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Critical Examination of Sputum Samples for Environmental Pollutants from Industrial Population of Bilaspur City

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Abstract

This study intends to determine how microscopic sputum analysis could potentially aid in identifying environmental toxins associated with breathing issues in order to develop better management approaches for lowering pollution's impact on the lungs. This project used a mixed method technique, which included both quantitative and qualitative research. The study included 50 participants from various socioeconomic backgrounds. A structured questionnaire was created to collect data from participants. The sputum specimens were examined under a bright field microscope. Microscopic investigation of the material using Giemsa stain determined the presence of environmental pollutants in the sample. It is clear that microscopic analysis is an effective technique for investigating the intricate interactions between environmental contaminants and respiratory hazards. Furthermore, microscopy provides a cost-effective and efficient method of assessing respiratory health in people exposed to pollutants, allowing for early identification and intervention to prevent the development of significant respiratory disorders. Thus, this study seeks to establish how microscopic sputum analysis could potentially aid in pinning down environmental toxins linked with breathing difficulties so as to come up with better management approaches towards reducing pollution impacts on lungs.

Introduction

In relation to respiratory health, environmental pollutants have become a growing concern, especially among individuals who are exposed to high levels of air pollutants.¹ Respiratory problems caused by these agents are a serious public health issue that subjects patients, to higher health care costs and poor quality of life.² For instance, microscopic analysis of sputum has emerged as an important diagnostic tool for detecting the presence of pollutants in the respiratory

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Keywords

Air pollution; Air borne particle; Health hazards; Respiratory risks; Sputum analysis. system which can shed light on how pollution affects human health.³ It also helps identify specific factors causing symptoms based on which regulations can be made for minimizing public danger from different types of these pollutants.⁴ Environmental pollution happens when there is an unwanted change in the environment that negatively impacts both plants & animals. The substances that pollute are called pollutants. They can be liquid, solid, or gas.⁵ When a substance's concentration goes beyond what is naturally present, it becomes a pollutant. Human actions or even natural events can cause this rise.6 Some pollutants can decompose quickly through natural processes, while others can stick around for decades and are hard to clean up once released. Examples include DDT, plastics, heavy metals, & nuclear waste.7

Particulate Matter Pollution

The term "particulate matter" describes the mixture of liquid droplets and solid particles that are present in the atmosphere. Some particles like dust, smoke, and soot can be seen with the naked eye; however, others are only detectable using an electron microscope.⁸ Emissions from power plants, industries, and vehicles create complex chemical interactions that generate particulate matter.⁹ PM2.5 & PM10 are commonly used methods to assess air quality by measuring particles in micrograms per cubic meter.¹⁰ There are numerous potential sources of PM2.5, and each may produce particles with unique chemical compositions and physical attributes. PM2.5 frequently contains ammonium, nitrates, black carbon, and sulphate.¹¹ Combustion engines, industrial processes, power generation, burning coal and wood, agricultural activities and other human-caused sources of PM2.5 building. Dust storms, sandstorms, and wildfires are examples of natural sources.

Air quality Index(AQI)

Emissions from eight major air pollutants are measured to calculate the Air Quality Index (AQI). These consist of lead (Pb), ammonia (NH3), carbon monoxide (CO), nitrogen dioxide (NO2), sulfur dioxide (SO2), ozone (O3), particulate matter (PM2.5 & PM10), and lead (Pb).¹² Every hour, readings are measured. Based on particular criteria, each country has its own air quality index.

Government agencies play a key role in informing the public about health risks related to air pollution. As part of the Swachh Bharat Abhiyan campaign, the National Air Quality Index was introduced in New Delhi in September 2014 to help to solve the issue. The Central Pollution Control Board (CPCB) established an expert committee that consists of State Pollution Control Boards (SPCBs), advocacy organizations, academic leaders, medical professionals, and experts in air quality. IIT Kanpur additionally received a technical study too. The recommendations for an Air quality index scheme for India came from the expert group and IIT Kanpur in 2014. Six major cities- Mumbai, New Delhi, Kolkata, Pune & Ahmedabad-have a system that monitors air quality continuously and collects data in real-time.13

AQI	Classification	Impacts on Health
0-50	Good/safe	Minimal effect
51-100	Satisfactory	Breathing problems are modest for sensitive people.
101-200	Moderately polluted	Patients with lung illness may have trouble breathing; others might experience discomfort.
201-300	Poor	Discomfort for heart disease patients; prolonged exposure causes breathing troubles.
301-400	Very poor	Long-term exposure-induced respiratory disease; serious consequences for people with heart and lung conditions.
401-500	Severe	Respiratory problems can affect healthy people, and patients with heart or lung diseases may face serious health consequences.

Table 1: Categories of Air Quality Index

Current Scenario of Air Quality Global Scenario

The countries, regions, and territories in Africa, Central Asia, and South Asia had the highest annual average PM2.5 concentrations in 2023 when weighted by population. Despite the fact that data on air quality in Africa is steadily growing, just 24 of the 54 countries had sufficient data for the 2023 research, leaving 30 countries missing.¹⁴

Since 2019, Afghanistan has constantly been among the top 15 most polluted countries. Afghanistan

and Oman, which were listed as the sixth most polluted nation in 2022, are noticeably absent from the list due to data availability issues.¹⁵ Twenty new countries were added in 2023, including Burkina Faso (ranked fifth in 2023) and Rwanda (ranked fifteenth in 2023) for pollution. In 2023, 10 nations, territories, and places met the WHO annual PM2.5 standard, with many of them located in Oceanic.¹⁶

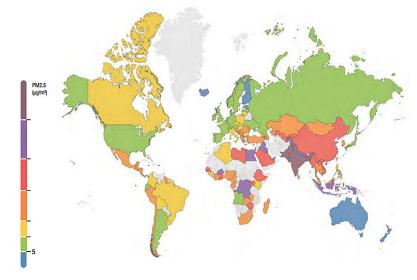


Fig. 1: PM2.5 concentration (µg/m3) over global area

National Scenario

India continues to suffer from poor air quality, coming in at number three in global level and in the region in 2023.¹⁷ In 2023, the average annual PM2.5 concentration increased little to 54.4 μ g/m³ contrasted with 53.3 μ g/m³ 2022. In Delhi, the National Capital Territory, PM2.5 levels increased by 10% in 2023, reaching a monthly average of 255 μ g/m³ in November, when levels peaked.¹⁸ It is approximated that PM2.5 levels in India exceed the WHO's yearly recommendation of 5 μ g/m³, affecting 1.36 billion people.

Also, 96% of the population, have PM2.5 levels that are seven times higher than this limit. More than 66% of the nation's cities report yearly averages higher than 35 μ g/m³, which is consistent with this trend in city-level statistics. India is home to more air quality monitoring stations than all other countries in the region combined, indicating the size of its large air quality monitoring network. In 2023, 256 cities contributed data to the vast monitoring network, accounting for 74% of all cities in Central and South Asia.Thirteen of the fifteen cities listed are in India, accounting for a nearly equal share of the cities in the region that are the most polluted.¹⁹

Role of Forensic Science in Controlling Environmental Pollution

Forensic science is essential in tackling environmental pollution. It uses scientific methods to identify, analyze, & reduce pollution incidents. This field is a vital part of the fight against pollution as it offers rigorous, evidence-based insights along with practical solutions. It is multidisciplinary in nature as it addresses environmental issues from detection all the way to remediation & policy creation. key roles include

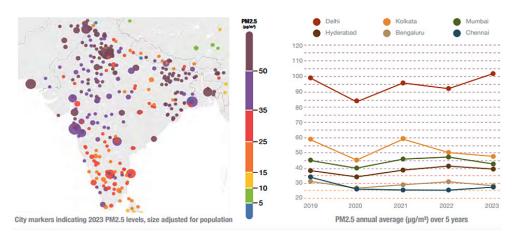


Fig. 2: PM2.5 concentration over Indian cities & Annual average over 5 years

Identification of Pollutants

Forensic scientists employ advanced techniques to find pollutants in various environmental samples-like air, soil, & water. These include harmful substances such as heavy metals, pesticides, & organic chemicals.

Source Attribution

determining the origin of pollution is crucial for effective control measures. Forensic methods can trace pollutants back to their sources using techniques like chemical fingerprinting, DNA analysis or isotopic analysis. This information helps to hold polluters accountable & aids in targeted interventions.²⁰

Environmental Monitoring

Ongoing monitoring programs rely on forensic techniques. These programs involve routine sampling &analyzing of environmental media to observe changes in pollutant levels over time. By spotting trends and catching emerging pollutants early on, forensic science plays a role in proactive pollution management.²¹

Legal & Regulatory Support

Forensic evidence matters in court cases linking to environmental pollution. Forensic experts may provide testimony and share opinions based on the examination conducted by them. Such evidence bolsters regulatory actions & enforcement of environmental laws, ensuring polluters face consequences for their actions.²²

Remediation Strategies

After identifying pollutants & determining their sources, forensic science helps create effective cleanup strategies. These strategies focus on cleaning contaminated sites and improving environmental quality. Forensic data directs choices regarding cleanup technologies and confirms the success of these efforts.²³

Policy Development

At all levels-local, national, and international forensic science informs the creation of environmental policies. Data and insights from forensic studies help set pollution standards, shape regulations, & design management plans for the environment. This effort prevents pollution and encourages sustainable practices.²⁴

Public Awareness & Education

By sharing clear scientific evidence, forensic science works to raise public awareness about pollution issues. This awareness fosters community engagement and motivates responsible actions by both industries and individuals. It also supports conservation projects.²⁵

Research & Innovation

Forensic scientists are always creating new techniques and technologies to better detect, analyze, & control pollution. Research in fields like forensic chemistry, biology, and environmental science leads to advancements that enhance efficiency and accuracy in tackling these investigations.²⁶

Bright Field Microscope

A bright field microscope, sometimes referred to as a compound light microscope, is an optical microscope that creates dark pictures on a light background by using light rays. Specimens that have been dyed with simple dyes to provide contrast between the image and the image backdrop are observed using this microscope. It is specifically made with a lens, which is a magnifying glass that alters the sample to produce an image that is seen through the eyepiece.

Principle

A specimen needs to travel through a uniformly lighting light beam in order to be focussed and create a picture. The microscope creates a contrasted image by using differential absorption and differential refraction. To make it easier to characterize contractions, the samples are first processed by staining. A combination of absorption and refraction contrast is represented by the refractive index, which sets color samples apart from their surroundings. A microscope's capacity to generate high-resolution images from a suitably supplied light source focused on the image is essential to its operation. To view the sample on a microscope slide, it is submerged in oil or covered with a cover slip.

Parts of A Bright Field Microscope

Eyepiece (ocular lens) – In order to help focus the picture from the objective lens, the microscope contains two eyepiece lenses at the top. This is where the image created by unaided eyes can be seen.

A clean image of the specimen or object being focused is provided by the objective lens, which is made up of six or more glass lenses.

The image can be focused by moving the stage using two focus adjustment knobs on the arm. They serve to guarantee the production of crisp, clear images. -The sample is placed on the stage, which is situated directly beneath the objective lens. The light is concentrated here, and the sample can be moved for improved visibility using flexible buttons.

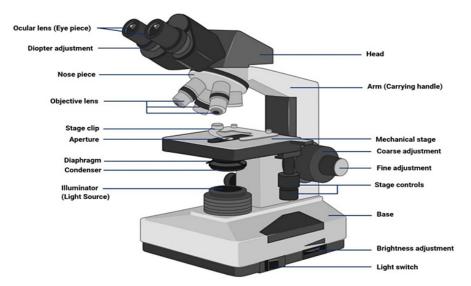


Fig. 3: Bright Field Microscope

- Condenser: It is mounted under the platform to focus the light beam on the sample. To adjust the light quality, it can be fixed or movable; but that depends entirely on the microscope.
- Arm:This is the solid metal backbone of the microscope, used to carry and move the microscope from one location to another. They contain a microscope base which is a

microscope stand. The arm and base combined hold all other parts together.

- There is a light illuminator or mirror placed at the base or tip of the microscope.
- The nosepiece has about two to five objectives with different magnifications. It can move to any position depending on the lens to focus the image.

 An Aperture (Contrast) Diaphragm: It controls the diameter of the light beam passing through the condenser. When the condenser is nearly closed, light passes through the centre of the condenser creating high contrast, and when the condenser is wide open, the image is very bright with very low contrast.

Environmental pollutants are increasingly affecting respiratory health, especially among those exposed to high pollution levels. These pollutants lead to serious health issues, raising healthcare costs and reducing quality of life. Sputum analysis is a key diagnostic tool for identifying pollutants in the respiratory system and can inform regulations to reduce public exposure. Pollution occurs when unwanted elements interfere with the environment, causing harm to plants and animals. Pollutants can be solid, liquid, or gas and can come from human activities or natural events. The Air Quality Index (AQI) measures eight major pollutants to inform the public about air quality. India faces significant air quality challenges, ranking high in pollution levels. The role of forensic science is crucial in combating environmental pollution by identifying pollutants, determining their sources, and supporting legal actions. Forensic techniques also help monitor pollution levels and develop effective clean-up strategies. The bright field microscope is an essential tool for observing specimens, using light to create contrast for analysis. It includes components like eyepiece lenses, objective lenses, focus knobs, a stage for samples, and a condenser to focus light. Addressing environmental pollution is vital for public health, requiring comprehensive monitoring, regulatory support, and community awareness through forensic science.

Materials and Methods Study Area

The district of Bilaspur, situated in the state of Chhattisgarh in central India, is renowned for its historical significance and rich cultural legacy. The coordinates of Bilaspur are 22.09°N 82.15°E. It is 264 meters (866 feet) above sea level on average. The district of Bilaspur is an area where about 2.5 million people live. The district is distinguished by its heterogeneous population, comprising a blend of indigenous cultures and additional ethnicities. The Bilaspur district is home to several enterprises that are important to the local economy, such as coal mines, steel mills, thermal power plants, and cement factories.²⁷

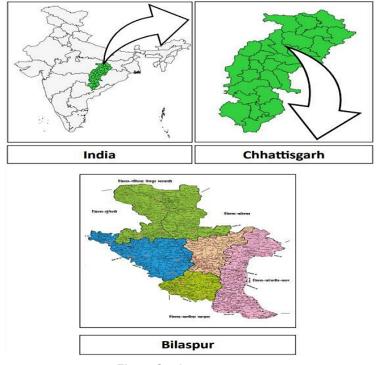


Fig. 4: Study area on map

This work was targeted on the population living near exposed arena or near industrial areas where they were more vulnerable to harmful environmental pollutants. The study was carried out on a total number of 50 participants ranging between 21-55 years of age, belonging to different socioeconomic classes. The participants were eventually selected from different groups namely, field labourers, teachers, housewives and students.

Data Collection

Through Questionnaire

a structured questionnaire was designed which constitute two parts. Part 1 contain socio demographic details including age, gender, marital status, residential area, eating habits, educational status, occupational status and duration of exposure at residential area. Along with socio-demographic data, it contained details regarding nicotine or alcohol addiction and the duration of addiction. Part 2 contain questions regarding issues related to various body systems such as respiratory system, cardiovascular system and dermal system of the participants.

Ethical clearance and consent from the participants were respectfully taken prior to obtaining their data. The aim and objectives of the work were clearly mentioned to the participants along with the importance of their responses for the prepared questionnaire. All the answers provided were noted down as self-reported.

Sample Collection Sputum Sampling Process

Each of the 50 participants received a sterile plastic container for collection of sputum sample. The participants were requested to provide sputum samples of about 1 ml in the sterile container with their name and category indicated on the provided container. These specimens were then analysed within three to five hours after collection.

Analysis

Sputum samples from the container were taken with the help of a dropper to a clean glass slide and then few drops of methanol were added to it. Subsequently, it was allowed to air dry and then photographs of the slide containing sample and methanol was taken. Then, one drop of Giemsa Stain solution was added to the slide. With the help of forceps, cover slips were placed onto the glass slide avoiding formation of bubbles and then photographs were taken.

Microscopic Analysis

The prepared glass slide was placed under the bright field microscope and then the sample was analysed at 10x and 40x magnification for determining foreign particles in the sputum sample. Then with the help of a software named Image View, the image of the sputum sample analysed was taken. Procedures were performed carefully and the images were taken accordingly.

Statistical Analysis

The entire data regarding socio-demographic profile and issues related to various systems were noted down in a tabularized format and the percentage and frequency were calculated accordingly. All the required data were precisely collected. The observations were noted down in tabularized format and the sputum sample viewed under microscope was represented in picture format.

Results

50 people participated in the study those were between the age group 21-55 years as mentioned below (Figure 5.a). 38 participants were males and 12 participants were females (Figure 5.b). The study population were divided into different groups i.e., 13 participants were teachers, 13 participants were students, 12 participants were labourers and 12 participants were housewives (Figure 5.c). 13 participants of the total study population were exposed to industrial emissions for less than or equal to 5 years, 7 participants of the study population were exposed between 6-10 years, 10 participants were exposed between 11-15 years and 20 participants were exposed for greater than 15 years (Figure 5.d). Self-reported data about various health related issues including respiratory issues, cardio-vascular issues and dermal issues are also noted down. It was noted that 23 participants of the total study population were not having any dermal system issues, whereas 17 participants showed allergic reactions and 10 participants showed other minor issues (Figure 6.c). It was seen that 20 participants of the group had symptoms of cough, cold and wheeze whereas 30 participants showed other respiratory

issues (Figure 6.b). Majority of the study population (40 participants) showed no signs of cardiovascular difficulties, and only a small percentage showed signs of hypertension (3 participants) and palpitation, chest pain, breathlessness (7 participants) (Figure 6.a).

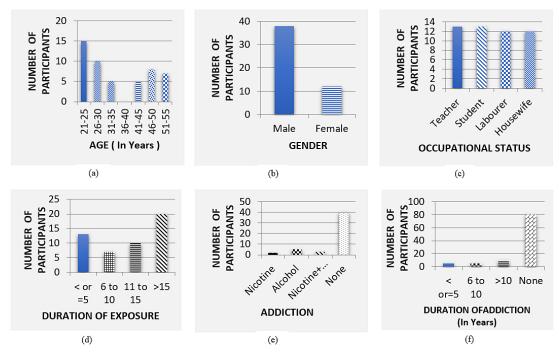
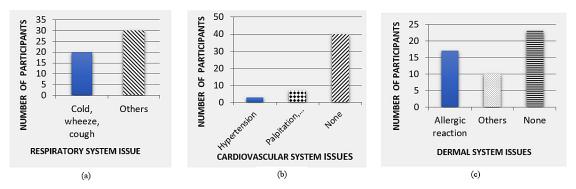
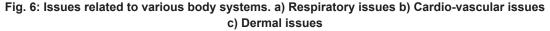


Fig. 5: Socio-demographic parameters under study. a) Age b) Gender c) Category of Population d) Duration of Exposure e) Addiction f) Duration of Addiction





Microscopic examination of sputum sample using bright field microscope showed accurate results regarding the presence of foreign particles within the sample.

Sputum Sample (teachers)

From the analysis of sputum samples (teachers), it was seen that majority of the sample were normal

and showed very minute or no cytological changes. As they were not directly exposed to heavy environmental pollutants, minimum or no cytological changes were observed.

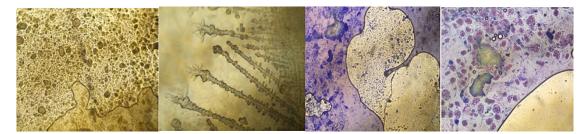


Fig. 7: Sputum sample of teachers observed under microscope showing presence of foreign particles

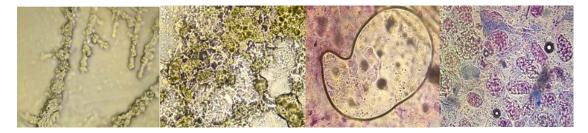


Fig. 8: Sputum sample of students observed under microscope showing presence of foreign particles

Sputum Sample (Students)

Most sputum samples from students showed normal results with few or no cytological disturbances due to low exposure to pollutants.

Sputum Sample (Labours)

From the analysis of sputum samples of labours, it was seen that majority of the sample showed

cytological disturbances and presence of foreign particles. As they are directly exposed to heavy environmental pollutants in the work area, different types of foreign particles such as fibres, heavy dust, etc., were observed. These foreign particles ranged from small to large sizes.

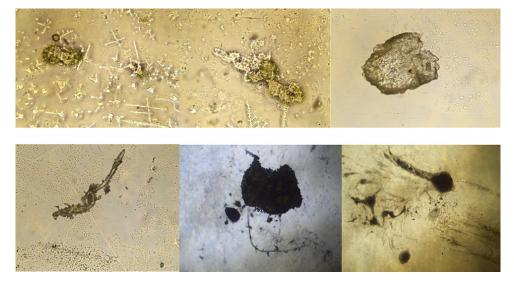


Fig. 9: Sputum sample of labours observed under microscope showing presence of foreign particles

Sputum Sample (Housewives)

From the analysis of sputum samples of housewives, it was seen that majority of the sample were normal and shown very minute or no cytological disturbances. As they had minimum exposure to heavy environmental pollutants, minute or no cytological changes were observed.

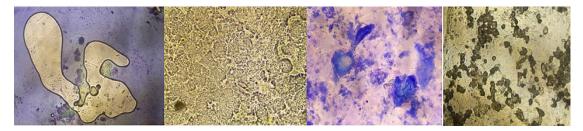


Fig. 10: Sputum sample of housewives observed under microscope showing presence of foreign particles

Under microscopic examination where most of the population's samples were normal, majority of the laborers' population showed presence of various foreign particles or environmental pollutants that varied in their shapes and sizes. Among the total sample population, laborers' population those are directly exposed to industrial and field works seemed to have high no. of foreign particles in their sputum samples compared to other samples.

Discussion

Microscopic analysis of sputum samples with Giemsa stain comes up as a critical method to unravel the connection between the environmental pollution and respiratory diseases.^{28,29} The study determined the relationship between the air pollution exposure in an industrial area and their effects on the individuals residing nearby. Among the study population, labourers who are directly exposed to air pollution show greater number of cytological changes or presence of foreign particles than those who are not directly exposed.³⁰ 12 participants of the total study population were of labourers where it showed significant increase in the presence of environmental pollutants that could cause respiratory issues and other major diseases. Additionally, occupation also had a crucial role in creating numerous cytological changes in sputum because laborers were directly exposed to the industrial region as opposed to students, housewives, and teachers.28

These results align with the research carried out by Magdi Mansour Salih and colleagues. In their study, cytopathological alterations in a control group residing in a rural residential area were compared to exposure to air pollution in an industrial sector of Sudan. When compared to the control group, the sputum sample obtained from people inhibiting the Khartoum North Industrial Area showed an elevation in the number of inflammatory cells and epithelial inflammatory alterations, indicating an inflammatory reaction.²⁸ Florence N. Schleicher and Mazzei's investigations showed that sputum analysis and inflammatory components can be employed to improve lung function in asthmatic patients.³¹ Additionally, a number of studies have linked pulmonary cytological alterations to air pollution. These two investigations demonstrated that allergic disorders can be brought on by diesel exhaust particle air pollution, which results in elevated IgE production and preferred Th2 cell activation.^{32,33} According to our study, the most significant contributing factor to these cytological alterations that would directly link to various health concerns along with being a worker in that field is living and working close to an industrial region and significant exposure to severe air pollution. Sputum cytology has a good diagnostic accuracy for endobronchial tumor detection, according to research by A. Van Rensburg.³⁴ Age was a predominant factor that also contributed to the changes. Teachers and pupils showed little to no signs of these contaminants, even when they were exposed to low levels of pollution. The study by W Merrill and colleagues, which discovered a connection between cytological alterations and lavage fluid, lends credence to this conclusion. According to them, sputum that is inflammatory qualifies as a significant indicator of

bronchial damage.³⁵ Madison et al.'s study came to the conclusion that abnormal pulmonary functional tests might be strongly predicted by sputum cytology. Additionally, they suggested that it might point to a connected subject's propensity for lung illness.36 Working close to an industrial region and being exposed to it on a daily basis may cause cytopathological alterations that could eventually result in lung cancer. Numerous other research on the effects of ambient air pollution have demonstrated a relationship between lung function and exposure duration.^{37,38} In this investigation, cytopathological alterations were substantially correlated with gender. Male participants showed a higher incidence of cellular atypical alterations than female participants when exposed to pollutants in an industrial location. According to a 2017 epidemiological study, there is insufficient evidence to establish a gender-specific effect of air pollution on lung function in children and adolescents.³⁷ But according to certain research, men who were exposed to air pollution showed more alterations in their respiratory systems than did women.³⁹ The respiratory system's capacity for regeneration and the immune system's progressive loss of effectiveness can account for the rise in cytopathological alterations that accompany aging. Furthermore, men experience cytopathological changes more frequently than women; this could be due to the fact that men are more susceptible than women to occupational exposure and indoor and outdoor pollution. As research in this area continues to evolve, incorporating advancements in technology and methodology, the use of sputum analysis holds great promise for enhancing our ability to safeguard respiratory health in the face of growing environmental challenges.⁴⁰ Moving forward, further research efforts should focus on standardizing sputum analysis techniques and expanding its application to different populations and environmental settings to maximize its potential for improving respiratory health outcomes.

Conclusion

In conclusion, the microscopic analysis of sputum for environmental pollutants and respiratory risks presents a valuable tool in assessing the impact of external factors on respiratory health. Through the examination of sputum samples, researchers can identify the presence of harmful pollutants which have been linked to various respiratory diseases. This method allows for a more comprehensive understanding of the sources and levels of pollutants that individuals may be exposed to in their environment, enabling targeted interventions to mitigate the associated health risks. This study conducted in the Bilaspur district of Chhattisgarh, India, targeted individuals living near industrial areas exposed to environmental pollutants. Fifty participants aged 21 to 55 were surveyed through structured questionnaires to gather sociodemographic information and health issues related to respiratory, cardiovascular, and dermal system. Further, sputum samples were collected and analysed showing that most samples from teachers, students and housewives had shown minimal changes, while laborers exposed to pollutants indicated significant cytological disturbances. This suggests that direct exposure to pollution correlates with respiratory health issues. The findings align with existing researches linking air pollution to respiratory diseases, emphasizing the need for effective pollution management strategies. The microscopic analysis of sputum offers a non-invasive and easily accessible means of monitoring respiratory health, making it a practical technique for large-scale studies aimed at assessing environmental impacts on public health. In light of the findings discussed in this paper, it is evident that microscopic analysis of sputum provides a valuable approach for investigating the relationship between environmental pollutants and respiratory risks. In order to lessen the burden of respiratory disorders linked to environmental pollution, public health policies and interventions must be informed by this information. Therefore, the microscopic analysis of sputum represents a powerful tool for exploring the complex interplay between environmental pollutants and respiratory risks. As technologies and methodologies evolve, the use of sputum analysis is poised to play an increasingly important role in safeguarding public health in the face of ongoing environmental challenges. Public health faces significant difficulties because of the complexity of environmental pollutants, especially in comprehending their effects on respiratory problems. The microscopic analysis of sputum comes up as a critical method to unravel the connection between the environmental pollution and respiratory diseases. Air pollutants and microplastics, which are some of the environmental contaminants, can result in respiratory health effects such as lung

dysfunction and cardiovascular diseases. With this technique, researchers are able to identify if these pollutants are present within the lungs thereby improving levels of knowledge regarding quantities encountered by people and possible dangers relating to them. Not only is this analytic approach useful in diagnosing respiratory diseases associated with pollution but also it assists in informing focused interventions alongside policy measures geared towards minimizing public health's vulnerability to environmental toxins.

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Conflict of Interest

The authors do not have any conflict of interest.

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This statement does not apply to this article.

Ethics Statement

Ethical clearance has been taken from the concerned ethical clearance committee of Guru Ghasidas Vishwavidyalaya.

Informed Consent Statement

Informed consent has been taken from all the participants participated in this study.

Author Contributions

Each author mentioned has significantly and directly contributed intellectually to the project and has given their approval for its publication.

- Sanjida Shabnam: Conceptualization, Methodology, Analysis, Writing - Original Draft.
- **P. Vishnupriya:** Data Collection, Analysis, Writing & Editing.
- Dr. T. L. Chandra: Resources, Supervision-Review & Suggestions.
- **Dr. Sudhir Yadav:** Visualization, Project Administration, Supervision- Final Comments.

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