

**EFFECTS OF FINE AEROSOL AND ITS TOXIC  
COMPONENTS ON  
HUMAN LUNG - AN INTEGRATIVE APPROACH**

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COMPONENTS ON  
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*by*

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*Submitted*

**in fulfilment of the requirements of the degree of Doctor of Philosophy**

*to the*



**INDIAN INSTITUTE OF TECHNOLOGY DELHI  
SEPTEMBER 2020**

*This Thesis is Devoted to Maa and*

*Baba*

*For their endless love, support and*

*sacrifices....*

## **CERTIFICATE**

This is to certify that the thesis entitled “**EFFECTS OF FINE AEROSOL AND ITS TOXIC COMPONENTS ON HUMAN LUNG- AN INTEGRATIVE APPROACH**” being submitted by **Miss. ANANYA DAS** to the Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy** is a record of the original bonafide research work carried out by her under my guidance and supervision. The thesis work, in my opinion, has reached the requisite standards fulfilling the requirement for the Degree of Doctor of Philosophy.

The results contained in this thesis have not been submitted in part or in full to any other University or Institute for the award of any degree or diploma.

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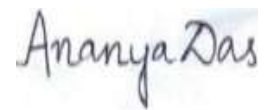
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## ABSTRACT

Delhi, India has been identified as one of the highly polluted cities in the world and recently associated with the highest population-weighted concentration of particulate matter of aerodynamic diameter less than equal to  $2.5\ \mu\text{m}$  ( $\text{PM}_{2.5}$ ). Increase in industrialization and traffic counts in the city stands as one very important reason for the immense air pollution in the city. However, the unavailability of the health risk estimations using long-term data for Indian cities and especially for Delhi has been pointed out as a difficulty in the conducting human health risk assessment.

The main objective of this study was to understand the health risk posed by particles of different aerodynamic sizes ranging from  $\text{PM}_{2.5}$  to  $\text{PM}_{0.25}$  (particulate matter less than or equal to  $0.25\ \mu\text{m}$  aerodynamic diameter) by theoretical risk calculation using realistic values and also by *in-vitro* toxicity analysis using lung epithelial cell line. The thesis had three sub-objectives. The first objective was to understand the difference between ambient concentrations of particles of different sizes at two heights (10 m and 1.5 m) using a year-long monitoring. The second objective was to understand cytotoxicity of particles of different sizes to lung epithelial A549 cell lines, by measuring the cell viability along with the generation of reactive oxygen species (ROS) and pro-inflammatory cytokines during exposures of fine ambient  $\text{PM}_{2.5}$  and all the other smaller sizes. The effect of mass concentration (monthly as well as seasonally) of particulate matter on the cellular cytotoxicity were compared. Lastly, the risk estimates due to exposures of particulate matter and associated metals were estimated for lungs (considering metal (singular) as well as binary mixtures of metals). Further, the respiratory deposition dose (RDD) of both the ambient fine and very fine particles were calculated using the human respiratory tract model and analyzed for estimating depositions of PM-associated metals to lungs and

different organs, such as gall bladder and other tissues using the physiologically based pharmacokinetic model (PBPK) models.

The main findings of the study include a higher deposition of particles less than 250 nm in the alveolar region, and also the difference between the mass concentrations of PM sampled at two different altitudes. The mean mass concentration plays a vital role in the calculation of both individual risk and respiratory deposition dose (RDD) values.

More chronic diseases can occur if this exposure remains for a long period. Cyto-toxicity studies showed seasonal- and mass-dependent variations of cytotoxicity and ROS generation potential, and the pro-inflammatory response of the cytokines (IL-6 and IL-8) due to particles of different sizes. Few metals, irrespective of the carcinogenicity, showed a strong positive correlation with the biological endpoints. The PM mass concentration in bioavailable form showed a positive correlation with Arsenic (As), Lead (Pb), Copper (Cu). Not all biological responses showed consistent correlations with other biological endpoints. Metals, such as As, Cr, and Cd governed the cytotoxicity of biological -points in winter season for Delhi. The pro-inflammatory responses indicated that Delhi ambient PM<sub>2.5</sub> particles showed high fold of difference (3-53 folds) from the unexposed samples for 2 kinds of cytokine responses.

The study also dealt with focusing on the following vital issues: (i) Though PM<sub><0.25</sub> is only a small fraction of total PM<sub>2.5</sub>, its impact has been seen more stronger on bio-points compared to PM<sub>2.5</sub> particles, (ii) increased cytotoxicity of PM<sub><0.25</sub> and the need of its monitoring. The *in vitro* toxicity data also showed that the dependency not only depends on mass concentration of both the fine and the very- fine size particles but also depends on the physiochemical characteristics of constituents as well.

## सार

दिल्ली, भारत की पहचान दुनिया के सबसे प्रदूषित शहरों में से एक के रूप में की गई है और हाल ही में सबसे अधिक जनसंख्या-भार वाले  $PM_{2.5}$  एकाग्रता के साथ जुड़ा हुआ है। शहर में औद्योगिकीकरण और यातायात में वृद्धि शहर में भारी वायु प्रदूषण का एक बहुत महत्वपूर्ण कारण है। हालांकि, भारतीय शहरों और विशेष रूप से दिल्ली के लिए लंबे समय के डेटा का उपयोग करके स्वास्थ्य जोखिम अनुमानों की अनुपलब्धता को मानव स्वास्थ्य जोखिम मूल्यांकन के संचालन में कठिनाई के रूप में बताया गया है।

इस अध्ययन का मुख्य उद्देश्य आकार के 2.5 माइक्रोन के पार्टिकुलेट मामले से उत्पन्न स्वास्थ्य जोखिम को समझना था, जो कि यथार्थवादी मूल्यों का उपयोग करते हुए सैद्धांतिक जोखिम गणना द्वारा और फेफड़ों के उपकला सेल लाइन का उपयोग करके इन-विट्रो विषाक्तता विश्लेषण द्वारा 0.25 से कम आकार का है। थीसिस के तीन उप-उद्देश्य थे। पहला उद्देश्य एक साल की निगरानी का उपयोग करके दो ऊंचाइयों (10 मीटर और 1.5 मीटर) पर विभिन्न आकारों के कणों की परिवेश सांद्रता के बीच अंतर को समझना था। दूसरा उद्देश्य ठीक-ठीक परिवेश  $PM_{2.5}$  के जोखिम के दौरान प्रतिक्रियाशील ऑक्सीजन प्रजातियों (आरओएस) और प्रो-भड़काऊ साइटोकिन्स की पीढ़ी के साथ सेल व्यवहार्यता को मापने के द्वारा फेफड़ों के उपकला  $A_{549}$  सेल लाइनों के लिए विभिन्न आकारों के कणों की साइटोटॉक्सिसिटी को समझना था। अन्य छोटे आकार। सेलुलर साइटोटोक्सिसिटी पर परिवेश और व्यक्तिगत पार्टिकुलेट मैटर दोनों के द्रव्यमान (मासिक के साथ-साथ मौसमी) के प्रभाव की तुलना की गई। अंत में, पार्टिकुलेट मैटर और संबंधित धातुओं के एक्सपोजर के कारण जोखिम का अनुमान फेफड़ों (धातु (एकवचन) के साथ-साथ धातुओं के द्विआधारी मिश्रण) के लिए लगाया

गया था। इसके अलावा, दोनों परिवेश ठीक और पराबैंगनी कणों की श्वसन बयान खुराक (आरडीडी) की गणना मानव श्वसन पथ मॉडल का उपयोग करके की गई और पीएम-संबद्ध धातुओं के फेफड़ों और विभिन्न अंगों, जैसे पित्ताशय और अन्य ऊतकों की जमा राशि के आकलन के लिए विश्लेषण किया गया।

अध्ययन के मुख्य निष्कर्षों में वायुकोशीय क्षेत्र में 250 एनएम से कम कणों का उच्च जमाव शामिल है, और दो अलग-अलग ऊंचाई पर नमूना किए गए पीएम के द्रव्यमान सांद्रता के बीच का अंतर भी है। माध्य सांद्रता व्यक्तिगत जोखिम और श्वसन चित्रण खुराक (RDD) दोनों की गणना में महत्वपूर्ण भूमिका निभाता है। अधिक पुरानी बीमारियां हो सकती हैं यदि यह जोखिम अवधि के एक बड़े कार्यकाल के लिए रहता है। साइटो-टॉक्सिकिटी के अध्ययनों में विभिन्न आकारों के कणों के कारण मौसमी- और द्रव्यमान पर निर्भर साइटोटॉक्सिसिटी और आरओएस पीढ़ी की क्षमता, और साइटोकिन्स (आईएल -6 और आईएल -8) की समर्थक-भड़काऊ प्रतिक्रिया दिखाई दी। कुछ धातुएं समूह से बेपरवाह हैं चाहे कार्सिनोजेनिक या नॉनकार्सिनोजेनिक ने जैविक समापन बिंदुओं के साथ एक मजबूत सकारात्मक सहसंबंध दिखाया। जैव-अनुपलब्ध रूप में पीएम द्रव्यमान सांद्रता आर्सेनिक (As), लीड (Pb), कॉपर (Cu) के साथ एक सकारात्मक सहसंबंध दिखाया। सभी जैविक प्रतिक्रियाओं ने अन्य जैविक समापन बिंदुओं के साथ निरंतर संबंध नहीं दिखाया। धातु, जैसे, सीआर, और सीडी ने दिल्ली के लिए सर्दियों के मौसम में जैविक-बिंदुओं के साइटोटॉक्सिसिटी को नियंत्रित किया। भड़काऊ समर्थक प्रतिक्रियाओं ने संकेत दिया कि दिल्ली के परिवेशी PM<sub>2.5</sub> कणों ने 2 प्रकार के साइटोकाइन प्रतिक्रियाओं के लिए अनपेक्षित नमूनों से अंतर (3-53 सिलवटों) की उच्च तह दिखाई। अध्ययन ने निम्नलिखित तीन महत्वपूर्ण मुद्दों पर ध्यान केंद्रित करने से भी निपटा: (ए) बढ़ी हुई पीएम की साइटोटॉक्सिसिटी <0.25 (बी) पीएम 2.5 स्तर और पीएम <0.25 के स्तर के बीच सहसंबंध की कमी और (ग) अल्ट्राफाइन कणों की निगरानी शुरू करने की आवश्यकता (यानी PM

<0.25) उन्नत PM<sub>2.5</sub> स्तरों वाले देशों में। PM<sub>2.5</sub> बनाम PM <0.25 के सहसंबंध परिणाम 0.08 के R<sup>2</sup> मान के साथ कम पाए गए, जिसने पीएम <0.25 के माप की आवश्यकता और इसके कम आकार और उन्हें विनियमित करने की आवश्यकता को समझने के लिए प्रयास शुरू करने पर जोर दिया। इन-विट्रो टॉक्सिसिटी डेटा से यह भी पता चला कि निर्भरता न केवल ठीक और पराबैंगनी आकार के कणों की सामूहिक एकाग्रता पर निर्भर करती है, बल्कि भौतिक रासायनिक घटक के भी निर्भर करती है।

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## LIST OF ACRONYMS

- PM: Particulate Matter
- RDD: Respiratory Deposition Doses
- RI: Risk for individual.
- BALF: Bronchoalveolar Lavage Fluid
- HRT: Human Respiratory Track Model
- PBPK: Physiologically based pharmacokinetic modelling
- HQ: Hazard Quotient
- HI: Hazard Index
- ECR: Excess Cancer Risk.
- HHRA: Human Health Risk Assessment
- SI: Stimulatory Index
- ROS: Reactive Oxygen Species
- IL-6 &IL-8: Interleukin
- NIST-UPM: National Institute of Standards and Technology Urban Particulate Matter.
- IRIS- EPA: Integrated Risk Information System – Environmental Protection Agency
- CPCB: Central Pollution Control Board
- US-EPA: United State-Environmental Protection Agency.
- IMD- Indian Meteorological Department
- ICRP: International Commission on Radiological Protection
- TLC: Total Lung Capacity
- ADD: Average Daily Dose
- ED: Exposure Duration
- EF: Exposure Frequency

- BW: Body weight
- AT: Average Time
- RfD: Reference Dose
- SFI: Slope Factor Index
- PAHs: Polycyclic aromatic hydrocarbons
- VOCs: Volatile Organic Carbon
- NAAQS: National Ambient Air Quality Standards
- MTT: [3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide] Assay
- DMEM: Dulbecco's Modified Eagle Media.
- PBS: Phosphate Buffer Saline
- MQW: Milli-Q water.

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