Photocopiers are indispensable tools in today’s world. They are also sources of indoor air pollution. Photocopiers emit toner particles, aerosols, carbon black, ultra fine, fine and coarse particles, toxic gases like ozone, nitrogen dioxide, volatile organic compounds, semi-volatile organic compounds, radiation, paper particles, extremely low-frequency electromagnetic fields and engineered nanoparticles during operation.

In India, photocopiers are ubiquitous. They are a source of income to thousands of young unskilled people. Although used photocopiers are classified under hazardous waste, majority of the multifunctional print and copying machines used in photocopier units in India are imported second hand machines. To minimize commercial space, photocopiers are often operated in congested spaces and combined with other office equipments without proper ventilation. In most units, photocopiers are poorly maintained with sub standard toners and spares. Therefore, air pollution burden from photocopiers in India may be high due to the above factors. Chronic exposure to air pollutants has been recognized to affect cardiovascular and respiratory systems, through the oxidative stress and inflammatory pathways. Hence, it is imperative to assess the health status of operators in photocopier units.

**Objectives of the study**

With the above mentioned facts, the present study was carried out with the following objectives:

1. To assess the pulmonary function among operators in photocopier units by spirometry.
2. To assess systemic inflammation among operators in photocopier units by selected serum/plasma biomarkers.
3. To analyse the relationship between duration of exposure to photocopiers, pulmonary function and the selected biomarkers.
4. To explore the use of exhaled breath condensate metabolomics as a non-invasive tool to assess the lung function.

This study was carried out in a cross sectional observational design in Coimbatore district of Tamilnadu, India. The present study was approved by the Human Ethical Committee of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore (HEC.2011.24). Written informed consent was given by all the participants before data and blood and exhaled breath condensate collection.

Selection of participants was based on the following criteria:

Inclusion criteria: Male and female participants in the age group of 18 – 50 years.

Exclusion criteria: Prevalence of systemic ailments namely cardiovascular diseases, diabetes mellitus and hypertension.

Operators who were working in photocopier units for ≥ 1 year were included in the photocopier operator group. Participants without any professional exposure to photocopiers were included in the control group.

An interview schedule was evolved for this study and validated by a pulmonologist. The interviewer collected personal, socio economic, demographic, professional, life style and general health details including medical history and current medication from all participants, by administering this interview schedule. Weight and height of the participants were recorded by standard procedures.

Pulmonary function of the selected participants was assessed by using Vitalograph Alpha 6000 spirometer, UK by the investigator who was trained for this purpose before initiating this study. Spirometry was performed according to the American Thoracic Society / European Respiratory Society guidelines. Each participant’s best trial (largest sum of FEV₁ and FVC) was included in analyses. The percentages of predicted values for all parameters were calculated using published values for Asians - ERS 1993.
Five ml of blood was collected from the participants by venipuncture. Plasma/serum was removed, aliquoted into labeled cryovials and stored at -80°C until analysis.

A total of 329 participants were approached to take part in the study. Two hundred and twenty seven participants accepted to take part in the study and completed the interview schedule. But, 66 were disqualified from the study. Eight participants withdrew from the study. Assessment of pulmonary function tests was carried out on 153 participants (110 photocopier operators and 43 controls). Blood samples could not be obtained from 13 participants. Thus, hematological, biochemical and inflammatory biomarkers were assessed in 100 photocopier operators and 40 control participants.

Complete blood count was assessed in the whole blood of all participants which included Hematocrit, Hemoglobin, Red Cell Distribution Width, Mean Corpuscular Volume, Red Blood Cell Count, Platelet Count, Mean Platelet Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Platelet Distribution Width and Platelet Large Cell Ratio. Differential count was also carried out.

Random plasma glucose was estimated. Total protein, albumin, thiobarbituric acid reactive substances (TBARS), Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant capacity (FRAC) were assessed in serum. Myeloperoxidase was assessed in lithium heparin plasma. Inflammatory and oxidative stress markers viz., Inter Cellular Adhesion Molecule-1 (ICAM-1), Clara cell protein (CC16), free 8-Isoprostane, Interleukin-6 (IL-6), Interleukin-8 (IL-8), Eosinophil Cationic Protein (ECP), Leukotriene B$_4$ (LTB$_4$), C Reactive Protein (CRP), total nitrates, thioredoxin reductase and glutathione peroxidase were assessed in K$_2$ EDTA plasma.

Genotoxicity in photocopier operators was assessed by single cell gel electrophoresis in a sub sample of the participants. Serum levels of Cadmium and Selenium were assessed in a sub sample of the study participants.
Breathing air through a cooling system results in condensation, thereby rendering collection of exhaled breath in a liquid form. Exhaled breath condensate was collected from a selected sub sample of participants using an improvised device assembled in the laboratory. Samples of approximately 1 ml were collected in 15 - 20 minutes. They were stored at - 80°C and \(^1\)H NMR spectra were obtained using 500 MHz FT NMR. \(\text{D}_2\text{O}\) was used as internal lock. 1 D spectra were obtained and water suppressed. This data was normalized and assessed by Partial Least Squares - Discriminate Analysis using the software Metaboanalyst 2.0.

Ambient air quality was monitored in five photocopier units during a work day to assess the levels of exposure of photocopier operators to indoor air pollutants. Carbon monoxide, nitrogen dioxide, ozone, fine particulate matter (PM\(_{2.5}\)), particulate matter (PM\(_{10}\)), sulphur dioxide, lead, arsenic, nickel, ammonia, benzene and benzpyrene were the parameters assessed.

Statistical analysis was performed using SPSS 16.0. Non parametric comparisons were carried out using Chi square test or Fisher’s exact test. Data were checked for normal distribution using Shapiro Wilk test. Normally distributed data were compared by student’s ‘t’ test. Data that were not normally distributed were compared by Mann Whitney test. Non parametric correlation was carried out using Spearman’s rank correlation. Significance is reported at p<0.05.

**Salient findings of the study**

Prevalence of respiratory symptoms and general health symptoms were not significantly different between the controls and photocopier operators. In the cross sectional study, none of the pulmonary function parameters namely Vital Capacity (VC), Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 Second (FEV\(_1\)), Maximal Mid Expiratory Flow (FEF\(_{25-75}\)) and Maximum Ventilatory Volume (MVV) were significantly different between the controls and photocopier operators. A significant negative correlation was observed between photocopier exposure and pulmonary
function indices namely FEV$_1$/FVC and FEF$_{25-75}$. This suggested that chronic exposure to photocopiers would have caused a decline in pulmonary function.

Among the differential count, no significant differences were found in the number of white blood cells, lymphocytes and neutrophils. However, mixed cells comprising monocytes, basophils and eosinophils were significantly lower in photocopier operators compared to controls. Photocopier operators might have possible airway and systemic inflammation. Among the red cell indices, hematocrit, mean corpuscular volume and red cell distribution were significantly higher ($p<0.05$) in photocopier operators compared to controls. No significant differences were found in the number of platelets and platelet indices between the two groups of participants. It could be suggested that photocopier operators are subjected to chronic hypoxia and possible systemic inflammation.

Total protein levels were not significantly different between the controls and photocopier operators. However, albumin levels were significantly lower and globulin levels were significantly higher among photocopier operators, altering their albumin:globulin ratio. Chronic exposure to photocopier emissions would have led to acute phase reactions.

Markers of lipid peroxidation, i.e., serum TBARS and plasma free 8-isoprostane were significantly higher in photocopier operators when compared to controls. Trolox equivalent antioxidant capacity was not significantly different between the two groups. However, ferric reducing antioxidant capacity was significantly less in photocopier operators. High oxidative stress could be observed among photocopier operators.

Among the non specific inflammatory proteins, IL-6 and IL-8 were significantly higher in photocopier operators. No such differences were observed in ECP, CC16 and CRP levels. Among the markers of endothelial function, total plasma nitrates were not significantly different between the two groups. ICAM-1 was higher among photocopier operators. LTB$_4$ was elevated in photocopier operators. No significant differences were found in the levels of myeloperoxidase. Exposure to photocopier
emissions was found to cause systemic inflammation and endothelial dysfunction, elevating the risk of cardiovascular disease.

CRP exhibited significant negative correlations with lung function markers namely FEV$_1$, FVC and MVV. Among the biomarkers studied, CRP, although a non specific inflammatory marker was found to be the best indicator of lung function.

Among the male photocopier operators, levels of inflammatory markers ICAM-1, LTB$_4$, ECP and IL-8 were significantly positively correlated with duration of photocopier exposure. Smoking by photocopier operators was found to be associated with increased systemic inflammation as seen by positive correlation between IL-8 and combined exposure to photocopiers and cigarette smoke.

Significant correlations were observed between the hematological indices, inflammatory markers and pulmonary function indices assessed in the study.

Selenium levels were significantly higher among photocopier operators, but selenoprotein levels were not different. Exposure to photocopier emissions could be suggested to lead to high selenium levels.

Photocopier operators had significantly high levels of DNA damage when compared to controls suggesting that DNA damage would have been caused by chronic exposure to photocopier emissions.

Characteristic differences were found in the $^1$H NMR spectra of exhaled breath condensate of healthy participants and participants with restrictive and obstructive lung diseases. However, discrimination between participants with restrictive / obstructive pulmonary function and healthy participants could not be done by partial least squares – discriminate analysis.

Ambient air quality levels in photocopier units showed high levels of particulate emissions (both PM$_{10}$ and PM$_{2.5}$) by photocopiers.

The following conclusions may be drawn from the present investigation:
The results of the study suggest that chronic exposure to photocopiers may cause obstructive pulmonary diseases. Hypoxemia, high oxidative stress and systemic inflammation and endothelial dysfunction were observed among photocopier operators. Photocopier exposure might predispose the operators to cardiovascular diseases. High levels of particulate emissions by photocopiers might increase the risk of cardiopulmonary diseases among the operators.

**Limitations of the study**

- Assessment of local airway inflammation is ideal in case of indoor air pollution studies. However, due to the practical difficulties of collecting sputum / nasal lavage / bronchioalveolar lavage samples from the large number of participants in the field, local airway inflammation could not be assessed.

- Exposure to photocopiers was self reported and was not monitored by levels of biomarkers in human biological fluids. As photocopiers are sources of a multitude of pollutants, biomonitoring of the pollutants in the participants was not possible.

**Recommendations for further research**

- Characterization of toners used in photocopying process and their association with pulmonary and cardiovascular ailments in the personnel involved in manufacture and handling of the toners.

- Characterization and chemical composition of particles emitted by photocopiers and their relationship to pulmonary and cardiovascular problems of personnel in this industry.

- Formulation of measures to minimize photocopier emissions.