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Review Article

Oxidative Potential of Ambient PM and Related Health Endpoints over South Asia: A Review

Anil Patel, Neeraj Rastogi*

Geosciences Division, Physical Research Laboratory, Ahmedabad-380009, India

***Corresponding author.**
Tel: +91 79 2631 4306
E-mail: nrastogi@prl.res.in

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ABSTRACT South Asia occupies only about 3.5% of the world's area but, about 25% of the average world's population lives here and is continuously exposed to severe air pollution. Unprecedented development activities in most of the South Asian cities emit primary and secondary pollutants into the atmosphere. Particulate matter (PM), a principal air pollutant, are tiny enough to remain suspended in the atmosphere for a long time (about a week). They can penetrate the human nasal airway and damage the lungs. PM effects on human health are assessed based on their mass concentration, size distribution, and chemical composition. Despite being critically important, studies related to PM effects on human health are limited over South Asia. In recent years, only a few South Asian research groups started studying the ability of atmospheric PM to cause human health hazards by generating *in situ* reactive oxygen species (ROS). The capability of atmospheric PM to produce ROS and/or deplete antioxidants is termed as their oxidative potential (OP). Though limited, efforts are made to identify particular species with the higher OP. Atmospheric aging of PM can also alter their OP. No studies from South Asia, except a few from India, investigated how the atmospheric aging changes the chemical and physical properties of PM and affect their OP over South Asia. These studies also showed that OP depends more on PM composition rather than its concentrations. Therefore, mitigation strategies for reducing PM mass concentrations alone may not be sufficient, and linking PM OP with significant health effects may be a better way to regulate specific sources of PM rather than overall PM mass. This review reports the necessities and limitations for PM OP studies in South Asia and future directions.

KEY WORDS Aerosols, Reactive oxygen species, Oxidative potential, Human health

1. INTRODUCTION

There are a variable concentrations and chemical composition of particulate matter (PM) in the Earth's atmosphere, with their size ranging from a few nm to several μm. They interact with the respiratory system of all living organisms. The majority of ambient atmospheric species are injected into the atmosphere by several natural sources (e.g., mineral dust, sea-salts, volcanic emissions, forest fires, etc.) (Fig. 1). Most of the natural gaseous and particulates species we breathe today are similar to what respired by our ancestors. After the pre-industrial era, anthropogenic activities

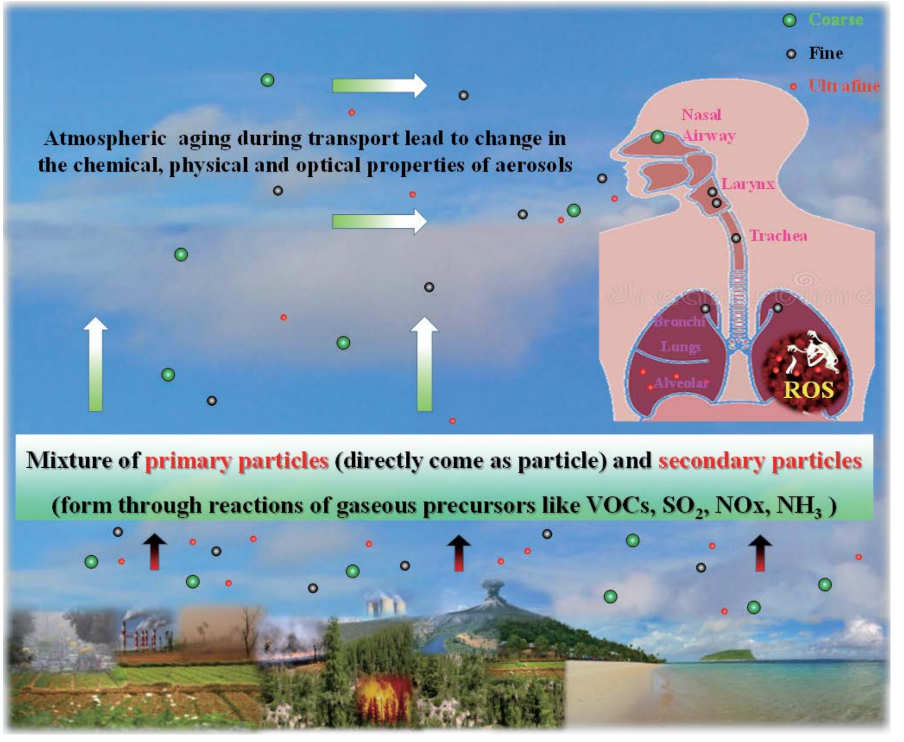

Fig. 1. A schematic representation of sources of atmospheric PM and their reach in the human body where they can generate reactive oxygen species and affect human health.

started pumping a large pool of toxic and redox-active species into the atmosphere via industrialization and urbanization, which started deteriorating the ambient air quality (Akatsu, 2015). Ambient PM (or aerosols) are the reactive as well as non-reactive species including organic carbon (OC), elemental carbon (EC), major inorganic ions, trace and heavy metals, etc., in variable sizes (Seinfeld and Pandis, 2006). Fig. 1 depicts a schematic representation of sources of atmospheric PM and their reach in the human body where they can generate reactive oxygen species and affect human health. Assessing the effects of PM on Earth's climate and human health is among major current research focus worldwide (Pöschl, 2005). The emission sources of PM and meteorological conditions over different regions vary drastically and thus, their concentrations and composition also show a large spatiotemporal variability. PM retain in the lower troposphere for about a week, depending upon their physiochemical properties and meteorological conditions. However, particles emitted at one place, depending upon the wind speed and wind direction, can travel thousands of kilometers before they get removed from the atmosphere. Thus, the toxic emissions in one place can affect many distant places downwind. During the long-range transport of PM, aging processes such as homogeneous and heterogeneous reactions, condensation, coagulation, evaporation, aggregation, oxidation, reduction, etc., may enhance/reduce particle toxicity (Pöschl, 2005).

PM mass concentrations are usually reported in microgram of PM per cubic meter of air ($\mu g\ m^{-3}$). The average adult inhales about 17 m^3 of air daily (~11.8 lpm) (Brochu, 2006). The amount of PM reaching the human respiratory system depends upon the ambient PM concentration levels, as a fraction of PM gets trapped in the system (Lyu *et al.*, 2018; Fang *et al.*, 2017; Oberdorster *et al.*, 2005). Human exposure to an environment with PM mass concentration higher than defined safe limits may result in chronic health problems (Pope *et al.*, 2020; Delfino *et al.*, 2006; Oberdörster *et al.*, 2005). Millions of death have been recorded worldwide due to fine PM exposure only, as shown by world health statistics - 2018 (Burnett *et al.*, 2014). However, the PM toxicity cannot be explained in terms of merely the amount of dose; rather, it depends upon the physicochemical properties of particles present in the dose (Patel *et al.*, 2021; Patel and Rastogi, 2020; Borlaza *et al.*, 2018; Charrier *et al.*, 2016; Valavanidis *et al.*, 2008). PM induced oxidative stress in human is one of the widely accepted mechanisms. It occurs when the defense mechanisms cannot control the enhanced level of oxidants produced by the PM in the human body. These oxidants are nothing but reactive oxygen species (ROS). Biologically relevant ROS members include but not limited to moderately reactive superoxide ($O_2^{\cdot -}$), less reactive hydrogen peroxide (H_2O_2), and highly reactive hydroxyl radical (·OH). On inhalation, atmospheric redox-active PM species interact with cells, such as macrophages, and produce ROS (Fig. 1). In this process, redox-active PM species such as transition metals and quinones catalytically transfer an electron from biological reducing respiratory complexes, e.g., reduced forms of nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH), to the molecular oxygen (O_2) (Kumagai *et al.*, 1997). *In situ* ROS formation is the sequential univalent reduction of the O_2. The capacity of PM to deplete anti-oxidants and/or oxidize the electron-rich enzymes through generating ROS in the human body is known as their 'oxidative potential (OP)', a metric of PM induce toxicity (Bates *et al.*, 2019). Further, NADH and NADPH enzymes are essential for maintaining a vast array of biological processes, and their deficiency or imbalance are associated with many pathological disorders (Xiao *et al.*, 2018).

The $O_2^{\cdot -}$ formation depends upon the cellular oxygen concentration. Mitochondria is so far known as one of the most suspicious places where the *in vivo* ROS generation occurs. Lungs are mostly affected by ROS due to their direct contact with the atmospheric oxygen. Particularly in tissues not exposed to O_2, the *in vivo* ROS production is likely to be smaller due to insufficient mitochondrial oxygen concentration (Turrens, 2003). Sometimes, however, hyperbaric conditions dissolve more oxygen in blood plasma, resulting in a hyperoxic environment. In this circumstance, the size of PM and their retention time in the human respiratory tract define the actual cytotoxicity (Oberdörster *et al.*, 2005). In contrast to the larger size PM, ultrafine PM have accessibility to the blood stream by different transfer routes and mechanisms, thereby reaching almost any organ in our body, including the brain and heart (Terzano *et al.*, 2010). In the hyperoxic environment, the brain is the most suspicious organ to show elevated levels of ROS (Turrens, 2003).

The present article reviews the current status of the work reported on the PM OP, and how it interacts with biological tissues in the human body over the different regions of South Asia. It also discusses the limitations and future directions for PM OP studies in South Asia.

2. AVAILABLE ASSAYS TO MEASURE PM OP AND ROS CONCENTRATION

Among several assays available in the literature for measuring PM OP, the majority measures the potential of PM to produce ROS (i.e., endogenous ROS, also known as OP). In contrast, a few assays measure PM-bound ROS concentration (exogenous ROS). These assays are nicely summarized in a recent study (Bates *et al.*, 2019). These assays can be acellular (cell-free) or cellular.

2.1 Acellular Assays

A variety of acellular assays such as dithiothreitol (DTT, Cho *et al.*, 2005), Glutathione (GSH, Shahpoury *et al.*, 2019), Ascorbic Acid (AA, Fang *et al.*, 2016), Electron Spin Resonances (ESR, Yang *et al.*, 2014), Chemiluminescent Reductive Acridinium Triggering (CRAT, Yang *et al.*, 2014), etc, are employed for assessing the PM induce ROS. However, each of these assays has a different sensitivity to the various PM components that produce ROS, and none respond to all such components. The degrees of their reactivity with PM vary from assay to assay (Bates *et al.*, 2019 and references therein). For example, DTT oxidation is executed by redox-active PM that further reduces O_2 to produce ROS. DTT assay responds to a pool of components that include a few metals and organic components; however, the role of Fe in the DTT assay is debatable (Bates *et al.*, 2019; Charrier and Anastasio, 2012).

On the contrary, Rao *et al.* (2020) and Bates *et al.* (2019) reviewed that other assays such as AA, GSH usually measure the capability of metals present in PM to deplete antioxidants with the adverse response to organic components. ESR assay measures the capacity of PM to induce ·OH, and it responds well to metals. CRAT has a high selectivity for superoxide ion, and it responds to ferric and cupric ions and organic species, such as quinones (Yang *et al.*, 2014). At present, no single acellular assay can assess the OP of all the toxic PM species; however, it would be nice to have one.

2.2 Cellular Assays

Cell-based assays such as dichloro-dihydro-fluorescein diacetate (DCFH-DA, Venkatachari *et al.*, 2005), and p-hydroxyphenyl acetic acid (POPHAA) dimerization (Hasson and Paulson, 2003) measures the particle-bound ROS, i.e., equivalent H_2O_2 concentration. Furthermore, Wardman (2007) reviewed several fluorescent and luminescent probes such as dichlorodihydrofluorescein, dihydrorhodamine, hydroethidine, etc., for the measurement of oxidative and nitrosative species in cells and tissues. However, dichlorodihydrofluorescein and dihydrorhodamine are shown to be unreactive towards superoxide ion, and less reactive towards the H_2O_2, a dismutation product. Further, background oxidation of H_2O_2 by metal impurities such as Fe (Fenton reaction) in the buffer solution can influence the outcomes; hence, chelating treatment to buffer solution before the reaction is performed (Shen and Anastasio, 2011).

Further, these probes require a catalyst for the reaction. As none of these assays cover the entire range of ROS family, it is better to measure the capability of PM to generate ROS (i.e., OP), rather than measuring the concentration of ROS. Among all the OP assays developed so far, the DTT assay is widely used because it responds to many organic components and trace metals (Charrier and Anastasio, 2012). The response of DTT assay also correlates well with a pool of biological endpoints such as hemeoxygenase-1 (HO-1) expression (Li *et al.*, 2003), a reduction activity of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Steenhof *et al.*, 2011), a fraction of nitric oxide in exhaled breath (Delfino *et al.*, 2013), markers of the airway and nasal inflammation (Janssen *et al.*, 2015), increased relative risk for asthma (Yang *et al.*, 2016; Bates *et al.*, 2015), and congestive heart failure (Bates *et al.*, 2015). Thus, a general conceptual framework of the toxicity associated with PM sources and emission characteristics can be represented by DTT-based PM OP. However, the development of one probe covering all the ROS is the need of the hour.

3. REVIEW OF PM OP OVER SOUTH ASIA

South Asia covers only 11.6% of the Asian continents and 3.5% of the world's land but, this region accounts for ~40% and ~25% of Asia's and world's population, respectively. The population density of the South Asian region is ~10 times higher than the average population density of the world (Demographia, 2020). Moreover, India is among the fastest-growing economies of the world (Subramanian, 2019), and occupies ~60% of the land area of the South Asian region along with ~12% higher population density. Besides, a variety of anthropogenic emissions are increasing unprecedently in several metropolitan cities of the developing countries of South Asia, which violate the international air quality standards (Marlier *et al.*, 2016; WHO, 2016; Brauer *et al.*, 2012; Gautam, 2010; Nandasena *et al.*, 2010). Winter conditions usually cause an unhealthy accumulation of particulates in the lower troposphere for an extended period of stagnation in many regions (Singh *et al.*, 2021). Wind transport can harm rural areas downwind. Despite being a hotspot, studies reporting PM composition and OP together over South Asia are despicably low.

3. 1 Link between PM Composition and OP over South Asia

In recent years, a very few groups from India reported the PM chemical composition along with its DTT-based OP (DTT-OP) over different regions dominated by various emission sources and/or meteorological conditions (Patel *et al.*, 2021; Patel and Rastogi, 2020, 2018a, 2018b; Puthussery *et al.*, 2020; Yadav *et al.*, 2019; Rastogi and Patel, 2017; Vreeland *et al.*, 2016). Though limited, these studies covered many important aspects including DTT-OP near to point source emission like trash burning (Vreeland *et al.*, 2016), seasonal variation and the effect of long-range transport of aerosols on chemical composition and DTT-OP over a mountain (Patel and Rastogi, 2018b) and an ocean (Patel and Rastogi, 2020), DTT-OP from mixed sources over big cities (Patel *et al.*, 2021; Puthussery *et al.*, 2020; Yadav *et al.*, 2019), and the plausible effect of meteorology (e.g., fog) on the chemical composition and DTT-OP over a semi-arid site (Patel and Rastogi, 2018a). Recently, Ahmad *et al.* (2021) reported DTT-OP over Lahore and Peshawar in Pakistan. PM mass and related volume-normalized and mass-normalized DTT-OP reported by these studies are depicted in Fig. 2.

Vreeland *et al.* (2016) reported the OP of extremely high concentrations of redox-active organic aerosols (mainly aromatic di-acids from plastic burning and levoglucosan from biomass burning) emitted from the roadside trash burning in Bangalore, India. The dose (volume-normalized DTT-OP) reaching the people passing by or living near to the trash burning location was significantly higher, as predicted, compared to the ambient samples, evidencing a significant public health hazard. However, organic carbon (OC) mass-normalized OP in

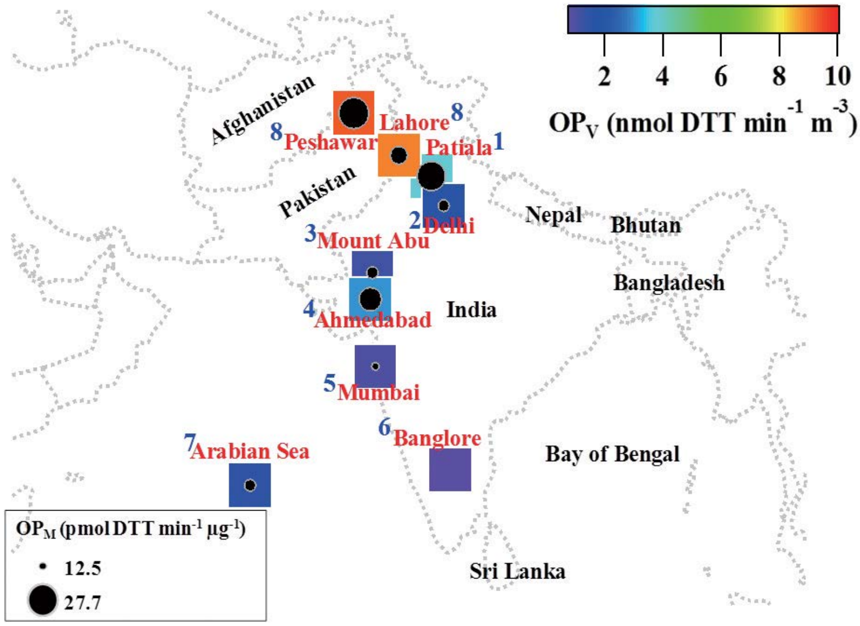

Fig. 2. Dithiothreitol (DTT)-based average volume-normalized oxidative potential (OP_V, color scale) and average mass-normalized oxidative potential (OP_M, size of bubbles) over South Asia. [1]Patiala; Patel and Rastogi, 2018a, [2]New Delhi; Puthuserry *et al.*, 2020, [3]Mount Abu; Patel and Rastogi, 2018b, [4]Ahmedabad; Patel *et al.*, 2021, [5]Mumbai; Yadav *et al.*, 2019, [6]Banglore; Vreeland *et al.*, 2016, [7]Arabian Sea; Patel and Rastogi, 2020, [8]Lahore and Peshawar, Ahmad *et al.*, 2021.

samples collected near trash burning was lower than those for ambient PM samples. After two years of that study, through a laboratory experiment, our group reported that secondary inorganic aerosols (SIA, the sum of NH_4^+, NO_3^- and SO_4^{2-}) do not respond to DTT-activity (Patel and Rastogi, 2018a). Thus, the mass-normalized OP can be significantly biased if there is a relatively similar or higher SIA fraction than water-soluble redox-active organic species in the ambient PM over a given study region. Patel and Rastogi (2018a) proposed to use variation in mass-normalized DTT-OP along with the WSOC/SIA ratio to overcome the bias introduced by SIA. The same study also reported that the biomass burning derived carbonaceous aerosols in $PM_{2.5}$ have higher OP than those derived from fossil fuel combustion. In the same year, seasonal variability in PM_{10} chemical composition and DTT-OP was reported over Mount Abu, a high altitude site in western India (Patel and Rastogi, 2018b). The highest PM_{10} mass during pre-monsoon over Mount Abu was ascribed to high dust loading from the surrounding Desert and Arab continents. However, the corresponding DTT-OP (both volume- and mass-normalized) was not higher in the pre-monsoon season. It was reported that anthropogenically emitted pollutants transported by the northeasterly air-masses were dominant during post-monsoon, and found to be associated with the highest DTT-OP (both volume- and mass-normalized). After that, a study from the same group over the marine environment (the Arabian Sea) observed that DTT-OP (both volume- and mass-normalized) of PM_{10} associated with the air-masses traveled from the Indian subcontinent was higher compared to PM_{10} traveled from the marine and desert environments, depicting the role of anthropogenic emission on the PM toxicity (Patel and Rastogi, 2020). These observations highlighted the poor reactivity of the dust components of PM_{10} with the DTT. Interestingly, EC mass fraction in $PM_{2.5}$ over Patiala (Patel and Rastogi, 2018a), and in PM_{10} over Mount Abu (Patel and Rastogi, 2018b) and the Arabian Sea (Patel and Rastogi, 2020) was found to be positively correlated with mass-normalized DTT-OP ($p < 0.05$). The slope from this linear relationship over different regions highlighted that atmospheric aging of species emitted along with EC makes them more DTT-active, as both the Arabian Sea and Mount Abu predominantly receive aged aerosols. A recent study by our group reported OP and a variety of chemical speciation of PM_{10} at the five sites representing different environments of a big city (Ahmedabad) in western India (Patel *et al.*, 2021). These sites include near traffic junction, bus transport hub, industrial, and two types of residential area in Ahmedabad. Narol, an industrial site, expectedly yielded the highest volume-normalized DTT-OP, highlighting a higher risk to Narol's inhabitants from potentially redox-active PM_{10} species. One of the interesting features was that volume-normalized DTT-OP varied with PM_{10} mass, but mass-normalized DTT-OP remained similar from site-to-site. As inferred from the optical properties of PM_{10}, nitrogenous organic compounds mainly emitted from the traffic-related sources in Ahmedabad have higher OC mass-normalized OP. Yadav *et al.* (2019) reported that volume-normalized DTT-OP for indoor $PM_{2.5}$ emitted from the combustion of crop residue, firewood, mixed, and biomass fuel was an order of magnitude higher than ambient $PM_{2.5}$. Further, a recent study by Puthussery *et al.* (2020) reported the real-time measurement of DTT-OP in $PM_{2.5}$ over New Delhi, India. They found that photochemically aged organic aerosols (OA) mostly drove the DTT-activity, whereas fresh $PM_{2.5}$ emitted from vehicles showed less influence on OP. Further, Yu *et al.* (2018) reported that there are elements in the PM components with synergistic and antagonistic effects in generating ROS based on the DTT assay, which also needs to be understood over South Asia. Ahmad *et al.* (2021), the only study on DTT-OP from Pakistan, reported that relatively higher fraction of carbonaceous aerosols and metals in $PM_{2.5}$ over Peshawar could be the reason for observed high DTT-based mass-normalized OP compared to that found over Lahore. Despite drastically different volume-normalized DTT-OP over Patiala (3.8 nmol DTT min^{-1} m^{-3}, India) and Peshawar (9.3 nmol DTT min^{-1} m^{-3}, Pakistan), mass-normalized DTT-OP was similar (Fig. 2). Such observations highlight that the PM OP over these nearby locations were not similar. Further, the another study from Lahore during 2007–2008 investigated DCFH-DA-based ROS-activity of water-soluble fine and coarse PM with the highest activity in fall and mid-late winter (Shafer *et al.*, 2010). The findings showed that the water-soluble Cu, Mo, Fe, and Ni are the most potential ROS-active metals.

3.2 PM and Heath of Biological Tissues in the Human Body over South Asia

A few studies from India investigated the role of PM exposure on the health of biological tissues using a variety of markers listed in Table 1 (Das *et al.*, 2021; Jan *et al.*, 2020; Roy *et al.*, 2015; Sambandam *et al.*, 2015; Dutta *et al.*, 2013, 2012; Banerjee *et al.*, 2012; Mondal *et al.*, 2011;

Table 1. Overview of ROS and their role in human health over South Asia.

Study	Site and exposure	Assays for Reactive Oxygen Species	Biological markers
Arif *et al.*, 1992 and Rahman *et al.*, 1997	India; Particles and Fibers	Luminol dependent and peroxide dependent chemiluminescence assays for ROS	Human and rat alveolar macrophages
Mondal *et al.*, 2011	India; Biomass smoke_85 pre-menopausal women from rural areas of West Bengal	ROS by flow cytometry and superoxide dismutase (SOD) by spectrophotometry	Comet assay for DNA single-strand breaks in Buccal Epithelial Cells (BECs)
Dutta *et al.*, 2012, 2013	India; Biomass smoke_Blood sample and Sputum sample	ROS by flow cytometry and SOD by spectrophotometry	Serum interleukin-6 (IL-6), C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-8 (IL-8) were measured by Enzyme-linked immunosorbent assay (ELISA)
Pant *et al.*, 2015	India; Oxidative potential of PM_{10} fraction of road dust in New Delhi	Depletion of Ascorbic acid (AA) and Glutathione (GSH)	–
Roy *et al.*, 2015	India; Urban and rural indoor environment in Pune	DTT assay	Plasmid DNA assay
Sambandam *et al.*, 2015	India; Respirable coal fly ash from power plant chimneys	Depletion of GSH and ROS generation using DCFH-DA assay	DNA fragmentation assay, MTT assay
Jan *et al.*, 2020	India; Atmosphere of Pune	DCFH-DA assay	MTT assay and % haemolysis of human red blood cells
Das *et al.*, 2021	India; Atmosphere of Delhi	DCFH-DA assay	MTT assay, IL-6, IL-8

Rahman *et al.*, 1997; Arif *et al.*, 1992). Here, Arif *et al.* (1992) and Rahman *et al.* (1997) investigated the toxic potential of various particles and fibers to the human and rat alveolar macrophages. They found that alveolar macrophages in humans produce more ROS compare to that in rats. A group from Chittaranjan National Cancer Institute investigated the role of PM in deoxyribonucleic acid (DNA) single-strand damage on pre-menopausal women who were engaged in cooking using bio-fuel/bio-mass in rural areas of West Bengal, India (Mondal *et al.*, 2011). They observed a sharp rise in DNA single-strand breaks in Buccal Epithelial Cells (BECs), which was positively associated with the ROS generation and PM levels, indicating oxidative stress resulted from biomass smoke. Similar type of studies were reported from West Bengal, wherein blood sample and sputum samples were measured for several health indicators including serum Interleukin-6 (IL-6), IL-8 by Enzyme-linked immunosorbent assay (ELISA), Tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP) from the women using biomass and compared with the women using cleaner fuel (as a control) (Dutta *et al.*, 2013; Dutta *et al.*, 2012). ROS generation, depletion of superoxide dismutase (SOD), and indoor concentration of PM were also measured. Biomass users had more indoor air pollutants compared to controlled one and their serum as well as sputum contained significantly elevated levels of the biological markers of inflammation, oxidative stress and hypertension such as IL-6, IL-8, TNF-α and CRP, and ROS generation; while SOD was depleted significantly. If the damage is not repaired, it may lead to several health problems, including cancer (Moustacchi, 2000).

Furthermore, a study from the urban and rural indoor environment in Pune, India, demonstrated the significant role of fine metals in the DTT oxidation and DNA damage, highlighting the importance of PM size in the indoor environment's toxicological mechanism (Roy *et al.*, 2015). After that, the positive effects of respirable coal fly ash, collected from power plant chimneys in Chennai, India, on the induced *in vitro* toxicity in different cell-lines were examined by DNA fragmentation assay, MTT assay, depletion of GSH, and ROS generation using DCFH-DA assay (Sambandam *et al.*, 2015). Moreover, a group from Pune University, India, carried out a study to asses the toxicological effects in the human body due to metals present in the atmosphere of Pune. They studied the response of various biological markers, including the cytotoxicity profiles measured by MTT assay on two different cell lines (epithelial cell line and human peripheral blood mononuclear cells) and % haemolysis of human red blood cells on exposure to different concentration of PM (Jan *et al.*, 2020). In addition, ROS measured using DCFH-DA in both cell lines were also found to be increasing with exposed PM mass concentration. Das *et al.* (2021) showed a year-long $PM_{2.5}$-metals catalyzed ROS generation by DCFH-DA assay along with toxicological parameters at Delhi, the hotspot for air pollution in India. A higher fraction of trace metals in post-monsoon and winter caused lower cell-viability in contrast to the pre-monsoon and monsoon periods. However, the $PM_{2.5}$ during pre-monsoon and winter were associated with higher ROS generation compared to the rest two seasons. This highlights that the metals damaging the cells are not the same which actually produce ROS in human body.

4. SUMMARY AND FUTURE SCOPE

It is indeed disappointing to notice that none of the studies related to health endpoints discussed above measured the complete PM composition, including the speciation of organic carbon, which is reported to be a highly responsive component for ROS generation in the human body (through redox-cycling of quinone type species). Further, the most of such studies reported over South Asia were carried out either in the indoor environments or near the source locations. However, the PM OP can be affected by several factors including photochemical aging, volatility, and pH of PM, and ambient meteorological conditions (Bates *et al.*, 2019 and references therein). On the other hand, the majority of the OP based studies carried out over regions dominated by different sources and meteorological conditions in outdoor environments did not consider any health endpoints. It is a compulsive requirement to provide a complete scenario of PM induce health effects in time and space over South Asia.

Further, such studies are virtually lacking in other South Asian countries such as Pakistan, Afghanistan, Nepal, Bhutan, Bangladesh, Sri Lanka, and Maldives. Several studies have reported air pollution as the potential risk factor for cardiovascular disease over India (Balakrishnan *et al.*, 2019; Dutta and Ray, 2013; Nautiyal *et al.*, 2007; Cropper *et al.*, 1991), Nepal (Adhikari *et al.*, 2020), Sri Lanka (Nandasena *et al.*, 2010), Afghanistan (Rana *et al.*, 2019; Falvo *et al.*, 2015) and Pakistan (Sughis *et al.*, 2012). However, none of these studies discussed specific species responsible for the pollution-borne diseases and the mechanism behind it. Yamamoto *et al.* (2014) made an effort to review air pollution as a risk factor for morbidity and mortality in South Asia; however, there was a bias as all of the studies were from India only.

As mentioned earlier, the OP is one of the most conspicuous health indicators for PM induced health effects in humans. A significant criticism of the studies that have modeled OP and associated health endpoints can be explained by the fact that PM composition varies drastically in time and space and thus their relation with OP shall also vary (Fang *et al.*, 2016; Bates *et al.*, 2015). It is vital to start assessing PM OP at least over selected strategic sites (e.g., megacities, rural areas, mountains, islands) dominated by different sources and/or meteorological conditions in South Asia. It is also crucial to characterize the PM chemical composition temporally and spatially along with their OP using different cellular and acellular assays, as no assay covers the entire PM components. It would be nice to develop a single acellular assay that responds to all the toxic species present in the ambient PM. In addition, the measurements of various biological markers that express the oxidative stress and other health endpoints should also be taken into account while considering the PM toxicity over South Asia. The involvement of epidemiologists and medical researchers with atmospheric scientists is critical to understand the underlying mechanisms of PM induced health effects.

It is worth mentioning that PM mass can be similar at multiple sites and/or at one location at different times. But, PM composition is highly variable in time and space as it strongly depends upon the emission sources, meteorological parameters, and atmospheric processing, and this fact cannot be ignored (Patel *et al.*, 2021; Patel and Rastogi, 2020, 2018a, 2018b; Verma *et al.*, 2015; Li *et al.*, 2009). Further, there are elements in the PM components with synergistic and antagonistic effects in generating ROS, and they need to be understood over South Asia. Recommended studies will be crucial in designing/developing mitigation strategies to reduce/stop the emissions of specific species that have a significant role in the oxidative burden. Reduction of PM mass concentrations alone may not be sufficient for better air quality for human health.

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