Biomonitoring potential of some local different terrestrial plants for monitoring polycyclic aromatic hydrocarbons (PAHs) and heavy metal pollution

Submitted by~

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CERTIFICATE OF APPROVAL

This is to certify that the project entitled "Biomonitoring potential of some local different terrestrial plants for monitoring Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metal pollution" carried out by Marshal Murmu, M.E, 2nd Year 2nd Semester (**Examination Roll No.: M4CHE24023**), 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: "Biomonitoring potential of some local different terrestrial plants for monitoring Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metal pollution".

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Abstract

In the current Anthropocene period of growing industrialisation and urbanisation, air pollution is one of the major issues confronting the globe. Most notably, heavy metal (HM) and polycyclic aromatic hydrocarbons (PAHs) pollution pose risks to public health as well as the environment. Thus, monitoring of HM and PAHs has become utmost necessary. But using traditional air monitoring methods is quite inconvenient because of their installation in large area, maintenance cost and manpower to handle. As a result, the alternate way to create a natural filtering barrier is to use green belts for biomonitoring of airborne contaminants. In this study, Jadavpur, a site of South Kolkata, is chosen as a study area based on pollution load data of CPCB and WBPCB. Two terrestrial plant species *B. spectabilis* and *M. indica* are chosen as biomonitors depending on their APTI value and abundance in the locality. *B. spectabilis* with APTI value >17 was found to be tolerant whereas *M. indica* with APTI value <17 found to be intermediately tolerant and can be bioindicator. The PAHs accumulation capacity and HM capturing capacity of both the plants are examined to establish the potential of the species in monitoring PAHs and HMs in and around the city. The relative water content (RWC), leaf extract pH, ascorbic acid, total chlorophyll content, protein, proline, sugar, wax, lipid, C, H, N, S, C/H, C/N content were determined to find the impact of PAHs and HMs on plant characteristics, which would help in identifying their tolerance potential and capturing capacity. Airborne PAHs and HMs were found to affect leaf biochemical and physiological parameters. For PAHs assessment, extractions were performed with toluene using Soxhlet extractor for 6 hrs. The samples were cleaned using silica gel column chromatography and analysed in HPLC. For metal concentrations, HMs were first extracted by digesting the sample in aqua regia for 24 hrs. The samples were then evaporated, filtered and analysed in ICP-OES. The findings revealed that both the plant species are good accumulators of PAHs (*B. spectabilis*: 198.82±3.66 μg g^{-1}; *M. indica*: 358.86±0.09 μg g^{-1}). The sources of PAHs were again

determined by diagnostic ratios. NAPH, BghiP, DahA, PHEN and BaP were found to be the dominant PAHs in the studied location. The anthropogenic activities, vehicular emission, fossil fuel combustion were the major contributors of PAHs in that area. Among all the PAHs found, 96-97% are carcinogenic and mutagenic (as determined from carcinogenic and mutagenic equivalent concentrations (BaP_{TEQ} and BaP_{MEQ})), having serious health impacts on human being. Based on HM accumulation, MAI was found higher in *M. indica* (35.11) than *B. spectabilis* (29.68). Ni, Zn, Pb, Cu, Cr, Al were the major HMs found in the area. The plant species showing high MAI value and high APTI value are suitable for planting where HM and PAHs pollution is higher and can be used to build green belt in and around the city for biomonitoring of PAHs from the air.

1. INTRODUCTION

1.1. Ambient Air Pollution: A global issue

Urban atmospheric pollution is one of the most serious global environmental issues of the past decade. The developing nations like India, which are switching from predominantly rural country to increasingly urban, have to face critical challenges in terms of climate action and sustainable development (Van Duijne, 2017; Singh C. et al., 2021). India is projected to have 53% of the national population as urban population by addition of 416 million urban dwellers by the year 2050 (UNDESA, 2018). Pollutants of ambient air are a threat to both human and environmental health. The major factors are intense population growth and industrialization (Pandey et al., 2015). Because of continued urbanisation, land use and cover patterns in metropolitan areas have changed, which has an impact on the regional climate by changing the characteristics of the surface and boundary layers of the atmosphere (Shepherd, 2005; Ren et al., 2008; Yang et al., 2012). Additionally, increasing anthropogenic emissions, intense precipitation (which may cause urban flooding), higher temperatures, and frequent heat waves with negative effects on human health are all caused by urbanization's shifting land use and cover (Chestnut et al., 1998; Ramanathan et al., 2001; Shastri et al., 2015). India, a nation experiencing tremendous development and population growth, is experiencing extreme air pollution; nine of the ten most polluted cities in the world are located in India [WHO Global Urban Ambient Air Pollution Database (Update 2016), 2016]. In recent times, there has been a lot of focus on the rising levels of air pollution in the majority of Indian megacities during the past few decades and the resulting effects on human health (such asthma and cardio-respiratory disease) (Sarath and Ramani, 2014; Gautam et al., 2020; Shaw and Gorai, 2020).

The health impacts and haze episodes linked to atmospheric particle matter (PM) are a major issue for government agencies and the general population among pollutants. PM has a great potential to absorb harmful metals, which might later infiltrate the body through the respiratory system and have detrimental physiological consequences. Polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) are two examples of toxic substances found in airborne particles. Metals are released into the environment by both natural and man-made processes. Natural processes include soil creation, evaporation from seas, volcanic eruptions, forest fires, and mobilisation from rock (Ugulu et al., 2012; Dogan et al., 2014). Rubber tyre wear, auto workshops, motor oil, electroplating industries and petrol combustion are some urban sources of pollution.

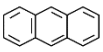
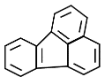
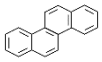
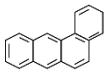
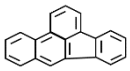
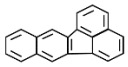
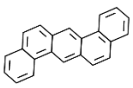
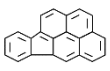
1.2. Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs: PAHs are a significant class of poisonous, mutagenic, genotoxic, and/or carcinogenic organic contaminants or pollutants. The term "PAHs" refers to a sizable class of lipophilic persistent organic pollutants (POPs) containing several fused aromatic rings arranged in a cluster, a linear arrangement, or both (Ghosal et al., 2016). In general, anthropocentric activities such industrial operations, waste incineration, home heating, and vehicle traffic emissions are the major source of incomplete combustion of organic compounds that results in the formation of PAHs. However, some natural processes can also cause localised accumulations of HMs; for instance, large concentrations of As in water are primarily caused by rock weathering, volcanic eruptions, or microbiological activity. PAHs may be roughly divided into two groups: those with low molecular weights (LMWs, having 2-3 rings) and those with high molecular weights (HMWs, having 4-7 rings) (Mukhopadhyay et al., 2020). Due to their mostly lipophilic and hydrophobic nature, PAHs have been difficult to breakdown (Kronenberg et al., 2017). The solubility of PAHs in water diminishes as their molecular weight rises, and their melting and boiling temperatures also rise (Agarwal et al., 2018). Anthracene, benz[a]anthracene, benzo[a]pyrene, fluoranthene, benzo[b]fluoranthene, benzo[k]fluoranthene, naphthalene, dibenz [a, h] anthracene, Indeno[1,2,3-cd]pyrene, fluorene, phenanthrene, and pyrene are among the important 16 PAHs listed by the United States Environmental Protection Agency (USEPA) are shown in Table 1. Due to their potential toxicity when exposed to living things, these 16 PAHs represent serious environmental problems (Bojes and Pope, 2007). HMW PAHs, which range in composition from chrysene to coronene, are well-known to be powerful carcinogens with long-term lethal, genotoxic, and mutagenic effects (Neff, 1979). Organisms are exposed to PAHs in the terrestrial environment by inhalation, digestion, and direct contact, or mostly through digestion in the aquatic environment (Bergman et al.,1979). Control of these substances and the determination of their sources are essential due to their significance to the ecosystem. The major sources of PAHs have been elaborated in Fig.1. There are several reliable environmental contamination indicators for this purpose, especially for PAH pollution, including organisms and plant species.

Both the gaseous phase (mostly for PAHs with greater vapour pressure) and the particle phase (primarily for PAHs with lower vapour pressure) may incorporate PAHs. Both gaseous and particle-bound PAHs are exposed to plants via stomatal absorption and/or diffusion via wax layers and membranes, substances from the vapour phase may enter the interior of leaves (Tian et al., 2019). The lipophilic cuticular wax layer is where PAH compounds concentrate, indicating that the amount of PAHs accumulated will rely on crucial leaf properties including the structure and thickness of the cuticular wax layer (Li et al., 2017).

Table 1.

Structure of the16 PAHs identified as priority organic pollutants by USEPA and WHO

16 Priority PAHs identified by USEPA and WHO	Structure	Formula	Molar mass (g/mol)
Napthalene		$C_{10}H_8$	128.17
Acenapthylene		$C_{12}H_8$	152.19
Acenapthene		$C_{12}H_{10}$	154.21
Flourene		$C_{13}H_{10}$	166.22
Anthracene		$C_{14}H_{10}$	178.23
Phenanthrene		$C_{14}H_{10}$	178.23
Flouranthene		$C_{16}H_{10}$	202.26
Pyrene		$C_{16}H_{10}$	202.25
Chrysene		$C_{18}H_{12}$	228.28
Benzo[a]anthracene		$C_{18}H_{12}$	222.28
Benzo[b]flouranthene		$C_{20}H_{12}$	252.30
Benz[k]flouranthene		$C_{20}H_{12}$	252.30
Benz[a]pyrene		$C_{20}H_{12}$	252.31
Dibenz[a,h]anthracene		$C_{22}H_{14}$	278
Benzo[g,h,i]pyrene		$C_{22}H_{12}$	276
Indeno[1,2,3-cd]pyrene		$C_{22}H_{12}$	276.33

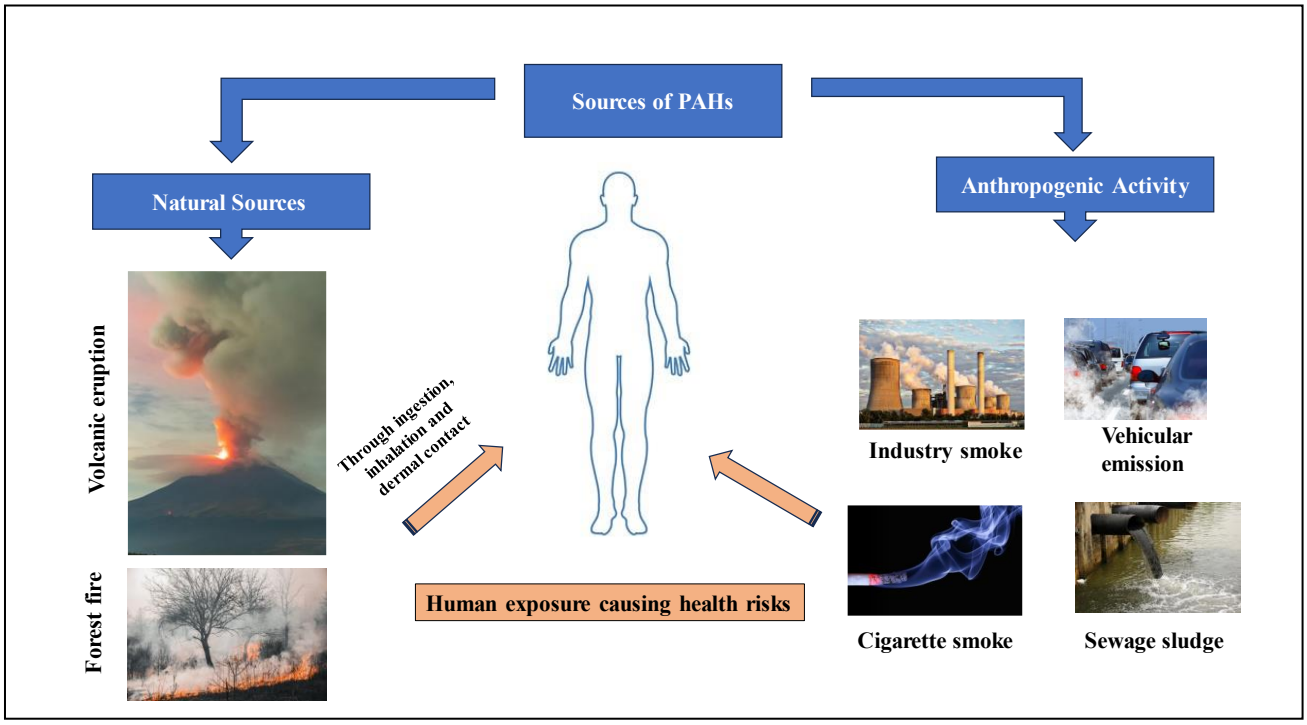


Fig. 1: Sources of PAHs in air and routes to human body

It has been demonstrated that the leaves of evergreen plants and shrubs can accumulate particle or vapour-phase PAHs either in the waxy cuticular tissues on the foliage surfaces or via stomatal movement for the diffusion of gaseous PAHs into leaf inner tissues (Fellet et al., 2016). The process of absorption by plant leaves is regulated by particulate or gaseous nature of PAHs, specific surface area of leaves, total lipid content in foliar tissues, octanol-air partition coefficient, foliar dust concentration, stomatal density and atmospheric conditions like pressure and temperature (Slaski et al., 2000). Influence of meteorological factors, specific leaf area (SLA), leaf area index (LAI) as well as leaf density on PAHs absorption by deciduous tree species (hazelnut and maple) was examined by Terzaghi et al. (2015). The authors observed that PAHs uptake rate varies directly with SLA, whereas LAI is species dependent. Singh et al. (1991) for the first time introduced a factor, namely Air Pollution Tolerance Index (APTI) with respect to plants to investigate their vulnerability towards airborne organic and inorganic pollutants. According to their study, the evaluation of APTI can be related to four morphological parameters of plants such as ascorbic acid, chlorophyll content, relative water content and leaf-extract pH, which signifies their tolerance or sensitivity to environmental changes.

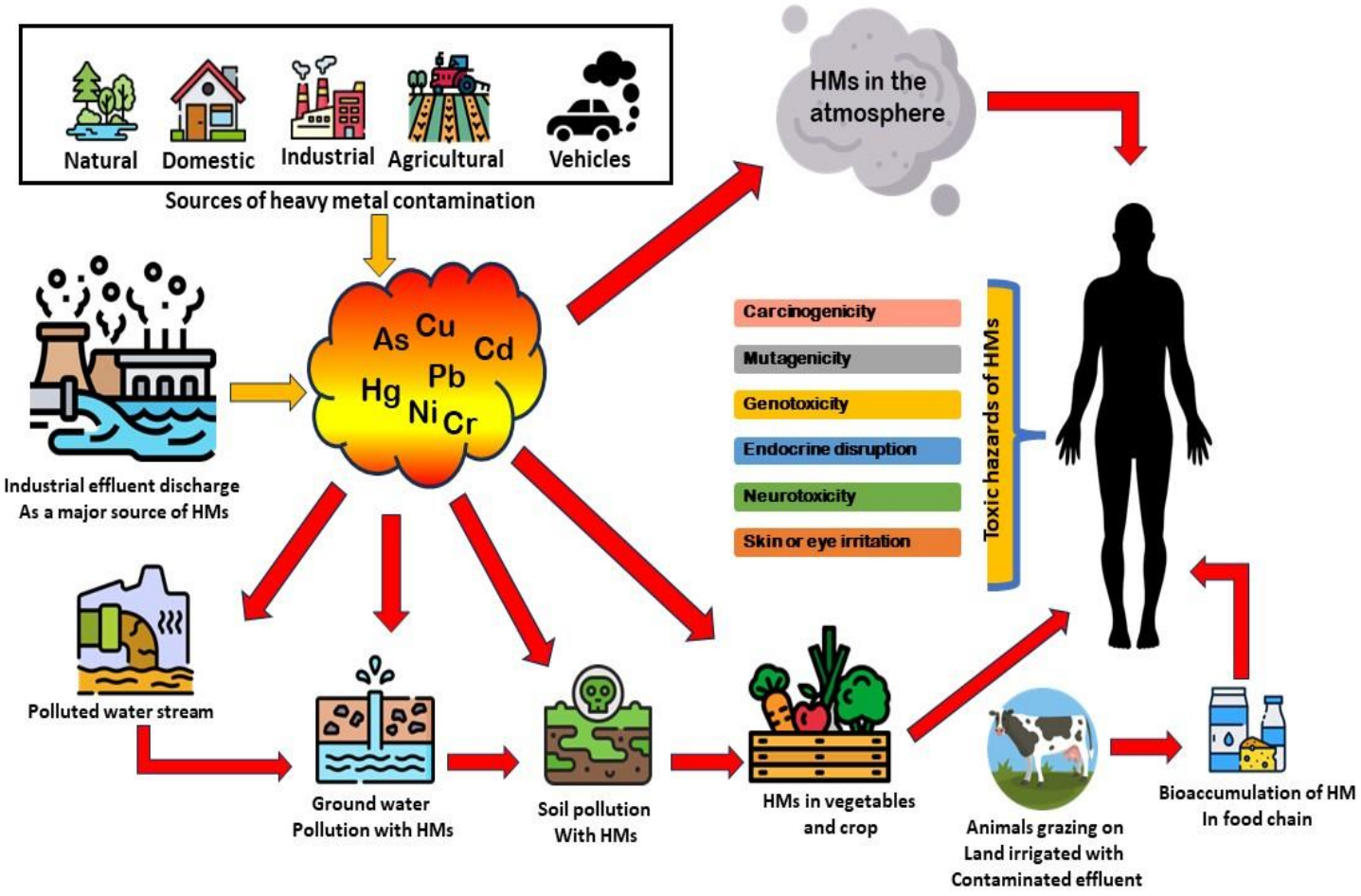


Fig. 2: Introduction of heavy metals in the environment and their bioaccumulation.

1.3. Heavy metals as air pollutants:

Heavy metal pollution of the environment has become a significant problem for both industrialised and developing nations worldwide (Chauhan and Chauhan, 2014). The atomic density of heavy metals is more than 4 g/cm^3, and they are found in diverse environmental media at varying concentrations. Heavy metals are classified as detrimental environmental contaminants because of their toxicity, persistence, and bioaccumulation (in plants, animals, soil, water, and sediments) (Martenez and Motto, 2000; Girmaye, 2014; Asati et al., 2016). Heavy metals can build up in the environment due to anthropogenic or natural factors, such as industrialization, urbanisation, mining, burning of fuel, waste incineration, and agricultural activity. Examples of natural factors include weathering of rocks, volcanic eruptions, leaching, soil formation, and forest fires (Chang et al., 2014; Adelasoye and Alamu, 2016; Wang and Zhang, 2018). According to several studies, agricultural inputs such pesticides, herbicides,

fertilisers, fungicides, and insecticides are the primary causes of the buildup of heavy metals in the environment (Atinafu et al., 2015; Mengistie et al., 2017). The environmental pollution caused by industrial wastes usually has high concentrations of several heavy metals such as Pb, Ni, Cd, Cu, and Cr (Arora et al., 1985). A number of biochemical and physiological processes are hampered by heavy metal toxicity. They have an impact on the well-being and standard of living of people, plants, and animals (Nagajyoti et al., 2010). Due to their ability to absorb heavy metals in their roots, leaves, fruits, or seeds, plants can be utilised as indicators of environmental contamination (Radulescu et al., 2013; Emamverdian and Ding, 2017).

Atmospheric heavy metals can reach soil and plant leaves by dry and wet deposition (Fig.2), resulting in changes in their concentrations in both matrices (Maisto et al., 2004). Dry deposition of heavy metals on plants increases during dry periods, and a stronger effect is detected after precipitation (Onder and Dursun, 2006). The materials deposited on leaves and needles affect chlorophyll, cell membrane, and stomata while they also reduce plant growth. Both dry and wet depositions cause the growth of main and side buds to stop, leaf color to fade, and some parts of trees to dry. These changes reduce the resistance of trees to drought, frost, insects, and fungi (Shanker et al, 2005; Onder and Dursun, 2006). Recently, many studies have used trees for monitoring elemental deposition from the atmosphere. Many plant groups, including evergreen trees such as *Phoenix dactylifera* (Al-Khlaifata and Al-Khashman, 2007; Al-Khashman et al., 2011), *Populus alba* (Madejón et al., 2004), *Cedrus libani* (Onder and Dursun, 2006), and *Nerium oleander* (Dongarra et al., 2003) have been used for monitoring environmental pollution. The use of vegetation as a passive sampler in biomonitoring has the advantage of high spatial and temporal distribution due to the excellent availability of plants and low sampling costs (Sawidis et al., 2011). Trees are usually easier to identify as compared to other organisms such as fungi, algae, lichens, or mosses (Berlizov et al., 2007).

1.4. Traditional monitoring of PAHs:

Both gas and particle-bound PAHs can be found in the surrounding air. The characteristics of the individual PAH, the atmospheric circumstances (such as relative humidity and ambient temperature), and the composition of the aerosol all affect their phase distribution (Wang et al., 2013; Lima et al., 2005., Geldenhuys et al., 2015). The four main steps in measuring air PAHs are sampling, extraction, sample clean-up, and analysis. Any measurement campaign for PAHs in ambient air has to include sampling as a crucial phase that determines whether it is successful or not (Munyeza et al., 2019). There are two main methods of PAHs sampling: Active Sampling (involves drawing a predetermined amount of air through the sorbent) and Passive Sampling

(allows vapour to permeate into the sorbent). Over the past few years many monitoring methods are used to monitor ambient PAHs in are which are listed in the table

Table 2.

Traditional method of air sampling for monitoring PAHs

Year	Country	Gas/ Particle phase	Sampling method /media	References
2000	Burundi	Gas and particle	Gilair pump (styrenedivinylbenzene adsorption tube, GFF)	Viau et al., 2000
2001	Ezypt	Particle	High volume active sampler	Hassanien et al., 2001
2006	Benin	Particle	Battery operated pump (GFF)	Fanou et al., 2006
2006	Kenya	Particle	Mini-pump (QFF)	Muendo et al., 2006
2007	Algeria	Particle	Medium volume air sampler (QFF)	Ladji et al., 2007
2009	Whole Africa	Gas	Passive sampler (PUF disks)	Klánová et al., 2009
2010	Algeria	Particle	Medium volume sampler (QFF)	Moussaoui et al., 2010
2011	Benin	Gas and particle	GFF	Ayi-Fanou et al., 2011
2012	Uganda	Gas and particle	High volume sampler (PUF, GFF)	Arinaitwe et al., 2012
2012	Nigeria	Particle	High volume air sampler (GFF)	Ana et al., 2012
2013	Algeria	Particle	Medium volume sampler (QFF)	Moussaoui et al., 2013
2014	Tunisia	Particle	Low volume air sampler (GFF)	Hassine et al., 2014
2015	Sudan	Gas and particle	High volume air sampler (PUF,QFF)	Osman et al., 2015
2016	Algeria	Gas and particle	Passive sampler (PUF)	Boudehane et al.,2016
2017	Nigeria	Particle	Low volume air sampler (QFF)	Salaudeen et al.,2017
2018	Rwanda	Particle	High volume sampler (GFF)	Kalisa et al., 2018

Due to the installation and maintenance of large instruments on-site, traditional equipment-based air sampling (Table 2) and air quality measurement are time-consuming and expensive operations, higher vascular plants, lichens, and bryophytes have provided a better, quicker, and

more affordable alternative to the traditional techniques of air quality monitoring, overcoming their limitations.

1.5. Biomonitoring of PAHs and HM using plant species:

1.5.1. Bioindicators and Biomonitoring:

A bioindicator is an organism that can identify the presence of an illegal substance in its environment by displaying quantitative and visible changes that are distinct from those brought on by other natural or artificial stresses. The term "bioindicator" is thus often exclusively applied to an organism that may be utilised to gather qualitative data about its immediate surroundings (Smodis and Parr, 1999). Bioindicators are organisms that function as ecosystem health indicators. The greatest bioindicators are plentiful, simple to gather, and some may be used to track changes in ecosystem health and quality. Different types of bioindicators have been shown in Fig. 3. Bioindicators are used by scientists to analyse air pollution together with other types of monitoring. On-going biomonitoring can provide information on how ecosystem health may advance or deteriorate over time.

- Lichens, to measure nitrogen and sulphur in the ecosystem.
- Plant leaves, to study ozone damage to plants.
- Dragonfly larvae, to study mercury and its effects on the ecosystem.

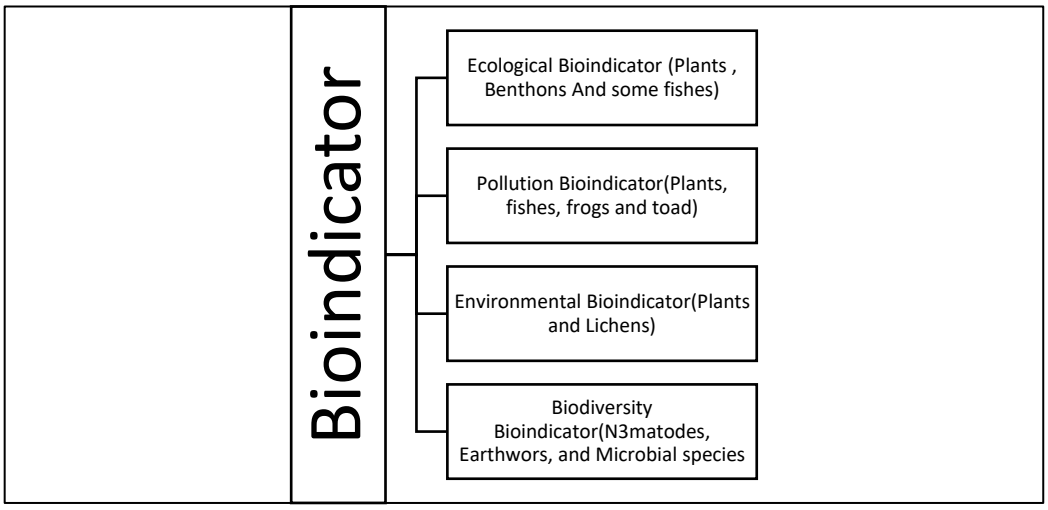


Fig. 3: Typical representation of the spread of bioindicators based on behavioural changes.

An organism responds to changes in environmental circumstances, such as those brought on by pollutants, by altering its morphological and/or metabolic-physiological modes of life. This is true of both bioindication and biomonitoring, while a "bio monitor" is a type of organism

that can be employed to gather quantitative data (Markert, 1993). Biomonitors have quantifiable data on an ecosystem's health. A biomonitor is similar to a bioindicator, with the difference being that it measures the effect or final result on an organism or environment.

Advantages of biomonitoring:

Additionally, biomonitors are present in remote places, can measure integrated exposure over a long period of time, and don't require expensive technological equipment to gather them (Smodis and parr, 1999). Polluted regions can be detected using extensive biomonitoring surveys, and by using the right statistical methods, information on the many types of pollution sources and the transboundary movement of air pollutants can be discovered (Smodis and Parr, 1999).

Criteria for selection of bioindicators:

In general, indicators must capture the complexity of the ecosystem yet remain simple enough to be monitored relatively easily over the long term. A set of indicators should possess some or all of the following qualities:

- Provide early warning signs, i.e., indicate an impending change in key characteristics of the ecosystem.
- Provide continuous assessment over a wide range and intensity of stresses. This allows the detection of numerous impacts to the resource of concern and also means that an indicator will not bottom out or level off at certain thresholds.
- Have a high specificity in response. This may be critical to establish causal relationships and, hence, appropriate management decisions.
- Be cost-effective to measure, i.e., amenable to simple protocols applicable even by non-specialists.
- Be easily communicated to non-scientists and decision makers. It was found that non-scientists better understand information on contamination of forest plants by air pollution than specific information on individual measures (e.g., foliar chemistry and lichen chemistry).

There are three broad categories of indicators: biological (e.g., species, populations, communities), structural (e.g., stand structure and patch configuration in the landscape), and process-based (e.g., frequency and intensity of fires or flooding events). But biological indicators tend to be the primary tool on which we rely to make management recommendations.

1.5.2. Terrestrial plants as bioindicators:

Similarly, the vegetation cover constitutes a basic component of the ecosystem providing multiple services to the livelihood community. Vegetation cover stands out as a proper indicator assigning a wide integrative data informing about the spatial variation of the surrounding conditions. The variation of the structure and plant communities contribute also in informing about the environmental factors and their associated impacts (Wei et al., 2018). In drylands, vegetation cover is submitted to different stresses related to low rainfall, drought and water availability, it plays, thus, an important key role in many environmental processes gathering several protection mechanisms (Omuto et al., 2010).

Due to their immovability and lengthened existence, higher plants are subjected to many categorized ecosystem pollutants and hence often seem to be worthy biological indicators (Ernst, 2003). The effect of pollutants on plant arrays from morphological fluctuations to biochemical and/or cellular alterations is frequently noticed through the overall impacts on their growth, performance, and population intensity. On the whole, external vegetative symptoms are considered as the first biological indicator (Saber et al., 2015a). But generally, it would be necessary to confirm such assumptions with further botanical and/or chemical analyses (Bleeker et al., 2003). Most PTEs could be monitored by certain higher plants (Malizia et al., 2012). Certainly, the quantitative response in plant growth is easier to recognize and express than the qualitative influences using the tolerance index (TI).

Urban trees have the potential to significantly reduce air pollution, which will enhance both the environment and public health (K. Boonpragob. "Using lichen as bioindicator of air pollution". Acid deposition Monitoring and Assessment. Third country Training, JFY. Bangkok, Thailand, 2003). An increasing number of higher plants, particularly various tree sections, are being used for air monitoring. Because they are in close proximity to air contaminants, roadside plant leaves have the potential to exacerbate these pollutants (JissyJyothi and D.S. Jaya. "Evaluation of Air pollution tolerance index of selected plant species along roadsides in Tiruvananthapuram, Kerala". J. Environ. Biol. Vol.1, pp. 379-386, 2010). Different parts of the plant like leaf, bark can be used for biomonitoring of PAHS and HMs. The leaves of *C. camphora* (Yin et al., 2021), the branches and leaves of olive tree in Bursa, Turkey (Sari et al., 2021) were used for monitoring PAHs.

It might be simpler to differentiate between external and internal quality criteria when qualitative impacts are well-thought-out. External factors such as form, color, and taste are often measured as subjective parameters that oblige skillful surveillance. Now there are abundant biological plant symptoms following pollution such as changes in pH recognized

after growing certain acidophytes, i.e., common ling (*Calluna vulgaris*), hair grass (*Deschampsia Flexuose*), or sunflower (*Drosera rotundifolia*); changes in nitrate content in ecosystems associated with growing wild barley (*Hordeum murinum*), French mercury (*Mercurialis annua*), or large nettle (*Urtica dioica*); and changes in total soluble salts content following growing certain plants such as the halophytes lead grass (*Salicornia europeae*), tripoly (*Aster tripolium*), and wild march beet (*Statice limonium*).

1.6. Air Pollution Tolerance Index (APTI):

The fast growth in traffic, industrial and vehicular emissions, and the decrease in urban vegetation cover have all contributed to the severe deterioration of air quality in major cities of developing nations in recent decades (Kim et al., 2015; Santos et al., 2015). High concentrations of contaminants that are detrimental to human health and wellbeing are frequently found in urban air (Lave and Seskin, 2013; Mayer, 1999). Environmentalists and policymakers have highlighted the importance of a permanent green belt in and around metropolitan areas as well as along roadways to lessen the impact of hazardous airborne pollution (Klumpp et al. 2004; Chaulya et al. 2001). In an ecosystem, plants are crucial to preserving the ecological equilibrium. Specifically, plant leaves operate as a sink by providing a vast surface area for the deposition of air contaminants (Kim et al., 2015). Different contaminants can impact plants directly through their leaves or indirectly by acidifying the soil (Steubing et al., 1989). On the other hand, some air pollutants can negatively impact plant development by changing the biochemical composition, photosynthetic rate, seed germination, number of flowers on florescence, pedicle length, and stomata (Chakraborty et al., 2009). Because different plant species exhibit variations in their sensitivity to various air contaminants, each plant reacts differently to pollutants of a similar kind. Certain plants are able to adjust their biochemical parameters, including those related to chlorophyll, ascorbic acid, leaf pH, and relative water content, to cope with changes in their surroundings (Kuddus et al., 2011; Flowers et al. 2007). The air pollution tolerance index is a single formulation that uses these parameters to compute the results (APTI). In general, the identification of plant species that are sensitive, intermediate, and tolerant to air pollution may be accomplished by APTI (Nayak et al. 2015; Leghari et al., 2011; Das and Prasad 2010; Liu and Ding 2008). The sensitive species act as indicators of pollution (Singh et al., 1991), whereas tolerant ones may be utilised to expand the green belt and serve as sinks for airborne pollutants (Rai and Panda 2014; Kousar et al. 2014; Kuddus et al. 2011; Agbaire and Esiefarienrhe 2009). An APTI value of ≤11 considered as sensitive, 12-16 as intermediate and ≥17 classified as tolerant (Bharti et al., 2018). Below some plant species and their corresponding APTI value are listed:

Table 3.

Terrestrial plant species and their corresponding APTI

Species	APTI	Remarks	Reference
P. longifolia	7.76±0.49	Sensitive, can be use as bioindicator	Bharti et al., 2018
A. nilotica	14.41	Intermediate	Bharti et al., 2018
F. religiosa	20.41	Tolerant, can be use as biomonitor	Bharti et al., 2018
I. palmata	23.86	Tolerant	Pandey et al., 2015
H. indicus Br.	8.10	Sensitive	Pandey et al., 2015
B. alba Linn.	13.79	Intermediate	Pandey et al., 2015
C. camphora	36.0	Tolerant	Shrestha et al., 2021
Ficus sp.	16.3	Intermediate	Shrestha et al., 2021
P. guajava	9.5	Sensitive	Shrestha et al., 2021
J. violacea	14.79	Intermediate	Pandey et al., 2015

2. Literature Review:

1. Determination of Heavy Metal Concentrations and Their Potential Sources in Selected Plants: Xanthium strumarium L. (Asteraceae), Ficus exasperata Vahl (Moraceae), Persicaria attenuata (R.Br) Sojak (Polygonaceae), and Kanahia laniflora (Forssk.) R.Br. (Asclepiadaceae) from Awash River Basin, Ethiopia, 2019, Tadesse, A.W., Gereslassie, T., Yan, X., Wang, J., Biological Trace Element Research 191, 231–242.

Summary: The main objective of the study is to measure the level of concentration of heavy metal in the plants *Xanthium strumarium* L. (Asteraceae), Ficus exasperata Vahl (Moraceae), *Persicaria attenuata* (R.Br) Sojak (Polygonaceae), and *Kanahia laniflora* (Forssk.) R.Br. (Asclepiadaceae). Identification of potential heavy metal sources in plants. Evaluation of risk factor on human and environment based on WHO (World Health Organization) and FAO (Food and Agricultural Organization) standard limits.

Experimental: Four plants sample are taken here namely *Xanthium strumarium* L. (Asteraceae), *Ficus exasperata Vahl* (Moraceae), *Persicaria attenuata* (R. Br) Sojak (Polygonaceae), and *Kanahia laniflora* (Forssk.) R.Br. (Asclepiadaceae) from Awash River Basin, Ethiopia.The sample are air dried and kept at -20 $^{\circ}$C for analysis. Then the samples are dipped into 10% Nitric Acid (HNO_3, 63%) for 12 Hours and to remove dust and contaminants washed three times with double-distilled water. Later the samples are Oven dried at 60-65 $^{\circ}$C for 24 Hours. 0.25 g of each sample is taken into Teflon vessels and digested with 2ml of H_2O_2 (Hydrogen Peroxide 70-72%) and 7ml of HNO_3(Nitric Acid 63%). The samples are digested in microwave digester for 2 Hours. The samples are heated at a temperature of 135 $^{\circ}$C after digestion and later cooled at room temperature. The samples are filtered and stored at -4 $^{\circ}$C until analysis. Finally the samples are analyzed using ICP-MS (ThermosXSERIES2).

Conclusion: This study showed that the Al, Cu, Ni, and Zn are found in the plants where as Cr, As, Cd, Pb, and Hg were below the detection limit in some plant samples.

2. Assessment of Heavy Metals Tolerance in Leaves, Stems and Flowers of *Stevia rebaudiana* Plant, 2014, Hajara, E.W.I., Sulaimanb, A.Z.B., Sakinahb, A.M.M., Procedia Environmental Sciences 20, 386 – 393.

Summary: In this study *Stevia rebaudiana* plants were used for the assessment of heavy metal tolerance in leaves, stems and flowers using Inductively coupled plasma mass spectrophotometry (ICP-MS). Fifteen heavy meatals like As, Cd, Cr, Cu, Fe, Mg, Pb, Se, Zn, Al, Ag were analyzed in this experiment.

Experimental: *Stevia rebaudiana* plants were collected from Malacca, Malaysia. The leaves, stems and flowers were dried in oven equipment and grinded to produce powder. These samples were measured using a vibrator sieve shoaker. Approximately 0.5g of each parts of the sample was taken into Taflon digestion vessel and added high purity concentrated Nitric Acid (65%) and high purity concentrated of hydrogen peroxide (30%). The analysis of sample was done by Inductively Coupled Plasma Mass-Spectrophotometer (ICP-MS) instrument Elan 9000 and Agilent 7500a.

Conclusion: Variety of elements such as As, Cd, Cr, Cu, Fe, Mg, Pb, Se, Zn, Al, Ag, Co, Ca, Mn and Ni were presented in the leaves, stems and flowers of Stevia plant and the determined concentration of heavy metals in leaves, stems and flowers were below the tolerance level. Range of elements presents in each part of the plants in mg/kg.

- Arsenic: 0.05316-3.909
- Cadmium: 0.169-0.6048
- Chromium: 3.87-6.1815
- Copper: 2.004-8.11
- Iron: 125.11-972
- Magnesium: 1.701-1760
- Plumbum: 0.4895-1.735
- Selenium: 0.0007644- 0.04536
- Zinc: 20.19-44.4235
- Aluminium: 58.87-494.4
- Silver: 0.0317-0.0676
- Cobalt: 0.0228-0.0842
- Calcium: 259.44-558
- Manganese: 6.03-12.48
- Nickel: 1.53-2.4

3. A critical review on plant biomonitors for determination of polycyclic aromatic hydrocarbons (PAHs) in air through solvent extraction techniques, 2020. Mukhopadhyay, S., Dutta, R., Das, P., Chemosphere 251, 126441.

Summary: In this study, the main focus has been given on different approaches of biomonitoring for air quality measurements with the help of plants or there parts, lichens and mosses replacing the standard conventional air sampling methods for their difficulties and high

cost in different polluted zones. The main method that is normally being used for assessment of airborne PAHs is solvent-extraction.

Experimental: Plants, lichens and mosses are collected from different polluted areas. Biomass homogenisation and air drying are done before PAH extraction. The solvent extraction techniques that are used are mechanical agitation, Soxhlet extraction, Ultrasonic agitation, ASE, PPE, SPE, MAE. The samples are cleaned up and concentrated using rotary evaporator and nitrogen stream and analysed with the help of HPLC/GC-MS.

Conclusion: Identification of different modern solvent extraction methods along with Soxhlet method and analytical methods for extraction of PAHs from accumulative bioindicators has been developed for accurate determination of PAHs. The choice of solvent, temperature, time of extraction, pressure matrix characteristics are the process parameters which are usually checked during the analysis.

4. Heavy metals accumulation and translocation in native plants grown on tailing dumps and human health risk, 2020,Pehoiu, G., Murarescu, O., Radulescu, C., Dulama, I.D.,Teodorescu, S., Stirbescu, R.M., Bucurica, I.A., Stanescu, S.G., Plant Soil 456, 405–424.

Summary: The main objective of the study is to determine the content of Cr, Mn, Ni, Cu, Zn, Cd, Pb by ICP-MS in plant tissues of *Ranunculus ficaria*, *Plantago major*, *Taraxacum officinale*, and *Achillea millefolium* collected from radioactive area of Romania. To evaluate the values of EDI, HRI and TTHQ in the plants to check the health hazards in human being if they are consumed.

Experimental: The sample of the plants *Ranunculus ficaria*, *Plantago major*, *Taraxacum officinale*, and *Achillea millefolium* are collected from the polluted and radioactive area of Romania. Freshly collected plants samples are carefully cleaned by deionized water. The samples are digested with 1ml H_2O_2 (Hydrogen peroxide, 31%, high purity) and 9ml HNO_3 (Nitric acid, 65%, high purity) in Teflon Vessels. The samples are cooled after digestion for one hour. Then the samples are transferred to 25ml volumetric flasks with distilled water. Finally, ICP-MS is used to analyze the clear solution.

Conclusion: The study showed the main source of heavy metals contamination, their effect on wild plants and human health. The research also suggest that the use of these plants species for the management of diseases will not cause any toxicity in terms of heavy metal.

5. Heavy metals and trace elements detected in the leaves of medicinal plants collected in the southeast part of Turkey, 2022, Ozyigit, I.I., Karahan, F., Ibrahim Ertugrul Yalcin, I. E., Asli Hocaoglu-Ozyigit, A. H., Ahmet Ilcim, A., Arabian Journal of Geosciences 15, 27.

Summary: The main objective of the study was to evaluate the contamination rate of heavy metal detected in 37 medical plant species that are commonly used in Southeast Anatolia in Turkey.

Experimental: The samples were collected from the industrial and mining area of South East Antalonia in Turkey. 50 g of each samples were taken and dried in an oven at 80 °C for 2 days. Then the sample are grounded and shifted using a 1.5 mm shieve for accomplishing the drying process. 0.2 g of each samples are taken in taflon vessels and treated with 10 ml of 65% Nitric acid. The samples were treated using 600 PSI pressure for pulverization. The final 50 ml volume of each samples were analyzed ICP-OES.

Conclusion: Anthropogenic activities are the main reasons of contamination of heavy metals in water, soil and air. The following heavy metals were found in the plants 8.68–278.83 for Al, 46.68–505.24 for B, 2430.0–15,561.3 for Ca, 0.04–1.11 for Cd, 0.94–21.67 for Cr, 13.7–44.81 for Cu, 12.1–456.2 for Fe, 1083.3–9026.98 for K, 344.8–3064.72 for Mg, 48.32–557.62 for Mn, 295.38–1416.02 for Na, 0.042–9.58 for Ni, 1.37–18.0 for Pb, and 68.82–220.29 for Zn. The concentration rate are in mg kg^{-1}.

6. Heavy metals accumulation and translocation in native plants grown on tailing dumps and human health risk, 2020. Pehoiu, G., Murarescu, O., Radulescu, C., Dulama, I.D., Teodorescu, S., Stirbescu, R.M., Bucurica, I.A., Stanescu, S.G., Plant Soil 456,405–424.

Summary: The main objective of the study is to evaluate the heavy metal concentration in the plant tissues of *Ranunculus ficaria, Plantago major, Taraxacum officinale, and Achillea millefolium*, frequently consumed or used in traditional medicine, collected from one of radioactive area of Romania.

Experimental: The samples are collected from the Banat Region is part of the Carpathian Mountains in Romania. Plants samples are collected according to Codex Methods of Sampling for about 8 weeks. Total 6 samples/ week are collected from each three selected areas. Collected samples are carefully cleaned with deionized water and each plant are separated by root, steam, leaves and flower. The samples are completely dried and grinded in order to obtain fine powder. About 400 mg of each samples are taken in Teflon vessels. 1mL H_2O_2(31%, high purity) and 9mL of HNO_3(65%, high purity) are added to the samples. After

digestion process the samples are cooled in the PTFE-TFM vessels for about one hour. Then the samples are analyzed by ICP-MS technique.

Conclusion: The research highlighted that the high amount of Cd, Mn, and Pb, are found in the tissues of *Taraxacum officinale and Plantago major.* The study also suggested that the use of the plant species for the management of dieses will not cause hazards in terms of heavy metal as these metals are found to be present in readily bioavailable form.

7. Assessment of heavy metal pollution in the agricultural soils, plants, and in the atmospheric particulate matter of a suburban industrial region in Dhaka, Bangladesh, 2021, Shammi, S.A., Salam, A., Khan, Md. A.H., Environ Monit Assess 193, 104.

Summary: The focus of the research is to study the concentration of the heavy metal ((Fe, Mn, Cr, Cu, Ni, Co, Zn, Pb, and Cd) in agricultural soils, plants and in atmospheric particulate matter (PM) in the suburban industrial zone located beside the Dhaka Export Processing Zone (DEPZ) at the Dhaka district in Bangladesh.

Experimental: Plant leave samples from rice (*Oryza sativa L.*), maize (*Zea mays L.*), cabbage (*Brassica oleracea var. capita*), pumpkin (*Cucurbita pepo var. pepo*), arum (*Arum palaestinum*), and red spinach (*Amaranthus dubius*) were also collected randomly from the agricultural lands. The samples are first air dried and then over dried at 80 °C. Then the samples are ground to pass through a 0.2-mm sieve. The samples are digested with the help of HNO_3 (Nitric acid) and $HCIO_4$ (Perchloric acid). The heavy metal content is determined with the help of flame atomic absorption spectrophotometer (AAS, model: Analyst 800, PerkinElmer)

Conclusion: The concentration of heavy metals in plant leaf samples in µg/g are Fe:1471.67, Mn 141.24, Cr 6.0, Cu 12.61, Ni 3.62, Co 13.5, Zn 54.03, Pb 6.65, Cd 0.02.

8. Heavy metal accumulation in leaves of selected plant species in urban areas of Delhi, 2022, Patel, K., Chaurasia, M., Rao, K. S., Environmental Science and Pollution Research 30, 27622-27635.

Summary: This study was conducted to evaluate the contamination of heavy metals (Pb, Cd, Cu, and Zn) in the environment including atmospheric dust, soil and leaves of plant species at different sites of Delhi.

Experimental: Samples from selected plant species at four various sites are collected. The leaves are washed and dust are collected through the gravimetric method. Then leaves are dried and crushed. The samples are digested using triple acid digestion method. The

concentration of heavy metals are determined with the help of atomic absorption spectroscopic (AAS).

Conclusion: Among all the heavy metal Pb, Cd, Cu and Zn are found in high concentration which are above WHO's permissible limits.

9. Assessment of Airborne Heavy Metal Pollution Using Pinus spp. and Tilia spp, 2013, Serbula, M. S., Kalinovic, T.S., Ana A. Ilic, A.A., Kalinovic, J.V., Steharnik, M.M., Aerosol and Air Quality Research 13,563-573.

Summary: This study focuses on the evaluate concentration of heavy metal, Cu, Pb, Zn and Mn, in the branches, roots and leaves of the plant *Tilia spp*. and *Pinus spp*. Evaluation of biomonitoring ability of this plant species in the surrounding areas of mining metallurgical complex, Serbia.

Experimental: Plant samples are collected from three different zones within the Bor region. All the plant samples are thoroughly washed with water to remove dust and air dried for 10 days in room temperature then dried in dryer for 24 h at 50 °C. The dried leaves are crushed. The samples are digested according to the U.S. EPA method 3050B and analysed by ICP-AES.

Conclusion: The average concentration of the element in (μg/g) for Cu, Pb, Zn, Mn is 427.12, 96.50, 104.87, 781 respectively. The study concluded that pine is more suitable for biomonitoring than linden.

10. Air biomonitoring of heavy metals and polycyclic aromatic hydrocarbons near a cement plant, 2014, Baldantoni, D., Flavia De Nicola, F.D., Anna Alfani, A., Atmospheric Pollution Research 5, 262-269.

Summary: The main air of the study is to compare the capabilities of the Mediterranean plant species to accumulate heavy metal and PAHs and to evaluate the depositions of heavy metal and PAHs at local scale due to cement plant or other anthropogenic activities happening in industrial area.

Experimental: The samples are collected from the different inside area of Italcementi Group cement plant. The samples are oven dried at 75 °C and characterized for their water content. The samples are stored at -20 °C. The leaf samples are extracted with the help of sonication in a dichloromethane-acetone mixture. The PAHs concentration are determined with the help of GC-MSD.

Conclusion: Different concentration of heavy metal and PAHs are found in the two species acenaphthylene, acenaphthene and anthracene of plants. In case of HMs the leaves of *Q. ilex* had the highest concentration. The concentration of PAHs acenaphthylene, acenaphthene and anthracene in cement area is lower than the urban area.

11. Heavy metal distribution in wheat plant components following foliar Cd application, 2023, Pengkun Liu, P., Li, L., James A. Ippolito, A.J., Xing, W., Wang, Y., Wang, Y., Cheng, Y., Qiu, K., Chemosphere 322, 138177.

Summary: The goal of the study is to evaluate the distribution and transport of Cd in ground plant tissue on application of foliar Cd. To study effect of Cd application on the distribution of heavy metal in wheat plants and the impact of the leaf's Cd absorption on wheat grain Cd accumulation throughout transportation.

Experimental: The wheat samples are cut just above soil level and washed with tap and then distilled water. Then the plants are divided into three parts from top to bottom and levelled as P1, P2, P3. All plants materials are oven dried at 75 °C and crushed and mix. The plant samples are digested in concentrated nitric acid (HNO_3) and hydrochloric acid (HCL) in the ratio of 3:1 in microwave digester. Then the samples are analysed in ICP-MS.

Conclusion: The study showed that the application of Cd foliar did not affect the Cd concentration in Wheat.

12. Monitoring of heavy metals in topsoils, atmospheric particles and plant leaves to identify possible contamination sources, 2007, Oliva, S.R., Espinosa, A.J.F., Microchemical Journal 86, 131-139.

Summary: The aim of the study is to evaluate the grade of soil contamination of the experimental site of Seville, compare the metal level of the soil where the plants *N. oleander L. and L. camara L.* grows with the metal level of PM_{10} particles and leaves of the plants and to understand the behaviour of elements in both species of plants.

Experimental: A total of 22 samples of PM_{10}, 22 of oleander leaves, 22 of lantana leaves, 22 of oleander soils and 22 of lantana soils were collected from Seville of Andalusia. From healthy looking plant several branches are cut off and taken to lab, about 300 g of fresh

mature leaves are taken and dried at 70 °C overnight. Then the leaves are grounded to fine powder. The sample is digested in a microwave oven Millestone, model Ethos 900 with a mixture of 15 ml of concentrated nitric acid (HNO_3) and concentrated perchloric acid ($HClO_4$) v/v 3:1. The concentration of the metals are evaluated by inductively coupled plasma atomic

emission spectrometry (ICP-AES), using a Fisons-ARL 3410 sequential multi-element instrument.

Conclusion: The mean concentration of the elements Pb, Ni, Mn, Cu, Fe, V^b, Ti, Ba in plants in mg kg^{-1} are 1.3 ± 0.3, 1.6 ± 0.2, 42.4 ± 2.6, 8.5 ± 0.8, 283 ± 11, 0.5 ± 0.1, 17.3 ± 2.2, 21.8 ± 2.5 respectively. The levels of all elements are under the maximum acceptable limit for residential, recreational and institutional areas. The vehicular traffic, sludge application and use of fertilizer are the main source of pollutants such as Ba, Cu, Ni and Pb.

13. Bioaccumulation of heavy metals in plant leaves from Yan'an city of the Loess Plateau, China, 2014, Hu, Y., Dexiang Wang, D., Wei, L., Zhang, X., Song, B., Ecotoxicology and Environmental Safety 110, 82-88.

Summary: The aim of the study is to evaluate the concentration of toxic metals (Pb, Cu, Cd, Cr and Zn) in the leaves of common green species in Yan'an. To determine the plants total metal accumulation capacities and to offer knowledgeable data for additional studies of heavy metal accumulating on the Loess Plateau.

Experimental: The current study was carried out at Yan'an city of the Loess Plateau, China. Eighty four samples from six different district including nine tree species and five shrub species were collected. All samples are kept at room temperature until analysis. The samples are put through a three-step washing sequence. The leaves were air dried first, killed out in an oven for five minutes at 105 °C, and then dried at 70 °C for 48 to 72 hours to get a uniform weight. For digestion, the dried leaves were weighed and mechanically ground in a stainless steel mill (particle diameter: 100 μm). To measure the concentration of heavy metals, 0.25 g of dried, homogenised samples (accurate to 0.0001 g) were put in 100-mL clean, dry pyrex digestion tubes, and 5 mL of strong nitric acid was added. A flame atomic absorption spectrophotometer (AA-7000A, Beijing East & West Analytical Instrument Inc., Beijing, China) was used to quickly determine the heavy metal contents in the digested solutions.

Conclusion: The range of heavy metal concentration (mg/g) at each site is shown in following tables 4 and 5.

Table 4.

The range of heavy metal concentration (mg/g) in site 1

Heavy metal	Range (mg/g)
Pb	2.15-15.91
Cd	0.48-2.75

Cr	0.071-0.549
Cu	2.49-7.03
Zn	5.26-11.29

Table 5.

The range of heavy metal concentration (mg/g) in site 2

Heavy metal	Range (mg/g)
Pb	2.83-12.57
Cd	0.86-2.28
Cr	0.097-0.385
Cu	1.53-7.38
Zn	4.21-19.21

The MAI should be a crucial indication when choosing tree species for phytoextraction and urban greening since species with the highest accumulation of one metal did not always have the maximum capability for overall metal accumulation.

14. Heavy metal in leaves of twelve plant species from seven different areas in Shanghai, China, 2017, J. Liang, J., Fang, H.L., Zhang, T.L., Wang, X.X., Liu, Y.D., Urban Forestry & Urban Greening 27, 390-398.

Summary: The objective of the study is to evaluate the differences between the concentration of heavy metal on the leaves of twelve plant species from seven different places; to compare unwashed and washed leaves, as well as learning about the density and structure of stomata in plant leaves, to understand why leaves have varying heavy metal concentrations and to determine the primary source (from the soil or the air) of heavy metals in plant leaves based on correlation studies of the metal concentrations in the washed leaves.

Experimental: This study was conducted at seven sites at different locations in Shanghai, China. Twelve plant species including seven trees and five shrubs are collected from the outer areas of the canopies using stainless steel. All the plant samples are divided into two parts. One part is washed three times with deionized water in ultrasonic cleaner to remove dust. The other part of the sample was not washed. The fresh leaves are weighed and oven dried at 75 °C. A polytetrafluoroethylene (PTFE) tube was filled with around 0.25 g of each leaf sample. The materials were gently mixed, adding concentrated HCl (hydrochloric acid), concentrated HNO_3 (nitric acid), and hydrogen peroxide (30%), and allowed to equilibrate for overnight. To

determine the concentration of heavy metals (Cu, Zn, Pb, and Cd) in the samples, an inductively coupled plasma-mass spectrometer was used to digest the samples.

Conclusion: When compared to unwashed leaves, the washed leaves of *Nerium indicum* had higher CRs for Cu (92.7%) and Zn (36.9%), those of *Magnolia grandiflora* had higher CRs for Pb (63.4%) and Cd (49.1%), and those of *Cedrus deodara* had lower CRs for Cu (0%), Zn (8%), Pb (5.93%), and Cd (2.97%).Additionally, correlation analyses of the mean metal contents in the soils and washed leaves, the concentration factors (CFs) of heavy metals, and the comparison of new and old leaves showed that the Cu, Zn, Pb, and Cd in plant needles may originate primarily from the soil, whereas the Cu, Zn, Pb, and Cd in the leaves of broad-leaved plants may originate primarily from bulk atmospheric deposition.

15. Assessment of air pollution tolerance index of *Murraya paniculata* (L.) Jack in Kolkata metro city, West Bengal, India, 2021, Mukhopadhyay, S., Dutta, R., Dhara, A., Urban Climate 39, 100977.

Summary: The current research is the first attempt to evaluate *Murraya paniculata* (L.) Jack's potential as a useful biomonitor of air quality impacted by PAHs at various study locations in South Kolkata, India. The primary goals of this study were to determine the APTI for the plant species and the impact of plant factors on APTI in order to show its use in biomonitoring. Additionally, it was intended to create an extraction method for separating PAHs from the leaves of the mentioned plant, which would be followed by appropriate cleanup and measurement by gas chromatography-mass spectrometry (GC-MS).

Experimental: The study was conducted in Kolkata, West Bengal, India. Leaves of the plant *Murraya paniculate* (L.) Jack are collected from 2-3 individual trees of same species by hand using sterile gloves, put in polythene bags and immediately transported to the lab for further analysis. Leaf samples are washed with distilled water and dried at -196 °C using alyophilizer under vacuum for 24h. To improve the kinetics of analyte extraction by minimising diffusion paths and increasing the contact surface area between the sample and solvent, dried leaves were manually crushed with a mortar and pestle. With the aid of the Soxhlet apparatus and 150 mL of toluene (a non-polar organic solvent), PAHs were extracted from homogenised plant leaves (less than 10 g dry weight) over the course of six hours, with the temperature of the extraction kept close to the boiling point of the solvent. The extracted solutions were purified using silica gel column chromatography (glass column of standard dimensions was packed with 10 g of silica gel, previously activated at 180 C for 4 h and 2 g of anhydrous Na_2SO_4) for precise and

trustworthy analysis, and the fractions enriched with PAHs were eluted with n-hexane. the dried residues were treated in HPLC grade methanol for GC-MS analysis.

Conclusion: The current study emphasises the ecophysiological characteristics of *Murraya paniculata* (L.) Jack in response to adverse environmental factors like presence of PM, PAHs, etc. influencing plant growth, cellular metabolism, reproduction, and sustenance, which have been examined to determine the plant's potential for biomonitoring. Due to its tolerance index being significantly higher than the limiting value (APTI > 17), it can be said that the tree species M. paniculata is a biomonitor of importance for evaluating and minimising environmental pollution.

3. Objectives and Plan of Work:

3.1. Overall and specific objectives:

Present study will be conducted to investigate levels of PAHs and some environmentally significant heavy metals in ambient air of south Kolkata, West Bengal, India. This preliminary research may help researchers and policy makers in terminating and managing air pollution of the city. The few researchers had reported the main contributors of air pollution in the city which were transport sector followed by power plants, industrial units and burning of debris. In recent years, many studies have been carried out to determine the concentrations of PAHs and heavy metals in respirable particulate matter phase (SPM); but reports on PAHs and heavy metals in ambient air phase in Kolkata city are very scanty.

Biomonitoring studies, based on pollutant accumulation analyses in tree leaves, allow evaluating the impact caused by air–dispersed pollutants on ecosystems, providing useful data, complementary to those obtained by instrumental monitoring. In particular, plant leaves present morphological characteristics, such as the presence of hairs and cuticle, making them particularly useful in bioaccumulation studies. Most of the studies related to air pollution in Kolkata are focused on direct air sampling and very few reports are available on PAHs and heavy metal profiles present in the ambient air of the city along with scanty information related to its source apportionment. There have been very few attempts in Kolkata so far for air pollution monitoring using plant bioaccumulators or biomonitors for determination of such airborne pollutants.

Based on above findings, the present study set a broad objective to assess the ambient concentration of PAHs and heavy metals in south Kolkata through biomonitoring approach. To satisfy the broad goal, following specific objectives are intended to carry out:

- Selection of sampling site based on pollution load data.
- To examine the biomonitoring potential of different terrestrial plant species growing in and around sample site through the estimation of air pollution tolerance index.
- Determination of ambient concentration of PAHs in terms of biomonitoring capacity of selected species: Solvent Extraction followed by HPLC analysis
- Determination of ambient concentration of heavy metals in terms of biomonitoring capacity of selected species: Acid digestion followed by analysis for target heavy metals by inductively coupled plasma atomic emission spectroscopy (ICP-AES).

- Evaluation of the impact of anthropogenic activities occurring in chosen urban areas causing heavy metal and PAHs deposition.

3.2. Plan of work:

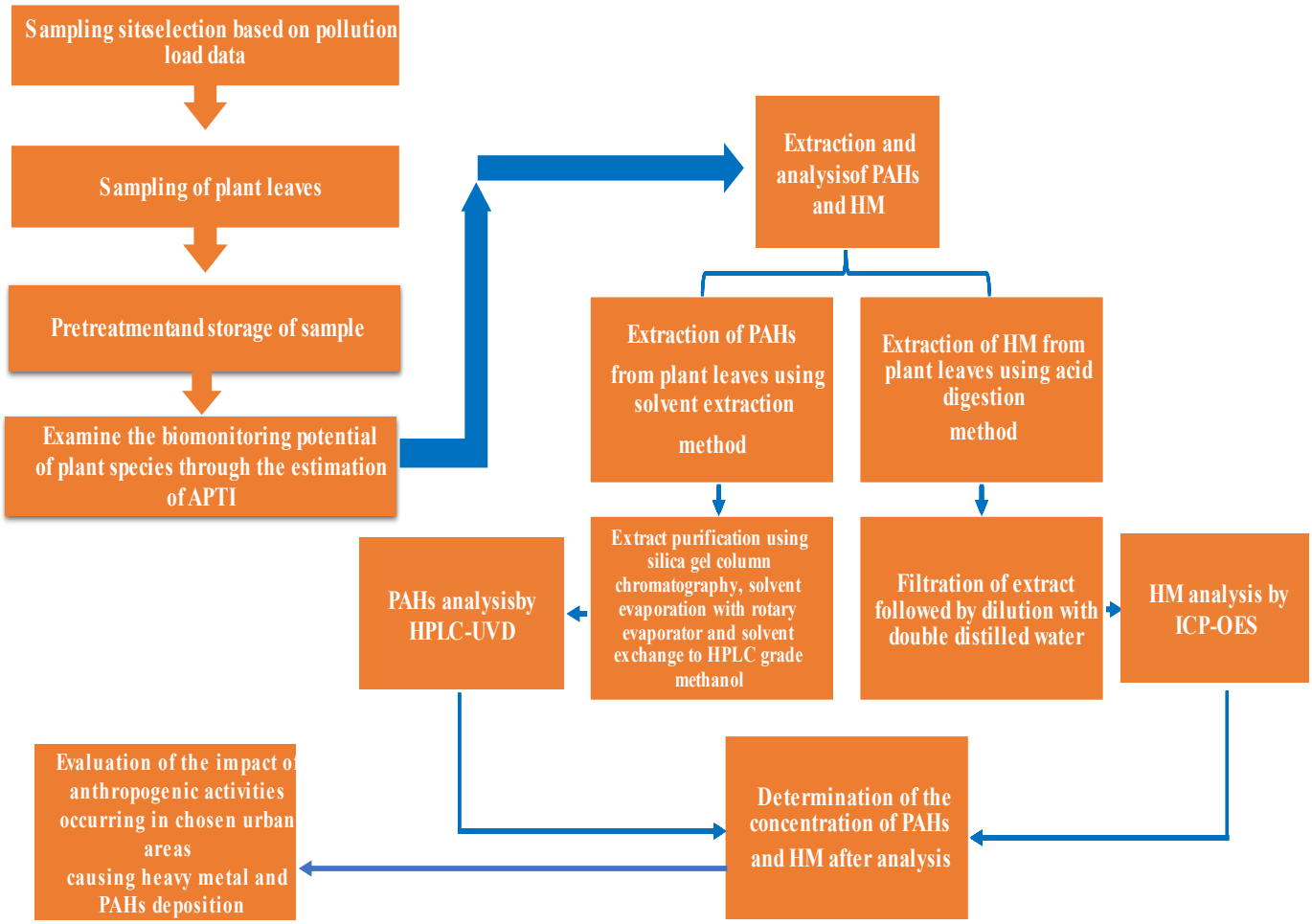


Fig. 4: Flowchart of plan of work

4. Materials and Methods:

4.1. Study area: The research was conducted in the city of Kolkata, West Bengal, India. The sampling was done during summer months (May-June, 2023). The study was done near Jadavpur University area (22º29'54"N, 88º22'12"E) Jadavpur (Fig. 5). The sampling site is chosen based on the rising level of pollution loads associated with disastrous impacts and mainly characterized by heavy traffic density, urban or residential settlements, commercial establishments, shops, restaurants, bakeries, etc. The site is also corresponded to a nearby automated ambient air quality monitoring station run by West Bengal Pollution Control Board (WBPCB), India, data of which has been shown in Table 6. Interiors of Contai (21.78ºN 87.75ºE) in East Medinipur (a remote area of semi-urban origin, approximately 150 km away from Kolkata) was chosen as a control site (Fig. 6).



Fig. 5: Location map of sampling site (polluted)

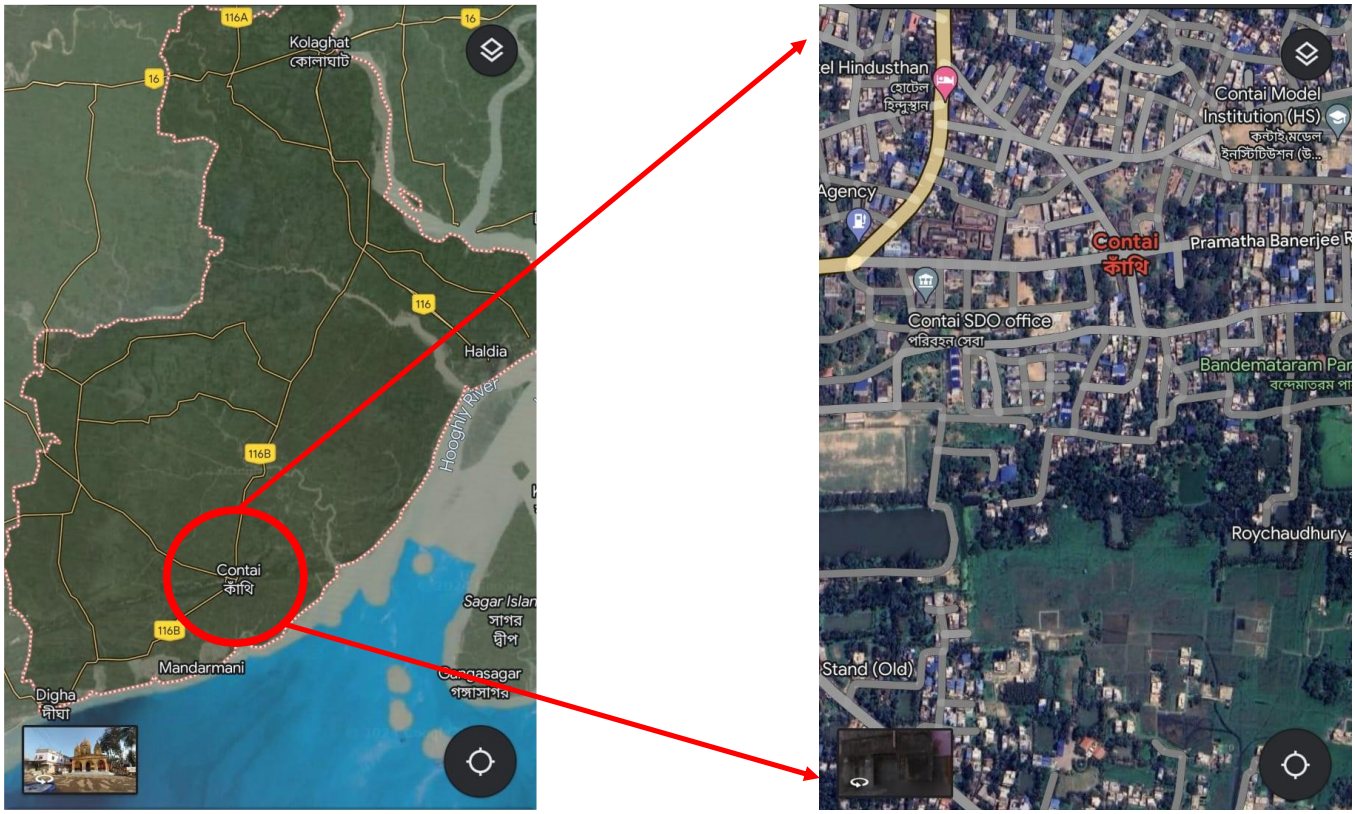


Fig. 6: Location map of control site (Contai, East Medinipur)

Table 6.

National ambient air quality monitoring (NAAQM) data (average) of the study sites during sampling period (as provided by CPCB and WBPCB) (Ref.: https://airquality.cpcb.gov.in/AQI_India_Iframe/)

Site	Geographical coordinate		Average NAAQM data								
			PM_{10} ($\mu g\ m^{-3}$)	$PM_{2.5}$ ($\mu g\ m^{-3}$)	NO_2 ($\mu g\ m^{-3}$)	SO_2 ($\mu g\ m^{-3}$)	NH_3 ($\mu g\ m^{-3}$)	CO ($\mu g\ m^{-3}$)	O_3 ($\mu g\ m^{-3}$)	Air quality index (with respect to $PM_{2.5}$)	Air quality status
JADAVPUR	22°29'54"N, 88°22'12"E	**Permissible limit (24-hourly average)**	**100**	**60**	**80**	**80**	**400**	**2000**	**100**		
			145	207	25	9.3	10	66.6	59	**244**	**Poor**

4.2. Sampling and processing of plant materials*:* *Bougainvillea spectabilis* (species 1) and *Mangifera indica* (species 2) have been selected for the study because of its wide occurrence, natural growth and abundant availability in the urban areas of Kolkata. Fresh and mature leaves of the selected plant from the branches of 2-3 individual trees of same species of the area are collected, put in polythene bags and immediately transported to lab for further analysis.

Bougainvillea is a genus of thorny ornamental vines, bushes, and trees belonging to the four o' clock family, Nyctaginaceae. It is native to eastern South America, found from Brazil, west to Peru, and south to southern Argentina. Different authors accept from 4 to 22 species in the genus. The inflorescence consists of large colourful sepal-like bracts which surround three simple waxy flowers, gaining popularity for the plant as an ornamental.

Bougainvillea spectabilis grows as a woody vine or shrub, reaching 15 to 40 feet (4.6 to 12.2 m) with heart-shaped leaves and thorny, pubescent stems. The flowers are generally small, white, and inconspicuous, highlighted by several brightly colored modified leaves called bracts. The bracts can vary in color, ranging from white, red, mauve, purple-red, or orange. Its fruit is a small, inconspicuous, dry, elongated achene.

Magnifera indica commonly known as mango, which belongs to the Anacardiaceae family, is considered to be one of the most commercially significant tropical fruit crops worldwide and a crucial traditional crop (Kumar et al., 2021). Mangoes are members of the genus Mangifera, which has around thirty species of tropical fruiting trees (Shah et al.,2010). The mango is indigenous to eastern India and Burma in southern Asia. Early on, it reached Malaya, eastern Asia, and eastern Africa. The mango is a massive evergreen tree that may grow to a height of 15 to 30 metres. The tree is held firmly in place by a mass of feeder roots as well as a long, unbranched taproot that can reach a depth of 6–8 metres. The leaves are 29-30 cm in length and 3-5 cm in width when on blooming branches and up to 50 cm when on sterile branches. They are oblong, lanceolate, simple, leathery, and alternating.

After collection, the leaf samples were carefully washed with distilled water three to five times to remove dust or soil particles and other debris. Unwashed leaves were also stored for the analysis of few leaf parameters. The leaf samples were dried in hot air oven for 24 hours at 80 °C (Fig. 7). To improve the kinetics of analyte extraction by minimising diffusion paths and increasing the contact surface area between the sample and solvent, dried leaves

were manually crushed using a mortar and pestle to produce a powdery homogenous sample (Mukhopadhyay et al., 2020).

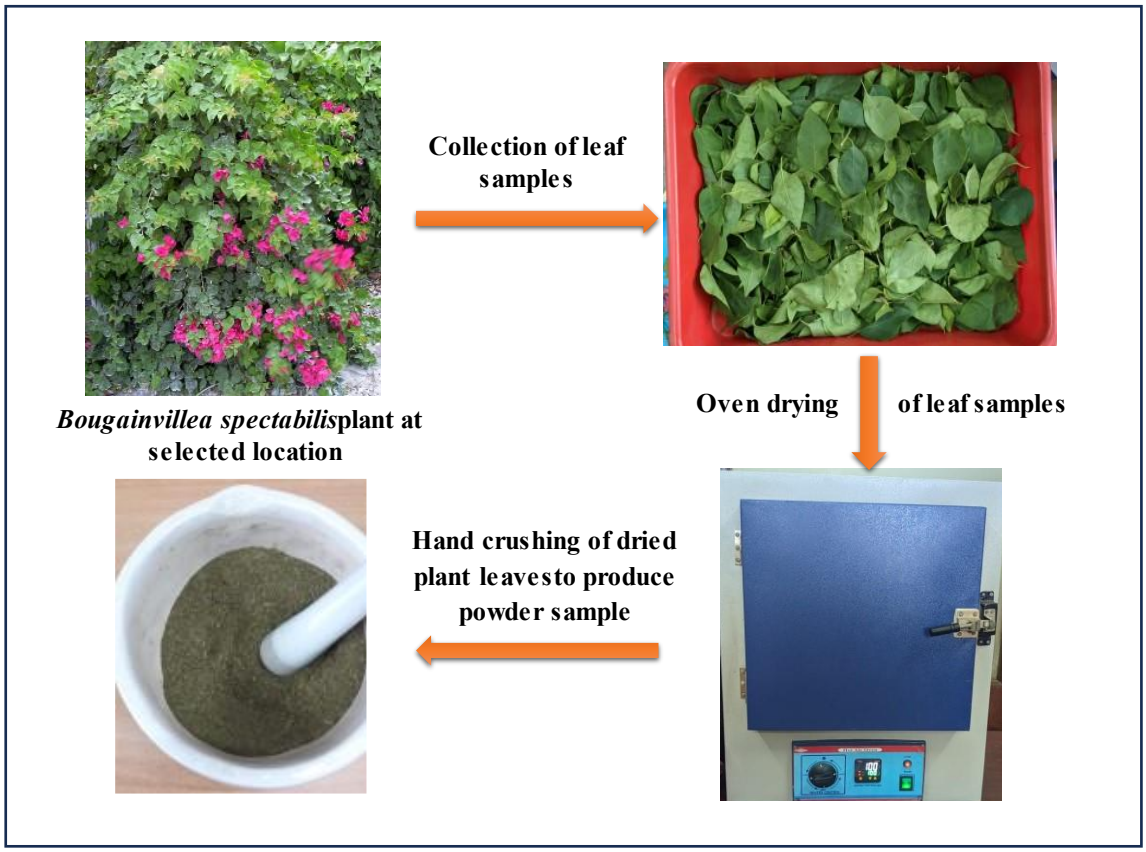


Fig. 7: Processing of sample

4.3. Selection of samples for biomonitoring of PAHs and HMs:

4.3.1. Air pollution tolerance index (APTI): Physiological, metabolic, and morphological traits of a plant determine its capacity to withstand air pollution (Singh and Verma, 2007). The ability of plants to withstand air pollution is described by the air pollution tolerance index (APTI). Based on their levels of tolerance or sensitivity to air contaminants, plants can be classified using this useful parameter. Total chlorophyll, relative water content (RWC), ascorbic acid, and pH of leaf extract are the four biochemical variables used for determining the APTI of plants (Badri et al., 2021). APTI can be calculated by following formula:

$$APTI = \frac{AA(TCH+pH)+RWC}{10} \quad \text{..... Eq. 1}$$

Where, AA = Ascorbic Acid content (mg/g); pH = Leaf extract pH; TCH = Total chlorophyll content (mg/g); RWC = Relative Water Content (%)

Tolerant plant species are those with APTI values greater than 17, whereas intermediately tolerant species have values between 10 and 16. The APTI of sensitive plant species varies from 1 - 10, with a value of 1 or less being regarded very sensitive as shown in Table 7.

Table 7.

Standard range of APTI (Bharti et al., 2018)

Sl. No	APTI value or Range	Level of Tolerance
1	>17	Tolerant
2	10-16	Intermediate tolerant
3	<10	Sensitive
4	<1	Highly sensitive

- **Determination of Relative Water Content (RWC):** To calculate RWC we measured three different weights of the leaves. Fresh weight has taken immediately after collecting leaves. Turgid weight has taken after immersing the leaves in water for 12 hours and dry weight has taken after drying in hot air oven at 80 °C overnight. The RWC is calculated using the following equation:

$$RWC = \frac{Fresh\ weight - Dry\ weight}{Turgid\ weight - Dry\ weight} x100 \quad \text{..... Eq. 2}$$

- **Determination of Leaf extract pH:** 2g of crushed leaf sample is mixed with 40mL of distilled water. The sample mixture is stirred for 20min. Then the mixture is filtered using a filter paper and the pH is measured with the help of digital pH meter (Labtronics, Model: LT-23).

- **Determination of Total Chlorophyll:** 2g of crushed leaf sample is mixed with 15mL of 80% acetone in a beaker. Then the sample is stirred for 15 min for thorough extraction with filter paper. The leaf extract so obtained taken into centrifuge tube. Then the absorbance is measured at 645nm for Chlorophyll a and 663nm for chlorophyll b using

a UV-VIS Spectrophotometer (Perkinelmer, Lamda-365, Sl No-365K5091709) (Fig. 8).

The total chlorophyll content can be calculated using the following equations:

$$Chl_a = \frac{(12.70 \times A663 - 2.69 \times A645) \times v}{1000 \times w} \quad \text{..... Eq. 3}$$

$$Chl_b = \frac{(22.90 \times A645 - 4.68 \times A663) \times v}{1000 \times w} \quad \text{.....Eq. 4}$$

$$Chl_t = Chl_a + Chl_b \quad \text{.....Eq. 5}$$

Where Chl_a=Amount of chlorophyll a; Chl_b=amount of chlorophyll b; Chl_t=Total chlorophyll;

A663= absorbance at 663nm; A645=absorbance at 645nm; W=weight of sample; v= volume of sample.



Fig. 8: UV-Vis Spectrophotometer

- **Determination of Ascorbic acid content:** 5mL of working standard solution is pipette out into a 100ml of conical flask. 10mL of 4% oxalic acid is added with it and titrated it against dye (v_1). Now the sample is extracted in 4% oxalic acid and make up to a known volume of 100mL. Pipette out 5mL of this supernatant and 10 mL of 4% oxalic acid is added and titrated against the dye (v_2). The amount of ascorbic acid content can be calculated by the following formula:

$$\text{Ascorbic acid content} = \frac{0.5\ mg}{v1\ ml} \times \frac{v2\ ml}{5\ ml} \times \frac{100\ ml}{wt.\ of\ the\ sample} \times 10\text{mg/g} \quad \text{.....Eq. 6}$$

4.3.2. Biochemical properties of biomonitor:

- **Wax content analysis**: Leaf wax content (Wax) is considered to be another important factor affecting PAH adsorption (Prajapati and Tripathi, 2008) and determines the capacity of leaf to sorb PAHs. A beaker of volume 100 mL was taken. The initial weight of beaker is taken. In the beaker 2 gm sample is extracted in 25 ml chloroform for 5min (Yang et al., 2017). Then the extract is evaporated and the final weight of the beaker is measured. The difference in the weight determined the wax content.
- **Lipid Content Analysis:** Leaf lipids act as the main reservoir or sink for the absorption of organic pollutants like PAHs. Initial weight of beaker was taken. In the beaker 2gm of samples were extracted with 10ml chloroform and 20 mL of methanol for 15min. Then 10 mL of chloroform was added and extracted for 10min and finally 10 mL of water was added and extracted for 20min. Then the extracts were collected using separating funnel. The lipid was evaporated using heating mantle and the final weight of the beaker was taken. The difference in weight determined the amount of lipid (Mukhopadhyay et al., 2021).
- **Total Soluble Sugar (TSS) Content Analysis:** TSS is typically regarded as an additional osmo-regulator which imparts stress tolerance to the plants. 1.5 g of crushed leaf sample is mixed with 10 mL of hot ethanol (80%) in a beaker. Then the sample is stirred for 5 min for thorough extraction with filter paper. The supernatant is stored in refrigerator. Then 2 mL of supernatant is mixed with 5 mL of $ZnSO_4$ and 4.7 mL of $Ba(OH)_2$ and centrifuged. Then the 0.5 mL of supernatant is added with 4ml of Anthrone reagent (extrapure AR, 98%; SRL, India). The sample is kept in boiling water for 10 min. Brought the sample in RT and absorbance is taken at 630nm (Sarabi and Arjmand-Ghajur, 2021).
- **Protein Extraction:** Protein molecules serve as the fundamental building blocks for several elements of plant development and also regulate the cellular stress responses to environmental gradients (Qazi et al., 2019). 1gm of sample is extracted in 10 mL extraction buffer and crushed to form a paste. Centrifuged the sample for 20 min. The supernatant is collected and stored in refrigerator. 0.1 mL of supernatant is added with 0.9 mL of Bradford reagent, mixed thoroughly and kept for incubation for 10 min. Then the absorbance is taken at 595 nm (Jones et al., 1988).
- **Proline Extraction:** Proline behaves as an osmoprotectant or cytoplasmic osmolyte. It improves the performance of plant in polluted atmosphere and regulates the biochemical cascades for stress recovery (Hayat et al., 2012; Raza et at., 2023). Proline content of plant leaves were determined according to the protocol proposed by Bates et al. (1973). 1g leaf

samples were added with 10 mL 3% sulphosalicyclic acid (extrapure 99%; SRL, India) and crushed well to make a paste. Then the sample were centrifuged at 2800 rpm for 10 min and the supernatants were collected thereafter in glass vials for analysis. After that in a different test tubes 2 mL of samples were mixed with 2 mL ninhydrin reagent (extrapure AR/ACS grade, 99%; SRL, India) prepared by dissolving 5mL 85% ortho-phosphoric acid (extrapure, assay: 85%; SRL, India), 15 mL deionized water and 1.25 g in a solution of glacial acetic acid (ACS reagent, ≥99.7%, Merck, Darmstadt, Germany of 30 mL and 2 mL 0f glacial acetic acid. Then the mixers were kept in boiling water for 1 h. The samples were then added with 4 mL of toluene after cooling them to room temperature. The samples were mixed vigorously, after that the organic layer was pipetted out and transferred to a glass cuvette for absorbance reading at 520 nm against blank sample with only toluene.

- **Determination of carbon, hydrogen, nitrogen and sulphur contents of plant leaves:** 4mg sample is taken in aluminium foil. At first blank run was done. Then the samples and the standards were measured for carbon, hydrogen, nitrogen and sulphur in CHNS combustion analyzer (Elementer, vario EL cube) (Fig. 9) (Cochavi et al., 2017).



Fig. 9: CHNS (Elementer)

4.4. Foliar PAHs extraction and analysis: Extraction of PAHs from homogenized plant leaves (5 g d.w.) were done with the help of Soxhlet apparatus using 100 mL of toluene (non-polar organic solvent) for 6 hours. The temperature of the extraction was kept near the boiling temperature of the solvent. The extracted solutions were purified using silica gel column chromatography. 10 g of silica gel which was previously activated at 180 ºC for 4 hours was packed in a glass column of standard dimensions. The chromatographic fractions were evaporated in heating mantle and the dried residue were dissolved in HPLC grade methanol for analysis in HPLC-UVD (Waters 2489 UV/Visible Detector) (Mukhopadhyay et al., 2021) (Fig. 10).

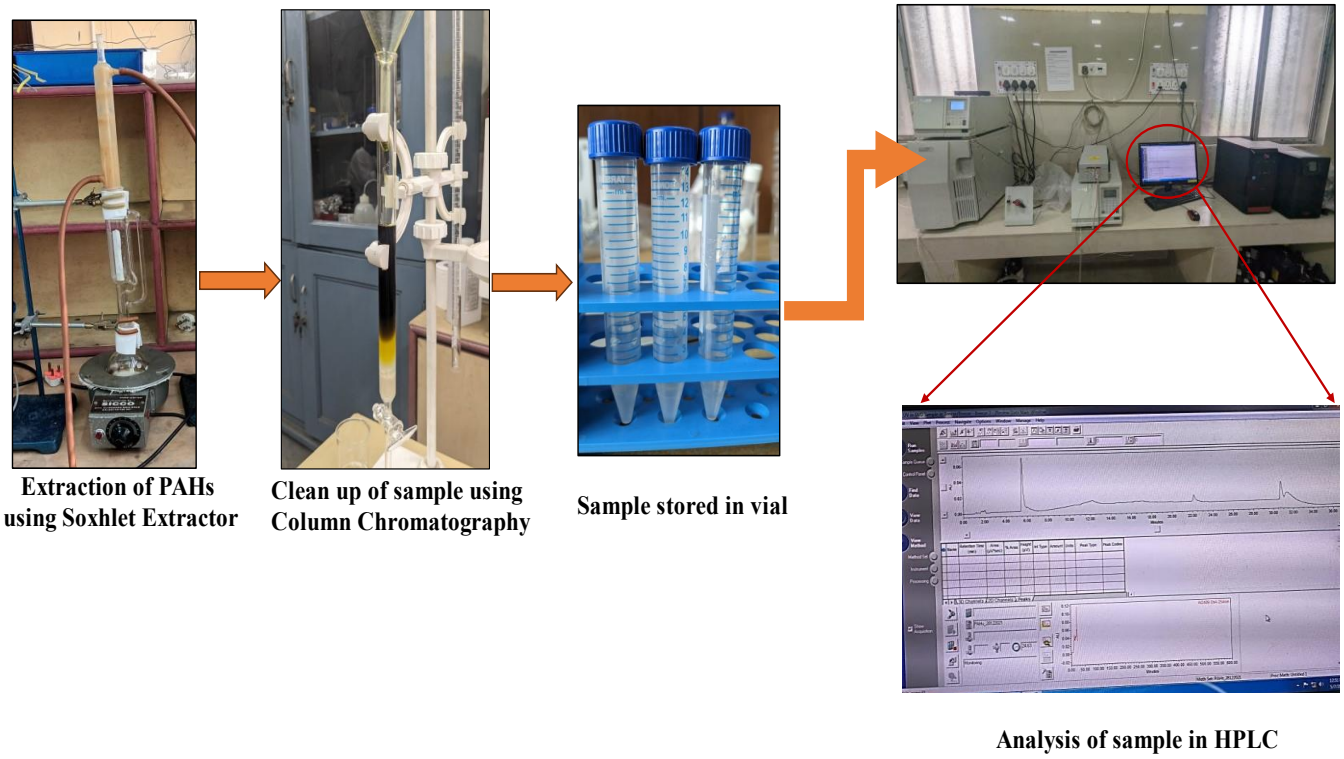


Fig. 10: Extraction and analysis of PAHs in leaf samples using HPLC

4.5. Source apportionment of PAHs using diagnostic ratios (DRs): PAHs diagnostic ratios (DRs) is an important tool for identifying and assessing the pollution emission sources. PAHs with same molar mass and physiochemical properties are involved as a pair for PAHs diagnostic ratios, as they undergo similar environmental fate processes. The PAHs diagnostic ratios that are used to calculate the PAHs sources are given in the Table 8.

Table 8.

Diagnostic ratios used with their typically reported values for processes

PAHs ratio	Value range	Source	References
∑LMW/∑HMW	<1	Pyrogenic	Zhang et al., 2008
	>1	Petrogenic	
∑COMB/∑PAHs	≈1	Combustion	Ravindra et al., 2008a
FL/(FL+PYR)	<0.5	Petrol emissions	
	>0.5	Diesel emissions	Ravindra et al., 2008b
ANT/(ANT+PHE)	<0.1	Petrogenic	Pies et al., 2008
	>0.1	Pyrogenic	
FLA/(FLA+PYR)	<0.4	Petrogenic	De La Torre-Roche et al., 2009
	0.4-0.5	Fossil fuel combustion	
	>0.5	Grass, wood, coal combustion	
BaA/(BaA+CHR)	0.2-0.35	Coal combustion	Akyuz and Cabuk, 2010
	>0.35	Vehicular emissions	
	<0.2	Petrogenic	Yunkar et al., 2002

	>0.35	Combustion	
BaP/(BaP+BeP)	≈0.5	Fresh particles	Oliveira et al., 2011
	<0.5	Photolysis (ageing of particles)	
IP/(IP+BghiP)	<0.2	Petrogenic	Yunker et al., 2002
	0.2-0.5	Petroleum combustion	
	>0.5	Grass, wood and coal combustion	
BbF/BkF	2.5-2.9	Aluminium smelter emissions	
BaP/BghiP	<0.6	Non traffic emissions	Katsoyiannis et al., 2007
	>0.6	Traffic emissions	

4.6. Risk analysis of PAHs:

- **BaP-TEQ & BaP-MEQ:** BaP_{TEQ} (carcinogenic equivalent concentration in µg g^{-1}) and BaP_{MEQ} (mutagenic equivalent concentration in µg g^{-1}) are the two factors associated with the PAHs risk assessment. They are evaluated with respect to BaP (considered as the indicator of carcinogenic PAHs). Individual BaP_{TEQ} and BaP_{MEQ} of each PAH was calculated by multiplying the measured foliar concentration of PAH compound (PAH_i) with its toxic equivalent factor (TEF) (Nisbet and LaGoy, 1992) and mutagenic equivalent factor (MEF) (Durant et al., 1996). Values of BaP_{MEQ} for BaA, CHR, BbF, BkF, BaP, D[ah]A, IP and B[ghi]P) and BaP_{TEQ} (for all 16 PAHs) were then added to calculate the mutagenic and carcinogenic potential of PAHs in the present matrix. It was calculated by the following equations. TEF_i

$$BaP_{TEQ} = \sum_{i=1}^{N} PAHi \times TEFi \quad \text{.....Eq. 7}$$

$$BaP_{MEQ} = \sum_{i=1}^{N} PAHi \times MEFi \quad \text{.....Eq. 8}$$

4.7. Foliar HMs extraction and analysis:

Extraction of HMs from homogenized plant leaves is accomplished with the help of ICP-OES. 0.5 g of leave samples were digested in 10 mL of aqua regia ($HCl:HNO_3$= 3:1) overnight (12 hours). The acid mixers were evaporated in heating mantle. The samples were cooled down to room temperature and volume make up was done by adding 50 mL of distilled water. The sample were filtered using syringe filter and stored in glass vial for analysis in ICP-OES.

The filtered samples were analysed by ICP-OES (Optima 8000, Perkinmer) for various metals. 2% of HNO_3 was used as a blank. Concentration of HM in the samples were determined from a standard curve made using a standard solution diluted at different concentration levels (Fig. 11). The samples introduced into the ICP-OES in a liquid form, is pumped into the sample introduction system, which is made up of a spray chamber and a nebulizer. The sample was dried, vaporised, atomized and ionised inside various heating zones within the plasma (a highly ionised gas). During this time, the sample is transformed from a liquid aerosol of solid particles.

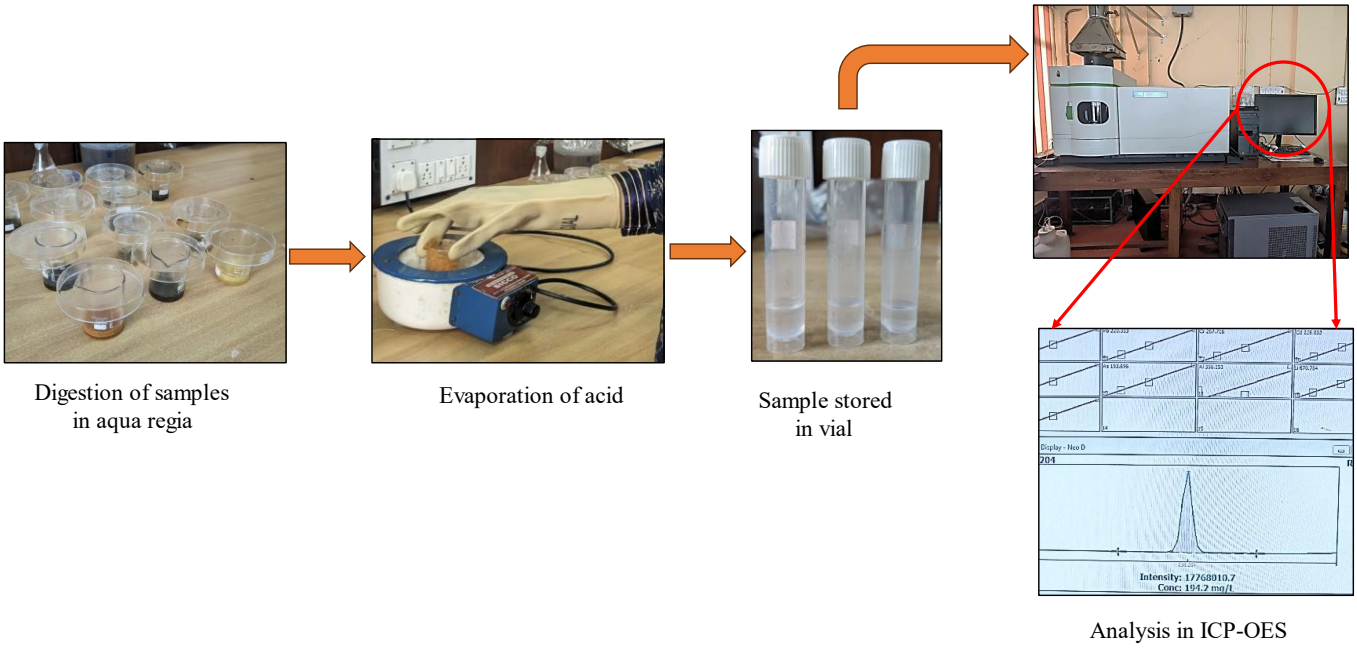


Fig. 11: Extraction and analysis of heavy metal

4.8. Estimation of metal accumulation index (MAI): Multiple heavy metals frequently contaminate the environment, and their interactions have distinct harmful impacts on ecosystems (Wang and Arne, 2003). Plant leaves can accumulate different heavy metals simultaneously, so to evaluate the performance of the plants in terms of metal accumulation, metal accumulation index (MAI) was calculated using the following formula.

$$MAI = (\frac{1}{N}) \times \sum_{j=1}^{N} Ij \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Eq. 9}$$

Where, N=Total number of heavy metals studied, Ij=Sub index of j gained by dividing the metal concentration by its standard deviation (Hu et al., 2014; Nadgorska-Socha et al., 2017).

4.9. Estimation of capturing rate (CR) of heavy metals: Capturing rate was used to separate the contribution of the adsorbed and captured heavy metals. CR was calculated using the following formula:

$$\text{CR } (\%) = \frac{[X]unwashed\ leaf - [X]washed\ leaf}{[X]unwashed\ leeaf} \times 100 \ldots\ldots\ldots\ldots\ldots \text{Eq. 10}$$

Where [X]unwashed leaf is the concentration of metal in the unwashed leaves and [X] washed leaf is the concentration of metals in the washed leaves (Liang et al., 2017).

5. Results and Discussion:

5.1. Sample selection based on APTI and Biochemical analysis of leaves:

5.1.1. Air Pollution Tolerance Index (APTI):

Studies on the establishment of green belts (Shannigrahi et al., 2004), reduction of traffic noise (Pathak et al., 2011), and reduction of pollutants near highways and industrial areas have all employed APTI. Considering the parameters of APTI, one of the typical consequences of air pollution that inhibits photosynthesis is the progressive loss of chlorophyll and yellowing of leaves. When it comes to photosynthesis, ascorbic acid is a crucial electron donor (Vanoy, 2014). The pH is a key factor in photosynthetic activity (Bergman et al., 2012). These biological factors relate to one another and are especially sensitive to air pollution. According to APTI values, low APTI plant species can be utilised as bio-indicators in low pollution regions, whereas high APTI plant species can be employed to reduce air pollution in highly contaminated areas (Molnar et al., 2020; Kanwar et al., 2016). The parameters of APTI for the two selected species have been represented in Table 9.

Table 9.

APTI values of *B. spectabilis* and *M. indica*

Parameter	*B. spectabilis*	*M. indica*
RWC (%)	85.92±0.05	92.61±0.27
pH	6.91±0.64	6.81±0.57
Ascorbic acid (mg/g)	10.22±0.55	7±0.70
Chlorophyll	3.044±0.03	0.522±0.33
APTI	18.6±0.07	14.39±0.36

RWC: Abiotic stress responses in plants are connected with leaf RWC (water status in plants), which aids in controlling physiological activities in plants, specifically growth and metabolism (Mukhopadhyay et al., 2021). A high pollution load makes protoplasm more permeable, which causes a loss of water and dissolved nutrients from plant cells and, eventually, an early onset of leaf senescence (Agarwal and Tiwari, 1997). Under stressful circumstances, particularly when plants are exposed to high quantities of airborne pollutants, the high RWC in plants governs the physiological activities of plants. Additionally, because of increased RWC, the acidity of plant leaf cell sap is lowered,

increasing the plants' resistance to pollution and assisting them in coping with drought conditions (Gupta et al., 2016; Rai 2016; Kaur and Nagpal, 2017; Sen et al., 2017; Mina et al., 2018).

It is seen that *Bougainvillea spectabilis* and *Magnifera indica* showed RWC (in %) values of 85.92 and 92.61 respectively (Table 9). RWC is found to be in the range of 60–70% depending on the plant species during the early stages of wilting (drooping, folding, and rolling of leaves due to loss of water from plant cells). Transpiring leaves have RWC of approximately 98% while in a state of full turgidity, and it decreases to about 30–40% in plant leaves during conditions of dehydration or at the stage of dying (Arndt et al., 2015). The result from current study showed that RWC of *M. indica* is higher than *B. spectabilis*. Because plants react differently to various contaminants, there may be variations in the RWC of the analysed plants. Achakzai et al. (2017) showed that the value of the RWC ranges from 43% - 90% for different plant species growing around brick kilns in Pakistan.

Leaf extract pH: Through detoxifying mechanisms evolved in the plant under contaminated circumstances, alkalinity influences the tolerance threshold of different plant species. The pH of the leaf tissue has an impact on several other processes as well, including the stomatal activity of plant leaves, correct operation of various enzymes, photosynthetic efficiency, and cell growth (Jai and Davies, 2007). Due to excessive levels of SO_X and NO_X in the surrounding air, the pH of plant leaves falls or turns acidic in a polluted environment.

In this study the value of leaf extract pH of *B. spectabilis* and *M. indica* is 6.91 and 6.81 respectively (Table 9) and found to be alkaline range. The study's findings are in line with those of Karmakar and Padhy (2019), who used tropical dry deciduous forest trees for urban greening (control site: 4.67± 0.07-5.72± 0.08 and polluted site: 4.49± 0.11-5.58 ±0.16) and Pandey et al. (2016), who used climber trees or vines for developing strategies of vertical gardens in Varanasi, India (rural site: 6.12–7.85 and urban site: 5.80–6.53). Therefore, plants with alkaline leaf extract pH are considered to be more tolerant and successfully combat air pollution, which is also obvious from the current study.

The current study showed that the pH value ranges from 6.81 to 6.91. Higher leaf extract pH helped plants tolerate air pollution by accelerating the pace at which hexose sugar gets converted into ascorbic acid (Escobedo et al. 2008; Joshi and Bora 2011). According to the

current study, the pH variance might be a result of differences in how plants react to exposure to the same contaminants.

Total chlorophyll: The total chlorophyll content for *B. spectabilis* and *M. indica* was found to be 3.044 mg/g and 0.522 mg/g respectively (Table 9). The current study found considerably lower total chlorophyll concentration in *M. indica* as compared to *B. spectabilis*. The difference in chlorophyll concentration is due to the variation in pollution load. Greater accumulations of particulate matter, such as dust and soot, clog the stomata holes and disrupt the plant's gas cycle, which is necessary for the mechanism of photosynthesis (Leghari and Zaidi, 2013).

Total chlorophyll is regarded as a measure of photosynthetic activity, growth, and biomass productivity (Joshi and Swami, 2009; Pavlovi et al., 2014). The degradation of photosynthetic pigments has been frequently utilised as a marker of air pollution, and a drop in chlorophyll concentration indicates that plants are more vulnerable to air pollution (Woo and Je, 2006). When under stress, chlorophyll pigments, which are highly organised, can go through a number of photochemical processes, including oxidation, reduction, pheophytinization, and reversible bleaching.

Pandey et al. (2016) examined 24 species of climbers in Varanasi, Uttar Pradesh, India, and found that the chlorophyll content was lower at polluted urban sites (2.98 0.03-11.82 0.84 mg g^{-1}), whereas it was higher (3.89 0.87-11.58 0.92 mg g^{-1}) at the control site. As a result, it may be inferred that decreasing plant chlorophyll content is a sign of severe air pollution, but high chlorophyll content in spite of pollution is a sign of a plant's resistance to harmful chemicals (Ogunkunle et al., 2015).

Ascorbic acid: Ascorbic acid is an antioxidant that provides resistance to plants in a stressed condition. Ascorbic acid level also rises with an increase in pollution to help combat the stressed state (Bharti et al., 2018). It acts as a powerful reductant, electron donor, scavenges free oxygen radicals, facilitates the reduction of sulphite to hydrogen sulphide and reduces the toxicity of SO_2 (Varshney and Varshney, 1984). In addition, it regulates plant development and growth as well as enzyme activity, photosynthesis, transpiration, redox signalling pathways in chloroplasts, the integrity and stability of plasma membranes for cell survival, RWC in plants, and leaf senescence or ageing (Akram et al., 2017).

From the study, we found that the ascorbic acid content in *B. spectabilis* is 10.22 mg/g and that of *M. indica* is 7 mg/g (Table 9). Bharti et al. (2018) studied 25 plant species of an industrial area of Central Lucknow, India, and found ascorbic acid contents of 4.20 mg/g and 9.70 mg/g for *B. spectabilis* and *M. indica* respectively. The authors found lower contents of ascorbic acid for many plant species like *C. procera* (5 mg/g), *D. lablab* (2.50 mg/g), *C. citrinus* (2.70 mg/g), *H. rosa-sinensis* (4.20 mg/g), etc. The ascorbic acid contents in different plant species were found to be in the range of 0.8 – 19 (mg/g) (Bharti et al., 2018). Mukhopadhyay et al. (2021) examined *Murraya paniculata* in different sites of Kolkata, India and found ascorbic acid content of the plant in the range of 18.18 ± 1.34–27.27 ± 1.20 mg/g in highly contaminated zones. Significant breakdown of chlorophyll at the polluted sites is a definite marker of plant leaf senescence under abiotic stressors which is effectively combatted and controlled by increased level of ascorbic acid content in the foliar tissues to help them thrive in the polluted environment. The above study has also been validated by the findings of Thawale et al. (2011), Gupta et al. (2016), Pandey et al. (2016), Bharti et al. (2018), Karmakar and Padhy (2019) and Roy et al. (2020).

High amount of ascorbic acid favours the tolerance of plants. In the present study, it is observed that the ascorbic acid content in *B. spectabilis* is more than *M. indica* (Table 9). It may be because different plants have different levels of tolerance; for example, more ascorbic acid is present in tolerant plants than in sensitive plants, which may explain the variation in sensitivity.

APTI: The study revealed that the APTI of *B. spectabilis* and *M. indica* was found to be 18.6 and 14.39 respectively (Table 9). The APTI has been used by several studies to distinguish plant species into three categories: sensitive, intermediate, and tolerant (Ogunkunle et al., 2015). Plants with APTI value ≤11 are considered to be sensitive, while those with APTI value ranging from 12 to 16 are classified as intermediate, and APTI value of ≥17 are known to be tolerant (Singh et al., 1991). Since the APTI value that we found for *B. spectabilis* is >17, it can be used as a biomonitor (i.e. air pollution tolerant species) and the APTI value we found for *M. indica* is 14.39, which indicates that the species is intermediate to air pollution tolerance, it can be used as a bioindicator.

5.1.2. Biochemical analysis of leaves:

The biochemical parameters are significantly negatively impacted by ambient air pollution, which further inhibits the general growth and development of plants. For many

years, research has been conducted on the effects of different air contaminants on physiology and biochemistry of plants (Agrawal and Agrawal, 1989; Rai, 2011a, b; Rai et al., 2013; Rai and Panda, 2014; Rai and Singh, 2015; Rai, 2016a, b). Short-term physiological reactions as well as long-term physiological, structural and morphological changes are all part for adaptation of plant to shifting environmental conditions. These alterations assist plants in reducing stress and making the best use of both internal and external resources (Dineva, 2004). The morphological, physiological, biochemical and genetic condition of plants all have been significantly impacted by the affected ambient environment brought about by PAHs and HMs pollution in metropolitan areas (Farooq et al., 2000a; Seyyednejad et al., 2011; Prajapati, 2012a, b; Younis et al., 2013; Rai et al., 2013; Rai and Panda, 2014; Rai and Singh, 2015; Rai, 2016a, b).

Table 10.

Biochemical analysis of plant leaves

Parameter	***B. spectabilis***	***M. indica***
Wax (mg/gm)	22±0.70	28.5±0.35
Lipid (mg/gm)	14.5±0.35	54±0.70
Sugar (mg/gm)	0.728±0.19	1.136±0.61
Protein (mg/gm)	5.098±0.06	4.509±0.35
Proline (µg/gm)	1790±7.07	0.3975±0.42

Wax content analysis: The physicochemical characteristics of various plant leaves are different as a result their capacity to adsorb polycyclic aromatic hydrocarbons also differ. By absorbing particulate particles onto their leaf surfaces, plants significantly contribute to ambient air filtration. Through molecular diffusion, PAHs can enter the mesophyll directly. To a large extent, the 1-octanol/water partition coefficient (K_{ow}) determines how well plant leaves adsorb particulate PAHs. The PAH wax/water partition coefficient varies from 104.1 to 1076, which is higher than or equal to K_{ow}, demonstrating the waxy layer's strong capacity to adsorb PAHs and encourage PAH absorption. But most organic contaminants, including

PAHs, may be retained by leaf wax, keeping them from penetrating the leaves and interfering with plant development (Lietal.,2010; Wildetal.,2010). In our study the wax content in *B. spectabilis* was found to be 22 mg/g and in *M. indica* was 28.5 mg/g, which is higher (Table 10). Also, it was observed that the highest PAHs accumulation was found in *M. indica* (Table 11). It could be concluded that the PAHs capturing capacity depends on the concentration of wax since PAHs are lipophilic in nature.

Lipid content analysis: Plant leaves have lipid-rich tissues that PAHs may penetrate into due to their lipophilic properties (Mukhopadhyay et al., 2021). In response to abiotic stressors, leaf lipids first provide signals about the onset of the stress and then mitigate cellular damage by remodelling the lipid membrane and restoring the membrane's stability, fluidity, permeability, and integrity (Balogh et al., 2013; Moradi et al., 2017). Higher plants may undergo changes in leaf lipid composition and content according to their physiology and different environmental, meteorological and anthropogenic factors (Kuiper, 1985). In the above study the lipid content was found to be 14.5 mg/g and 54 mg/g for *B. spectabilis* and *M. indica* (Table 10) respectively. It was also observed that the bioaccumulation of PAHs in *M. indica* is higher than *B. spectabilis.* Hence, we can conclude that the lipid content might be a controlling factor for uptake mechanism and diffusion rate of organic pollutants in plant leaves (Li et al., 2017).

Total soluble sugar (TSS) analysis: Total soluble sugar increases plant cell turgor and stability while simultaneously triggering the defence mechanisms against ROS, safeguarding cellular constituents and regulating signalling pathways during stress (Rai 2016). The weakened chlorophyll molecule and inadequate pollution sequestration and translocation capabilities of the sensitive plants result in reduced TSS levels (Banerjee et al., 2021). The total soluble sugar was found 0.728±0.19 mg/gm for *B. spectabilis* and 1.136±0.61mg/gm for *M. indica* (Table 10). This lower level of total soluble sugar indicates that the species undergo a stress condition. Also, sensitive plant showed lower total soluble sugar level due to deformed chlorophyll molecule and poor performance in pollutants sequestration (Banerjee et al., 2022).

Protein content analysis: Protein molecules not only serve as the fundamental building blocks for several elements of plant development, including expansive expansion, structural growth with biomass change, cell formation and proliferation, etc., but they also regulate the cellular stress responses 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 in that species (Rai 2016). In our study, the protein content was found to be higher in *B. spectabilis* i.e. 5.098 mg/gm than *M. indica* i.e. 4.509 mg/gm (Table 10). The protein concentration of *M. indica* showed a decline due to the presence of various air pollutants and this decrease is higher in the sensitive plants. Similar trend was found by Mukhopadhyay et al (2024).

Proline content analysis: Proline is responsible for osmotic regulation, maintaining cell membrane integrity, clearing ROS, stabilizing proteins and enzymes in plants (Ashraf and Foolad, 2007; Kavi Kishor and Sreenivasulu, 2014). In the study, it has been seen that the concentration of proline in *B. spectabilis* is 1790 µg/gm and that of *M. indica* is 0.3975 µg/gm (Table 10). Proline helps to resist stress in plant in polluted environment (Mukhopadhyay et al., 2024). In this experiment the PAHs accumulation is high in *M. indica* but proline content is lower and in *B. spectabilis,* the PAHs accumulation is lower (Table 11) but proline content is high. From the results, it is inferred that proline content decreased with increase in pollution load in that area.

5.1.3. Elemental C-H-N-S-O analysis:

Carbon (C) analysis:

Carbon plays a vital role in plant growth and development, avoids stress, reduces damage and most importantly in photosynthesis, it is one of the most versatile and abundant elements of plant metabolism (Hartman and Trumbore, 2016). The uptake of CO_2 by plants reduces due to pollution-induced stomatal blockage, which hampers photosynthesis (Saxena and Kulshrestha, 2016) and hence causes the plant to reallocate its requirement for C for combatting stress (Kobe et al., 2010). Because of this, the C content of plant leaves serves as a reliable biomarker of the health of the plant against contamination (Mukhopadhyay et al., 2024). The leaf C content of the studied plant species is shown in Fig. 12. The C content of different evergreen and deciduous species varies from 43.64–52.43%, suggesting higher carbon allocation and synthesis of carbon compounds in leaf tissues for resistance to stress (Mukhopadhyay et al., 2024). In the present work, the C content was found to be higher in *B. spectabilis* (55.45%, Fig. 12) than *M. indica* (36.37%, Fig. 12). Therefore, it can be said that the C content > 35% might indicate their differential survival capacity against abiotic stress.

Hydrogen (H) analysis:

Physiological processes including seed germination, seedling growth, adventitious rooting, root elongation and post-harvesting are regulated by H in plants (Xu et al., 2013, Dai et al., 2017, Wu et al., 2015, Zhang et al., 2015). H also enhances the resistance of plant to environmental stimuli (Dai et al., 2017, Zhang et al., 2015). In our present study, the H content was found to be 4.95% (Fig. 12) for *B. spectabilis* and 9.42% (Fig. 12) for *M. indica,* which is in the normal range of H content of plant leaves, i.e. 6% (Pettersen, 1984; Bert and Danjon, 2006) and helped them thrive in the polluted environment.

Nitrogen (N) analysis:

Nitrogen helps in plant physiological metabolism and growth. N is also the primary constituent of chlorophyll and other compounds. Rate of photosynthesis of plants decreases when there is a deficiency of N, resulting in the reduction of yield and quality of photosynthetic product (specially protein) as well as yellowing of leaves (Zhang et al., 2023). The average N content of plant leaves ranges from approximately 1-5% as stated by Gislum and Griffith (2004) and Muratore el al. (2021). In our study, the N content was found as 5.36% and 1.25% for *B. spectabilis* and *M. indica* respectively (Fig. 12). High N content in *B. spectabilis* imparts high tolerance to the plant by maintaining the structure and function of chlorophyll molecules (as can be confirmed from the high values of chlorophyll content (Table 9) as compared to *M. indica*.

Sulphur (S) analysis:

S is an essential component of proteinaceous amino acids such as methionine and cysteine, glutathione, vitamins (biotin and thiamine), chlorophyll, coenzyme A, and S-adenosyl-methionine (Li et al., 2020; Nakai et al., 2020; Scherer et al., 2008). S helps plants to combat against different stress, promote biological nitrogen fixation and increase NPK uptake nutrients (Om et al., 2023). In this study the concentration of S found to be 0.9% for *B. spectabilis* and 0.16% for *M. indica* (Fig. 12). Linzon et al (2012) analysed 34 plant species and found the S content in the range of 0.13-1.72%, which support our present study.

C/N and C/H ratio analysis:

The presence of N and C are important parameters in the regulation of plant development and metabolism (Coruzzi and Zhou 2001). The C/N balance, or the ratio of C metabolites to N metabolites in the cell, is crucial for controlling plant growth and development in addition to the individual significance of C and N (Coruzzi and Zhou 2001; Aoyama et al., 2014; Martin

et al., 2002). C/N ratio also helps to regulate leaf senescence under normal conditions. High C and low N availability, a common C/N imbalance can cause leaf senescence or aging (Aoyama et al., 2014; Wingler et al., 2006). In our study, it has been seen that the C/N ratio of *B. spectabilis* is 10.34 and that of *M. indica* is 29.16 (Fig. 12) i.e., the ratio is higher in *M. indica*. It has been also observed that the sugar content is higher in *M. indica* (Table 10) and the N content is lower (Fig. 12). From these results, it can be interpreted that there is a C/N imbalance in *M. indica* (Daoqian et al., 2015) which indicates high pollution load in that area imparting stress to the plant species.

Estimating the quantity of hydrogen in the photosynthetic product in plants is mostly dependent on the carbon to hydrogen ratio (C/H ratio) (Satoh et al., 20222). The C/H ratio is found to be higher in *M. indica* (7.34) than *B. spectabilis* (5.88) (Fig. 12).

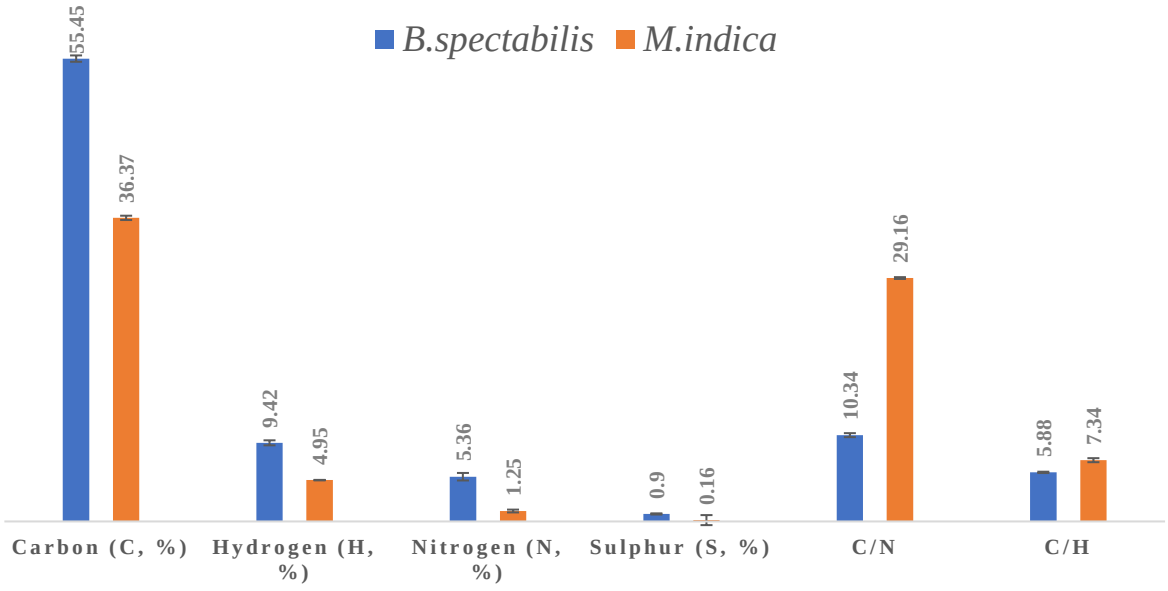


Fig. 12: Elemental characterization of plant leaves with respect to C, H, N, S, C/N and C/H.

5.2. Foliar PAHs determination:

PAHs are released in the atmosphere by incomplete combustion of fossil fuels or biomass (Wild and Jones, 1995). Because of their semi volatile nature, they can travel great distances through atmosphere by splitting into the particulate phase and gaseous phase, based on their unique physiochemical properties (Desalme et al., 2013). PAHs can be accumulated by plant cells or in intercellular spaces due to their lipophilic nature (Lankin et al., 2014). After being

captured and held by plant leaves, PAHs can either be adsorbed onto the cuticle surface or absorbed and translocated into interior tissues after getting diffused through the stomatal openings (Desalme et al., 2013).

Table 11.

Concentration of PAHs in the plant leaves of *B. spectabilis* and *M. indica* in polluted and control sites

PAHs	Concentration			
	B. spectabilis		***M. indica***	
	Polluted site	**Control site**	**Polluted site**	**Control site**
NAPH	39.46±0.38	6.4±0.28	32.5±0.35	1±0
ACY	6.46±0.32	5.8±0.14	9.48±0.36	5.8±0.14
ACE	0.26±0.52	4.2±0.14	5.22±0.15	5±0.70
FLU	12.08±0.05	2.6±0.28	32.64±0.24	2.2±0.84
PHEN	21.18±0.57	4±0.70	37.34±0.24	-
ANT	0.04±0.67	1±0.70	7.62±0.26	2±0.70
FLT	0.68±0.22	-	23.14±0.09	0.20±0.56
PYR	1.22±0.55	4.8±0.56	23.02±0.01	5.4±0.28
BaA	16.02±0.01	8.2±0.56	27.48±0.36	-
CHR	0.14±0.60	-	16.84±0.59	6.2±0.14
BbF	2.26±0.18	7.4±0.28	33.2±0.56	-
BkF	10.2±0.56	-	20.62±0.43	-
BaP	18.04±0.02	-	36.34±0.46	-
DahA	33.14±0.60	-	13.10±0.07	-
BghiP	34.98±0.69	-	28.76±0.16	-
IP	2.66±0.24	4.2±0.56	11.56±0.39	9.6±0.28
Total	**198.82±3.66**	**48.6±1.69**	**358.86±0.09**	**37.4±0.28**

In the study, concentrations of 16 priority USEPA PAHs are measured. In the polluted site, the concentration of PAHs for *B. spectabilis* is ranked as NAPH > BghiP > DahA > PHEN > BaP > BaA > FLU > BkF > ACY > IP > BbF > PYR > FLT > ACE > ANT (Table 11) and for *M. indica* the concentration of PAHs ranked as PHEN > BaP > BbF > FLU > NAPH > BghiP > BaA > FLT > PYR > BkF > CHR > DahA > IP > ACY > ANT > ACE (Table 11). The total PAHs concentration is higher in *M. indica* (358.86 μg g^{-1}) than *B. spectabilis* (198.92 μg g^{-1}).

The contributions of LMW PAHs and HMW PAHs for both the species *B. spectabilis* (40% and 60% respectively) and *M. indica* (35% and 65% respectively) are nearly same. This indicates that the HMW PAHs are captured significantly by the plant species. From the above result, it can be concluded that the PAHs capturing capacity of *M. indica* is higher than that of *B. spectabilis*. For *B. spectabilis*, major PAHs contributors are NAPH, BghiP, DahA and PHEN, indicating diesel combustion, coal burning, gasoline combustion, cooking oil combustion and fumes and garbage burning as the major sources of PAHs in the air. Whereas, *in M. indica*, PHEN, BaP, BbF, FLU are the major PAHs contributors. This suggests that wood fire, vehicle exhaust, combustion of organic materials, combustion of fossil fuels, cigarette smoke and cooking soot are the sources of PAHs in the ambient air of that area. On the other hand, concerning the air quality of the control site, foliar PAHs concentrations (48.6 μg g^{-1} for *B. spectabilis* and 37.4 μg g^{-1} for *M. indica*) were observed to be much less as compared to the urban location and also, most of the HMW PAHs were not detected, which may be due to the differential pattern of exposure to air pollution of a semi-urban site, socioeconomic differences and variations in the atmospheric concentrations of PAHs. Based on the above facts, it can be understood that the PAHs pollution load in the urban area of South Kolkata region is very high than that of the semi-urban control area. Again, foliar accumulation of carcinogenic PAHs confirmed the biomonitoring potential of the two species and hence, both the species can be employed for reducing ambient PAHs load in polluted urban sites.

5.2.1. Source Apportionment using PAHs diagnostic ratio:

The PAHs diagnostic ratio is one of the most widely used and simple methods for determining the origins of PAHs. This method is frequently applied to qualitative assessment of samples and/or variations among sampling locations (Konstantinova et al., 2019). The Diesel and petrol combustion emission differences have been identified using PAH diagnostic ratios (Ravindra et al., 2008a), various byproducts of refining crude oil and methods of burning biomass, such as grass, savanna, and bush fires (Yunker et al., 2002). Natural Phenomenon such as biomass burning, volcanic eruption can be the reasons of PAHs in the environment (Wang et al., 2007). However, the anthropogenic activities like coal and wood burning, petrol and diesel oil combustion and industrial processes can be the reasons of PAHs in heavily urbanized or industrialized regions (Mostert et al., 2010). A number of diagnostic ratios are employed to evaluate the precise sources of certain PAH, which have been proposed as indicators or tracers of processes that release PAH into the atmosphere (Oliveira et al., 2011). Due to the variety of substrates, routes, and circumstances involved in PAH production,

various sources of PAHs produce distinct molecular distribution patterns (2, 5, 7) (Yan et al., 2005). The ∑LMW/∑HMW, ∑COMB/∑PAHS, FLU/(FLU+PYR), ANT/(ANT+PHE), FLT/(FLT+PYR), BaA/(BaA+CHR), IcdP/(IcdP+BghiP), BbF/BkF, BaP/BghiP are used in this study (Table 12). These ratios helped to recognize the origin of PAHs in the sampling site. The ∑LMW/∑HMW ratio used to locate the petrogenic or pyrogenic sources of PAHs. If the ratio is >1 this indicates the source is petrogenic, <1 indicates the source is pyrogenic (Zhang et al., 2008). The ANT/(ANT+ PHE) ratio is typically used to locate sources of oil and combustion. The efficacy of petroleum sources is indicated if this ratio is less than 0.1, and if it is greater than 0.1, the combustion sources are dominating (Yunker et al., 2002).

Table 12.

Source apportionment using PAHs diagnostic ratio for *B. spectabilis* and *M. indica*.

PAHs Ratio	*B. spectabilis*			*M. indica*		
	Diagnostic ratio value	**Standard Range**	**Source**	**Diagnostic ratio value**	**Standard Range**	**Source**
∑LMW/∑HMW	O.67±0.00	<1	Pyrogenic	0.53±0	<1	Pyrogenic
∑COMB/∑PAHS	0.34±0.06	<1	-	0.61±0	<1	-
FLU/(FLU+PYR)	0.98±0.09	>0.5	Diesel emission	0.59±0	>0.5	Diesel emission
ANT/(ANT+PHE)	0.001±0.03	<0.1	Petrogenic	0.17±0.007	>0.1	Pyrogenic
FLT/(FLT+PYR)	0.36±0.02	<0.4	Petrogenic	0.5±0.00	>0.4	Fossil fuel combustion
BaA/(BaA+CHR)	0.99±0.04	>0.35	Vehicular emission	0.6±0.02	>0.35	Vehicular emission
IcdP/(IcdP+BghiP)	0.07±0.007	<0.2	Petrogenic	0.29±0.007	0.2-0.5	Petroleum combustion
BbF/BkF	0.22±0.03	-		1.6±0.007	-	-

BaP/BghiP	0.51±0.01	<0.6	Non traffic emission	1.26±0.01	>0.6	Traffic emissions

The obtained ∑LMW/∑HMW ratio is 0.67 for *B. spectabilis* and 0.69 for *M. indica* (both <1) (Table 12), this conclude that pyrogenic activities are the main reasons of PAHs. FLU/(FLU+PYR) was found to be greater than 0.5 for both the species (Table 12), which concluded that the diesel emission could be the sources of the corresponding PAHs. Again, we found that ANT/(ANT+PHE) was 0.001 (<0.1) for *B. spectabilis* and 0.17 (>0.1) for *M. indica* (Table 12). This indicating that the petrogenic sources might be the reasons for the corresponding PAHs in the air for *B. spectabilis* and pyrogenic sources might be the reasons for the corresponding PAHs in the air for *M. indica*. Major sources of PAHs in ambient air include residential and commercial heating with wood, coal or other biomasses (oil and gas heating produce much lower quantities of PAH), other indoor sources such as cooking and tobacco smoke, and outdoor sources like motor-vehicle exhaust (especially from diesel engines), industrial emissions and forest fires. Benz[a]anthracene is a product of incomplete combustion of oil, plant, or animal matter and is a carcinogen. Benz(a)anthracene is primarily found in gasoline and diesel exhaust, tobacco and cigarette smoke, coal tar and coal tar pitch, coal combustion emissions. Dibenzo (a, h) anthracene is more likely to be found in coal tar, coal tar pitch, creosote, incomplete combustion of fuels in engines, during coal coking.

5.2.2. BaP equivalent toxicity:

The toxicity of the PAHs was studied in this study in context of BaP equivalent concentration (Bap-TEQ). ΣBaP-TEQ_{16} (sum total of carcinogenic or toxic equivalent concentrations of 16 PAHs) were found to be 54.72 µg/gm for *B. spectabilis* and 59.42 µg/gm for *M. indica* (Table 13) and For *B. spectabilis* the major toxicity contributors are DahA, BaP, BkF, BaA (Fig. 13a) and for *M. indica* are BaP, BbF, BaA (Fig. 13b). ΣBaP-TEQ_{car} (sum total of 7 carcinogenic PAHs having 4- ,5-, 6- rings) were found to be 54.3 µg/gm for *B. spectabilis* (Fig. 14a) and 58.9 µg/gm for *M. indica* (Fig 14b). The major contribution (94-99%) of the seven carcinogenic PAHs having 4-6 rings was found with the dominance of BaA, BbF, BkF, BaP, DahA and IP for both the species. Vehicular sources i.e. emission of petrol, diesel, kerosene oil, natural gas might be the main sources of carcinogenic PAHs in the areas. Similar result was found in the study conducted by Mukhopadhyay et al. (2024) where the dominance of 4-

6 rings PAHs were found. Among 16 PAHs the 7 carcinogenic PAHs were contributed about 94-99% for the species. These results suggest that the assessment and management of HMW PAHs must be given top priority in order to preserve a healthy and liveable environment and lower potential health hazards. Numerous studies have actively evaluated the possible dangers of PAHs for hazard assessments owing to exposure (Blaszczyk et al., 2017; Fred-Ahmadu and Benson, 2019; Ihunwo et al., 2021; Li et al., 2014) which is in line with the findings of the present investigations.

Table 13.

BaP equivalent toxicity for *B. spectabilis* and *M. indica*.

PAHs	TEF	*B. spectabilis*		*M. indica*	
		BaP_{TEQ} (µg/gm)	**%Contribution**	**BaP_{TEQ} (µg/gm)**	**%Contribution**
NAPH	0.001	0.039±0.0007	0.072	0.032±0.002	0.054
ACY	0.001	0.006±0.0007	0.0123	0.009±0.0007	0.0105
ACE	0.001	0.0003±0.0001	0.0005	0.005±0.001	0.008
FLU	0.001	0.012±0.001	0.038	0.032±0.006	0.054
PHEN	0.001	0.021±0.0007	0.038	0.037±0.005	0.062
ANT	0.01	0.0004±0.003	0.0008	0.076±0.003	0.001
FLT	0.001	0.0007±0.0001	0.001	0.023±0.002	0.038
PYR	0.001	0.001±0.003	0.002	0.023±0.005	0.038
BaA	0.1	1.602±0.07	2.94	2.748±0.18	4.624
CHR	0.01	0.001±0.006	0.002	0.168±0.05	0.282
BbF	0.1	0.226±0.02	0.414	3.32±0.13	5.587
BkF	0.1	1.02±0.05	1.872	2.06±0.04	3.467
BaP	1	18.04±0.03	33.124	36.34±0.95	61.15
DahA	1	33.14±0.6	60.85	13.10±0.63	22.04
BghiP	0.01	0.349±0.03	0.642	0.287±0.01	0.484
IP	0.1	0.266±0.02	0.488	1.156±0.11	1.945
$\sum$BaP-TEQ_{16}		**54.72±0.61**		**59.42±0.11**	

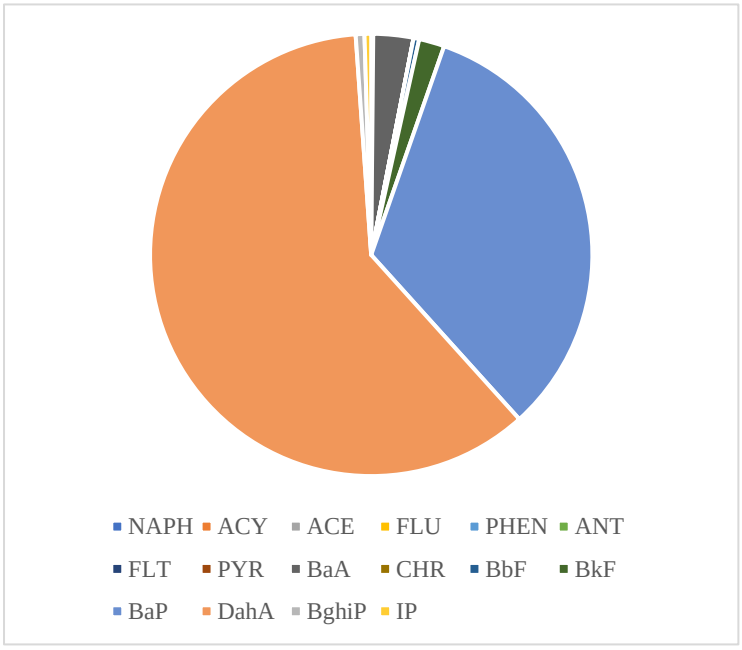


Fig. 13a: BaP_{TEQ} concentration for *B. spectabilis*

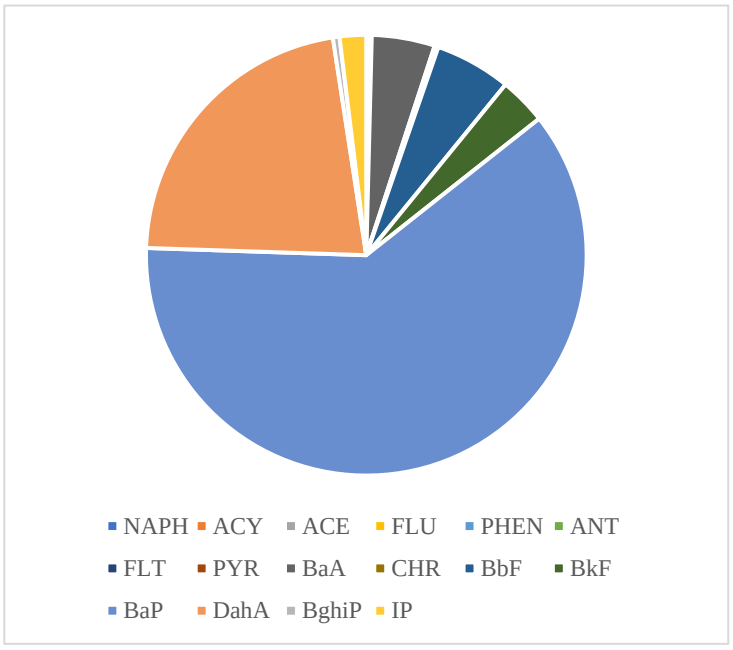


Fig.13b:BaP_{TEQ} concentration for*M.indica*

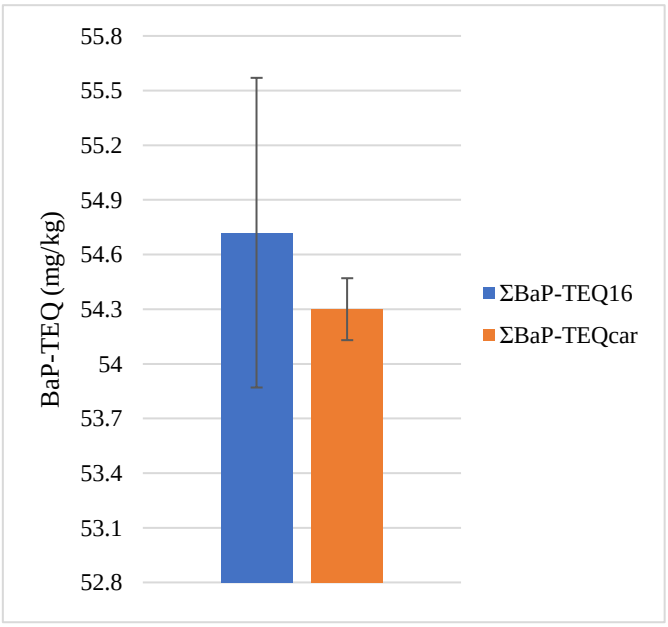


Fig. 14a: Concentration of 7 carcinogenic PAHs of *B. spectabilis*

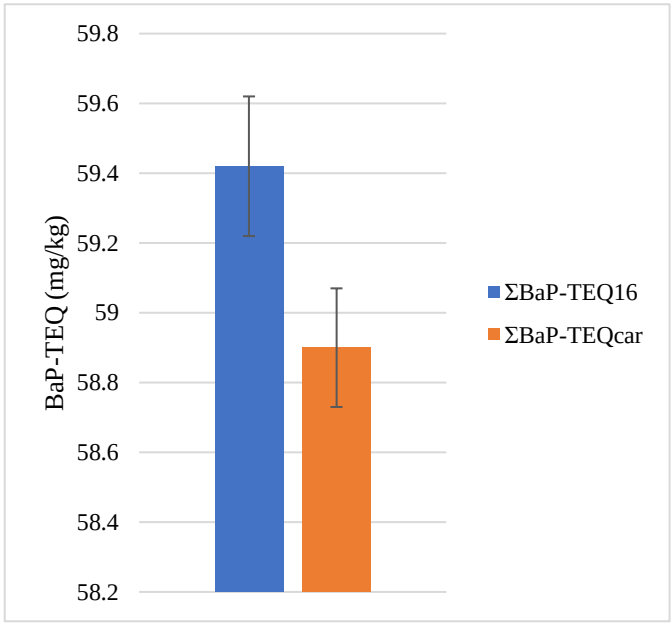


Fig. 14b: Concentration of 7 carcinogenic PAHs for *M. indica*.

5.2.3. Mutagenic equivalent concentration (MEQ):

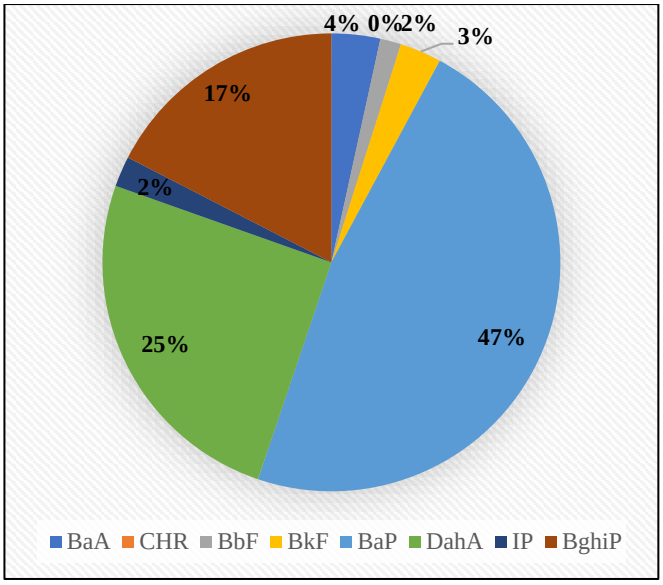
The risk assessment of PAHs in terms of mutagenicity is calculated with respect to BaP equivalent toxicity (BaP_{MEQ}). The concentration is calculated for eight mutagenic PAHs are shown in Table 14 for both the species. The rank of concentration for *B. spectabilis* is BaP > DahA > BghiP > BaA > BkF > IP > BbF > CHR and for *M. indica* is BaP > BbF > BghiP >

DahA > IP > BkF > BaA > CHR (Table 14). In the study, it has been seen that the major BaP_{MEQ} contributors are BaP, DahA, BghiP and BaA for *B. spectabilis* and BaP, BbF, BghiP, DahA and IP respectively. The highest mutagenic threats posed by 5-6 rings PAHs accounting for 96-97% in both the species. The total BaP_{MEQ} concentration in *M. indica* is higher (62.27 µg/gm) than the *B. spectabilis* (38.26 µg/gm). This indicate that the mutagenic PAHs have high concentration in that area. Anthropogenic activities, Vehicular sources (i.e. petrol, diesel, natural gas emission) might be the reasons for the release of mutagenic PAHs in the atmosphere.

Table 14.

Concentration of BAP_{MEQ} (µg/gm) for *B. spectabilis* and *M. indica*

PAHs	**MEF**	**BaP_{MEQ} (µg/gm)**	
		B. spectabilis	***M. indica***
BaA	0.082	1.31±0.007	2.25±0.03
CHR	0.017	0.002±0.0007	0.28±0.007
BbF	0.25	0.56±0.04	8.3±0.14
BkF	0.11	1.12±0.06	2.26±0.04
BaP	1	18.04±0.03	36.34±0.46
DahA	0.29	9.61±0.18	3.8±0.07
IP	0.31	0.82±0.08	3.58±0.12
BghiP	0.19	6.64±0.12	5.46±0.03
Total		**38.10±0.11**	**62.27±0.43**



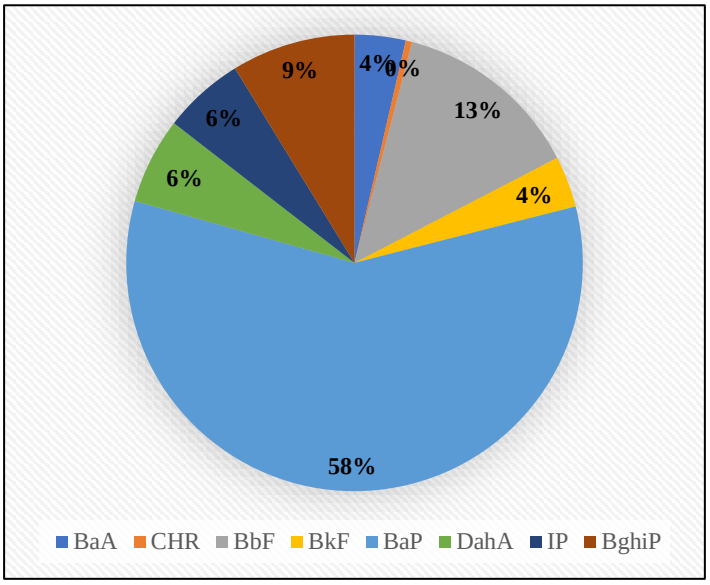


Fig. 15a: Percentage (%) of BAP_{MEQ} for *B. spectabilis* **Fig. 15b**: Percentage (%) of BaP_{MEQ} for *M. indica*

5.3. Foliar HMs analysis:

Heavy metals are regarded as one of the most harmful categories of anthropogenic environmental contaminants because of their toxicity and enduring nature in the environment (Sayyed and Wagh, 2011; Tiwari, 2011; Anim et al., 2012; Gyamfi et al., 2012; Prajapati et al., 2012). There is a significant risk to human health from the presence of heavy metals along the side of road and in ambient air (Rai, 2015). The nearby plants and ecosystem may also be negatively impacted by the presence of heavy metals above their threshold level. The tendency that plant leaves may accumulate heavy metals by dry or wet deposition or absorption is dependent on a number of variables, including the geographic distribution of plants and unique characteristics including leaf area, surface texture, environment, plant physiology and biochemistry (Gill, 2014). There are two probable ways that heavy metals can enter and accumulate in plant leaves. The first is by surface deposition of the metal, which allows it to enter leaf tissues through cuticular and stomatal apertures, channels and wounds in the leaf (Shahid et al., 2015). HMs can also enter plants through other possible pathway, by the roots taking up the metals from the soil, moving them through the xylem to different plant sections and storing them in the leaves (Tomasevic et al., 2004; Tangahu et al., 2011; Shahid et al., 2015; Gupta et al., 2015). Through the food chain, heavy metals have an adverse effect on health (Kananke et al., 2015b; Alkhader, 2015). Moreover, eating food contaminated with heavy metals can results in weakened immune systems, stunted growth, psychiatric disorder, diseases linked to malnutrition and a higher risk of upper gastrointestinal malignancies (Turkdogan et al., 2003; Khan et al., 2008; Lu et al., 2015).

Table 15.

Concentration of heavy metals in *B. spectabilis* and *M. indica* with permissible limit and MAI

Heavy metal	*B. spectabilis*	*M. indica*	Permissible limit (mg/kg)
As	18.7±1.2	36.8±1.3	
Mn	92.2±2	70.1±1.7	
Al	15740±481	1061±41.9	
Cd	7.9±0.2	12.3±0.6	0.02 (WHO, 1996)
Co	-	21.7±0.3	10 (WHO, 1996)
Cr	10.4±0.3	30.8±1.3	1.30 (WHO, 1996)
Mo	22.7±0.6	26.8±0.8	
Cu	-	12.6±0.5	10 (WHO, 1996)
Fe	798±44.7	409.5±13.5	
Hg	860±28.6	448±23.8	0.001-0.04 (WHO, 1996)
Li	41.8±2	51.9±2.3	
Ni	-	21.2±0.8	10 (WHO, 1996)
Pb	-	24.4±0.3	0.3- 10 (WHO, 1996)
Zn	334±15.4	308±13.1	10- 150 (Padmavathiamma and Li, 2007)
MAI		**35.11**	

The concentrations of As, Mn, Al, Cd, Co, Cr, Mo, Cu, Fe, Hg, Li, Ni, Pb, Zn are shown in Table 15. The rank of concentration of heavy metal for *B. spectabilis* is Al > Hg > Fe > Zn > Mn > Li > Mo > As > Cr > Cd and for *M. indica* is Al > Hg > Fe > Zn > Mn > Li > As > Cr > Mo > Pb > Co > Ni > Cu > Cd. For both the species the top five highest concentration of heavy metals are Al, Hg, Fe, Zn, Mn. The concentration of Al in *B. spectabilis* is 15740±481 mg/kg and for *M. indica* is 1061±41.9 (Table 15). The concentration of Cd, Co, Cr, Cu, Ni, Pb, and Zn exceeded WHO permissible limit (WHO, 1996). This is due to burning of fossil fuels, tire wear from moving vehicles, especially Cu-Cd contamination may take place due to the brake pad wear or engine (Padmavathiamma and Li, 2007). Heavy traffic load may be ascribed to vehicle emissions from chromium trioxide (CrO3) in the rubber used in automobile tires and catalytic converters, which may contain as much as 25 ppm of Cr (Galvagno et al., 2004). In the study, the concentration of Cr was found to be 10.4 mg/kg for *B. spectabilis* and 30.8 mg/kg for *M. indica*, which is very high. This might be due to fossil fuel combustion and solid waste incineration as mentioned earlier. The Cu only found in *M. indica* (12.6 mg/kg) (Table 15) is

slightly higher than the permissible limit (10 mg/kg, WHO, 1996). Arpita et al., (2020) found similar concentration of Cu ranging from 4.85-29.25 mg/kg in commercial site. Generally, concentration of Pb in plant species should be <10 mg/kg (Padmavathiamma and Li, 2007; Kabata-pendias and Pendias, 2001), in *M. indica* it is found to be 24.4 mg/kg (Table 15), which is very high. This can be due to the escalated anthropogenic activities, vehicular emissions and combustion of coal (Arpita et al, 2020). Similar concentration of Pb was found is *M. indica* (24.80 mg/kg) in commercial site by Arpita et al., 2020. Zn is an important element for plants, since it helps in biosynthesis (Serbula et al., 2012). The normal range of Zn should be 10-150 mg/kg (Padmavathiamma and Li, 2007). Both plant species showed high concentration of Zn (334 mg/kg in *B. spectabilis* and 308 mg/kg in *M. indica*) (Table 15), which is very high. This can be due to the municipal wastes (Wu et al., 2003). The concentration of Ni was found to be 21.2 mg/kg in *M. indica* (Table 15) which is twice the permissible limit (10 mg/kg, WHO, 1996). The high concentration of Ni might be due to the anthropogenic activities, including traffic and industrial activities (Shahrukh et al., 2023).

Plant captured different metals in the polluted environment (Khalid and Khanoranga, 2019). MAI was used to evaluate the metal accumulation capacities of plant species. According to current study, the MAI value is 29.68 for *B. spectabilis* and 35.11 for *M. indica* (Table 15). Here MAI is higher in *M. indica* than *B. spectabilis*. The HM accumulation is also higher in *M. indica*. Similar result was found by Khalid and Khanoranga (2019). Plants showing a higher value of MAI can be used as a barrier between polluted and non-polluted environment. The plant can be more efficiently grown in HM contaminated areas and considered as tolerant species if the plants exhibit higher MAI values (Jamil et al., 2009; Nadgorska-Socha et al., 2016). According to Liu et al. (2007), plant species with high value of MAI would be ideal for planting areas where HM pollution is high. In this investigation, the species with highest total capacity for accumulation of all metals were not necessarily those with the maximum accumulation of any one metal. Similar result was found by Sharukh et al. (2023). From these findings, it has been concluded that the importance and significance of MAI for species selection for phytoextraction and urban greening.

5.4. Capturing Rate (CR) analysis of HM:

In order to distinguish the respective contributions of the adsorbed and captured heavy metals, the capturing rate (CR) was employed. The identified heavy metals can either be absorbed by the leaves through precipitation or be taken up by the roots from the soil and transferred to the leaves. It was challenging to determine the precise elemental concentrations that came from

the soil or the air. To differentiate between the metals that the leaves absorbed from the air and those that they deposited on their surface, it was helpful to wash the leaves in distilled water (Alfani et al., 2000). The CR values of the HMs are represented in Table 16. The study showed that the highest CR value was found in Cu (100%) and Pb (100%) for *B. spectabilis* and Al (94.47%) for *M. indica*. The CR values of Fe ranges from 20.83% (*B. spectabilis*) from 33.30% (*M. indica*). The CR value of Mn was found to be 85.51% for *B. spectabilis* and 72.03% for *M. indica* (Table 16.). The CR values for Hg ranges from 42.59% (*B. spectabilis*) to 54.52% (*M. indic*a). These findings also showed that the properties of the leaf surface are crucial in determining the quantity of airborne particles that are captured and linked to trace elements (Dzierzanowski et al., 201; Saebo et al., 2012; Simon et al., 2014). Additionally, the wax layer and leaf structure play a critical role in the retention of heavy metals (Dzier˙ zanowski et al., 2011), and higher concentration of heavy metals retained by *M. indica* (Table 15.). Furthermore, according to Shuman (1994), zinc and copper are widely acknowledged as necessary microelements for plant growth, and plant leaves have the ability to acquire these elements from both the roots and the leaves.

Table 16.

Capturing rate of HMs for *B. spectabilis* and *M. indica*

Heavy metal	**Capturing Rate (%)**	
	B. spectabilis	***M. indica***
Fe	20.83	33.30
Cu	100	-
Zn	-	-
Pb	100	-
Mn	85.51	72.03
Al	48.42	94.47
Li	-	-
Mo	-	-
Hg	42.59	54.52

6. Conclusion:

The current work has concentrated on the selection of abundant yet local terrestrial plant species as tolerant forms of air pollution biomonitors under pollution load based on their APTI value, PAHs and HMs capturing abilities. The plant species *B. spectabilis* was found to be tolerant (APTI 18.6±0.07) and *M. indica* was found to be intermediately tolerant (APTI 14.39±0.36). Dust-borne air pollutants disrupted the atmosphere, which had a negative influence on the biochemical and physiological characteristics of the plant leaves in the urban area of South Kolkata. The present study highlighted the ecophysiological features of the species in reaction to detrimental environmental circumstances such as presence of HMs and PAHs influencing plant growth, cellular metabolism, reproduction, which have been examined to ascertain the plant biomonitoring potential. A number of reactions, including enhanced ascorbic acid synthesis, high leaf extract pH, and high RWC, which had a significant influence on plant tolerance, enabled the plant to survive in the harsh environment. Biochemical analysis of plant leaves demonstrated that because of their lipophilicity, the leaf lipids and waxes served as controlling factors in modulating PAHs concentrations. Total soluble sugar content increased with the pollution load and played a role in the enhancement of stress tolerance in plants. On the other hand, high pollution stress induce reduction in protein and proline contents. Elemental characterization (C, H, N and S) of plant leaves also directly or indirectly indicated differential tolerance and survival capacities of the species under variable abiotic stresses.

The PAHs concentration was determined in the plant species in both polluted and control sites and it is evident that high PAHs pollution load is present in the sample site. It is also observed that *M. indica* can capture PAHs almost double (358.86±0.09 µg g^{-1}) than that by *B. spectabilis* (198.82±3.66 µg g^{-1}). The HMW PAHs were predominant and contributed about 60% for both the plant species. Among the 16 priority PAHs, NAPH, BghiP, DahA, PHEN, BaP, BaA were the major contributors of total PAHs concentration in for *B. spectabilis,,* while in *M. indica*, PHEN, BaP, BbF, FLU, NAPH, BghiP, BaA were the dominant in PAHs reservoir. Analysis of diagnostic ratios revealed that diesel combustion, coal burning and vehicular emission were the major sources of PAHs in the sample site. The major contribution (94-99%) of the seven carcinogenic PAHs having 4-6 rings was found with the dominance of BaA, BbF, BkF, BaP, DahA and IP for both the species, whereas, the highest mutagenic threats were posed by 5-6 rings PAHs accounting for 96-97% in both the species. The total mutagenic metal concentration is higher is *M. indica* (62.27 µg g^{-1}) than *B. spectabilis* (38.26 µg g^{-1}). In the context of HM biomonitoring, As, Mn, Al, Cd, Co, Cr, Mo,

Cu, Fe, Hg, Li, Ni, Pb, Zn were found in the plant species with the concentration range varying between 7.9-15740 mg kg^{-1} in *B. spectabilis* and 12.3-1061 mg kg^{-1} in *M. indica*. Among them, the major contributors were Al, Hg, Fe, Zn, Mn, Li for *M. indica* and Al, Hg, Fe, Zn, Mn for *B. spectabilis*. Burning of fossil fuels, tyre wear of moving vehicle, solid waste incineration, anthropogenic activities, vehicular emission, coal combustion were the responsible sources of HMs causing elevated concentrations in the study area. Comparing the values of MAI between the two species, superior performance in metal accumulation was exhibited by *M. indica* (MAI: 35.11) as compared to *B. spectabilis* (MAI: 29.68). The highest value of CR was found in Pb and Cu for *B. spectabilis* and Al for *M. indica*. From the above findings, it can be concluded that the selected plant species are resistant to harsh polluted condition and can be used as biomonitors. Also, as the species are fast growing in nature and have abundance in the urban areas of Kolkata, the plant species can be used to build green belts in and around the city for monitoring PAHs and HMs.

The metric used to identify any species, whether it can be a bioindicator or a biomonitor, is called APTI. According to our research, *M. indica* has an APTI of 14.39±0.36, indicating that it is an intermediately tolerant species that might serve as a bioindicator. However, it is evident that the species has a far higher capability for collecting PAHs and HMs than the tolerant species *B. spectabilis*. Furthermore, we can see that the species' wax, lipid, sugar content, C/H, and C/N ratios are more appropriate than those of *B. spectabilis*, further supporting the idea that the former can endure extremely contaminated environments. The present detailed biomonitoring study of two terrestrial, abundant plant species confirms that plants' biomonitoring potential and their performance is subject to their quality based on both relevant biochemical parameters and APTI parameters. Survival of species under pollution stress is judged by APTI parameters and pollutant capturing efficiency depends on relevant biochemical properties like lipid content for PAHs capturing. Thus, based on our research, we can say that *M. indica*, a species with an intermediate tolerance, has been proved to be a good performer in biomonitoring job and can be employed as a biomonitor to be used in green belting plan in and around Kolkata.

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