Virology: Current Research

The Emergence of Ten SARS-CoV-2 Variants and Airborne PM$_{2.5}$

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Abstract

Background: The atmospheric pollutant PM$_{2.5}$ has been implicated in the pathogenesis of COVID-19. Some of the variants of SARS-CoV-2 possess the attributes of increased transmissibility and immune escape and appear to have been naturally selected to promulgate the pandemic. Variants of SARS-CoV-2 resulted due to a number of persistent SARS-CoV-2 mutations found in widely disparate and distant regions. This paper examines a possible association between airborne pollutant PM$_{2.5}$ and the emergence of ten SARS-CoV-2 variants.

Methods: The daily mean levels of PM$_{2.5}$ of a number of cities, where SARS-CoV-2 variants were detected, were obtained from the World Air Quality Index (WAQI). The mean daily PM$_{2.5}$ levels were evaluated just before the occurrence of the first cluster of PM$_{2.5}$ peaks' atmospheric concentration, till after the emergence of the SARS- CoV-2 variants. Where available the daily number of new cases of COVID-19 diagnosed was matched to the PM$_{2.5}$ levels.

Results: There appears to be a common pattern of PM$_{2.5}$ in most of the regions prior to the emergence of the SARS-CoV-2 variants. An initial cluster of PM$_{2.5}$ peaks was noted on average 50 days prior to the emergence of the variants and another group of smaller peaks in PM$_{2.5}$ were noted just before or contemporaneous with the emergence of the SARS-CoV-2 variants. In the regions where the quantity of daily new cases was available, a number of significant correlations were obtained between PM$_{2.5}$ levels and the number of new cases of SARS-CoV-2 variants.

Conclusion: In most regions two clusters of PM$_{2.5}$ peaks were noted prior to the emergence of SARS-CoV-2 variants. The first cluster of PM$_{2.5}$ peaks may suggest that anthropogenic activity was increased possibly reflecting augmented human to human contact. Due to elevated levels of PM$_{2.5}$, a consequent propagation of the respiratory ACE-2 receptor (port of viral entry into the cell) ensued. Coronavirus- laden PM$_{2.5}$ may have induced intra-host mutagenesis in the SARS-CoV-2 genome, contemporaneously diminishing pulmonary immunity. With the second cluster of PM$_{2.5}$ peaks, this airborne pollutant may have also acted as a viral vector. The above findings suggest that antecedent peaks in PM$_{2.5}$ prior to SARS-CoV-2 variants' emergence not only contributed to transmission, but also impacted the immediate viral environs which may have led to SARS-CoV-2's natural selection.

Keywords: PM$_{2.5}$ • SARS-CoV-2 • COVID-19 • Mutations • Variant emergence

Introduction

The COVID-19 pandemic has reappeared in subsequent waves in the form of more transmissible and potentially more virulent variants. This increase in COVID-19 incidence has been attributed to the reversal of regional lockdowns and measures, endorsing physical distancing which were legally enforced in most countries. Following lockdown, a contemporaneous reduction in COVID-19 rates and atmospheric pollution including particulate matter (PM$_{2.5}$) were noted only to be partially reversed once lockdown was ceased. This reversal of lockdown also involved a recrudescence of elevated levels of the pollutant particulate matter PM$_{2.5}$.

The first study suggesting an association between PM$_{2.5}$ and SARS-CoV-2 was noted in the United States, whereby a link between long-term exposure to particulate matter PM$_{2.5}$ and COVID-19 related mortality was demonstrated [1]. A recent preprint has confirmed that PM$_{2.5}$ was a robust variable in connection with increasing SARS-CoV-2 rates [2]. Morphological evidence also confirmed that genes coding for SARS-CoV-2 were found attached to particulate matter [3]. In a large number of Chinese cities, a 2% increase in COVID-19 new cases was demonstrated with every 10 µg/m³ increment in airborne PM$_{2.5}$ [4]. The deleterious effects of particulate matter on pulmonary microbial defences may encourage SARS-CoV-2 colonization of the respiratory epithelium [5,6]. Particulate matter increases

the number of ACE-2 receptors, the point of host cell entry of SARS-CoV-2 [7]. Particulate matter may actually act as a vector for transmission of COVID-19 infection by increasing its airborne reach surrounding the human habitat [8]. Contini and Costabile and also aiding deeper penetration into the respiratory tract [9,10].

It has been suggested that particulate matter PM$_{2.5}$ was not only responsible for SARS-CoV-2 transmission, but may also have been involved in this virus' evolution. Acting as a SARS-CoV-2 vector, PM$_{2.5}$ may have been responsible for exerting selective pressure determining the emergence of the first variant called the G614 variant [11].

A recurring set of mutations, mainly E484K, N501Y and K417N suggests SARS- CoV-2 may be undergoing convergent evolution [12]. This convergent evolution may be catalyzed by the presence of a common environmental mutagen such as the ubiquitous pollutant PM$_{2.5}$ which has repeatedly been shown to be a robust co-factor in the SAR-CoV-2 Pandemic [2]. Contrasting effects by PM$_{2.5}$ on viral infectivity have been shown on two bacterophages Φ6 and ΦX174 [13]. Aerosol admixture of PM$_{2.5}$ with bacteriophages Φ6 and ΦX174 (a non-enveloped bacteriophage) showed a reduction of ox 6 infectivity but a contrastingly superior ΦX174 infectivity compared to controls. This paper examines the possibility that pollution with PM$_{2.5}$ changes augmented COVID-19 infection and had a hand in SARS-CoV-2's natural selection, strongly suggesting that particulate matter may

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have acted co-factor in a multi-modal manner catalyzing the SARS-CoV-2 pandemic through the emergence of its variants.

METHODS

The average levels of particulate matter $PM_{2.5}$ of a number of cities were obtained from the World Air Quality Index (WAQI). This Air Quality Index is a real-time measurement of atmospheric pollutants, including $PM_{2.5}$ (EPA Environmental Protection Agency 2020-2021). The daily average $PM_{2.5}$ levels were assessed prior to a prominent spike in $PM_{2.5}$ till beyond the emergence of the SARS-CoV-2 variants in each region. During 2020, the regions noted to have COVID-19 variants included Beijing, Valencia, Nelson Mandela Bay in South Africa, Bexely (UK), Los Angeles, New York, Louisiana, and New Mexico in the USA and Sao Paolo in Brazil. In mid-March 2021, two variants emerged in Nagpur, in the Maharashtra region of India and in Eastern Kentucky USA respectively. In Beijing, the period between January 20th and mid-February 2020 was chosen when the G614 variant [Figures 1 and 2] appears to have been detected [14]. In Valencia the first peak of $PM_{2.5}$ [Figures 3 and 4] was noted around the 11th of April and the 20A.EU1 variant appears to have been detected at the end of May 2020 [15]. The $PM_{2.5}$ in Bexely, UK peaked on the 12th August 2020, 40 days before the B.1.17 variant was detected on the 20th of September [Figures 5 and 6]. In Nelson Mandela Bay the origins of the B.1.351 variant in South Africa were noted in early October, 60 days after the $PM_{2.5}$ peak on the 19th July 2020 [Figure 7]. In Los Angeles the first case of the B.1.429 variant was noted during a solitary $PM_{2.5}$ spike on the 6th of July which was followed by another two wider spikes in mid-September and the beginning of October after which a surge in COVID-19 cases was noted in November [Figures 8 and 9]. The B.1.526 variant was detected in the in New York at the end November, 15 days after a spike in $PM_{2.5}$. The surge in the B.1.526 variant followed in mid-December contemporaneous with a $PM_{2.5}$ spike [Figures 10 and 11]. In Louisiana and New Mexico, the B.1.2 variant followed a similar pattern with three spikes in $PM_{2.5}$, the first and most prominent 45 days before the variant was detected in September, the second spike at detection in November and the third smaller spike in early December when COVID-19 cases surged Figures 12-15. In Sao Paolo Brazil two peaks of $PM_{2.5}$ were noted during September to be followed by the emergence of the B.1.1.248 variant in early December [Figure 17].

Two variants emerged in mid-March 2021, one occurring in Nagpur India in Figures 18 and 19 and the second in Eastern Kentucky, USA in Figure 16. Prior to the B.1.617 variant in India a wide peak of $PM_{2.5}$ was noted during the first three weeks of January and 50 days later, during another smaller peak, the B.1.617 variant emerged in mid-March 2021. After a series of $PM_{2.5}$ peaks in February in Kentucky the R.1 variant was detected 45 days later, contemporaneous with another larger $PM_{2.5}$ spike in mid-March 2021.

The viral samples first determining the G614 variant in China were obtained from Beijing. This does necessarily mean that the variant first emerged in Beijing, however for lack of further evidence the $PM_{2.5}$ in Beijing was utilized in this study. It may well be that with the sudden rise in cases the G614 variant first emerged in Wuhan, the paper by Hodcroft, et al. point to the North-East of Spain as being the origin of the 20A.EU1, and following a super spreader event in Bergamo, the $PM_{2.5}$ levels from Valencia were assessed [33]. The B.1.1.7 appears to have originated in Kent, UK. and the $PM_{2.5}$ levels in Bexely were utilized in this study. The B.1.351 variant in South Africa has been suggested to have possibly originated in Nelson Mandela Bay where although $PM_{2.5}$ levels are available, the daily new case counts could not be collated from the East Cape. In Los Angeles the first case of the B.1.429 variant was noted during a solitary $PM_{2.5}$ spike while the B.1.526 variant was detected in the Washington Heights in New York. In Louisiana and New Mexico, where the B.1.2 variant was first detected, the $PM_{2.5}$ levels of these states' capital cities were assessed. The $PM_{2.5}$ levels in East Kentucky were used in this study as this is where the R.1 variant is reported to have been first detected. The $PM_{2.5}$ levels of Sao Paolo

were assessed as this was pollution data available from Brazil, although the B.1.1.248 variant appears to have been first detected in Manaus which however did not have any $PM_{2.5}$ levels available on WAQI [16]. The pattern of elevated levels of particulate matter pollution in Brazil appears to perennially occur in October due to anthropogenic and natural factors. The B.1.617 variant appears to have been first detected in India in November 2020, but its emergence as a VOC was noted in Nagpur in Mid-March 2021. As may be noted in the absence of solid evidence, in some cities certain assumptions had to be taken to decide the site from which $PM_{2.5}$ levels had to be assessed.

The data was analysed for normality and all the data were found to be nonparametric. The Mann Whitney U test was applied for comparing nonparametric variables of both groups of cities and the Spearman Rank test was applied for nonparametric correlations.

Results

There appears a common pattern of $PM_{2.5}$ in most of the regions assessed prior and during the emergence of the COVID-19 variants. An initial spike/s of $PM_{2.5}$ was noted on average 50 days prior to the surge of the variants and another smaller spike/s in $PM_{2.5}$ was noted just before or contemporaneous with the emergence of the variant in Figures 1-18. Prior to the emergence of the G614 variant in Beijing, the average $PM_{2.5}$ level during its initial spike was 153.4 μg/m³ (SD+/-63.9) to settle to 94.4 μg/m³ (SD+/-47.8) (p<0.001) [Figure 1]. With the data available the number of new cases of COVID-19 in Beijing decreased with diminishing $PM_{2.5}$ levels in Figure 2. Before the appearance of the 20A.EU1 variant in Valencia the $PM_{2.5}$ three spikes averaged at 61.3 μg/m³ (SD+/21.8) to decrease to a mean of 41.2 μg/m³ (SD+/-15.5) (p<0.04) in Figure 3. Similar but to a lesser extent COVID-19 new cases in Valencia decreased with decreasing $PM_{2.5}$ levels in Figure 4. In Kent, U.K. the single initial $PM_{2.5}$ spike averaged 82 μg/m³ (SD+/-29) before the detection of the B.1.1.7 when the $PM_{2.5}$ averaged 27.8 μg/m³ (SD+/-18) (p<0.03) [Figure 5]. As regards COVID-19 cases in Kent there was a weak correlation with $PM_{2.5}$ levels in Figure 6. In Nelson Mandela Bay where B.1.351 was first detected, the $PM_{2.5}$ mean level was reported as 40.4 μg/m³ (SD+/-14) while prior to this variant's emergence, the $PM_{2.5}$ spike averaged 85.1 μg/m³ (SD +/-17.3)(p<0.0001) in Figure 7. In Sao Paolo Brazil the average $PM_{2.5}$ during its peak was 107.4 μg/m³ (SD+/-34.2) before B.1.1.248 variant emerged when the baseline $PM_{2.5}$ was 48.3(SD+/-18) (p<0.0001) (Figure 17). In the USA the average $PM_{2.5}$ spike levels prior to the emergence of the SARS-CoV-2 variants were 118(SD+/-28.8)μg/m³ in Los Angeles (baseline 66.1(SD+/-25.1)μg/m³, 75+/-27.8 (baseline 43.3(SD+/-14.4)μg/m³ in Louisiana, 71.4+/-11.3 μg/m³ (baseline 43.6(SD+/-12.4)μg/m³ New Mexico, 54.3+/-13.8)μg/m³ (baseline 34.4(SD+/-11.6) in New York and 37.7+/-7(baseline 28.5(SD+/-6.8)μg/m³ in Eastern Kentucky, all of which were significant (p<0.0001) in Figures 8-16. Prior to the emergence of the variant in India, the $PM_{2.5}$ peak in Nagpur averaged 166.8+/-10.8 μg/m³ (baseline 123.2 SD+/-16.9 μg/m³) (p<0.0001) in Figures 18. There was no correlation between COVID-19 new cases and $PM_{2.5}$ levels [Figure 19] for the Indian variant.

Where available for assessment a number of variables correlations resulted between new cases SARS-CoV-2 and $PM_{2.5}$ levels. In Beijing the strongest correlation between $PM_{2.5}$ concentrations and the number of new cases was noted (R=0.53) (p<0.001). A weaker correlation between $PM_{2.5}$ concentrations and the number of new cases was obtained in Valencia (R=0.25 p<0.05). No significant correlations between $PM_{2.5}$ concentrations and the number of new cases in Bexely UK, Louisiana and New York, USA. Negative significant correlations resulted in los Angeles (R=-0.36 p<0.001) and Mew Mexico (R=-0.35 p<0.0001). No correlation between COVID-19 new cases and $PM_{2.5}$ levels, however it must be mentioned that the new cases were retrieved from the Maharashtra province whereas the $PM_{2.5}$ levels were obtained from one of its cities, Nagpur the putative origin of the Delta variant (Indian variant).

Discussion

This paper indicates that a common pattern appears to occur in relation to $PM_{2.5}$ levels in most of the locations where SARS-CoV-2 variants emerged. Approximately 50 days prior to the emergence of the ten most persistent SARS-CoV-2 variants, a spike/s in the atmospheric levels of the airborne pollutant $PM_{2.5}$ was noted for most of the variants that appear to have continued to promulgate human infection. Just before or contemporaneous with the variant surge, another smaller spike/s in $PM_{2.5}$ was also noted for most of the SARS-CoV-2 variants.

Similar to this study, a lag phase in the emergence of influenza infection has been noted following exposure to particulate matter $PM_{2.5}$. A study in Montana showed that the rate of influenza in winter increased following wildfires in summer. Elevated daily mean $PM_{2.5}$ concentrations during the summer wildfire season positively correlated with increased rates of influenza in the following winter. With every 1 μg/m³ increase in average daily summer $PM_{2.5}$, two analyses indicated a 16% and 22% increase in influenza rates respectively [17,18].

Sources of $PM_{2.5}$

The sources of $PM_{2.5}$ very much depend on the characteristics of the regions' activities. In the industrial regions the carbonaceous sources of $PM_{2.5}$ are coal combustion, vehicles' gasoline and diesel exhaust [19]. A prime example is the elevated levels of $PM_{2.5}$ in China due to the daily combustion of 80,000 tonnes of coal [20]. Wuhan, the site thought to be SARS-CoV-2's origin, is considered the Chinese hub of transportation earning the moniker as the "Chicago of China". Residential and commercial areas have similar sources of $PM_{2.5}$ in the form of gasoline and diesel exhausts and biomass combustion due to cooking and heating. In a trans-seasonal study done in Amsterdam and Helsinki, the sources of indoor and outdoor $PM_{2.5}$ were very similar. In a sizable proportion (41%), the major sources of $PM_{2.5}$ were secondary pollution, whereby primary pollutants interacted with atmospheric molecules. The remaining 59% of $PM_{2.5}$ sources were motor vehicles' exhaust, calcium-rich particles, biomass burning, soil and road dust, and marine aerosols. A similar pattern was noted for median personal, indoor, and outdoor $PM_{2.5}$ concentrations, whereby in Amsterdam the levels of this pollutant were 13.6 μg/m³, 13.6 μg/m³, and 16.5 μg/m³ respectively, while in Helsinki the $PM_{2.5}$ concentrations were 9.2 μg/m³, 9.2 μg/m³, and 11.1 μg/m³. In both Amsterdam and Helsinki, the personal and indoor $PM_{2.5}$ concentrations highly correlated with outdoor concentrations (median R=0.7-0.8) [21].

Specific habitats have their own species of $PM_{2.5}$ depending on the activities carried out in these areas. In a study of schools in Barcelona, on average 47% of indoor $PM_{2.5}$ was due to soil particles (13%) and organic sources (34%) and calcium-rich particles from chalk and building deterioration. The remaining 53% of $PM_{2.5}$ in schools was derived from emissions from outdoor sources including week-day traffic [22]. Significant pollution with particulate matter may also occur emanating from natural causes such as wildfires [23]. Of particular relevance especially in the USA, in regions where there are perennially very low levels of $PM_{2.5}$, this pollutant suddenly shoots up due to wildfires. This aspect is of significance because, whereas most by-products of wildfires are rapidly transformed while suspended in the atmosphere, $PM_{2.5}$ remains unchanged for several days [24].

$PM_{2.5}$ and SARS-CoV-2 related infection and mortality: The relevance of particulate matter $PM_{2.5}$ and PM10 during the pandemic became evident when epidemiological studies demonstrated that a large proportion of sporadic cases of COVID-19 could not be explained through direct human to human contact. Epidemiological studies indicated that aerosol and droplet contact could not explain these sporadic cases and the regional differences in the transmission of SARS-CoV-2 [25].

Long-term exposure to $PM_{2.5}$ was associated with increased the SARS-CoV-2 related infection and mortality rates. A study in the USA showed that an increment of just 1 μg/m³ in $PM_{2.5}$ exposure, correlated with an 8% augmentation in the COVID-19 mortality rate [1]. Another study in the USA demonstrated an increase of only 1 μg/m³ in $PM_{2.5}$ exposure was associated with a 13% increase in COVID-19 related mortality rate [26]. In a large Chinese study of 120 cities, an increase of 10 μg/m³ in $PM_{2.5}$ was linked to with a 2.24% (95% CI: 1.02-3.46) rise in the daily new cases of COVID-19 respectively [4]. Another study in China showed that a 10 μg/m³ increment in atmospheric $PM_{2.5}$ increased the SARS-CoV-2 case-fatality ratio by 0.24% (0.01%-0.48%) [27]. In the Lombardy region of Italy, $PM_{2.5}$ levels as high as 38.31 μg/m³ have been recorded (WHO acceptable upper limit 25 μg/m³). Atmospheric concentrations of $PM_{2.5}$ in Northern Italy correlated with SARS-CoV-2 infection incidence (R=0.67, p<0.0001), the COVID-19 related death rate (R=0.65, p<0.0001) and the case-fatality rate (R=0.7, p<0.0001). An international study from 63 countries over five continents similarly demonstrated a connection between $PM_{2.5}$ and COVID-19 cases. This study showed that a 10 μg/m³ increase of $PM_{2.5}$ pollution level was associated with 8.1% (95% CI 5.4%-10.5%) increase in the number of COVID-19 cases during the 14 day period of assessment [28].

$PM_{2.5}$ and the emergence of the G614 variant in China: The G614 variant was the first prominent variant that appears to have originated in China early in 2020. Prior to the emergence of the G614 variant in Beijing, the average $PM_{2.5}$ level during its peak was 153.4 μg/m³ (SD+/-63.9) and later settled to 94.4 μg/m³ (SD+/-47.8) (p<0.001) when the G614 variant surge occurred (Figure 1).
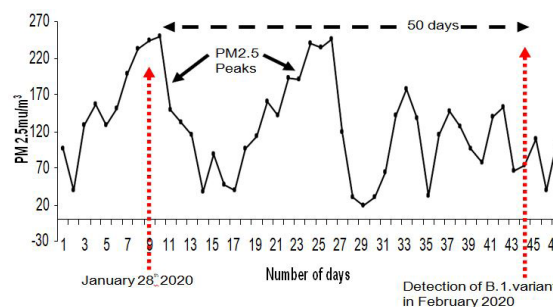

Figure 1. Emergence of G614 variant in Beijing after atmospheric $PM_{2.5}$ spikes in the mid-January 2020.

In Beijing the downward trend of the number of daily new cases mirrored that of the $PM_{2.5}$ levels, however it must be mentioned that the number of new cases available were at latter part of the bell-shaped curve (Figure 2).
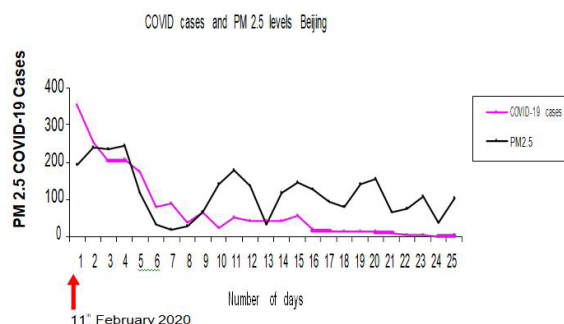

Figure 2. Emergence of G614 variant and new cases of SARS-CoV-2 in Beijing after atmospheric $PM_{2.5}$ spikes in the mid-Febuary 2020.

Interestingly the $PM_{2.5}$ levels in Wuhan preceded those of Beijing by a month. This may suggest that the G614 mutation may have occurred in Wuhan with the elevated $PM_{2.5}$ levels having "primed" the population as an ideal reservoir. The population in Wuhan may have acted as the ideal reservoir due the presence of a high viral load of the progenitor Wuhan 1, with a $PM_{2.5}$ weakened pulmonary immunity, a $PM_{2.5}$-induced increase in the

respiratory viral point of entry (ACE receptor) in the presence of a multitude of people crammed together in constrained $PM_{2.5}$ replete places such as train platforms or restricted spaces indoors during the Chunyun Spring Festival [29]. The G614 variant may have replaced the original Wuhan 1 during the mass movements of the Chinese population attending the Chunyun Spring festival which is held 15 days before the New Year and lasts a total of 40 days. The Chunyun festival is the largest mass movement occurring on the globe, involving nearly 400,000,000 individuals and over 2.9 billion journeys are made during the internal migration. A study between January 6th and February 6th, 2020 indicated that the diagnosis of COVID-19 was more likely within 11-12 days after people moved from Wuhan to 16 nearby cities in the Hubei Province. Following the spike in COVID-19 diagnoses, the number of cases declined after the implementation of cities' lockdown [30]. This mass movement of the Chinese population during Chunyun Spring festival overlapped with the $PM_{2.5}$ peaks in Beijing noted in this study. Thereafter the incidence of COVID-19 in China dropped dramatically possibly due to strict social distancing, face protection and a possible innate immunity of the Chinese populations towards the G614 variant [31].

The G614 variant which dominated and persisted throughout the SARS-CoV-2 genomic variant landscape involved a mutation whereby an amino acid alteration in the viral spike protein gene occurred at D614G position. This mutation involved a single genomic modification with the replacement of aspartic acid by glycine at the amino acid G614 position of the spike protein. *In vitro*, the G614 mutation has demonstrated increased cellular infectivity; however this does not seem to be consistently the case *in vivo* [14]. Genomic alteration at the G614 position appears to have led to a modification in the phenotypic configuration and functionality of the spike protein peptide. The original Wuhan 1 coronavirus had its spike protein's three peptides aligned in a "closed" configuration whereas the G614 variant's spike protein peptides are consistently found in an "open" orientation.

SARS-CoV-2 adherence to the respiratory angiotensin II receptors (ACE) receptors on the pneumocyte II and goblet cells depends on spike protein's trimeric peptide configuration. The spike protein adheres to respiratory cells' ACE receptors if at least two of its three peptides are positioned in an "open orientation" [32]. This may also be the case impacting SARS-CoV-2 adherence to particulate matter if the latter acts as its vector. G614 variant's different spike protein tripeptide orientation may determine significant changes to the functionality of the receptor binding domain. Molecular dynamic computer simulations have demonstrated a variety of components, all of which may singly or in concert determine the adherence potential of the receptor binding domain. These simulations demonstrated a complex network of salt bridges, hydrophobic sites, hydrogen bonding and electrostatic interactions between the receptor-binding domain of the SARS-CoV-2 Spike protein and the angiotensin II receptor [33].

$PM_{2.5}$ levels and the emergence of the 20A.EU1 variant in Spain: Three spikes in $PM_{2.5}$ in Valencia were noted before the appearance of the 20A.EU1 variant. The $PM_{2.5}$ spikes' averaged 61.3 µg/m³ (SD+/-21.8) to decrease to a mean of 41.2 µg/m³ (SD+/-15.5) (p<0.04) when the 20A.EU1 variant emerged (Figures 3 and 4).
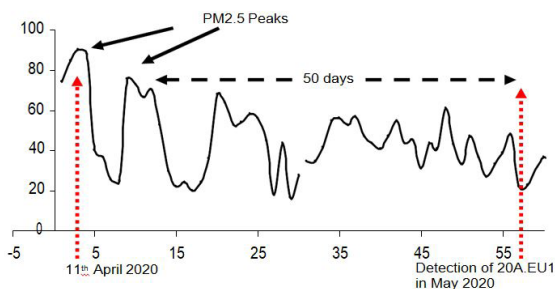
Figure 3. Emergence of 20A.EU1 variant in valencia spain after atmospheric $PM_{2.5}$ spikes in the mid-April 2020.
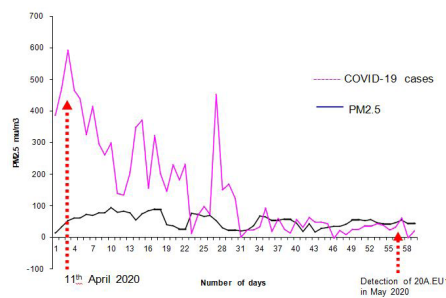
Figure 4. Emergence of 20A.EU1 Variant and New Cases of COVID-19 in valencia Spain after atmospheric $PM_{2.5}$ spikes in the mid-April 2020.

Similar to the pattern in China, the trend of the number of new cases of COVID- 19 mirrored that of the $PM_{2.5}$ levels in Valencia. These spikes in $PM_{2.5}$ overlapped with the population load in Spain following the tourist influx after travel restrictions were relaxed during May-June 2020. A research group in Basle detected a persistent mutant designated as 20A.EU1 of SARS-CoV-2 which has spread specifically in the European Continent [34]. The initial stages of the variant appear to have originated in the North East region of Spain. Two outbreaks of infection with this variant were detected in farmers coming from the provinces of Aragon and Catalonia in late June 2020. The 20A.EU1 variant may have originally infected the Aragonese and Catalan farmers who possibly transmitted the virus to the mink population in the North Eastern regions of Spain. Similar to humans, where population density is a risk factor for high COVID-19 infection rates, the mass confinement of minks led to widespread infection of the caged animals. This undoubtedly led to a high Ro factor in the confined animal population due to the exponential infection rate presenting fertile ground for the inception of variants [35]. High reproduction rates are a prerequisite for the occurrence of mutations which eventually may flourish adapting to natural selective pressures [36].

Spain following Italy was one of the first European countries to have succumbed to the COVID-19 pandemic. The first Italian residents noted to have contracted SARS- CoV-2 infection were in a small town near to Milan on the 21st of February [37]. The suggestion is that a super spreader event occurred when a well-attended (50,000) football match between the Spanish team of Valencia and the Italian team of Atalanta was played in the stadium of Bergamo on the 19th February 2020. Unknowingly early in February, Bergamo was already the focus for seeding COVID-19 throughout the Lombardy region of Northern Italy. Following Italy, not unexpectedly, coronavirus made its appearance in Valencia and soon after the rest of Spain was plunged into lockdown as the pandemic engulfed the whole nation leading to high mortality rates in the elderly and vulnerable individuals [38].

Following high mortality rates, lockdown was enforced in mid-March in both Italy and Spain [39]. Social distancing succeeded in reducing the Ro in May, encouraging diminution of restrictions in June. Spain reopened its borders to most European tourists on the June 21 a week later than most of the European Union member states. With travel restrictions relaxed, tourists in their thousands including those from the UK, crowded on to the Spanish coastal resorts. The holiday mood may have also relaxed the restriction on social distancing and any semblance of face protection of sun-seeking tourists. Consequently the 20A.EU1 variant spread throughout the Spanish peninsula and later the European Continent in particular the UK. Due the paucity of transatlantic travel, the 20A.EU1 variant was not detected in the Americas.

$PM_{2.5}$ and the emergence of the B.1.1.7 variant in the UK: Following the 20A.EU1 variant spread throughout the Spanish peninsula, Hodcroft et al. indicated that this variant spread throughout Europe [32]. The peak of new cases of the 20A.EU1 variant was achieved in Spain in mid-August when this variant had just been detected in Kent UK. Subsequently one month later in mid-

September this variant increased exponentially in the South East of England, to soon become the predominant variant in the UK. The B.1.1.7 variant and the 20A.EU1 variant appear to evolve from the 20A progenitor clade. Alternatively, possibly under the influence of an environmental factor, (possibly $PM_{2.5}$) the 20A.EU1 variant may have reversed its mutation to the progenitor which went on the produce the B.1.1.7 variant. Two peaks of $PM_{2.5}$ were noted prior to the emergence of the B.1.1.7 variant (Figure 5).
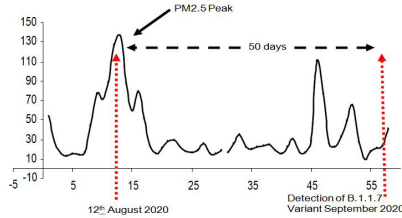
Fligure 5. Emergence of B1.1.7 variant in Bexely UK after spikes of atmospheric $PM_{2.5}$.

In mid-August the first spike in $PM_{2.5}$ was noted in Kent UK., 50 days before the emergence of the B.1.1.7 variant. A second peak in $PM_{2.5}$ occurred just before the detection of the B.1.1.7 variant. The $PM_{2.5}$ peaks in the South East of England averaged 82 µg/m³ (SD+/-29) before the detection of the B.1.1.7 and later decreased to a mean of 27.8 µg/m³ (SD+/-18) (p<0.03). This rise in $PM_{2.5}$ possibly coincided with increased exposure to motor vehicle exhaust, as the Kent area is a busy thoroughfare during this period. Elevated levels as high as 77.2 µg/m³ in upper stone street in Maidstone has been detected in central Kent (Friends of the Earth).

The number of new cases of the 20A.EU1 variant in Kent coincided with the elevated levels of $PM_{2.5}$ in this region (Figure 6).
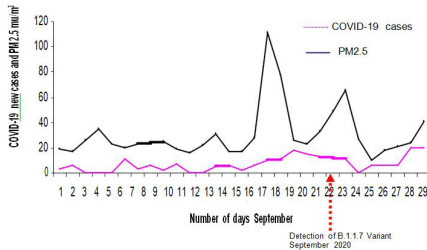
Fligure 6. Emergence of B1.1.7 variant and new cases of COVID-19 in Bexely UK after spikes of atmospheric $PM_{2.5}$ in September 2020.

Having gained a foothold in September, the B.1.1.7 variant continued to slowly rise throughout October, until it exponentially increased in mid-November emerging as the dominant variant in the U.K. B.1.1.7 appears to be resistant to monoclonal antibody neutralization to the N-terminal domain of spike protein and moderately resistant a few monoclonal antibodies to the receptor-binding domain [40].

$PM_{2.5}$ and the emergence of the B.1.351 variant in South Africa: The B.1.351 variant was first detected in Nelson Mandela Bay, South Africa in early October 2020. The $PM_{2.5}$ mean level in Nelson Mandela Bay during this period was reported as 40.4 µg/m³ (SD+/-14) while approximately 60 days prior to this variant's emergence, a $PM_{2.5}$ spike averaging 85.1 µg/m³ (SD+/17.3) (p<0.0001) was noted (Figure 7).
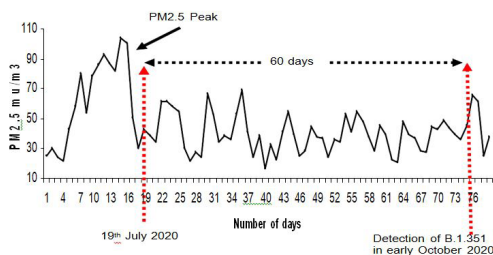
Fligure 7. Emergence of B1.351 variants in South Africa after spikes of atmospheric PM2.5.

The East Cape of South Africa has been hit by a 6 year drought. In July 2020 the water reservoir in Nelson Mandela Bay was down to 18% of its capacity due to the paucity of rainfall. Severe drought has a significant impact on atmospheric pollution and acts as one of largest causes of airborne $PM_{2.5}$. $PM_{2.5}$ aerosol concentration in the Owens Lake area in California's southwest increases abruptly from less than 5 µg/m³ to 25 µg/m³ during the drought period [41].

The B.1.351 is considered a variant of concern as it possesses the E484K mutation. The E484K mutation appears to confer some resistance to antibodies against the SARS-CoV-2 spike protein. The B.1.351 variant appears not only refractory to neutralization by most N-terminal domain monoclonal antibodies but also by multiple individual monoclonal antibodies to the receptor-binding motif on receptor binding domain due to an E484K mutation [42].

$PM_{2.5}$ and the Emergence of 5 Variants in the USA: Five persistent SARS-CoV-2 variants were detected in the USA. Early in May 2021 the USA variants B.1.429 and B.1.427 in Los Angeles were considered viruses of concern (VOC) while the B.1.526 and B.1.525 variants found in the Washington Heights were considered viruses of interest (VOI) (CDC 2021). Prior to May, the B.1.2 variant in Louisiana and New Mexico and the R.1. Variant in Eastern Kentucky were also detected with the former soon displaced by the B.1.1.7 variant and the latter causing an outbreak in a Nursing home facility (CDC 2021). On the 6th of July the B.1.429 variant was detected in Los Angeles coinciding with a solitary peak in $PM_{2.5}$ (Figures 8 and 9).
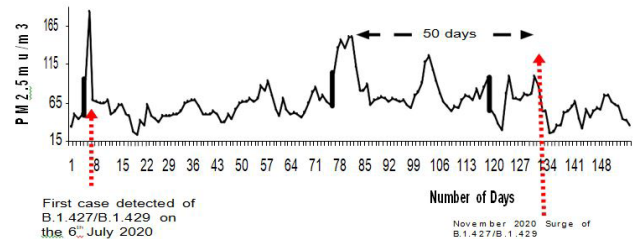
Figure 8. Emergence of B1.429 variants los angeles after spikes of atmospheric $PM_{2.5}$.
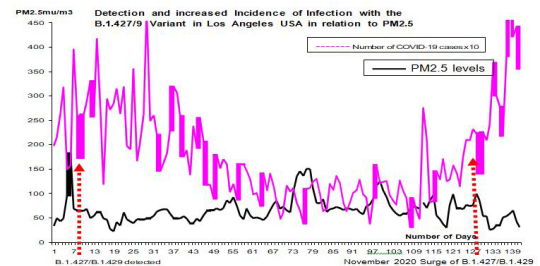
Figure 9. Emergence of B1.429 variants and new cases of COVID-19 los angeles after spikes of atmospheric $PM_{2.5}$.

This peak also coincided with the use of a multitude of fireworks in the Angeles area. In Los Angeles the average $PM_{2.5}$ peak levels prior to the emergence of the SARS-CoV-2 variants was 118(SD+/-28.8)µg/m³ in Los Angeles while its baseline was 66.1(SD+/-25.1)µg/m³. Two peaks of COVID-19 cases in Los Angeles occurred approximately 50 days apart. The first peak in COVID-19 cases occurred following the first spike in $PM_{2.5}$ on the 6th July and the second peak of COVID-19 cases occurred after two spikes in $PM_{2.5}$ in September. Simultaneous with this latter peak in $PM_{2.5}$ there existed an on-going wildfire called the "Bobcat fire" which started on 6th September, and quickly spread after being fanned by irregular strong Santa Ana and Diablo winds. This wildfire was one of the largest in the history of wildfires in the vicinity of Los Angeles and may have contributed to the $PM_{2.5}$ spike noted prior to the emergence of the B.1.429 variant. The persistent drought in South-West USA has further increased the $PM_{2.5}$ levels [43]. A $PM_{2.5}$ spike in the Washington Heights averaging 54.3+/-13.8 µg/m³

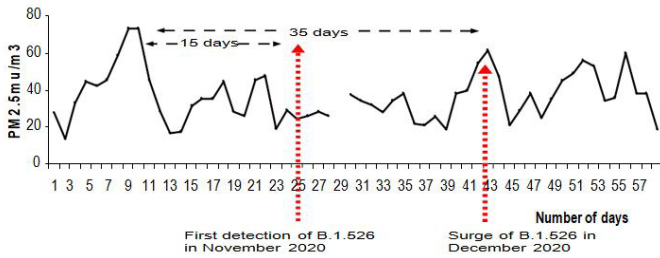(baseline 34.4(SD+/-11.6) was noted prior to the emergence of the B.1.526 variant (Figures 10 and 11).

Figure 10. Emergence of B1.526 variants in washington heights New York after spikes of atmospheric $PM_{2.5}$.
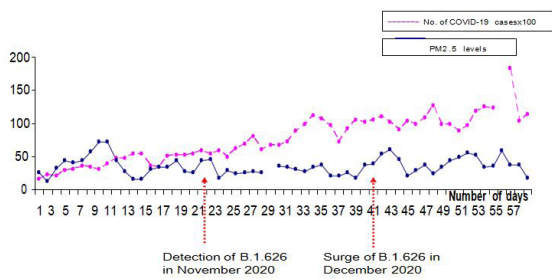

Figure 11. Emergence of B1.526 variants and new cases of COVID-19 in Washington heights, New York after spikes of atmospheric $PM_{2.5}$.

This spike in $PM_{2.5}$ in Washington Heights was noted in early September and from then on the new cases of COVID-19 progressively increased. A further rise in $PM_{2.5}$ was noted in December coinciding with an increase in the positive gradient in COVID-19 new case diagnoses. The main sources of outdoor air pollution in New York are motor vehicles and biomass combustion especially from wildfires. A recent study showed that pollutants from the smoke of wildfires from as far as Canada and the South-East USA caused significant increases in pollution concentrations in Connecticut and New York City. Most of the reactive components from biomass burning are usually chemically transformed in the vicinity of the wildfire; however $PM_{2.5}$ has a longer half-life ranging from a few days to about a week.

Prior to May 2021 the B.1.2 variant in Louisiana and New Mexico was briefly conspicuous only to be soon displaced by the more transmissible B.1.1.7 variant. The $PM_{2.5}$ levels in Louisiana and New Mexico were very similar whereby a peak of $PM_{2.5}$ was noted 45 days before the detection and surge of the B.1.2 variant and another peak just before the variant's surge. The $PM_{2.5}$ levels in Louisiana and New Mexico reported atmospheric $PM_{2.5}$ levels of 75+/-27.8 µg/m³ (baseline 43.3 SD+/-14.4) µg/m³ and 71.4+/-11.3 µg/m³ (baseline 43.6 SD+/-12.4) µg/m³ respectively (Figures 12 and 13).
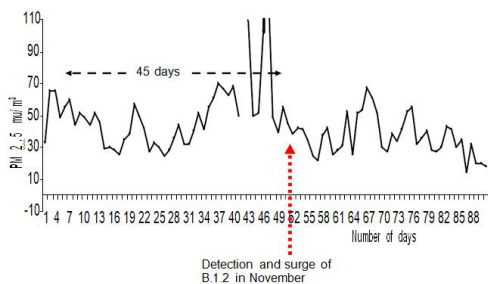

Figure 12. Emergence of B1.526 Variants in Louisiana USA after spikes of atmospheric PM2.5.
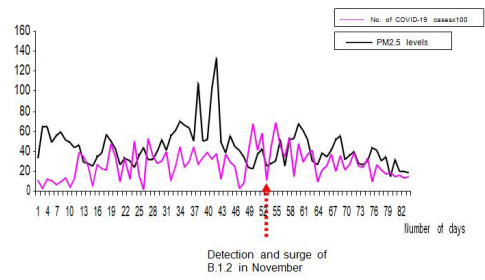

Figure 13. Emergence of B1.2 Variants and New cases of COVID-19 in Louisiana, USA after spikes of atmospheric PM2.5.

The baseline $PM_{2.5}$ following the B.1.1.7 emergence was significantly lower 27.8 µg/m³ (SD+/-18.0) suggesting that this variant acts more favourably at lower levels of $PM_{2.5}$ as opposed to other SARS-CoV-2 variants. An increase of COVID-19 new cases occurred following the $PM_{2.5}$ in Louisiana. In New Mexico a spike of COVID-19 new cases occurred approximately 45 days after the $PM_{2.5}$ spike (Figures 14 and 15).
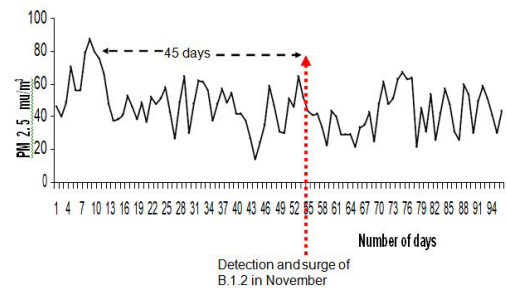
Figure 14. Emergence of B.1.2 variants in New Mexico, USA after spikes of atmospheric PM2.5.
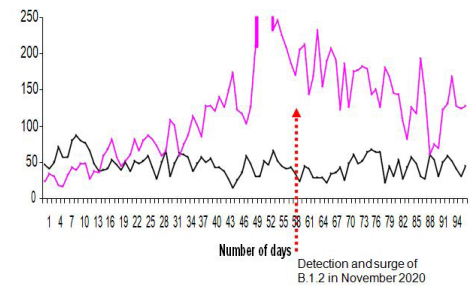
Figure 15. Emergence of B.1.2 variants in New Mexico, USA after spikes of atmospheric PM2.5.

The R1 variant was detected in Eastern Kentucky during an outbreak in a Nursing home facility in mid-March 2021. This too seems to have been preceded by a $PM_{2.5}$ spike recording an average of 37.7+/-7 µg/m³ with a background baseline of 28.5 µg/m³ (SD+/-6.8) (Figure 16).
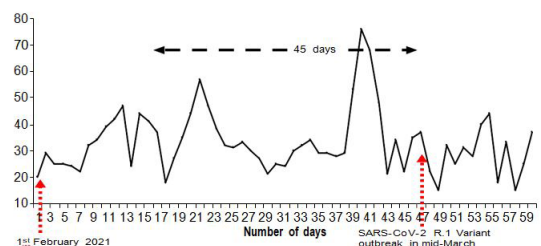
Figure 16. Emergence of R.1.variant in kentucky, USA after spikes of atmospheric PM2.5.

Potential contributors to elevated PM$_{2.5}$ levels and the consequent spread of SARS- CoV-2 in the U.S.A. were a number of holidays encouraging mass gatherings. These included Independence Day 4th of July, Labor Day in early September and Thanks giving day in late November.

PM$_{2.5}$ and the emergence of the B.1.1.248 variant in Brazil: In Sao Paolo Brazil, two peaks of PM$_{2.5}$ were noted prior to the emergence of the B.1.1.248 variant in early December (Figure 17).
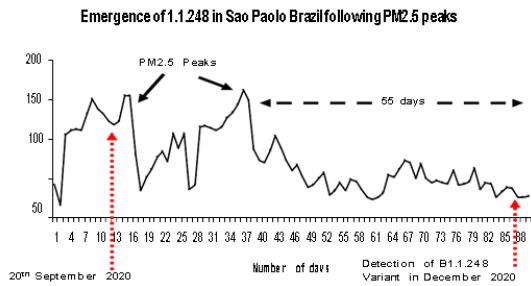
Figure 17. Emergence of B1.1.248 variant in Brazil after spikes of atmospheric PM2.5.

The first peak of PM$_{2.5}$ started in mid-September and another peak in PM$_{2.5}$ occurred in mid-October 2021. The average PM$_{2.5}$ during its peak in Sao Paolo was 107.4 µg/m^3 (SD+/-34.2) before B.1.1.248 variant was detected when the baseline PM$_{2.5}$ was 48.3 µg/m^3 (SD+/-18). The main cause of air pollution in Sao Paolo appears to be vehicular exhaust [16].

This is on the increase due to mass urbanization, increasing the population density which in turn elevates vehicular usage. The elevated population density in itself significantly increases human to human contact encouraging further SARS- CoV-2 transmission. Biomass combustion by the population and wildfires also contribute to the PM$_{2.5}$ atmospheric load.

Wildfires in south-east Brazil produce smoke that aggravates air pollution in major cities such as Sao Paulo. In a study on asthma in children the average dose of PM$_{2.5}$ was 1.95 µg/kg.day (CI: 1.62-2.27) during the dry season and 0.32 µg/kg.day (CI: 0.29-0.34) with the arrival of rain. During the dry season, children and adolescents showed a toxicological risk to PM$_{2.5}$ of 2.07 µg/kg.day (95% CI: 1.85-2 .30) [44].

Depending on the weather, long-range transport of wildfire smoke affects the air quality of sao paolo. Combustion of biomass produces increased quantities of low-lying pollution exacerbated in part, to the South Atlantic subtropical high pressure system.

Transported over considerable distances from wildfires, this pollution further contributes to poor air quality and smog in Sao Paulo [45].

PM$_{2.5}$ and the emergence of the B.1.617.2 variant in India: A broad peak in PM$_{2.5}$ was noted during the first 3 weeks in January in Nagpur India where the B.1.617 variant emerged (Figure 18).
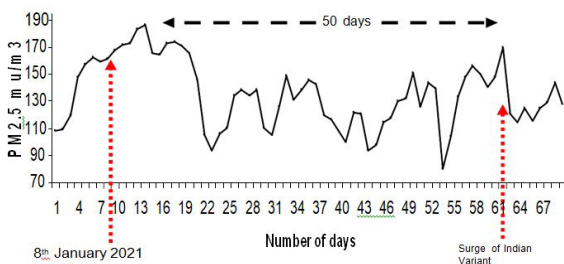

Figure 18. Emergence of B1.617 variant in Nagpur India after spikes of atmospheric PM2.5.

Prior to the emergence of the B.1.617 variant in India, the PM$_{2.5}$ peak in Nagpur averaged 166.8+/-10.8 µg/m^3 in January 2021, with a baseline of 123.2 µg/m^3 (SD+/-16.9). Between the 14th January and the 27th April 2021, an estimated 9.1 million pilgrims attended the religious Hindu pilgrimage and festival called the Kumbh Mela. The peaks in PM$_{2.5}$ coincided with the gatherings throughout the Kumbh Mela Festival however the size of crowds decreased significantly towards the end of April possibly due to the increasing incidence and mortality of COVID-19. The beginnings of the surge of new cases with the Indian variant occurred in Maharashtra state of which Nagpur is a city, at the end of February (Figure 19) [46].
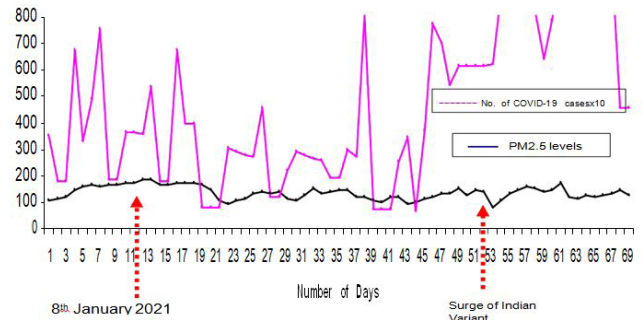

Figure 19. Emergence of B1.617 variant and new cases of COVID-19 in Marahashtra, India after spikes of atmospheric PM2.5 in Nagpur.

In a study by Singh et al. 2021, amongst all cities assessed, Delhi was found to have the highest air pollution, followed by Kolkata, Mumbai, Hyderabad, and Chennai. A common pattern was noted in most of these cities except for Chennai, whereby the highest concentrations of PM$_{2.5}$ occurred in the winter while the lowest levels of this pollutant were noted during the monsoon season. PM$_{2.5}$ levels in the cities exceeded WHO safety cut-off levels for 50% and 33% of days annually except for Chennai. In New Delhi for more than 200 days in a year exceeded the WHO safety cut-off levels. Compared to the previous years a decrease has been noted and can be attributed to the recent policies and regulations implemented in Indian cities attenuating air pollution. PM$_{2.5}$ levels are however still elevated requiring stricter compliance to the Indian National Clean Air Program to further accelerates the reduction of the pollution levels [47].

PM$_{2.5}$ and the angiotensin II converting enzyme receptor (ACE-2): The occurrence of PM$_{2.5}$ peaks a few weeks prior to the emergence of SARS-CoV-2 variants may have made the effected populations more susceptible to COVID-19. The point of entry of the SARS-CoV-2 virus is the Angiotensin II Converting Enzyme Receptor (ACE-2) which is commonly found on type-2 pneumocytes responsible for gaseous exchange. Specifically the ACE-2 protein acts as the receptor for the attachment of the SARS-CoV-2 spike protein, consequently increasing the risk for invasive disease. Preclinical studies utilizing the murine model exposed to particulate matter affected both the ACE-2 and TMPRSS-2 proteins (transmembrane protease serine type 2). Both the ACE-2 and TMPRSS-2 proteins are required for the entry of SARS-CoV-2 into pulmonary host cells. Immuno histochemical assessments indicate that exposure to particulate matter increased the expression of both ACE-2 and TMPRSS-2 proteins. Moreover image cytometry demonstrated increased expression of ACE-2 and TMPRSS-2 proteins specifically in the type-2 pneumocytes which are potential targets for SARS-CoV-2. In another mouse pulmonary model characterized by human ACE-2 receptors, the bronchial instillation of particulate matter augmented the expression of ACE-2 and TMPRSS-2 proteins in the lungs. Furthermore, particulate matter was shown to exacerbate the pulmonary lesions caused by SARS-CoV-2 infection in the murine model [4]. In humans, during the COVID-19 pandemic in Italy, bioinformatic analysis by Borro et al (2020) showed increased DNA sequences encoding for the ACE-2 receptor in response to exposure to PM$_{2.5}$. The bioinformatic analysis of the ACE-2 gene identified nine nucleic acid sequences for the aryl hydrocarbon receptor. In the same study significant correlations were

noted between $PM_{2.5}$ levels and COVID-19 incidence (R=0.67, p<0.0001), the mortality rate (R=0.65, p<0.0001) and the case-fatality rate (R=0.7, p<0.0001) [48]. Increased preponderance of the ACE-2 receptors in the lung was noted following exposure to atmospheric pollution to both nitrous oxide and $PM_{2.5}$. Both nitrous oxide and $PM_{2.5}$ induce an inflammatory change in the respiratory epithelium resulting in both acute and chronic inflammation. The concentration of mucus-producing goblet cells increase in an effort to cleanse the bronchial tree from pollutants and consequently an increase in goblet cells ACE-2 receptors is noted [49].

The above evidence suggests an association between exposure to particulate matter and the increase in the ACE-2 receptor, the viral point of cell entry. The $PM_{2.5}$ peaks may have acted differentially according to the time of variant emergence. The peaks in $PM_{2.5}$ approximately 50 days before the variant emergence demonstrated in this study may have instigated an inflammatory response with a consequent induction in ACE-2 receptors in the respiratory tract [50]. The $PM_{2.5}$ peaks immediately prior to the emergence of the variant may have indicated elevated human to human contact increasing viral load, which in the presence of readily available points of cell entry, increased infection rates. In the presence of high infection rates and a soaring viral load, the possibility of viral mutation became a greater possibility (Figure 20).
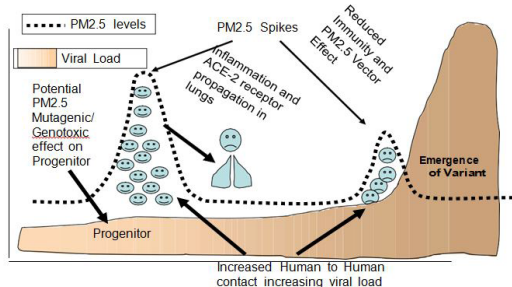

Figure 20. Medium to short-term effects of PM2.5 Prior to emergence of SARS-CoV-2 variants. Schematic view of Atmospheric PM2.5 levels with contemporaneous viral load leading to Emergence of SARS-CoV-2 Variant. First PM2.5 peak associated with anthropogenic effect due to increased human activity and consequent contact. The first peak causes lung inflammation and increased pulmonary ACE-2 and TMPRSS-2 receptor concentrations. There may also be a potential PM2.5 viral mutagenic induced variant emergence, on one hand causing genotoxicity to the progenitor expediting the latter's eventual displacement and favouring the emergence of the more transmissible variant. Second PM2.5 spike also related to diminished population immunity and potential PM2.5 vector effect.

Particulate matter acting as a vector for SARS-CoV-2 transmission: The timeline of the COVID-19 pandemic indicates that it appears to have spread early in 2020, from Wuhan in China, then to Qom in Iran and soon later to the Lombardy region in Northern Italy [51]. An environmental variable common to all these three cities was the presence of elevated atmospheric pollution levels of particulate matter prior to the surge in COVID-19 cases in these regions. Atmospheric particulate matter in the Lombardy region showed that out of 34 RNA extractions for the genes E, N and RdRP coding for SARS-CoV-2, twenty detected one of these genes [52]. Elevated transmissions rates of SARS-CoV-2 infection were evident from outset of the COVID-19 pandemic. At the pandemic's peak, the highest R0 SARS-CoV-2 achieved was estimated at 5.7 [53,54], significantly higher than the R0 2.2 for SARS-CoV-1 [56]. The substantially elevated R0 suggests that besides human to human transmission, other variables including airborne particulate matter may accelerate SARS-CoV-2 spread.

Setti et al. demonstrated that COVID-19 spread in 110 Italian provinces, correlated with atmospheric concentrations of both $PM_{2.5}$ and PM10 [3]. It was suggested that SARS-CoV-2 transmission could be further accelerated by particulate matter carriage beyond the social distance of two metres. In a similar study $PM_{2.5}$ and PM10 atmospheric levels correlated with COVID-19 rates in Italy and reaffirmed the hypothesis that particulate matter may also

act as a vector for SARS-CoV-2 in Figure 20 [8]. In Kuala Lumpur Malaysia, a study showed that the highest concentrations of SARS-CoV-2 RNA on $PM_{2.5}$ in the wards correlated not only with the number of patients with COVID-19 but also with absence of air purifiers. High levels (74 ± 117.1 copies µL^{-1}) of SARS-CoV-2 genes on $PM_{2.5}$ were detected in the single room ward without an air purifier compared to the general ward equipped with an air purifier (10 ± 7.44 copies µL^{-1}).

The association between atmospheric pollution and infectious disease is not a novel one. Measles with an R0 of 18, has the highest R0 of all infectious diseases and has been closely linked with airborne pollution. "Dust events" in the Gansu region (China), have been linked with increased incidence of measles [56]. Greater atmospheric pollution during the dry season in the Niger is associated with a hike in measles-related childhood deaths which subside at the onset of the rainy season [57]. The state of Kansas in 1935 experienced the most severe measles epidemic in American history during the "Dust Bowl" phase [58]. Outbreaks of polio in the USA initiated at the beginning of summers following World War II and declined with the arrival of September rains [59].

Mutational and genotoxic potential of $PM_{2.5}$ on SARS-CoV-2 resulting in viral gain of function: Early in 2020, the SARS-CoV-2 genome was sequenced demostrating that it consisted of a single RNA strand containing approximately 29,000 nucleotide bases. Following this finding, numerous SARS-CoV-2 genomes have been sampled. The vast majority of variants do not survive, however if a mutation confers an adaptation advantage, then through natural selection it will survive to outpace the incumbent [60-62].

Mutational potential of SARS-CoV-2 may occur through selective pressure as an adaptation to the environmental milieu which may possibly be either mutagenic or possibly genotoxic. One such environmental factor may include atmospheric particulate matter which may act as both a vector and mutagen for SARS-CoV-2 [63,64]. Particulate matter may furthermore differentially favour variants by not only acting as a mutagen resulting in variant emergence, but actually be genotoxic to the progenitor virus expediting the latter's disappearance. A recurring set of mutations, namely E484K, N501Y, and K417N may suggest that SARS-CoV-2 is undergoing convergent evolution. This convergent evolution of SARS-CoV-2 may be due to the presence of a common mutagenic catalyst in the form of the omnipresent pollutant $PM_{2.5}$ which has repeatedly been shown to be a consistent co-factor in the SAR-CoV-2 Pandemic [65-67].

Contrasting effects by $PM_{2.5}$ on viral infectivity have been shown possibly due to mutations in viruses and bacteriophages. Viral transmission may be considered a phenotypic reflection of genomic mutation. During the 1968 influenza pandemic, mutations were noted in the influenza virus hemagglutinin serotype H3 molecule and appear to have provided an advantage to the resultant variant to evade antibodies and consequently this gain of function caused disease in previously immune individuals [68].

RNA viruses unlike DNA viruses lack the gene proofreading function of polymerase enzymes. This lack of gene proofreading function may be due to the hypothesized evolutionary precedence of RNA (RNA World), which appears to have emerged well before the inception of DNA [69]. As opposed to DNA, RNA may have both attributes of acting as both a genetic carrier and as an enzyme. RNA viruses have higher mutation rates (10-3 to 10-4 errors per incorporated nucleotide) than DNA viruses' error rate (10-8 to 10-11 errors per incorporated nucleotide). SARS-CoV-2 variants' increased transmissible behaviour may therefore be dictated by these relatively frequent (compared to DNA viruses) RNA viral mutations resulting in gain of function.

Viral mutagenicity and gain of function due to particulate matter $PM_{2.5}$ has been suggested in an experimental study on bacteriophage transmissibility in the presence of this airborne pollutant. A study suggested differential effects on the transmissibility of two bacteriophages Φ6 and ΦX174 in the presence of $PM_{2.5}$. Whereas the aerosol admixture of $PM_{2.5}$

with ΦX6 reduced this bacteriophage's transmissibility, a diametrically opposite effect was noted on the bacteriophage ΦX174, demonstrating superior infectivity compared to controls.

Particulate matter concentrations were significantly higher in the earth's atmosphere during the "RNA World", 4 billion years ago (Haldean Eon). The Haldean Eon was a time in earth's evolution whereby elevated volcanic activity contributed particulate matter in the presence of dense water vapour to the earth's hydrosphere in a significant manner, possibly providing the ideal "primordial soup" for the inception of the RNA molecule [70]. Molecular cooperation in the right environment may have presented the ideal conditions for an autocatalytic feedback mechanism encouraging RNA replication [71]. There are other hypotheses delineating the appropriate ambience resulting in the origins of RNA and life itself. The currently sustained elevated levels of airborne particulate matter may be mimicking the atmosphere earlier in the earth's history providing the appropriate conditions for RNA virus transmission and evolution.

Adverse effects of particulate matter on pulmonary immunity: Particulate matter has a negative influence on affects pulmonary immunity at all levels of its defences. This is particularly evident with $PM_{2.5}$ as its micrometre diameters facilitate its passage through narrow bronchioles eventually depositing in the alveoli. The function of the mucociliary system is impaired by particulate matter disturbing its cleansing capabilities of the respiratory tract. Similar to tobacco smoking, respiratory tract exposure to particulate matter causes mucociliary paresis and promotes increased goblet cell mucus [72]. Particulate matter renders the respiratory epithelium more permeable at the cell junctions and cell membranes. Epithelial permeability to viral invasion is further encouraged by inducing particulate matter-induced pro inflammatory mediators which weaken the baso-lateral aspect of pulmonary host cells and diminishing the concentration of tight junction [73,74].

Particulate matter may instigate uncontrolled viral dissemination by causing macrophage dysfunction. Inefficient clearance of viral phagocytosis may result due to deficient Human leucocyte antigen recognition, diminished cytokine production and dysfunction of toll-like receptor genes expressed on cell membranes [75]. Exposure to particulate matter may influence the ability of Natural Killer cells to eliminate cells infected by viruses [76,77]. Particulate matter $PM_{2.5}$ has been linked to increased levels of antiangiogenic and proinflammatory cytokines [78-80].

Particulate matter exposure effects CD4+ and CD8+ T cells leading to the diminution of interleukin IL-2 and interferon 1-γ production [58]. In severe cases of COVID-19 infection due to resultant significant loss of CD4+ and CD8+ T cells early in the infection, adaptive immune responses are delayed, resulting in prolonged viral clearance [81,82].

Increased infectiousness and antibody escape due to sars-cov-2 variants: a mutagenic role of Particulate Matter? Evidence is emerging that SARS-CoV-2 variants are more transmissible and are able to evade antibody neutralization by previous infection and vaccines. *In vitro* assessments of serum samples from patients who recovered from COVID-19 or received a vaccine could not completely neutralize new variants such as the South African B1.351 variant which possesses the E484K mutation [83]. The N105Y mutation, is also associated to weakened antibody activity against the SARS-CoV-2 [84]. Two other mutations, the K417N and K417T mutations, act in concert with the combination of the E484K and the N105Y mutations to accelerate the lethality of SAR-CoV-2 [47]. Viral transmissibility and antibody evasion are the main characteristics of increased infectiousness. More comprehensive studies have shown that most predominant mutations strengthen the binding between the spike protein's receptor binding domain and the ACE-2 receptor. More recently found mutations of the receptor binding domain, such as the N439K, S477N, S477R, and N501T, further cement viral RBD and ACE-2 binding, increasing infectiousness [85]. Genetic and protein-protein binding analysis elucidating that antibody evasion may be due to a number of mutations in the spike protein's receptor binding domain. Besides the E484K and the N501Y mutation involved in

Alpha, Beta, and Gamma variants, many more variants have been found to weaken the binding between the receptor binding domain and neutralizing antibodies. Further disruption of receptor binding domain linkage with neutralizing antibodies was noted with the mutations L452R and E484Q found in the Delta variants. The L452R mutation is found in the California variant B.1.427. Receptor binding domain mutations may pose a threat to vaccine escape, diminishing the efficacy the current vaccines available. A list of most likely vaccine escape mutations include S494P, Q493L, K417N, F490S, F486L, R403K, E484K, L452R, K417T, F490L, E484Q, and A475S. The mutation T478K appears to make the B.1.1.222 in Mexico the most infectious variant and this may account for the high case-fatality ratio noted in this country (John Hopkins Resource Centre). Wang et al. suggested that the genetic evolution of the SARS-CoV-2 receptor binding domain may result from the combination of four factors. These factors include host cell gene editing, viral genome proof reading and random genetic drift, with natural selection over-arching all these three factors [40]. It would be interesting to explore whether atmospheric pollution including the presence of airborne particulate matter in elevated levels, as noted in the peaks of this pollutant preceding the variants' emergence, may actually catalyze the former three factors, introducing the environmental element of natural selection. The effect of particulate matter on increasing the pulmonary concentrations of the ACE-2 and TMPRSS-2 receptor has already been confirmed. In concert with this pollutant's effect, a chain reaction may result to influence both host cell gene editing and viral proof reading and instigate the catalyzed genetic drift.

A way of circumventing immune escape towards the spike protein may involve a vaccine targeting critical relatively "immutable" regions of the viral spike protein. A new vaccine targets nonhuman-like antigen components found in spike protein, which possesses a unique distributed charge including the presence of a charged furin-like cleavage site [86,87].

Limitations of the study: The average daily $PM_{2.5}$ levels are taken in this study and it must be mentioned that there may be wide variations between the maximum and minimum levels of particulate matter throughout the day including when there may be human exposure to this pollutant. Exposure to atmospheric outdoor $PM_{2.5}$ was carried out in this review, does not necessarily equate that to humans being similarly exposed to the same levels indoors. A collation of five studies demonstrated that indoor SARS-CoV-2 transmission was very high compared to outdoors (18.7 times; 95% confidence interval, 6.0-57.9) studies compared to outdoor spread (<10%). Although this study utilized outdoor $PM_{2.5}$, the study by mentioned earlier, demonstrated that indoor $PM_{2.5}$ concentrations highly correlated with outdoor concentrations (median R=0.7-0.8) [19]. In some countries, such as in China, indoor levels may actually be higher if fossil fuels are used for heating purposes. In 2020, natural gas consumption for heating purposes in Wuhan increased 2.8 times fossil fuel utilization by the traditional stove and water heater, potentially producing another indoor source for particulate matter [88].

There was a significant difference in new case counts obtained between different states included in this review. This suggests the strong possibility of variation in the frequency and availability of diagnostic testing for SARS-CoV-2. From the Beijing data available only the latter part of the bell-shaped curve for the new case counts could be assessed. These COVID-19 new cases in both Wuhan and Beijing were preceded by spike in $PM_{2.5}$. In this study not all the states assessed had daily COVID-19 new case counts available.

The detection and emergence of variants has its drawbacks especially from the temporal aspect. There is a great variation in the availability and access to RNA sequencing processes. The USA (less than 1% of global COVID-19 sequencing) has the greatest capacity for sequencing but the absence of a single Health System as in the U.K. (9% of global sequencing) hampers the co-ordination of sampling and its availability for sequencing. There is also a significant cost to sequencing procedures [89]. A model has been proposed that within the SARS-CoV-2 perspective, 5% sampling of all

positive tests in a population would allow the detection of emerging variants at a prevalence between 0.1% to 1.0%. Attenuating the risk of vaccine escape and the prevention of future coronavirus pandemics very much depends on the availability and easy access to genomic surveillance [90].

Conclusion

There appears to be a link between atmospheric $PM_{2.5}$ and the emergence of SARS- CoV-2 variants. In most regions assessed, two clustersof $PM_{2.5}$ were noted prior to the emergence of SARS-CoV-2 variants. These spikes in $PM_{2.5}$ spike may suggest that the combination of a number of factors including, a). Anthropogenic activity increasing the viral burden, b). $PM_{2.5}$-induced propagation of the ACE-2 and TMPRSS-2 receptor (the viral point of host cell entry), c). Potential $PM_{2.5}$-induced viral mutagenesis resulting in variant emergence and genotoxicity to the progenitor, d). $PM_{2.5}$ toxicity diminishing host pulmonary immunity and e). Possible $PM_{2.5}$ vector effect, increased the prospect of the emergence SARS-CoV-2 variant. The above findings suggest that significant changes in $PM_{2.5}$ levels may not only contribute to transmission, but also to the evolution of SARS-CoV-2.

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