



## A COMPARATIVE ANALYSIS OF METAL POLLUTION USING POLLEN GRAINS AS BIOINDICATORS IN RURAL AND URBAN HABITATS

LAVANYA GIRIDHAR<sup>1</sup>, CHETAN H. C.<sup>2</sup>, HASEENA RAFATH<sup>3\*</sup>

<sup>1,3</sup>Department of Botany, Annamalai University, Annamalai Nagar, India

<sup>2</sup> Centre of Conservation of Natural Resources, The University of Trans-Disciplinary Health Sciences and Technology, Yelahanka, Bengaluru, Karnataka, India

\*Address for Correspondence:

**Dr.Haseena Rafath**

Department of Botany, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu-608002, India.

### Article History

Volume 6, Issue Si4, 2024

Received: 1 July 2024

Accepted: 20 July 2024

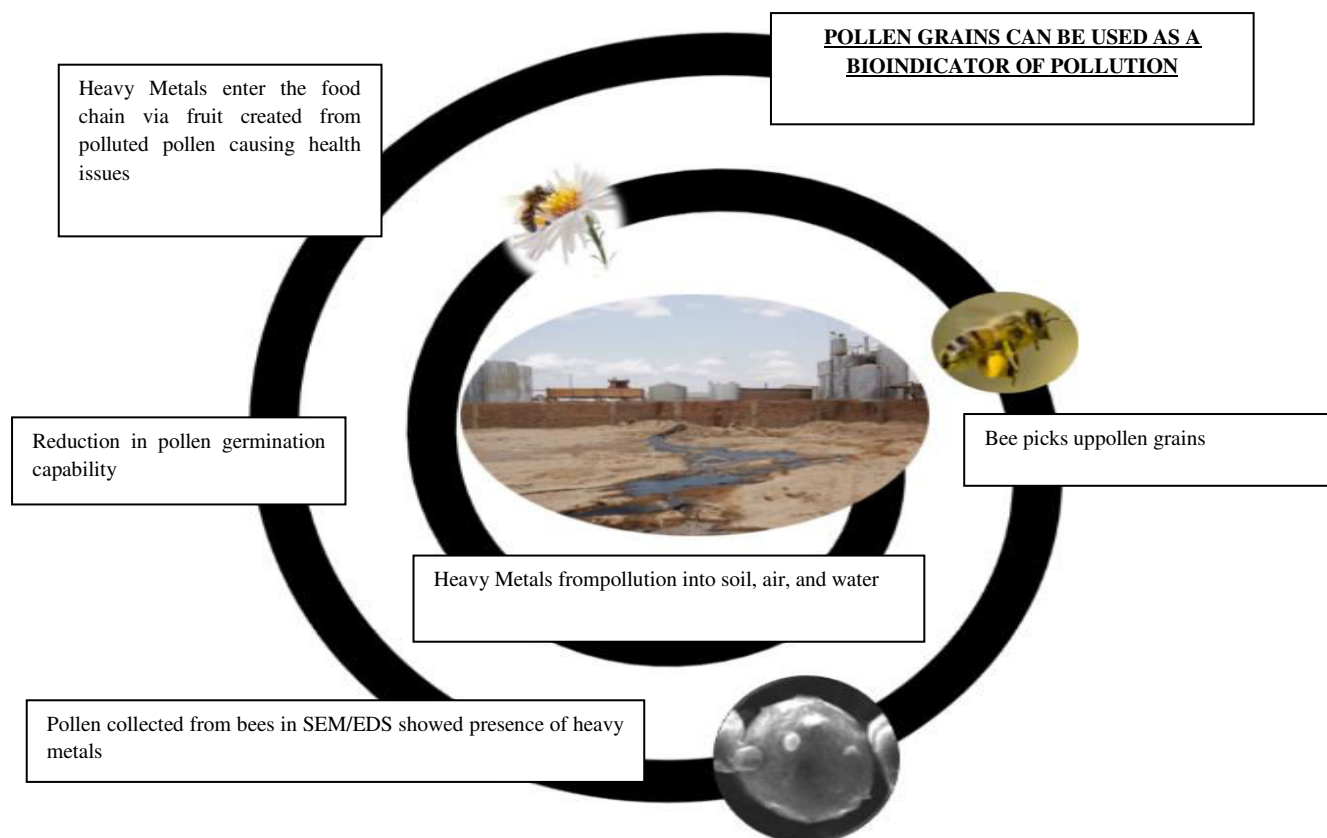
Doi:

10.48047/AFJBS.6.Si4.2024.4829-4840

### Abstract

This study was conducted to identify heavy metal pollutants using pollen grains as bioindicators of environmental contamination. The experiment was conducted on pollen grains collected from foraging honey bees, *Apis cerana* from an industrial suburb, Peenya and a rural area in Hesaraghatta in Bengaluru. Scanning electron microscopy and energy dispersive X-ray spectroscopy (SEM/EDS) were conducted on the pollen grains to detect the particulate matter and the metals present in them. The findings revealed a stark contrast between urban industrial and rural non-industrial areas. Pollen collected from urban industrial zone exhibited elevated levels of particulate matter, hosting heavy metals such as aluminium, molybdenum, mercury, chromium, nickel, copper, zinc, lead, and iron. In contrast, pollen from rural non-industrial area exhibited lower levels of particulate matter and a notable absence of heavy metals. Pollen grains are contaminated due to pollutants present in the environment. Pollutants can be found adhering to the pollen grains.

### Graphical Abstract



**Keywords:** *Apis cerana*, Bee pollen, Energy-Dispersive X-ray Spectroscopy, Scanning electron microscopy, heavy metals

## 1. INTRODUCTION

The escalating environmental contamination attributed to the presence of heavy metals has emerged as a significant concern in recent times, casting a pronounced impact on public health. The intensification of anthropogenic activities has inflicted deleterious consequences on humanity, as unchecked emissions from rapid industrialization and urbanization have evolved into a global quandary. Amidst the myriad environmental contaminants, heavy metals play a pivotal role, with their concentrations in the air, soil, and water displaying a consistent upward trajectory [1]. The presence of heavy metals can be directly quantified in the air, soil, or water, while alternatively; biological entities such as airborne pollen grains can serve as discernible indicators of pollutants.

Pollen grains, functioning as male gametophytes in angiosperms, contribute to male gametes for sexual reproduction. Following anther dehiscence, pollen grains are exposed to the atmospheric milieu [2]. Pollen grains assume critical significance in the plant life cycle and bear paramount importance for humans, considering the role of angiosperms as a source of sustenance and diverse resources. During their journey from anther to stigma, pollen grains may come into contact with particulate matter (PM), with atmospheric particles adhering to the external wall of pollen grains, thereby inducing pollution. Both pollen and PM act as prevalent triggers for asthma [3]. Beyond the deleterious effects on human health, the attachment of particles to pollen grains can also perturb pollen germination processes. Pollutants are recognized to impede pollen germination, typically resulting in a reduction in pollen germination capability [4,5].

Even during transportation by bees, pollen grains remain susceptible to atmospheric exposure. Worker honeybees utilize a feeble electrostatic field to attract thousands of pollen

grains while visiting flowers [6]. In the foraging process, honey bees amalgamate freshly collected pollen with nectar, packaging it into their corbiculae or pollen baskets. Pollen plays a pivotal role in bee colonies, providing essential nutrients such as proteins, lipids, and minerals for their development [7]. Bee pollen is acknowledged as a natural superfood for humans, attributed to its nutritional and medicinal properties [8]. The pollution of pollen by particulate matter containing metals directly or indirectly impacts plants, pollinators, and humans.

Numerous studies have delved into the examination of pollen grains exposed to polluted environments. Some studies involve the direct collection of pollen from trees, subjecting them to polluted air, while others utilize various types of pollen collectors to gather airborne pollen [3]. However, there are very few studies where pollen grains have been directly sampled from both polluted and nonpolluted sites to investigate the deposition of particulate matter on them and evaluate the chemical composition of the particulate matter but still a commendable amount of work in this regard is scarce. Recognizing this critical gap in existing research, the researchers have collected pollen grains directly from pollinators like honey bees that can potentially serve as indicators of air pollution, particularly particulate matter with specific heavy metals.

## 2. MATERIALS AND METHODS

The study was conducted in a park in Peenya Industrial Suburb (SITE A) in Bengaluru and a rural area, Hesaraghatta (SITE B) on the city's outskirts. Foraging honey bees *Apis cerana* with pollen baskets were collected from both sites randomly. Bee collection was done between 9 am to 10 am. Bees were caught by the method described by Shivanna and Tandon [2]. Bees were directly collected in a clean wide-mouthed vial of suitable size of 50 ml capacity. When the insect entered the flower, the vial was held in the mouth of the flower. The insect entered the vial when it was exiting the flower. The lid was immediately closed to prevent the escape of the bee. The bees were immobilised by placing the vials on ice. The pollen baskets were removed from the hind limbs. The pollen pellets were dried using silica gel in a desiccator. The dried pollen grains were observed under a Scanning Electron Microscope [9]. Particulate matter adhered to the surface of pollen grains was selected for Energy-Dispersive X-ray Spectroscopy (EDS) and metals and non-metals found were noted. The number of occurrences of metals and non-metals was taken into account in urban and rural sites (Table 3). A one-way ANOVA was used to compare the means.

## 3. RESULT AND DISCUSSION

Pollen collected from pollen baskets of bees foraging in polluted areas showed the presence of particulate matter as seen in Figures 1A to 1E. The EDS of these particles showed the presence of various metals and non-metals, as shown in Table 1. Pollen collected from rural areas exhibited lower levels of particulate matter shown in Figures 2F to 2I, and EDS analysis revealed an absence of metals in the detected particulate matter as seen in Table 2. Statistical analysis revealed significant difference in the means of occurrence of metals and non-metals in the two sites ( $P < 0.05$ ). The pollen grains collected from site A (urban) ( $M = 1.43$ ,  $SD = 0.65$ ) compared to pollen grains collected from site B (rural) ( $M = 0.14$ ,  $SD = 0.36$ ) demonstrated significantly higher levels of metals and non-metals,  $p = 7 \times 10^{-7}$ .

The corresponding EDS Spectra Reports can be found in the Appendix. It is important to note that silicon had not been taken into account, as it might be present due to the utilization of silica gel in the pollen grain processing.

In a comprehensive investigation of site A (urban) and site B (rural), it was observed that pollen grains collected from site A exhibited the presence of various metals, including Aluminium, Molybdenum, Sodium, Calcium, Mercury, Nickel, Lead, Potassium, and non-

metals such as Sulphur and Phosphorus. Conversely, in pollen grains collected from site B, the analysis revealed minimal quantities of Aluminium, Mercury, Lead, Chromium, Iron, Nickel, and Copper. Notably, non-metals were absent in the pollen grains from site B. This stark contrast underscores the impact of environmental pollution on the composition of pollen, highlighting the accumulation of metals in urban regions compared to their relatively negligible presence in cleaner rural environments.

Pollen from urban site A contains elevated levels of metals like Aluminium, Mercury, Lead, and Nickel, indicative of urban pollution sources such as industry and vehicle emissions. Non-metals like Sulphur and Phosphorus were also present, likely from industrial and agricultural activities. In contrast, pollen from rural site B showed minimal metals and lacked non-metals, reflecting cleaner environmental conditions. This disparity underscores the impact of urban pollution on pollen composition, highlighting the accumulation of harmful pollutants in urban environments and the comparatively cleaner conditions in rural areas. Further understanding of differences in the deposition of particulate matter on pollen grains collected from different types of plants or vegetation would pave the way to a better understanding of pollution impacts on plants, ecosystems, and potentially human health through pollen-mediated pathways.

The research on heavy metal pollution's potential effects on honey bees can give greater insights into how heavy metals like lead, cadmium, mercury, and arsenic impact bee health, leading to increased mortality, immune suppression, and developmental issues. Behavioural studies would elaborate on the disruption of navigation, foraging, and communication, such as the waggle dance, and their effects on reproductive behaviours. This kind of research can have a more futuristic exploration of bioaccumulation and the ecological impacts on pollination efficiency and biodiversity and could be a potential topic to be investigated in the near future.

Non-biodegradable heavy metals acknowledged as the most hazardous and toxic pollutants, have proliferated in the environment due to their extensive application in various sectors like domestic, industrial, medical, agricultural, and technological domains. This surge has prompted concerns regarding their detrimental effects on the environment [10].

The pollen and the ovary form potential food after fertilization. If these gametophytes carry pollutants of any form, it would result in severe health hazards not only in humans but in all macro-organisms, specifically animals that rely on plants as food. The accumulation of these heavy metals beyond the threshold level through biomagnification would be fatal and would remain an obscure threat to animal diversity, consequently affecting their health and causing mortality.

Several studies have been made in recent times showing the presence of heavy metals in soils and groundwaters of Peenya Industrial Area urban area from where we had collected pollen from honey bees for our studies (site A). In the case study made by [11], it was found that the quality of groundwater in Peenya is contaminated by industrial discharge containing heavy metals such as cadmium, chromium, copper, iron, lead and nickel. Many water samples showed heavy metals to be crossing the permissible limits. The case study also included testing of samples of topsoil which showed the concentration of heavy metals to be high at topsoil in most of the sampling stations.

Abiotic materials used to indicate pollutants include soil and water, similarly living matter like pollen grains could also be used for finding the presence of pollution. Several studies have shown particulate matter adhered to pollen which has been collected from plants growing in industrial areas or from air using various sampling techniques. Pollen obtained from industrial areas has also shown morphological deformities along with various pollutants. Air pollutants can cause allergic symptoms, but when associated with allergen pollen grains, their allergenicity power is increased [12]. The quality and composition of particles attached

to pollen grains express theoretically a measure of the quality of the local and regional environment [13].

In present SEM studies, pollen grains obtained from honey bees have been used to detect metallic pollutants. A review of studies on bee pollen by Thakur and Nanda [8] indicates that bee pollen naturally contains potassium, phosphorus, calcium, magnesium, zinc and iron. The present SEM/EDS findings have shown metals like aluminium, mercury, chromium, molybdenum, nickel, lead and copper present in the particles adhered to the pollen samples.

Analyzing the data from both Site A and Site B provided valuable insights, confirming the validity of assertion of the researchers that pollen grains serve as indicators of air pollution. When comparing the data from polluted sites with that from non-polluted sites, the pollen grains exhibited a substantial deposition of heavy metal particulate matter. In contrast, Site B, categorised as non-polluted, displays only a minimal presence of organic elements such as carbon and oxygen. In the pollen collected from the Site B, particulate matter was adhered to pollen but no significant metals were found in them.

This supports the notion that pollen grains indeed serve as reliable indicators of air pollution. Pollen grains collected from the contaminated area reveal the presence of Chromium. When this element infiltrates or impregnates the edible portion and reaches the food web, it has the potential to induce toxicity, leading to various pathophysiological complications. These may include allergic reactions, anaemia, burns, and sores, particularly in the stomach and small intestine. Moreover, chromium exposure can adversely impact the male reproductive system, causing damage to sperm and affecting multiple biological systems [14]. The ramifications of chromium pollution extend beyond the immediate health concerns, significantly impacting the water and soil environments. During rainfall, these pollen grains, laden with heavy metals, can be washed into nearby water bodies. This phenomenon may be identified as a major contributor to the global burden of cancer in humans. Specific emphasis is placed on the development of malignant neoplasms in the lung, liver, stomach, and genitourinary system [15].

Beyond chromium, numerous other heavy metals, including Molybdenum, Mercury, Lead, Nickel, and Zinc, as well as non-metals such as Sulfur and Phosphorus, were identified, each exerting significant effects. Molybdenum, for instance, exhibits heightened gastrointestinal absorption in both animals and humans. Single exposure studies in animals have demonstrated that hexavalent molybdenum is readily absorbed from the gastrointestinal tract. The absorption rates range between 40% and 85% in guinea pigs, rats, and goats [16]. The observed health impacts of molybdenum exposure include common complaints such as joint pains, headaches, backaches, and nonspecific hair and skin changes [17].

The presence of lead deposition in pollen grains, which might potentially originate from vehicle and industrial exhaust, raises concerns given that lead toxicity is among the most perilous metal toxicities. Children, in particular, are susceptible to developing lead toxicity. Lead exerts its effects by instigating oxidative stress, a result of the inefficient replenishment of glutathione. Additionally, lead can induce hemolytic anaemia by disrupting cellular membranes through lipid peroxidation. The impact of lead toxicity extends to the disturbance of neurotransmitter levels, contributing to severe health issues linked to organ damage, some of which may result in fatalities [18].

Specific heavy metals like nickel fall into the categories of soluble and insoluble nickel compounds, classified as Group 1 (carcinogenic to humans) and Group 2B (possibly carcinogenic to humans), respectively [19]. This nickel has the potential to directly enter the human food system through unfiltered honey obtained from polluted locations. Water-soluble nickel compounds, once absorbed by the lungs, are eliminated by the kidneys. They can induce irritation in the nose and sinuses, potentially causing the loss of the sense of smell and nasal septum perforation. In contrast, insoluble nickel compounds persist in the lungs for an

extended period and are the forms of nickel responsible for cancer development. Epidemiological studies have revealed increased mortality rates from lung cancer and cancer of nasal cavities among nickel refinery workers due to chronic exposure to nickel-containing dust and fumes[20].

A multitude of studies consistently highlight the release of sulphur compounds as by-products of fossil fuel combustion in vehicles and industries. Sulphur dioxide, a prominent emission resulting from the combustion of sulphur-containing fuels like coal and specific types of oil, is recognized for its adverse effects on both the environment and human health [21]. Scientific investigations have identified SO<sub>2</sub> as a significant contributor to air pollution. Once released into the atmosphere, SO<sub>2</sub> undergoes chemical reactions leading to the formation of sulphate aerosols. Inhalation of these aerosols can have detrimental effects on respiratory health, potentially causing or exacerbating conditions such as asthma and bronchitis [22].

Beyond its impact on human health, sulphur compounds in vehicle exhaust play a role in the generation of acid rain. Sulphur dioxide, upon release into the atmosphere, reacts with water vapour to form sulphuric acid. This acidic precipitation falls to the ground as acid rain, inflicting severe consequences on ecosystems. Acid rain can negatively impact soil quality, water bodies, and vegetation, with extensive ecological implications [23,24]. The adverse effects of sulphur in vehicle exhaust continue to be a central concern in environmental science. Mitigation efforts involve the adoption of cleaner fuel technologies, such as low-sulphur fuels, and the integration of emission control devices in vehicles. These measures aim to reduce the release of sulphur compounds into the air, with the overarching goal of minimizing their negative impact on both the environment and human health [25].

Anemophilous plant pollen is more prone to cause allergies by inhalation as they are smaller in size and are present in the air. As entomophilous pollen grains are larger, they may not be directly inhaled by people. Nevertheless, contaminated bee pollen can contaminate the bee hives and the products obtained from bees like honey and bee pollen with harmful metals. More studies are required to determine the accumulation and effects of these metals in the bee hives. The pollen grains may also become the cause of the spread of pollutants from one area to another.

#### **4. CONCLUSION**

In the present study, the pollen grains were collected from bees; hence, the exact mode of contamination cannot be ascertained. The pollen may have these metals when they were present in the anther or may have come in contact with these pollutants in the air. Bees can also have been the cause of contaminating the pollen grains and it cannot be ignored. The lethality of any form of heavy metals is evident, and one convenient method for evaluating pollutants is through pollen grains. They offer a less combustible and straightforward approach. The significance of pollen grains as indicators of the deposition of heavy metal cannot be disregarded. SEM studies reveal the existence of contaminants but do not provide a quantitative measure of their concentration. Nevertheless, the presence of particulate matter containing metallic pollutants on the pollen is indicative of industrial pollution in the area. Therefore, pollen can be deemed a bio-indicator of pollution.

#### **5. AUTHORS' CONTRIBUTION**

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

#### **6. ACKNOWLEDGEMENTS**

The authors are thankful to Department of Botany, Annamalai University for providing all the facilities required to carry out this research.

#### **7. FUNDING**

There is no funding to report.

## 8. CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest in this work.

## 9. ETHICAL APPROVAL

This study does not involve experiments on animals or human subjects.

## 10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

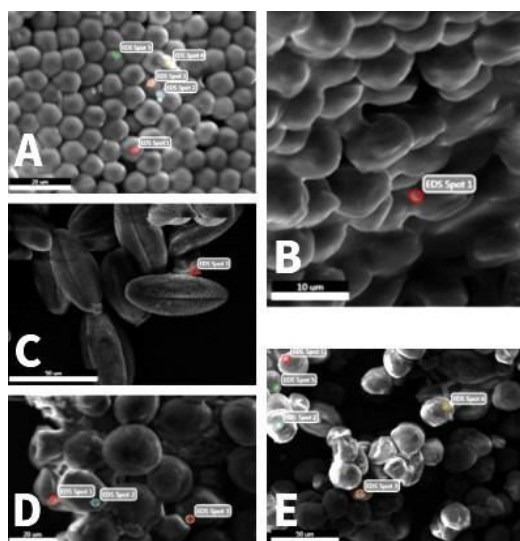
## 11. CONSENT TO PUBLISH

All authors agree to publish the paper in African Journal of Biological Sciences.

## REFERENCES

1. TimothyN, Tagui Williams E. Environmental Pollution by Heavy Metal: An Overview. *Int. J. of Environ. Chem.* 2019; 3(2):72.
2. ShivannaKR, Tandon R. Reproductive ecology of flowering plants: a manual, New Delhi: Springer India; 2014.
3. VisezN, Ivanovsky A, Roose A, Gosselin S, Sénéchal H,PoncetP,*et al.* Atmospheric particulate matter adhesion onto pollen: a review. *Aerobiologia* 2020;36:49-62.
4. SénéchalH, Visez N, Charpin D, Shahali Y, Peltre G, Biolley JP,*et al.* A review of the effects of major atmospheric pollutants on pollen grains, pollen content, and allergenicity. *The Scientific World J.*2015.
5. Wolters JHB, Martens MJM.Effects of air pollutants on pollen. *The Botanical Review* 1987; 53:372-414.
6. Clarke D, Morley E, Robert D. The bee, the flower, and the electric field: electric ecology and aerial electroreception. *J. of Comparative Physiology A* 2017;203:737-748.
7. BogdanovS. The bee pollen book. Bulgaria: Bee Product Science; 2011.
8. ThakurM, Nanda V. Composition and functionality of bee pollen: A review. *Trends in Food Sci. & Tech.* 2020;98:82-106.
9. Papa G,Capitani G, Pellicchia M, Negri I. Particulate Matter Contamination of Bee Pollen in an Industrial Area of the Po Valley (Italy). *Appl. Sci.* 2021; 11(23):11390.
10. Shankar BS.: A critical assay of heavy metal pollution index for the groundwaters of Peenya Industrial Area, Bangalore, India. *Env. Monitoring and Assessment* 2019; 191(5):289.
11. RamakrishnaiahC, Manasa N. Distribution and migration of heavy metals in Peenya industrial area, Bangalore, Karnataka, India-a case study. *Journal of Geography, Environ. and Earth Sci. Int.* 2016; 6(2):1-13.
12. ChehreganiA, Majde A, Moin M, Gholami M, Shariatzadeh MA,Nassiri H. Increasing allergy potency of Zinnia pollen grains in polluted areas. *Ecotoxicology and Environ. Safety* 2004; 58(2):267-272.
13. KalbandeDM, Dhadse SN, Chaudhari PR, Wate SR.Biomonitoring of heavy metals by pollen in urban environment. *Environ. Monitoring and Asmt.* 2008; 138:233-238.
14. Hossini H, Shafie B, Niri AD, Nazari M, Esfahlan AJ, Ahmadpour M, *et al.* A comprehensive review on human health effects of chromium: Insights on induced toxicity. *Environ. Sci. and Pollution Res.* 2022; 29(47):70686-70705.
15. Georgaki MN, Charalambous M, Kazakis N, Talias MA, Georgakis C, PapamitsouT, *et al.* Chromium in water and carcinogenic human health risk. *Environ.* 2023; 10(2):33.
16. TallkvistJ,Oskarsson A. Molybdenum. In*Handbook on the Toxicology of Metals*, Academic Press; 2015,p. 1077-1089.
17. WalravensPA, Moure-ErasoR, SolomonsCC, Chappell WR, Bentley G.Biochemical abnormalities in workers exposed to molybdenum dust. *Archives of Environ. Health: An Int. Journal* 1979; 34(5):302-308.

18. Debnath B, Singh WS, Manna K. Sources and toxicological effects of lead on human health. *Indian J. of Med. Specialities* 2019; 10(2):66-71.
19. Genchi G, Carocci A, Lauria G, Sinicropi MS, Catalano A. Nickel: Human health and environmental toxicology. *Int. J. of environ. Res. and Public Health* 2020; 17(3):679.
20. Seilkop SK, Oller AR. Respiratory cancer risks associated with low-level nickel exposure: an integrated assessment based on animal, epidemiological, and mechanistic data. *Regulatory Toxicology and Pharmacology* 2003; 37(2):173-190.
21. Cao Y, Chen M, Dong D, Xie S, Liu M. Environmental pollutants damage airway epithelial cell cilia: Implications for the prevention of obstructive lung diseases. *Thoracic Cancer* 2020; 11(3):505-510.
22. Tseng CY, Huang YC, Su SY, Huang JY, Lai CH, Lung CC *et al.* Cell type specificity of female lung cancer associated with sulfur dioxide from air pollutants in Taiwan: an ecological study. *BMC public health* 2012; 12(1):1-8.
23. Fatima F, Fatima N, Amjad T, Anjum A, Afzal T, Riaz J *et al.* A review on acid rain: An environmental threat. *Pure and Appl. Biol.* 2020; 10(1):301-310.
24. Tripathi AD. Environmental impact of acid rain: A review. *Asian J. of Multidimensional Res.* 2021; 10(11):592-597.
25. Ng KH, Lai SY, Jamaludin NFM, Mohamed AR. A review on dry-based and wet-based catalytic sulphur dioxide (SO<sub>2</sub>) reduction technologies. *J. of Hazardous Materials* 2022; 423:127061.

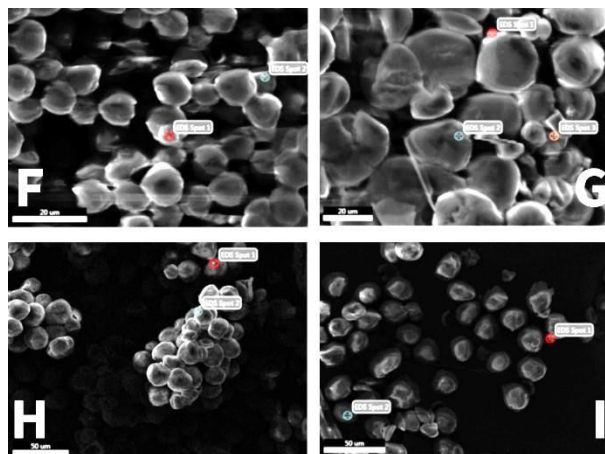


Figures 1A to 1E: Pollen grains from SITE A, indicating the spots on which EDS was performed.

Table 1. Metals found by EDS analysis of the particulate matter adhering to the pollen grains from SITE A.

Figure	Metals present	Non-metals present
Figure A		
Spot 1	Aluminium	None
Spot 2	Molybdenum	None
Spot 3	Sodium, calcium	Sulphur
Figure B		
Spot 1	Mercury, Nickel	None
Figure C		

Spot 3	Mercury, lead, potassium	Phosphorus
Figure D		
Spot 1	Aluminium	None
Spot 2	Mercury	None
Figure E		
Spot 4	Chromium, Iron, Nickel, Copper, Zinc	None
Spot 5	Copper, Zinc	None



Figures 2F to 2I: Pollen grains from SITE B, indicating the spots on which EDS was performed.

Table 2. Metals found by EDS analysis of the particulate matter adhering to the pollen grains from SITE B.

Figure	Metals present	Non-metals present
Figure F		
Spot 1	Calcium	None
Spot 2	None	None
Figure G		
Spot 1	Aluminium (negligible)	None
Spot 2	Mercury (negligible)	None
Spot 3	Aluminium	None
Figure H		
Spot 1	None	None
Spot 2	None	None
Figure I		
Spot 1	None	None
Spot 2	Mercury, Lead, Chromium, Iron, Nickel, and Copper ( all negligible)	None

Table 3: Recurrence of metals and non-metals pollutants on pollen grains collected from honey bees from SITE A (Urban) and SITE B (Rural)

<b>METALS AND NON-METALS</b>	<b>SITE A</b>	<b>SITE B</b>
Sodium	+	-
Calcium	+	+
Potassium	+	-
Aluminium	++	+
Molybdenum	+	-
Mercury	+++	-
Nickel	++	-
Lead	+	-
Chromium	+	-
Copper	++	-
Iron	+	-
Zinc	++	-
Phosphorous	+	-
Sulphur	+	-

**APPENDIX - EDS Spectra Reports**

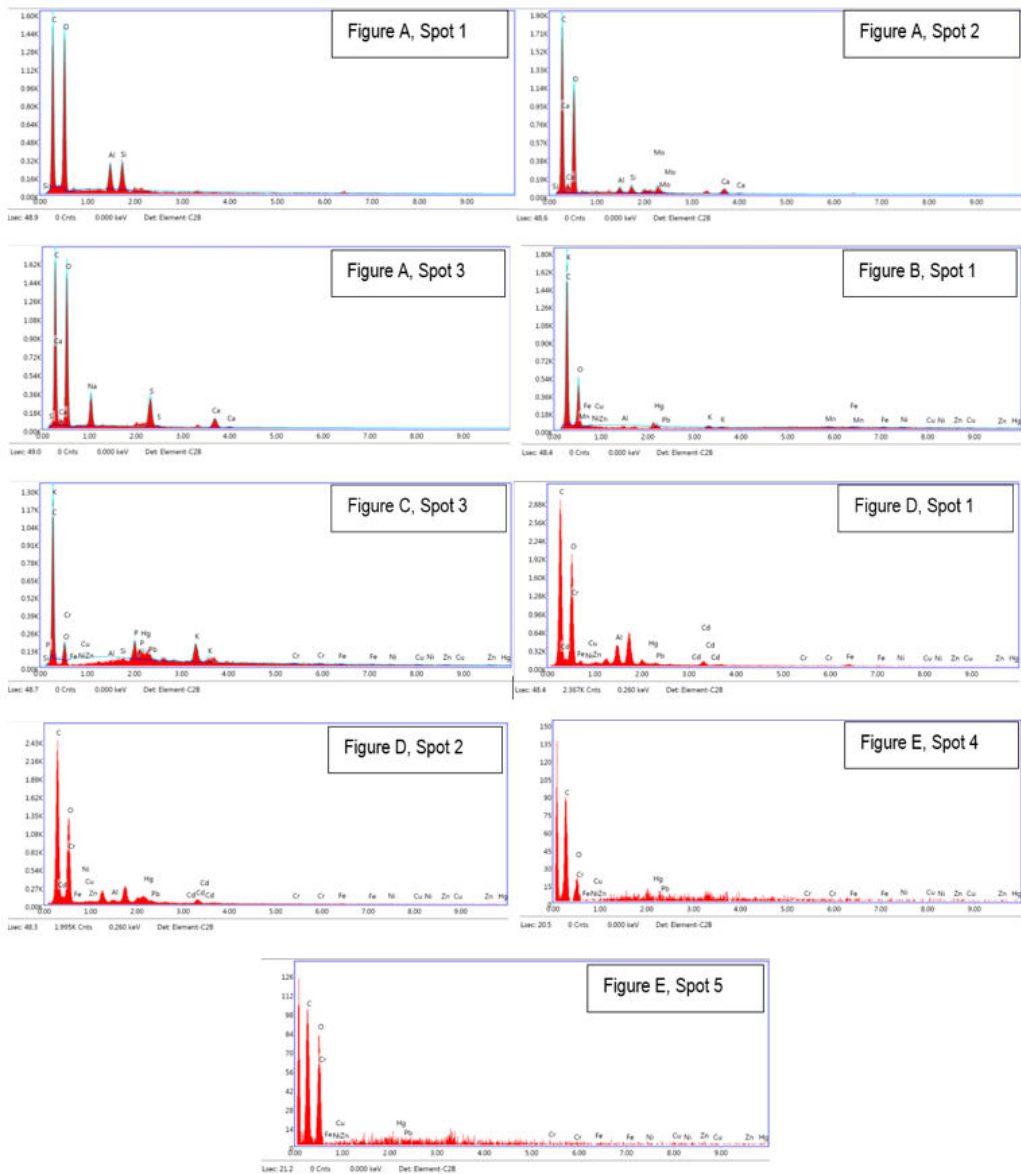


FIGURE 3: EDS Spectra reports of pollen grains collected from SITE A as given in TABLE 1

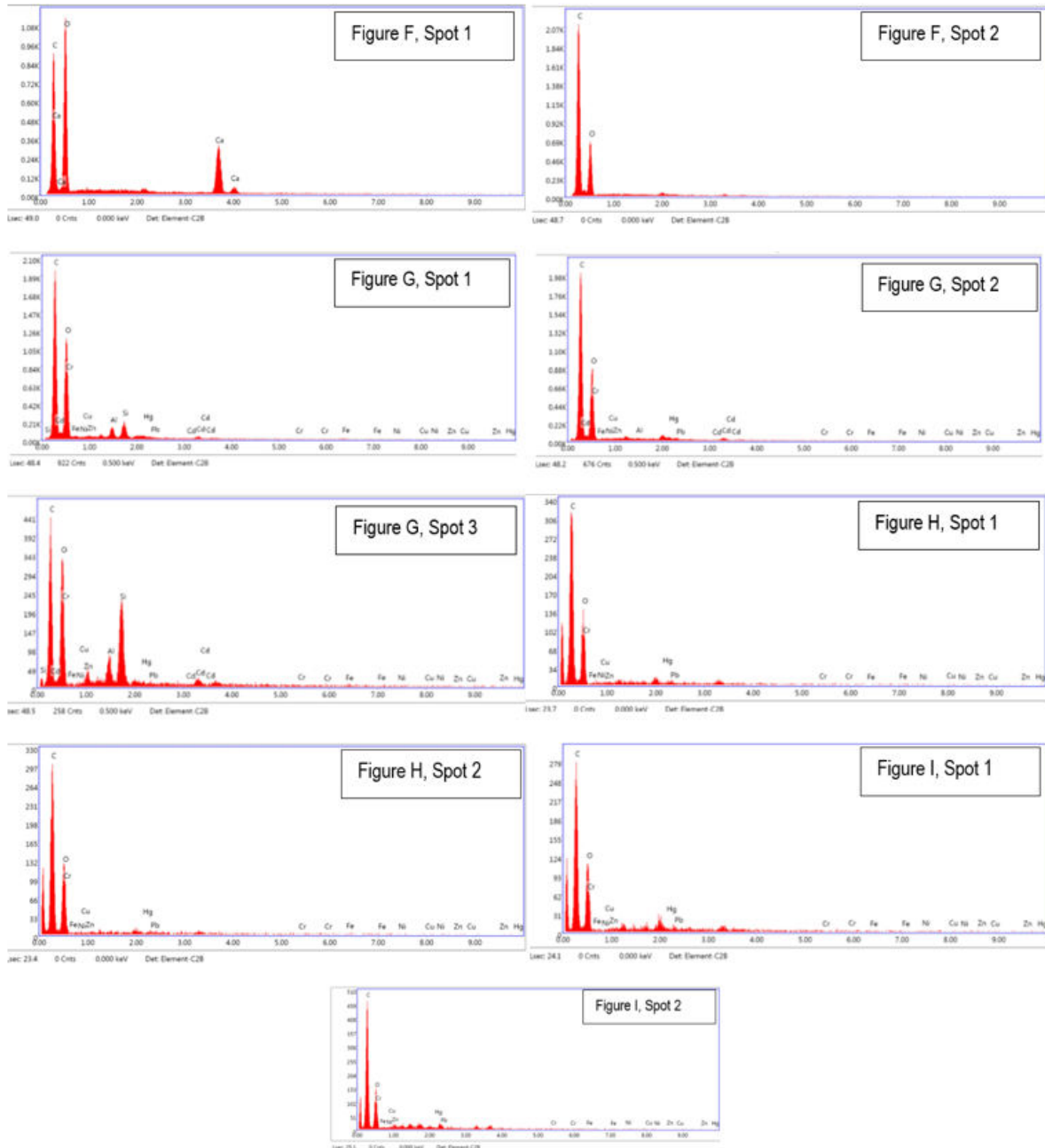


FIGURE 4: EDS Spectra reports of pollen grains collected from SITE B as given in TABLE