Polycyclic Aromatic Hydrocarbons (PAHs) in particulate phase (adsorbed) and mesophyll tissue phase (absorbed) of the leaves of a wetland plant bioindicator: A Focus on distribution, risk assessment, and sources.

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CERTIFICATE OF APPROVAL

This is to certify that the project entitled "Polycyclic Aromatic Hydrocarbons (PAHs) in particulate phase (adsorbed) and mesophyll tissue phase (absorbed) of the leaves of a wetland plant bioindicator: A Focus on distribution, risk assessment, and sources". carried out by Marshal Kisku, M.E, 2nd Year 2nd Semester (**Examination Roll No.: M4CHE24024**), Department of Chemical Engineering, Jadavpur University, Kolkata-32, under the supervision of **Dr. Ratna Dutta**, Associate Professor, Department of Chemical Engineering, Jadavpur University, Kolkata-32, India, is hereby approved and presented in a satisfactory manner to narrate its acceptance as prerequisite to the degree for which it is being submitted.

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Declaration of Originality and Compliance of Academic Ethics

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of his "Master of Chemical Engineering" studies. All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to research work.

Thesis Title: "Polycyclic Aromatic Hydrocarbons (PAHs) in particulate phase (adsorbed) and mesophyll tissue phase (absorbed) of the leaves of a wet land plant bioindicator: A Focus on distribution, risk assessment, and sources".

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are persistent and resistant to bio-degradation and making them lipophilic environmental carcinogens with detrimental effects on public health. Considering PAHs as priority pollutants, their distribution in soil of the studied area was investigated. The result revealed that the investigating area is highly polluted with soil PAHs (283.14 μg g^{-1}) contamination. The comparison of soil PAHs pollution level with sediment quality guidelines (SQGs) including the effect range low (ERL) and effect range medium (ERM) values, indicating adverse biological effects on the research region ecosystem. Using M-ERM-Q (mean effect range medium-quotient) and M-PEL-Q (mean probable effect level-quotient) in the situation, the biological risk or anticipated combined toxicity for exposure to a mixture of PAHs in the soil was assessed which again implies toxicological effects to the aquatic creatures to the studied area. Additionally, risk quotient (RQ) were used to assess the ecological risk that pollutants provide to aquatic biota, where RQ-$PAHs_{(NCS)}$ <800 and RQ-$PAHs_{(MPCs)}$ >1 in the research locations suggest low to moderate and high levels of ecological risk, respectively. This finding led to urgent biomonitoring of PAHs pollution in the study area (Panchasayar Lake in South Kolkata). Moreover, research on the biomonitoring of Particulate matter (PM) and PAHs pollution in wetland has been lacking up until the date. Panchasayar Lake in South Kolkata, India is considered as study site in the present study, and it was selected based on WBPCB pollution load data. In this context, two wetland plant species namely ***Mikania micrantha kunth*** and ***phyla nodiflora (L.) Greene*** was selected as bio-monitoring species based on air pollution tolerance index (APTI). The species called ***phyla nodiflora (L.) Greene*** was found to be intermediately tolerant and potential bioindicator, while ***Mikania micrantha kunth*** was found to be tolerant with APTI value > 17 with expected biomonitoring potential. To ascertain the influence of PAHs pollution load on the chosen plant species some physical and biochemical

properties are assessed relative water content (RWC), pH of the leaf extract, ascorbic acid, total chlorophyll content, protein content, proline content, sugar content, wax, and lipid content. To evaluate the presence of PAHs in soil and both bioindicator plant species, six hours of toluene extractions were carried out using a Soxhlet extractor. Both the studied plant species were found to be effective PAHs accumulators, according to the research findings (***Mikania micrantha kunth*** : 161.74 μg g^{-1} and ***Phyla nodiflora (L.) Greene*** : 209.04 μg g^{-1}).Using diagnostic ratios, the contribution of various sources of PAHs in the ambient were checked and confirmed. Vehicular emissions, anthropogenic activities, and the combustion of fossil fuels are found to be the major responsible factors. High levels of PAHs have been found to be carcinogenic and mutagenic (as shown by their carcinogenic and mutagenic equivalent concentrations, or BaP-TEQ and BaP-MEQ) and have been linked to major health effects in humans. Additionally, Particulate matter captured by both the plant species and their associated PAHs levels were investigated in this work, where BaP, NAPH, BghiP, BbF, BkF were identified mainly sourced from vehicular and diesel/gasoline emission. High accumulation level of PAHs in these plant species suggested them as suitable bio-monitor to assess the PAHs pollution status in the study area and for further remediation activities to ensure environment sustainability.

CHAPTER: 1

1. INTRODUCTION-

1.1. Background of the study:

Due to the rapid growth in traffic, industrial and vehicular emissions, and the decrease in urban vegetation cover, the quality of the air in large cities in developing countries has drastically declined in the last few decades (Bharti et al., 2022). Air pollution is one of the worst urban pollution issues. It damages ecosystem sustainability and human health, resulting in 7 million premature deaths and $2.9 trillion in annual global economic losses (IQAir, 2020). A complex blend of substances makes up air pollution. Other ingredients might be left out, although some substances are regulated because of their known toxicity. Polycyclic aromatic hydrocarbons (PAHs) are one such class of the most prevalent and plentiful pollutants. Their many configurations comprise a vast array of chemical molecules, ranging from two to seven fused benzene rings (Di Toro et al., 2000; Kim et al., 2013).Poisonous air effects are imposed on residents as well as native flora and fauna due to the deteriorating state of the environment caused by the diverse presence of xenobiotics, gaseous pollutants, heavy metals, persistent organic pollutants (POPs) like polycyclic aromatic hydrocarbons (PAHs), dust, or particulate matter (PM) (Haque and Singh, 2017). Additionally aggravating the situation are the ceaseless efforts to build industries and infrastructure, the power sector, dense traffic, the expansion of impoverished settlements, the reduction of open spaces, and the limitless emission of air pollutants (both local and transboundary), which raises their concentration in the atmosphere significantly (Haque and Singh, 2017). Deathly air pollutants that are genotoxic in nature include air suspended PM and PAHs. According to the International Agency for Research on Cancer, PM is a Group I human carcinogen (IARC,2013).The presence of PAHs in the environment is harmful to human health and wellbeing because of their mutagenic and carcinogenic qualities (Jia et al., 2011; Zhang et al., 2009). As a result, in the last few decades, scientists have worked to improve, create, and apply methods for monitoring and correcting air quality in response to public alarm over the growing risks that polycyclic aromatic hydrocarbons (PAHs) represent to the environment and human health (Mukhopadhyay et al., 2020). Different sources of PAH pollution have been brought about by human activity (waste drainage from industry and

agriculture), and each source can cause a unique pattern of PAH contamination that results in a different distribution pattern in the soil of the wetland (Baumard et al., 1998).

1.2. Polycyclic Aromatic Hydrocarbon (PAHs):

Polycyclic Aromatic Hydrocarbons (PAHs) are risky compounds with many benzene rings bonded together in various structural arrangements. PAHs are resistant to breakdown because of their hydrophobic and lipophilic characteristics (Kronenberg et al., 2017). As a result, they can be found in various environmental media, such as water, soil, sediments, and air (Lawal, 2017; Kucuksezgin et al., 2020). Of the hundreds of known PAHs, sixteen have been designated as High Priority Pollutants by the Environmental Protection Agency (EPA).Naphthalene (NAPH), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLU), phenanthrene (PHEN), anthracene (ANTH), fluoranthene (FLTH), pyrene (PYR), benzo[a] anthracene (B[a]A), chrysene (CHRY), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo[a]pyrene (B[a]P), benzo[g,h,i]perylene (B[ghi]P), Indeno[1,2,3-c,d]pyrene (IND), and dibenz[a,h]anthracene (D[ah]A) are among them. These 16 PAHs are a concern for the ecosystem due to their ubiquity and persistence in the environment, as well as their potential toxicity for people and other organisms (Hussar et al., 2012).

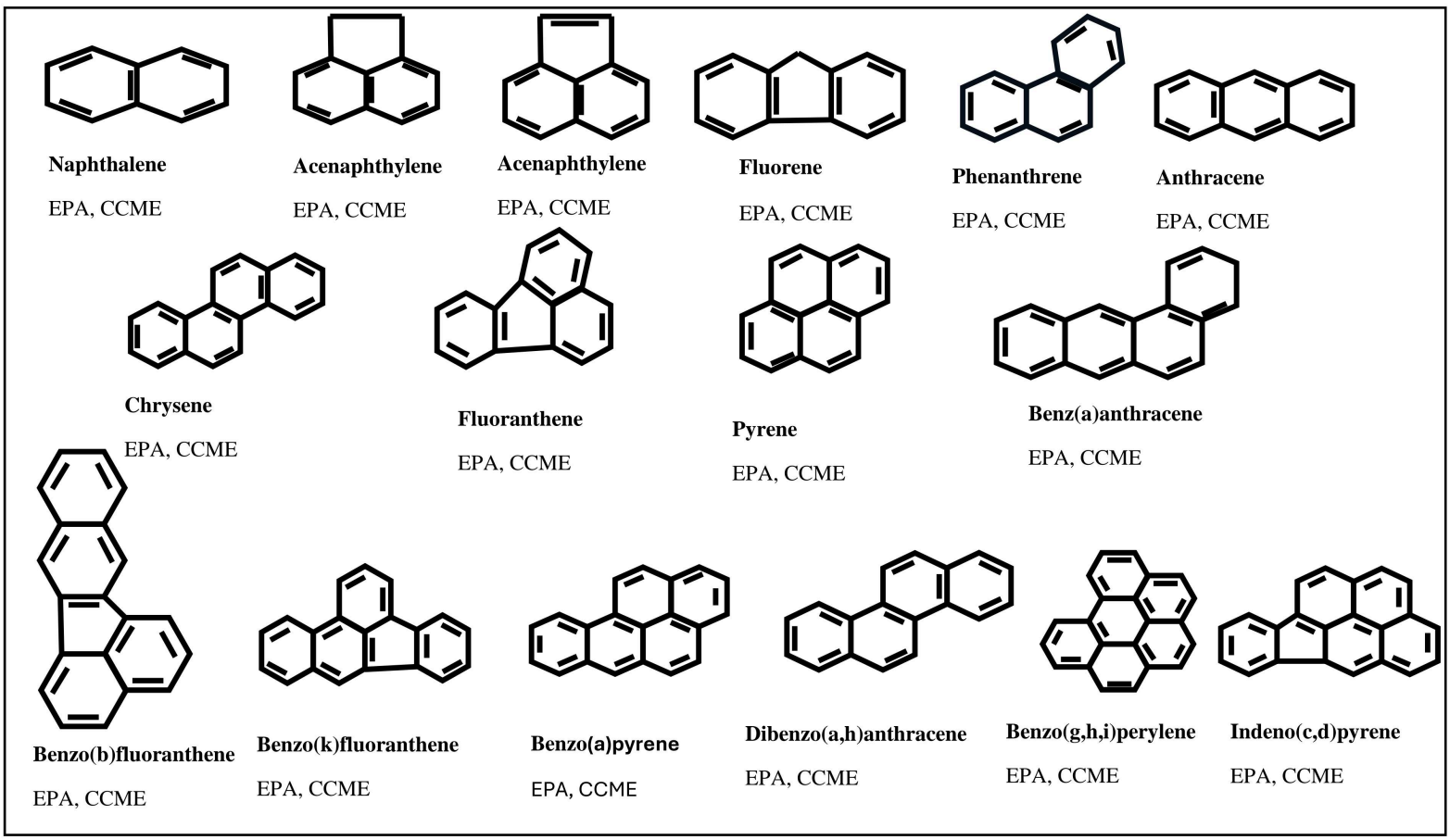


Figure 1. The United States Environmental Protection Agency (US EPA) has classified 16 priority pyrogenic PAHs (Huang, 2014).

1.2.1. Physical and chemical properties of PAHs:

Low-molecular-weight PAHs (two and three rings) are found in the environment mostly in the vapor phase, while multi-ringed PAHs (five rings or more) are mainly associated with particles. Intermediate-molecular-weight PAHs (four rings) are dispersed across the vapor and particle phases, contingent upon the temperature of the environment. Particle-bound PAHs are regarded to pose a major threat to human health. Benzo[a]pyrene (B[a]P) is widely used as a marker for total exposure to carcinogenic PAHs since it contributes significantly to the total carcinogenic potential (one study reported that it was in the range of 51–64%) (Choi et al., 2010).

Table 1. Physical and Chemical characteristic of 16 USEPA PAHs (Tang et al., 2021)

Name	Formula	Rings	Molecular Weight (D)	Phase distribution	Boiling point (°C)	Vapor pressure (mmHg)
Naphthalene	$C_{10}H_8$	2	128.18	Gas	218	4.5×10^{-1}
Acenaphthene	$C_{12}H_{10}$	3	154.21	Gas	96	1.5×10^{-4}
Acenaphthylene	$C_{14}H_{10}$	3	152.21	Gas	265~275	2.1×10^{-4}
Fluorene	$C_{14}H_{10}$	3	166.23	Gas	295	3.8×10^{-4}
Anthracene	$C_{14}H_{10}$	3	178.24	Gas/Particle	340~342	1.5×10^{-6}
Phenanthrene	$C_{14}H_{10}$	3	178.34	Gas/Particle	340	1.5×10^{-6}
Fluoranthene	$C_{16}H_{10}$	4	202.26	Gas/Particle	375	2.6×10^{-8}
Pyrene	$C_{16}H_{10}$	4	202.26	Gas/Particle	393~404	2.6×10^{-8}
Benzo(a)anthracene	$C_{20}H_{12}$	4	228.29	Particle	438	1.3×10^{-9}
Chrysene	$C_{18}H_{12}$	4	228.29	Particle	448	1.3×10^{-9}
Benzo(a)pyrene	$C_{20}H_{12}$	5	252.32	Particle	495	2.8×10^{-12}
Benzo(b)fluoranthene	$C_{20}H_{12}$	5	252.32	Particle	461	2.8×10^{-12}
Benzo(k)fluoranthene	$C_{20}H_{12}$	5	252.32	Particle	480	7.0×10^{-11}
Dibenzo(a,h)anthracene	$C_{22}H_{12}$	5	278.35	Particle	487	6.3×10^{-2}
Benz(g,h,i)perylene	$C_{22}H_{12}$	6	276.34	Particle	550	1.6×10^{-12}
Indeno(1,2,3-cd) pyrene	$C_{22}H_{12}$	6	276.34	Particle	530	1.8×10^{-13}

1.2.2. Sources of PAHs:

The environment has several, well-known sources of PAHs (Figure 2). Primarily, real-world environmental samples are used to calculate the PAH content. To help differentiate between different PAH source groups, chemical fingerprinting employs a number of methods. Examples of this type of source include wood, coal, and oil. Examining certain chemical markers present in a range of samples will help achieve this. Thus, studies aiming at defining general "background" ranges of PAHs in the environment are often complemented by source identification techniques. Identification and localization of non-point sources of PAHs in residential and industrial areas can also be facilitated by chemical fingerprinting (Patel et al., 2020).

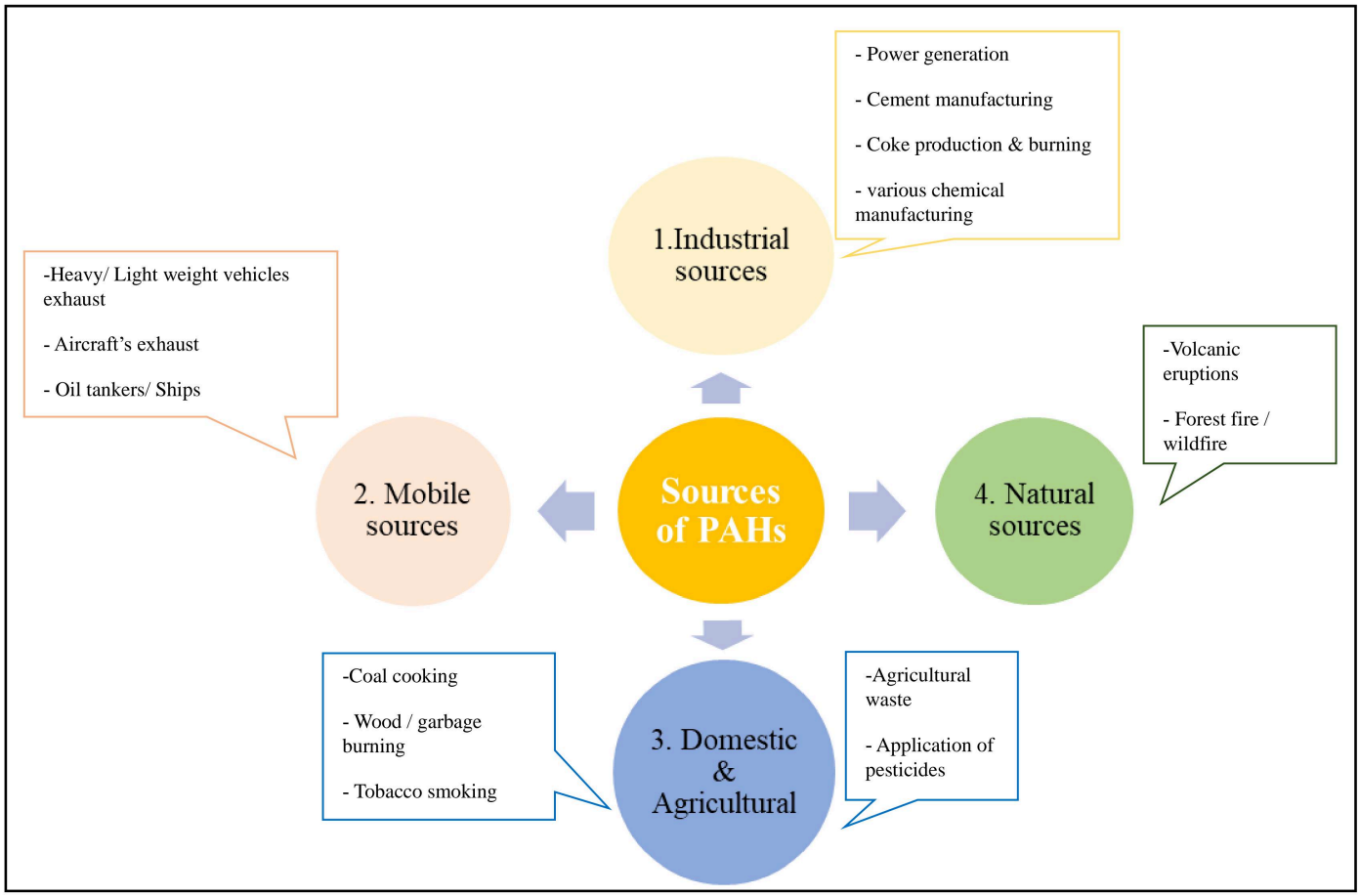


Figure 2. Sources of PAHs in the environment (Patel et al., 2020)

1.3. Bio-monitors:

Biomonitoring is the process of keeping an eye on environmental health using living things or bioindicators. The quality of the environment and the effects of various pollutants are quantified using bio sensors. There are several approaches to biomonitoring that are intended to modify the environmental health only a little (Marques, 2001). Using bioindicators, biomonitoring can be carried out. An ecosystem's natural state is tracked in the environment through the use of bioindicators. For example, plankton is a significant biomarker for detecting water quality and an indicator of water contamination. Plankton is an organism inside a biological system that provides information about the health of its surroundings. Plankton responds swiftly to changes in the environment. In terrestrial, marine, and atmospheric environments, high amounts of PAHs are detected. Bioindicators generate a comprehensive assessment of the environmental effects of ecosystem stresses by identifying signals over a wide range of temporal and geographical scales. Certain plants can help us assess how well the ecosystem in our surroundings is doing. However, by taking toxins into their tissues, they are also well known for lowering pollution levels in ecosystems (Roy et al., 2022). Many organizations utilize and support bioindicators as a means of managing biomonitoring and evaluating the effects of humans (the World Conservation Union, International Union for Conservation of Nature) (Figure 2). To assess the health of a particular ecosystem, bioindicators can be used at a variety of scales, from the cellular to the ecological level. They combine knowledge from the biological, physical, and chemical elements of our world that result in alterations in population size, population density, community makeup, and ecological activities. From a management standpoint, bioindicators guide our decisions regarding what is and is not biologically sustainable (Emily et al., 2010).

1.3.1. Criteria for selection of bioindicators:

It is always appropriate to choose the bioindicator species or groups of species based on established species and local disturbances in a given ecosystem. Recently, ecologists have developed a comprehensive set of standards for choosing reliable biological indicators.

- ✓ Very accessible
- ✓ Affordable
- ✓ Available in a variety of areas throughout the year
- ✓ Resistance to unfavorable weather conditions;

- ✓ sensitivity to a particular concentration of pollutants;
- ✓ high rate of reproduction;
- ✓ innocuous to the environment (Pattanayak et al., 2020).

1.3.2. Types of bioindicators:

Based on the many uses of bioindicators, four groups can be identified (Figure 3):

- **Pollution indicator:** Pollution indicators are living things, such as bacteria, plants, insects, and microbes, that can accumulate pollutants and provide information about hazardous substances or the level of pollution in various environmental media, such as water, air, and soil (Maqbool Geelani et al., 2012).
- **Environmental indicator:** Species or groups of species that exhibit predictable responses to environmental perturbations or changes include bioassay organisms, sentinels, detectors, exploiters, accumulators, and others. A collection of indicators intended to diagnose the condition of the environment for the purpose of environmental policy making is known as an environmental indicator system (https://www.environmental-mainstreaming.org/documents/EM%20Profile%20No%2010%20-%20Indicators%20(20%20November%2009).pdf).
- **Biodiversity indicator:** For determining the species richness of a community, the species richness of an indicator taxon is employed. But now, "measurable parameters of biodiversity" are included in the definition, which includes things like species richness, endemism, genetic parameters, population-specific parameters, and landscape parameters (http://eolss.net/sample-chapters/c09/E6-38A-01-07.pdf).
- **Ecological indicator:** There is information on this species' susceptibility to several pressures, including habitat fragmentation and pollution. The indicator's reaction is typical of the community (Gerhardt, 2002).

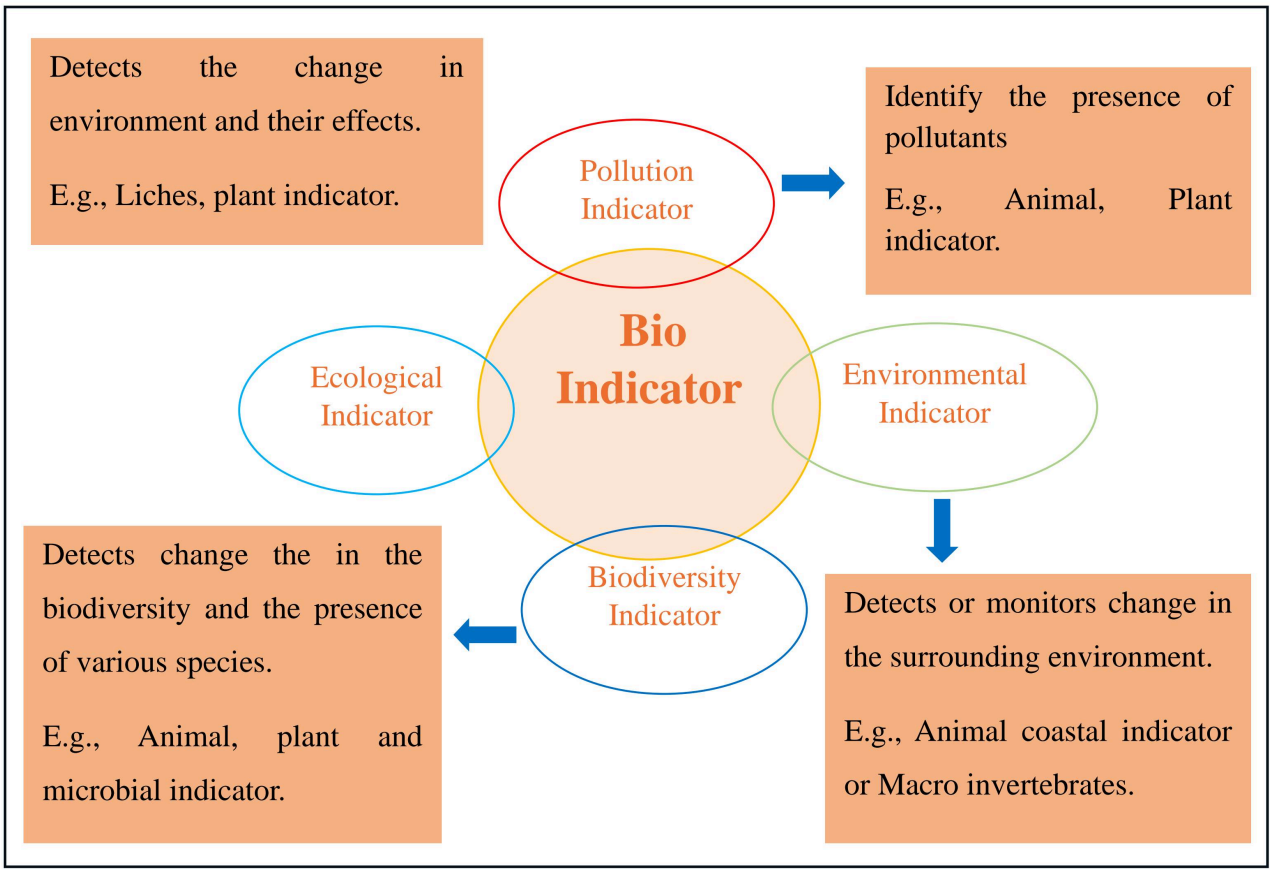


Figure 3. Types of Bioindicators (Parmar et al., 2016)

1.3.3. Advantages of bioindicators:

The use of Bio-indicators has the following benefits:

a) It is possible to determine the biological effects.
b) Helps track the competing and complementary effects of different pollutants on the ecosystem.
a) Early toxicology monitoring is possible, as is the monitoring of the harmful effects of toxins on both plants and people.
b) They are easily counted since they are so numerous.
c) It is more cost-effective than other specialized measuring system (Pattanayak et al., 2020).

1.3.4. Plant as a potential bioindicator for biomonitoring of PAHs pollution:

Identification of PAH concentration by tree component samples has a crucial role in determining the environmental health. Through stomata or external cuticular lamellae tree leaves may directly absorb PAHs in the dissolved phase. The flow of CO_2 from the atmosphere to the main carbon-fixation sites in the chloroplasts of mesophyll cells is necessary for leaf photosynthetic function. Leaf surfaces feature adjustable stomatal pores that are connected to mesophyll cells by a complex web of air channels in order to handle this passage of CO_2 in and water vapor out (Lundgren et al., 2019). Mesophyll is the term for the ground tissue that lies underneath a leaf's upper and lower epidermis. The parenchyma cells that make up mesophyll tissue has chloroplasts. Chlorenchyma is a type of parenchyma with chloroplasts that carries out photosynthesis (Sari et al., 2021). In addition to being bonded to particles, PAHs can also exist in the gaseous phase (mostly PAHs with greater vapour pressure). Both particle- and gaseous-bound PAHs are a source of exposure for plants. Stomatal absorption and/or diffusion through wax layers and membranes can allow compounds from the vapour phase to enter the interior of the leaves (Hubai et al., 2022). But several studies have also revealed that particle-phase PAHs assemble on the surface of leaves and may subsequently contribute to lipid-rich cataracts. In the particulate phase, solid airborne particles including dust, soot, and aerosols can get adsorbed or absorbed with PAHs (Sari et al., 2021). In relation to plants, the term "particulate phase" describes solid or liquid particles that are present in or connected to plant tissues or fluids. These particles may have several sources and functions inside the plant (Sari et al., 2021). Typically, when we talk about the particulate phase, we're talking about the fine and ultrafine particles that are in the air. These particles can come from a variety of sources, such as combustion processes, industrial emissions, and organic materials like pollen and soil particles.

1.4. Wetlands:

Wetlands are home to a large number of species that are important to both the environment and commerce. For instance, commercially valuable fish and shellfish including prawns, blue crab, oysters, salmon, trout, and sea trout are either supported by or linked to wetlands. For migratory birds and waterfowl like ducks, egrets, and geese, wetlands offer a crucial habitat. In fact, nearly half of all threatened or endangered species in United States live fully in

wetlands, and over one-third use them at some time during their life cycle (USEPA 1995). Because of this, numerous wetlands are commonly recognized as essential objectives for conservation or restoration (Cherry et al., 2011).

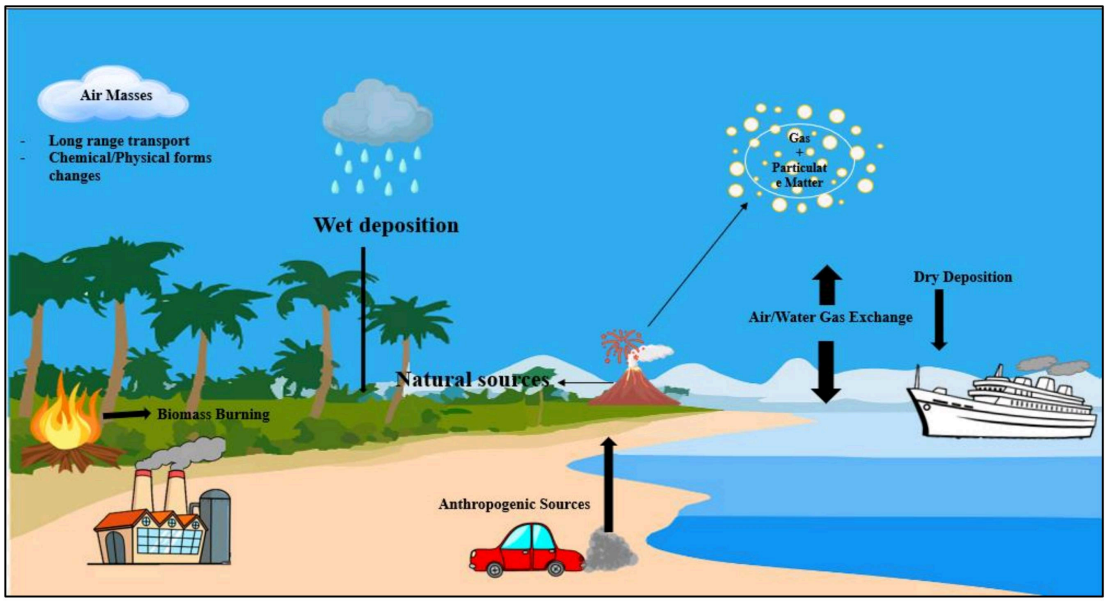


Figure 4. Sources of PAHs into wetland (Hussain et al., 2018)

1.4.1. Significance of wetlands:

The creation of wetlands is essential to sustainable development. Nonetheless, unchecked urban growth is causing an unparalleled loss of these wetlands. which altered the scenario of pollution in urban wetland areas. In this regard, biomonitoring is an important method that offers a quantitative and qualitative evaluation of the wetland ecosystem's state of health. Nevertheless, the amount of research on biomonitoring of PAH pollution in wetlands is still quite little. In this situation, the collection of PAHs is greatly aided by plant bio monitors. The standard air monitoring method has a number of serious drawbacks, including high cost, maintenance difficulties, and power supply requirements (Mitsch et al., 2007).

To date, there has been a lack of research on the biomonitoring of PAH pollution in wetlands. In this situation, the collection of PAHs is greatly aided by plant bio-monitors. The present study conducted to determine the tolerance of wetland plant species towards air pollution growing near Kolkata (South Kolkata) in India by calculating their APTI and effect

on plant parameters on APTI to prove its worth in biomonitoring. Therefore, we are concentrating on the fact that the wetland plant is used to track the level of PAH contamination in the atmosphere, locate PAH point sources, and assess the risk to the environment and human health.

CHAPTER: 2

2. LITERATURE REVIEW

1. Accumulation of polycyclic aromatic hydrocarbons and trace elements in the bioindicator plants *Tillandsia capillaris* and *Lolium multiflorum* exposed at PM10 monitoring stations in Stuttgart (Germany), 2010. Rodriguez, H, J., Pinata,L,M., Fangmeier, A., Klumpp, A., Chemosphere 80, 208–215.

- **Summary**- The main objective of this study is to compare the accumulation of trace elements in T. capillaris with that of the standardized biomonitor L. multiflorum, and to analyze the relationship between PAH accumulation and trace element accumulation in the bioindicator plant *T. capillaris* with various levels of vehicular traffic in the city of Stuttgart (Germany).
- **Experiment-**
  - **Sample-** In this experiment *Tillandsia capillaris* Ruiz and Pav. form *capillaris* were collected from tree trunks in San Isidro, Cordoba Province at Argentina.
  - **Extraction-** Using 80 g of NaCl, the homogenized material was combined with an aliquot of internal standard solution, water, acetone, and petroleum ether (1:2:1 v/v), and then the extraction technique was carried out. The extracts were homogenized, and the organic supernatant was dried with sodium sulfate, filtered, and concentrated to 0.5 mL in a rotary evaporator with the thermostatic bath set to a maximum of 40 C.
  - **Analysis-** In this experiment all extracts were analysed through HP-5 MS.
- **Conclusion -** The suitability of T. capillaris (Ruiz and Pav.) form capillaris as an accumulation bioindicator for trace elements was further supported in this study in a region lacking the transplanted species, which displayed a similar response pattern to the standardized bioindicator L. multiflorum (LAM) cv. Lema. T. capillaris additionally shown to be a reliable and sensitive biomonitor for the buildup of polycyclic aromatic hydrocarbons.

2. Improved biomonitoring of airborne contaminants by combined use of holm oak leaves and epiphytic moss,2013. Nicola, F., Spagnuolo, V., Baldantoni, V., Sessa, L., Alfani, A., Bargagli, R., Monaci, F., Terracciano, S., Giordano, S., Chemosphere 92, 1224 – 1230.

- **Summary:** The main objective of this research was to compare the ability of native epiphytic moss *Leptodon smithii* and leaves of *Quercus ilex* (holm oak) to accumulate a

wide spectrum of elements and PAHs not routinely monitored in automatic devices and discuss about the result of their different morpho-physiological characteristics.

- **Experiment:**
  - **Sample-** The moss shoots and holm oak leaves were collected from sites, including Italian Regions: Campania and Tuscany with different anthropogenic activities.

  - **Extraction-** 5g of fresh holm oak leaves and 3g of epiphytic moss were mixed with equal quantities with anhydrous sodium sulphate and extracted by three consecutive sonication's (Misonix, XL2020 sonicator) with 300 mL of a mix dichloromethane: acetone (1:1 = v:v). The extracts were concentrated by vacuum rotary evaporator to 4 mL, then it was filtered and dried under a gentle nitrogen stream.
  - **Analysis-** Freshly collected samples were spiked with internal standard of 0.4 ml and mixed in cyclohexane making the volume to 4 ml and analysed by GC– MSD for PAHs determination.
- **Conclusion:** Concentrations of 16 EPA-PAHs were determined in *Quercus ilex* leaves and the epiphytic moss *Leptodon smithii* which have been seen at urban, periurban and extraurban holm oak stands areas in Campania and Tuscany. And urban areas have higher levels of environmental contaminants and highest PAH content in holm oak leaves and moss than preurban and extra urban ones.

3. Spatial distribution and source apportionment of polycyclic aromatic hydrocarbons (PAHs) in Camphor (Cinnamomum camphora) tree bark from Southern Jiangsu, China,2014. Zhou, L., Dong, l., Huang, Y., Shi, S., Zang, L., Zhang, X., Yang, W., Li, L., Chemosphere 107, 297–303.

- **Summary-** The main objective of this study is to determine the levels of PAHs in the bark of camphor trees and to look into their distribution in Southern Jiangsu. The potential PAH sources were discovered in this study. The association between tree bark and PUF disks was established as they serve as passive air samplers for each other.
- **Experiment-**
  - **Sample-** In Southern Jiangsu, all camphor tree bark samples were collected from the cities of Suzhou, Wuxi, Nantong, Changshu, Zhangjiagang, Kunshan, Jiangyin, and Jingjiang.

- **Extraction-** The Accelerated Solvent Extraction procedure was used to extract about 18 g of the bark sample together with 3 g of diatomite. Using a 1:1 acetone-to-hexane mixture, do the following procedures: static, 5 min; flushing, 50%; purge, 60 s; two cycles**.** The extract was then filtered and concentrated using a rotary evaporator. Hexane was substituted for the original solvent, and 15 ml of it was evaporated. Three grams of MgSiO3 were added, and the extract was then frozen in the refrigerator for one night to eliminate the bark lipids.
- **Analysed**- In this experiment all samples were analysed by GCMS-QP2010 plus.

- **Conclusion-** Tree bark absorbed significantly more particulate-phase PAHs than PUF samplers, which both tended to accumulate gas-phase PAHs. Good For each particular PAH congener, a correlation between tree bark and PUF samplers was discovered, demonstrating that both of them respond well to the monitoring of gas-phase PAHs.

4. Uptake and phytotoxicity of anthracene and benzo[k]fluoranthene applied to the leaves of celery plants (*Apium graveolens var. secalinum L.*) 2015. Wieczorek, J., Sienkiewicz, S., Pietrzak, M., Wieczorek, Z., Ecotoxicology and Environmental Safety 115, 19 – 25.

- **Summary:** The purpose of this research was to determine the effects of long-term foliar application of anthracene (ANT) and benzo[k]fluoranthene (BkF) of celery plants. The both PAHs were applied separately to determine whenever in combination correct and addictive effects on celery plants to despite their structural differences.
- **Experiment:**
- **Sample-** The celery plant was collected from large area and a natural high-level compound that participates in non-enzymatic reaction of plant cells to above – ground table and chemical stress and oxidative stress.
- **Extraction-** The plants were washed with ultrapure water was allowed to dried with filter paper. After dividing into four parts as leaf blades, stalks, roots and bulbs it was freeze dried and homogenized. Every sample was triplicated extracted by an ultrasonic disintegrator with a solution of 1:1 acetone and v/v n-hexane. The extracted samples were combined and applied to a filled column with absolute sodium sulphate and eluted with n-hexane and acetone. The extracts samples were 2ml evaporated in vacuum

evaporator. Then condensed residues were dissolved in n-hexane to a 1 ml final volume.

- **Analysis:** In this experiment Synchronic Spectro-fluorimetry used to determined PAHs.Varian Cary 5000 spectrophotometer used to measure absorption spectra in the range of 220700 nm. And the fluorescence was measured using the Perkin-Elmer LS 50B luminescence spectrometer.

- **Conclusion:** In treatments where above-ground plant were exposed to separately and combination of two PAHs: ANT and BkF. In this experiment ANT and BkF applied regularly for 43 days to the above-ground part of celery plant. After experimental days of exposure (Overall dose of 1321 mg/plant) ANT and BkF keeps only 1.4% and 17.5% of the total roots. The average concentration of ANT was twofold higher to compared of BkF levels on leaf blade.

5. Lipid-content-normalized polycyclic aromatic hydrocarbons (PAHs) in the xylem of conifers can indicate historical changes in regional airborne PAHs 2015. Kaung, Y, W., Li, J., Hou, E, Q., Environmental Pollution 196, 53-59.

- **Summary:** The main objective of this research was the distribution of PAHs-DW from the oldest to the youngest xylem did not grow progressively Urbanization and industrialization in the PRD region, indicating that xylem PAHs-DW did not reflect historical growth in anthropogenic PAHs.

- **Experiment-**

- **Sample-** xylem of Masson pines collected from Dinghushan natural reserve which is located in the northwest of PRD of southern China.

- **Extraction-**3 g of fresh xylem chip were extracted from lipids content of xylem using through dichloromethane: hexane (1:1, v/v) for 48 h with Soxhlet- extraction.

- **Analysis:** This samples were quantified using Hewlett-Packed 5890 gas chromatography and 5972 mass selective (GC-MSD) detector.

- **Conclusion-** The study conducted an analysis on the fluctuation of PAHs concentrations and lipid content in Masson pine trees xylem. The data collected were then compared with historical PAHs reported in sedimentary cores and the days of haze occurrence. Based on the analysis the PAHs-LC in the xylem of conifers could be

trustworthy indicator of past variations in airborne PAHs, when using dendrochemical monitoring to study airborne PAHs in historical and comparative research.

6. Towards more ecologically realistic scenarios of plant uptake modelling for chemicals: PAHs in a small forest, 2015. Terzaghi, E., Zacchello, G., Scacchi, M., Raspa, G., Jones, K, C., Cerabolini, B., Guardo, A, D., Science of the Total Environment 505,329-337.

- **Summary:**The purpose of this work is to examine how the fluctuation of meteorological and ecological factors, specifically SLA and LAI, can affect the absorption and release of organic pollutants in plants, ultimately impacting air pollution levels.
- **Experiment:**
  - **Sample-** In this experiment two different sampling campaigns were collected:1. From march to July 2007 leaves of two tree species like cornel, Corn's mas and maple, *Acer pseudoplatanus*. And air samples collected for PAH measurements. 2. From to December 2012 leaves of the previous species plus for additional one was sampled for SLA and LAI determinations.
  - **Extraction-** During weekly 100 g of leaves were sampled from sampling campaign. Using dichloromethane and purified following a gel permeation and silica-alumina clean up procedure PAHs were extracted from gas and particle phase also in leaves.
  - **Analysis-** These extracts were analysed in GC-MS.
- **Conclusion-** This research paper is to analysed the dynamics of changes in the concentrations of particular chemical in the air and leaves of plants. The study was conducted weekly and correlated with relevant meteorological and ecological parameters to determines the extends of their impact.

7. PAH detection in *Quercus robur* leaves and *Pinus pinaster* needles: A fast method for biomonitoring purpose,2016. Nicola, F. D., Grana, E. C., Aboal, J. R., Carballeira, A., Fernandez, J, A., Mahia, P. L., Rodriguez, D. P., Lorenzo, S. M., Talanta 153, 130-137.

- **Summary:** As described in this experiment the set-up and optimization of analytical methods for the extraction of 19 PAHs in pedunculate oak leaves and maritime pine needles and discussing about analytical performance characteristics.
- **Experiment:**

- **Sample-** In this experiment pedunculate oak leaves and maritime pine needles were collected from Galicia.
- **Extraction-** Powdered leaf/ needle of 250 mg with addition of 50 ml of working solution of the D-la-beled surrogate standards were blended in an agate with 0.5 g of C18 with during 3 min. After that homogeneous dispersion was deposited in the top of a Envi-Florisil SPE tube, where0.5 g of anhydrous sodium sulphate and PSA was added for oak leaf also there 0.5 g of partially deactivated silica for pine needle samples.
- **Analysis-** In this experiment all the analyses were perform by PTV-GC-MS/MS.

- **Conclusion-**A matrix solid-phase dispersion extraction method, previously used for moss samples, has been improved and modified for the analysis pedunculate oak leaves and maritime pine needles were widely used in biomonitoring studies throughout Europe due to the complexity and heterogeneity of plant. Use of Florisil combined with additional clean-up sorbents 10% deactivated silica for pine needles and PSA oak leaves are improvements over previous method because their matrices are high in interfering compound as demonstrated by PTV-GC-MS/MS in full scan mode.

8. PAH related effects on fish in sedimentation ponds for road runoff and potential transfer of PAHs from sediment to biota,2016. Grung, M., Petersen, K., Fjeld, E., Allan, I., Christensen, J, H., Malmqvist, L, M.V., Meland, S., Rannekev, S., Science of the Total Environment 566-567, 1309-1317.

- **Summary:** The purpose of this experiment was to investigate if fish exposed to road and traffic related pollutants from sedimentation ponds as PAHs related effects and to identifying the source of PAHs in sediment, plant and biota from the sedimentation from pond.
- **Experiments:**

- **Sample-** In this experiment *phoxinus phoxinus* (common minnow) was selected from sedimentation pond. o **Extraction-** In an IKA 11 R sample mill, all samples were homogenised using the same amount of hydromatrix and then extract using pressurised liquid extraction in ASE200 system. The extraction cell for the plant sample was made up of a bottom layer of 4 g of activated silica followed by 5-10 g of sample mix then Ottawa sand was placed on top.
- **Analysis-** In this experiment all the analyses were perform by HPLC and GC-MS.

- **Conclusion-** Sedimentation ponds have been put in place to reduce the effects on the aquatic environment of road runoff a significant source of pollution. This experiment aimed determine whether road pollution had an impact on a fish from sedimentation ponds and the transfer of PAHs from road runoff material to aquatic organisms was substantial.

9. PAHs uptake and translocation in *Cinnamomum camphora* leaves from Shanghai, China,2017. Yang, B., Liu, S., Liu, Y., Li, X., Lin, X., Liu, M., Liu, X., Science of the Total Environment 574, 358-368.

- **Summary:** The main objective of this research was in shanghai spatial-temporal patterns and sources apportionments of PAHs to determine in entire camphor leaves.
- **Experiment:**
  - **Sample-** *Cinnamomum camphora* leaves was selected for this research. o **Extraction-** In this experiment the sample was extract in three major steps. First foliar dust was removed from leaves, then leaves drained their surface moisture after that 10 g leave were immersed in 500 ml flask with 150 ml dichloromethane for 2 minutes to extract cuticular waxes. After 2 nd steps of cuticular waxes, that leaves were freeze and dried and ground. Then 2g crushed dried samples used for PAHS measurements in leaf mesophyll tissue. After three steps done the sample on the foliar dust and mesophyll samples were Soxhlet extract in 120 ml mixture of 1:1 on dichloromethane and acetone for 18 h**.**
  - **Analysis-** In this study Gas Chromatography/ Mass Spectrometry (GC/MS) were used to analysed 16 kinds of in individual PAH prioritized.
- **Conclusion-** This work compared the foliage uptake and inner-leaf translocation of PAHs from various urbanised locations by *Cinnamomum camphora.* The diversity of leaf wax contents sampled throughout various seasons and locales probably contributed to the observed spatial and seasonal variations of 16PAHs in leaves. The main mechanism of PAH accumulation in leaves was their movement from foliar dust to cuticular wax and measurement of the TFf-w showed that low molecular weight had a propensity to increase.

10. Biomonitoring, status and source risk assessment of polycyclic aromatic hydrocarbons (PAHs) using honeybees, pine tree leaves, and propolis, 2017. Kargar, N., Matin, G., Matin, A, A., Buyukisik, H, B., Chemosphere 186, 140-150.

- **Summary:** The purpose of this work is identifying better indicators of airborne PAHs and provide comprehensive assessment of relative abilities of honeybees, propolis and pine leaves in simplify source identification and ecological risk assessment of atmospheric PAHs through by applying sampling, chemical and statistical analysis.
- **Experiment:**
  - **Sample-** In this experiment 200 worker honeybees were collected from four different places of turkey.
  - **Extraction-** 3g of the freeze-dried samples in vials with 3 ml of mixing of 1:1, v/v acetone/ hexane. Then 5 min of ultrasonic waves for all samples. After that 2 ml of upper phase injected of each vial in to Gas-chromatography.
  - **Analysis-** These extracts were analysed through Agilent 6890 N gas chromatograph (GC).
- **Conclusion-** This study correlation factor analysis, and principal components analysis (PCA) results indicate that airborne emissions are potential sources of PAH pollution in pine leaves and honeybees, but the result of study discovered that propolis is contaminated by PAHs as a result of the replacement of herbal resin sources with synthetic gummy substances from paving materials.

11. Direct determination of surfactant effects on the uptake of gaseous parent and alkylated PAHs by crop leaf surfaces,2018. Sun, H., Guo, S., Nan, Y., Ma R., Ecotoxicology and Environmental Safety 154, 206-213.

- **Summary:** The main objective of this research was to investigated the uptake of gaseous phe,3-MP and Retune by living crop leaf surfaces for effects of surfactants using fiber-optic fluorimetry.
- **Experiment:**
  - **Sample-** In this experiment living maize, potato and soybean were collected as representative crop species.

- **Extraction:** These three samples are cultivated for a 22 °C in a 16 h light or 20 °C in an 8 h dark cycle for 21 days in a controlled climate chamber programmed with 20000 lx at daytime with light intensity and relevant humidity of 35% and continuous air circulation system for ventilation.
- **Analysis:** The extracts leaf-wax samples were analysed through GC-MS and their mixture were analysed using GC-FID to measure wax compound.

- **Conclusion:** Segregation of atmospheric PAHs in crop systems is of increasing concern about their potential and human health. To assess alkylated and parent PAHs accumulation precisely, taking separate Retene and 3-MP and Phe in living maize, soybean and potato leaf surfaces as well as effects of CTMAB and SDBS were examined in situ using through fiber-optic fluorimetry in this study.

12. Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: Morphological characteristics have the greatest impact, 2019.Tian, L., Yin, S., Ma, Y., Kang, H., Zang, X., Tan, H., Meng, H., Liu, C., Science of the Total Environment 652,1149–1155.

- **Summary-**The main objective to determine the elements influencing the uptake and accumulation of PAHs by leaves.
- **Experiment**-
- **Sample-** In this experiment *Sabina chinensis, Cedrus deodara, Magnolia grandiflora, Metasequoia glyptostroboides, Ginkgo biloba, Cinnamomum camphora, Salix babylonica and Platanus acerifolia* were selected from Shanghai.
- **Extraction-** Anhydrous sodium sulfate (2 g) and silica gel (5 g) with a mesh size of 60–100 was first packed into the SFE cell before the precise weight of entire needles was added. As internal standards, the sample was spiked with standard perdeuterated naphthalene-d8, phenanthrene-d10, acenaphthene-d10, chrysene-d12, and perylene-d12 at concentrations of 2 ggl, 250 L each. The SFE system was attached to the cell for extraction.
- **Analysed-** In this experiment all extractions were analysed using GC-MS.
- **Conclusion-** In this study, this paper assessed how various leaf features affected their capacity to accumulate PAHs. These studies discovered that the proportions of various

benzene rings varied greatly and that the mean concentrations of PAHs ranged from 300 to 2000 ng/g1. The number of PAHs contained in the coniferous species S. chinensis and C. deodara was substantially higher than in species with broad leaves. MMW PAHs predominated the chemical profiles, followed by HMW PAHs; the concentration of LMW PAHs was incredibly low**.**

13. Impact factor assessment of the uptake and accumulation of polycyclic aromatic hydrocarbons by plant leaves: Morphological characteristics have the greatest impact, 2019.Tian, L., Yin, S., Ma, Y., Kang, H., Zhang, X., Tan, H., Meng, H., Liu, C., Science of the Total

Environment 652, 1149-1155.

- **Summary:** The purpose of this experiment was to determine the content of PAHs in plant leaves without washing the specimens and asses the effects of different leaf characteristics on the uptake and accumulation of PAHs in 8 common landscaping tree species in shanghai.
- **Experiment:**
  - **Sample**- In this experiment *Sabina chinensis, Cedrus deodara, Magnolia grandiflora, Metasequoia glyptostroboides, Ginkgo biloba, Cinnamomum camphora, Salix babylonica* and *Platanus acerifolia* were selected as representative of the common ever greening species in Shanghai.
  - **Extraction**- 5 g of fresh leaves was used for this extraction. 2 g of anhydrous sodium sulphate and 5 g of 6100 mesh silica gel with first packed of the SFE cell.
  - **Analysis**- All extractions were analysed using GC-MS.
- **Conclusion**-This study has primarily focused on effects of leaf morphology and physiological features. Here 8 common tree species choose to assessed the PAH content. In this study they investigated the variables influencing leaf uptake and PAHs build up using supercritical fluid extraction technique.

14. Characterization, source apportionment, air/plant partitioning and cancer risk assessment of atmospheric PAHs measured with tree components and passive air sampler.2021, Sari, F. M., Esen, F., , Tasdemir, Y., Environ Res,194:110508.

- **Summary-** To determine the types and concentrations of PAHs obtained from tree components and PASs, and compare the ability of olive tree components to accumulate PAHs (particle-phase),on the leaf, in the dissolved phase in the leaf, and in 1-year-old and 2-year-old branches), discuss their temporal variations, pinpoint the potential PAH sources, gauge the concentration of PAHs in the atmosphere using a bark-air partitioning model, and determine the likelihood that cancer will develop in adults and children who inhale PAHs.
- **Experiment-**
  - **Sample-** The olive tree from which the branch and leaf samples was located near Bursa, Turkey was collected for this experiment.
  - **Extraction-** The lipid content of the tree component samples (leaf, branch 1, branch 2) was determined by adding 50 mL of a petroleum ether: dichloromethane (PE: DCM, 1: 1) solution to 5 g of the sample. The sample was shaken all night before undergoing a 15-minute ultrasonic extraction. The sample was then ultrasonically extracted for an additional 30 minutes with 40 mL of an acetone: hexane (ACE: HEX, 1: 1) mixture after the PE/DCM mixture had been carefully emptied into a different bottle.
  - **Analysis**- In this experiment all extract were analyzed through mass spectrometer (Agilent 5975) and gas chromatograph (Agilent 7890A).
- **Conclusion**- In this study, polycyclic aromatic hydrocarbons (PAHs) in ambient air and olive tree components (leaf and branch) were simultaneously collected and analysed to determine their levels and accumulations, temporal variations, potential sources, air-plant partitioning, and cancer risks over a 12-month period. This work was able to determine the relationship between the levels of PAH in the sampling materials and the weather by using the Pearson correlation coefficient. It was discovered that the concentrations were significantly influenced by temperature and mixing layer height. With the help of a bark-air exchange model, atmospheric PAH levels were also projected.

15. Bioaccumulation of polycyclic aromatic hydrocarbons (PAH) in Polychaeta Marphysa sanguinea in the anthropogenically impacted Tunis Lagoon: DNA damage and immune biomarkers.2022, Mdaini, Z., Telahigue, K., Hajji, T., Rabeh, I., Pharand, P., Cafsi, M, E., Tremblay, R., Gagne, J, P., Marine Pollution Bulletin 184,114104.

- **Summary:** The main objective of this work are evaluation of the impact of PAH on *M.sanguinea* physiology and the polychaeta as bioindicator of PAH pollution in Tunis lagoon. And purposed of this paper was the spatio-temporal distribution in Tunis lagoon and concentrations of 15 Pah in *M.sanguinea* body.
- **Experiment:**
  - **Sample-** Tunis Lagoon well known as the Lake of Tunis, which is located of Tunis, this huge wetland was selected for this experiment. And M.*sanguinea* also collected for this experiment.
  - **Extraction-** In this experiment three method were used for this extraction. First method, acetonitrile, toluene and hexane were as a solvent where 16 ml of this mixture (1:1:1) were added to 4 g of freeze0-dried tissue. After shaking 30 min the sample was placed in 25 min sonication bath. Second method was a modified Quencher's where it used to improve PAH extraction. And the third method was traditional Quencher's extraction method.
  - **Analysis-** In this experiment all extracts were analysed in HPLC-FD.
- **Conclusion-**This study evaluated the effects of PAHs on the polychaete M.*sanguinea* in Tunis lagoon. The highest PAH concentrations were accumulated at sampling zone with a maximum PAH of 6028,87 ng/g DW. Animal physiological modifications were unmistakably connected to bioaccumulated PAH.

16. Plant-air partition coefficients for thirteen urban conifer tree species: Estimating the best gas and particulate matter associated PAH removers.2022, Giraldez, P., Aboal, R. Jesus., Fernandez, A. J., Guardo, D, A., Terzaghi, E., Environmental Pollution 315, 120409.

- **Summary-** The purpose of this study was to investigate the removal of gas and particle-associated PAHs from urban settings by 13 decorative conifer species that are often used in parks and streets around the world. This would make it possible to choose the best conifers for removing PAH from cities.
- **Experiment-**
  - **Sample-** Twenty-three trees corresponding to thirteen species (plus 1 cultivar) and 3 families were sampled at the University Campus of the University of Santiago de Compostela.

- **Extraction-** Five branches from each tree, cut at a height of 5 m and in five different directions, were stored in a tray at 5 °C for up to four days. These and the leaves were chosen from the current year. The full green (photosynthetically active) portion of the leaf was chosen when the leaves were grouped together in scaly clusters (i.e., Cupressus species). After each tree was chosen, a composite sample (weighing between 100 and 200 g f.w.) was created. These samples were homogenized in an ultra-centrifugal mill (ZM 200; Retsch®) at 12,000 rpm, dried in separate paper envelopes at 30 °C in an all-purpose precision oven (model Digitronic-TFT), and then kept at 20 °C in complete darkness in hermetically sealed glass containers.
- **Analysed-** In this experiment all sample were analysed by using MSPD-PTV-GC–MS/MS method.

- **Conclusion-** The study revealed a near to an order of magnitude difference in total PAH uptake between the most efficient tree *(Pinus pinaster*) and the least efficient tree (*Picea abies*). Considering the capacity of the various species to increase the fraction of PAHs associated with particulate matter (PM) on their needles, Cupressus lusitanica was the most significant PAH species (34% of all PAHs), and Pinus pinaster was the least significant (3.4% of total PAHs). The PAH absorption of a particular amount of plant biomass on air concentration reduction can be fine-tuned using the new KPA equations.

17. Spatio-temporal distribution and sources of polycyclic aromatic hydrocarbons in Tunis Lagoon: Concentrations in sediments and *Marphysa sanguinea* body and excrement,2023. Mdaini, Z., Telahigue, K., Haijji, T., Pharand, P., Cafsi, M, E., Tremblay, R., Gagne, J, P., Marine Pollution Bulletin 189,114769.

- **<u>Summary</u>:** The objective of this work was to evaluate the toxicity, spatiotemporal distribution and sources of PAHs in the Tunis Lagoon complex. The aimed of this work was offer fresh perspectives on the most likely pathway for PAHs.
- **<u>Experiment</u>:**
- **Sample-**Tunis Lagoon well known as the Lake of Tunis, which is located of Tunis, this huge wetland was selected for this experiment.

- **Extraction-** For this extraction of PAHs in sediments acetonitrile, toluene and hexane were used as solvent. 500 ml of the mixture (1:1:1) was added to 250 mg of dried sediments. After shaking samples for 15 min, it centrifuged at 10000 g foe 5 min.
- **Analysis-** In this experiment all extracts were analyzed through GC-MS.

- **Conclusion-** This work provides important information about the sources, toxicity and spatiotemporal distribution of PAHS in the Tunis lagoon complex. Surface sediments, *Marphysa Sanguinea* excretions and bodily fluids were all tested for PAHs amounts. According to this research PAHs have a pyrogenic source that predominates. PAHs recovered from polychaetes were clearly distinguished from detected in sand and faeces by principal component analysis.

18. Polycyclic aromatic hydrocarbons in mullet (*Chelon auratus*) from two lagoons of great ecological and economic importance in Tunisia: Levels, sources and human health risk implications.2023, Ameur, W, B., Annabi, A., Mhadhbi, T., Hassine, S, B., Ghanmi, S., Megdiche, Y, E., Khadija, M., Ennaceur, S., Trabelsi, S., Hammami, B., Touil, S., Driss, M, R., Journal of Sea Research 192,102325.

- **Summary:** The main purpose of this work was residue levels of PAHs in a benthopelagic fish species known as the golden grey mullet. The PAH levels in this commercially valuable fish species from Tunisian lagoon are also being evaluated fro first time in this experiment.
- **Experiment:**

- **Sample-** In this experiment 30 mullet fish were collected from two Lagoon (Bizerte and Ghar El Melh).
- **Extraction-** 40 ml of a 1:1 v/v hexane/acetone mixture was used to extracts three times is an ultrasonic bath. Evert extraction took 30 min. By evaporating the resulting extract in rotary evaporator to a volume of 20 milliliters.

- **Conclusion-** The muscles of (mullet) were measured. The mullet's total PAH levels were on par with or lower than those discovered in fish specimens from other nations. Using a variety of compositional ratios to analyse the nature of the observed compounds, it was discovered that both of the tested lagoons had petrogenic and pyrolytic anthropogenic pollution.

CHAPTER: 3

3. Objectives and Plan of Work:

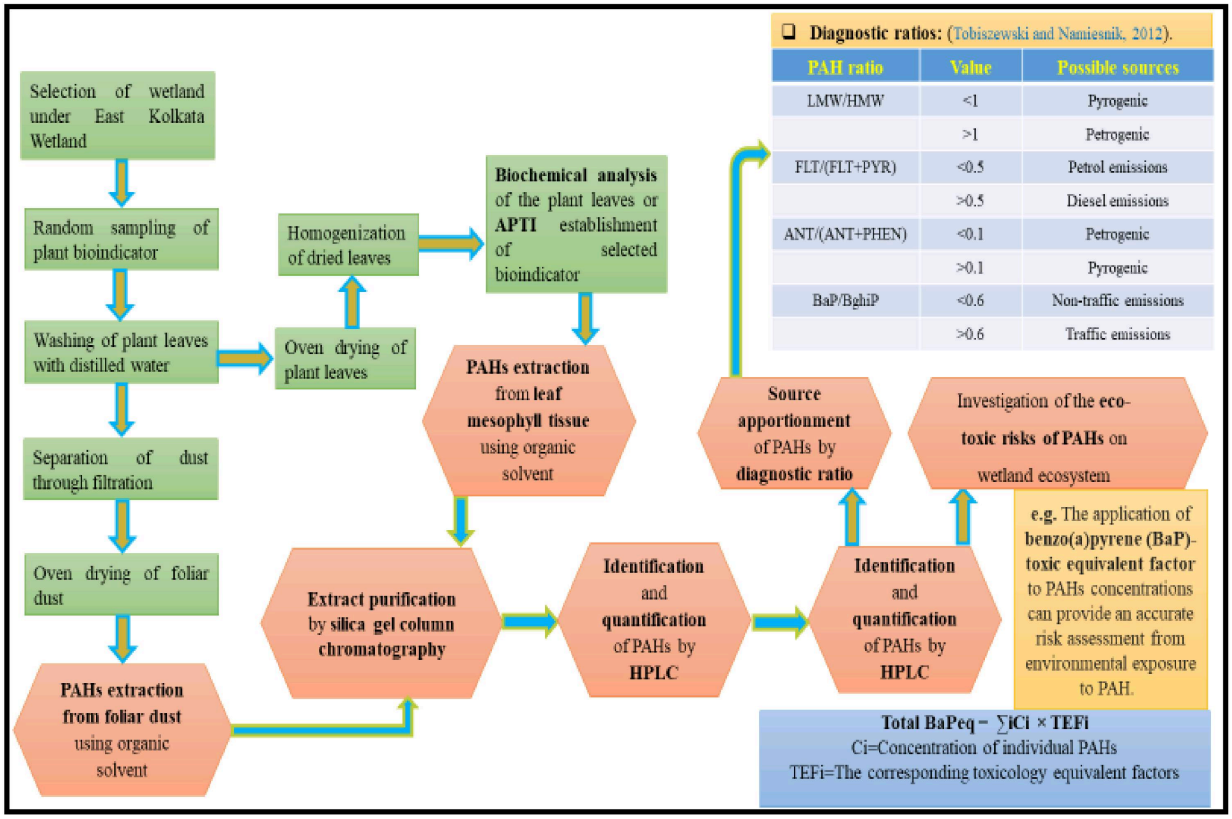
3.1. Overall and specific objectives:

Wetlands are highly abundant, bio diverse ecosystems and considered as natural sinks for various contaminants like criteria and priority pollutants. Wetlands not only provide territory, food and environment for living for numerous plants and animals; but also control the climatic condition in large extent. With advancement of societal condition primarily based on industrialization, PAHs pollution causes serious threat to wetland ecosystem which includes environment, soil and associated biota through food web. In this context, biomonitoring is a promising approach to provide the qualitative and quantitative of the health of wetland ecosystem by monitoring PAHs. It is noteworthy that there is limited number of studies on biomonitoring of PAHs pollution in the wetlands till date. Uptake and distribution of PAHs in plant bio-monitors are basically diverse in pattern. The uptake mechanisms of PAHs by vegetation include the air-to-leaf pathway and the soil-to-root pathway in which we are focusing on the first pathway. After deposition to foliage, polycyclic aromatic hydrocarbons (PAHs) may remain on the leaf surface, can accumulate in the cuticular wax and/or diffuse into the remaining interior mesophyll tissue. A great portion of PAHs uptake can be achieved through accumulated PM (both PM2.5 and PM10) on leaves.This study aims to identify a suitable wetland, plant bioindicators followed by to determine total PAHs accumulation (through both foliar PM bound phase and mesophyll tissue bound) and distribution pattern. Therefore, in the present work, the following specific objectives are planned to accomplish the broad aim of the study:

- To select sample wetland area enlisted in East Kolkata Wetlands based on soil pollution load by PAHs and to determine risk associated with soil PAHs contamination based on different soil quality guidelines (SQGs).
- To select two wetland plant species for biomonitoring of ambient PAHs based on APTI and relevant biochemical properties
- To develop the protocol for isolation of foliar dust-laden PAHs and PAHs present in the leaf inner mesophyll tissues from available bioindicators
- To determine the distribution of PAHs (HMW and LMW) in particle adsorbed phase and mesophyll tissue absorbed phase

- To determine the bioconcentration factor (BCF) of PAHs on leaves
- To identify the potential PAHs sources using diagnostic ratios in wetlands

3.2. Plan of Work:



CHAPTER: 4

4. Materials and Methods:

4.1. Study area: The study was conducted in the city of Kolkata (South Kolkata), West Bengal, India. For the present work, samples were collected from Panchasayar Lake, one of the wetlands in South Kolkata. Sampling spot (Figure 5) was selected based on the anthropogenic activities in the proximity of the study area including the heavy vehicular emissions, dense population and activities related to the ongoing construction of a metro station. Moreover, Table 2 shows the air quality data provided by WBPCB for the four criterion pollutants-PM_{10}, $PM_{2.5}$, SO_2, and NO_2 - near the site throughout the sampling period in order to identify the areas in the Kolkata metro area with exacerbated air quality.

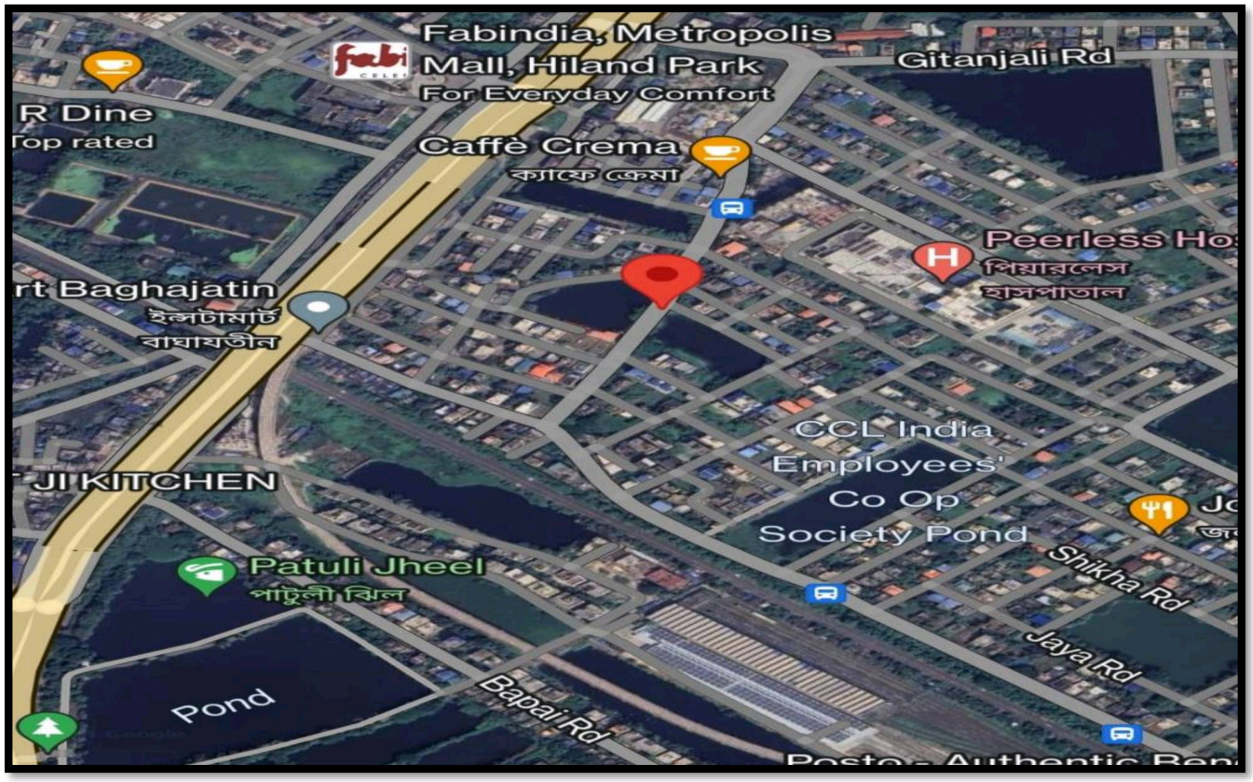


Figure 5. Location map of the sample site Krishak Pally Road, New Garia, West Bengal, India.

Table 2. Air Quality Index (AQI) near study site
(http://emis.wbpcb.gov.in/airquality/filter_for_aqi.jsp)

Pollutant	Concentration (µg/m³)	Sub Index
NO_2	27	34
PM_{10}	63	63
$PM_{2.5}$	33	55
SO_2	4	5



Figure 6. Sampling site

4.2. Sample collection, pre-treatment, storage, identification & description:

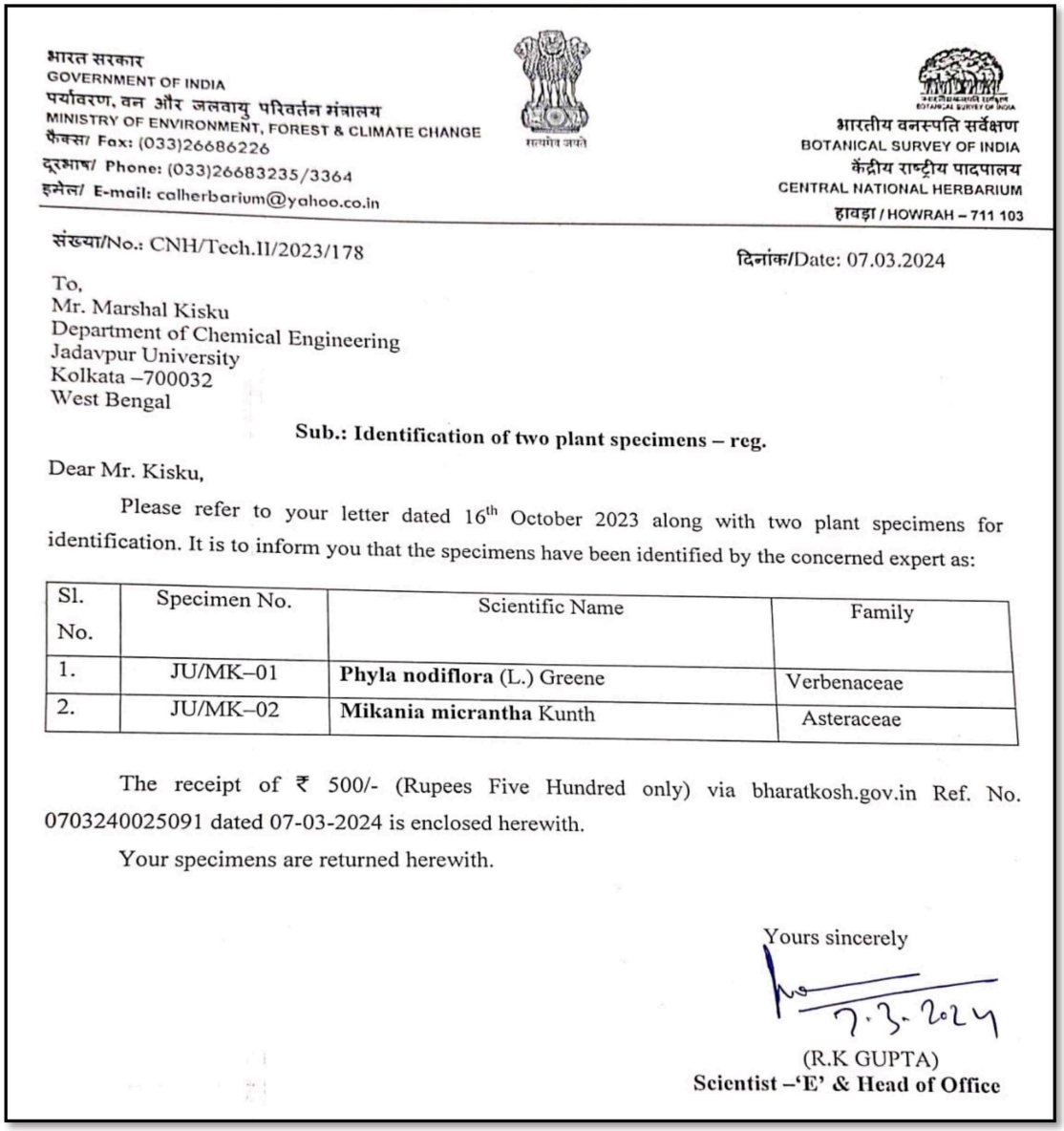
The sampling campaign was carried out in the summer (5^{th} may, 2023) season (Figure 6). The collected soil was kept in plastic ziplock bag and brought to the laboratory. Soil samples were then freeze-dried, homogenized and stored at refrigerator for analysis. Whereas, three replicates of fully grown leaves of both the species were collected and sealed in ziplock bags to prevent the moisture loss and contamination following sampling. In order to reduce the impact of rainfall on PM deposition on plant leaves, it was made sure that over 15 days had passed since the last rain event. Identification of plant species was done from Central National Herbarium (CNH), Botanical Survey of India (BSI), Howrah, West Bengal, 711103. We selected the identified species ***Mikania micrantha kunth*** (Figure 7) and ***Phyla nodiflora (L.) Greene*** (Figure 8) for the study due to their widespread distribution, growth and plentiful availability in the study area. Here we have attached the identification certificate (Figure 9).



Figure 7. *Mikania micrantha* kunth



Figure 8. *Phyla nodiflora (L.)* Greene



भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
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इमेल/ E-mail: calherbarium@yahoo.co.in

सत्यमेव जयते

भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA
केंद्रीय राष्ट्रीय पादपालय
CENTRAL NATIONAL HERBARIUM
हावड़ा / HOWRAH – 711 103

संख्या/No.: CNH/Tech.II/2023/178

दिनांक/Date: 07.03.2024

To,
Mr. Marshal Kisku
Department of Chemical Engineering
Jadavpur University
Kolkata –700032
West Bengal

Sub.: Identification of two plant specimens – reg.

Dear Mr. Kisku,

Please refer to your letter dated 16th October 2023 along with two plant specimens for identification. It is to inform you that the specimens have been identified by the concerned expert as:

Sl. No.	Specimen No.	Scientific Name	Family
1.	JU/MK–01	**Phyla nodiflora** (L.) Greene	Verbenaceae
2.	JU/MK–02	**Mikania micrantha** Kunth	Asteraceae

The receipt of ₹ 500/- (Rupees Five Hundred only) via bharatkosh.gov.in Ref. No. 0703240025091 dated 07-03-2024 is enclosed herewith.

Your specimens are returned herewith.

Yours sincerely

7.3.2024

(R.K GUPTA)
Scientist –'E' & Head of Office

Figure 9. Identification certificate

***Mikania micrantha* kunth** (Figure 7), a climber with long, flexible stems, hanging branches and are frequently rooted in the earth. The large leaves of this species are characterized with long petiole and leaf blade is roughly triangular in shape with an acute tip and a deeply cordate base that has been shortened and a nearly hairless underside (Table 3). Whereas, the leaves of ***Phyla nodiflora (L.)* Greene** (Figure 8) are green, ovate or oblanceolate and have a serrate leaf margin extending from the middle to tip of the leaf. This plant grows to a height of about 15 cm and its stem can reach up to 0.9 meters; as it ages, it turns woody at the base and is primarily yellow-green, but it become rubbish near the tip and leaf nodes. (Phyla nodiflora (L.)

Greene (worldfloraonline.org)) (Table 3). Following their collection, leaf samples were thoroughly cleaned three to five times in distilled water to get rid of any dirt or dust had accumulated on the leaf surfaces, as well as spider webs, aphids, insects' eggs and other debris from the foliage. The samples were then dried in a hot air oven at 80°C under hoover for a full day to avoid damaging of the matrix's volatile organic compounds, pigments and analytes (Mukhopadhyay et al., 2021). In order to increase the kinetics of analyte extraction by reducing diffusion routes and increasing the contact surface area between the sample and solvent, dried leaves were then manually pulverized with a motor and pestle to generate a homogeneous powder (Figure 10) (Mukhopadhyay et al., 2021).

Table 3. Plant Sample Characteristic (https://indiabiodiversity.org/species/show/230405) (https://indiabiodiversity.org/species/show/230730)

Plant	Family	Habit	Leaf Shape	Venation	Characteristic	Benefits
Mikania micrantha kunth	Asteraceae	A fast-growing, multi-branching, perennial vine having a twining, climbing, or creeping habit. Up to 6 meters or more can be grown on its stems.	Heart-Shaped or somewhat triangular	3 to 7 palmate veins extending from the base; subentire, wavy, or coarsely serrated margin; small petiole almost equal in length to the blade.	Long, climbing, variable development, sparse pubescence nearly hairless.	mental health, anti-inflammatory, wound dressing, and healing of sores.

Phyla nodiflora (L.) Greene	Verbenaceae	Forb/Herb	Ovate or oblanceolate with serrate leaf margin from the middle to the tip of the leaf.	Petioles are normally undetectable or hardly noticeable on both surfaces; they are 2-8 mm long or obsolete and frequently have such widely cuneate margins that they seem to be a part of the blade.	15 cm tall, green leaves, ovate or oblanceolate with serrate leaf margin from the middle to tip of the leaf.	treat constipation and knee pain.

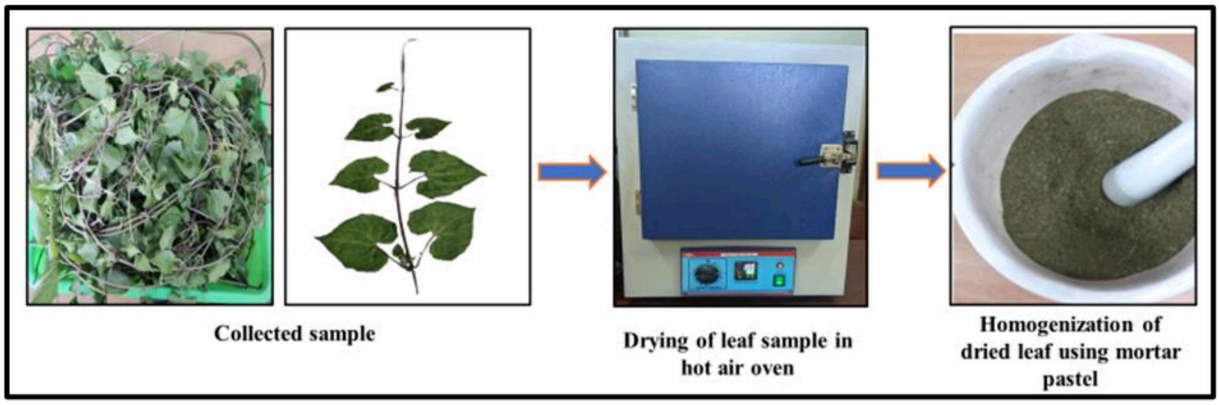


Figure 10. Drying and homogenization of collected leaf samples

4.3. Soil PAHs extraction, purification and identification:

Soil PAHs were extracted by the Soxhlet extraction method suggested by Sarkar et al. (2012) with slide modification using 120ml of n-Hexane for 6 hr (Figure 11). Using silica gel column chromatography, extract clean-up was accomplished by separating PAHs from matrix interferences (Figure 12). A chromatographic column fitted to a retort stand was loaded with the extracts, and 10 g of heated silica gel (10-200 mesh, chromatographic quality) was poured into it (Mukhopadhyay et al., 2021). But each time, before the column was packed, a silica gel slurry was made using the same organic solvent that was used to extract the sample. To ensure a constant quantity of silica gel throughout the procedure, cotton plugging was also carried out at the bottom of the spiral. Taking care to prevent the formation of fissures in the silica bed, extra solvent was carefully drained away after it took shape. Before purification, toluene was used to condition the column. The silica column was used in each instance to filter an 8 mL leaf sample's extract. The filtrates were concentrated using a Buchi R-3 Rotavapor rotary evaporator at low pressure. They were then resuspended in methanol (HPLC grade, assay≥99.9%) to bring the final volume up to 4 mL and refrigerated until analysis (Mukhopadhyay et al., 2023). In the end, PAHs were measured using a high-performance liquid chromatograph (HPLC, Waters, USA) coupled to UV detection at 254 nm (Waters 2489 UV/Visible detector) and combined with a binary pump (Waters 1525) to draw two solvents together under high pressure (maximum limit: 5000 psi) to create a gradient and a reversed phase Waters PAH C18 Column, S-5 µm (4.6 mm i.d.×250 mm length) for PAH separation (Figure 13). 20 µL loop with 20 µL sample injection volume was connected to the HPLC system's injector port. The following methodology was used to program the gradient elution at a flow rate of 1.5 mL min−1 using acetonitrile

(ACN) and water of HPLC grades as mobile phases: 50% ACN during the first five minutes, then raised to 100% in 20 minutes and kept for 28 minutes, then dropped to 50% once more in 32 minutes and held for 37 minutes (Waters Corporation 2008). The column was running for a total of 37 minutes at a temperature of 30 °C. The EPA Method 550.1 rules served as the foundation for the above-described procedure. Software called Breeze2 was used to collect and process the data.

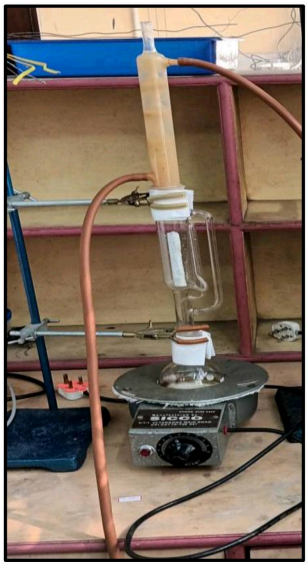


Figure 11. Soxhlet extraction

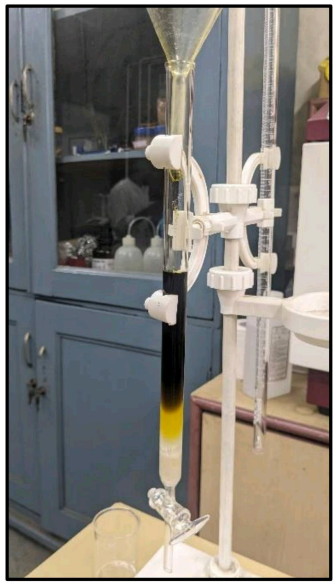


Figure 12. Silica gel column chromatography

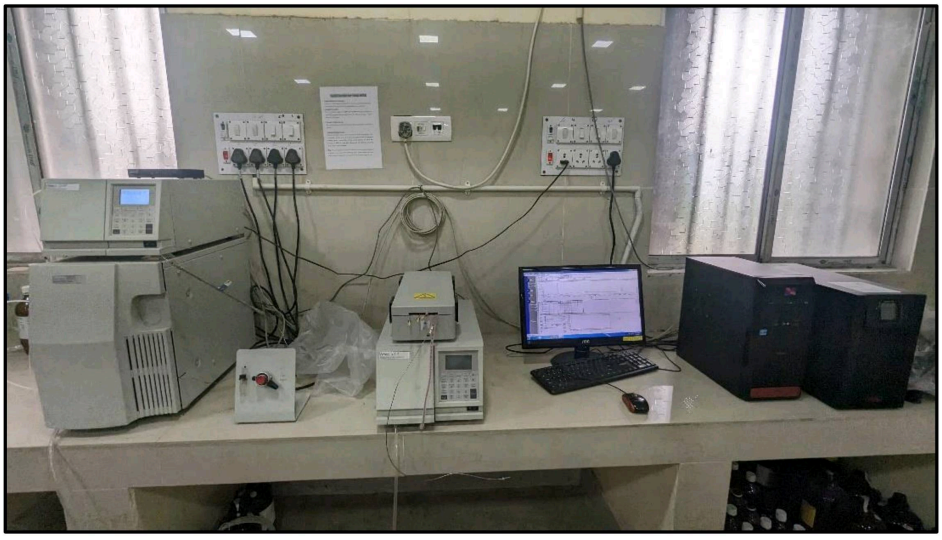


Figure 13. High-Performance liquid Chromatograph (HPLC)

4.4. Risk assessment related to soil PAHs contamination:

Both short- and long-term hazardous threats to wildlife and human health are associated with contaminated soil. Polycyclic aromatic hydrocarbons (PAHs) are known carcinogens of both humans and animals and are frequently found in soil. They are found in fuel spills, coal tars, creosotes, and urban runoff. Chlorinated pesticides have the potential to cause damage to the central nervous system and interfere with the endocrine systems of both humans and wildlife. In this context, assessment of risk associated with soil PAHs contamination will help the scientific society in designing of remediation strategies.

4.4.1. Soil toxicity guidelines:

The National Oceanic and Atmospheric Administration (NOAA) published a globally recognized guideline that uses two different values to determine the biological toxicity threshold of soil: the effects range low (ERL) and the effects range median (ERM) (Buchman, 1999).

An indicator of the contamination level below which effects are rarely observed is the Effects Range-Low (ERL), and an indicator of the contaminant level above which effects are typically observed is the Effects Range-Median (ERM) (Wolfe et al., 1994). The values indicate that soil with these levels of a specific contaminant typically cause biological effects in different tests, depending on the cumulative pollution load in the soil. However, they do not imply a causal connection between a specific contaminant and the observed effects. Also, the effect range-low values/effect range-median values (ERL/ERM) and the threshold Effect levels/Probable effect levels (TEL/PEL) are the SQGs (Soil Quality Guidelines) that were used to investigate. These coefficients were calculated according to the following formulas (Durant et al., 1996; Kapsimalis et al., 2014):

$$M - ERM - Q = \frac{\sum \frac{C_i}{ERM_i}}{n}$$

$$M - PEL - Q = \frac{\sum \frac{C_i}{PEL_i}}{n}$$

Where *ci* stand for level of compound *i*, ERM/PEL is the and ERM*i* /*PELi* same stand for compound *i* and *n* is the total number of different compounds. M-ERM-Q and M-PEL-Q is the

ERM and PEL quotient (Ashayeri et al., 2018). There is no harmful biological effect there is no harmful adverse biological effect (9% probability of toxicity) when, m-ERM-Q ≤ 0.1; potential adverse effect (21% probability of toxicity) when 0.1 < m-ERM-Q ≤ 0.5; moderate adverse effect (49% probability of toxicity) when 0.5 < m-ERM-Q ≤ 1.5; and significant harmful effect (76% probability of toxicity) when m-ERM-Q > 1.5. ERM and PEL standard value has been mentioned Table 9.

4.4.2. Risk Quotient:

Toxic equivalency factors (TEFs) and risk quotient (RQ) are the foundation of an approach that was developed to correctly and sensitively evaluate the ecosystem danger posed by PAHs (Cao et al., 2010). The risk quotient (RQ) of the PAHs under research and the associated environmental quality values were compared in order to evaluate the ecological toxicity of the PAH compounds in the soils. For PAHs in agricultural soil, there is not enough toxicological information available (Crommentuijn et al., 2000). The ecological danger of Σ16-PAHs was frequently evaluated using TEQ_{BaP} (Shi et al., 2021). RQ is able to evaluate the ecological risk of PAH monomers in addition to the overall ecological danger of Σ16-PAHs. Additionally, risk quotients (RQ) were utilized to evaluate the ecological hazards associated with chemicals to aquatic biota, as well as the overall ecological risks and possible ecological risks of PAH monomers (Zhang et al., 2022; Kalf et al., 1997; Sun et al., 2009; Wang et al., 2014). The risk level posed by certain PAHs were characterized by RQ calculated as :

$$RQ = \frac{C_{PAHs}}{C_{QV}}$$

where C_{QV} represents the equivalent quality values of specific PAHs in the medium and C_{PAHs} represents the concentration of specific PAHs in the medium. The quality values in the medium are derived from the Maximum Permissible Concentrations (MPCs) and Negligible Concentrations (NCs) of PAHs in water as reported by Kalf (1997). RQ_{NCs} and RQ_{MPCs} can therefore be described as follows:

$$RQ_{NCs} = \frac{C_{PAHs}}{C_{QV(NCs)}}$$

$$RQ_{MPCs} = \frac{C_{PAHs}}{C_{QV(MPCs)}}$$

where $C_{QV(NCs)}$ was the quality values of the NCs of PAHs in the medium, and $C_{QV(MPCs)}$ was the quality values of the MPCs of PAHs in the medium (Cao et al.,2010). NC_s and MPC_s standard value has been mentioned in Table 10. Risk classification of individual PAHs and total PAHs is presented in Table 4 (Cao et al., 2010). This classification system allows for the comparison of the ecosystem risk levels of PAHs across various media and may be used to evaluate the ecosystem risk of PAHs in any media (Cao et al., 2010).

Table 4. Individual PAH and ∑PAH risk classification (Cao et al., 2010).

Individual PAHs			∑PAHs		
	RQ_{NCs}	RQ_{MPCs}		$RQ_{\sum PAHs\ (NCs)}$	$RQ_{\sum PAHs\ (MPCs)}$
Very low risk	0		Very low risk	0	
			Low-risk	≥1; <800	0
Moderate-risk	≥1	<1	Moderate risk1	≥800	0
			Moderate-risk2	<800	≥1
High-risk		≥1	High-risk	≥800	≥1

4.4.3. BaP equivalent toxicity and mutagenicity:

The BaP toxic equivalency factors (TEFs) were used to estimate the toxic equivalent quantity of the BaP (TEQ_{BaP}) in order to quantify the probable toxicity of PAHs in soil by using the following formula (Durant et al., 1996; Nisbet & Lagoy, 1992):

$$BaP - TEQ = \sum C_i * TEF_i$$

where Ci is the concentration of the individual PAHs compound and TEF*i* (refer Table 8) represents the respective toxicity equivalency factor for each PAHs.

On the other hand, BaP mutagenic equivalent factors (MEF) were used to compute the mutagenic equivalent quantity (MEQs) of eight carcinogenic PAHs by the formula mentioned below (Durant et al., 1996; Lang et al., 2015; Masiol et al., 2012):

$$MEQs = \sum C_i * MEF_i$$

where C*i* is the concentration of the individual compound and MEF*i* (Table 8) represents the mutagenic equivalency factor for each PAHs.

4.5. Air Pollution Tolerance Index (APTI) of the plant's leaves:

The earth's surface has lost its green cover as a result of the rapid urbanization and industrialization of developing nations like India. An imbalance in nature is being caused by this situation. The toxicity of the air is increased by cumulative exposure to various contaminants. The Air Pollution Tolerance Index (APTI) is used to assess the amount of resistance or susceptibility of plants to air pollution. Through biochemical changes, some plants can respond to their environment, especially in the areas of chlorophyll, ascorbic acid, leaf pH, and relative water content. The air pollution tolerance index (APTI) of a certain plant species can be calculated by combining these modifications to biochemical traits. In general, APTI can be used to determine which plant species are sensitive, intermediate, and tolerant to air pollution (Bharti et al., 2018). Plants could be classified in four different groups on the basis of their tolerance level, described in Table 5. It takes into consideration four factors: ascorbic acid content (AA), total chlorophyll content (TCH), relative water content (RWC), and pH of leaf extract. To determine and compute these variables collectively, the plant's APTI is obtained (Pandey et al., 2015).

Table 5: Classification of plant on the basis of APTI value (Shrestha et al., 2021)

S.N.	APTI Value/range	Response/Degree or Level of Tolerance
1	>17	Tolerant
2	10-16	Immediately tolerant
3	<10	Sensitive
4	<1	Highly sensitive

These four factors RWC, pH, TCH, and AA were used to calculate the plant species' APTI using the equation below:

$$APTI = \frac{AA[(TCH + pH) + RWC]}{10}$$

It has the following units: AA in mg/g, TCH in mg/g, and RWC in percentage.

Leaf relative water content (RWC): Leaf samples (about 11 g) (FW) were floated in 150 ml distilled water for 12 hours. Then, it was dried and its turgidity weight (T) was determined. After then, it was oven dried at 75 °C for 24 hours to determine its dry weight (DW). RWC was calculated using the following formula (Shrestha et al., 2021):

$$RWC = \frac{Fresh\ weight - Dry\ weight}{Turgidity\ weight - Dry\ weight} \times 100$$

Leaf extract pH: 2 g of fresh leaf was crushed. After it is mixed in 40 ml distilled water. Then, it was stirred at 800 rpm at 25 °C for 20 min in a REMI.2MLH machine to determine its pH with a pH meter (Bharti et al., 2018).

Total chlorophyll content (TCH): Crushed 2 g leaf sample was extracted by 15 ml of acetone 80% in a beaker and stirred for 15 minutes. After that it was subjected to a spectrophotometer to be read at 645 mm for chlorophyll a and 665 mm for chlorophyll b. Total chlorophyll content was calculated using the following formula (Bharti et al., 2018):

$$Chla = \frac{(12.70 \times A663 - 2.69 \times A645)}{100 \times W} \times V$$

$$Chlb = \frac{(22.60 \times A645 - 4.68 \times A663)}{100 \times W} \times V$$

Where, total chlorophyll content (Chl_t) = (Chl_a+Chl_b), Chl_a=Amount of chlorophyll a; Chl_b=amount of chlorophyll b; A663= absorbance at 663nm; A645=absorbance at 645nm; W= weight of sample, and V= volume of sample.

Ascorbic acid (AA) content: In a 100 ml conical flask, pipette 5 ml of the working standard solution. When titrating against the dye (V1 ml), add 10ml of 4% oxalic acid. The arrival of pink color, which lasts for a few minutes, is the end point. Now, the consumption of dye is similar to the ascorbic acid quantity. The material is extracted (0.5–5g, depending on the sample) in 4% oxalic acid, diluted to a specified volume (100ml), and then centrifuged. After that, take 5 ml of this supernatant using a pipette, mix with 10 ml of 4% oxalic acid, and then titrate it against the dye (V2 ml). The formula below can be used to determine the ascorbic acid content (Shrestha et al., 2021):

$$AA = \frac{0.5mg \times V2\ ml \times 100ml}{V1\ ml \times 5ml \times weight\ of\ the\ sample} \times 100mg/g$$

4.6. Biochemical characterization of the plant's leaves:

In order to characterize plant leaves biochemically, a variety of chemicals and molecules that are important to the growth, development, and defense systems of the plant are analyzed in the leaves. In this study we found plant biology and ecology by characterizing these biochemical components and learning more about the physiological state, stress reactions, and metabolic pathways functioning within plant leaves (Iverson et al., 2001; Rozentsvet et al., 2022).

Wax: 2 g of crushed leaf sample is mix with 25 ml chloroform in a beaker. Then the sample is stirred for 5 min for through extraction with filter paper. Then the leaf extract is evaporated, and the final weight of the beaker is measured. The difference in the weight determined the wax content (Yang et al., 2017).

Lipid: The beaker's initial weight is determined. 20ml of methanol and 10ml of chloroform are used to extract 2g of material in a beaker for 15 minutes. After adding 10 milliliters of chloroform and extracting for 10 minutes, 10 milliliters of water are added and extracted for 20 minutes. After that, a separating funnel is used to gather the extract (Mukhopadhyay et al., 2021).

Sugar: Soluble sugars were extracted from plant ***Mikania micrantha kunth*** and ***Phyla nodiflora (L.) Greene***. Initial 1.5 g crushed sample is mixed with 10 ml hot ethanol in a beaker. Then the sample is stirred for 5 minutes for through extraction with filter paper. After the leaf extract stored in refrigerator. Then 2 ml of supinated sample centrifuge with 5 ml ZnS04 and 4.7 ml of Ba $(OH)_2$. Then, 4 ml of anthrone solution is added to 0.5 milliliters of supernatant. For ten minutes, the sample is maintained in boiling water. the sample in RT, and the absorbance at 630 nm is measured (Sarabi and Arjmand-Ghajur, 2021).

Protein: In 10 ml of extraction buffer, 1 gram of sample is extracted and crushed into a paste. The sample was centrifuged for 20 minutes. After being collected, the supernatant is kept in a refrigerator. After adding 0.1 ml of supernatant and 0.9 ml of Brad form reagent, everything is well combined and incubated for 10 minutes. At 595 nm, the absorbance is then measured (Jones et al., 1988).

Proline: Proline content of plant leaves determined according to the protocol proposed by Bates et al. (1973). 1 gm sample is mixed with 10 ml 3% sulfosalicylic acid and crushed well. Then the mixer is centrifuged and supernatant is collected. 2ml sample is brought to room temperature. 4ml toluene is added to the sample and mixed properly. Again, the mixed is centrifuged and colored layer is separated and absorbance is taken at 520 with the help of UV-VIS.

4.7. Bioconcentration factor (BCF):

Bioconcentration means a process through which an organism accumulates substances in response to the concentration of those substances in an external medium (Paraiba et al.,2010). The Bioconcentration factor, BCF is the quotient of the pollutant concentrations in the organism with respect to that of external medium. (Paraíba et al.,2010). Thus, the BCF provides the information about the bioconcentration of the polluting substance in the biological material under a specific environment as well as its accumulative potential (Paraiba, 2007; Paraiba and Kataguiri, 2008). The BCF calculated by using the following formula:

$$BCF = {}^{C_{leaves}}/_{C_{soil}}$$

Where, C_{leaves} and C_{soil} represent the concentration of PAHs in plant leaves and soil respectively (Oleszczuk et al., 2005).

4.8. Isolation and quantification of PM with different size fractions:

Particulate matter (PM) in the atmosphere is categorized in different ways. Fine PM is defined as smaller than 10 µm in diameter ($PM_{10\text{-}100}$), and ultrafine PM is defined as smaller than 2.5 µm in diameter ($PM_{2.5\text{-}10}$) (Kang and Kim, 2014). Both $PM_{10\text{-}100}$ and $PM_{2.5\text{-}10}$ can adsorb a wide range of toxic and harmful components on their surface, which can not only enter the human respiratory tract for deposition but also penetrate the alveoli into the blood circulation, posing a serious threat to public health (Liu et al., 2022; Stolzel et al., 2007).

Surface PM was measured from the particles deposited on plant leaves using the methodology developed by Dzierzanowski et al. (2011). To remove surface-adsorbed particles, leaf samples were placed in Erlenmeyer flasks and extensively cleaned with 250 mL of deionized water while being stirred for one minute (Mukhopadhyay et al., 2024). The washed-out solutions were first filtered using a metal sieve to remove particles larger than 100 µm. Gravimetric analysis was used to assess the amount of dust accumulated on leaves. Using distilled water, the fresh leaves were thoroughly cleaned. The cleaned solutions were then filtered through Whatman Grade 1 cellulose filters, which collected large fractions of PM (between 10 and 100 µm), and Whatman Grade 42 filter paper, which retained coarse PM (2.5–10 µm). The particles had previously been weighed dry to constant weights (Mukhopadhyay et al., 2021). Wet filter papers covered in dust were once more dried in an oven and weighed. The amount of dust (mg) on the plant leaves was determined by the difference in weights between the two stages of the filters. According to Prajapati and Tripathi (2008), leaf surface areas (cm2) were also ascertained by drawing the impression of a single leaf on graph paper. Using the ratio of leaf area to leaf dry mass in cm^2 g^{-1}, specific leaf area was calculated (Mukhopadhyay et al., 2021). The weight of the dust divided by the total leaf area was then used to evaluate the plant leaves' capacity to capture or retain dust (Jiao et al., 2004; Yang et al., 2017). The final unit of measurement for foliar dust concentration or dust load was mg cm^{-2} and mass of each PM fraction was noted.

4.9. Extraction, purification and analysis of PAHs from leaves and PM:

A sample weighing 5 g (d.w.) was Soxhlet extracted for 6-8 hours in 120 mL of n-hexane. The primary extractor chamber of the device was filled with a filter paper thimble containing the solid plant matrix for continuous extraction by solvent reflux; the exhaustive extraction cycles were performed multiple times at the solvent's boiling point temperature (Figure 12). After that, the final extract was chilled to aid in additional separation and cleanup, resulting in the active PAHs fraction of the leaves (Mukhopadhyay et al., 2023). The size fractionation of collected PM was done for two different groups including $PM_{2.5\text{-}10}$ and $PM_{10\text{-}100}$ by using Whatman Grade 1 cellulose filters paper (Figure.14) and Whatman Grade 42 filter paper (Figure.15) respectively. Then, PM-bound PAHs were extracted by mechanical stirring using 150ml of toluene for 4hrs (Dzierzanowski et al., 2011) process the data. In both cases, silica gel column chromatography and HPLC analysis was performed for extract purification and PAHs concentration determination respectively. The methods have been described elaborately in the section 4.3.



Figure 14. Whatman Grade 1 cellulose filter paper

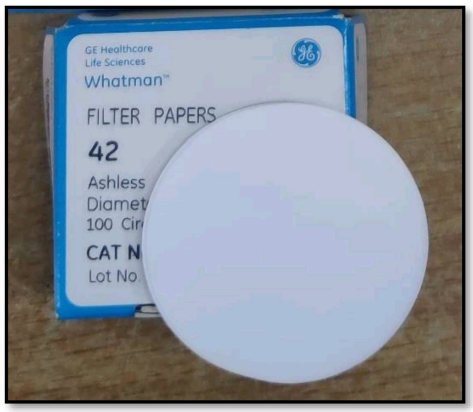


Figure 15. Whatman Grade 2 cellulose filter paper

4.10. PAHs source identification by diagnostic ratios:

The majority of polycyclic aromatic compounds found in the environment are either pyrogenic or petrogenic, with human activity being the primary cause of both. Pyrogenic compounds are discharged into the atmosphere during the burning of fossil fuels (Younis et al., 2023). PAHs with a petrogenic origin are released from crude oil and its refined fuels, including kerosene, gasoline, diesel, lubricating oils, and asphalt, at temperatures between 100 and 300 °C (Flores et al., 2022). At temperatures between 350 °C and above 1200 °C, pyrogenic sources release

particulate matter at high temperatures when incomplete combustion occurs in organic materials (coal, wood, petroleum, etc.), fossil fuels are used in power plants and other industrial operations, waste is burned, gasoline or diesel fuel is used in cars, and forest fires occur (Abdel-Shafy and Mansour, 2016; Wolska et al., 2012). The temperature at which each source formed is related to the PAH profiles. PAH diagnostic ratios have been widely documented in the literature and are useful for identifying sources (Ari et al., 2020; Elorduy et al., 2016; Javed et al., 2019; Tobiszewski and Namie´snik, 2012). However, the diagnostic ratios should be used at sampling locations near the emission sources when PAHs may undergo photochemical reactions in the atmosphere (Javed et al., 2019). As a first diagnostic ratio, the PAHs released by combustion sources (such as Flu, Pyr, Chr, BbF, BkF, BaA, BaP, IP, and BghiP) can be used to determine how much of the overall PAH content is attributable to combustion sources (Zhang et al., 2016). The diagnostic ratio was determined for different PAHs of interest, as summarized in Table 6.

Table 6. Diagnostic ratio reported for PAHs in soil (Hwang et al., 2003)

PAHs	Diagnostic Ratio	Sources
∑LMW/∑HMW	>1	pyrogenic
	<1	petrogenic
FLU/(FLU+PYR)	<0.5	Petrol emission
	>0.5	Diesel emission
ANT/(ANT+PHE)	<0.1	petrogenic
	>0.1	pyrogenic
FLT/(FLT+PYR)	<0.4	petrogenic
	0.4-0.5	pyrogenic
	>0.5	biomass, coal combustion
BaA/(BaA+CHR)	<0.2	petrogenic
	0.2-0.35	Petroleum comb.
	>0.35	Biomass, coal comb.
IcdP/(IcdP+BghiP)	<0.2	petrogenic
	0.2-0.35	Petroleum comb.
	>0.5	Grass, wood and coal

		comb.
BbF/BkF	2.5-2.9	Aluminium smelter emission
BaP/BghiP	<0.6	Non-traffic emission
	>0.6	Traffic emission

CHAPTER: 5

5. Result and Discussion:

5.1 Distribution of PAHs in Soil:

PAHs are the environmental toxins, widely distributed and have a moderate to low solubility in water. This allows them to attach themselves to particles and then accumulate in soil (McGrath et al., 2019). In water, PAHs often adsorb on particulate matter before settling and building up in soil. Water disturbance causes PAHs that have accumulated in soil to be resuspended and released into the water, so posing a risk to human health before they are transferred to the food chain (Yim et al., 2007; Luo et al., 2008; Li et al., 2009; Arias et al., 2010; Sarkar et al., 2012; Keshavarzifard et al., 2014; De et al., 2005). In addition to being important sources of food and shelter for the majority of benthic animals and overlying aquatic organisms, soil also acts as accumulation reservoirs for persistent organic pollutants (Unlu et al., 2010; Zhao et al., 2014; Mu et al., 2015; Liu et al., 2017; Viguri et al., 2002; An et al., 2016; Varnosfaderany et al., 2015; Zhang et al., 2012). A comprehensive understanding of the distribution of PAHs in soil is crucial to analyze the degree of pollution and ecological risks (Han et al., 2019). According to the present work, the total concentration of the 16 USEPA PAHs in the chosen wetland soil was 283.14 ± 6.27 µg. g^{-1} (Table 7).The contamination level of soil can be categorized as low (0-0.1 µg. g^{-1}), moderate (0.1-1 µg. g^{-1}), high (1-5 µg. g^{-1}) and very high (>5 µg. g^{-1}) according to Baumard et al. (1998). Another study of Maliszewska-Kordybach et al. (1996) classified PAHs pollution level of soil as not contaminated (<0.2 µg. g^{-1}), weakly contaminated (0.2-0.6 µg. g^{-1}), contaminated (0.6-1 µg. g^{-1}) and heavily contaminated (>1 µg. g^{-1}). Our survey site is highly polluted based on these two scales presented by Baumard et al. (1998) and Maliszewska-Kordybach et al. (1996). Another group of researchers reported that (Han et al. (2019) and Guang Gu et al. (2020)) the level of PAHs pollution level in the soil were 0.4035 µg. g^{-1} and 0.2464 µg. g^{-1}, respectively, which is significantly lower than the result obtained in our study. On the other hand, the recorded concentration of total PAHs (1286.01±374.07) in Woji Creek in the Nigeria Delta of Nigeria was much higher than the present study (Ihunwo et al., 2021). PAHs like DahA, ACE, and ACY were not found in our samples during this investigation. The percentage contribution of individual congener of PAHs compounds in the total concentration of PAHs are BbF (0.32 %) > PYR (1.010%) > CHR (1.123%) > IP (1.81%) > BghiP (8.05%) > NAPH (8.548%) > PHEN (8.76%) > FLU (9.72%) > BkF (11.29%) > FLT (11.42%) > ANT

(11.91%) > BaA (12.18%) > BaP (13.83%). As per the percentile contribution, HMW PAHs (4-rings: 25.74%, 5-rings: 25.45%, 6-rings: 9.87%) predominated over LMW PAHs (2-rings:8.54%, 3-rings:30.39%) in the study area. This trend is consistent with the study conducted in Woji Creek in the Nigeria Delta of Nigeria (Ihunwo et al., 2021) and Lake Macquarie in Austria (Idowu et al., 2020). In order to achieve the objective of the present research work, the high level of PAHs pollution in the soil sample directs us to select the area as study zone and consequently for conducting the biomonitoring study of PAHs contamination.

Table 7: Concentration of PAHs in the soil collected from chosen wetland

PAHs	Concentration (µg g^{-1})	Contribution (%)
NAPH	24.2 ± 0.85	8.548
ACE	Nd	nd
ACY	Nd	nd
FLU	27.52 ± 1.76	9.71
PHEN	24.8 ± 0.14	8.76
ANT	33.74 ± 0.19	11.91
FLT	32.34 ± 1.18	11.42
PYR	2.86 ± 0.09	1.010
BaA	34.5 ± 0.36	12.18
CHR	3.18 ± 0.58	1.123
BbF	0.92 ± 0.06	0.32
BkF	31.96 ± 0.74	11.29
BaP	39.18 ± 0.58	13.83
DahA	Nd	nd
BghiP	22.8 ± 0.85	8.05
IP	5.14 ± 0.61	1.81
∑ TOTAL	**283.14 ± 6.27**	100

*nd: not detected; *na: not available

5.2. Risk Assessment:

5.2.1 BaP equivalent toxicity (BaP-TEQ) & mutagenicity (BaP-MEQ):

In the present study, we assess the BaP-TEQ and BaP-MEQ to determine carcinogenic and mutagenic equivalents, respectively for the soil of the study zone. The BaP-TEQ and BaP-MEQ values of studied PAHs are summarized in Table 8. The estimated value of ∑ BaP-TEQ was 47.1102 ± 2.98 μg. g^{-1}. According to the Canadian soil quality guidelines for protection of the environment and human health based on PAH carcinogenic effects, a safe ∑BAP-TEQ value should be less than 0.6 μg g^{-1} (Nahar et al., 2023). The result indicated that the studied area is highly prone to biological risk for exposure to the PAHs concentration in Soil. The studied value of ∑BAP-TEQ in this case was higher than that reported in the soil from Southwest Nigeria (0.3074 μg. g^{-1}) and sediment of Khark Island of SW Iran (0.00655 μg. g^{-1}), reported by Akhbarizadeh et aal., 2016 and Parra et al., 2020. However, the value was lower than that of recorded in the sediment sample collected from Woji Creek in the Nigeria Delta region of Nigeria (115.29 μg. g^{-1}) (Ihunwo et al., 2021). BaP was the largest contributor in causing toxicity whereas PYR contributed the least to toxicity risk (Table 8). In this study, the average BaP-TEQ contribution from 16 PAHs (Table 8) were ranked in the following order: BaP (83.16%) > BaA (7.32%) > BkF (6.78%) > IP (1.09%) > ANT (0.71%) > BghiP (0.48%) > BbF (0.19%) > FLT (0.06%) > FLU (0.0573%) > PHEN (0.0526%) > NAPH (0.0509%) > CHR (0.006%) > PYR (0.004%). Therefore, the most of the toxicological issues of that wetland area had their roots in BaP, BaA and BkF. This was also confirmed by the BaP-TEQ assessment of the sediment collected from Egyptian red sea (Younis et al., 2023), Woji Creek in the Nigeria Delta region of Nigeria (Ihunwo et al., 2021), Wadi El Bey Watershed in Tunisia (Gadara et al., 2017) and Shandong Peninsula (Li et al., 2014). Therefore, BaP concentration serves as a marker for carcinogenic effects of PAHs in the soil. BaP-TEQ of HMW PAHs (46.697 μg. g^{-1}) is higher than that of LMW PAHs (0.4132 μg. g^{-1}). The dominance of HMW PAHs thus implied their highest potential of toxicity. According to Yu et al. (2015), the primary sources of these PAHs during summer months were grass, wood and orcoal combustion or vehicular emission. The high concentration of HMW PAHs in the research site suggests the substantial potential risk caused by carcinogenic and toxic properties of HMW PAHs, especially BaP (Jafarabadi et al., 2021).

The total BaP-MEQ value calculated for 8 PAHs was 51.733 ± 3.06 µg. g^{-1} (Table 8). In comparison to other studies, the calculated BaP-MEQ value recorded in the present work is much higher than those reported for Khark Island, SW Iran (0.00518 µg. g^{-1}; Akhbarizadeh et al., 2016), Liaohe delta reed wetland, China (0.0333 µg. g^{-1}; Lang et al., 2015), while much lower than Woji creek in the Nigeria Delta region of Nigeria (143.67 µg. g^{-1}; Ihunwo et al., 2021). The percentage contribution of the following 8 PAHs in the total BaP-MEQ was ranked as follows: BaP (75.73%) > BghiP (8.373%) > BkF (6.794%) > BaA (5.468%) >IP (3.079%) > BbF (0.444%) > CHR (0.104%). The BaP, BghiP and BkF are found to be the primary causes. In addition, Ihunwo et al. (2021) reported that BaP and CHR were the primary and least contributors of mutagenic effects in the Woji creek in the Nigeria Delta region of Nigeria. BaP concentrations were discovered to be greater in their respective research fields by other studies most notably Parra et al. (2020) and Lang et al. (2015). The result obtained showed that there is a significant biological risk of exposure to PAHs concentrations in the study area indicated by BaP-MEQ value, which points to a highly contaminated local source that may be dependent on sources of vehicle emissions.

Table 8. BaP-TEQ and BaP-MEQ of Soil PAHs

PAHs	TEF (Nisbet et al., 1992)	BaP - TEQ	Contribution %	MEF (Durant et al., 1996,1999)	BaP-MEQ	Contribution %
NAPH	0.001	0.024 ± 0.005	0.0509	--	--	--
ACY	0.001	--	--	--	--	--
ACE	0.001	--	--	--	--	--
FLU	0.001	0.027 ± 0.003	0.0573	--	--	--
PHEN	0.001	0.0248 ± 0.02	0.0526	--	--	--
ANT	0.01	0.3374 ± 0.3	0.71	--	--	--
FLT	0.001	0.032 ± 0.006	0.06	--	--	--
PYR	0.001	0.002 ± 0.02	0.004	--	--	--
BaA	0.1	3.45 ± 0.39	7.32	0.082	2.829 ±	5.468

					0.83	
CHR	0.01	0.003 ± 0.005	0.006	0.017	0.054 ± 0.005	0.104
BbF	0.1	0.092 ± 0.006	0.19	0.25	0.23 ± 0.55	0.444
BkF	0.1	3.196 ± 0.57	6.78	0.11	3.515 ± 0.35	6.794
BaP	1	39.18 ± 0.58	83.16	1.0	39.18 ± 0.58	75.73
DahA	1	--	--	0.29	--	--
BghiP	0.01	0.228 ± 0.55	0.48	0.19	4.332 ± 0.48	8.373
IP	0.1	0.514 ± 1.06	1.09	0.31	1.593 ± 0.29	3.079
TOTAL	**-**	**47.1102 ± 2.98**	**100**		**51.733 ± 3.06**	**100**

5.2.2. Soil quality guidelines:

The impact evaluation on aquatic soil was conducted using the Effects Range Low (ERL) and Effects Range Median (ERM) values, which were ranked from low to high impact values (Long et al. 1995). Using two benchmarks as a guide, the effects of PAH on organisms are categorized based on their concentrations. ERM (or PEL) and ERL (or TEL), while levels between these two standards are occasionally hazardous to organisms, amounts over ERM (or PEL) are frequently identified as dangerous, and amounts below these two benchmarks are expected to be safe. The current ERL and ERM values were compared to the observed concentrations of PAHs (He et al., 2014; Long et al., 1995; Sun et al., 2017; Zhang et al., 2016). As shown in Table 9, all the individual PAHs congeners exceeded the ERM/PEL values. Hence, adverse biological effects are often linked to the soil of the study site. In this context, the biological risk or predicted combined toxicity for exposure to a mixture of PAHs in soil can be evaluated using M-ERM-Q and M-PEL-Q. The mean value M-ERM-Q and M-PEL-Q were found to be 12.76 µg. g^{-1} and 43.57 µg. g^{-1} respectively. Additionally, IP was also detected in the studied soil, however its ERM/PEL limits are still unknown. Hence, the risk related to IP in comparison to these levels was not described in the present work.

Table 9. Standard values of ERM and PEL of 16 PAHs & corresponding result obtained in the soil of study site

PAHs	Concentration i ($\mu g\ g^{-1}$)	ERMi (Long et al., 1995) ($\mu g.\ g^{-1}$)	$\frac{Ci}{ERMi}$	$\sum\frac{C_i}{ERM_i}{n}$ ($\mu g.\ g^{-1}$)	PELi (CCME,1999) ($\mu g.\ g^{-1}$)	$\frac{Ci}{PELi}$	$\sum\frac{C_i}{PEL_i}{n}$ ($\mu g.\ g^{-1}$)
NAPH	24.2 ± 0.85	2.1	11.5		0.391	61.89	
ACE	nd	0.64	Nd		0.128	nd	
ACY	nd	0.5	Nd		0.0889	nd	
FLU	27.52 ± 1.76	5.1	5.3		1.494	18.42	
PHEN	24.8 ± 0.14	1.5	16.5	21.76	0.544	45.58	43.57
ANT	33.74 ± 0.19	1.1	30.67		0.245	137.71	
FLT	32.34 ± 1.18	5.1	6.34		1.494	21.64	
PYR	2.86 ± 0.09	2.6	1.1		1.398	2.04	
BaA	34.5 ± 0.36	1.6	21.56		0.693	49.78	
CHR	3.18 ± 0.58	2.8	1.13		0.846	3.75	
BbF	0.92 ± 0.06	1.88	0.48		na	nd	
BkF	31.96 ± 0.74	1.62	19.72		na	nd	
BaP	39.18 ± 0.58	1.6	24.48		0.763	51.34	
DahA	nd	0.26	Nd		0.135	nd	
BghiP	22.8 ± 0.85	1.6	14.25		0.763	29.88	

*nd: not detected; *na: not available

5.2.3. Risk Quotient:

The risk quotient (RQ), which has gained popularity in recent times, is also utilized for evaluating the possible risks associated with PAHs in soil. The study area can be considered into various degrees of ecological risk, ranging from low, medium to high risk level, based on the values of $RQ_{(NCs)}$ and $RQ_{(MPCs)}$ of individual PAHs and total PAHs. The results of the current study indicate that the $RQ_{(NCs)}$ for each individual PAH is greater than 1 (Figure 16(a)), indicating a significant level of ecological risk. Furthermore, all the $RQ_{(MPCs)}$ >1, with the exception of CHR and IP (Figure 16(b)), not only indicate ecological danger but necessitates the prompt deployment of control measures and corrective action. At the study location, high levels of ecological risk are found by **$RQ_{\sum PAHs(NCs)}$ (844.4) >800** and **$RQ_{\sum PAHs(MPCs)}$ (8.444) >1** (Table 10) where Cao et al. (2010) showed their results in soil $RQ_{\sum PAHs\ (NCs)}$ had 0.0403, which is less than 1 and $RQ_{\sum PAHs\ (MPCs)}$ had 0.00, which is equal to 0. Moreover, the absence of ACE, ACY, and DahA (Figure 16(b)) in the soil suggests that these PAH molecules do not appear to be associated with the threat with the area. The findings indicated a substantial ecological hazard that may be brought on by a variety of pyrogenic and petrogenic activities, such as burning, heavy vehicle emissions, etc which are dangerous for both humans and the environment.

Table 10. Standard values of RQ_{NCs} and RQ_{MPCs} and result obtained for the soil collected from study site

PAHs	**NCs (µg. g^{-1})** (Cao et al., 2010)	**MPCs (µg. g^{-1})** (Cao et al., 2010)	**RQ_{NCs}**	**RQ_{MPCs}**	**$RQ_{\sum PAHs(NCs)}$**	**$RQ_{\sum PAHs(MPCs)}$**
NAPH	0.0014	0.14	17285.0	172.85		
ACE	0.0012	0.12	nd	nd		
ACY	0.0012	0.12	nd	nd		
FLU	0.0012	0.12	22933.3	229.33		
PHEN	0.0051	0.51	48627	48.62		
ANT	0.0012	0.12	28116.6	281.16		
FLT	0.026	2.6	1243.84	12.43		

PYR	0.0012	0.12	2383.3	23.83	**844.4**	**8.444**
BaA	0.0036	0.36	9583.33	95.833		
CHR	0.107	10.7	29.71	0.29		
BbF	0.0036	0.36	255.5	2.55		
BkF	0.024	2.4	1331.6	13.31		
BaP	0.027	2.7	1451.1	14.51		
DahA	0.027	2.7	nd	nd		
BghiP	0.075	7.5	304	3.04		
IP	0.059	5.9	87.11	0.87		

*nd: not detected;

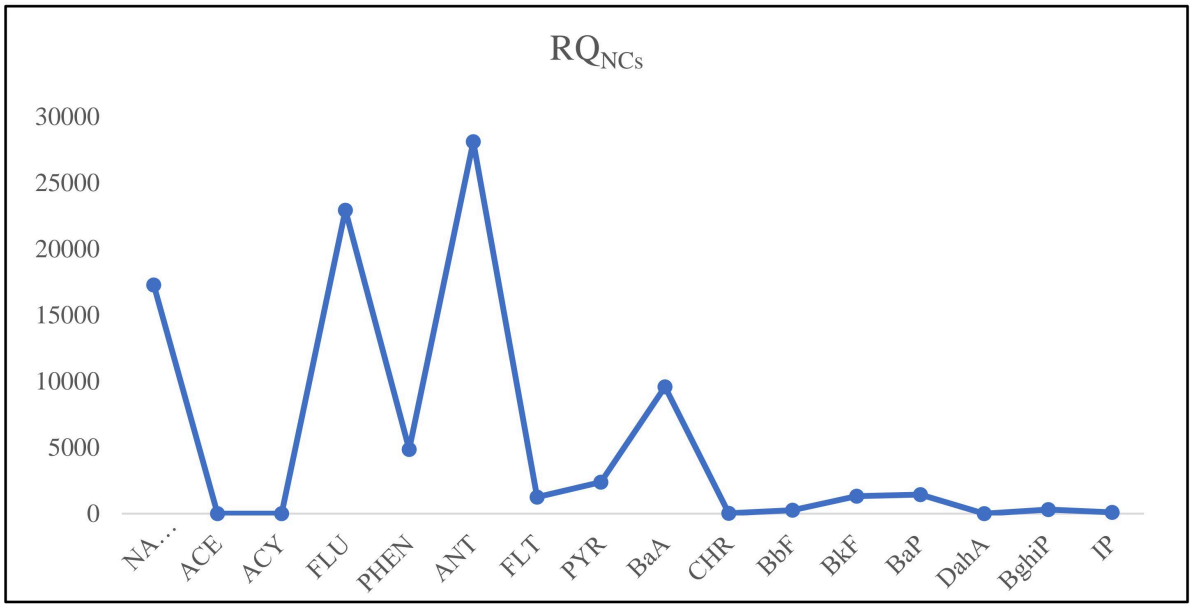


Figure 16(a). RQ_{NCs} of individual PAHs congeners present in soil

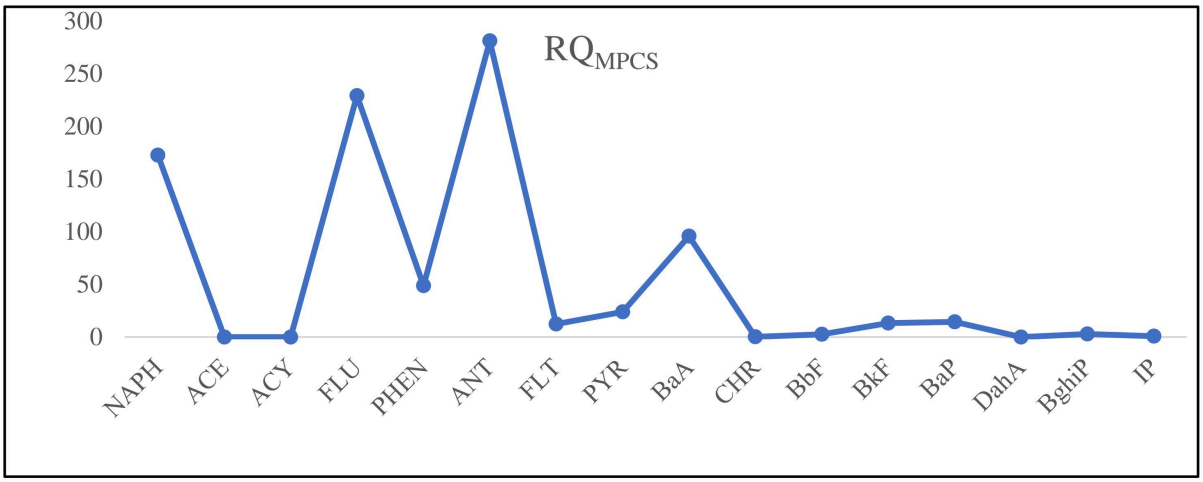


Figure 16(b). RQ_{MPCs} of individual PAHs congeners present in soil

5.3. Biomonitoring potential of plant species and monitoring of PAHs pollution:

5.3.1. APTI of plant leaf:

The earth's surface has lost its green cover as a result of the rapid urbanization and industrialization of developing nations like India. An imbalance in nature is being caused by this situation. The toxicity of the air is increased by cumulative exposure to various contaminants. The Air Pollution Tolerance Index (APTI) is used to assess the amount of resistance or susceptibility of plants to air pollution. Through biochemical changes, some plants can respond to their environment, especially in the areas of chlorophyll, ascorbic acid, leaf pH, and relative water content. The air pollution tolerance index (APTI) of a certain plant species can be calculated by combining these modifications to biochemical traits. In general, APTI can be used to determine which plant species are sensitive, intermediate, and tolerant to air pollution (Bharati et al., 2018). Plants could be classified in four different groups on the basis of their tolerance level, described in (Table 5). It takes into consideration four factors: ascorbic acid content (AA), total chlorophyll content (TCH), relative water content (RWC), and pH of leaf extract. To determine and compute these variables collectively, the plant's APTI is obtained (Pandey et al., 2015).

5.3.1.1. RWC:

Relative water content is defined as the proportion of the water in a leaf at the moment of sample to the maximum amount of water that leaf can hold. It is a crucial factor in water relationship studies since it, for instance, enables the computation of the osmotic potential at full turgor

(Arndt et al., 2015). RWC is found to be in the range of 60–70% depending on plant species (Mukhopadhyay et al., 2021). In this study we found relative water content is 85.236 % for ***Mikania micrantha kunth*** and 86.30 % for ***Phyla nodiflora (L.) Greene*** (Table.11). The high RWC in plants regulates the physiological functions of plants under stress condition, particularly, when plants get exposed to high levels of airborne pollutants (Bharti et al., 2018). According to the result, the RWC value of ***Phyla nodiflora (L.) Greene*** is higher than that of ***Mikania micrantha kunth***. The result showed that the RWC differed between the two species. Pandey et al. (2015) found highest RWC (75%) for ***Adenocalymma comosum*** and lowest RWC (50%) for ***Hemidesmus indicus Br.*** The capacity of plants to tolerate air pollution is better when the RWC is higher (Bharti et al., 2018).

5.3.1.2. Leaf extract pH:

Alkalinity affects the tolerance threshold of several plant species through detoxifying mechanisms developed in the plant under polluted conditions. Other processes that are impacted by the pH of the leaf tissue include cell proliferation, the effectiveness of photosynthetic reactions, the stomatal activity of plant leaves, and the proper operation of numerous enzymes (Jai and Davies, 2007). In a polluted environment, plant leaves' pH decreases or becomes acidic as a result of excessive quantities of SOX and NOX in the air around them. In this study the value of leaf extract pH of *Mikania micrantha kunth* is 5.5 and ***Phyla nodiflora (L.) Greene*** is 6.53(Table.11). The study's findings are consistent with those of Bharti et al. (2018) who considered *S. cumin* and found lowest pH of leaf extract (4.5) at control site and Pandey et al. (2015) who found the highest pH of leaf extracts (8.3) in *Vernonia elaeagnifolia* at control site. According to the results, Phyla nodiflora (L.) Greene has a higher pH of leaf extract value than ***Mikania micrantha kunth.*** This study found that low pH at residential sites decreased photosynthesis by impairing gaseous exchange through stomata, whereas higher leaf extract pH at the study site improved plants' tolerance to air pollutants by accelerating the rate of hexose sugar to ascorbic acid conversion (Bharti et al., 2018).

5.3.1.3. Ascorbic acid content:

According to Bharti et al. (2018), the ascorbic acid concentration (mg/g) ranged from 0.6 to 19.6 on average. Another study of Shahrukh et al. (2023) showed ascorbic acid content of leaf extract of *F. benghalensis* was found to be 2.63 ± 0.47 mg/g at their study site. According to our study Ascorbic acid content of ***Mikania micrantha kunth*** was to be 8.863 ± 0.09 mg/g and ***Phyla***

nodiflora (L.) Greene was to be 7.5 mg/g(Table 11). In present study both species (***Mikania micrantha kunth*** and ***Phyla nodiflora (L.)***) as compared to previous study Shahrukh et al. (2023) to be higher. To fight the stressful situation, ascorbic acid levels also increased along with pollution. It performs several different jobs, including being a powerful reductant, an electron donor, a free-radical scavenger, a catalyst for converting sulphite to hydrogen sulphide, and a chemical that lowers the toxicity of SO2 (Shrestha et al., 2021). One of the variables that may be held liable for the varying levels of plant tolerance to various air contaminants at both places is variation in ascorbic acid concentration.

5.3.1.4. Total chlorophyll content (TCH):

Plants capable of producing high chlorophyll content even under polluted conditions are commonly tolerant to air pollutants (Shahrukh et al., 2023). The total chlorophyll (TCH) content (mg/g) is a popular indicator of how air pollution impacts the rate of photosynthesis in plant leaves. According to Bharti et al. (2018), TCH is used as a proxy for biomass productivity, growth, and photosynthetic activity. Plants from the control location were found to have more chlorophyll than those from the wetland section of the residential site. In their study of 24 species of climbers in Varanasi, Uttar Pradesh, India, Pandey et al. (2015) found that the chlorophyll content was lower at polluted urban sites (2.98 0.03-11.82 0.84 mg/g) and higher at the control site (3.89 0.87-11.58 0.92 mg/g). According to our study the outcome of the total chlorophyll content of ***Mikania micrantha kunth*** is 5.043 mg/g and ***Phyla nodiflora (L.) Greene*** is 2.035 mg/g (Table 11). Therefore, it can be concluded that ***Phyla nodiflora (L.) Greene*** has lower plant chlorophyll content than ***Mikania micrantha kunth***, indicating greater atmospheric pollution, whereas Mikania micrantha kunth has higher plant chlorophyll content, indicating greater tolerance to harmful contaminants (Ogunkunle et al., 2015).

As potential bio-indicators of pollution, higher APTI values make plants more tolerant of air pollution than lower APTI values. According to our study, the value of ***Mikania micrantha kunth*** is 17.86 and ***Phyla nodiflora (L.) Greene*** is 15.05 (Table 11), where ***Mikania micrantha kunth*** is tolerant and ***Phyla nodiflora (L.) Greene*** is immediately tolerant from Table 5.

Table 11. APTI parameters and its value for two wetland plant leaves: *Mikania micrantha kunth* & *Phyla nodiflora (L.) Greene*

Parameter	*Mikania micrantha kunth*	*Phyla nodiflora (L.) Greene*
RWC (%)	85.236 ± 0.55	86.30 ± 0.49
pH	5.5 ± 0.36	6.53 ± 0.34
Ascorbic acid	8.863 ± 0.09	7.5 ± 0.36
Chlorophyll (mg/g)	5.043 ± 0.68	2.035 ± 0.69
APTI	**17.86 ± 1.45**	**15.05 ± 3.22**

As a result, it is clear that the ***Mikania micrantha kunth*** of the collected sample is tolerable to air pollution with APTI > 17 at study site. This makes it advantageous to use the ***Mikania micrantha kunth*** as a bio monitor that enables both qualitative and quantitative assessment of eco-health for accurate estimates of air pollutant exposure. Higher index values imply high tolerance of plants, competent enough to withstand environmental pollution with less visible damage or external injury (Mukhopadhyay et al., 2021). Another result of ***Phyla nodiflora (L.)*** **Greene** of collected samples is immediately tolerable to air pollution with APTI (10-16) at study site.

5.3.2 Biochemical characteristic of plant leaf:

In response to abiotic stressors, leaf lipids first provide signals about the onset of the stress and then mitigate cellular damage by remodeling the lipids in the membrane and restoring the stability, fluidity, permeability, and integrity of the membrane integrity (Balogh et al., 2013; Moradi et al., 2017). Due to their lipophilicity, these have also been shown to be the primary domains for the accumulation, absorption, partitioning, and digestion of organic pollutants (like PAHs) in plant leaves from the air (Hwang and Wade, 2008; Zhang and Zhu, 2009), with temperature variations having an impact on the process (Simonich and Hites, 1994). In this study, leaf lipid in ***Mikania micrantha kunth*** and ***Phyla nodiflora (L.) Greene*** was 61 mg/g and 37 mg/g (Table.12). High content of plant leaf lipids of ***Mikania micrantha kunth*** at the polluted site make it good predictor of PAHs pollution load than ***Phyla nodiflora (L.) Greene*** and it is a salient feature of plant tolerance to pollution stress.

Table 12. Leaf characteristic of *Mikania micrantha kunth* and *Phyla nodiflora (L.) Greene*

Parameter (mg/g)	*Mikania micrantha kunth*	*Phyla nodiflora (L.) Greene*
Wax	30.5 ± 0.36	23 ± 0.71
lipid	61 ± 0.71	37 ± 0.71
Sugar	0.550 ± 0.04	0.186 ± 0.58
protein	6.807 ± 0.14	4.172 ± 0.59
Proline	1.968 ± 0.73	13.4 ± 0.43

Total Soluble Sugar (TSS) is commonly recognized as an additional osmo-regulator, apart from proline, that enhances plant cell stability and turgor. Additionally, it activates the defense mechanisms against reactive oxygen species (ROS), safeguarding cellular constituents and regulating signaling pathways during stress (Rai, 2016). According to Banerjee et al. (2021), structural degradation of leaf chlorophyll and somewhat lower pollutant bioaccumulation capacity are the secondary effects that link plant sensitivity and low TSS. Plants acquire resilience to stress through the combined effects of TSS and antioxidants at the cellular and tissue levels (Bolouri-Moghaddam et al., 2010). In this study TSS accumulation was highest in ***Mikania micrantha kunth*** than ***Phyla nodiflora (L.) Greene*** with leaf concentration of 0.550 mg/g and 0.186 mg/g (Table.12). High TSS concentrations were also found in the plant leaves of contaminated areas by Jeddi et al. (2021) and Kamble et al. (2021), indicating the many functions of soluble sugars.

Protein molecules are the fundamental building blocks of many aspects of plant growth, including expansive growth, structural growth with biomass change, cell formation and proliferation, etc. They also control how cells respond to stress in reaction to environmental gradients (Qazi et al., 2019). Depending on a plant species' innate capacity to withstand abiotic stress, pollution stress-mediated toxicity may have a role in the increase or decrease of protein content (Rai, 2016). The current study found that ***Phyla nodiflora (L.) Greene*** plant leaves' protein concentration decreased to compared ***Mikania micrantha kunth*** (Table.12) in response to stress. Due to the existence of different air contaminants, protein content ***Phyla nodiflora (L.) Greene's*** decreased relative to ***Mikania micrantha kunth***, and this decline was greater in

sensitive plants. Pollutants may be considered to be the primary cause of the proteolytic cleavage of peptide bonds, which breaks down protein structures into little amino acids and enhances protein reduction. This cleavage may also be considered as an inhibitor of *de novo* protein synthesis (Iqbal et al., 2000).

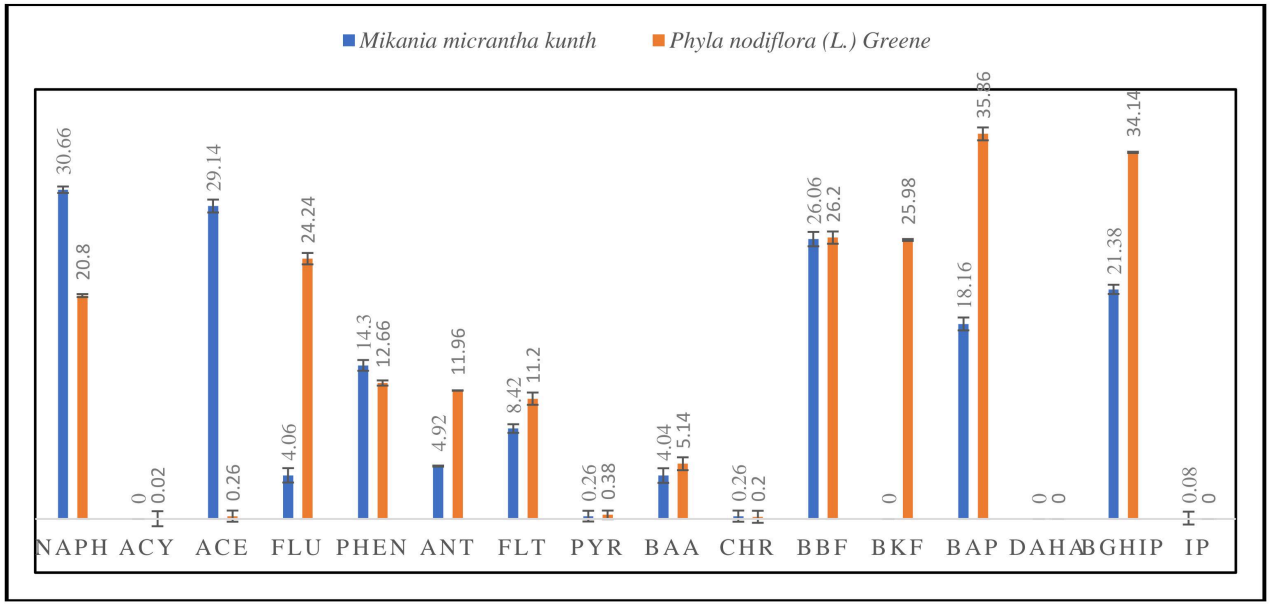
Proline helps plants regulate osmotic flow, regulate proteins and enzymes, remove reactive oxygen species, and preserve the integrity of their cell membranes (Kavi Kishor and Sreenivasulu, 2014). In a contaminated environment, proline helps plants withstand stress (Mukhopadhyay et al., 2024). According to the study, proline concentrations in ***Phyla nodiflora (L.) Greene*** and ***Mikania micrantha kunth*** are 13.4 mg/g and 1.968 mg/g (Table 12), respectively.

Proline helps plants regulate osmotic flow, regulate proteins and enzymes, remove reactive oxygen species, and preserve the integrity of their cell membranes (Kavi Kishor and Sreenivasulu, 2014). In a contaminated environment, proline helps plants withstand stress (Mukhopadhyay et al., 2024). Same observation was also obtained in the present study where ***Mikania micrantha kunth*** with low PAHs accumulation (161.74 µg g^{-1}) showed a lower proline content (1.968 mg g^{-1}) and ***Phyla nodiflora (L.) Greene*** exhibiting high PAHs concentration (209.04 µg g^{-1}) showed higher proline content (13.4 mg g^{-1}) (Table 12). Thus, based on the findings, it can be concluded that proline might be a factor for inducing stress tolerance to ***Phyla nodiflora (L.) Greene*** though the species exhibited intermediate tolerance with respect to APTI.

5.3.3. PAHs concentration of plant leaves:

Human activity and weather patterns have a significant influence on particulate matters' (PAHs) in urban air. These factors directly affect humidity, ambient temperature, mixing height, cloudiness, severity and frequency of rain, and wind speed, which in turn controls how PAHs spread from various emission sources (Mukhopadhyay et al., 2021). In this study bio monitor plant ***Mikania micrantha kunth*** and ***Phyla nodiflora (L.) Greene*** in the urban study sites revealed remarkable differences in PAHs contamination (Figure.17). In this study the average leaf PAHs contribution from 16 PAHs (Figure.17 were ranked as follows: NAPH (0.19%) > ACE (0.18%) > BbF (0.16%) > BghiP (0.13%) > BaP (0.11%) > PHEN (0.09%) > FLT (0.05%) > ANT (0.03%) > FLU (0.03%) > BaA (0.024%) > PYR (0.001%) > CHR (0.001%) > IP (0.001%) for ***Mikania micrantha kunth*** and ACY (9.57%) > BaP (0.17%) > BghiP (0.16%) >

BbF (0.12%) > BkF (0.12%) > FLU (0.12%) > NAPH (0.09%) > PHEN (0.06%) > ANT (0.06%) > FLT (0.05%) > BaA (0.02%) > CHR (0.01%) > PYR (0.002%) > ACE (0.001%) for ***Phyla nodiflora (L.) Greene***. Therefore NAPH, ACE and BbF were showed high contribution and PYR, CHR, IP were low contribution of PAHs in ***Mikania micrantha kunth***. And another side ACY, BaP and BghiP were showed high contribution and PYR and ACE has lower contribution of PAHs in ***Phyla nodiflora (L.) Greene***. This study showed that total leaf PAHs concentration of ***Mikania micrantha kunth*** and ***Phyla nodiflora (L.) Greene*** is 161.74 µg g^{-1} and 209.04 µg g^{-1} (Figure.17). Where, ***Phyla nodiflora (L.) Greene*** had greater leaf PAHs concentration than ***Mikania micrantha kunth***. According to Mukhopadhyay et al. (2023) showed leaf PAHs concentration to be 200.98 µg. g^{-1} higher than ***Mikania micrantha kunth*** and lower than ***Phyla nodiflora (L.) Greene***. ***Phyla nodiflora (L.) Greene*** showed maximum concentration enriched with high level of HMW PAHs (139.1 µg. g^{-1}). Another, ***Mikania micrantha kunth*** reflect the maximum contribution of LMW PAHs (83.08 µg. g^{-1}). In this study PAHs like ACY and BkF was not found in ***Mikania micrantha kunth*** and IP was not found in ***Phyla nodiflora (L.) Greene***. Whereas, DahA were not recorded in both the plant species. It observed that the total concentration of PAHs at the research site is influenced to some extent by human activity and vehicle emissions.



***All concentration in µg g^{-1}**

Figure 17. Concentration of PAHs on leaves of two wetland plant species

5.3.4 PM associated PAHs:

Multiple studies using plant leaves, including theoretical, experimental, computational, and simulation methods, have led to the development of a clear understanding of plant-air partitioning and Phyto monitoring for PM and PAH cleanup (Borgulat and Borgulat, 2023; Pleijel et al., 2022; Redondo-Bermudezet al., 2021; Xie et al., 2022). According to Heiminki and Pershagen (1994), PM_{10} and $PM_{2.5}$ bounding PAHs account for most of the risk of lung cancer. The majority of PAHs are so bounded the size of the particles to which they are linked effects the health impacts of airborne particles differently (Yin et al., 2018). Notably, there are few studies in Kolkata (particularly in East Kolkata) that examine the potential of biomonitoring for air quality monitoring, with a focus on atmospheric PM and PAH concentrations, as well as the long-term effectiveness of bio-monitors in determining the location of urban plantations (Mukhopadhyay et al., 2024). The leaf surface area and PM specific concentration is presented in Table 13a which confirms both PM2.5 and PM10 concentration are higher in ***Phyla nodiflora (L.) Greene*** and that supports their PAHs uptake. The concentration of PM bound PAHs is determined as presented in Table 13b with higher in ***Phyla nodiflora (L.) Greene*** ($PM_{2.5}$-∑PAHs: 1328.736 μg g^{-1} and PM_{10}-∑PAHs: 1080.864 μg g^{-1}) than that of in ***Mikania micrantha kunth*** ($PM_{2.5}$-∑PAHs: 135.872 μg g^{-1} and PM_{10}-∑PAHs: 357.888 μg g^{-1}). The concentration of $PM_{2.5}$ bound individual PAHs congeners in the ***Phyla nodiflora (L.) Greene*** followed the order of BaA > BbF > CHR > ANT > BaP > NAPH > FLT > PYR > PHEN > BkF > BghiP > FLU and that in ***Mikania micrantha kunth*** is NAPH > ANT > PHEN > BAP > BghiP > ACY >BaA > IP > CHR > BbF > FLU > BkF >ACE > PYR. The concentration of $PM_{10\text{-}100}$ -bound individual PAHs in the ***Phyla nodiflora (L.) Greene*** followed the following order: BaA > ANT > PYR > CHR > BaP > BbF > FLU > NAPH > DahA > BkF > FLT > PHEN > IP and that in ***Mikania micrantha kunth*** is BbF > NAPH > BghiP > BaP > CHR > FLU > PHEN > FLT > ANT > BaA > ACY > IP > PYR > ACE. By comparing the concentrations of $PM_{2.5}$-∑PAHs and $PM_{10\text{-}100}$-∑PAHs, it was found that $PM_{2.5}$-bound PAHs were significantly higher than PM_{10}-bound PAHs in ***Phyla nodiflora (L.) Greene***, while the other studied species ***Mikania micrantha kunth*** showed completely opposite trend. It was observed that BbF, NAPH, BghiP and BaP have highest $PM_{10\text{-}100}$-bound accumulation of ***Mikania micrantha kunth.*** On the other hand, BaA, ANT, PYR, CHR and BaP have highest $PM_{10\text{-bound}}$ accumulation of ***Phyla nodiflora (L.) Greene***. Also, in this

study (Table. 13b) showed that NAPH, PHEN, BaP and BghiP have highest $PM_{2.5}$-bound in ***Mikania micrantha kunth*** and BaA, BbF, CHR, ANT and NAPH have highest $PM_{2.5}$-bound in ***Phyla nodiflora (L.) Greene***. BaP, NAPH, BghiP, BbF, BkF were identified for main sources of vehicular and diesel/gasoline emission in ***Mikania micrantha kunth*** and ***Phyla nodiflora (L.) Greene*** (Guo et al., 2003; Teixeira et al., 2013).

Table 13a: Concentration of $PM_{2.5}$ and PM_{10} on the leaves of two wetland plant species

Plant Species	**Leaf surface area cm^2**	**PM_{10}**		**$PM_{2.5}$**	
		Weight of PM (gm)	**PM Concentration/Load (mg/cm^2)**	**Weight of PM (gm)**	**PM Concentration/Load (mg/cm^2)**
Mikania micrantha kunth	270.882	0.624	2.3	0.131	0.48
Phyla nodiflora (L.) Greene	235.36	0.841	3.57	0.159	0.68

Table 13b. Concentration of PM_{10} bound PAHs and $PM_{2.5}$ bound PAHs in both species

PAHs	***Mikania micrantha kunth***		***Phyla nodiflora (L.) Greene***	
	PM_{10} Concentration (ug/g^{-1})	$PM_{2.5}$ Concentration (ug/g^{-1})	PM_{10} Concentration (ug/g^{-1})	$PM_{2.5}$ Concentration (ug/g^{-1})
NAPH	59.072 ± 0.66	39.104 ± 0.64	57.152 ± 0.6	60.896 ± 0.08
ACY	4 ± 0.71	3.104 ± 0.64	nd	nd
ACE	0.064 ± 0.005	0.48 ± 0.36	nd	nd
FLU	29.632 ± 0.97	1.056 ± 0.67	58.752 ± 0.89	34.08 ± 0.66
PHEN	21.088 ± 0.65	22.24 ± 0.54	39.328 ± 0.48	51.488 ± 0.37
ANT	16.16 ± 0.6	32 ± 0.71	207.68 ± 0.23	182.976 ± 0.02
FLT	18.592 ± 0.99	nd	40.608 ± 0.99	57.952 ± 3.51
PYR	0.256 ± 5.53	0.192 ± 0.57	68.096 ± 0.64	52.64 ± 0.26

BaA	6.4 ± 0.43	2.048 ± 0.68	236.736 ± 0.89	243.776 ± 0.16
CHR	33.248 ± 0.54	1.376 ± 0.45	139.552 ± 1.03	214.016 ± 0.69
BbF	59.616 ± 0.98	1.152 ± 0.6	59.072 ± 0.66	237.28 ± 0.51
BkF	nd	0.8 ± 0.08	43.072 ± 0.66	50.88 ± 0.09
BaP	53.504	15.808 ± 0.14	61.376 ± 0.45	101.12 ± 0.63
DahA	nd	nd	51.776 ± 0.87	nd
BghiP	55.264 ± 0.53	15.744 ± 0.89	nd	41.632 ± 0.97
IP	0.992 ± 0.006	1.824 ± 0.13	17.664 ± 0.95	nd
∑Total	**357.888 ± 8.62**	**135.872 ± 7.79**	**1080.864 ± 9.29**	**1328.736 ± 4.43**

*nd: not detected

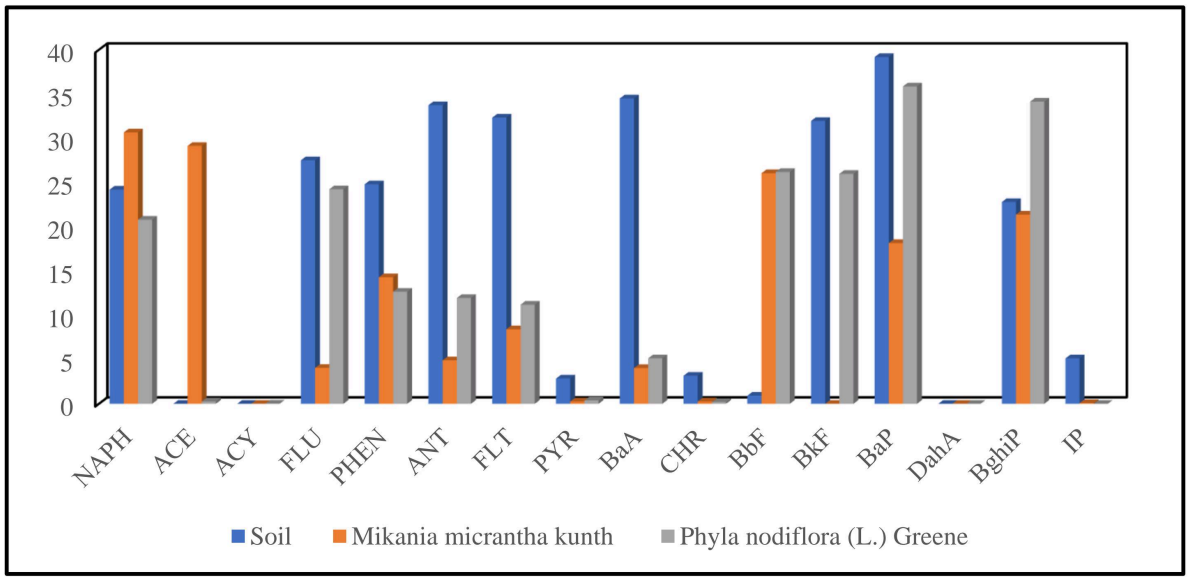
5.3.5. Bioconcentration factor (BCF):

Based on total PAHs concentration on plant leaves and associated soil (Figure 17), it is observed that BCF for ***Mikania micrantha kunth*** and ***Phyla nodiflora (L.) Greene*** with respect to soil is 32.43 and 33.65 (Table 14), respectively though most of the congeners individually show very low BCF value. Among 16 PAHs congeners, strikingly BbF shows highest BCF value in both the plant species and NAPH has also impressive value. The BCF contribution from 16 PAHs were found as follows for ***Mikania micrantha kunth***: BbF > NAPH > BghiP > PHEN > BaP > FLT > ANT > FLU > PYR > BaA > CHR > IP; but the order is slightly different in ***Phyla nodiflora (L.)*** **Greene**: BbF > BaP >BkF > FLU > NAPH > PHEN > ANT > FLT > CHR > BaA > PYR > BghiP. Actually, PAHs with two and three rings in particular may readily volatilize from contaminated soil onto foliage but HMW PAHs are directly captured from ambient. In general, highly lipophilic PAH molecules (heavy PAHs) show lower accumulative potential than the less lipophilic ones (light PAHs). Exceptionally, very high value of BCF for BbF (28.48) was found in both the plant species which suggests Basir et al. (2017) rate of absorption of that PAH is faster than that at which the substance is lost by catabolism and excretion. According to our result we found that BCF for total PAHs 32.43 in ***Mikania micrantha kunth*** and 33.65 in ***Phyla nodiflora (L.) Greene*** which is much greater than 1, indicate that both species have almost equal (different due to leaf morphology or difference in biochemical properties) but appreciable accumulation potential which is evident from their biochemical properties.

As BCF of other PAHs congeners except BbF are not too high, it can be concluded that other congeners are not only volatilized less for foliage uptake but also translocated in minimal amount from root. Therefore, although the investigation of air–plant and soil–plant partitioning relationships will be the guiding route to interpret the BCF results, but a comprehensive model describing uptake, transport and accumulation of PAHs from surrounding soil and atmosphere will work well.

On the other hand, as the PM accumulation is significantly high by both the plant species, therefore it can uptake and accumulate high proportion of ambient PAHs resulting low Bioconcentration factor (BCF) in leaves with respect to PM (calculated using following similar expression) (Table 15).

$$BCF = \frac{Concentration\ of\ PAHs\ on\ leaves}{Concentration\ of\ PAHs\ on\ PM}$$



***All concentration in µg g^{-1}**

Figure 17. Concentration of PAHs of two wetland plant species and associated soil of the study site

Table 14. Bioconcentration factor for 16 PAHs in both wetland plant species w.r.t. wetland soil

	BCF	
PAHs	***Mikania micrantha kunth***	***Phyla nodiflora (L.) Greene***
NAPH	1.27	0.86
ACE	nd	nd
ACY	nd	nd
FLU	0.15	0.89
PHEN	0.58	0.51
ANT	0.15	0.35
FLT	0.26	0.35
PYR	0.09	0.13
BaA	0.11	0.15
CHR	0.08	0.06
BbF	28.32	28.48
BkF	nd	0.81
BaP	0.46	0.91
DahA	nd	nd
BghiP	0.94	0.15
IP	0.02	nd
∑Total	**32.43**	**33.65**

*nd: not detected

Table 15. Bioconcentration factor for 16 PAHs in both wetland plant species w.r.t. PM

PAHs	**BCF**	
	Mikania micrantha kunth	***Phyla nodiflora (L.) Greene***
NAPH	0.31	0.17
ACY	nd	nd
ACE	53.56	nd
FLU	0.13	0.26
PHEN	0.33	0.13

ANT	0.10	0.03
FLT	0.45	0.11
PYR	0.58	0.003
BaA	0.47	0.01
CHR	0.007	0.0005
BbF	0.42	0.08
BkF	nd	0.27
BaP	0.26	0.22
DahA	nd	nd
BghiP	0.30	0.82
IP	0.02	nd
∑Total	**56.93**	**2.103**

*nd: not detected

5.4. Source Apportionment by Diagnostic ratio:

According to our study's PAHs ratio findings, the primary sources of PAHs in soil are mixed pyrogenic activities, including the burning of coal, biomass, petroleum, and automobile emissions. Study site soil and both species had the following characteristics based on the diagnostic ratios and sources of PAHs (Table 16): soil and *Phyla nodiflora (L.) Greene* had ∑LMW/∑HMW values <1, indicating pyrogenic sources and *Mikania micrantha kunth* has value >1, indicates petrogenic. Soil and both species had ANT/(ANT+PHE) values >0.1 indicating pyrogenic source and FLU/(FLU+PYR) values were >0.5 indicating Diesel emission, FLT/(FLT+PYR) values were higher than 0.5 suggested that wood, grass coal and combustion sources, BaA/(BaA+CHR) value more than 0.35 is attributed to vehicular emission sources. IcdP/(IcdP+BghiP) value of soil is higher than 0.2 represent a Grass, wood and coal combustion sources and *Mikania micrantha kunth* has value below 0.2 represent petrogenic source, other hand *Phyla nodiflora (L.) Greene* didn't found any sources. Soil and both species had BbF/BkF value 0.02, 0 and 1 but they didn't find any sources. Soil and both species had BaP/BghiP values higher than 0.6 which indicates to traffic emission sources. The research area's PAH diagnostic

ratios show that pyrogenic origin is the predominant source. However, it is also verified that there are sources of biomass and coal combustion. Nonetheless, the diagnostic ratios show that the majority of PAHs in the study region had a petrogenic origin. The primary sources of PAHs in the study field are traffic and vehicle emissions. Since PAHs are persistent, human exposure through particle inhalation, dietary consumption of contaminated food products including vegetables, and direct contact with contaminated water, sludge, and soil will continue to be a problem due to this pollution.

Table 16. Source Apportionment of Soil PAHs and both species using Diagnostic Ratio

PAHs ratio	Sample	Value	Standard Range (Hwang et al., 2003)	Source
∑LMW/∑HMW	Soil	0.63	<1	Pyrogenic
	Mikania micrantha kunth	1.05	>1	Petrogenic
	Phyla nodiflora (L.) Greene	0.5	<1	Pyrogenic
FLU/(FLU+PYR)	Soil	0.9	>0.5	Diesel emission
	Mikania micrantha kunth	0.9	>0.5	Diesel emission
	Phyla nodiflora (L.) Greene	0.9	>0.5	Diesel emission
ANT/(ANT+PHE)	Soil	0.57	>0.1	Pyrogenic
	Mikania micrantha kunth	0.2	>0.1	Pyrogenic

	Phyla nodiflora (L.) Greene	0.5	>0.1	Pyrogenic
	Soil	0.91	>0.5	Grass, wood, coal combustion
	Mikania micrantha kunth	0.9	>0.5	Grass, wood, coal combustion
FLT/(FLT+PYR)				
	Phyla nodiflora (L.) Greene	0.9	>0.5	Grass, wood, coal combustion
	Soil	0.91	>0.35	Biomass, coal combustion
	Mikania micrantha kunth	0.9	>0.35	Biomass, coal combustion
BaA/(BaA+CHR)				
	Phyla nodiflora (L.) Greene	0.9	>0.35	Biomass, coal combustion
	Soil	1	>0.5	Grass, wood and coal combustion
	Mikania micrantha kunth	0.004	<0.2	Petrogenic
IcdP/(IcdP+BghiP)				
	Phyla nodiflora (L.) Greene	--	--	--
	Soil	0.02	--	--
	Mikania micrantha kunth	0	--	--
BbF/BkF				

	Phyla nodiflora (L.) Greene	1	--	--
BaP/BghiP	Soil	1.7	>0.6	Traffic emission
	Mikania micrantha kunth	0.8	>0.6	Traffic emission
	Phyla nodiflora (L.) Greene	1.05	>0.6	Traffic emission

CHAPTER: 6

6. Conclusion:

The present study investigated the potential of two different wetland plant species in biomonitoring of PAHs at particulate phase and also that absorbed within the plant leaves. Although being classified as an intermediately tolerant species, **Mikania micrantha Kunth** demonstrated the PAHs concentration of 161.74 μg g^{-1} and ***Phyla nodiflora (L.) Greene***, on the other hand, showed 209.04 μg g^{-1}rich with a high degree of HMW PAHs. However, ***Mikania micrantha Kunth*** represent the highest level of LMW PAHs enrichment. ***Phyla nodiflora (L.) Greene's*** tendency is reflected in the soil matrix, which indicates the main nature of HMW PAHs. Yet, the high concentration of PAH pollution in the soil sample (283.14 ± 6.27 μg. g^{-1}), where LMW PAH predominated and contributed 30.29% , directs us to choose the study area for biomonitoring study for PAHs pollution. As toxicity rises with increasing molecular weight of PAHs congeners, higher values of BaP-TEQ (47.1102 ± 2.98 μg. g^{-1}) attributable to HMW PAHs, especially BaP, in the soil matrix, indicating significant toxicity potential. The total mutagenic (BaP-MEQ) concentration for eight PAHs is 51.733 ± 3.06 μg. g^{-1} ,which points to a highly contaminated local source that may be dependent on sources like vehicular emissions. In the impact evaluation of PAHs pollution on the soil of the study zone, the Effect Range Low (ERL) and Effect Range Medium (ERM) values were calculated and resulted high impact. It is possible to evaluate the biological risk or expected combined toxicity for exposure to a mixture of PAHs in soil by using M-ERM-Q and M-PEL-Q (12.76 μg. g^{-1} and 43.57 μg. g^{-1}). This suggests that the toxicity caused by PAHs was rather constant. Evaluation of the ecological toxicity of PAH compounds in soils where RQ_{NCS} >800 and RQ-PAHs(MPCs) >1 in the research locations suggest high levels of ecological risk, indicating a substantial ecological hazard that may be caused by a variety of pyrogenic and petrogenic activities, such as burning, heavy

vehicle emissions, etc. The main sources of PAHs in soil are mixed pyrogenic activities, which include burning coal, biomass, petroleum, and vehicle emissions, according to the PAHs ratio results of our research. The primary sources of PAHs were found to be diesel combustion, coal burning, and vehicle emissions, according to an analysis of diagnostic ratios. Plant leaves subjected to biochemical research revealed that the lipophilicity of the lipids and waxes on the leaves acted as a regulating factor in varying the quantities of PAHs. This study did not find a trend between the concentration of PAHs in leaves and their lipid content; nevertheless, the high level of lipids in the leaves of ***Mikania micrantha kunth*** plants at the contaminated site, which is thought to be a better indicator of the presence of persistent organic pollutants, such as PAHs, than ***Phyla nodiflora (L.) Greene***. Total Soluble Sugar (TSS) and antioxidants work together to strengthen plants' resistance to stress at the cellular and tissue levels. ***Mikania micrantha kunth*** had a higher Total Soluble Sugar (TSS) than ***Phyla nodiflora (L.) Greene*** (0.550 mg/g vs 0.186 mg/g). As ***Phyla nodiflora (L.) Greene*** plant leaves were subjected to stress, the current study discovered that their protein concentration dropped in ***Phyla nodiflora (L.) Greene*** compared to ***Mikania micrantha Kunth***. If the drop is more pronounced in plants, then that were considered sensitive. The study reveals that the PM (both PM_{10} and $PM_{2.5}$) capturing capability of the two plant species is highly impressive and different due to their leaf size-shape-morphology. The striking feature of the two plant species is PM bound PAHs is almost 3 times higher than that of absorbed in mesophyll tissue phase in ***Mikania micrantha Kunth*** and 11 times more in **Phyla nodiflora (L.) Greene**. The fact is also reflected in BCF value in case of accumulation in PM to leaf showing negligible chance of bioaccumulation of PAHs on leaves from PM. The total PAHs capturing capability of the two chosen plant species is presented in **Table 17**. The relevant

source apportionment study confirms the relevant sources of PAHs emission around the study zone which needs to control for sustainment of the wetland health.

Table 17: Total uptake of PAHs (PM bound and mesophyll tissue bound) by plant leaves

Plant species name	**PAHs Concentration in wetland soil** ($\mu g/g^{-1}$)	**PM bound PAHs concentration**($\mu g/g^{-1}$)	**Mesophyll tissue absorbed PAHs concentration** ($\mu g/g^{-1}$)	**Total uptake of PAHs by leaves** ($\mu g/g^{-1}$)
Mikania micrantha kunth	283.14	493.76	161.74	655.5
Phyla nodiflora (L.) Greene		2408	209.04	2617.04

Therefore, following conclusion can be drawn from the current study:

- Both ***Mikania micrantha kunth*** and ***Phyla nodiflora (L.) Greene*** sensitive to tolerant plant biomonitoring species with appreciable uptake rate of PAHs in foliar dust phase and mesophyll tissue phase.
- Among two species, ***Phyla nodiflora (L.) Greene*** acts as superior indicator as it can capture more PM and can absorb higher amount of PAHs.
- Leaf morphology along with size-shape-leaflet pattern need to be investigated further to ascertain the reason of higher uptake rate by ***Phyla nodiflora (L.) Greene.***
- Wetland PAHs pollution (ambient and soil pollution) can be controlled by plantation in presence of abundant, tolerant and efficient biomonitoring species.

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