

# Pollen as a Bioindicator of Vehicular Pollution: A Case Study of Chapra Town (Bihar)

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## ABSTRACT

Environmental health monitoring relies on crucial bioindicators like pollen grains. Vehicular pollution, a significant threat poses risks to ecosystems and air quality. Pollen grains, as reproductive microspores of seed plants, serve as sensitive biological indicators due to their direct interaction with the atmosphere. Chapra as a town characterized by urban growth and traffic, is an ideal case to study the link between pollen patterns and vehicular emissions. The present work aimed to assess the impact of vehicular pollution on the local environment of Chapra using pollen as a bioindicator. Surveys of airborne pollen concentrations in various zones of Chapra town were done and trapped pollen was isolated and identified. Fluctuation of pollen grain density throughout different seasons was studied. Protein content was determined. The correlation between the toxicological effects and the ecological dimension was examined, considering the distribution patterns of pollen grains. A pollen calendar was provided. The findings revealed heightened sensitivity of pollen grains from plants located in the traffic-prone areas of Chhapra. Substantial decrease in both the quantity and dimensions of pollen grains were also shown as well as reductions in pollen tube lengths and viability in contrast to the control site. Furthermore, the contaminated site displayed the most pronounced abnormalities in pollen structure, accompanied by reduced levels of pollen protein content.

**Keywords:** Bioindicator, Pollen grains, Pollen calendar, Pollen protein content, Vehicular pollution.

## Highlights

Vehicular pollutants present in atmosphere may directly affects pollen grains by following mechanism:

- Modifications of the morphological structure of pollen grains.
- Modification of the biological function of pollen grains such as rate of germination and pollen tube growth.
- Vehicular emission can enhance the allergenic potential of pollen grains.
- Occurrence of damaged male gametes in polluted atmosphere with increasing vehicular emissions can be used for pyto indication of the degree of atmospheric pollution.
- Impact of vehicular emission on physiological and metabolic Processes taking place in pollen grains is needed to analyses the changes taking place in structural changes in protein remain responsible for enhanced allergenicity leading to harmful impact on human health.

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## INTRODUCTION

Urbanization and increasing vehicular traffic raise concerns about air quality and public health. The surge in urbanization and the escalating volume of vehicular traffic have raised significant concerns about air quality and its extensive effects on the environment and public health. In understanding vehicular pollution, the significance of airborne pollen grains as bioindicators becomes prominent in revealing the complexities of vehicle emissions. Fresh pollen can provide valuable information about plant phenology, ecophysiology, population dynamics and gene flow (Vasilevskaya, 2022). Biological indicators, which show an integrated response to air pollution and other environmental factors, can be used as a complementary system to monitor the effects of air pollutants and to provide reliable indications of the quality and characteristics of the environment. (Carreras *et al.*, 2009). The fertility of plants and their successful pollination largely hinge on pollen grains. Under conditions of severe air pollution, plant fertility diminishes due to direct and indirect impacts on the reproductive mechanisms. Developed pollen grains, when released from the flower buds in the contaminated air absorbed humidity and some pollutants, which influenced their viability, henceforth, plants reproductive system (Leghari *et al.*, 2018).

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Pollen grains, which act as the male gametophytes, are crucial for the reproduction of flowering plants, and the fertility of plants heavily relies on them. Intense air pollution in an area can greatly affect the fertility of the surrounding plant life. Vehicular emissions are a major contributor to air pollution, leading to abnormalities in anthers and reducing both the quantity and quality of male fertility in plant species. It is well understood from studies that pollen has become a biomonitoring tool of atmospheric pollution. Pollen grains, the male reproductive organs, are relatively more exposed to air pollutants than any other organs of plants. Biomonitoring with pollen can certainly

be accomplished with simple tool and procedures (Hemavathi *et al.*, 2020). The objective of this research was to investigate the impact of vehicle emissions on the pollen grains of flowering plants. The current research involved a comparative analysis of pollen grains from specific plant species collected from both polluted and non-polluted areas in Chapra town, Bihar. The study focused on examining protein levels and the viability of pollen grains. The findings indicated that pollen grains from plants in polluted areas were highly responsive to vehicular exhaust emissions. A distinct decline in fertility rates, a notable decrease in the number of pollen grains, and a significant reduction in the length of pollen tubes were noted. Additionally, there was an increasing level of asymmetry in the external features of pollen grains, along with lower protein content. Biomonitoring involves utilizing plant reactions to assess environmental alterations. It identifies or predicts particular plant species that are sensitive to a certain pollutant or a mix of pollutants. Plant organs are more susceptible to air pollutants compared to other living beings. The consequences of air pollution extend to significant losses in agriculture, the economy, and the overall environment. Bio-monitoring offers a straightforward, rapid, and convenient method to assess environmental health. Conducting a seasonal analysis of pollen grains in the polluted atmosphere of Chapra Town can serve as a cost-effective and valuable tool to identify health issues, especially among residents. Surprisingly, there is currently no existing report on pollen grains in the air of Chapra Town. This study, spanning one year analyzed angiospermic plant species' pollen grains through lab investigations and on-site visits. Pollen collection was done by a rotating drum at polluted and reference sites. The selection was based on varying vehicular traffic density. Preceding sample collection, a comprehensive eco-floristic survey was undertaken including climate and meteorological data analysis. The research aimed to evaluate the significance of pollen in assessing air pollution, explore its impact on vegetation, and emphasize the influence of pollen germination on fruit setting and productivity.

Investigation into pollen germination under different stimuli provided insights into air pollutants and plant adaptability.

## MATERIAL AND METHOD

### Workplace

The study is conducted in Chapra town, the headquarters of Saran district in Bihar, India. Chapra is positioned at 25.7848°N latitude and 84.7274°E longitude, with an average elevation of 36 meters, encountering a tropical environment characterized by a yearly temperature of 26°C and an average precipitation of 1059 mm. With a population of 567,023 inhabitants, Chapra is traversed by the Ganges and Ghaghara Rivers, facing heavy traffic comprising various vehicles. The location map of the study area is shown in Fig. 1. Despite not being an industrial area, Chapra contends with pollution primarily from automobile emissions. The selection of study areas considered factors like traffic density, smoke presence, road dust, and visible vehicle emissions. Chapra boasts diverse flowering and fruit plants, such as roses, orchids, mangoes, litchis, and berries. The research categorized areas into reference and polluted sites, factoring in ecological conditions and thriving plant species. Data were sourced from Rail Wheel Plant, Bela (Saran), Shiva Test House, Patna, Central Pollution Control Board, New Delhi, and Bihar State Pollution Control Board, Patna. Meteorological and climatic data were obtained from the Chapra town meteorological observatory records.

### Reference Site (Site-I)

As shown in Fig. 2, a suburban area with minimal traffic activity has been identified as a reference site (site-I).

### Polluted site (site-II)

As shown in Fig.3, the city area has been designated as a polluted Site (Site- II).



Fig. 1: Atlas of the work area



Fig. 2: Suburban areas with minimal traffic activity



Fig. 3: City area (City Centre and along highway)

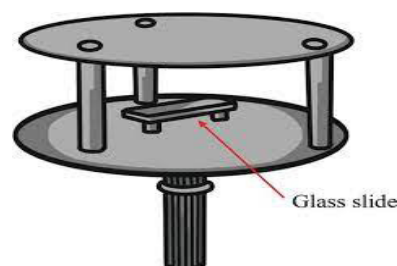


Fig. 4: Rotating drum

### Climate of the Study area

Chapra, located in the northern hemisphere, has a warm and temperate climate with lower winter rainfall compared to summer. The mean temperature stands at 25.5°C, with an annual rainfall of 1134 mm. September sees the highest humidity at 83.25%, while April has lower humidity at 35.66%. July experiences frequent heavy rainfall, and winter sets in around mid-October. Summer spans from mid-March to June, with peak heat in May and June. Meteorological data for Chapra town, including temperature, rainfall, and humidity, were compiled from observatory records. The town experiences dense cloud cover during the monsoon, occasional cloudiness in winter, and generally clear or lightly clouded skies throughout the rest of the year. Monsoons bring intense rainfall and thunderstorms, influenced by cloud movement from depressions in the Bay of Bengal.

### Field Survey

Before sampling, a preliminary examination assessed expected emission levels based on vehicular traffic. Monitoring vegetation diversity and flowering seasons was done across Chapra over a year. Pollen grains, sensitive biomonitors, were used to evaluate air pollution impact. Different locations with varying traffic levels were compared.

### Instrumental Techniques for Pollen Sampling

Specimens were collected during 2019, covering winter, summer, and the rainy season. Pollen sampling was done in the morning, avoiding rain and wind. City center, highway area, and suburb area in Chapra were chosen based on traffic and emissions. Pollen grains were collected using a 20mm clear adhesive tape attached to a rotating drum coated with petroleum jelly (Fig. 4). The tape segments were changed every eight days, mounted on microscopic slides using glycerin jelly, and examined under a light microscope at a magnification of 400X to observe larger dust particles.

### Morphological Analysis of Pollen Grains

The study involved identifying pollen types and species by examining distinctive morphological features such as shape, size, exine sculpture, and aperture presence. The Palynological glossary was used as a reference. After identification, pollen samples were isolated to assess fertility, germination, and protein content. Morphological irregularities like black spots, exine breakage, and cytoplasmic granules were investigated. Pollen was collected from polluted (town center and roads) and controlled (rural suburban) areas. Light microscopy was used at a magnification of 400 times to investigate how atmospheric

pollution affected pollen surface and exine under different conditions of air pollution.

### Pollen Morphometrics Analysis

Quantitative analysis, including size determination and regularity assessment of pollen grains, was conducted using micrometry and morphological examination through a light microscope. Pollen from polluted and control sites underwent size determination using an ocular micrometer calibrated with a stage micrometer on a light microscope. The investigation was conducted thrice, and the measurements of size were recorded in millimeters (equivalent to  $1 \times 10^{-6}$  meters), and the average was calculated. The evaluation of regularity involved assessing the proportion of organized and characteristic pollen grains, with an examination of 10 grains for each species. Pollen diameter measurements were observed from three sampling sites. All experiments were triplicated, and mean data were recorded. Photographs of collected pollen grains from the city center, highway vicinity, and the control area (suburban region) were taken.

### Preparation of Pollination Calendar

Before sampling, a preliminary examination of the study area assessed for emission levels based on vehicular traffic volume. Anticipating high emissions near roadsides, moderate in the city center, and low in suburban areas, different locations in Chapra were regularly visited. The study aimed to prepare a pollen calendar for Chapra, which is crucial for healthcare professionals advising on managing allergic conditions. This information is valuable for aerobiologists, given the significant role of flowering plants in causing inhalation allergies. The pollen calendar plotted occurrences of various plant species' pollen grains, identifying peak months for each type. During these peak months, pollen grains were collected for further experimental investigations.

### Pollen Fertility/Sterility Analysis

Pollen fertility was assessed using the iodine method on temporary pressure preparations, screening for the Palynotoxic effects of motor vehicle emissions. Palynomorphological assessment observed anomalies in pollen grain shape and size, indicating the impact of motor transport emissions. For fertility examination, 2, 3, 5 – triphenyl tetrazolium chloride (TTC) staining technique was employed. A 1% TTC solution in 60% sucrose was used for staining. Pollen grains on glass slides were stained with TTC, covered, and airtightly sealed with didutylphthalate xylene (DPX). After allowing incubation in daylight for a duration of 3 hours, the slides were observed using a light microscope at a magnification of 400X. Pollen fertility was categorized based on the count.

### Pollen germination (Viability)

Before sampling, a preliminary examination of the study area was conducted, assessing expected emission levels. Pollen germination was observed through *in-vitro* experiments using the standing drop method in Brewbaker and Kwack's culture medium (1963). The experiment was replicated three times, and averages of the data were utilized. Pollen grains from sampling sites were placed on a culture medium with specific conditions, and after 24 hours, developed and undeveloped

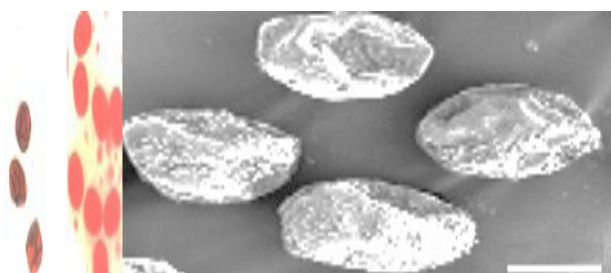


Fig. 5: Shows the viability and non-viability of microspores

pollen grains were counted under a light microscope. The percentage of germination was determined by assessing pollen grains that exhibited pollen tubes surpassing their individual lengths. The viability of pollen grains was also checked using the germination method. These findings provide valuable insights for further scientific research on using pollen grains as bio-indicators for vehicular pollution. Before sampling, a preliminary examination of the study area assessed expected emission levels. Pollen morphological abnormalities, fertile and sterile grain percentages, and germinability reveal how airborne pollutants impact generative structures, affecting biodiversity and allergenic potential. Motor vehicle emissions' palynotoxic effect is evaluated through pollen fertility indices, identifying specific plant species as bioindicators of vehicular pollution.

Pollen Germination (%) = Number of Germinated pollen grains / Total no. of observed pollen grain ×100

### Pollen Protein Examination

Loading buffers containing sodium dodecyl sulfate, glycerol, bromophenol blue, and mercaptoethanol were prepared with the following composition: 63.4mM Tris-HCl (pH 7.2), 2% SDS, 26% glycerol, 0.01% bromophenol blue, and 5% β-mercaptoethanol. Ninety microliters of pollen extracts, which included 10 μg of protein, were obtained from both control and polluted site areas. After vortexing and heating in boiling water for 3 minutes, the samples were separated using 12% SDS-PAGE, following the method described by Laemmli 1970. Proteins were set at 70 V and stored at 50 V for a whole night through the vertical slab gel system (Akhtarian, Ps-2000, Model 75, Iran). Electrophoresis was conducted 3 to 4 times (Rezanejad, F; 2013). Protein was identified by staining through the solution of the Coomassie brilliant blue R- 250. For the de-staining of proteins, the composition of ethanoic acid, methanol and distilled water was used in the ratio of [1:1:8].

## RESULTS AND DISCUSSION

In this context, detailed and elaborate studies of air biomonitoring using passive plant samplers have been the prime concern for the recognition and estimation of even small-scale

**Table 1:** Month-wise average temperature of Chapra Town

S. No.	Month	Average maximum temp (°C)	Average minimum temp (°C)
1.	January	22.5	10.5
2.	February	26.4	14.90
3.	March	25.3	18.5
4.	April	31.2	23.5
5.	May	32.8	25.5
6.	June	31.1	26.9
7.	July	28.6	26.2
8.	August	28.1	25.9
9.	September	27.5	25.1
10.	October	30.6	21.0
11.	November	27.7	16.6
12.	December	23.4	11.8

environmental changes in the spatio-temporal trends of the pollutants in air (Mukhopadhyay *et al.*, 2021). Major pollutants, such as sulfur dioxide and nitrogen dioxide, were found to significantly damage pollen, with sulfur dioxide having a more pronounced effect. The collected data, presented in Table 1 to Table 11 offers valuable insights into the relationship between vehicular pollutants, the atmosphere, and pollen grains in Chapra town. The highest average maximum temperature, 32.8°C, occurred in May, while the lowest, 22.5°C, was recorded in January. January also registered the lowest average minimum temperature at 10.5°C (Table 1). The data presented in Table 2 shows that PM 2.5 is the predominant air pollutant in Chapra, exceeding standard limits annually. PM 10, although generally above the standard limit, remains lower from June to October. Gaseous pollutants from vehicular sources (CO, CO<sub>2</sub>, NO<sub>2</sub>, and SO<sub>2</sub>) are consistently below standard limits, but high automobile density contributes to elevated particulate pollutants. Vehicular pollution, exacerbated by traffic congestion and roadside activities, is a significant factor. Vehicular activities contribute to PM 2.5 becoming the predominant pollutant. Dangerous levels of PM 10 are recorded in January, February, March, April,

**Table 2:** Status of air quality of Chapra

S. No.	Month/ year	Air quality parameters (μg/m <sup>3</sup> )					
		PM 10	PM 2.5	NO2	SO2	O3	CO
1.	January	138	107	12.6	16.1	09.2	2.9
2.	February	170	119	13.0	16.8	11.4	2.0
3.	March	159	251	17.6	21.2	14.3	01.9
4.	April	104	64	17.2	13.9	19.1	01.8
5.	May	101	53	17.4	14.2	11.3	02.9
6.	June	89	101	14.0	13.7	07.6	02.4
7.	July	71	35	09.5	11.0	03.0	02.0
8.	August	62	41	08.0	17.3	03.1	02.0
9.	September	69	74	11.4	16.5	03.2	02.4
10.	October	81	65	14.2	17.2	05.4	03.0
11.	November	173	132	31.5	24.3	08.0	05.3
12.	December	187	318	30.3	22.0	02.5	07.0
Annual average		117	113	15.7	17.0	08.2	03.0
Max. standard		100	60	80	80	100	04

**Table 3:** Structure and size of pollen grains observed

S. No.	Species of pollen	Structure and size of pollen grain
1.	<i>Acacia arabica</i>	8 celled compound, Psilate Exine, Polyad, 30–38 μm
2.	<i>Amaranthus spinosus</i>	Pentoporate, Spinulose, Reticulate Exine, 20–26 μm
3.	<i>Argemone mexicana</i>	3-Zonocalpate, Foveolate, Reticulate Exine, 22–25 μm
4.	<i>Azadiracta indica</i>	4-Zonocalpate, Psilate and reticulate exine, Prolate, 35–40 μm
5.	<i>Brassica campestris</i>	3-Zonocalpate, Reticulate Exine, 18–21 μm

May, November, and December. In January, February, March, June, November, and December, PM 2.5 exceeds the hazardous threshold. NO<sub>2</sub>, SO<sub>2</sub>, O<sub>3</sub>, and CO levels are within standard limits. Table 3 provides details about the number of cells, exine

ornamentation (surface features), and size of pollen grains for each of the mentioned plant species. This information is valuable for understanding the diversity in pollen morphology among different plants. The pollen grain of *Acacia arabica* has a compound structure with 8 cells. The exine is psilate, and it forms a polyad. The size of the pollen grain is in the range of 30 to 38 µm. *Amaranthus spinosus* having the pollen grain of pentoporate, with a spinulose surface and a reticulate exine. Its size ranges from 20 to 26 µm. The pollen grain of *Argemone mexicana*: is 3-zonocalpate, with a foveolate surface and a reticulate exine. Its size is approximately 22 to 25 µm. *Azadiracta indica*: The pollen grain has a 4-zonocalpate structure, with a psilate and reticulate exine. It is prolate in shape and has a size of 35 to 40 µm. *Brassica campestris*: The pollen grain is 3-zonocalpate, with a reticulate exine. Its size falls within the range of 18 to 21 µm. In Table 4, species names with their family name were given. Table 5 provides information on the peak months of occurrence for the given plant species *Acacia arabica* reaches its peak occurrence in October. *Amaranthus spinosus* is most prominent in September. *Argemone mexicana* exhibits its peak occurrence in March. *Azadiracta indica* also has its peak in March and *Brassica campestris* is most noticeable in February. In Table 5, species and peak month of occurrence were seen that is *Acacia arabica* has an 8-celled compound structure with a psilate exine featuring polyads, and a size of 30 to 38 µm. *Amaranthus spinosus*

**Table 4:** Species and family-wise details of observed pollen

S. No.	Name of species	Family
1.	<i>Acacia arabica</i>	Mimosae
2.	<i>Amaranthus spinosus</i>	Amaranthaceae
3.	<i>Argemone mexicana</i>	Papaveraceae
4.	<i>Azadiracta indica</i>	Meliaceae
5.	<i>Brassica campestris</i>	Crusiferae (Brassicaceae)

**Table 5:** Peak month of occurrence of pollen grains

S. No.	Name of plant species	Peak month of occurrence
1.	<i>Acacia arabica</i>	October
2.	<i>Amaranthus spinosus</i>	September
3.	<i>Argemone mexicana</i>	March
4.	<i>Azadiracta indica</i>	March
5.	<i>Brassica campestris</i>	February

**Table 6:** Pollen calendar of Chapra Town and its suburb

S. No.	Name of the species	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1.	<i>Acacia arabica</i>	√	×	×	×	×	×	×	√	√	√	√	√
2.	<i>Amaranthus spinosus</i>	√	×	×	×	×	×	×	√	√	√	√	√
3.	<i>Argemone mexicana</i>	√	√	√	√	√	√	√	√	√	√	√	√
4.	<i>Azadiracta indica</i>	×	×	√	√	√	×	×	×	×	×	×	×
5.	<i>Brassica campestris</i>	√	√	√	×	×	×	×	×	×	×	×	×

**Table 7:** Pollen deformities (%) found in pollen grains collected from polluted areas

S. No.	Name of species	Abnormal shape	Reduced size	Black spots on exine	Broken exine	Pollen with cytoplasmic granules on surface
1.	<i>Acacia Arabica</i>	07	00	07	00	00
2.	<i>Amaranthus spinosus</i>	06	16	08	06	14
3.	<i>Argemone mexicana</i>	08	00	11	00	00
4.	<i>Azadiracta indica</i>	02	12	03	14	11
5.	<i>Brassica campestris</i>	02	05	00	00	00

**Table 8:** Mean pollen fertility (%)

S. No.	Name of plant species	City centre		Highway area		Suburb (Control)	
		Fertile (%)	Sterile (%)	Fertile (%)	Sterile (%)	Fertile (%)	Sterile (%)
1.	<i>Acacia arabica</i>	79.4	20.6	81.2	18.8	92.0	08.0
2.	<i>Amaranthus spinosus</i>	60.6	39.4	64.7	35.3	90.0	10.0
3.	<i>Argemone mexicana</i>	88.0	12.0	90.0	10.0	94.5	05.5
4.	<i>Azadiracta indica</i>	68.2	31.8	60.3	39.7	89.0	11.0
5.	<i>Brassica campestris</i>	80.0	10.0	81.0	09.0	94.0	06.0

**Table 9:** Mean pollen viability (Germination%)

S. No.	Name of plant species	City centre	Near highway	Suburb (Control)
1.	<i>Acacia arabica</i>	1.1	1.0	1.2
2.	<i>Amaranthus spinosus</i>	1.3	1.7	4.5
3.	<i>Argemone mexicana</i>	2.4	2.2	2.7
4.	<i>Azadiracta indica</i>	3.2	2.9	5.8
5.	<i>Brassica compestris</i>	5.2	5.5	6.2

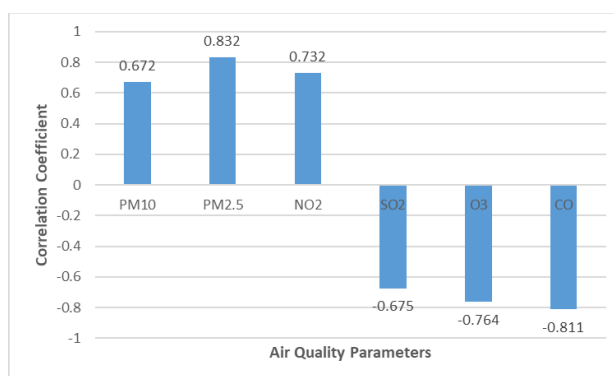
**Table 10:** Pollen protein content (mg/g)

S. No.	Name of plant species	City centre	Near highway	Suburb (Control)
1.	<i>Acacia arabica</i>	76.0	81.0	91.0
2.	<i>Amaranthus spinosus</i>	54.0	61.0	80.0
3.	<i>Argemone mexicana</i>	73.0	83.0	89.5
4.	<i>Azadiracta indica</i>	66.5	58.0	83.0
5.	<i>Brassica compestris</i>	75.5	78.0	87.5

**Table 11:** Correlation of various air quality parameters with pollen abundance

Air quality parameter	Correlation with pollen abundance	Significant correlation?
PM10	Positive (0.672)	Yes
PM2.5	Positive (0.832)	Yes
NO <sub>2</sub>	Positive (0.783)	Yes
SO <sub>2</sub>	Negative (-0.675)	Yes
O <sub>3</sub>	Negative (-0.764)	Yes
CO	Negative (-0.811)	Yes

exhibits a pentoporate structure with a spinulose, reticulate exine and measures 20 to 26  $\mu\text{m}$ . *Argemone mexicana* displays a 3-zonocalpate structure with a foveolate, reticulate exine, and a size of 22 to 25  $\mu\text{m}$ . *Azadiracta indica* showcases a 4-zonocalpate structure with a psilate and reticulate exine, a prolate shape, and a size of 35 to 40  $\mu\text{m}$ . *Brassica compestris* features a 3-zonocalpate structure with a reticulate exine and a size of 18 to 21  $\mu\text{m}$ . In Table 6 Pollen Calendar of Chapra Town and its suburbs were shown. The percentage of pollen deformities found in pollen grains collected from polluted areas is estimated as *Acacia Arabica* exhibits an abnormal shape of 7, with no reduced size, seven black spots on the exine, no broken exine, and no pollen with cytoplasmic granules on the surface. *Amaranthus spinosus* displays an abnormal shape of 6, a reduced size of 16, 8 black spots on the exine, a broken exine count of 6, and 14 instances of pollen with cytoplasmic granules on the surface. *Argemone mexicana* shows an abnormal shape of 8, no reduced size, 11 black spots on the exine, no broken exine, and no pollen with cytoplasmic granules on the surface. *Azadiracta indica* has an abnormal shape count of 2, a reduced size of 12, 3 black spots on the exine, 14 broken exine, and 11 instances of pollen with cytoplasmic granules on the surface. *Brassica compestris* features an abnormal shape count of 2, a reduced size of 5, and no


**Fig. 6:** Correlation between air quality parameters and pollen abundance

black spots on the exine, no broken exine, and no pollen with cytoplasmic granules on the surface (Table 7). The percentage of mean pollen fertility of given plant species collected from contaminated and uncontaminated regions are shown in Table 8. The viable pollen grains exhibited color differentiation: red at the center of mass and pink with a slight orange hue at the edges as shown in Fig. 5. Most grains showed good viability, but some were sterile. Pollen staining intensity decreased towards the coverslip margin and in areas with air bubbles, indicating reduced air availability. This inconsistency in staining may be due to air-inhibiting TTC reduction. Mean pollen viability (Germination%) is given in Table 9. Pollen Protein Content (mg/g) is shown in Table 10 that is *Acacia arabica* has values of 1.1 in the City Centre, 1.0 Near the Highway, and 1.2 in the Suburb (Control). *Amaranthus spinosus* shows values of 1.3, 1.7, and 4.5 in the City Centre, Near Highway, and Suburb (Control), respectively. *Argemone mexicana* exhibits values of 2.4, 2.2, and 2.7 in the City Centre, Near Highways, and Suburbs (Control). *A. indica* has values of 3.2, 2.9, and 5.8 in the City Centre, Near Highways, and Suburb (Control). *Brassica compestris* shows values of 5.2, 5.5, and 6.2 in the City Centre, Near Highway, and Suburb (Control).

The results of the analysis revealed intriguing insights into the relationship between air quality parameters and pollen abundance in Chapra Town. As shown in Table 11 and Fig. 6, correlation analysis unveiled significant positive correlations between certain air pollutants, such as PM10 and No<sub>2</sub>, and the abundance of pollen grains, particularly during the spring and autumn months. Conversely, negative correlations were observed between pollen abundance and other pollutants like CO and O<sub>3</sub>, suggesting a potential inhibitory effect of these pollutants on pollen production. These findings underscore the intricate relationship between anthropogenic activities, air quality, and plant reproductive health, highlighting the importance of targeted interventions to mitigate the adverse effects of pollution on ecosystem functioning.

## CONCLUSION

Bio-indicators, particularly pollen grains, are sensitive indicators of atmospheric pollution, reflecting changes in allergenicity and morphological characteristics. Pollutants alter the contents of

allergenic molecules in pollen grains, increasing their overall allergenic potential. The shape and size of pollen grains vary under different environmental stresses, making their study crucial for environmental scientists and plant breeders. Pollen grains, with diagnostic features such as shape, size, and exine sculpture, provide insights into the effects of pollutants like SO<sub>2</sub>, NO<sub>2</sub>, O<sub>3</sub>, and CO. Vehicular emissions, including harmful gases and heavy metals, affect the morphology, viability, and allergenic potential of pollen grains. Different plant species exhibit variable sensitivity to vehicular pollution, impacting their physical and chemical properties. Pollen grains, being sensitive to changing environmental conditions, reflect the adverse effects of pollutants, providing valuable information for air quality management. The use of pollen grains as bioindicators offers a cost-effective and practical approach to monitoring the biological impact of air pollution.

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### AUTHOR CONTRIBUTION

Analysis, Interpretation of data, and manuscript writing done by Naira Nayab. Overall supervision, critical revision and review of paper approved by Md Anzer Alam.

### CONFLICT OF INTEREST

No conflicts of interest are present among the authors.

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