# ASSESSMENT OF IMPACT OF SULPHUR OXIDE AND NITROGEN OXIDE ON AIR MICROFLORA AT OCTROI NAKA IN ULHASNAGAR AND MIDC IN DOMBIVALI

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**Abstract:** Sulphur Oxide (SOx) and Nitrogen Oxide (NOx) are well known pollutants responsible for various respiratory disorders in humans such as asthma and bronchitis. Their impact on humans is well documented and it is proven that both have a key role in inflammation of lung epithelial cells. However their impact on microbial air flora is not yet fully understood. Hence in the present study we investigate, two highly polluted areas namely, Octroi Naka area in Ulhasnagar and MIDC area in Dombivali for outdoor airborne microflora (bacteria and fungi).Concentrations of air borne viable bacteria averaged between $3.1x10^5$CFU/m$^3$ and $2.4x\ 10^6$CFU/m$^3$ for MIDC area while $3.1x10^3$ CFU/m$^3$ and $5.95x10^3$ CFU/m$^3$ for Octroi area. Similarly concentrations of air borne fungi averaged between $1.2x10^5$ CFU/m$^3$ and $1.9x10^6$ CFU/m3 for MIDC area and for Octroi area in range of $1.12x10^3$ CFU/m$^3$ and $2.8x10^6$ CFU/m$^3$. Airborne Gram positive bacteria were most abundant, with more than 90% of the measured population at each location. It was observed that when the concentration of NOx and SOx was high, there was a significant reduction in bacterial and fungal count. The reduction of bacterial and fungal load was found to be 1 log to 2 log cycles. This indicates that increase in the NOx and SOx concentration causes reduction in microbial load.

**Keywords:** NOx, SOx, air microflora, air pollution.

**Introduction:** Exposure to aerosol pollution is now an almost inescapable feature of urban living throughout the world, which is associated with a wide range of adverse health effects including contagious, infectious diseases, allergies and cancer [1]. Elevated concentrations of $SO_2$ can be indicated by an odor of 'burning matches' and is associated with human health impacts, including respiratory (breathing) effects, especially asthma. Vegetation, especially lichens, are very sensitive to $SO_2$ at relatively low concentrations [2]. The gas irritates airways and eyes and is known to cause longer-term heart diseases, other cardiovascular ailments, and bronchitis. It also readily causes shortness of breath and coughing amongst asthma sufferers [3]. $SO_2$ is also a major contributor to acid rain, which damages the environment and upsets ecosystems [2]. Nitrogen oxides causes severe respiratory problems, especially in children. When combined with water, it forms nitric acid and other toxic nitrates. $NO_2$ is also a main component in the formation of ozone at the surface level [4]. The gas irritates the lungs and has been known to lower the immune system [1]. It may cause acidification and eutrophication harmful to health (mainly the respiratory system), materials, cultural artifacts, vegetation and crops [5]. Elevated concentrations of $NO_2$ can also affect visibility though creation of a 'reddish brown' haze. However, the effects of NO on vegetation is coming under increasing investigation in Europe, while NOx is also a concern due to the role it plays as a precursor pollutant for PM 2.5 formation and its association with acid deposition [2]. Similarly TSP is associated with aesthetic and environmental impacts such as soiling of materials or smothering of vegetation [6]. It may pose the greatest threat to human health because, for the same mass, they absorb more toxic and carcinogenic compounds than larger particles and penetrate more easily deep into the lungs [7]. The increase in particulate matter has been shown to cause small, reversible decrements in lung function in normal asymptomatic children, and in both adults and children who have some form of pre-existing respiratory condition, particularly asthma. These changes were often accompanied, especially in adults, by increases in symptoms such as chronic bronchitis or cough [8].

Among the microorganisms present in the atmosphere, bacteria usually dominate in number despite their high death rate, due to environmental factors, major being dehydration stress. Bacterial cell wall components, such as endotoxin (most prevalent in gram negative bacteria) and peptidoglycans (most prevalent in gram positive bacteria) are crucial agents with important inflammatory properties that may induce respiratory symptoms. It is important to note that pollution levels and climate play an important role in determining the outdoor air microbial concentrations because the transport of bioaerosol is primarily governed by kinetic factors, while their fate is dependent on their chemical makeup and the atmospheric factors to which they are exposed [9]. Hence, the monitoring of outdoor airborne microorganisms is necessary in the context of evaluating the risk on health and to study its evolution. Most of these studies were carried out on airborne fungi. But up to now there are very few studies available on monitoring of the bacteria and fungi found in the highly polluted atmosphere [10]. The present study aims to investigate the current atmospheric load of airborne bacteria at different locations in Octroi Naka area in Ulhasnagar and MIDC area in Dombivali, where no survey of air borne bacteria has been attempted till now. Also to estimate the influence of atmospheric and seasonal factors on bio-aerosol pollution.

**Materials and Methods:**

**Study area:**

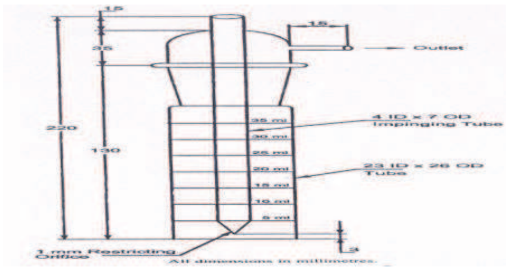
Octroi Naka, Ulhasnagar is a heavy traffic area situated in Thane district of Maharashtra state in India at an altitude of 14m (19.2200° N latitude; 73.1500°E longitude). MIDC area, Dombivali is an industrial area situated at an altitude of 11m (19.1200° N latitude; 73.0600°E longitude) with tropical type of climate with three distinct seasons: winter, summer and monsoon. It represents an urban area surrounded by major industrial and residential activities.

**Sampling:**

**Sampling of Air pollutants:**

Sulpur dioxide and oxides of nitrogen being major air pollutants were selected for the study. For sampling of gaseous pollutant Respirable Dust Sampler (Envirotech) was used along with gaseous attachment [11].

30 ml of respective absorbing solution for Sulphur dioxide and Oxides of nitrogen was added in an impinger tube and sampling was done for four hours at the flow rate of 1 L/min.




**Impinger tube**

**Estimation of $SO_2$:**

For chemical estimation of $SO_2$, an absorbing chemical, potassium tetra chloromercurate (TCM) was used. The air sample was bubbled through the solution containing absorbing medium with the help of gaseous attachment (APM 460N, Envirotech) to RDS. Once the sampling was done, the amount of $SO_2$ was measured by Modified West & Gaeke Method [12]. Sulphur dioxide from air was absorbed in a solution of potassium tetrachloro mercurate (TCM). A dichlorosulphito mercurate complex, which resists oxidation by the oxygen in the air, was formed. The complex was made to react with para-rosaniline dye and formaldehyde to form the intensely colored pararosaniline methylsulphonic acid. The absorbance of this compound was measured by means of a spectrophotometer at 560 nm.

**Estimation of $NO_X$:**

For chemical estimation, the air sample was bubbled through the absorbing medium (containing sodium hydroxide and sodium arsenite) with the help of gaseous attachment (APM 460NL, Envirotech) to RDS. After sampling was done, the amount of NOx was measured by Modified Jacobs & Hochheiser Method (IS 5182 Part 6 Methods for Measurement of Air Pollution: Oxides of nitrogen) [12]. Ambient nitrogen dioxide ($NO_2$) was collected by bubbling air through a solution of sodium hydroxide and sodium arsenite. The concentration of nitrite ion ($NO^{-2}$) produced during sampling was determined colorimetrically by reacting the nitrite ion with phosphoric acid, sulfanilamide, and N-(1-naphthyl)-ethylenediamine di-hydrochloride (NEDA) and measuring the absorbance of the highly coloured azo-dye at 540 nm [13].


Respirable dust Sampler

**Estimation of SPM and RSPM:**

The measurement of RSPM (Respirable suspended particulate matter) and SPM (Suspended particulate matter) was done by cyclonic flow technique and gravimetric analysis with the help of specific filter paper [13].

**Microbiological sampling:**

For sampling respirable dust sampler (Envirotech) was used along with gaseous attachment (APM 460NL).

30 ml of sterile nutrient broth for bacteria and sterile Sabourauds broth for fungi respectively were added in an impinger tube and sampling was done for four hours at the flow rate of 1 L/min during end of rainy season till winter season. Before each sampling, the sampler surface and impinger tubes were disinfected with an ethanol solution.

**Total Microbial Load:**

Total microbial load was checked by serial dilution method. In this method the processed broth was diluted using sterile saline and then the appropriate dilutions were spread on to sterile Nutrient agar and Sabourauds agar plates. Plates incubated for 24 hours and the isolates obtained thereafter were streaked and maintained on to sterile slants till further identification.

**Identification of the Isolates:**

Identification of the isolates was done with the help of morphological and biochemical tests as per Bergey's manual of determinative bacteriology [14].

**Results and Discussion:**

**Table 1** presents the average levels of total culturable bacteria along with results for SOx, NOx, RSPM and SPM results for Octroi Naka location in Ulhasnagar during the study period (August, 2013 to January, 2014). Concentration of airborne bacteria varied in the range of $3.1x10^3$ to $2.4x10^6$ CFU/$m^3$ and for fungi the count was in the range of $1.12x10^3$ to $2.8x10^6$CFU/$m^3$ **(Fig 1)**.Correspondingly the SOx values were in the range of 19 to 65 μg/$m^3$, NOx values 26 to 69μg/$m^3$, RSPM values in the range of 49 to 125μg/$m^3$, and SPM values in the range of 64 to 156μg/$m^3$.It is clear that levels of SOx, NOx, RSPM and SPM have a high impact on microbial load. The reading taken on 9/10/13 shows low levels of pollutants and increased levels of bacterial and fungal counts $2.4x10^6$ and $2.8x10^6$CFU/$m^3$ respectively. The increase was up to two log cycles (**Table 2**). Similarly readings taken on 8/1/14 shows highest levels of pollutants, correspondingly the bacterial and fungal counts have reduced $3.1x10^3$ and $3.2x10^3$ CFU/$m^3$ respectively. This clearly indicates that seasonal variation and level of pollutants have direct impact on microbial load. **Table 3** presents the average levels of total culturable bacteria along with results for SOx, NOx, RSPM and SPM results for MIDC area Dombivali during the study period (October, 2013 to January, 2014). Concentration of airborne bacteria varied in the range of $3.1x10^5$ to $2.4x10^6$ CFU/$m^3$ and for fungi the count was in the range of $1.2x10^5$ to $6x10^6$CFU/$m^3$. **(Fig 2)**. Correspondingly the SOx values were in the range of 18 to 93 μg/$m^3$, NOx values 34 to 87 μg/$m^3$, RSPM values in the range of 59 to 156μg/m, and SPM values in the range of 73 to 190 μg/$m^3$.The reading taken on 19/10/13 shows low levels of pollutants and increased levels of bacterial and fungal counts $2.4x10^6$ and$1.9x10^6$CFU/$m^3$ respectively. The increase was up to one log cycle (**Table 4**). Similarly readings taken on 9/1/14 and 29/1/14 shows high levels of pollutants, correspondingly the bacterial and fungal counts have reduced $3.1x10^5$ and $1.2x10^5$ CFU/$m^3$ respectively. The trend in results obtained for Octroi Naka in Ulhasnagar and MIDC area in Dombivali are same. It is clear that environmental pollutants not only impact humans but they are also important in decreasing microbial counts in air. The concentration of airborne Gram positive bacteria were significantly larger than airborne Gram negative bacteria and is in agreement with other reports. It was previously demonstrated that Gram positive bacteria have greater resistance and survival ability outdoors than Gram negative bacteria under strong sunlight [9]

**Table 1 Observation table for Octroi**

| | Sox µg/m³ | NOx µg/m³ | RSPM µg/m³ | SPM µg/m³ | Nutrient Agar CFU/ m³ | Sabouraud Agar CFU/m³ |
|---|---|---|---|---|---|---|
| 14/08/13 | 31 | 52 | 92 | 117 | $5.95x10^3$ | $1.12x10^3$ |
| 09/10/13 | 19 | 26 | 49 | 64 | $2.4x10^6$ | $2.8x10^6$ |
| 08/01/14 | 65 | 69 | 125 | 156 | $3.1x10^3$ | $3.2x10^3$ |

**Table 2: Microbial load of Octroi Naka, Ulhasnagar**

| Dates | Nutrient Agar CFU/m3 (log 10 ) | Sabouraud Agar CFU/m3 (log 10 ) |
|---|---|---|
| 14/08/2013 | 3.774516966 | 3.049218023 |
| 9/10/2013 | 6.380211242 | 6.447158031 |
| 8/1/2014 | 3.491361694 | 3.505149978 |

**Fig 1: Graphical representation of all the parameters of Octroi station:**

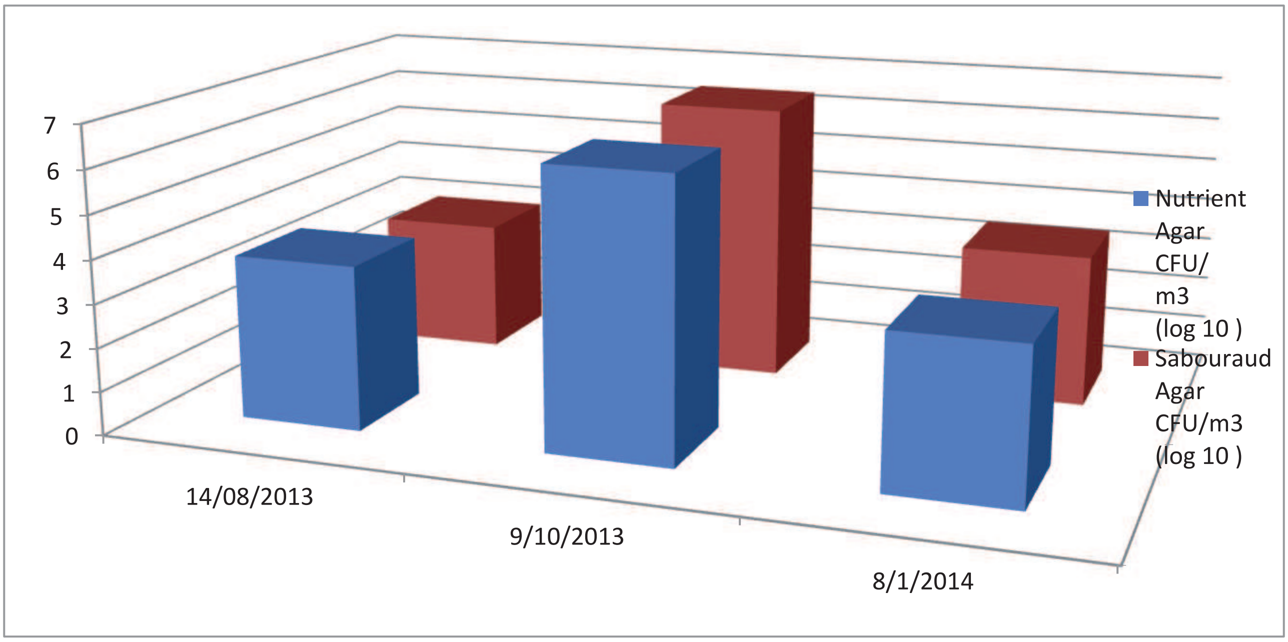


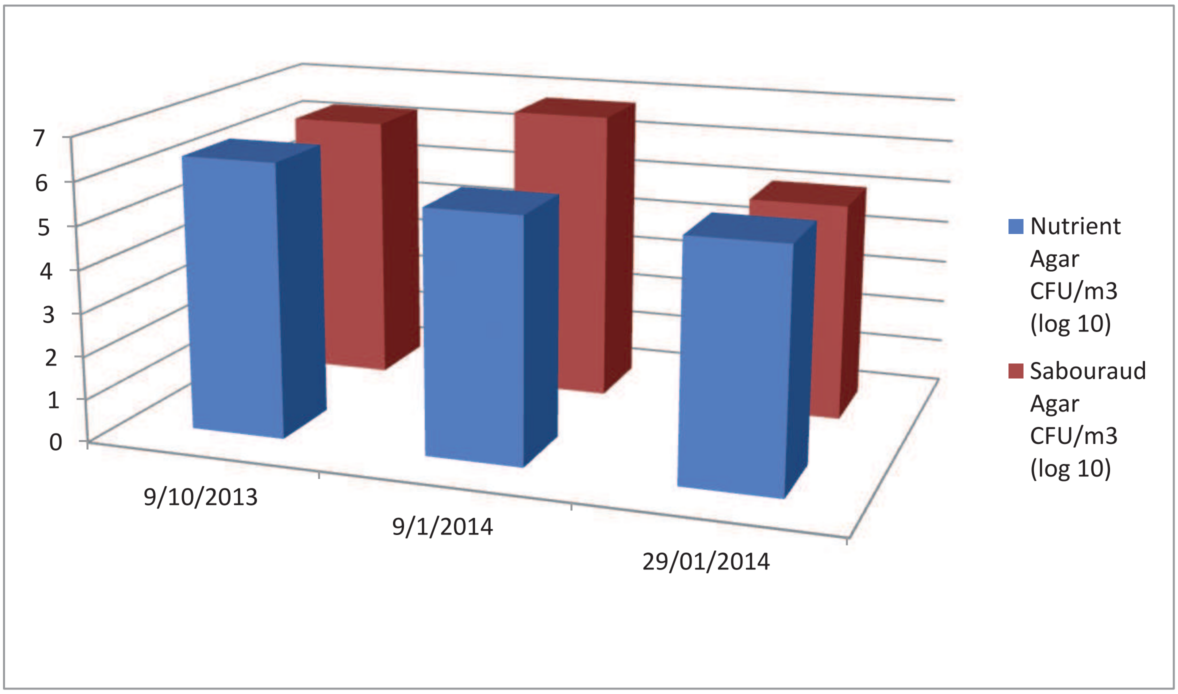
**Table3: Observation table for MIDC area of Dombivali**

| | Sox µg/m³ | NOx µg/m³ | RSPM µg/m³ | SPM µg/m³ | Nutrient Agar CFU/m³ | Sabourauds Agar CFU/m³ |
|---|---|---|---|---|---|---|
| 09/10/13 | 18 | 34 | 59 | 73 | $2.4x10^6$ | $1.9x10^6$ |
| 09/01/14 | 72 | 87 | 156 | 190 | $4.4x10^5$ | $6x10^6$ |
| 29/01/14 | 93 | 51 | 147 | 179 | $3.1x10^5$ | $1.2x10^5$ |

**Table 4 Microbial load of MIDC, Dombivli**

| Date | Nutrient Agar CFU/m3 ($\log_{10}$) | Sabouraud Agar CFU/m3 ($\log_{10}$) |
|---|---|---|
| 9/10/2013 | 6.380211242 | 6.278753601 |
| 9/1/2014 | 5.643452676 | 6.77815125 |
| 29/01/2014 | 5.491361694 | 5.079181246 |

**Figure 2 Graphical representation of microbiological counts at MIDC area of Dombivali**



**Conclusion:**

A study on air levels of bacteria and fungi and the influence of polluting factors on airborne bacteria concentration was carried out at two locations of industrially important towns near Mumbai. Highest bacterial and fungal levels were observed at Octroi naka ($2.4x10^6$ and $2.8x10^6$ CFU/$m^3$) when the level of all pollutants was low. Similar results were also seen in Dombivali ($2.4x10^6$ and $1.9x10^6$ CFU/$m^3$). Almost all organisms were found to be Gram positive spore forming rods, most organisms, obtained belonged to ***Bacillus spp***. It has been observed that, as the concentration of NOx, SOx, RSPM, SPM increases, there is decrease in microbial and fungal count. Seasonal analysis shows that, just after rainy season, due to sedimentation microbial and fungal counts attain lower values. This indicates that pollutants have a strong impact not only humans but also on microbes.

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