENT ACT, 2019 | National Environment Management Authority, n.d.).

By further optimizing the developed biosensor, there will be cost substitution for the existing detection mechanisms PAHs thereby increasing the adoption of the National Environment Management Authority (NEMA) guidelines (ENVIRONMENTAL IMPACT ASSESSMENT GUIDELINES FOR WATER RESOURCES RELATED PROJECTS IN UGANDA THE REPUBLIC OF UGANDA, 2011). There will be an increment in the number of individuals and organizations carrying out the required environmental impact assessments (EIAs) before undertaking development projects that may have significant impacts on the environment. Ultimately, the population will benefit from development activities that are carried out in an environmentally conscious manner.

Research question

1. What is the most abundant toxic Poly Aromatic Hydrocarbon within the environment?

2. What is the most feasible design of biosensor appropriate for detecting the most abundant toxic Poly Aromatic Hydrocarbon within the environment?

General Objective

To develop a feasible biosensor as a proof of concept for further optimization for detecting toxic polyaromatic hydrocarbons within the environment.

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Specific Objectives

1. To Screen the most abundant toxic polyaromatic hydrocarbon within the environment

2. To develop a feasible biosensor for detecting the most abundant toxic Poly Aromatic Hydrocarbon within the environment

3. To validate the developed biosensor

Scope

This study worked towards developing a feasible biosensor for detecting the most abundant toxic Polyaromatic Hydrocarbon within the environment.

Project Goals

1. **Innovative Biosensor Development**: Exhibit high sensitivity, specificity, and reliability.

2. **Accessible Technology:** To bridge the gap in environmental monitoring capabilities.

3. **Collaborative Network:** Collectively working towards mitigating the impact of PAHs on the environment and public health.

OUR APPROACH TO DEVELOPING THE BIOSENSOR

- **i.** Identify the highest priority PAHS
- **ii.** Design to provide a proof of concept
- **iii.** Validate the biosensor to determine operating conditions, specificity & sensitivity

Survey and Sampling

We surveyed ten (10) sites and sampled five (5) sites mainly due to resource constraints.

PRELIMINARY RESULTS

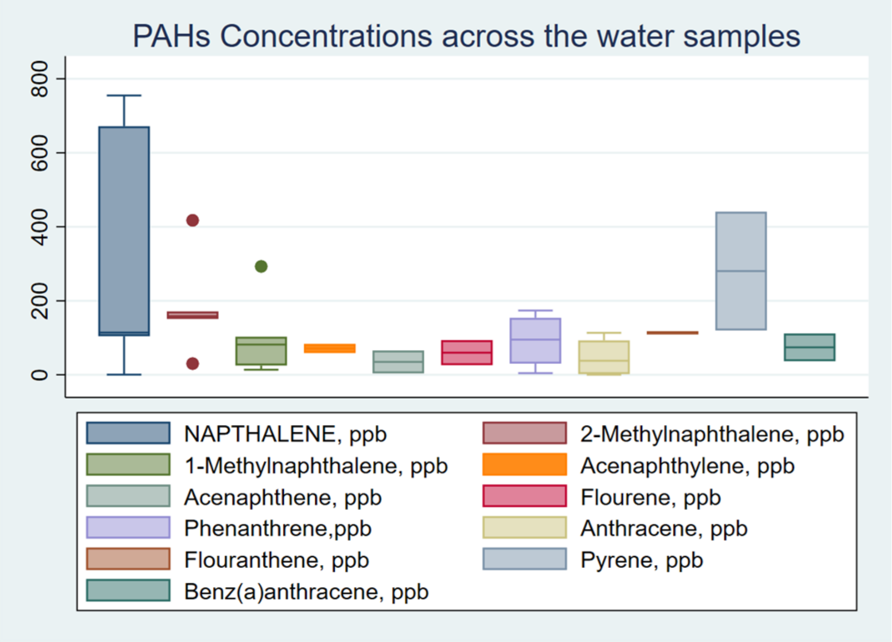


Figure 1. Distribution of PAHs across different samples with a box plot shows phenanthrene is fairly distributed.

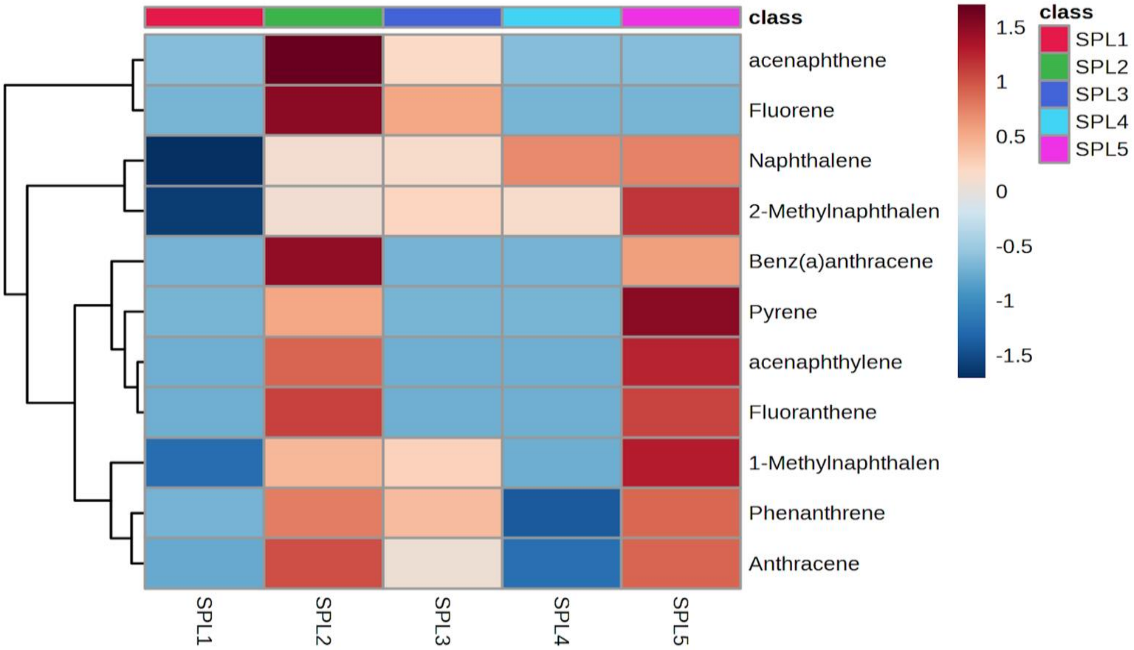


Figure 2. A heat map showing the abundance of each PAHs across each sample shows pyrene scored highest.

Scoring of the Different PAHs using the Environmental Protection Agency Recommendations, Pyrene and Phenanthrene ranked highest

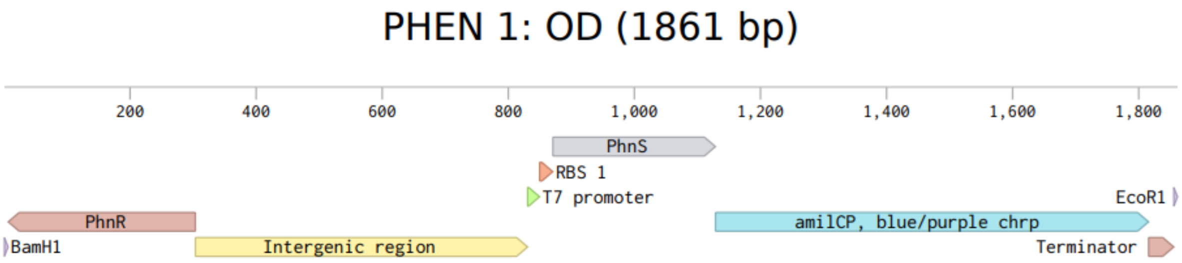
PAH	Persistence in Environment	Prevalence	Application	Toxicity	Priority List	Substance of High Concern
Pyrene	High	Widely distributed	Industrial & combustion processes	Moderate to high toxicity	EPA Priority chemical	Yes
Phenanthrene	High	Widespread in soil, water & air	Industrial & combustion process	Moderate to high toxicity	EPA Priority chemical	Yes

Therefore, pyrene and phenanthrene became our two biosensor candidates.

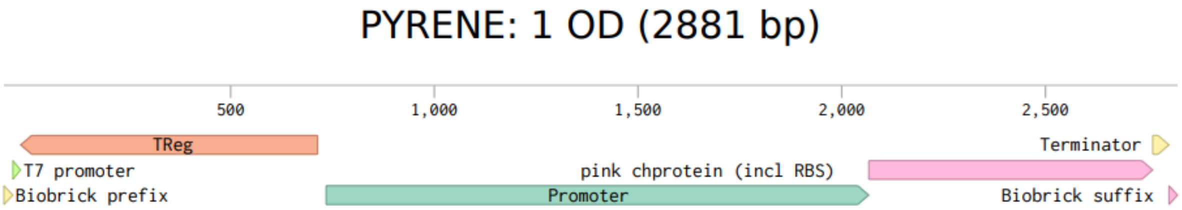
Biosensor Design

1. Genetic Design of the Detection Machinery

A. Phenanthrene Construct

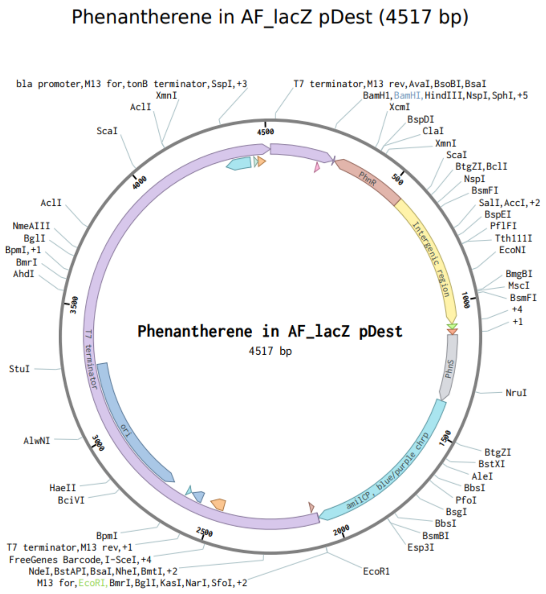


B. Pyrene Construct



2. Cloning of the constructs into the Plasmid

A. Phenanthrene Assembly



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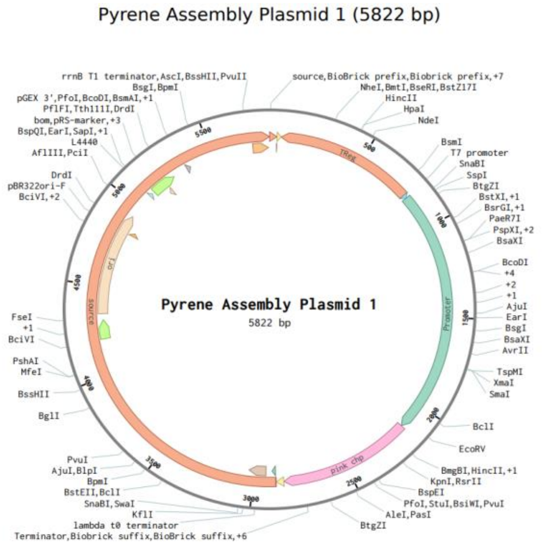

HOW THE MODEL WILL WORK