4

Results and Discussion

Indoor air pollution is a global health concern in today's modernised world. Workplace air quality is receiving greater attention nowadays due to technological transformations in advent of hi-tech sophisticated office devices that includes photocopier machines, computers and laser printers. These devices release both primary and secondary pollutants that increase the pile of pollutants mix inside the office set-up. Chemical reactions between primary pollutants and singlet oxygen species release secondary pollutants.

Comment [K1]: Second paragraph from original removed as per comments of inappropriateness for results para

The present study was aimed to identify whether there is pollutants exposure in xerographic units among service personnel and whether it affects the health of the workers, with special reference to alteration in lung function and biomarkers levels and to evaluate whether there is any relationship between effect and exposure. A metabolomics study was done to identify the excretory urinary biomarker in occupational settings.

Comment [K2]: reframed the sentence and removed the word pilot

The results of the present study are discussed under the following heads:

Phase I: Air Quality Monitoring of Xerographic Units

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4.12 Binary logistic regression of general health symptoms with photocopier exposure

4.13 Regression analysis between lung function parameters, haematological indices, biomarkers and independent continuous variables pack years and cumulative photocopier exposure

PHASE 1

4.1 Ambient Air Quality Monitoring

4.1.1 Physical Characteristics of the Xerographic Units

The physical characteristics of the xerographicunits (n = 12) where indoor air quality was measured are listed in Table 4. A typical photocopier centre consists of concrete building of approximate dimension 3 X 3 X 2.4 m^3 with walls on three sides and a retractable shutter at the entrance side except for two centres that have two way or three way entrances. The air flow rate of the photocopier centre ranged between 107 - 250 cfm. The common interior materials used in photocopy centres include cement floor, concrete ceiling and

painted cement walls. The only ventilation outlet is the front retrac Table shutter which remains open during business hours. Xerographic centres also have other VOC emitting sources like computers, printers, fax machines, incense, carpets, paper cutting machine, binding machine and unworkable machines in the limited space of the photocopy shops and furniture like metal or plastic chairs and wooden Table.

4.1.2 Characteristics of the Xerographic Machines

Table 5 lists the physical characteristicfeatures of xerographicmachines and toners (n = 12). Each xerographic centre was given numeric identification 1 through 12. Xerographic centres varied in the number of machines, manufacturers, models and also in their copy loads. All the centres had business working time up to 12 – 13 hours. Each copy centre had 2 – 3 xerographic machines with at least two monochrome (black) models. Five centres were also found to have one colour model in addition to monochrome model. The make of the machines found in the copier centres weremostly Canon, both for mono and colour cartridgesexcept for two centres. The average speed was found to be 35 copies / minute in all the xerographic centres and the average daily work load among the xerographic centre varied between 1,500 – 10,000 copies based on the customer demand, copier speed and machine capacity. Xerographic centres used either global or local toners. Global toners are produced by the xerographic machine manufacturers where as in contrary local toners are compatible toners manufactured locally in India and not by the manufacturers of the xerographic machines. All 12 xerographic centresstudied used locally manufactured toner named A, B and C. Centre No. 12 alone used global toner D in addition to local toner A.

Comment [K3]: toner brand replaced as toner named A,B and C

Table 4

Physical characteristics of xerographic units

Xerographic centre No.	Room dimension (m^3)	Flooring	Roof	Walls	Ventilation	Other VOC emitting sources	Furniture
1	3 X 3 X 2.4	Cement	Cement	Cement, painted	1 way opening Front metal shutter	Computer (1), Printer (2), Scanner (1), Fax Machine (1)	Chair (1), Table (1)
2	5 X 3 X 2.4	Cement	Cement	Cement, painted	1 way opening Front metal shutter	Computer (1) Paper Cutting Machine (1)	Table (1), Chair ((1)
3	6.0 X 2.4 X 2.4	Cement, vinyl sheet	Cement	Cement, painted	1 way opening Front metal shutter	Computer (3),Printer (1), Carpet (1),Incense (2),Fax machine (1)	Chairs (9), Table (2)
4	6.0 X 2.4 X 2.4	Cement	Cement	Cement	1 way opening Front metal shutter	Computer (1), Printer (1)	Chair (2),Table(1)
5	3.7 X 2.4 X 2.4	Cement	Cement varnish	Cement, oil painted	2 way opening Front / back metal shutter	Computer (3),Printer (2),Paper Cutting Machine (1)	Chair (3),Table (1)
6	4.9 X 2.4 X 2.4	Mosaic	Cement	Cement, painted	3 way opening Front / back/ side metal shutter	Computer (1),Printer (2),Paper Cutting Machine (1) unworkable Photocopier (3),Incense (2)	Chair (3),Table (2)
7	4.9 X 2.4 X 2.4	Mosaic	Cement	Cement, Painted	1 way opening Front metal shutter	Computer (1),Ink jet printer (1), Fax Machine (1), unworkable Printer (2), Paper Cutting Machine (1)	Chair (2),Table (2)
8	4.9 X 2.4 X 2.4	Cement	Cement	Cement, painted	1 way opening Front metal shutter	Incense (1), Computer (2)	Chair (3)
9	4.9 X 2.4 X 2.4	Ceramic	Cement	Cement , painted	1 way opening	Computer (3), Laptop (2), Printer (3), Carpet (1), Fax machine (1), unworkable Photocopier (1)	Chairs (3), Table (3)
10	3.4 X 2.4 X 2.4	Cement	Cement	Cement, painted	1 way opening	Incense (2)	Chair (5)
11	4.9 X 2.4 X 2.4	Marble	Cement	Cement, painted	1 way opening	Computer (1), Printer (1),Lamination unit (1), Paper cutting Machine (1), unworkable Photocopiers (2) and Printer (1)	Chair (7),Table (1)
12	4.9 X 2.4 X 2.4	Mosaic	Cement	Cement	1 way opening	Computer (1) , Printer (1), Lamination Unit (1), Paper cutting Machine (1),	Table (1), Chair (2),

Comment [K4]: Adjacent ACR value column in the table removed as per comment as they were reference values and not actual values

Table 5

Characteristic features of xerographic machines and toners

Copy centre	Business hours	No. of Xerographic Machines	Machine Make	Copy Speed Copy / Min	Average Copies / day	Toner Type	Toner Make
1	12 .00	2	Canon IR3530, Canon 6000	35 60	1500	Black	A
2	12 .00	2	Canon 6000, Canon IR3170C	60 31	1500	Black, Color	A
3	13.00	3	Canon IR 3530, Canon 6000, Kyocera 3510i	35 60 35	1500	Black	A, B
4	13.00	3	Canon 6060 [2], Canon IRC3200	65 32	3000	Black, Color	A
5	13.00	4	Canon IR105 [2], Canon IR 6570, Canon IRC3200	105 65 32	10000	Black, Color	A
6	13.00	3	Canon 6000 [3]	60	3000	Black	A
7	13.00	3	Canon IR6000 [2], Canon NP6060	60 60	5000	Black	A
8	13.00	4	Canon IR6020i [2], Canon 7086, CanonIRC3220	60 86 32	7000	Black	A
9	12.00	3	Canon IR 6570 [2]	65	1500	Black	A
10	12.00	3	Canon IR 6570 [2], Canon MF5750	65 20	2000	Black, Color	A
11	13.00	3	Canon NP 6060 [2] Canon IRC3200	32 60	5000	Black, Color	A, C
12	13.00	3	Canon IR 7200, Canon 8500, Kyocera 3010	72 80 30	2000	Black, Color	A, D

4.1.3 Ambient Air Quality Analysis

The levels of ambient air quality parameters were assessed in a representative sample of twelve photocopy units which are shown in Table 6.

Table 6

Ambient air quality parameters analysed in xerographic units

Parameters	Background levels Mean ± SD (n = 12)	Mean levels Mean ± SD (n = 12)	NAAQS limits
Fine Particulate Matter ($PM_{2.5}$) ($\mu g/m^3$)	**130 ± 10.1**	**227. 49 ± 16.31**	60
Carbon monoxide (mg/m^3)	< 1.02	< 1.16	2.0
Nitrogen-di-oxide ($\mu g/m^3$)	< 8.92	< 13.72	80
Sulphur-di-oxide ($\mu g/m^3$)	< 3.50	< 4.5	80
Ozone ($\mu g/m^3$)	< 9.45	< 9.76	100
Ammonia ($\mu g/m^3$)	4.0 ± 1.5	4.5 ± 0.5	400
Lead ($\mu g/m^3$)	< 0.1	< 0.1	1.0
Arsenic (ng/m^3)	< 0.1	< 0.1	6.0
Nickel (ng/m^3)	< 0.1	< 0.1	20
Benzene ($\mu g/m^3$)	< 0.1	< 0.1	5.0
Benzo (a) pyrene ($\mu g/m^3$)	< 0.1	< 0.1	1.0

Values are expressed as Mean ± SD; National Ambient Air Quality Standards (**NAAQS, 2009**)

Comment [K5]: Background levels inserted to show that study conduct areas were residential areas and were within the air quality index

Comment [K6]: typographical errors SD values removed

Comment [K7]: typographical errors removed

Comment [K8]: typographical errors removed

The background levels of outdoor air emissions in the selected residential places in Coimbatore (n=12) were within the permissible limits except for the particulate matter emissions. These results are in confirmation with reports by Saravanan *et al*. (2017) who stated that air quality index of Coimbatore district were found to occur in moderate level of health zone except industrial locations that requires stringent action and also at low levels during monsoon season. Ambient air quality in xerographic units shows the presence of high levels of fine

particulate matter ($PM_{2.5}$) emissions. These levels are about 3.8 fold higher than the daily permissible levels in these work places during machine operation. Similar results were obtained by numerous other studies in different parts of the world (Lee and Hsu, 2007; Kagi *et al*., 2007; Schripp *et al*., 2008; Wensing *et al*., 2008; Adentunji *et al*., 2009; Morawska *et al*., 2009; He *et al*., 2010; Tang *et al*., 2012 and Gosman *et al*., 2015). According to Lee and Hsu (2007), any of these reaction mechanisms: condensation, oxidation and ion induced nucleation of volatile organic compounds may possibly contribute to the formation of ultra fine particles during photocopying process. It was also noted that the key inducer of ultra fine particulate formation is corona devices which can generate oxidants namely ozone, nitric oxide radicals and ions during the photocopying process.

The levels of other air quality parameters namely nitrogen dioxides, sulphur dioxides, ozone, lead, arsenic, nickel and VOCs (Benzene and Benzo (a) pyrene were within Indian ambient air quality standards in all the monitored xerographic units.

Similar to the present study, Han *et al*. (2011) also observed only a minimal change in the levels of nitrogen dioxide in the indoor air of xerographic units assessed in six days continuous assessment period. Quaternary ammonium salt dispersed in polymeric resin of the toner have many utilities as charge control agents for toners, serve as adhesion promoters between toner and receiver sheets and also as toner fusing temperature reducers (Wilson and Bermel, 1993). Their emission rates in indoor air was within the permissible levels of Indian standards

It was claimed by Ewers and Nowak (2006) that the ozone levels generated from the modern copiers that use transfer roller technology is not increased in indoor air. However, according to Kagi *et al*. (2007), the ozone levels from the equipment may oxidize emitted VOCs into other contaminants.

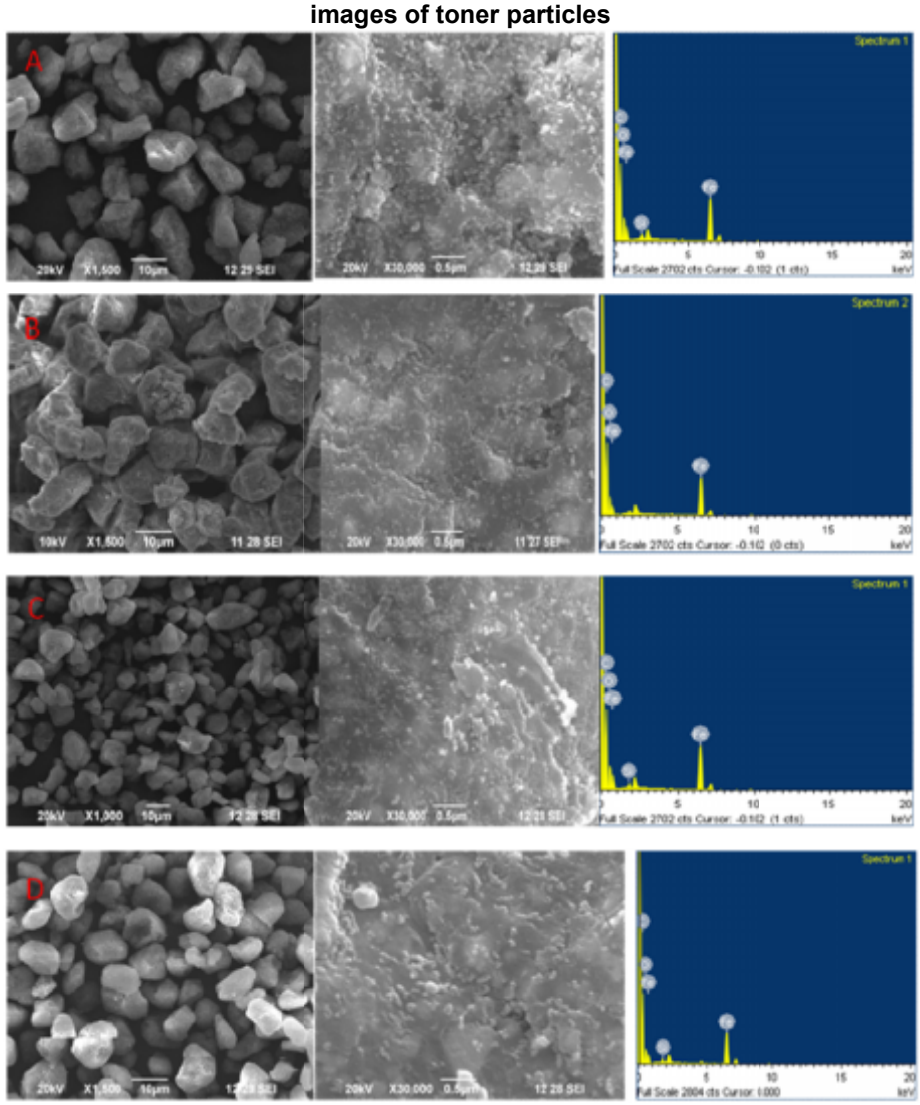
The coatings of the photocopier drums are an important source of arsenic dust and fumes (www.hse.gov.uk/pubns/indg441.htm) but their emission levels were also very minimal in the present study.

4.1.4 Characterisation of Toner

The particle size, morphology and composition of the toner particles as revealed by Scanning Electron Microscopy Energy Dispersive X ray spectra (SEM - EDAX) are indicated in Plate 3.

Plate 3

Scanning Electron Microscopy Energy Dispersive X ray spectra (SEM EDX) images of toner particles



From Plate 3, it is clear that the four selected brands of toner particles size ranged between 2 and 0 µm as depicted in Plate 3. The particles in local toners A, B and C appear slightly elongated and rough shaped with jagged margin. They were also not uniform both in their shape and size. The particles size of toners A, B and C were on an average 9 ± 1.41 µm, 9.3 ± 0.67 µm and 4.0 ± 0.84 µm respectively where as the global toner D appears to contain smooth round shaped and uniform sized (5 ± 0.71 µm) agglomerates.

Rough edges with non uniform sized particle distribution in the local toners indicate that they were manufactured through conventional mechanical pulverization technique in contrast to global toners manufactured through chemical preparation technique that involves polymerisation technique. These results are in accordance with Kiatkamjornwong and Pomsanam (2003) who stated that polymerized toners for electrophotographic printing were found to be smooth on their spherical surfaces and the particle sizes were 4 - 10 µm.

All these toner particles bear carbon as the base element covered by sub-micrometer sized particle (nano-sized) that is iron rich. From Plate 3 and Figure 4, it is evident that original toner D comparatively contained smoother surface which was also implied in the higher composition of elemental carbon (58.21 %) in contrast to the local toner A that contained high iron rich particles (30.86 %) which are indicated by denser cloud of nanosized particles embedded on the smooth surface. These results are in confirmation with the previous results obtained for toner particle size, morphology and element composition by Gminski *et al*. (2011)

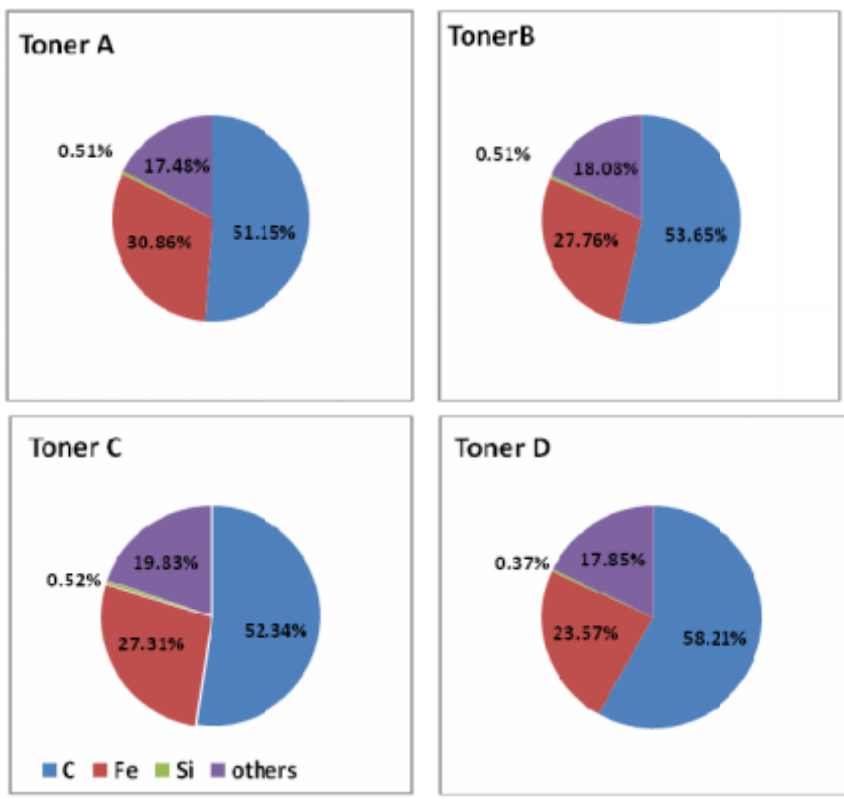
The element composition as measured by EDX (Figure 15) showed that carbon is the most abundant element in the investigated monochrome toner materials that constitutes the toner core resin with > 50% , followed by iron in the range between 23% - 30% and silicon in the range between 0.37 – 0.51%. The other elements constituted between 17% - 19%.

The differences in the elemental composition between the manufacturers are also depicted in Figure 15. Global toner D contained the highest carbon

content of 58.21% in comparison to the local toners A, B and C that constituted 51.15%, 53.65% and 52.34% Local toner A was found to be iron rich (about 30.86%) followed by toner B and C (27.76% and 27.31%) in comparison to the global toner D which contained lesser iron (23.57%). The silicon content was found to be least (0.37%) in toner D in comparison to local toners A, B and C that constituted 0.51% and 0.52%. According to Pettersson and Fogden (2005), the iron originates from the black colorant and silica particles are used as charge control additives. Gminski *et al*. (2011) concluded that the iron rich particles that contained considerable amount of oxygen may be magnetite. This also implies that all the toners screened were magnetic toners.

Figure 15

Element composition of the four selected brands of toner



The toner was characterised qualitatively for the emission compounds by screening test using Gas Chromatography Mass Spectrometry (GC MS) head space analysis. GC MS Spectra of the toners A, B, C and D with their peaks and retention time (RT) in minutes (min) are depicted in Figures 16, Figure 17, Figure 18 and Figure 19 respectively. Table 7 enumerates the compounds that were identified by head space GC / MS analysis in each of the four toner samples A, B, C and D under investigation. All the compounds of the toners that had a matching retention time (RT) from minimum 35% up to maximum 100% when compared against the available computer library of mass spectra (NIST 11) are listed in Table 7.

Figure 16

GC MS spectra of toner A

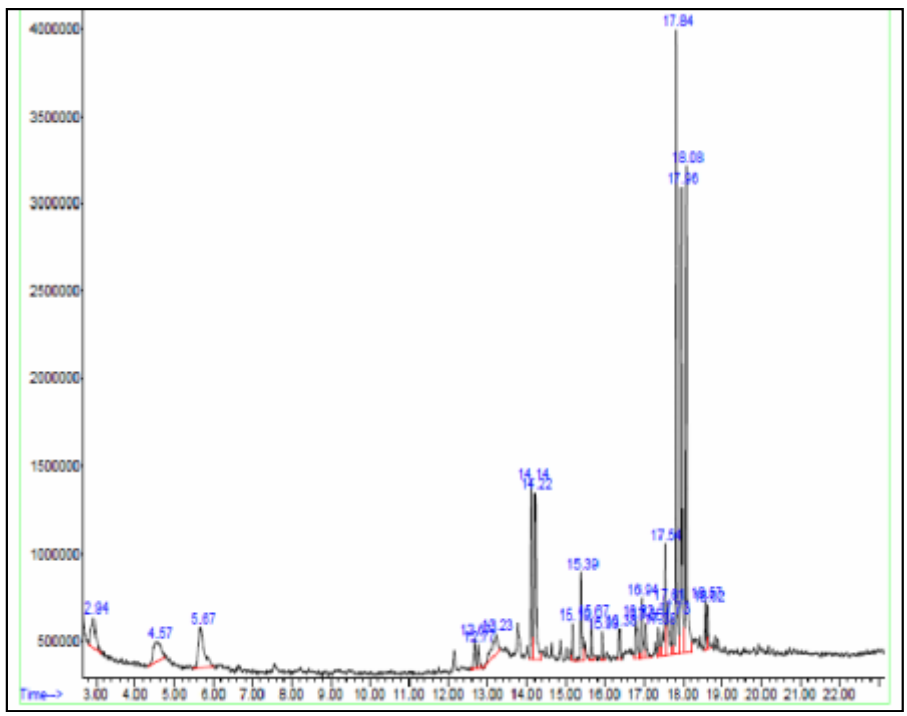


Figure 17

GC MS spectra of toner B

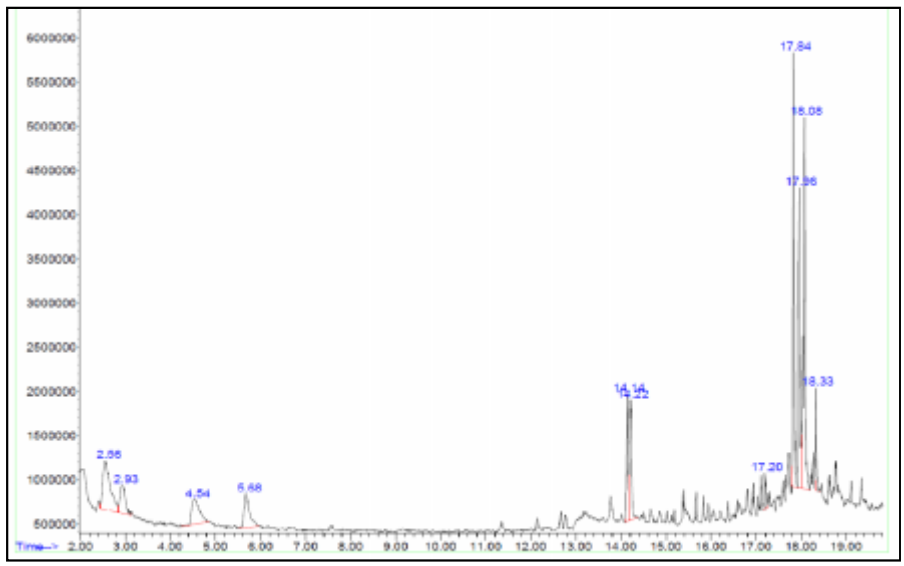


Figure 18

GC MS spectra of toner C

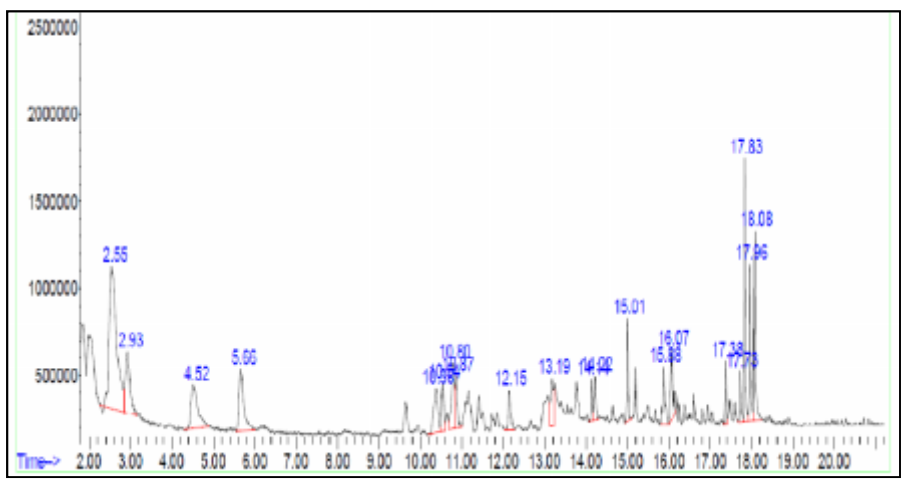


Figure 19

GC MS spectra of toner D

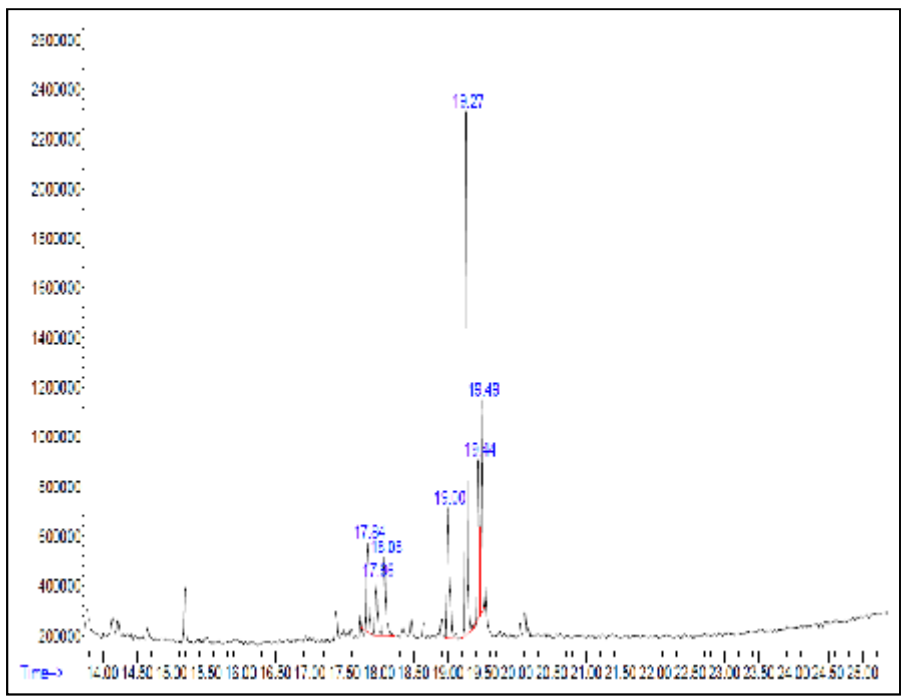


Table 7

Compounds identified by GC/MS headspace analysis in the four selected brands of toners

Compound Classification	Retention Time (minutes)	Compounds tentatively identified in toner samples	A	B	C	D	Matching quality (%)
PAH	19.48	Longifolene				✓	99
	19.27	2H-2,4a-Methanonaphthalene, 5,6,7-hexahydro-1,1,5,5-tetramethyl-, (2S)-				✓	98
	18.99	1,2,4-Methenoazulene, decahydro-1, 5,5,8a-tetramethyl-,[1S-(1.alpha., 2.alpa.,3a.beta, 4.alpha,8a.beta.,9R*)]-				✓	95
	15.88 & 16.07	Napthalene, decahydro-2,6-dimethyl			✓		50
	16.38	3-Azabicyclo(3.2.2) nonane	✓				50
Aromatic Hydrocarbons	**4.53**	**Toluene**	✓	✓	✓		76
	13.19 ± 0.03	**Benzene, 1,4-dichloro**	✓		✓		90
Aliphatic hydrocarbons							
n – alkanes	2.92	Heptane			✓		43
	16.94	Dodecane, 4,6-Dimethyl	✓				52
Cycloalkanes	**10.8**	Cyclohexane, 1-ethyl-1,4-dimethyl-, trans-			✓		81
	15.01	**Adamantane, 1,3-dimethyl-**			✓		60
	10.54	Cyclohexane, 1,1,3,5-tetramethyl-,cis-			✓		50
	15.67	Cyclohexane, 1,2-diethyl-3-methyl	✓				43
	17.37 ± 0.01	Cyclohexane carboxylic acid, 4-nitrophenyl ester	✓		✓		47
	17.84 & 17.96	Cyclohexane, 1,2,4-trimethyl-	✓	✓	✓	✓	38
	10.86	Cyclohexane, 1,1'-(1,2-dimethyl-1,2-ethanediyl)bis-			✓		36
Olefin	17.21	2, 4, 4, 6, 6, 8, 8-Heptamethyl-1-nonene		✓			37
	14.14	**2-Undecene,4-methyl-**	✓	✓	✓		43
	10.39	2-Nonene, 2-methyl-			✓		43

Table 7 (contd…)

Compounds identified by GC/MS headspace analysis in the four selected brands of toners

Compound Classification	Retention Time (minutes)	Compounds tentatively identified in toner samples	A	B	C	D	Matching quality (%)
Aliphatic hydrocarbons							
Halo olefin	5.68	**tetrachloroethylene**	✓	✓	✓		99
Alkyl ketone	14.23	**Ethanone, 1-cyclopentyl**	✓	✓	✓		38
	17.96	**2-Acetylcyclopentanone**	✓	✓	✓	✓	35
Cyclo siloxane	**12.14**	Cyclotetrasiloxane, octamethyl-			✓		64
Cyclic ketone	**18.08**	**4-Isopropyl-1,3-cyclohexanedione**	✓		✓	✓	64
Alkyl ester	17.53	propionic acid,2 dimethyl-,pentyl ester	✓				35
α - Primary Amine	15.94	Metaraminol	✓				38
n-aliphatic amide	15.2	Propanamide	✓				35
Alkanol	2.55	1-Butanol		✓	✓		52
Aliphatic dicarboxylic acid	18.56	Adipic dihydroxamic acid monohydrate	✓				40
Hetero cyclic Compounds	15.39	3-Methylbenzylthiocyanate	✓				43
	12.68	2-piperidinone,1-methyl	✓				40
	2.94	Piperazine-2-methyl	✓	✓			43
	12.76 & 16.81 & 17.04	3,3-Dimethyl piperidine	✓				43
	17.6	Cyano acetyl urea	✓		✓		43
	18.62	1-methyl-2-pyrrolidone - 4-carboxamide	✓				43

In addition to these identified compounds, each sample also contained two or more significant peaks that could not be identified. Some unidentified peaks were also noticed in reports by Henschel *et al*. (2001).

From Table 7, it is found that the global toner D had a unique set of > 99%, 98% and 95% polycyclic aromatic hydrocarbons (PAH) called longifolene – natural terpene compound (RT 19.48 min), methanonaphthalene (RT 19.27 min) and methanoazulene (RT 18.29 min) respectively which were not found in the other local toners. According to Ohmura *et al*. (2007), these natural terpene based substances are used as chain transfer reagents for polymerization reaction in the make of toner resin particles in order to reduce the odor emitted during heat fixing process. In contrast, the local toners A and C were found to contain PAH namely napthalene, decahydro-2, 6-dimethyl (RT 15.88 min and 16.07 min) and 3-Azabicyclo(3.2.2) nonane (RT 16.38 min) respectively as chain transfer agents. *In vitro* studies by Gminski *et al*. (2011) reported that low amounts of PAHs in toners were responsible for the genotoxic effects noticed in epithelial cells.

Usage of inexpensive industrial solvent especially toluene could be noticed in the formulation of local toners A, B and C with 76% matching identity. According to Sugiyama and Shinkai (1987), organic solvent toluene is used in toner composition to disperse the binder resin in order to produce pressure fixable free flowing electrophotographic toners. D It was concluded by Baelum (1991) that exposure to toluene among workers occupationally exposed to printers cause irritative and prenarcotic symptoms and possibly a lowered performance due to neurotoxic effects within the occupational limit in several countries.

In addition to this, usage of benzene 1,4 dichloro could also be noticed with > 90% identity in toners A and C. A study by denBesten *et al*. (1991) indicated significant acute toxic effects on liver, kidney and thyroid with series of chlorinated benzenes intraperitonial administration among rats. Acute exposure to 1,4-dichlorobenzene via inhalation in humans results in irritation to the eyes, skin and throat according to hazardous substances data bank (HSDB, 1993).

Toluene, benzene and cyclohexane are used in electrophotography toner to increase the liquid property of high volume resistivity in order not to destroy the latent image according to Schein (1992).

Higher n-alkanes namely heptane and dodecane and cycloalkanes could also be noticed in toner formulation of C and A as indicated in Table 7.

Toxicosis resulting from exposure to hydrocarbons has been associated with a variety of clinical signs, including dermal (dermatitis, skin eruptions, burns, epidermal necrosis), ocular (conjunctivitis, corneal irritation, corneal necrosis), gastrointestinal (nausea, vomiting, diarrhea, abdominal pain), pulmonary (vascular epithelium damage, petechiation, hemorrhage, atelectasis, respiratory distress) and central nervous system (euphoria, seizures, coma) disorders (Murphy *et al*., 2003).

Alkylated olefins especially 2-Undecene, 4-methyl-peak with 43% identity could be noticed in all three three local toners whereas in addition to undecene, 2-Nonene, 2-methyl- was also present with 43% identity. According to Hohner and Bayer (2006), these olefin monomers are present in the toner as formulation components of polyolefin waxes that act as release and anti-offset agent so as to aid the detachment of the photocopier toner from the fixing roller, act as adhesion promoters in the transfer to the paper and, in the production of the toner, contribute to homogeneous distribution of the pigments by acting as dispersion aid. They are essential in maintenance of melting temperature between 130^{o} C and 160^{o} C that is desirable for fusion of the resultant toner onto the paper (Kasuya *et al.,* 1985).

Olefins (alkenes) ranging in carbon number from C6 to C24, alpha (linear) and internal (linear and branched) demonstrate low acute toxicity by the oral, inhalation and dermal routes of exposure. These chemicals possess properties indicating hazards to human health (reversible mild skin and eye irritation; mild respiratory tract irritation to the lower chain length members) and the environment (Machado, 2004).

Halo olefin peak with 99% identity for tetra chloroethylene could be noticed in all local toners A,B and C (Table 7). These are used as copolymer with olefin monomers to produce toners powders of appropriate size as according to Kasuya *et al*. (1985). Among the other aliphatic hydrocarbons, the presence of alkyl ketone especially 2 acetyl cyclopentanone could be noticed in all toners with 35% identity (Table 7). It is used as an ink composition medium among all the toners (Uhlir-Tsang *et al*., 2007). In addition to this, the presence of another alkyl ketone, Ethanone, 1- cyclopentyl with 38% identity could also be noticed in local toners A, B and C which was not present in global toner D.

The presence of octamethyl cyclotetrasiloxane, as anti-blocking agent for free flowing of the toner could also be noticed in the toner C with 64% identity (Table 7). Siloxane (D4) is classified as persistent, bioaccumulative and toxic compound. Its high rate of volatility makes it harmful to respiratory system according to environmental risk assessment report by Brooke *et al*. (2009).

There were also other aliphatic hydrocarbons with 35 – 43% identity that includes, alkyl ester, primary amine, amide, aliphatic dicarboxylate and heterocyclic compounds noticed in local toner A formulation except for two heterocyclic compound piperazine-2-methyl and cyano acetyl urea which were also found in toner B and C. Aliphatic alcohol, 1-butanol peak was found to have 52% identity. This peak could be found in toner B and C composition. Use of halogenated aliphatic hydrocarbons involves serious health problems. They possess many local as well as systemic toxic effects; the most serious include carcinogenicity and mutagenicity effects on the nervous systems and injury to vital organs according to Stellman *et al*. (1998).

Thus, it is understood that basically all the toners whether local or global, release different hazardous organic compounds that include, polycyclic aromatic hydrocarbons, volatile organic compounds that include aromatic, aliphatic and heterocyclic compounds as a by-product of xerographic process during heat fixing process at a temperature of 180°C. These volatile organic compounds were not detectable in workplace air (table 6) which might be due to their less stability

and more photo-reactive nature of these compounds. Hence, toners were analysed by head space GC MS. These significant differences noticed among the emitted compounds from the toners according to Henschel *et al*. (2001), is mainly due to the differences in the process and / or polymer feedstock used in toner manufacture. Toners can be classified as belonging to the group of "granular bio-durable particles without known significant specific toxicity" (GBP). These kinds of dusts are called "low toxicity poorly soluble particles" according to reports by Ewers and Nowak (2006). Reports are available that claim the components of the toners namely styrene, benzene derivatives, PAHS, aliphatic hydrocarbons and ozone to cause either mutagenic or carcinogenic effects in humans (Vaghef and Hellman, 1998; Somrovska *et al*., 1999; Andreoli *et al*., 1997; Popp *et al*., 1997; Indulski *et al*., 1997; Garciduenas *et al*.,1997 and Goud *et al*., 2001).

Phase II

Health surveillance of photocopier service professionals

4.2. Demographic Profile of the Selected Participants

4.2.1 Socio Economic Status

According to Winkleby et al. (1992), socio economic status is a commonly used measure in epidemiological studies which is determined by education, income, occupation or a composite of these dimensions. The current practice in the analysis and interpretation of occupational data in epidemiology studies would be improved by the acknowledgement and examination of the relation between socio economic status, working conditions and health through multiple pathways (social, behavioural, physiologic and environmental) (MacDonald *et al*., 2009). Hence, in the present study, educational level and socioeconomic status were documented to ensure there is no influence of these factors on the outcome measures. The socioeconomic status and educational level of the participants are presented in Table 8.

Table 8

Socio economic status of the selected participants of the study

Socio economic Status[#]	Control (n = 77)	Exposed (n = 100)	p value
Monthly income (Rs.)			
< 3000	11	4	0.075
3000 – 8000	41	52	
8000 – 13000	11	21	
>13000	14	23	
Educational qualification			
< S.S.L.C	10	20	0.217
S.S.L.C	14	36	
Higher Secondary	15	17	
Diploma	27	57	
Graduation	3	9	

[#] Fishers exact test

The average income per month among both the groups is above Rs. 3000. Majority of them have completed a diploma course. Hence, it could be suggested that the study participants included similar socioeconomic status ($p<0.05$). Socioeconomic status (education, occupation, income) is often a confounder in long term pollution health effects according to reports by Laurent *et al.* (2007). In the present study, the influence of these risk factors [(< Rs.3000) and (< [10 years of study] S.S.L.C)] on respiratory function, the primary outcome measure of the study were eliminated as they did not significantly differ between the two groups.

4.2.2 Anthropometric Measurements of the Selected Participants

Anthropometric measurements namely the mean age, weight, height and Body Mass Index (BMI) are presented in Table 9.

Table 9

Anthropometric measurements

Anthropometric Measurement	Control (n = 77)	Exposed (n = 100)	p value
	Mean ± S.D	Mean ± S.D	
Age ‡	30.71 ± 8.23	29.19 ± 7.24	0.101
Weight ‡	68.26 ± 11.61	66.04 ± 8.36	0.141
Height ‡	168.78 ± 7.98	168.45 ± 5.48	0.744
Body Mass Index (BMI) ‡	23.94 ± 3.49	23.29 ± 2.95	0.184

‡ independent t test, values are expressed as **Mean ± S.D**

The participants in both the control and exposed groups did not significantly vary in the age. Afonso *et al.* (2011) observed that the incidence of respiratory disease increased with age. According to Zammit *et al.* (2010), BMI and its contributors (weight and height) are also important confounders that influence lung function. Weight gain and rising BMI are important risk factors associated with decrease in lung volumes and thereby respiratory diseases. However, in the present study, height, weight and BMI were also not different between the participants of the two groups. This minimizes the influence of these anthropometric measurements on the various lung and blood parameters studied.

4.2.3 Smoking Status and Alcohol Consumption

Smoking has a direct effect on the respiratory system. The rate of cigarette smoking among young people has continued to increase steadily as reported by Tantisuwat and Thaveeratitham (2014). Cigarette smoking is the principal cause of reduced lung function, the major factor of chronic obstructive pulmonary disease (COPD) (Hurd, 2000). Hence, smoking is an important confounder that needs to be measured in occupational exposure studies as stated by Richardson *et al.* (2014). Risk of occurrence of chronic obstructive pulmonary disease has been found to increase with pack years (Reilly *et al*., 2008). For this reason,

record of smoking and pack years of all participants help in the analysis of the effects of smoking on the different outcomes of occupational health studies.

In the present study, pack years of smoking is calculated as: Average number of cigarettes per day / 20 x number of years smoked. Ever smokers include both the current and the former smokers (S) (those who had quit smoking prior to three months of study period). Subjects who had never smoked cigarette in their lifetime were defined as non smokers (NS).

Table 10

Smoking status and alcohol consumption

Confounder	Control (n = 77)	Exposed (n = 100)	p value
Smoking status			
Ever smokers (S) †	37	52	0.356
Pack years ‡	1.07 ± 0.92	2.35 ± 2.9	0.050*
Alcohol consumption†	36	50	0.668

† Chi square test, ‡ independent t test, values are expressed as Mean ± S.D, $p < 0.05$*

Table 10 presents the smoking status and the alcohol consumption of the study participants. There were no significant differences in smoking habit and in alcohol consumption between control and exposed groups. Hence, the effect of these confounders on the study outcome was found to be minimum whereas pack years were found to be significantly different between control and exposed groups. Hence, the effects of pack years of cigarettes smoked on the different outcome of lung function and biomarkers are analyzed.

Alcohol consumption is also a frequent risk factor that merits attention as confounder in environmental and occupational exposure epidemiological studies (Bloom and Vena, 2015). Hence, self-reported alcohol consumption status of all participants was documented through the interview schedule.

4.2.4 Status of other Confounders

Exposure to biomass cooking fuel is reported to be associated with a multitude of respiratory and other diseases (Kim *et al*., 2011b). The type of cooking fuel usage was studied in the present study as biomass cooking fuel may be a confounder. This is presented in Table 11. Effect of confounder namely biomass fuel smoke was nullified since 99% of the study population used liquefied petroleum gas as household cooking fuel.

Table 11

Other confounder – Type of cooking fuel used

Cooking fuel#	Control (n = 77)		Exposed (n = 100)		p value
	n	%	N	%	
LPG	77	100	99	99	1.00
Firewood	-	-	1	1	

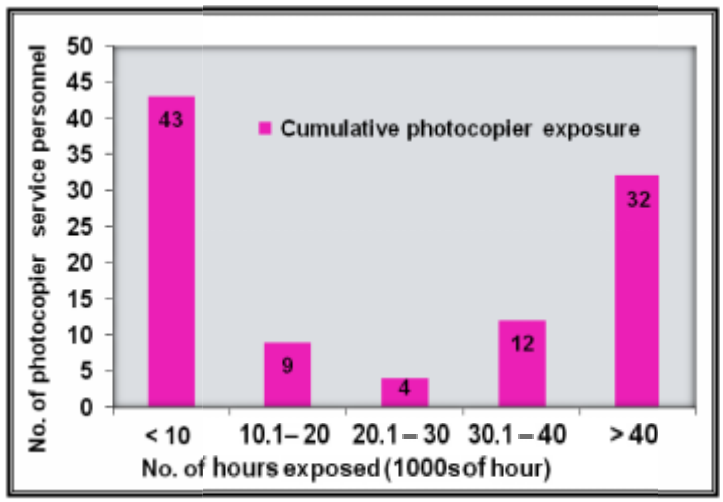
Fisher's exact test

4.2.5 Photocopier Exposure

Exposure to photocopier machines in general among photocopier service personnel includes exposure to spilled toner powder, cleansers and other emissions from photocopier machines in general as part of their machine maintenance process. It was self reported by the photocopier service personnel during administration of the interview schedule. Since the number of working hours varied, the cumulative exposure was calculated using the formula according to Elango *et al*. (2013): cumulative exposure = abc x 50, where a = No. of working hours/day; b = No. of working days/week; c = No. of years of exposure and the number 50 denotes 50 weeks/year. The cumulative exposure to photocopier machine maintenance is shown in Figure 20.

Figure 20

Cumulative photocopier exposure among photocopier service personnel



It is evident from Figure 20 that the number of participants with less than 10,000 hours of cumulative exposure (fresher in the industry) was at the maximum of 43% in the present study.

4.3 Respiratory and General Health Symptoms

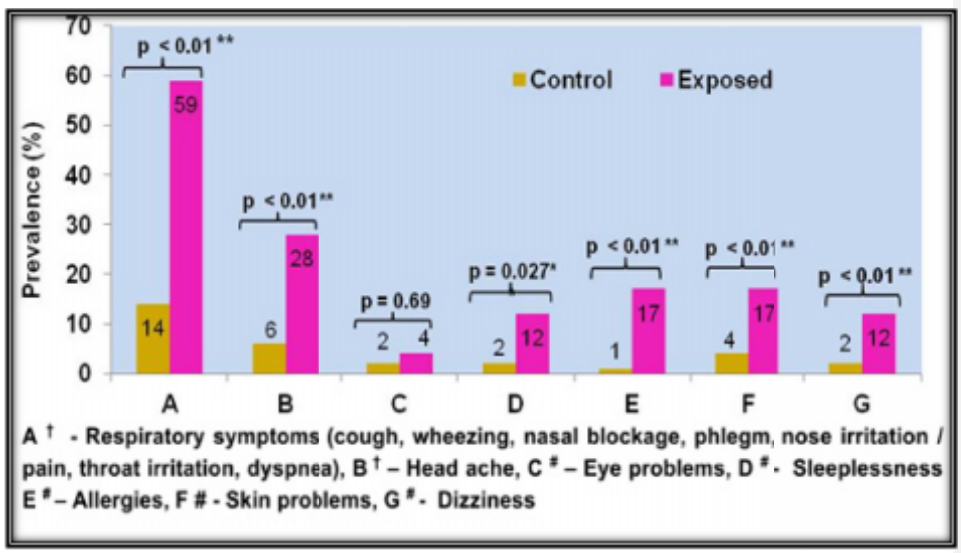
The prevalence of respiratory and general health symptoms among the study population is represented in Figure 21. The incidence of any one respiratory symptom such as cough, wheezing, nasal blockage, excessive sputum production, nose irritation, throat irritation and dyspnea was significantly higher ($p<0.01$) among photocopier service personnel when compared to controls.

A case study by Muñoz *et al*. (2007) reported chest tightness and dyspnea on exposure to toner. Higher incidence of respiratory symptoms among photocopier workers due to particulate matter exposure in photocopier units was also reported in study by Elango *et al*. (2013). Kitamura *et al*. (2014) also reported higher prevalence of breathlessness/dyspnea among the toner handling group of workers in a cohort study. Even in the present study, the significant differences between the study groups in respiratory symptoms might

be due to the exposure to particulate matter and volatile organic gaseous pollutants in the photocopy shops.

. **Figure 21**

Prevalence of respiratory and other general health symptoms



Chi square test, *p < 0.05, **p < 0.01

Among the prevalence of general symptoms namely headache, sleeplessness, allergies, skin problems and dizziness were found to be significantly different between the two study groups. All these factors emphasize a decline in the general health of the photocopier service personnel due to occupational exposure to pollutants especially toners, particulate matter, volatile organic compounds and cleansing organic solvents. Magari *et al*. (2001) reported that high level of inhaled particulates may affect the autonomic nervous system that would bring a drop in their sleeping period. Symptoms of toxicity on exposure to dry cleaning solvents namely tetrachloroethylene and toluene include fatigue, dizziness, headache, vomiting and nausea, signs of central nervous system dysfunction and narcosis (Garnier *et al*., 1996 and Malaguarnera *et al*., 2012).

4.4 Lung Function Profile

Spirometry is a test most frequently used to measure lung function and it is a measure of lung air flow volume (Litre) against time in seconds (Ranu *et al*., 2011). It is commonly interpreted in comparison to predicted normal value, based on a patient's sex, height, age and race, with the observed value expressed as a percent of predicted (Pakhale *et al*., 2009).

Lung function indices of spirometry include parameters namely Vital Capacity (VC), Forced Vital Capacity (FVC), Forced Expiratory Volume in 1 sec (FEV1), FEV1/ FVC or FEV1 ratio, $FEF_{25,\ 50,\ 75\%}$ and FEF $(_{25\ -\ 75\%})$ and MVV (Maximum Voluntary Ventilation). Table 12 gives the levels of lung function indices analyzed among the participants in the present study.

Table 12

Lung function Indices among the selected participants

Lung Function Parameters (% predicted)	Control n = 77	Exposed n = 100	p value
VC	**80.00 (73.0 – 85.00)**	**77.00 (71.00 – 83.00)**	**0.018 ***
FVC	84.00 (82.00 – 96.00)	87.50 (83.00 – 98.00)	0.624
FEV_1	84.00 (80.00 – 94.00)	88.00 (83.00 – 94.75)	0.102
FEV_1 / FVC	102.00 (95.00 – 105.00)	103.00 (93.25 – 109.75)	0.459
PEF	94.00 (84.00 – 105.00)	86.00 (83.00 – 107.00)	0.849
FEF_{25-75}	76.00 (61 .00 – 93.00)	80.00 (58.50 – 92.50)	0.820
PIF	45.00 (34.00 – 62.00)	42.00 (30.00 – 54.50)	0.106
MVV	**102.00 (92.50 – 110.00)**	**93.50 (77.00 – 102.00)**	**0.001****

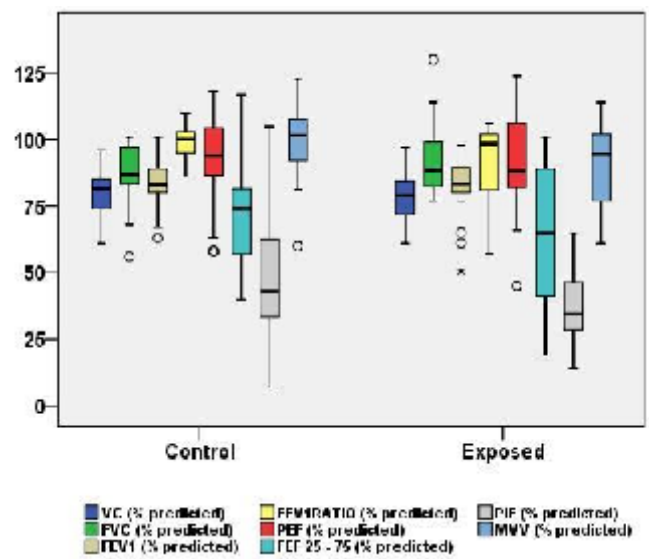
Values are expressed as median with (inter quartile range in parenthesis), p values for Mann Whitney test, *p<0.05, ** p<0.01

Mann Whitney test is a nonparametric test used to compare two populations when data are not normally distributed (Kühnast and Neuhäuser, 2008)

According to Weiss (1991), Vital capacity is a very suitable index for Vital capacity is the maximum volume of gas that can be slowly exhaled from the lungs after a maximal inspiration to full inspiration (Haynes, 2015). It is an important measure in patients with respiratory muscle weakness which is characterization of the restrictive ventilatory pattern (Hrist and Walsh, 2016).

Figure 22

Lung function indices of the selected participants



The box plots show the sample median (central vertical line), and the range within which the central 50% of values fall (box length), with the box edges at the first and third quartiles. The whiskers show the range of values, and outsider values are indicated by small "o"s. Far out value is indicated by asterisks.

It is evident from Table 12 and Figure 22, that there was a significant decrease in VC among exposed participants in comparison to controls which suggest restrictive pattern. VC is reduced in relation to the extent of loss of functioning lung parenchyma in many non obstructive lung disorders (Pellegrino *et al.,* 2005).

In contrast to this, Elango *et al*. (2013) reported no variation in any of the pulmonary function parameters among photocopier operators suggesting a possibility that photocopier workers are less toner handling group in comparison to the photocopier service personnel.

Forced Vital Capacity (FVC) is the total volume of air expelled by a forced expiration after maximal inspiration (Davey, 2010). FVC is an important parameter of the lung function test, which is used to diagnose airway obstruction and to rule out a restrictive process (Aaron *et al*., 1999). Forced Expiratory Volume in first second (FEV1) is the volume of air exhaled during the first second of a forced vital capacity manoeuvre. FEV1 % is the ratio of forced expiratory volume in first second to forced vital capacity (Aggarwal, 2011).

In contrast to significant decrease in % Predicted Vital Capacity as presented in Table 12 and Figure 22, there were no changes in % Predicted Forced Vital Capacity and % FEV_1 and FEV1/ FVC which indicates the absence of airway obstruction. These results were in tune with those observed by Nakadate *et al*. (2006) and Iwasawa *et al*. (2012) among workers in toner manufacturing units. Elango *et al*. (2013) reported no change in FEV1 and FEV1 / FVC lung function parameter among photocopier operators. Yanagi et al. (2014) also noticed no change in % predicted FEV1 values among toner handling workers in a four year follow-up cohort study.

According to Pellegrino *et al*. (2005), when VC is reduced, the FEV_1/ VC is normal or increased, a restrictive pulmonary defect may be suspected. The results of the present study support the same. Muhle *et al*. (1991) also reported minimal to moderate lung fibrosis in rats chronically exposed to high levels of respirable toner particles. Gallardo *et al*. (1994) described a rare case of restrictive lung disease and siderosilicosis due to photocopier toner dust.

Forced Expiratory Flow ($FEF_{25-75\%}$) is the average forced expiratory flow from the point at which 25% of the FVC has been exhaled to the point at which 75% of the FVC has been exhaled out (Johnson and Theurer, 2014). According to Ciprandi *et al*. (2012), FVC forced expiratory flow at 25 - 75% could be more

sensitive than FEV1 to detect slight airways obstruction. The results of the present study showed no difference in terms of PEF, FEF_{25-75} and PIF between the two groups namely control and photocopier service personnel. In contrast, Gardiner *et al.* (2001) found a significantly decreased FEF 25 - 75% in a cross sectional study among workers occupationally exposed to carbon black in European carbon manufacturing plant.

Peak Expiratory Flow (PEF) rate is the maximal rate that a person can exhale during a short maximal expiratory effort after a full inspiration (Abraham *et al*., 2014). PEF is reflective of expiratory muscle strength, especially the abdominals and the dimensions of the intra and the extrathoracic airways (Quanjer *et al*., 1997). PEF monitoring is a confirmatory test for the diagnosis of occupational asthma (Acton, 2011). There were no significant differences noticed in % predicted PEF rates between both groups as evidenced in Table 12 and Figure 22. In contrast, Zhang *et al*. (2014) reported significant difference in PEF % among workers exposed to carbon black in a cross sectional study.

Peak Inspiratory Flow (PIF)(% predicted) is presented in Table 12 and Figure 22. PIF is the highest inspiratory maneuver. It is the lowest point on the inspiratory curve (Whitman and Holland, 2012). No significant changes in PIF (% predicted) were found between the two groups.

The Maximal Voluntary Ventilation (MVV) maneuver can be used to confirm obstructive and restrictive conditions. A low MVV can occur in obstructive disease but is more common in restrictive conditions (Barreiro and Perillo, 2004).

There is a significant decline in the MVV of photocopier service personnel (exposed group) in comparison to control group (Figure 22). In tune with the decrease in VC, decrease in MVV among photocopier service personnel indicates lung disease on exposure to toner and gaseous pollutants in xerographic units. Styrene being an important volatile organic component of toner might have lead to decline in lung function especially VC and MVV. Since, it has been reported by Sati *et al*.(2011), that exposure to styrene in plastic factory workers leads to significant reduction in most of the lung volumes, capacities

(FVC, FEV(1), VC, ERV, IRV and IC) and flow rates (PEFR, MEF(75%) and MVV) in the styrene exposed workers in plastic factory.

4.4.1 Ventilatory Patterns Among the Selected Participants

According to Global Initiative for Chronic Obstructive Lung Disease (2010) there are three basic spirometric ventilary patterns that are Normal [(FEV1 and FVC above 80% predicted); (FEV1/FVC ratio above 0.7)], Obstruction [(FVC can be normal or reduced – usually to a lesser degree than FEV1); FEV1/FVC ratio below 0.7] and restriction [(FEV1 normal or mildly reduced); (FVC below 80% predicted)].

Table 13 presents the incidence of ventilatory pattern among the present study participants. The participants of both control and exposed group were categorized specifically as smokers and nonsmokers to assess the incidence of ventilatory pattern since according to Tantisuwat and Thaveeratitham (2014), smoking has a direct effect on the respiratory system. Spirometric normal ventilatory pattern was found to be 80% among Control Nonsmoker (CNS) and 78.4% among Control Smoker (CS) in comparison to 66.8% and 59.6% among Exposed Nonsmoker (ENS) and Exposed Smoker (ES) respectively followed by the prevalence of abnormal ventilatory defect which were 20% among CNS and 24.3% among CS in comparison to 35.4 % and 48% among ENS and ES respectively.

Table 13

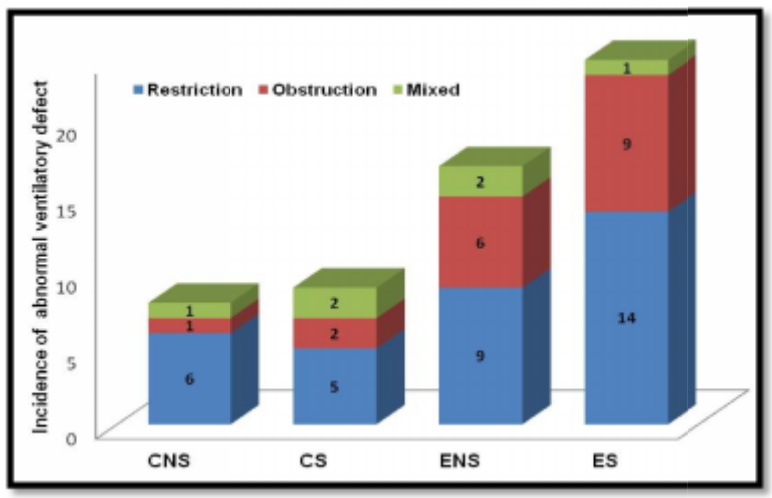
Incidence of ventilatory pattern

Lung Function	Control				Exposed			
	CNS n = 40	%	CS n = 37	%	ENS n = 48	%	ES n = 52	%
Normal	32	80	28	78.4	31	66.8	28	59.6
Abnormal	8	20	9	24.3	17	35.4	24	48
a. Restriction	6	15	5	13.5	9	18.8	14	26.9
b. Obstruction	1	2.5	2	5.4	6	12.5	9	17.3
c. Mixed	1	2.5	2	5.4	2	4.2	1	1.9

The abnormal ventilatory defect pattern includes restriction, obstruction and mixed type (includes both restriction and obstruction). From Figure 23, it was inferred that there is higher incidence of restrictive ventilatory followed by obstructive and mixed ventilatory pattern defect among photocopier service personnel (exposed group) in comparison to control group. This restrictive ventilatory pattern is also emphasized by significant decrease in VC and MVV among the photocopier service personnel in Table 12.

Figure 23

Incidence of abnormal ventilatory pattern among the selected participants



4.4.2 Correlation between Lung function and Cumulative Photocopier Exposure among Photocopier Service Personnel

Table 14 presents the correlation between lung function and cumulative photocopier exposure of the service personnel in xerographic units. There exists significant negative correlation between the following lung function parameters namely VC, FVC, FEV1 and MVV with cumulative photocopier exposure. These significant negative correlations clearly indicate that photocopier exposure among service engineers cause restrictive lung diseases. Similarly, Gardiner *et al*. (2001) in a cross sectional study found a significant association between exposure and decrement in FEV1, among participants exposed to

carbon black in European carbon black manufacturing industry in a cross sectional study.

Table 14

Correlation between lung function indices and cumulative photocopier exposure

Lung function parameters (% predicted) Vs Cumulative photocopier exposure (1000s of hour)	Correlation (n = 100)	
	r_s	p value
VC	**-0.279**	**0.005****
FVC	**-0.260**	**0.009****
FEV_1	**-0.309**	**0.002****
FEV_1/FVC	-0.001	0.996
PEF	0.002	0.984
FEF_{25-75}	-0.188	0.061
PIF	-0.033	0.743
MVV	**-0.287**	**0.004****

r_s - Spearman's rank correlation coefficient ** $p < 0.01$

Spearman's correlation coefficient (rs), a non parametric statistic is used in case of non normal data distribution (Mukaka, 2012)

A negative correlation of MVV with cumulative photocopier exposure is noticed (Table 14). These results were in contrast to results obtained by Elango *et al*. (2013) among photocopier operators who showed strong negative associations between FEV1/ FVC ratio and MMEF with cumulative photocopier exposure that predisposed the photocopier operators to chronic obstructive pulmonary disease. A negative correlation of MVV with occupational exposure to woollen dust particles was noticed among workers of woollen industry (Purohit *et al*., 2014). Meo *et al*. (2004) found a decrease in MVV which was further decreased with increase in duration of exposure to cement dust particles. Statistically significant decrements in FVC, FEV1, MVV and PEFR on chronic exposure to petrol fumes that consist of benzene, petrol and diesel exhaust, particulate matter and air pollutants were revealed among petrol pump workers (Hulke *et al*., 2012).

These significant negative correlation of lung function indices namely VC, FVC, FEV1(% predicted values) on long term exposure with % predicted FEV1/ FVC ratio indicates causal effects of cumulative xerographic exposure on lower airways with restrictive ventilatory pattern and decline in lung function is an indicator of progressive lung inflammation.

These results are in conformation with a case study by Gallardo *et al*. (1994) who reported siderosilicosis due to iron and silicon dust emission in female photocopier operators with predominantly bronchial obstruction, gradual pulmonary restriction and reduced lung diffusion of carbon monoxide that did not improve in pulmonary function even after treatment with oral corticosteroids except for clinical symptoms. Ambruster *et al*. (1996) also reported granulomatous pneumonitis and mediastinal lymphadenopathy lung diseases due to respirable toner dust emission of copper, silicon and iron. Song *et al*. (2009) stated that polyacrylate nanoparticulate emulsion exposed workers suffered from restrictive ventilatory dysfunction with nonspecific and progressive pulmonary inflammation, fibrosis and foreign-body granulomas of pleura whereby the nanoparticles lodge into the cytoplasm and caryoplasm of pulmonary epithelial and mesothelial cells causing progressive inflammation. Toner being a hybrid formulation of adhesive organic carbon nanoparticulate component (polyacrylate) (> 50%) and inorganic component [iron (23 – 30%) and silicon (0.37 – 0.52%) in Figure 15], might have caused this restrictive lung disease among xerographic service personnel due to exposure to particulate emissions (Table 6) from toners during machine maintenance cycles.

Ventilation plays a key role in the adequacy of the external gas exchange, the ultimate lung function. The appropriateness of the ventilatory pump response to a given metabolic load, however, is intrinsically linked to the ability of the force-generator units (i.e., the respiratory muscles) to provide the required output. The strength of the respiratory muscles could be inferred from dynamic maneuvers, maximal voluntary ventilation (MVV) (Neder *et al*., 1999).

Thus significant reduction and negative correlation of Vital Capacity and MVV with cumulative photocopier exposure in the present study might be attributed to decline in the involuntary movement of the respiratory muscles on

long time exposure to organic solvents namely toluene, benzene and tetrachloroethylene in toner formulation (Table 7) among photocopier service personnel and this is further substantiated by higher incidence of restrictive ventilatory pattern as recorded in Table 13. These results are coherent with the reports by Uzma *et al*. (2008) who concluded toxic deleterious effect on respiratory system on long exposure to organic solvents and polluted air in petrol filling workers.

4.4.3 Correlation between Lung function, Cumulative Photocopier Exposure and Pack Years among Photocopier Service Personnel who are Smokers

The influence of smoking as a confounder was studied among exposed photocopier service personnel smokers. Correlation between pulmonary function and pack years of cigarettes smoked among photocopier service personnel is presented in Table 15. A new variable was created by multiplying cumulative photocopier exposure (PE) and pack years (PY) based on Elango *et al*. (2013).

Table 15

Correlation between lung function and cumulative photocopier exposure and pack years among photocopier service personnel who are smokers

Lung function parameters (% predicted)	Cumulative Photocopier exposure (PE) (n = 52)		Pack year (PY)		PE X PY	
	r_s	p value	r_s	p value	r_s	p value
Vital Capacity (VC)	-0.065	0.648	0.076	0.590	-0.039	0.785
Forced Vital Capacity (FVC)	-0.151	0.285	0.040	0.777	-0.095	0.505
Forced Expiratory Volume in 1 second (FEV_1)	-0.236	0.092	0.116	0.414	-0.093	0.513
FEV_1/FVC	-0.056	0.696	0.038	0.791	0.004	0.975
Peak Expiratory Flow (PEF)	-0.121	0.392	0.152	0.281	-0.045	0.751
Maximum Mid Expiratory Flow (FEF_{25-75})	**-0.284**	**0.041***	0.184	0.192	-0.119	0.401
Peak Inspiratory Flow (PIF)	0.015	0.919	**-0.283**	**0.042***	-0.125	0.377
Maximal Ventilatory Volume (MVV)	-0.195	0.166	-0.249	0.075	-0.237	0.090

r_s - Spearman's rank correlation coefficient *p<0.05

It is obvious that there was a significant inverse relationship between % predicted lung function parameter namely Maximum Mid Expiratory flow and cumulative exposure and between Peak Inspiratory Flow and pack years individually. However, there was no significant correlation noticed between any of the other lung function parameters with the combined exposure variable (PE X PY).

4.4.4 Causative Effect of Lung Dysfunction

The study of causal relationships is important when addressing questions of etiology of the disease and the efficacy of treatment intervention in an epidemiological study (Daya, 2003). The evaluation of a cause-and-effect relationship between exposure to putative causal factor and outcome was undertaken in the present study to validate the hypothesis whether photocopier exposure leads to lung dysfunction.

In order to study this causative effect of abnormal ventilatory pattern, binary logistic regression was performed with normal and abnormal lung function as dependent variables. Odds ratio (O.R) of 3.1 at confidence interval of 1.19 to 8.14 (Table 16) indicated that there was a significant causal effect of photocopier exposure on the abnormal ventilatory pattern and it was found to be 3.1 times more likely to positively influence the lung function among photocopier exposed service personnel. It is obvious that the likelihood of lung dysfunction is merely because of occupational exposure and not because of synergistic effect of smoking and occupational exposure to photocopier machines and its components at their present exposure levels. In tune with this, Kitamura *et al*. (2014) declared that toner being a particulate chemical of about 6 to 8 microns inhalable in size could not be denied for its cause for occurrence of both restrictive diseases namely pneumoconiosis and chronic obstructive pulmonary disease among toner handling workers. Thus higher exposure and proximity to respirable toner size of 2 to 10 µm (Plate 3) with pollutant levels (227.49 ± 16.31 µg /m^3) of $PM_{2.5}$ (Table 6) in ambient air of xerographic units and organic solvent composition of toner (Table 7) among photocopier service personnel might be the reason for increased restrictive ventilatory pattern.

Table 16

Results of binary logistic regression with normal and abnormal lung function indicator as dependent variable

Factor	ß	p	O.R^	95% CI	
				Lower	Upper
Photocopier Exposure (PE)	1.135	0.021*	3.1	1.19	8.14
Smoking (S)	-0.069	0.905	0.933	0.301	2.889
PE X S (Synergistic / Combined effect)	-0.491	0.491	0.612	0.152	2.473

Value indicated as (^) denotes significant odds ratio (O.R) at p ≤ 0.05 at 95% Confidence Interval (CI).

Odds ratio (OR) is the measure of association between an exposure and an outcome. Odds ratio represents the odds than an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. In calculation of logistic regression, the regression coeffiecinet (ß) is the estimated increase in the log odds of the outcome per unit increase in the value of the exposure (Szumilas, 2010).

OR = (odds of disease in exposed / odds of disease in the control).

4.5 Complete Blood Count among the Participants

Blood can provide an early clue of environmentally induced illness, as it serves as conduit for harmful agents and also as an organ system it may be adversely affected by occupational exposure to potentially harmful agents. It serves as a biological monitor of exposure and provides a way to assess the effects of occupational exposure on lymphohaematopoietic system and other body organs (Goldstein, 1998). This necessitates complete blood count (CBC) as routine diagnostic test in workplace occupational health surveillance studies.

Rapid, accurate and relevant laboratory testing is essential in an era of cost-effective medicine. With the advent of automated haematoanalyzers and the principle of nonoptical electrical impedence that offers "state of the art" precision and accuracy in counting more than 10,000 cells per blood sample that includes RBC, WBC and platelet data, as well as histograms that characterize hematologic data (Rappaport *et al.,* 1988). Routine labour intensive complete blood count (CBC) has become easier with 7-parameter CBC and three-part

differential CBC obtained from a single aspiration on a stand-alone, bench-top instrument without uncapping the sample in automated haematoanalyzers (Urrechaga *et al.,* 2014).

According to Nakadate *et al*. (2006), machine maintenance subjects had longer duration of toner exposure than workers of toner production unit and machine development. Photocopier service personnel are exposed to toners particulate matter, toxic gaseous emissions and organic solvents viz., benzene and butanediol either while cleaning the machine or replacing the toner.

4.5.1 Red Blood Cell Indices and Platelet Indices

Table 17 presents the red blood cell indices and platelet indices. The Red cell indices continue to provide an essential support to the diagnosis and monitoring of hematological diseases, which include Mean Cell Volume (MCV), Mean Cell Haemoglobin Concentration (MCHC) and Mean Cell Haemoglobin (MCH) derived from precisely measured values of Haemoglobin (Hb) and Red Blood Cell (RBC) count collectively called the Wintrobe indices (Brugnara and Mohandas, 2013).

Mean Cell Volume (MCV) defines the size of the red blood cells and is expressed as femtoliters. Mean Corpuscular Haemoglobin (MCH) quantifies the amount of hemoglobin per red blood cell. Mean Corpuscular Haemoglobin concentration (MCHC) indicates the amount of hemoglobin per unit volume. MCHC correlates the hemoglobin content with the volume of the cell. It is expressed as g / dl of Red Blood Cells (RBC). RDW represents the coefficient of variation of the red blood cell volume distribution (size) (Sarma, 1990).

Table 17 present the levels red blood cell indices and platelet indices. It is evident that RBC count and Haemoglobin levels were not significantly different between the two groups. The results obtained were similar to the reports of Kitamura *et al.* (2009) who found no differences in the blood counts between workers in toner manufacturing units and referents. Even the photocopier operators did not show significant differences in the haematological parameters in comparison to the control subjects (Awodele *et al.,* 2015 and Elango *et al.,*

2013) whereas Moro *et al*. (2015) reported early haematological decrease in RBC count and Haemoglobin levels in gasoline exposed group in comparison to control group. Schnatter *et al.* (2010) also reported a decrease in haemoglobin levels and increase in MCV of participants exposed to benzene.

Table 17

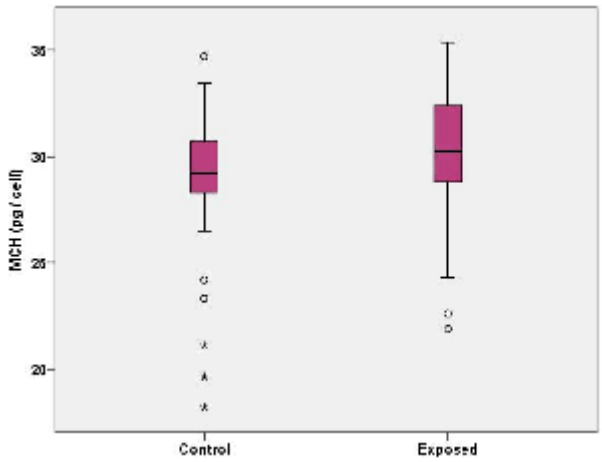
Red blood cell indices and Platelet indices

Haematological indices	Control n = 73	Exposed n = 90	p value
RBC (10^6/µl)	5.3 (4.6 – 5.8)	5.2 (4.9 – 5.6)	0.552
Hb (g/dl)	15.7 (13.4 – 16.9)	15.8 (14.8 – 16.7)	0.226
Hematocrit (%)	44.6 (39.4 – 49.3)	46.4 (43.10 – 48.7)	0.477
MCV (fL)	86.2 (83.2 – 89.5)	88.1 (84.0 – 89.8)	0.102
MCH (pg)	**29.2 (28.3 – 30.8)**	**30.3 (28.8 – 32.4)**	**0.007****
MCHC (g/dl)	**34.0 (32.5 – 34.5)**	**34.9 (33.3 – 36.1)**	**0.003****
RDW (fL)	43.4 (41.9 – 45.6)	44.2 (41.6 – 45.8)	0.751
Platelets(10^3/µl)	251 (226.0 – 295.5)	277 (233.5 – 301.5)	0.103
PDW (fL)	12.1 (11.1 – 13.8)	12.6 (11.5 – 14.3)	0.075
MPV (fL)	9.8 (9.4 – 11.0)	10.2 (9.6 – 10.9)	0.212
P-LCR (%)	23.4 (20.0 – 31.9)	26.8 (21.5 – 32.2)	0.136

Values are expressed as median with (inter quartile range in parenthesis), p values for Mann Whitney test, ** $p<0.01$

Figure 24

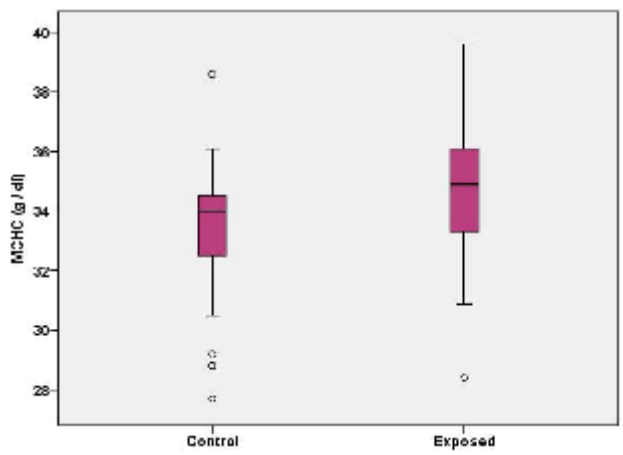
Mean Corpuscular Haemoglobin



Mean Corpuscular Haemoglobin (Figure 24) and Mean Corpuscular Haemoglobin Concentration (Figure 25) levels were found to be significantly increased among the photocopier service personnel when compared to the controls.

Figure 25

Mean Corpuscular Haemoglobin Concentration



Haufroid *et al.* (1997) reported significant increase in MCHC among workers exposed to low levels of 2-butoxyethanol. Even the liquid petroleum gas filling workers were found to have increased levels of MCH, MCHC when compared with the controls (Sirdah *et al.,* 2013). In contrast to this, Bogadi-Sare *et al.* (1997) reported decreased levels of MCH among workers in shoe making industry exposed to solvents like benzene and toluene. Gaharwar and Paulraj (2015) reported that increase in MCHC among nano iron oxide treated rat groups is mainly because of reduced number of blood cells, enhanced oxidative stress and reduction of cellular antioxidants and involvement of immune cells in the immune process.

Hence, it is predicted that the change in MCH and MCHC among photocopier service personnel is also due to oxidative stress induced haemolysis by exposure to complex mixture of toner pollutants that includes particulate matter and volatile organic compounds as indicated in Table 7, that induce secondary chemical reactions to produce free radicals .

Mean Platelet Volume (MPV) is the measurement of average size of the platelet (Lewis *et al*., 2006). It is calculated by dividing the platecrit (PCT), by the number of platelets. Platelet Distribution Width (PDW) is the width of the size distribution curve at 20% level of the peak. Platelet large cell ratio (P- LCR) is the number of the platelets falling above the 12 femtolitre threshold divided by the total number of platelets (Briggs and Machin, 2012).

Platelet indices namely platelet count, PDW, MPV and P-LCR were not significantly different between the control and photocopier service personnel. In contrast to present study reports, benzene exposed workers showed higher levels of PDW, MPV and P-LCR in comparison to controls (Huang *et al*., 2014).

4.5.2 Correlation between Red Blood Cell and Platelet indices and Cumulative Photocopier Exposure

Table 18 presents the correlation between red blood cell and platelet indices with cumulative photocopier exposure. Cumulative photocopier exposure was found to have significant positive correlation only with Haemoglobin (Hb) and not with any other haematological indices.

Table 18

Correlation between red blood cell and platelet indices and cumulative photocopier exposure

Hematological Indices Vs Cumulative photocopier exposure (1000s of hours	Correlation (n = 90)	
	r_s	p value
RBC (10^6/µl)	0.049	0.649
Hb (g/dl)	**0.217**	**0.040***
Hematocrit (%)	0.090	0.399
MCV (fL)	0.062	0.560
MCH (pg)	0.050	0.642
MCHC (g/dl)	0.053	0.623
RDW (fL)	0.016	0.881
Platelets (10^3/µl)	-0.086	0.418
PDW (fL)	0.051	0.631
MPV (fL)	0.001	0.994
P-LCR (%)	0.041	0.700

r_s – Spearman's rank correlation coefficient, *p<0.05

It could be predicted that (Table 18) positive correlation between haemoglobin (Hb) and cumulative exposure to photocopier pollutants on long term would lead to chronic hypoxia and change in haemoglobin concentration though an immediate significant increase in haemoglobin levels were not noticed in Table 17 among photocopier service personnel.

Sørensen *et al*. (2003) also reported positive correlation between haemoglobin and $PM_{2.5}$ exposure. A significant increase in haemoglobin (Hb) concentration was also noted by Gaharwar and Paulraj (2015) in petrol filling workers exposed to longer duration to benzene and carbon monoxide of environmental pollutants in their workplace. Subjects exposed to cigarette smoke had high Hb concentration due to the fact that smoking causes excessive production of carbon monoxide (CO), which leads to formation of carboxy haemoglobin that results in a shortage of Hb for oxygen carriage, shifting the Hb-oxygen dissociation curve to the left leading to hypoxia and stimulation of

erythropoiesis with increased levels of RBC cells, Haemoglobin and MCHC (Metta *et al*., 2015). The same might be the reason for increased levels of red blood cell indices among photocopier service personnel as the toner composition contains high percentage of organic carbon.

4.5.3 Correlation between Red Blood Cell Indices, Platelet Indices and Cumulative Photocopier Exposure, Pack Years and Synergistic Effect of both among Photocopier Service Personnel who are Smokers

Table 19 presents the correlation between red blood cell indices, platelet indices and photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers.

Table 19

Correlation between red blood cell indices, platelet indices and cumulative photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers

Hematological Indices	Photocopier Exposure (PE)		Pack Years (PY)		PE x PY	
	r_s	p value	r_s	p value	r_s	p value
RBC (10^6 cells/µl)	0.147	0.319	0.153	0.300	0.162	0.273
Hb (g/dl)	**0.323**	**0.025***	0.037	0.801	**0.309**	**0.033***
Hematocrit (%)	0.197	0.179	0.196	0.182	0.254	0.082
MCV (fL)	0.117	0.427	0-.007	0.960	0.101	0.492
MCH (pg)	0.078	0.601	-0.132	0.371	0.048	0.747
MCHC (g/dl)	0.030	0.839	-0.251	0.086	-0.070	0.637
RDW (fL)	0.001	0.994	0.154	0.295	0.139	0.345
Platelets (10^3/µl)	-0.183	0.212	-0.047	0.749	-0.162	0.270
PDW (fL)	0.066	0.654	0.131	0.375	0.070	0.637
MPV (fL)	0.079	0.595	0.188	0.202	0.116	0.434
P-LCR (%)	0.066	0.657	0.190	0.196	0.106	0.473

r_s – Spearman's rank correlation coefficient, *p<0.05

There was a positive correlation of haemoglobin not only with photocopier exposure but also with combined exposure to both photocopier exposure and

pack years of cigarette smoked indicating that there is significant positive synergistic effect of both photocopier and cigarette smoke exposure among smoking photocopier service personnel in haemoglobin levels.

In contrast, it was shown by Kamal and Malik (2012) that smoking enhances the exposure risk manifolds among automobile mechanics indicating combined effects along with occupational exposure to organic solvents indicated by reduced levels of RBC count and Haemoglobin. None other parameters of red blood cell indices and platelet indices were found to have significant correlations with either individual or combined exposure to cigarette smoke or photocopier exposure.

4.5.4 Differential Blood Count

White blood cells, lymphocytes, mixed cells (composed of monocytes, basophils and eosinophils) and neutrophils in the study participants are shown in Table 20.

There was no significant differences noticed between the control and exposed participants in differential blood count parameters namely WBC, lymphocytes and mixed cells except for a significant increase in neutrophil counts among photocopier service personnel in comparison to control participants (Table 20).

In harmony with our present study reports, Morimoto *et al*. (2013) reported persistent neutrophil infiltration in rat groups exposed to high dosage of nanoparticulate printer toners on intratracheal instillation and inhalation. Similarly, Kim *et al*. (2005) reported increase in WBC and neutrophils on exposure to high levels of welding fume consisting of particulate matter ($PM_{2.5}$) among a relatively young, healthy working population due to acute systemic inflammation. It was reported by Pirela *et al*. (2013) that neutrophils are key players in systemic inflammatory response post- exposure to a foreign substance. Neutrophil count is increased in bronchialveolar lavage fluid of mice exposed to $PM_{0.1}$.

Therefore, a substantial increase in neutrophil population in the present study indicates systemic inflammation, an adverse biological response caused due to exposure to fine particulate matter emissions in the xerographic centres.

Table 20

Differential blood count

Cell Count (103/µl)	Control n = 73	Exposed n = 90	p value
WBC	7.1 (5.8 – 8.0)	6.6 (6.0 – 8.0)	0.389
Lymphocytes	2.2 (1.8 – 3.1)	2.3 (2.0 – 2.6)	0.788
Mixed cells	0.9 (0.6 – 1.0)	0.8 (0.7 – 1.1)	0.653
Neutrophils	**3.4** (**2.7 – 3.8**)	**3.7** (**2.9 – 4.3**)	**0.004****

Values are expressed as median with (inter quartile range in parenthesis), p values for Mann Whitney test, ** p<0.01

Mixed cells were found to significantly decrease among photocopier operators in comparison to control participants (Elango *et al*., 2013) whereas in the present study no such variation in mixed cell counts could be found among the photocopier service personnel. These are in confirmation of the reports by Pirela *et al*., 2013 who also did not notice any change in the levels of lymphocytes, basophils and eosinophils in bronchoalveolar lavage fluid of mice exposed to toner particulate matter.

4.5.5 Correlation between Differential Blood Count and Cumulative Photocopier Exposure

Table 21 presents the correlation between differential cell count and cumulative photocopier exposure. There exists a significant positive correlation between neutrophil count and cumulative exposure.

The results are coherent with the report by Leonardi *et al*. (2000) that stated that long term exposure to airborne particulate matter leads to inflammation and activation of the cellular and humoral immune system. No other significant association of differential blood count with cumulative photocopier exposure could be noticed other than the significant positive association of lymphocytes with cumulative exposure.

Table 21

Correlation between differential cell count and cumulative photocopier exposure

Cell count Vs Cumulative photocopier exposure (1000s of hours)	Correlation (n = 90)	
	r_s	p value
WBC	0.037	0.728
Lymphocytes	**0.214**	**0.043***
Mixed cells	-0.097	0.365
Neutrophils	-0.094	0.380

r_s - Spearman's rank correlation coefficient, *p<0.05

4.5.6 Correlation between Differential Blood Count and Cumulative Photocopier Exposure, Pack Years and Synergistic Effect of both among Photocopier Service Personnel who are Smokers

Correlation between differential blood count and cumulative photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers is presented in Table 22.

Table 22

Correlation between differential blood count and cumulative photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers

Cell count (103/µl)	Photocopier Exposure (PE)		Pack Years (PY)		PE x PY	
	r_s	p value	r_s	p value	r_s	p value
WBC	-0.189	0.199	0.018	0.903	-0.162	0.273
Lymphocytes	-0.017	0.906	-0.054	0.713	0.008	0.959
Mixed cells	0.007	0.963	0.005	0.973	0.029	0.844
Neutrophils	-0.214	0.144	0.118	0.425	-0.144	0.327

r_s - Spearman's rank correlation coefficient

No significant correlations were observed between differential cell count, photocopier exposure, pack years and combined exposure to cigarette smoke and photocopier exposure

4.5.7 Correlation between Hematological Indices and Pulmonary Function

Correlation between haematological indices and percentage predicted lung function are shown in Table 23. Percentage predicted MVV was found to be positively correlated with red blood cell count and haematocrit where as it was found to be negatively correlated with MCV, MCH, MCHC, PDW, MPV, P-LCR and neutrophil count. Hence, percentage predicted MVV, could be suggested as the most sensitive marker that should be studied further in epidemiological studies. In addition to this, percentage predicted vital capacity was found to be negatively associated with Haemoglobin, MCV, MCH and MCHC.

Table 23

Correlation between hematological indices and pulmonary function

Hematological indices Vs Lung function indices (% predicted)	Correlation	
	r_s	p value
RBC Vs MVV	0.181	0.021*
HGB Vs VC	-0.210	0.007**
HCT Vs MVV	0.198	0.011*
MCV Vs VC	-0.156	0.047*
MCH Vs VC	-0.201	0.010**
MCHC Vs VC	-0.159	0.042*
MCHC Vs MVV	-0.189	0.016*
PDW Vs MVV	-0.202	0.010**
MPV vs MVV	-0.190	0.015*
P-LCR Vs MVV	-0.212	0.007**
Neutrophils Vs MVV	-0.200*	0.009**

r_s – Spearman's rank correlation coefficient, *p<0.05, **p<0.01

4.6 Biochemical Markers in the Selected Participants

Finn, (2015) reported that biochemical biomarkers are small molecular species, naturally varying or experimentally induced, which are measurable in body fluids and which may provide alternative or complementary tools to describe disease processes or to assess responses to pharmacological treatment. Levels of plasma glucose, serum total protein, albumin and globulin in the participants are shown in Table 24. Plasma glucose levels in the photocopier personnel were

on par with those of control participants. The level of random blood glucose was assessed to exclude diabetic participants in the study.

According to Cray *et al*. (2009) in basic health assessments, total protein and albumin are commonly measured on automated chemistry analyzers and globulin is a value calculated from these measurements. Monitor of the albumin: globulin ratio had been a standard oldest conventional method in human medicine to monitor inflammatory processes.

Table 24

Levels of glucose, total protein, albumin and globulin

Parameters	Control (n = 73)	Exposed (n = 90)	p value
Glucose (mg/dl)	87.20 (78.50 – 99.30)	86.70 (78.48 – 109.30)	0.923
Protein (g/l)	69.63 (65.67– 76.31	74.57 (71.60– 83.70)	0.003**
Albumin (g/l)	40.40 (37.5 – 45.0	40.20 (32.71 – 45.0)	0.273
Globulin (g/l)	27.50 (25.00 – 34.27	33.28 (28.67 – 41.59)	0.002**

Values are expressed as median with (inter quartile range in parenthesis),
p values for Mann Whitney test, ** p<0.01

Levels of total protein varied significantly between both the groups. Total protein levels were significantly higher in the photocopier personnel though within their normal reference levels of 60 to 83 g/L (https://www.nlm.nih.gov/medlineplus/ency/article/003483.htm).

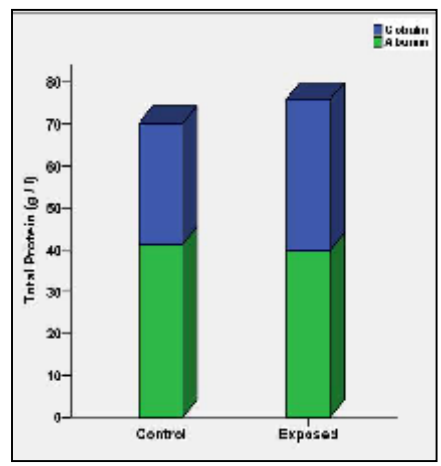


Figure 26

Albumin : Globulin Ratio

Photocopier service personnel showed a significant increase in globulin levels in comparison to control

participants. This variation in globulin levels alters the albumin globulin ratio and it was found to be lower among photocopier service personnel (1.1) when compared to controls (1.4) as depicted in Figure 26. These results are in tune with reports by Elango *et al*. (2013) that stated that globulin levels were found to significantly increase among photocopier operators chronically exposed to photocopier emissions.

Hyperglobulinemia noticed in photocopier service personnel might be a positive acute phase reaction caused by progressive decline in lung function (Figure 26) and the simultaneous inflammation as evidenced by the increase in neutrophils and correlation of lymphocytes with cumulative photocopier exposure (Table 20 and Table 21).

These results are in concurrence with Hunninghake and Crystal (1981) reported that the systemic hyperglobulinemia of pulmonary sarcoidosis, a kind of restrictive lung disease of unknown etiology may be due to the presence of activated T lymphocytes in excess in the lung which is perpetuated by the helper lymphocytes (Crystal *et al*., 1981)**.**

4.6.1 Correlation between Biochemical Markers and Cumulative Photocopier Exposure

Table 25 presents the correlation between biochemical markers and cumulative photocopier exposure**.** A significant negative correlation was found between albumin and cumulative exposure. This articulates the fact that long term exposure to photocopier pollutants might lead to decrease in albumin levels.

Albumin is a negative acute phase protein whose level decreases as a systemic response to inflammatory disorders according to Gabay and Kushner (1999). Albumin levels were significantly reduced in photocopier operators as reported by Elango *et al*. (2013). Other than this, there were no other significant correlation noticed between biochemical markers and cumulative photocopier exposure.

Table 25

Correlation between biochemical markers and cumulative photocopier exposure

Biochemical Markers Vs Cumulative photocopier exposure (1000s of hours)	Correlation (n = 90)	
	r_s	p value
Glucose (mg/dl)	0.193	0.068
Protein (g/l)	-0.142	0.182
Albumin (g/l)	-0.272	0.010**
Globulin (g/l)	0.083	0.436

r_s – Spearman's rank correlation coefficient **p<0.01

4.6.2 Correlation between Biochemical Markers and Cumulative Photocopier Exposure, Pack Years and Synergistic Effect of both among the Photocopier Service Personnel who are Smokers

Table 26, presents the correlation between biochemical marker and cumulative photocopier exposure, pack year and synergistic effect of both among photocopier service personnel who are smokers. A significant negative correlation of albumin was indicated both due to individual effect of photocopier exposure and combined effect of exposure with cigarette smoke. The effects of cigarette smoking are synergistic with several occupational and environmental hazards according to Vallyathan and Shi (1997).

Table 26

Correlation between biochemical markers and cumulative photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers

Biochemical markers	Photocopier Exposure (PE)		Pack Years (PY)		PE x PY	
	r_s	p value	r_s	p value	r_s	p value
Glucose (mg/dl)	0.047	0.751	0.251	0.085	0.118	0.426
Protein (g/l)	-0.157	0.287	-0.153	0.299	-0.241	0.099
Albumin (g/l)	**-0.307**	**0.034***	-0.020	0.894	**-0.301**	**0.038***
Globulin (g/l)	0.095	0.519	-0.176	0.233	-0.033	0.825

r_s – Spearman's rank correlation coefficient *p<0.05

4.6.3 Correlation between Biochemical Markers and Hametological Indices

Table 27, shows the significant correlations recorded between the biochemical markers and haematological indices.

Table 27

Correlation between biochemical markers and red blood cell indices

Biochemical Markers Vs Haematological Indices	Correlation	
	r_s	p value
Total Protein Vs Hb	0.226	0.004**
Total Protein Vs Haematocrit	0.194	0.013*
Total Protein Vs MCH	0.192	0.014*
Total Protein Vs PDW	0.168	0.032*
Total Protein Vs Mixed Cells	0.234	0.003**
Globulin Vs PDW	0.168	0.032*
Globulin Vs P-LCR	0.162	0.039*
Globulin Vs Neutrophils	0.176	0.025*

r_s – Spearman's rank correlation coefficient *p<0.05 **p<0.01

Total protein was found to be associated positively with haematological indices namely haemoglobin, haematocrit, MCH, PDW and mixed cells. Similarly globulin was found to be positively associated with PDW, P-LCR and neutrophils.

4.6.4 Correlation between Biochemical Markers and Lung Function Indices

No significant correlations were found between various biochemical markers assessed and lung function indices in photocopier service personnel.

4.7 Oxidative Status in the Selected Participants

Air pollutants are potent oxidants that trigger oxidative stress. Lungs are more susceptible to oxidant injury than any other organ in the body (Vallayathan and Shi, 1997). Oxidative stress is caused by an imbalance between the production of reactive oxygen (free radicals) and the body's ability (antioxidant

capacity) to readily detoxify the reactive intermediates or easily repair the resulting damage (Arrigo *et al*., 2015). It can trigger redox-sensitive pathways that lead to inflammation and cell death. However, it does appear that the susceptibility of target organ to oxidative injury also depends upon its ability to up regulate protective scavenging systems (Lodovici and Bigagli, 2011).

Inflammatory cells such as macrophages, neutrophils and eosinophils are the most important endogenous generators of oxidants. These oxidants are a part of the normal cellular metabolism and are critical for cell homeostasis (Lin and Thomas, 2010).

4.7.1 Lipid Peroxidation Status

Serum Lipid peroxidation status is presented in Table 28. Malondialdehyde (MDA), a cell membrane lipid peroxidation product is one of the most commonly reported oxidative stress biomarkers in clinical studies. It is generally detected as reaction of Thiobarbituric acid (TBA) with MDA. TBA_2-MDA (Moselhy *et al*., 2013).

Table 28

Biomarkers of lipid peroxidation

Parameters	Control (n = 73)	Exposed (n = 90)	p value
TBARS (µM)	1.3 (0.8 – 1.7)	2.1 (1.5 – 3.1)	**0.003****
Free 8-isoprostane (pg/ml)	29.1 (18.9 – 45.9)	40.8 (26.5 – 52.8)	**0.042***

Values are expressed as median with (inter quartile range in parenthesis),

p values for Mann Whitney test, *p<0.05, ** p<0.01

Isoprostanes are a family of stable, prostaglandin-like compounds generated by cyclooxygenase independent enzymes from the peroxidation of arachidonic acid, a polyunsaturated fatty acid present in phospholipids of cell membranes (Ho *et al*., 2013). It has been opined by Montuschi *et al*. (2004) that measurement of F2-isoprostanes is the most reliable approach to assess

oxidative stress status *in vivo*, providing an important tool to explore the role of oxidative stress in the pathogenesis of human disease. In addition, products of the isoprostane pathway have been found to exert potent biological actions and therefore may be pathophysiologic mediators of disease. Hence, isoprostanes are *in vivo* biomarkers and mediators of oxidative stress.

Table 28 presents the biomarkers of lipid peroxidation. Levels of serum TBARS and plasma free 8-isoprostane were significantly increased in photocopier service personnel in comparison to control participants.

Increased levels of TBARS (Figure 27) among photocopier service personnel in the present study are in accordance with reports of Kleinsorge *et al*. (2011) and Elango *et al*. (2013) who reported increased levels of TBARS indicating oxidative stress and damage in occupationally exposed photocopier operators when compared to control participants

Increased levels of free 8-isoprostane (Figure 28) among photocopier service personnel in the present study are in accordance with the observations by Miller *et al.* (2013) that stated exposure to diesel exhaust nanoparticles leads to increased plasma levels of 8-Isoprostane in mice indicating lipid peroxidation and oxidative stress Oxidative stress is enhanced in patients with interstitial lung diseases as reflected by increased concentrations of 8-Isoprostane in bronchoalveolar lavage fluid (Montuschi *et al*., 1998). In contrast, Elango *et al.* (2013) reported no change in the levels of free 8-Isoprostane among photocopier operators who were at higher risk of predisposition to cardiovascular diseases.

Thus occupational photocopier exposure leads to oxidant stress among photocopier service personnel as indicated by the increase in the biomarkers of lipid peroxidation levels (Figure 27 and 28).

Figure 27

Serum Thiobarbituric Acid Reactive Substances

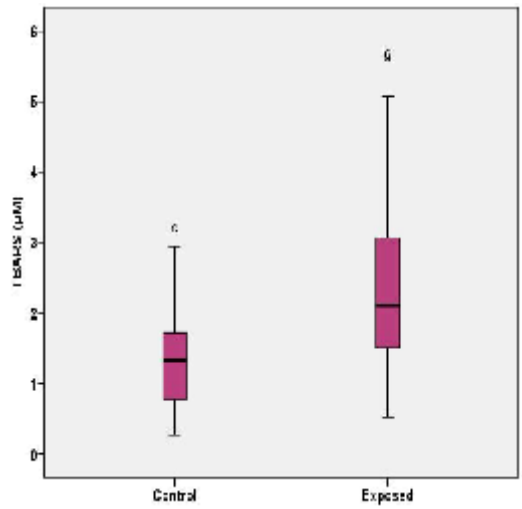
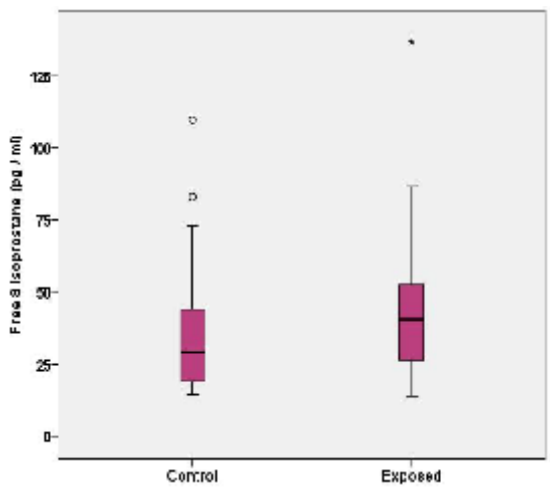


Figure 28

Plasma free 8-isoprostane levels



4.7.2 Correlation between Lipid Peroxidation and Cumulative Photocopier Exposure

Correlation between lipid peroxidation and cumulative photocopier exposure is presented in Table 29. There exists a strong positive association between TBARS and cumulative photocopier exposure though there was no positive correlation between photocopier exposure and free 8-isoprostane levels. In contrast, Kleinsorge *et al*. (2011) did not find any positive correlation between photocopier exposure and TBARS.

In the present study, this positive association of TBARS with cumulative photocopier exposure might prove TBARS as a best sensitive indicator of systemic oxidative stress rather than the free 8-isoprostane levels among photocopier service personnel.

According to Proudfoot *et al.* (1999), the levels of 8 isoprostane derived from enzyme immunoassay method measures only a specific form of isoprostane, though many isoforms of them are formed as by-product of lipid peroxidation in living systems. Hence, this might be the reason for the lack of association between free 8-isoprostane and cumulative photocopier exposure among photocopier service personnel.

Table 29

Correlation between lipid peroxidation and cumulative photocopier exposure

LPO Vs Cumulative photocopier exposure (1000s of hours)	Correlation n = 90	
	r_s	p value
TBARS (µM)	0.440	0.005**
Free 8-isoprostane (pg/ml)	0.045	0.673

r_s – Spearman's rank correlation coefficient **p<0.01

4.7.3 Correlation between Lipid Peroxidation Status and Cumulative Photocopier Exposure, Pack Years and Synergistic Effect of both among the Photocopier Service Personnel who are Smokers

Table 30 presents the association of lipid peroxidation with individual photocopier exposure, pack years and combined effect of both among photocopier service personnel who are smokers. Individual exposure to xerography and cigarette smoke and synergistic effects of both are positively associated with TBARS levels. This indicates that not only the individual exposure to xerography and cigarette smoke but also the combined effect of both leads to oxidative stress among the photocopier service personnel. Premanand *et al*. (2007) reported that smokers had higher TBARS levels in comparison to non-smokers which might reflect increased lipid peroxidation that occurs by the release of hydroxyl radicals by the cigarette smoke. This concludes that cigarette smoke is an established confounder in occupational studies. No such significant associations were noticed with 8-isoprostane levels.

Table 30

Correlation between lipid peroxidation status and cumulative photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers

Lipid peroxidation marker	Photocopier Exposure (PE)		Pack Years (PY)		PE X PY	
	r_s	p value	r_s	p value	r_s	p value
TBARS (µM)	**0.444**	**0.004****	**0.286**	**0.049***	**0.501**	**0.002****
Free 8-isoprostane (pg / ml)	0.211	0.151	-0.014	0.923	0.119	0.419

r_s – Spearman's rank correlation coefficient *p<0.05

4.7.4 Antioxidant Status

Trolox equivalency antioxidant capacity (TEAC) is used as a benchmark assay to assess total antioxidants due to difficulties in measuring individual antioxidant components of a complex mixture (Lin and Thomas, 2010). Ferric reducing antioxidant capacity (FRAC) or ferric reducing / antioxidant power (FRAP) are indicative of the total amount of non-enzymatic antioxidants (Chandramathi *et al*., 2009). It is based on the reduction of ferric tripyridyltriazine complex (Fe^{3+}) to ferrous complex (Fe^{2+}) ion at low pH (Emin *et al*., 2010).

Plasma total antioxidant capacity and serum ferric reducing antioxidant capacity of the participants is shown in Table 31. It is evident that TEAC was not significantly different between the two groups whereas the levels of FRAC were significantly lower among the exposed group. Increased levels of biomarkers of lipid peroxidation (Table 28) and decreased levels of FRAC (Figure 29) indicated the imbalance in overall oxidative status and higher levels of oxidative stress.

Table 31

Markers of antioxidant status

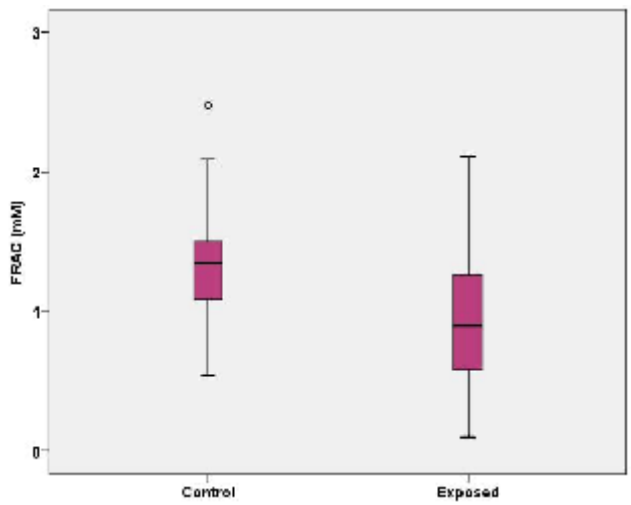
Parameters	Control n = 73	Exposed n = 90	p value
Trolox Equivalent Antioxidant Capacity (TEAC) (mM)	12.8 (10.6 – 14.9)	12.6 (11.0– 14.7)	0.514
Ferric Reducing Antioxidant Capacity (FRAC) (mM)	1.4 (1.1 – 1.5)	0.9 (0.6 – 1.3)	0.001**

Values are expressed as median with (inter quartile range in parenthesis),
p values for Mann Whitney test, ** p<0.01

According to Wang *et al*. (2006), a decrease in total antioxidant activity *in vivo*, may be the result of an acute increase in production of reactive species, although the production of reactive species would probably have to be extensive to disturb the systems steady state level of antioxidants. Therefore total plasma antioxidant activity may not be the tool of choice for measuring systemic oxidative stress. Plasma also contains endogenous chelating proteins such as albumin which binds ferric or cupric ions and hence prevents the formation of free radicals from hydrogen peroxide. Determination of antioxidant activity in plasma may not necessarily reflect the activity in target compartments where oxidative stress is greatest. In addition the accumulation of antioxidants in selective biological sample may not be apparent from plasma measurements. Thus lack of decrease in plasma TEAC levels might be due induction of antioxidant enzymes to compensate for the oxidant stress caused due to photocopier exposure. Hence, in the present study, FRAC measured in serum is more reflective of total antioxidant capacity rather than plasma TEAC.

. **Figure 29**

Serum Ferric Reducing Antioxidant Capacity



4.7.5 Correlation between Antioxidant Status and Cumulative Photocopier Exposure

Table 32

Correlation between antioxidant status and cumulative photocopier exposure

Antioxidant status Vs Cumulative photocopier exposure (1000s of hours)	Correlation n = 90	
	r_s	p value
Trolox Equivalent Antioxidant Capacity (TEAC) (mM)	- 0.142	0.183
Ferric Reducing Antioxidant Capacity (FRAC) (mM)	- 0.298	0.004**

r_s – Spearman's rank correlation coefficient, **p<0.01

Correlation between antioxidant status and cumulative photocopier exposure is tabulated in Table 32. Significant negative correlation occurred between FRAC and cumulative photocopier exposure whereas no such significant correlation was obtained between TEAC and cumulative photocopier exposure. Zhou *et al*. (2003) reported significant negative correlation between antioxidants such as vitamin E, vitamin C, beta carotene, superoxide dismutase, catalase and ozone levels in photocopier operators.

4.7.6 Correlation between Antioxidant Status and Cumulative Photocopier Exposure, Pack years and Synergistic Effect of both among photocopier Service Personnel who are Smokers

Table 33 presents the correlation between antioxidant status, cumulative photocopier exposure, pack years of cigarette smoked and synsergistic effect of both among the photocopier service personnel who are smokers.

Table 33

Correlation between antioxidant status and cumulative photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers

Antioxidant status	Photocopier Exposure (PE)		Pack Years (PY)		PE X PY	
	r_s	p value	r_s	p value	r_s	p value
Trolox Equivalent Antioxidant Capacity (TEAC) (mM)	-0.104	0.483	0.278	0.056	0.036	0.810
Ferric Reducing Antioxidant Capacity (FRAC) (mM)	-0.146	0.323	0.009	0.950	-0.180	0.221

r_s – Spearman's rank correlation coefficient

. None of the antioxidants were found to significantly correlate with either individual exposure to photocopier pollutants or pack years of cigarette smoke or due to synergistic effect of both among photocopier service personnel who are smokers. This concludes that smoking is not a confounder in the study of antioxidant status among photocopier service personnel.

4.7.7 Correlation between Oxidative Status and Pulmonary Function

Table 34 presents the association between oxidative status and lung function indices. Significant negative correlations were noticed between lipid peroxidation bioamrkers and lung function. TBARS was negatively associated with % predicted FVC among general male population, incoherence to the results of Ochs-Balcom *et al*., (2005) indicating the relationship of oxidative stress and

pulmonary function. Similarly free 8-isoprostane levels were inversely correlated with % VC. The results obtained were comparable to the results observed by Ogawa *et al*., (2006) among systemic sclerotic patients.

. **Table 34**

Correlation between oxidative status and lung function indices

Parameters	r_s	p value
TBARS Vs % predicted FVC	- 0.219	0.005**
Free 8-Isoprostane Vs % predicted VC	- 0.178	0.023*
FRAC Vs % predicted MVV	0.241	0.002**
TEAC Vs % predicted MVV	0.196	0.012*

r_s – Spearman's rank correlation coefficient *p<0.05 **p<0.01

Significant positive correlations were noticed between antioxidants FRAC and TEAC with % predicted MVV. All the above significant correlations of oxidative stress biomarkers with lung function indices might be due to pathophysiological relevance of these biomarkers. These associations indicate the present pathological situation of lung dysfunction (Table 13) thus occupational exposure among photocopier service personnel might have triggered oxidative stress that induced lung dysfunction.

4.7.8 Correlation between Oxidative Status and Hematological Indices

Table 35 shows the significant correlation between oxidative status and haematological indices.

Lipid peroxide biomarker TBARS was found to be positively correlated with MCV, MCH and neutrophils. Antioxidant marker TEAC was found to be negatively correlated with lymphocyte count and FRAC was found to be positively correlated with RBC count, MCV, MCH, MCHC and RDW. Semba *et al*., (2010) stated an inverse relationship between RDW and several serum antioxidants. Free 8-isoprostane was not found to be correlated with any of the haematological indices.

Table 35

Correlation between oxidative status and hematological indices

Parameters	r_s	p value
TBARS Vs MCV	0.172	0.029*
TBARS Vs MCH	0.203	0.009**
TBARS Vs Neutrophil count	0.236	0.002**
TEAC Vs Lymphocyte count	-0.192	0.014*
FRAC Vs RBC count	0.236	0.002**
FRAC VS MCV	-0.178	0.023*
FRAC Vs MCH	-0.205	0.009**
FRAC Vs MCHC	-0.166	0.034*
FRAC Vs RDW	-0.156	0.047*

r_s – Spearman's rank correlation coefficient *p<0.05 **p<0.01

FRAC levels were found to be positively correlated with albumin (r_s = 0.167, p = 0.033) and negatively associated with TBARS (r_s = 0.309, p < 0.05). This inverse association explains the role of oxidant-antioxidants in severity of lung dysfunction. Depletion of antioxidants might occur to counteract the damage caused to plasma membrane lipid peroxidation induced by photocopier exposure among photocopier service personnel.

4.8 Inflammatory Biomarkers in the Selected Participants

Biomarkers are used as a research tool that links exposure to air particles and respiratory health. However, its application in assessment of adverse health effects in both environmental and occupational exposure is unclear (Suhaimi and Jalaludin (2015).

Biomarkers are detectable characteristic changes that reflect exposure associated events at biochemical and molecular levels. It provides a potential to

develop high throughput screening methods for medical surveillance of workers exposed to pollutants in occupational settings. Increased levels of proinflammatory cytokines, cytokine receptors and C-reactive protein have been considered the biomarkers associated with air pollution induced systemic inflammation; platelet activation and increased expression of adhesion molecules have been identified as the biomarkers for the adverse cardiovascular effects of air pollution (Li and Nel, 2011).

4.8.1 Levels of Clara Cell Protein and Leukotriene B_4

Table 36 presents the levels of Clara Cell protein and Leukotriene B_4. Clara cell secretory protein (CC16) is an immunoregulatory molecule produced largely by epithelial cells of the lung that has been ascribed with anti-inflammatory properties also known as CC10 or uteroglobin (Johansson *et al*., 2007). The determination of CC16 in serum is a new non-invasive test to detect Clara cell damage or an increased epithelial permeability in various acute and chronic lung disorders (Broeckaert and Bernard, 2000).

Table 36

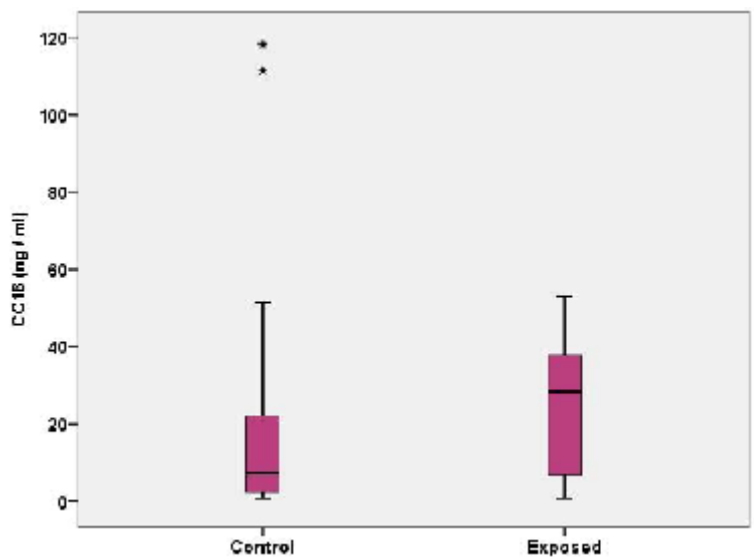
Plasma Clara Cell Protein and Leukotriene B_4

Inflammatory markers	Control n =73	Exposed n = 90	p value
CC16 (ng/ml)	7.3 (2.2 - 22.3)	28.4 (6.7 - 37.9)	**0.001****
LTB_4 (pg/ml)	16.5 (9.3 - 40.5)	131.0 (79.0 - 167.9)	**0.001****

Values are expressed as median with (inter quartile range in parenthesis),
p values for Mann Whitney test, ** p<0.01

Figure 30

Plasma Clara Cell Protein Levels



The levels of CC16 (Figure 30) were found to be significantly increased among the photocopier service personnel.

According to Bernard (2014) increased levels of CC16 in serum, is due to increased airway permeability and is also peripheral marker of events taking place in the deep lung. This means that circulating levels of CC16 are determined not only by the intrapulmonary pool of CC16, but also by the rate at which the protein leaks from the lungs and at which it is cleared from plasma. Because of its small size, CC16 is rapidly eliminated from plasma by glomerular filtration and as a corollary its serum level rises in parallel with serum creatinine.

CC16 levels were found to be increased in sarcoidosis (Hermans *et al*., 2001), interstitial lung disease (Olewicz-Gawlik *et al*., 2015) emphysema, fibrosis and combined pulmonary fibrosis and emphysema (Kokuho *et al*., 2014). In contrast, their levels were found to be decreased in chronic bronchitis, asthma and in smokers (Lomas *et al*., 2008).

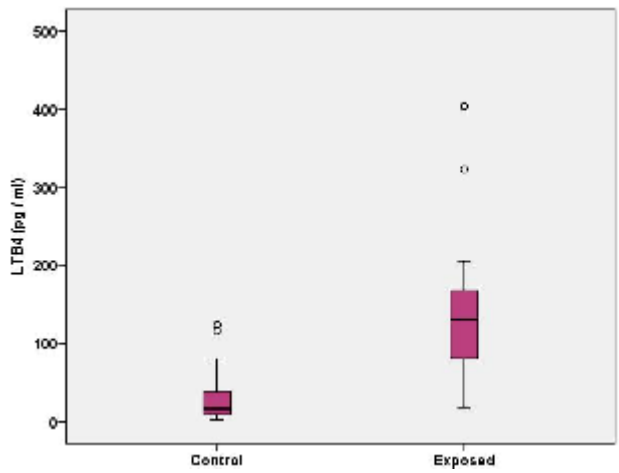
Age is an important factor that influences the levels of CC16 (Lakind *et al*., 2007). In the present study, age was matched between the groups. Hence, their confounding effect on study outcome is ruled out. Increase in CC16 levels among the exposed participants on exposure to photocopier emissions might be due to chronic disruption of the bronchoalveolar blood barrier integrity and its passive leakage by transduction into plasma leading to lung dysfunction (Table 13) on exposure to photocopier pollutants. The results of the present study is analogous to the effects of several air pollutants smoke (Bernard *et al*., 1997) diesel exhaust (Beamer *et al*., 2015) and tobacco smoke exposure (Ma *et al*., 2015).

Leukotriene B4 is one of the most potent chemoattractants for polymorphonuclear neutrophils (PMN) and is known to stimulate adhesion of leukocytes to endothelium, chemotaxis and PMN degranulation. LTB4 has been implicated in the pathology of chronic inflammatory diseases like asthma and rheumatoid arthritis as well as in acute inflammation. High levels of LTB4 have been found in acute and chronic inflammatory lesions (Kavelaars *et al*., 2003).

Levels of leukotriene B4 were significantly higher in photocopier service personnel (Figure 31). Incoherence to this, in the present study the levels of neutrophils were also increased due to their activation by photocopier pollutants (Table 20). These activated neutrophils might release leukotriene B4 that result in further inflammation. The results obtained are also analogous to the reports of Nemmar *et al*. (2009) who observed increased plasma levels of LTB4 in diesel exhaust particulate exposed hypertensive rats which were noticed as a result of pulmonary inflammation that further exacerbate to systemic inflammation by passage of particulate pollutants from lungs to systemic circulation. It is also in sync with the levels of systemic cytokines, leukotriene B4 levels also increased in patients hospitalized with COPD excacerbations (Pinto-Plata *et al*.,2007). Increased levels of leukotrienes were also noticed in bronchoalveolar lavage fluid of patients with restrictive lung dysfunction, systemic sclerosis (Kowal-Bielecka *et al*., 2003).

Figure 31

Plasma Leukotriene B_4 levels



4.8.2 Levels of Interleukin 6 and 8

The plasma levels of interleukins 6 and 8 of the study participants are presented in Table 37.

Table 37

Plasma interleukins

Inflammatory markers	Control n =73	Exposed n = 90	p value
IL-6 (pg/ml)	8.2 (4.6 - 10.7)	13.7 (7.4 - 17.3)	**0.001****
IL-8 (pg/ml)	12.6 (9.0 - 18.6)	21.3 (16.0 - 30.9)	**0.002****

Values are expressed as median with (inter quartile range in parenthesis),
p values for Mann Whitney test, ** p<0.01

Interleukins represent a class of immunomodulatory cytokines, small intercellular signaling proteins that are critically involved in the regulation of immune responses. Interleukins can have pro- and anti-inflammatory functions or even both which is dependent on the inflammatory stimulus, their source and the target cell type. These cell types include leukocytes, mainly lymphocytes (Hammerich and Tacke, 2014). Their primary functions are to stimulate

inflammatory cell chemotaxis and to induce maturation and proliferation of immune cells including neutrophils, macrophages, T helper cells, B-cells and natural killer cells (Lin and Thomas, 2010).

Interleukin-6 (IL-6) is a cytokine that acts in a pro and anti-inflammatory way. It is involved in inflammation and infection responses in addition to the regulation of metabolic, regenerative and neural process (Scheller *et al*., 2011). Interleukin-8 (IL-8) is an oxidative stress-responsive pro-inflammatory chemokine. It is a potent chemoattractant and activator of neutrophils that leads to neutrophil influx and inflammation (Qazi *et al*., 2011).

The levels of IL-6 and IL-8 were significantly higher among photocopier service personnel or exposed group (Figure 32). Karoly *et al*. (2007) opined that exposure to ultrafine particulate matter increased the expression of IL-6 and IL-8 genes in human pulmonary artery endothelial cells. Kido *et al*. (2011) stated that exposure to PM_{10} in mice induces translocation of pulmonary inflammatory mediator mainly IL-6 from lungs to the systemic circulation leading to systemic inflammation with downstream effects of vascular dysfunction. Increased levels of inflammatory markers, IL-6 and IL-8 were also noted in serum and/or bonchoalveolar lavage fluid of COPD subjects as a systemic inflammatory response (El-Shimy *et al*., 2014). In tune with the present study it was reported by (Elango *et al*., 2013) that chronic exposure to photocopier particulate matter emissions leads to increased levels of plasma IL-8 among photocopier operators. Thus, higher levels of IL-6 and IL-8 in plasma among photocopier service personnel might indicate lung dysfunction and systemic inflammation that progress to vascular dysfunction in latter conditions.

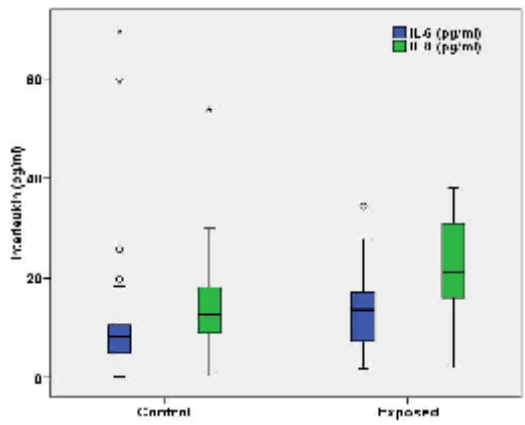


Figure 32
Plasma Interleukin levels

4.8.3 Levels of Eosinophil Cationic Protein, C-Reactive Protein and Total Nitrates

The plasma levels of Eosinophilic Cation Protein (ECP), C-Reactive Protein (CRP) and total nitrates among the study participants are presented in Table 38.

Table 38

Levels of Eosinophil Cationic Protein, C-Reactive Protein and Total Nitrates

Inflammatory markers	Control n = 73	Exposed n = 90	p value
ECP (ng/ml)	135.3 (88.1 - 176.3)	229.5 (125.9 – 282.2)	0.001**
CRP (µg/ml)	1.3 (0.9 - 2.2)	2.4 (1.8 - 2.8)	0.001**
Total Nitrates (µM)	12.6 (9.0 - 18.6)	21.3 (16.0 - 30.9)	0.171

Values are expressed as median with (inter quartile range in parenthesis),
p values for Mann Whitney test, ** p<0.01

The Eosinophil Cationic Protein (ECP) is released from activated eosinophils and is one of the important mediators of allergic inflammation in respiratory tract mucosa. ECP levels usually correlates with eosinophil activity in allergic rhinitis, asthma, conjunctivitis and atopic dermatitis (Dodig *et al*., 2011).

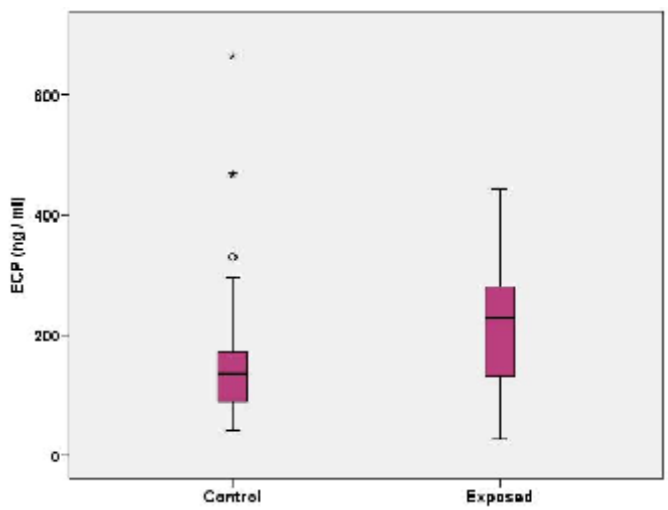
It is illustrated from Figure 33, that levels of ECP were significantly increased among photocopier service personnel or the exposed group. The significant difference between the two groups exposed and control might be due to the increase in inflammatory cells which upon activation on exposure to particulate allergens present in photocopier emissions might have triggered degranulation of inflammatory cells and increased levels of ECP. In harmony to this result, even in the present study, the levels of inflammatory cells, especially neutrophils were increased as noted in (Table 20).

The results are coherent to observation by Monteseirín (2009) who stated that ECP is also found in neutrophils and their levels are more closely related to the presence/activation of neutrophils than to that of eosinophils in various IgE mediated allergic processes. Elango *et al*. (2013) also reported significant

increase in ECP among photocopier operators upon chronic exposure to photocopier particulate emissions.

Figure 33

Plasma Eosinophilic Cationic Protein levels

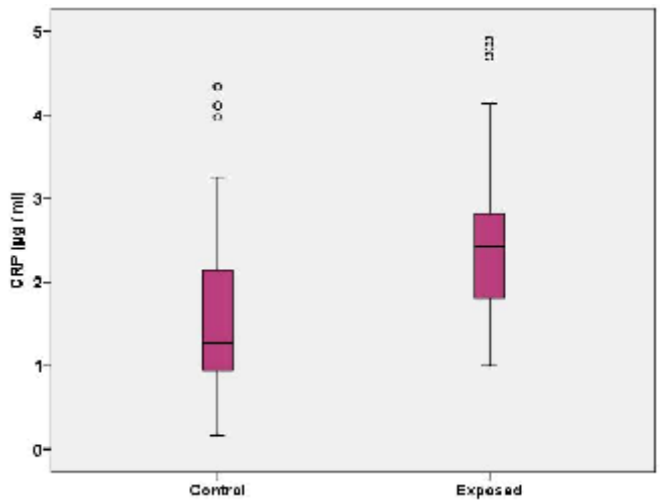


C-reactive protein (CRP) is one of the acute phase plasma reactants whose levels increase in response to inflammation, infection and tissue damage (Ólafsdóttir, 2011). CRP is a systemic biomarker to study ongoing lung inflammation (Dahl *et al*., 2007).

It is depicted from Figure 34 that levels of CRP were significantly increased in the exposed group. The significant increase might be due to increase in systemic inflammation among photocopier service personnel triggered by exposure to photocopier particulate matter ($PM_{2.5}$) emissions. In concurrence with the findings of the present study, Hoffmann *et al*. (2009) reported an increase in the levels of systemic marker, CRP on long term residential exposure to high levels of $PM_{2.5}$. A moderate increase in serum CRP is also implicated in sarcoidosis according to Drent *et al*. (1999).

Figure 34

Plasma CRP levels



Measurement of nitrate and nitrite (total nitrates) in extracellular fluids is a window to assess systemic nitric oxide metabolism. Activation of the immune system, whether locally or systemically, is associated with an increased production of nitric oxide (NO) as measured by increases in plasma and/or urinary levels of nitrate (NO_3^{-}) and nitrite (NO_2^{-}). Their measurements in plasma may provide an important tool for monitoring NO production and possibly disease activity *in vivo* (Grisham *et al*., 1995). Nitric oxide has been suggested as a sensitive marker of airway inflammation (Kato *et al*., 2013).

No significant rise in plasma total nitrate levels could be speculated due to diffusion of endogenously produced nitric oxide before their metabolism to final end products (total nitrates) to the local site of airway inflammation which might be essentially to induce vasodilation in pulmonary hypertensive airways caused due to restrictive lung dysfunction by photocopier particulate emissions among photocopier service personnel. In contrast to the present study, Shah *et al*. (2008) found lower levels of plasma nitrate among participants on exposure to carbon ultra fine particles in comparison to exposure to filtered air.

4.8.4 Activity of Myeloperoxidase and Levels of Inter Cellular Adhesion Molecule-1

Table 39 presents the plasma activity of Myeloperoxidase (MPO) and level of ICAM-1 (Inter Cellular Adhesion Molecule).

Table 39

Activity of MPO and level of ICAM-1

Inflammatory markers	Control n = 73	Exposed n = 90	p value
MPO (ng/ml)	385.7 (323.0 – 497.8)	387.6 (320.6 – 506.8)	0.780
ICAM-1 (ng/ml)	112.0 (96.8 – 135.5)	163.3 (113.7 – 212.6)	0.001**

Values are expressed as median with (inter quartile range in parenthesis),
p values for Mann Whitney test, ** $p<0.01$

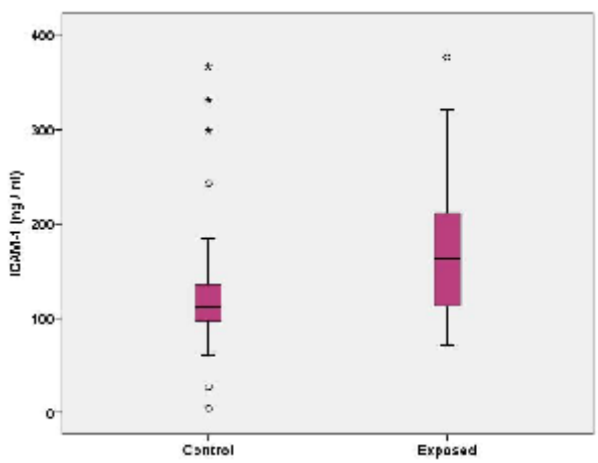
MPO is the most abundant component in the azurophilic granules of leukocytes and it is found not only in neutrophils but also in monocytes and in some subtypes of tissue macrophages (Comandini *et al*., 2009). It is secreted by leukocytes in response to oxidative stress and inflammation (Kaya *et al*., 2012). Plasma MPO activity is a useful biomarker in predicting coronary artery diseases (Salonen *et al*., 2012); ischemic heart disease and acute coronary syndromes (Loria *et al*., 2008).

The activities of MPO were not different between the groups of control and exposed participants in contrast to the increased neutrophil count observed among the photocopier service personnel. However, based on observations by Reid *et al*. (2003), it would be expected that MPO levels would increase with IL-8 levels, although endogenously occurring inhibitors of neutrophil activation such as secretory leukocyte protease inhibitor may have blunted this response in asthmatic subjects. However, this observation is speculative and their use as biomarker of lung dysfunction is not preferable as noted in the present study.

Intercellular adhesion molecules (ICAMs) are cell surface glycoproteins expressed on a wide variety of cell types, with distinct patterns of gene regulation and effector functions. They are constitutively expressed at low levels on vascular endothelial cells and on some lymphocytes and monocytes. Their increased expression is stimulated by both inflammatory and oxidant species on other cell types namely bronchial epithelial cells, mesangial cells and fibroblasts (Hubbard and Rothlein, 2000). ICAM-1 levels are elevated in the serum of patients with cardiovascular disease, autoimmune disorders, cancer (Lawson and Wolf, 2009) and acute lung injury (Calfee *et al*., 2009).

Figure 35

Plasma ICAM-1 levels



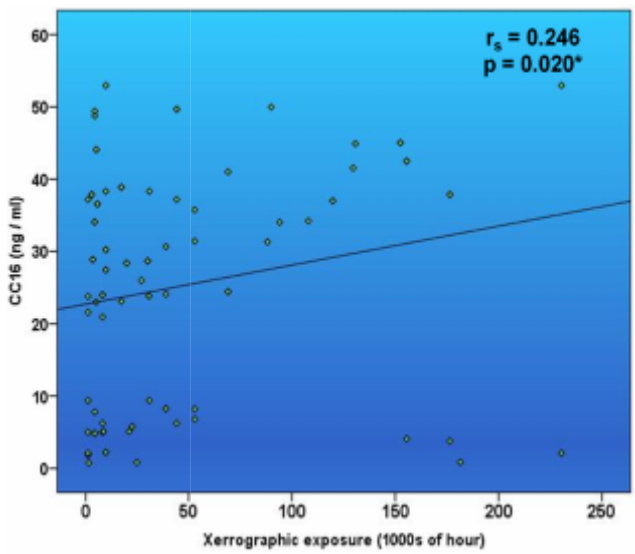
4.8.5 Correlation between Inflammatory Markers and Cumulative Photocopier Exposure

Plasma CC16 levels were found to be positively correlated with (r_s = 0.246, p = 0.020*, Figure 36) photocopier exposure. Provost *et al*. (2014) observed a strong positive association between short term exposure to particulate matter and serum CC16 levels among adolescents. Similarly, Vattanasit *et al*. (2014) also showed positive correlation between serum CC16 levels and traffic related lead particulate emissions. The coherence of this association in the present study implies that CC16 could be used as an efficient biomarker to assess adverse

effects of photocopier particulate emissions on the cellular integrity or the permeability of the lung epithelium or the lung damage among the photocopier service personnel.

Figure 36

Correlation between cumulative photocopier exposure and plasma CC16 levels



*correlation is significant at 0.05 level

4.8.6 Correlation between Inflammatory Markers and Lung Function Indices

Table 40 presents the correlation between inflammatory markers and lung function indices. CC16 was found to be negatively associated with the lung function parameter % predicted VC. Serum levels of CC16 were significantly inversely correlated with vital capacity (VC) in subjects with interstitial pulmonary disease especially systemic sclerosis as reported by Hasegawa *et al*. (2011). CRP levels were found to be negatively correlated with % FEV1. In lung function impairment, it is an established fact that CRP levels are associated with lung function indices (Fogarty *et al*., 2007 abd Montaño *et al*., 2014). Hancox *et al*., (2016) reported that lower lung volumes were associated with higher CRP indicating that pulmonary restriction may be a risk factor for systemic

inflammation. In addition to the association of CC16 and CRP levels with lung function indices, plasma total nitrates levels were also found to be positively correlated with percentage predicted peak expiratory flow.

Table 40

Correlation between inflammatory markers and lung function indices

Parameters	r_s	p value
CC16 Vs % predicted VC	-0.181	0.021*
CRP Vs % predicted FEV1	-0.198	0.011*
CRP Vs % predicted MVV	-0.172	0.028*
Total Nitrates Vs % predicted PEF	0.181	0.021*

r_s – Spearman's rank Parameters coefficient; *p<0.05

4.8.7 Correlation between Inflammatory Markers and Haematological Indices

Table 41 presents the correlation between inflammatory markers and haematological indices.

Table 41

Correlation between inflammatory markers and haematological indices

Parameters	r_s	p value
CC16 Vs neutrophil count	0.185	0.018*
LTB4 vs MPV	0.181	0.021*
IL-6 Vs mixed cell count	0.199	0.011*
IL-8 Vs neutrophil count	0.171	0.029*
CRP Vs lymphocyte count	0.170	0.030*
CRP Vs neutrophil count	0.203	0.009**
CRP Vs HCT	0.161	0.041*
CRP Vs MCV	0.207	0.008**
CRP Vs MPV	0.226	0.004**
Total Nitrates Vs WBC	-0.163	0.038*
Total Nitrates Vs mixed cell count	0.305	0.001**
Total Nitrates Vs haematocrit	0.157	0.046*
MPO Vs neutrophil count	0.188	0.016*

r_s – Spearman's rank correlation coefficient; *p<0.05; **p<0.01

There occurs a multitude of correlations between inflammatory markers and haematological indices that includes the following: CC16, IL-8, CRP and MPO were found to be positively associated with neutrophil count. In addition to this, inflammatory markers CRP was positively correlated with lymphocyte count where as total nitrates were negatively correlated with WBC count. Similarly inflammatory markers IL-6 and Total nitrates were also positively associated with mixed cell count. All these correlations indicate that haematopoietic cells are under stress due to photocopier pollutant emissions among photocopier service personnel. This might have a triggering effect on the haematopoietic cells to release systemic chemokines or inflammatory mediators in response to the lung inflammation as presented in Table 13.

According to Takizawa *et al.* (2012), it was reported that during systemic infection and inflammation, immune effector cells are in high demand and are rapidly consumed at sites of need. Although adaptive immune cells have high proliferative potential, innate immune cells are mostly postmitotic and need to be replenished from bone marrow hematopoietic stem and progenitor cells with the signals sensed through cytokines, chemokines. These signals lead to divisional activation, proliferation, differentiation and migration of hematopoietic stem and progenitor cells, all aimed at efficient contribution to immune responses and rapid reestablishment of hematopoietic homeostasis. Thus chronic inflammatory processes might impinge on hematopoiesis.

LTB_4 and CRP levels were found to be positively associated with MPV. According to Wasilewska *et al*. (2010), high-sensitivity C-reactive protein positively correlated with mean platelet volume in paediatric hypertension. In addition to this, CRP levels were also found to be positively correlated with haematocrit, MCV and MPV. In tune with this, CRP levels were found to be positively correlated with hematocrit levels in patients with Systemic Lupus Erythematosus (Yang *et al.,* 2015). Total nitrates were also also found to be positively correlated with haematocrit in the present study.

Further studies with a larger group of participants and longer follow-up are needed to confirm the results of the present study and to evaluate whether the levels of neutrophils could be used as a cheaper sensitive haematological biomarker of systemic inflammation progression and prognosis of lung dysfunction among photocopier service personnel..

4.8.8 Correlation between Inflammatory Markers and Oxidative Status and Biochemical Markers

The concept that inflammation and oxidative stress lead to bronchial hyper-responsiveness, airflow limitation and mucus hypersecretion in lung diseases have led to a widening search for the types of inflammatory cells and mediators that are responsible for the cascade of events linking the initial stimulus to the final abnormality in airway function (Corradi and Mutti, 2005). Table 42 presents the correlation between inflammatory markers and oxidative status in addition to correlation between inflammatory markers and biochemical markers. There are a number of correlations that are noticed between inflammatory markers and oxidative status and between inflammatory markers and biochemical markers which need to be investigated further for their application as sensitive and cheap biomarker.

ICAM-1 and ECP were found to be positively correlated with total protein content of the photocopier service personnel. Similarly ICAM-1 was found to be positively correlated with albumin content. Jin *et al*. (2009) also observed a positive correlation between glycated albumin levels and ICAM-1 concentration among diffuse coronary artery disease patients with type-2 diabetes mellitus. ECP and total nitrates were found to be correlated with the globulin content. α_2 - macroglobulin and ECP of nasal lavage fluid levels were found to be well correlated in children with allergic seasonal rhinitis (Meyer *et al*., 1999). Total protein is a reliable true cheap biomarker of the inflammatory status in photocopier service personnel that was found to be correlated with the levels of inflammatory markers namely ICAM-1 and ECP.

Plasma CC16 levels were found to be correlated positively with the levels of lipid peroxide indicator TBARS. CC16 being an important protective anti-inflammatory and antioxidant protein in airway inflammation as reported by Irander *et al*. (2012) and Tanaka *et al.* (2012) might have increased in response to oxidative stress indicator TBARS to counteract the effect of photocopier pollutants among the service personnel.

Table 42

Correlation between inflammatory markers and oxidative status and biochemical markers

Parameters	r_s	p value
ICAM-1 Vs Total protein	0.314	0.003**
ECP Vs Total Protein	0.212	0.045*
ICAM-1 Vs albumin	0.303	0.004**
ECP Vs Globulin	0.252	0.017*
Total Nitrates Vs Globulin	0.247	0.019*
CC16 Vs TBARS	0.302	0.001**
LTB_4 Vs 8-Isoprostane	0.192	0.014*
LTB_4 Vs FRAC	-0.449	0.001**
IL-6 Vs TBARS	0.175	0.025*
CRP Vs TBARS	0.434	0.001**
CRP Vs FRAC	-0.220	0.005**
Total nitrates Vs TEAC	0.214	0.006**

r_s – Spearman's rank correlation coefficient, *p<0.05, **p<0.01

LTB_4 was found to be correlated with lipid peroxide marker 8-isoprostane levels. Similar results were also observed by Ciebiada *et al*, (2012) in bronchoalveolar lavage fluid and in EBC samples among patients with primary lung cancer. A negative correlation was observed between LTB_4 levels and antioxidant marker FRAC.

A strong positive correlation was noted between IL-6 and TBARS among photocopier service personnel. In tune with this, Pestka *et al.* (2004) also reported the same observation in cardiovascular risk patients. It might be due to

the downstream progression of inflammation associated pathological events from lungs to heart through systemic circulation.

Acute phase reactant protein, CRP was found to be positively correlated with lipid peroxide indicator TBARS among photocopier service personnel. A similar observation was noted between high-sensitivity C-reactive protein and thiobarbituric acid reactive substances in patients with acute coronary syndromes (Ragab *et al.,* 2005). In tune with this, Majewska *et al*. (2004) also reported correlation of serum CRP with TBARS in exhaled breath condensate of subjects with acquired pneumonia. Total nitrate levels were correlated with Trolox equivalent antioxidant capacity among photocopier service personnel.

4.8.9 Correlation among Inflammatory Markers

Table 43 presents the correlation between inflammatory markers investigated in present study. A horde of correlation exists between the inflammatory markers. However, many such correlations were not identified in earlier research studies with the inclusions of few that include CC16 Vs LTB_4, LTB_4 Vs IL-6 and IL-8, LTB_4 Vs CRP, IL-6 Vs IL-8, CRP Vs IL-6 and IL-8. CC16 levels were found to be positively correlated with LTB_4.

A positive correlation between LTB4 in EBC and CC16 in nasal lavage was observed among asthmatic children (Schoeters *et al*., 2009). LTB_4 positively correlated with IL-6 and IL-8 levels due to a positive feedback which was postulated by Jennewein *et al*. (2001) as LTB_4 induced production of IL-6 and IL-8. Ding *et al*. (2005) also reported positive correlation of LTB_4 with IL-8 levels in animal models with COPD. LTB4 positively correlated to the CRP level in children with Henoch-Schonlein purpura (Liao and Wu, 2006). Pine *et al*., (2011) found a positive correlation between IL-6 and IL-8 levels in lung cancer risk patients. Correlation between IL-6 and CRP was reported in subjects with adenoviral respiratory infections (Kawasaki *et al*., 2002). A correlation between plasma CRP concentration and bronchoalveolar lavage interleukin levels (IL-6 and IL-8) was present in bronchiolitis obliterans syndrome (Vos *et al*., 2009). The positive relationship of ICAM1 with LTB_4 might indicate the adhesion kinetics mediated by LTB_4.

ICAM-1 was positively associated with IL-8 in the present study. Ohaga *et al.* (2003) also observed similar association between ICAM-1 and IL-8. Among the infammatory markers studied, CC16 was proved to be an important reliable marker in the present study as it indicates the lung inflammation and their associated downstream events of systemic circulation. In contrast, CC16 is also criticised as a biomarker in both acute and chronic pulmonary effects due to its transient nature (Lakind *et al*., 2007). Hence, in the present study, there is also a possibility to use LTB_4 as a promising biomarker in addition to CC16.

Table 43

Correlation among inflammatory markers

Biomarker	CC16	LTB4	IL6	IL8	ECP	CRP	Nox	MPO	ICAM-1
CC16	1.000								
LTB4	0.320**	1.000							
IL6	0.196*	0.290**	1.000						
IL8	0.187*	0.413**	0.239**	1.000					
ECP	0.283**	0.322**	0.151	0.265**	1.000				
CRP	0.145	0.371**	0.205**	0.260**	0.018	1.000			
Nox	0.105	0.228**	0.109	0.190*	0.270**	0.188*	1.000		
MPO	0.209**	0.091	0.080	0.056	0.024	-0.039	0.146	1.000	
ICAM-1	0.121	0.344**	0.182*	0.166*	0.319**	0.157*	0.113	-0.001	1.000

r_s – Spearman's rank correlation coefficient, *p<0.05, **p<0.01

4.8.10 Correlation between Inflammatory markers and Cumulative Photocopier Exposure

Among the inflammatory markers, only CC16 levels were found to be positively correlated with (r_s = 0.246, p = 0.020*, Figure 36). The other parameters were not found to be associated significantly with the cumulative photocopier exposure.

4.8.11 Correlation between Inflammatory Markers and Cumulative Photocopier Exposure, Pack Years and Synergistic Effect of both among Photocopier Service Personnel who are Smokers

The results of correlation between inflammatory markers, cumulative photocopier exposure and pack years among the photocopier service personnel who are smokers are presented in Table 44. IL-8 was found to be associated with zcigarette smoke which became stronger on photocopier exposure. Similarly, MPO activity were also associated with pack years of cigarette smoked, whereas ECP and CRP were not only associated with individual exposure to photocopier pollutants and pack years of cigarette smoked but also were found to have an effect on combined exposure to both.

Table 44

Correlation between inflammatory markers and cumulative photocopier exposure, pack years and synergistic effect of both among photocopier service personnel who are smokers

Inflammatory Markers	Photocopier Exposure (PE) (1000s of hours)		Pack Years (PY)		PE x PY	
	r_s	p value	r_s	p value	r_s	p value
CC16	0.032	0.830	-0.034	0.820	0.008	0.955
LTB4	-0.177	0.228	0.104	0.481	-0.077	0.601
IL6	0.030	0.839	0.113	0.446	0.040	0.787
IL8	0.202	0.169	0.565	<0.01**	0.355	0.013*
ECP	-0.427	0.002**	-0.312	0.031*	-0.513	<0.01**
CRP	0.429	0.002**	0.074	0.619	0.349	0.015*
Total Nitrates	-0.006	0.967	0.062	0.676	-0.022	0.880
MPO	0.074	0.616	-0.321	0.026*	-0.062	0.677
ICAM1	-0.170	0.248	0.012	0.938	-0.183	0.214

r_s – Spearman's rank correlation coefficient, *p<0.05, **p<0.01

4.9 Selenium, Cadmium and Selenoproteins among the Selected Participants

Selenium (Se) plays a key role in the maintenance of normal health in human populations. The cellular biochemistry of selenium involves the expression of a variety of selenoproteins. Selenium is part of the active site of Glutathione peroxidase (GPx), an antioxidant enzyme (Safaralizadeh *et al*., 2005). Table 45 presents the levels of serum selenium and cadmium among the participants.

Table 45

Serum selenium and cadmium levels

Trace element	Control n = 24	Exposed n = 14	p value
Selenium (µg / L)	**116.7 (106.5 – 129.8)**	**132.0 (114.5 – 137.8)**	**0.001****
Cadmium (µg / L)	9.7 (6.0 – 18.25)	12.6 (11.5 – 14.3)	0.075

p values for Mann Whitney test, **p<0.01

The levels of selenium were found to be significantly increased among the exposed participants whereas no significant variations were noticed in serum cadmium levels. This might be due to the exposure to selenium drums in photocopier machines during machine maintenance activity among photocopier service personnel. George and Wagner (2009) opined that amorphous selenium is used in the photodectors of the photocopier machines.

4.9.1 Activities of Glutathione peroxidase (GPx) and Thioredoxin Reductase

Plasma activities of glutathione peroxidase and thioredoxin reductase are presented in Table 46. The varied sample size for thioredoxin reductase is indicated [control (n = 14) and Exposed (n =10)] since in many participants the levels of thioredoxin reductase were below detecTable limits.

There was significant increase in the plasma activities of glutathione peroxidase among photocopier service personnel in contrast to non variations in thioredoxin reductase. In coherence, with increase in selenium levels (Table 45), among photocopier service personnel the increase in GPx activities might

indicate their regulation. Selenium availability regulates glutathione peroxidase enzyme activity due to their presence as selenocysteine at the catalytic site of glutathione peroxidase (Baker *et al*., 1993).

Table 46

Activities of Glutathione peroxidase and Thioredoxin reductase

Hematological Indices	Control	Exposed	p value
Glutathione peroxidase (nM/min/ml)	**n = 73**	**n = 90**	
	145.7 (123.6 – 169.6)	163.2 (127.5 – 163.2)	0.001**
Thioredoxin reductase (nM/min/ml)	**n = 14**	**n = 10**	
	4.2 (1.58 – 23.10)	15.7 (5.12 – 28.74)	0.305

Values are expressed as median with (inter quartile range in parenthesis),

p values for Mann Whitney test, ** p<0.01

4.9.2 Correlation between Glutathione peroxidase activity (GPx) and Biochemical Parameters and Haematological parameters

Table 47 presents the correlation between glutathione peroxidase acivity and biochemical markers as well as haematological parameters.

Table 47

Correlation between Glutathione peroxidase activity, biochemical parameters and haematological parameters

Parameters	r_s	P value
GPx VsTotal Protein	0.321	0.002**
GPx Vs Albumin	0.550	0.001**
GPx Vs RBC	0.287	0.006**
GPx Vs Haemoglobin	0.218	0.039*
GPx Vs Hematocrit	0.285	0.006**

r_s – Spearman's rank correlation coefficient, *p<0.05, **p<0.01

Activities of GPx were found to be positively correlated with total protein and albumin levels. Activities of GPx were increased with increase in RBC count

to counteract the oxidative stress caused due to photocopier pollutants. According to Cho *et al*. (2010), GPx are the primary enzymes involved in peroxide elimination in RBC. Glutathione peroxidase was also found to be positively associated with haematocrit.

4.9.3 Correlation between GPx activity and Oxidative Status and Inflammatory Markers

Table 48 presents the correlation between GPx activity and oxidative status as well as the correlation between GPx activity and inflammatory markers.

Table 48

Correlation between Glutathione peroxidase activity and oxidative status and inflammatory markers

Parameters	r_s	P value
GPx Vs TEAC	0.224	0.034*
GPx Vs TBARS	-0.243	0.021*
GPx Vs ICAM1	0.376	0.001**
Gpx Vs IL-8	-0.219	0.038*

r_s – Spearman's rank correlation coefficient, *p<0.05, **p<0.01

Activities of glutathione peroxidase, an antioxidant enzyme was found to be increased with increase in trolox antioxidant equivalent capacity. It also showed negative association with lipid peroxidation marker, TBARS. This inverse relationship between antioxidant GPx and oxidant TBARS is predictable which is to balance the oxidative stress that occurred due to lung dysfunction and inflammation. Among the inflammatory markers studied ICAM-1 and IL-8 were positively correlated with GPx. These associations indicate that GPx is induced under oxidative stress in the present study that arises due to lung inflammation which might progress downstream to cardiovascular problems.

4.10 Genotoxicity Assessment

Among the DNA damage parameters investigated in general, the percentage of DNA in the tail has been proposed by several authors to be the

most generally useful parameter since it uses a quantitative measure of damage (from 0 to 100%). Furthermore, this parameter is less variable across studies (Collins, 2004; Olive *et a*l., 1992 and De-Boeck *et al*., 2000). Tail moment has the disadvantage that it does not have standard units and given a particular tail moment it is impossible to visualise the level of damage being described. For all these reasons % tail DNA is increasingly considered the preferred metric of DNA strand breakage in the comet assay.

Table 49, Figure 37 and Plate 4 indicate genotoxic effects of occupational exposure among photocopier service personnel using the comet assay. Significant differences ($p < 0.01$) were observed between control and photocopier service personnel (exposed) participants for each of the following parameter: tail length, tail intensity, % DNA in tail, tail moment and olive moment. Cumulative exposure to photocopiers was found to be positively correlated with tail length, tail intensity, % DNA in tail, tail moment and olive moment. The results demonstrated the genotoxic effect of photocopier exposure in service personnel as evidenced by increased levels of DNA damage.

Table 49

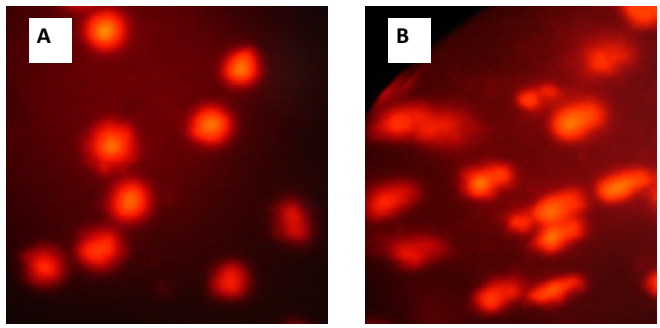
DNA damage parameters as assessed by comet assay

Comet parameters	Control (n = 45)	Exposed (n = 62)	p value
Tail Length (px)	3.5 (0.00 – 6.00)	5.50 (2.75 – 8.00)	<0.001**
Tail Intensity (px)	381.00 (6 – 1091.00)	1110.00 (251.50 – 180.10)	<0.001**
%DNA in Tail	0.00 (0.00 – 1.00)	3.50 (1.00 – 8.00)	<0.001**
Tail Moment	0.00 (0.00 – 0.00)	0.11 (0.00 – 0.483)	<0.001**
Olive Moment	0.00 (0.00 – 0.00)	0.294 (0.009 – 1.00)	<0.001**

Values are expressed as median with (inter quartile range in parenthesis),
p values for Mann Whitney test, *p<0.05, ** p<0.01

Plate 4

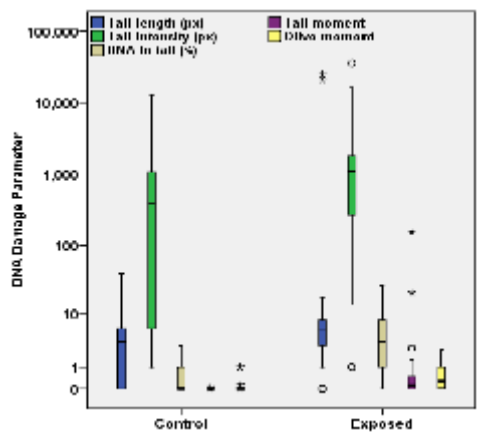
Blood Cells as Assessed by Comet Assay



A. Control **B. Exposed**

Figure 37

DNA damage parameters as assessed by comet assay



Earlier studies by Goud *et al*. (2001), Gadhia *et al*. (2005) and Kleinsorge *et al*. (2011) used meagre sample sizes for a biomonitoring study (Collins *et al*., 2014). The study by Manikantan *et al*. (2009) reported a significant increase in the incidence of DNA damage among 74 experimental subjects (male and female) when compared to their respective controls by comet assay. Balakrishnan and Das (2010) reported higher chromosomal aberrations among lymphocyte cultures of 66 workers exposed to photocopying machines when

compared to 40 controls. Hence, the observation of high DNA damage among photocopier service personnel is consistent with earlier biomonitoring studies with adequate sample size. This is mainly attributed to the toner composition as reported by Gminski *et al*. (2011) who stated that metals and metalloids as components of magnetite, or Polycyclic aromatic hydrocarbon materials as components of the carbon-bearing material, are responsible for the *in vitro* genotoxic effects. Apart from the above pollutants, UV light and particulate matter have been proved to be genotoxic agents (Griffiths *et al.,* 1998 and Gilli *et al*., 2007). Since, photocopier emissions are a mixture of all these pollutants at different concentrations, the genotoxic effect might be due to their individual and synergistic effects.

4.10.1 Correlation between Comet parameters and Cumulative Photocopier Exposure

Table 50 presents the correlation between comet parameters and cumulative photocopier exposure.

Table 50

Correlation between comet parameters and cumulative photocopier exposure

Comet Parameters	r_s	p value
Tail Length	0.217	0.090
Tail intensity	0.347	0.006**
DNA tail (%)	0.308	0.015*
Tail moment	0.264	0.038*
Olive moment	0.391	0.002**

r_s – Spearman's rank correlation coefficient *p<0.05, **p<0.01

Among photocopier service personnel, cumulative exposure to photocopier machines was found to be positively correlated with % DNA in tail, tail moment, tail intensity and olive moment. Even though we could not identify the individual compounds or their levels that may be responsible for the possible adverse effects associated with photocopier exposure, the findings of this biomonitoring study suggest particulate photocopier emissions as genotoxic hazards and their genotoxic effect was found to increase with exposure.

4.10.2 Correlation between Comet parameters, Cumulative Photocopier Exposure and Pack Years and Synergistic Effect of bothamong Photocopier Service Personnel who are Smokers

Table 51 presents the correlation between comet parameters, cumulative photocopier exposure and pack years and synergistic effect of both among photocopier service personnel who are smokers.

Table 51

Correlation between comet parameters, cumulative photocopier exposure and pack years and synergistic effect of both among photocopier service personnel who are smokers

Comet Parameters	PE		PY		PE X PY	
Tail Length	0.271	0.060	0.512*	0.043	0.013	0.961
Tail inensity	0.219	0.131	0.400	0.125	0.255	0.341
DNA tail	0.306	0.032*	0.196	0.468	0.390	0.135
Tail moment	0.293	0.041*	-0.440	0.088	-0.046	0.866
Olive moment	0.389	0.006**	0.340	0.198	0.399	0.126

r_s – Spearman's rank correlation coefficient *p<0.05, **p<0.01

No significant correlations were noticed in Table 50 between any of the studied comet parameters except for a positive association between tail length and pack years of cigarette smoked whereas significant correlation was noticed between all of the comet parameters and photocopier exposure among service personnel. This indicates that smoking is not a confounder in the present study of genotoxicity assessment among the photocopier service personnel.

4.11 Determination of metabolomic differences in excretory urinary biomarkers – A non invasive approach

Molecular biology methods have become very useful in occupational health and biomonitoring studies to provide more accurate and opportune diagnostics with non invasive metabolomics approach (Muñoz and Albores, 2010).

Figure 38

Urine ^{1}H NMR spectra of Control Nonsmoker (CNS)

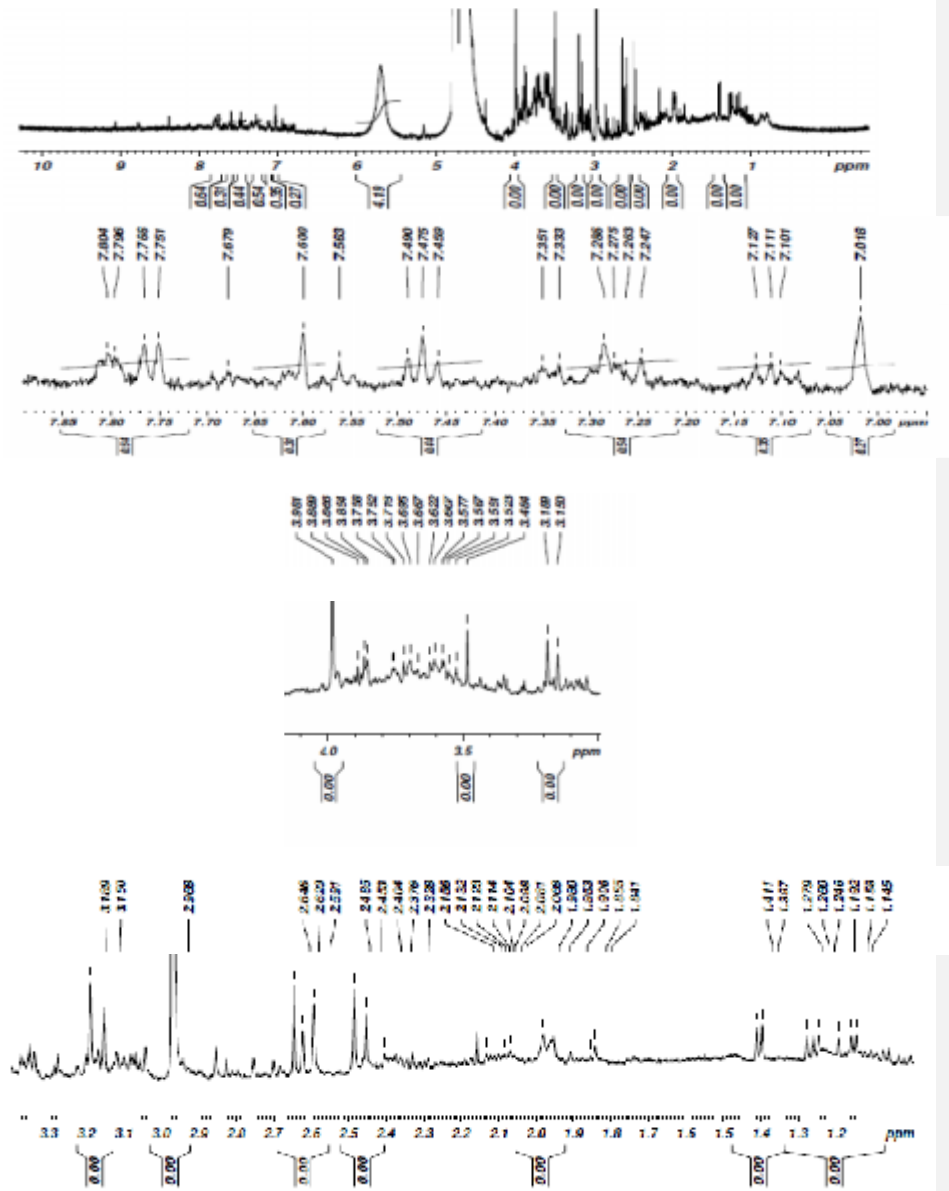


Figure 39

Urine ^{1}H NMR spectra of Control Smoker (CS)

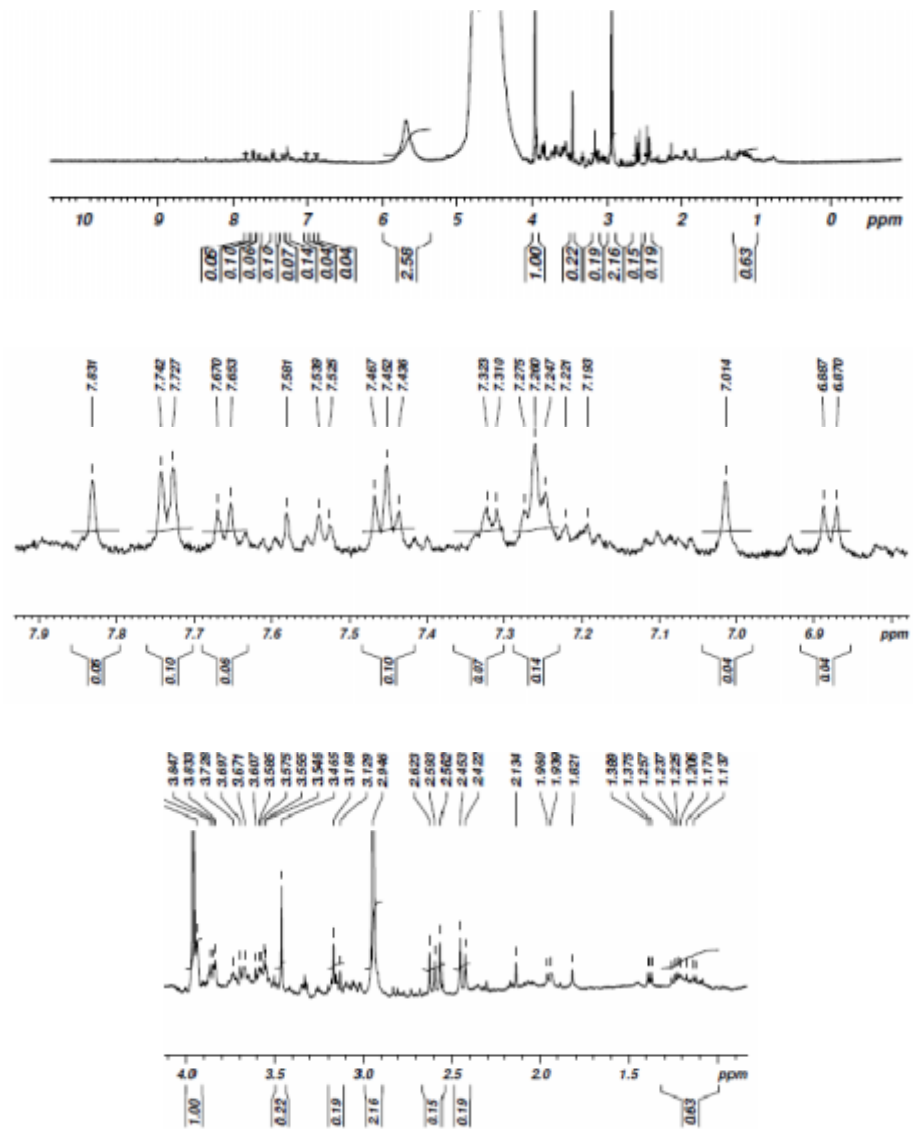


Figure 40

Urine ^{1}H NMR spectra of Exposed Nonsmoker (ENS)

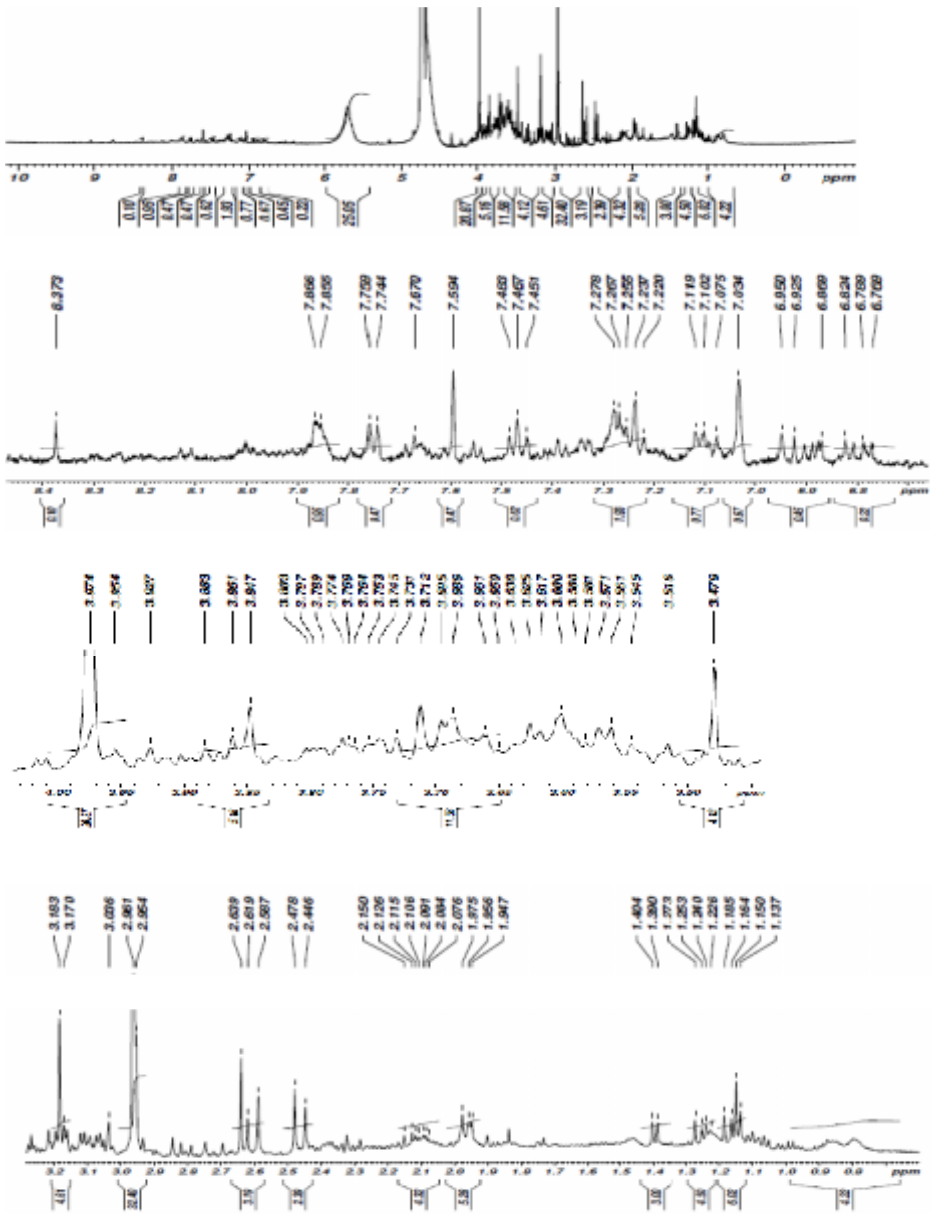
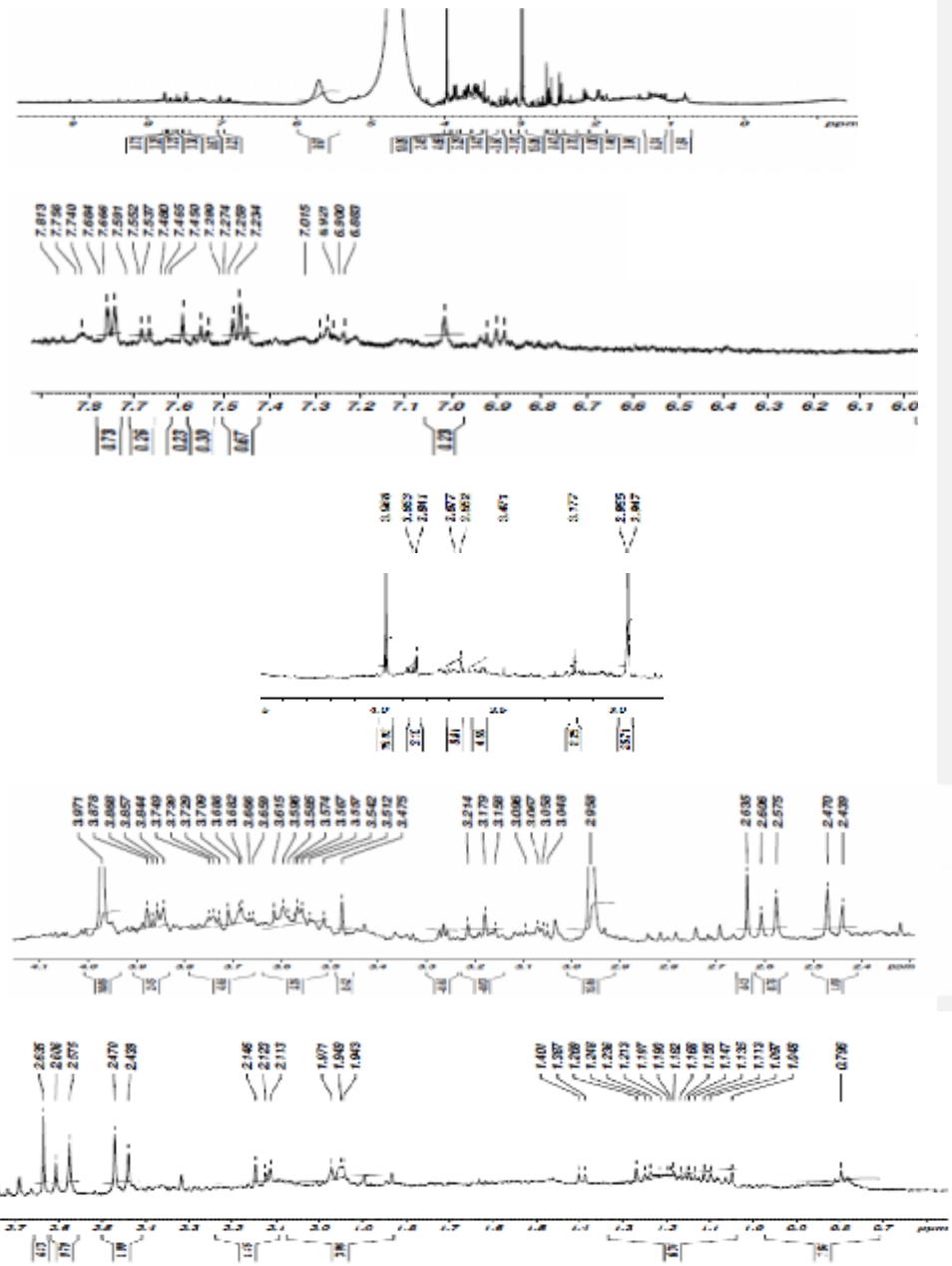


Figure 41

Urine ^{1}H NMR spectra of Exposed Smoker (ES)



Non-invasively collected matrices especially urine can offer opportunities to study additional aspects of exposure to and effects from environmental contaminants, such as repeated sampling, historical overview of exposure, mother-child transfer of substances, or monitoring of substances with short biological half-lives (Smolders *et al*., 2009).

Chemometric analysis was used to differentiate the complex spectra obtained from one dimensional ^{1}H NMR spectra of urine samples (Spectra of one subject in each group is presented in Figure 38, Figure 39, Figure 40 and Figure 41 representing the metabolomics pilot study participants Control Non smoker (CNS) (n =18), Control smoker (CS) (n = 9), Exposed Non smoker (ENS) (n = 10) and Exposed Smokers (ES), (n = 7) for metabolomic discrimination. As far as urine metabolomics study, is concerned, it is a unique finger print of each individual which depends specifically on the type of exposure. Hence, subjects were categorized specifically as smokers and non-smokers in addition to their differentiation as control and exposed participants. After data transformation by log normalization and autoscaling, univariate analysis by two way ANOVA was performed with Tukeys Honestly Significant Difference as Post Hoc test for urine samples followed by Multivariate analysis of Principal Component Analysis and PLS-DA (Partial Least Square Discriminate Analysis) to identify the putative marker in urine with higher discriminatory power between the groups of CNS, CS, ENS, ES for urine samples.

The univariate analysis of urine samples of the four different groups is represented with their log p value and significance in Table 52. One-way ANOVA followed by the post-hoc Tukey's HSD test of the ^{1}H -NMR dataset confirmed the significance of the selected features between the groups compared as indicated in Figure 42. Univariate analysis provides a preliminary overview about the NMR resonance peaks that are potentially significant in discriminating the metabolic differences between the four groups under study.

Figure 42

Important features identified in urine by one way ANOVA

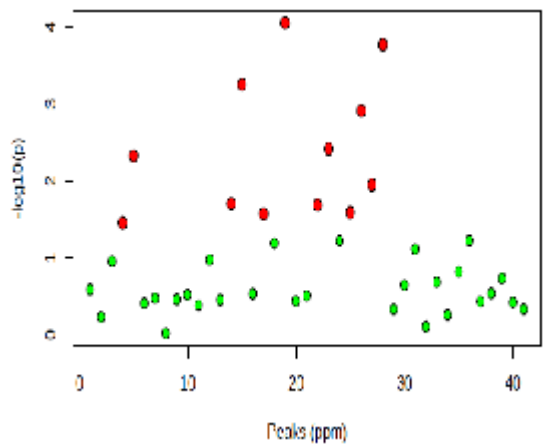


Table 52

Details of important features identified in urine by one way ANOVA and Post hoc test (Tukey's Honestly Significant Difference)

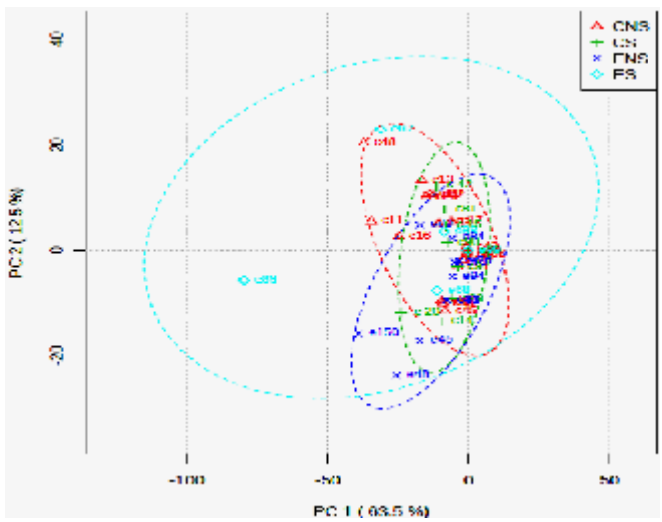
(δH 1 ppm)	-log 10 (p value)	p value	FDR	Post hoc test Tukeys HSD (Significant Comparisons)
3.48	8.73e-05	4.0588	0.003437	ES-CNS; ES-CS; ES-ENS
3.774	0.000168	3.7756	0.003437	ES-CNS; ES-CS; ES-ENS
2.973	0.000553	3.2572	0.007559	ES-CNS; ES-CS; ES-ENS
3.721	0.001217	2.9148	0.012472	ES-CNS; ES-CS; ES-ENS
3.616	0.003805	2.4196	0.031204	ES-CNS; ES-CS; ES-ENS
1.9775	0.004694	2.3285	0.032076	ES-CNS; ES-CS; ES-ENS
3.7505	0.011186	1.9513	0.06552	ES-CNS; ES-CS; ES-ENS
2.943	0.019658	1.7065	0.093243	ENS-CNS
3.585	0.020468	1.6889	0.093243	ES-CS
3.693	0.025698	1.5901	0.099902	ES-CNS; ES-CS
3.195	0.026803	1.5718	0.099902	ES-ENS
1.955	0.03491	1.4571	0.11928	*ns*

ns - non significant, δ proton chemical shift is usually expressed in parts per million (ppm)

Pattern recognition techniques are useful in discriminating different group of data. Principal component Analysis (PCA) is a well-known unsupervised multivariate technique for exploratory data analysis, which projects the data in a reduced hyperspace, defined by the principal components. These are linear combinations of the original variables, with the first principal component having the largest variance, the second principal component having the second-largest variance and so on. Partial Least Square Discriminate Analysis (PLS DA) is a supervised method, an important tool for statistical regression in chemistry (Ballabio and Todeschini, 2009). PLS - DA is used to distinguish two or more classes by searching for variable (X matrix) that are correlated to class member (Y matrix). In this approach the axes are calculated to maximize the separation between groups and can be used to examine the separation that would otherwise be across three or more principal components (Pears *et al.*, 2005).

Figure 43

2 D PCA score plot between the selected components of urine

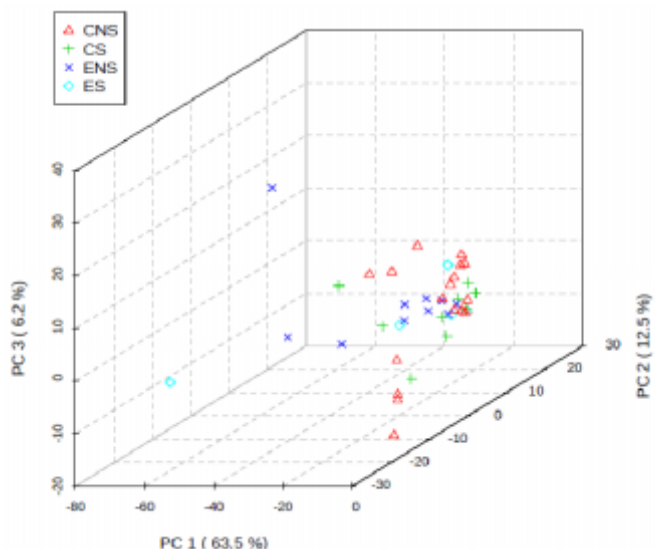


PCA, multivariate analysis was performed with the first three principal component sets on the urine obtained from the spectra. Through this analysis PC1 explained 63.5 %, PC2 12.5 % and PC3 6.2 % for urine (Figure 43 and Figure 44). PLS DA, supervised method was used for maximum discrimination of

intensities between the four predefined groups of CNS, CS, PCNS, PCS in urine samples. A regression analysis of the original data was performed against the Y Table for urine samples.

Figure 44

3 D PCA score plot between the selected components of urine



In the PLS-DA scores plot of urine (Figure 45 and Figure 46), the first three partial least squares components showed distinction of PLS1 50.4 %, PLS2 10.6 % and PLS3 11.3 % between the Y variable of four different classes of CNS, CS, PCNS.

The robustness of the model is double validated by the performance parameters namely R^2 and Q^2 obtained from Leave One Out of Cross Validation method (LOOCV) and by permutation test based on separation distance by 100 iterations. The fraction of variance is explained by R^2 and the total variation predicted by the model is Q^2 with discrimination significance at $P<0.05$. A model with $R^2 > 0.7$ and $Q^2 > 0.4$ is regarded as good for biological data (Lundstedt *et al*., 1998).

Figure 45

2 D PLS-DA score plot between the selected components of urine

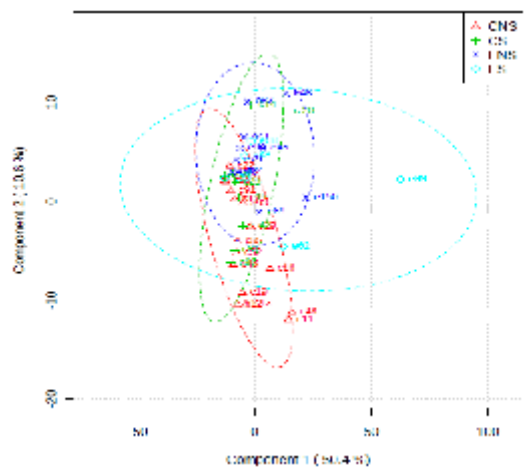
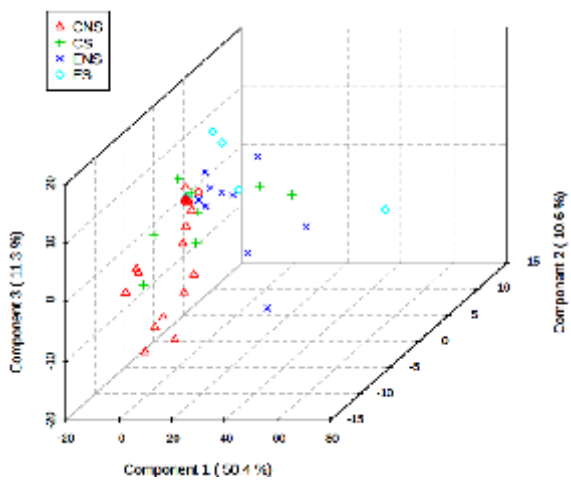


Figure 46

3 D PLS-DA score plot between the selected components of urine



The model generated for urine was found to be R^2 = 0.7 and Q^2 = 0.2 (Table 53) for principal component PC5 in PCA (Figure 47) at $p < 0.01$(Figure 48) for discrimination. These results indicate that model generated for metabolite changes in urine were moderate. Hence, the discrimination of metabolic

variations were moderate for urine samples between the groups CNS, CS, PCNS, PCS for urine samples

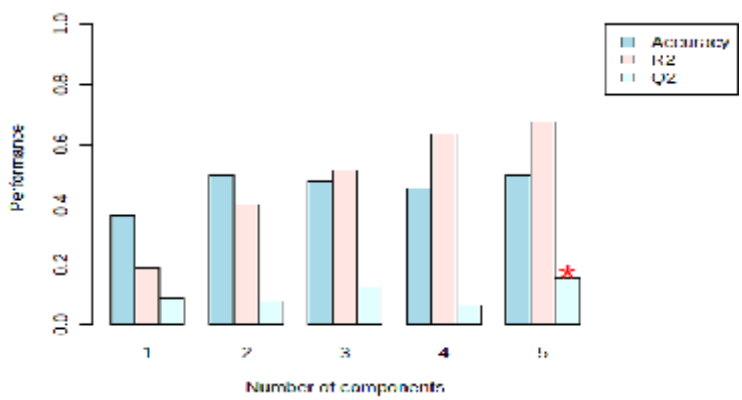
Table 53

Performance indicator table of the PLS- DA model validation for urine samples

Measure	1 Comps	2 Comps	3 Comps	4 Comps	5 Comps
Accuracy	0.4	0.5	0.5	0.5	**0.5**
R^2	0.2	0.4	0.5	0.6	**0.7**
Q^2	0.1	0.1	0.1	0.1	**0.2**

Figure 47

Performance indicator of the PLS- DA model validation using different number of components for urine.

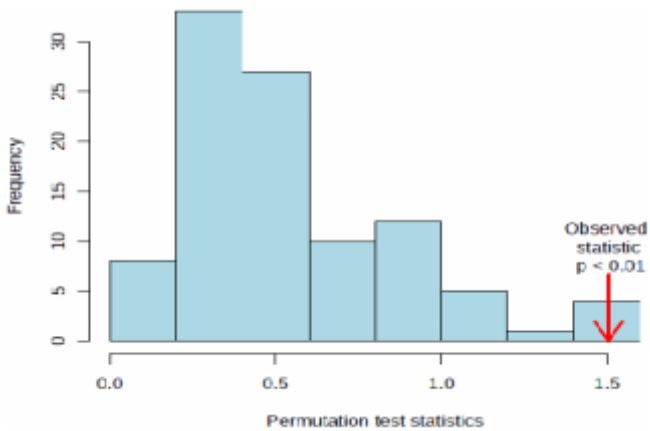


* **indicates the best classifier**

Identification of metabolites in addition to the classification, PLS- DA was used to select important features depending upon the variable importance in projection (VIP) score which is weighed sum of squares of the PLS loadings. Higher the VIP score in PLS - DA, higher is the importance of a variable in the present case of metabolite or a group of metabolites. To identify the important features we set the VIP score > 2.0 for urine samples.

Figure 48

Performance indicator of the PLS- DA model validation by permutation tests based on separation distance



The resonances corresponding to those metabolite peaks whose relative concentrations were significantly different in urine samples among the study group of CNS, CS, PCNS and PCS are listed based on their high, low and intermediate colour indications in Figure 49.

Figure 49

PLS-DA VIP score list for urine data sets

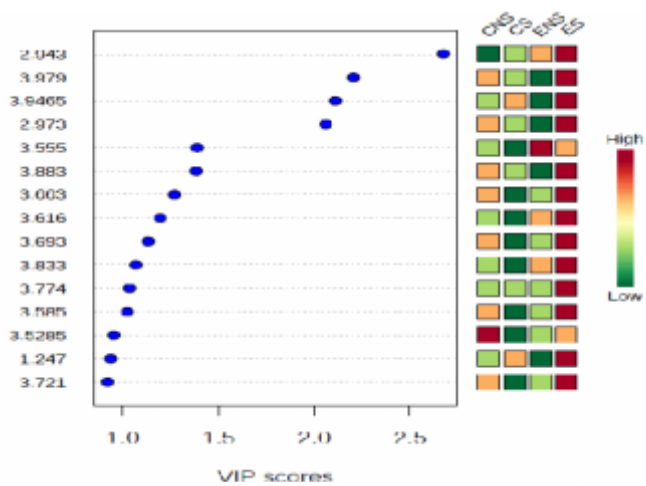
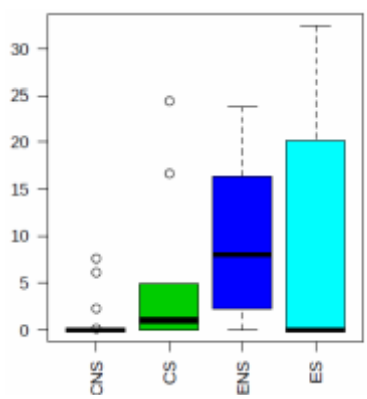


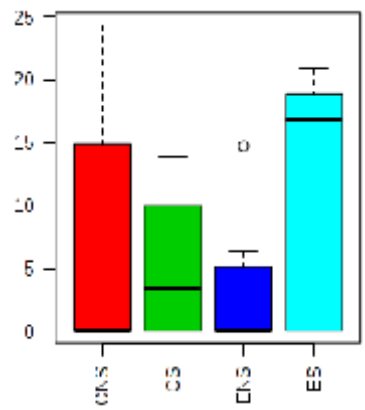
Figure 50

Concentration of metabolites in arbitrary units for urine data sets

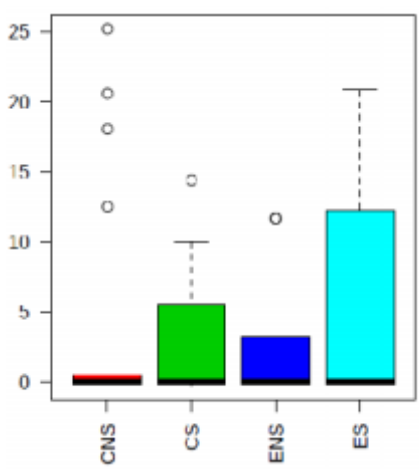
3 – Aminoisobutanoic acid



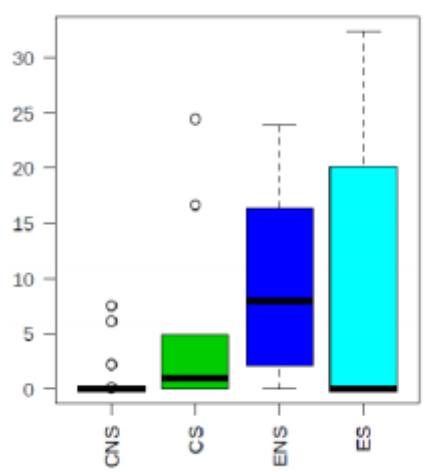
L - Histidine



Hippurate



Oxoglutarate



From the VIP scored peaks, the catalogue of the putative metabolites that could possibly play an important role in the discrimination was identified by searching the peaks in Human Meatabolome Database (HMDB) library and is represented in Table 54 and Figure 50. It was found, that urinary metabolite; 3 – aminoisobutanoic acid (**δH 1ppm, 2.943**) with higher VIP score of 2.7 was found to be moderately altered between the four groups namely CNS, CS, ENS

and ES followed by L-Histidine (**δH 1ppm, 3.979)** with VIP score of 2.2**,** Hippurate (δH 1ppm, 3.9465) with VIP score of 2.1 and Oxoglutarate (δH 1ppm, 2.973) with VIP score of 2.1 among photocopier service personnel.

3- Aminoisobutanoic acid is a break down metabolic product of thymine (Gartler, 1959). Their level excretion levels were found to be increased in urine of human subjects on exposure to lead (Tomokuni *et al*., 1992) ionizing radiations (Bawden, 1963), UV B radiation (Nakamura *et al*., 1973). In the present study the increased level of the same might be due to exposure to UV rays among photocopier service personnel as part of their maintenance activity. Thus the pilot study conducted with few subjects give new insight into the use of 3-Aminoisobutanoic acid, as efficient non invasive biomarker for bio-monitoring studies among the photocopier service personnel.

Table 54

List of putative metabolites with VIP Score > 2 for urine sample

(δH 1ppm)	VIP Score	Peak Search Metabolite	Human Metabolome Database
2.943	2.7	3-Aminoisobutanoic acid	**HMDB03911**
3.979	2.2	L- Histidine	**HMDB00177**
3.9465	2.1	Hippurate	**HMDB00714**
2.973	2.1	Oxoglutaric acid	**HMDB00208**

In addition, there were also other metabolites identified with lesser VIP score namely Histidine, hippurate and oxoglutarate.

According to reports by Eaton *et al*. (1998), the levels of urinary histidine excretion were found to increase in patients with classical allergy (type A allergy). It was also stated by him that allergy, when active, involves the release of histamine from mast cells and gut basophils this causes a loss of histamine to the system. This must be replaced by a continuing synthetic process from histidine and as blood histidine levels are homeostatically controlled. Histidine synthesized above body requirements is excreted in the urine.

Hence, increased excretion of histidine among photocopier service personnel in the present study might be due to allergic reactions caused by the particulate emissions of toner powder.

Increased urinary hippurate excretion among photocopier service personnel in the present study is related to lung function. According to the reports by McClay *et al*. (2010) increased levels of hippurate in chronic obstructive pulmonary disease subjects.

Increased excretion of oxoglutarate indicates the oxidative stress observed among the photocopier service personnel. According to Fukuhara *et al.* (2013), oxoglutarate urine excretion in rat model indicates oxidative stress.

Hence, it could be suggested that these occupational non-invasive biomarkers are very useful in the diagnosis of oxidative stress induced lung dysfunction, progonosis of occupational exposure to UV rays and particulate emissions among photocopier service personnel.

4.12 Binary logistic regression of general health symptoms with photocopier exposure

Table 55

Binary logistic regression of general health symptoms with Photocopier exposure

Independent Variable	Photocopier exposure		O.R	95% CI	
Dependent variable	ß	P		Lower	Upper
Respiratory Symptoms	-1.95	< 0.01**	0.14	0.05	0.40
Head ache	-2.051	<0.05*	0.13	0.028	0.601
Allergies	-2.148	<0.05*	0.12	0.014	0.956
Skin Problems	-1.339	<0.05*	0.26	0.84	0.817

Value indicated as (**,*) denotes significant odds ratio (O.R) at p≤ 0.01 or p ≤ 0.05 at 95% Confidence Interval (CI).

The odds ratio of less than 1 indicated in table 55, suggests that occupational exposure among photocopier service personnel were found to be negatively associated with adverse outcome of respiratory symptoms (14%),

headache (13%), and allergy (11.7%) and skin symptoms (26.2%) No significant association was noticed between general health symptoms namely dizziness, sleeplessness, eye problems and photocopier exposure. Similarly there were no significant association of general health symptoms with smoking as independent variable. Hence, were not indicated in table 55.

4.13 Regression analysis between lung function parameters, haematological indices, biomarkers and independent continuous variables pack years and cumulative photocopier exposure

Table 56 displays the significant association between photocopier exposure and the regression analysis interpretation after adjustment for the confounding variable. Pearson's correlation coefficients (r) were used for continuous dependent variables (pack years of cigarette smoked and cumulative photocopier exposure) and various lung function parameters, haematological indices and biomarkers. Those dependent parameters that were found to have significant correlations were further analysed by step-wise linear regression and expressed as significant regression equation with F value after adjustment for potential confounders within the allowed degrees of freedom (df). Non significant association were not expressed in the table 56.

Table 56

Regression analysis between lung function parameters, haematological indices, biomarkers and independent continuous variables pack years and cumulative photocopier exposure

S.No	Dependent variable	Status	Independent Variable				Regression Analysis				
			PY		CE						
			r	P	r	P	R^2	adjusted R^2	df	F value	p value
1.	VC (% pred)	↓	ns	ns	-0.225	0.003**	0.051	0.045	(1,175)	9.369	<0.001**
2.	FEV1(% pred)	↓	ns	ns	-0.155	0.040*	0.024	0.018	(1,175)	4.289	<0.05*
3.	MVV (% pred)	↓	ns	ns	-0.316	<0.001**	0.100	0.095	(1,175)	19.448	<0.001**
4.	HGB (g/dl)	↑	ns	ns	0.182	0.020*	0.033	0.027	(1,161)	5.543	<0.05*
5.	MCV (fL)	↑	0.217	0.005**	ns	ns	0.047	0.041	(1,161)	7.925	<0.05*
6.	RDW (fL)	↑	ns	ns	0.385	<0.001**	0.148	0.143	(1,161)	27.953	<0.001**
7.	MPV (fL)	↑	0.174	0.026*	ns	ns	0.030	0.024	(1,161)	5.026	<0.05*
8.	P-LCR (fL)	↑	0.213	0.006**	ns	ns	0.046	0.040	(1,161)	7.684	<0.05*
9.	Lymphocytes(10^3/µl)	↓	-0.182	<0.000**	ns	ns	0.033	0.027	(1,161)	5.491	<0.05*

Pearson's correlation coefficient (r) significant at **p < 0.001,*p<0.05; ns – non significant

CE – Cumulative years of photocopier exposure (1000s of years), PE – Pack years of cigarette smoked

Table 56 (Contd...)

S.No	Dependent variable	Status	Independent Variable				Regression Analysis				
			PY		CE						
			r	p	r	P	R^2	adjusted R^2	df	F value	p value
10.	Globulin (g/L)	↑	ns	ns	0.215	0.006**	0.046	0.040	(1,161)	7.830	<0.05*
11.	TBARS (µM)	↑	ns	ns	0.579	<0.001**	0.336	0.040	(1,161)	81.299	<0.05*
12.	FRAC (mM)	↓	ns	ns	-0.189	0.016*	0.036	0.331	(1,161)	5.976	<0.001**
13.	CC-16 (ng/ml)	↑	ns	ns	0.180	0.021*	0.032	0.030	(1,161)	5.407	<0.05*
14.	IL-6 (pg/ml)	↑	ns	ns	0.195	0.013*	0.038	0.032	(1,161)	6.355	<0.05*
15.	IL-8 (pg/ml)	↑	ns	ns	0.496	<0.001**	0.224	0.221	(1,161)	4.323	<0.05*
16.	CRP (µg/ml)	↑	ns	ns	0.417	<0.001**	0.032	0.026	(1,161)	5.407	<0.05*
17.	ICAM-1 (ng/ml)	↑	ns	ns	0.167	0.033*	0.028	0.026	(1,161)	4.616	<0.05*
18.	DNA in tail (%)	↑	ns	ns	0.339	<0.001**	0.115	0.109	(1,161)	20.881	<0.001**

Pearson's correlation coefficient (r) significant at **p < 0.001,*p<0.05;

CE – Cumulative years of photocopier exposure (1000s of years), PE – Pack years of cigarette smoked

The evaluation of a cause-and-effect relationship between exposure and outcome was undertaken in the present study to understand whether cumulative photocopier exposure leads to any lung dysfunction after adjustment for important confounder pack years, by step-wise linear regression modelling. The significant negative correlations of % predicted lung function parameters namely VC ($r = -0.225$; $p < 0.001$), FEV1 ($r = -0.155$; $p < 0.05$) and MVV ($r = -0.316$; $p<0.001$) suggests that cumulative photocopier exposure is the only contributor for the significant decrease of % predicted lung function parameters. These significant negative correlations of lung function indices indicate causal effects of cumulative xerographic exposure on lower airways with restrictive ventilatory pattern, an indicator of progressive lung inflammation on long time exposure to particulate matter and organic solvents in toner formulation among photocopier service personnel.

Gardiner *et al*. (2001) found a significant association between exposure and decrement in FEV1, among participants exposed to carbon black in European carbon black manufacturing industry in a cross sectional study. Statistically significant decrements in FVC, FEV1, MVV and PEFR on chronic exposure to petrol fumes that consist of benzene, petrol and diesel exhaust, particulate matter and air pollutants were revealed among petrol pump workers (Hulke *et al*., 2012).

The regression analysis of haematological indices is presented in table 56. It is evident that there exists a significant positive association between haemoglobin and RDW with cumulative exposure ($r = 0.18$; $p<0.05$, $r = 0.39$; $p<0.001$) among photocopier service personnel. Cumulative photocopier exposure is the only contributor for increase in haemoglobin and RDW as indicated by significant regression equation for haemoglobin (hb) and RDW. This indicates hypoxic environments among photocopier service personnel due to cumulative occupational exposure to photocopier machines. Sørensen *et al*. (2003) also reported positive correlation between haemoglobin and PM2.5 exposure. A significant increase in haemoglobin (Hb) concentration was also noted by Gaharwar and Paulraj (2015) in petrol filling workers exposed to longer

duration to benzene and carbon monoxide of environmental pollutants in their workplace. Exposure would have caused had high Hb concentration due to the fact that toner composition contains high percentage of organic carbon that would have formed carbon monoxide. Excessive production of carbon monoxide (CO), which leads to formation of carboxy haemoglobin that results in a shortage of Hb for oxygen carriage, shifting the Hb-oxygen dissociation curve to the left leading to hypoxia and stimulation of erythropoiesis with increased levels of RBC cells and Haemoglobin (Metta *et al*., 2015).

RDW has been reported as an inflammatory biomarker in different conditions such as cardiovascular diseases (Tonelli *et al*. (2008) and Arbel et al. (2014), chronic pulmonary diseases (Balta *et al*., 2014) and prognostic biomarker in idiopathic pulmonary fibrosis a kind of restrictive lung disease (Nathan *et al*., 2013).

Among the other blood indices MCV, MPV, lymphocytes count and P-LCR were found to be influenced only by pack years and not by cumulative photocopier exposure as indicated in table 56.

Hyperglobulinemia noticed in table 56, among photocopier service personnel might be a positive acute phase reaction caused by progressive decline in lung function (figure 26) also evidenced by significant positive association between cumulative exposure ($r = 0.215$; $p<0.05$) and globulin content with significant regression equation ($R^2 = 0.046$; adjusted $R^2 = 0.040$; $F(1,161) = 7.830$; $p<0.05$). Among the oxidative stress markers studied a strong positive association was observed between TBARS and cumulative photocopier exposure (table 56) at $p<0.001$. In the present study, this positive association of TBARS with cumulative photocopier and significant regression equation after adjustment for confounder might prove TBARS as a best sensitive indicator of systemic oxidative stress rather than the free 8-isoprostane levels among photocopier service personnel. In contrast, Kleinsorge *et al*. (2011) did not find any positive correlation between photocopier exposure and TBARS where as Increased levels of TBARS were reported by Elango *et al*. (2013) among

photocopier operators. Increased levels of TBARS (Table 28) and decreased levels of FRAC (Figure 29) with significant associations and regression equation noted in table 56 indicated the imbalance in overall oxidative status. Significant negative correlation occurred between FRAC and cumulative photocopier exposure whereas no such significant correlation was obtained between TEAC and cumulative photocopier exposure as indicated in table 56. The results are in tune with those of Zhou *et a*l. (2003) who reported significant negative correlation between ozone levels and antioxidants such as vitamin E, vitamin C, beta carotene, superoxide dismutase, catalase in photocopier operators.

Plasma CC16 levels were found to be positively correlated with ($r = 0.180$, $p < 0.05$, Table 56) cumulative photocopier exposure with significant regression equation. Provost et al. (2014) and Vattanasit et al. (2014) observed a strong positive association between exposure to particulate matter emissions and serum CC16 levels. The consistency of this association in the present study implies that CC16 could be used as an efficient biomarker to assess adverse effects of photocopier particulate emissions on the cellular integrity or the permeability of the lung epithelium or the lung damage among the photocopier service personnel. According to Bernard (2014) increased levels of CC16 in serum, is due to increased airway permeability and is also peripheral marker of events taking place in the deep lung. This means that circulating levels of CC16 are determined not only by the intrapulmonary pool of CC16, but also by the rate at which the protein leaks from the lungs and at which it is cleared from plasma. Because of its small size, CC16 is rapidly eliminated from plasma by glomerular filtration and as a corollary its serum level rises in parallel with serum creatinine. CC16 levels were found to be increased in sarcoidosis (Hermans *et al*., 2001), interstitial lung disease (Olewicz-Gawlik *et al*., 2015) emphysema, fibrosis and combined pulmonary fibrosis and emphysema (Kokuho *et al*., 2014). In contrast, their levels were found to be decreased in chronic bronchitis, asthma and in smokers (Lomas *et al*., 2008). Age is an important factor that influences the levels of CC16 (Lakind *et al*., 2007). Increase in CC16 levels among the exposed participants might be due to chronic disruption of the bronchoalveolar blood

barrier integrity and its passive leakage by transduction into plasma leading to lung dysfunction on exposure to photocopier pollutants. The results of the present study is analogous to the effects of several air pollutants smoke (Bernard *et al*., 1997) diesel exhaust (Beamer *et al*., 2015) and tobacco smoke exposure (Ma *et al*., 2015) on CC16 levels.

The positive association levels of IL-6 and IL-8 with cumulative exposure among photocopier service personnel with significant regression equation (table 56) indicates that cumulative photocopier exposure is the significant contributor of interleukin levels. Karoly *et al*. (2007) opined that exposure to ultrafine particulate matter increased the expression of IL-6 and IL-8 genes in human pulmonary artery endothelial cells. Kido *et al*. (2011) stated that exposure to PM10 in mice induces translocation of pulmonary inflammatory mediator, mainly IL-6 from lungs to the systemic circulation leading to systemic inflammation with downstream effects of vascular dysfunction. Increased levels of inflammatory markers, IL-6 and IL-8 were also noted in serum and/or bonchoalveolar lavage fluid of COPD subjects as a systemic inflammatory response (El-Shimy *et al*., 2014). In tune with the present study it was reported by Elango *et al* (2013) that chronic exposure to photocopier particulate matter emissions leads to increased levels of plasma IL-8 among photocopier operators. Thus, higher levels of IL-6 and IL-8 in plasma among photocopier service personnel might indicate lung dysfunction and systemic inflammation that progress to vascular dysfunction in latter conditions

C-reactive protein (CRP) is one of the acute phase plasma reactants whose levels increase in response to inflammation, infection and tissue damage (Ólafsdóttir, 2011). From table 56, it is evident that there is significant positive association ($r = 0.417$; $p<0.001$) and significant regression equation only between cumulative photocopier exposure and CRP and not with pack years of cigarette smoked. CRP is a systemic biomarker to study ongoing lung inflammation (Dahl *et al*., 2007). The significant increase might be due to increase in systemic inflammation among photocopier service personnel triggered by exposure to photocopier particulate matter (PM2.5) emissions. In concurrence with the findings of the present study, Hoffmann et al. (2009) reported an increase in the

levels of systemic marker, CRP on long term residential exposure to high levels of PM2.5. A moderate increase in serum CRP is also implicated in sarcoidosis according to Drent *et al*. (1999).

From table 56, it is observed that there is significant positive association ($r = 0.167$; $p<0.05$) only between cumulative photocopier exposure and ICAM-1 and not with pack years of cigarette smoked. ICAM-1 levels are elevated in the serum of patients with cardiovascular disease, autoimmune disorders, cancer (Lawson and Wolf, 2009) and acute lung injury (Calfee *et al*., 2009).

In addition to these biomarkers, there were also significant positive association of % DNA in tail with cumulative photocopier exposure (Table 56) indicating genotoxic effects among photocopier service personnel.