IMPACT OF AIR POLLUTION ON THE MICROBIAL CONCENTRATION OF NEW TOWN

A thesis

Submitted in partial fulfilment of the requirements for the award of the degree of
Master of Technology in Environmental Biotechnology

by

AINDRILA PANDA
Environmental Biotechnology

Roll Number: 002130904012
Exam Roll Number: M4EBT23006

SCHOOL OF ENVIRONMENTAL STUDIES

FACULTY OF INTERDISCIPLINARY STUDIES, LAW AND MANAGEMENT (F.I.S.L.M)

JADAVPUR UNIVERSITY

JADAVPUR, KOLKATA

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DECLARATION

I hereby declare that this thesis report titled "**Impact of air pollution on the microbial diversity of New Town**" is prepared and submitted to Jadavpur University, Kolkata in partial fulfilment of the requirements for the partial fulfilment of the continuous assessment of **Master of Technology in Environmental Biotechnology** course of Jadavpur University for the session 2021-2023. It is declared that no part of this thesis report, have been submitted elsewhere for the award of any degree or diploma.

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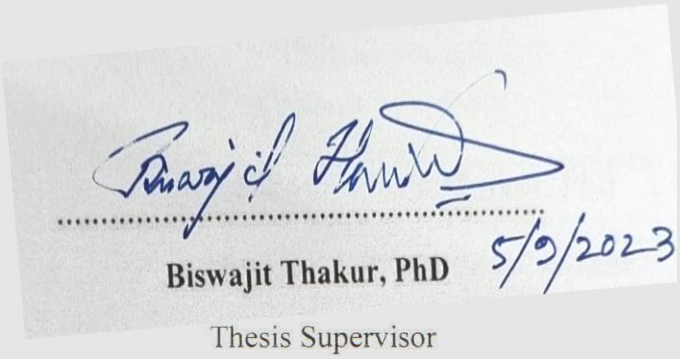
It is hereby certified that this thesis titled "**Impact of Air Pollution on Microbial Concentration of New Town**" has been prepared and submitted for the partial fulfillment of the continuous assessment of Master of Technology in Environmental Biotechnology course of Jadavpur University by Aindrila Panda(Roll No.: 002130904012),a student of said course for the session 2021-2023 under the joint supervision and guidance of the undersigned and Dr.Subarna Bhattacharyya, Assistant Professor, of School of Environmental Studies, Jadavpur University.



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Final examination for evaluation of thesis

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Signature of Examiner

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ABSTRACT

In this study, bioaerosol concentrations are collected from various locations at Newtown, Kolkata. For the first part of the study, correlation between bioaerosol concentrations with respect to the levels of criteria pollutants was observed. It was found that with increase in criteria pollutants, an average decrease in concentration of fungi and bacteria was prevalent at all sampling locations. However, the seasonal variation of these microorganisms was not dependant solely on the levels of criteria pollutants, but also on meteorological factors such as temperature and humidity. The microbial colonies have shown a general trend of sparse growth in the winter months of November and December and have shown relatively more growth in monsoon season. Further, crustal enrichment of the sampling sites were also studied in order to study the impact of heavy metals on microbial concentration. It was observed that, while areas with Ca enrichment promoted fungal growth and Zn promoted bacterial growth in some areas, areas with maximum Cr enrichment showed a marked decrease in the microbial concentrations.

In the second part of the study, one criteria pollutant (SO_2) and UV-C were selected to observed change in enzyme activities on isolated strains of fungi and bacteria in laboratory condition. Activities of enzymes in these microorganisms depend on their intrinsic physicochemical properties and are sensitive to SO_2 and UV-C, hence they are used as indicators of pollution in ambient air. Airborne bacteria and fungi were collected by impaction onto agar plates placed in an Andersen Two Stage Sampler following which four species of bacteria and fungi each were identified and purified based on their uniqueness in the locations. 25ppm of SO_2 and 625 $\mu W/cm^2$ UV-C were applied for 3, 6 & 12 hours after inoculation in two enzyme-substrate broths to study the effect of stress on the production of three enzymes like protease, RNAse and catalase. It was found that greater amount of enzyme was produced on application of SO_2 stress than on application of UV-C. Catalase enzyme, though produced in least amount was found to have the least variation when impact of hourly exposure was studied. Preference towards growth media in case of both fungi and bacteria remained the same in case of SO_2 stress, as that in the non-stress condition while it changed in case of application of UV-C stress. Lastly, while all the four strains of bacteria showed

similar characteristics on application of stress, *Aspergillus fumigatus* was found to be the most resilient under every stressed condition.

Keywords: ambient air pollution, enrichment factor, sulfur dioxide pollution, UV-C pollution, microbial physiology, microbial enzyme

LITERATURE REVIEW

A. Effect of SO_2 on fungi

A study on the effects of SO_2 on fungi, conducted on two strains of Alternaria, *Alternaria alternate* and *Alternaria brassicola* showed a significant decrease in the radial growth of both fungi at varied concentrations for different durations. (Wanp et al., 1997) A similar decrease in colony size has been displayed by *Sporobolomyces spp*. and *Cryotococcus spp*. at low temperatures. (Magan & McLeod, 1988) Wanp et al. suspects that the decrease in colony radii could have been caused due to unfavorable conditions affecting the mycelial growth in particular. This phenomenon could be responsible for the antifungal effect of SO_2 on pathogenic fungi. On the contrary, the disease-causing capacity of *Drechslera sorokiniana, D. teres* and most significantly, *Ascochyta sp.* showed a hike due to SO_2 exposure. (Fehrmann, 1986)

SO_2 affecting the mycelial growth inhibits acid phosphate, inactivating the sulphydial enzyme groups. (Saunders, 1966) This biochemical abnormality triggers the change in spore morphology in fungi. *Alternaria alternata* has shown an increase in the spore length at a lower concentration of exposure with a slight decrease when the concentration was increased. The spore width in both cases showed no significant variation when compared to the control. In the case of *Alternaria brassicola*, a marked decrease in spore width was observed for all duration at higher concentrations. Spore length however has shown a common increase in all cases except for 12 hour exposure at the higher concentrations of 200µg/m^3 which showed a decrease due to higher concentration exposure (Wanp et al., 1997) .

In six recorded powdery mildew fungi, the increased concentration of SO_2 showed inhibition of conidial germination percentage (Khan & Kulshrestha, 1991). The spore germination capacity and length of the germ-tube length in *Verticillium lecanii* and *Cladosporium herbarum* were significantly reduced, even though the former remained unvaried in *Alternaria alternata, Epicoccum nigru,* and *Fusarium culmorum* (Magan & McLeod, 1988). Lower concentrations of SO_2 taken as control, have shown an increase in spore germination of *Alternaria sp*. which decreased with an increase in concentration.

The sporulation of *Cladosporium* was negatively influenced when the concentration of SO_2 exposure increased (Payá Vicens & Suárez Fernández, 1984), unlike *Alternaria alternate* and *Alternaria brassicola* which has shown an increase in the sporulation. Even though, *A. brassicola* showed a slight decrease at higher levels of concentration. (Wanp et al., 1997) In *Botrytis cinerea*, the effect of SO2 was influenced by temperature, with an inhibitory effect on spores and mycelium at a lower concentration when at 20ºC than at 0ºC. (Smilanick &

Henson, 1992), contradicting the statement that the influence of factors such as humidity, temperature, dust, or sulphur dioxide are barely responsible for the behaviour of fungi.(Boyacioglu et al., 2007)

B. Effect of SO_2 on bacteria

The effect of SO_2 emission has shown varied effects in the case of bacteria and their metabolism. (Azoulay-Dupuis et al., 1982) found that female mice infected by *Klebsiella pneumonaie* and exposed to SO_2 for a prolonged period reduced the virulence of bacteria and increased the mortality of mice.

The response of soil bacteria under the effect of atmospheric SO_2 was studied and an increase in pigmented cultures was noted. These cultures adapted to ~~a~~ lower pH and on the application of SO_2 and eliminated the Pseudomonas colonies. Bacteria when grouped on the basis of nutrition and growth parameters showed an overall decrease in concentration with marked decrease of neutrophilic bacteria compared to acidophilic species. (Lettl, 1984)

The effect of industrial exposure of SO_2 has been studied mostly on lactic acid bacteria involved in the process of wine fermentation. Dose-dependent study on bacterial communities showed in increase of *Gluconobacter* below the exposure concentration of 25mg/l. (Bokulich et al., 2015) (Fang & Dalmasso, 1993) studied the impact of sulphur dioxide toxicity on laboratory strains of hetero and homo lactic acid bacteria and found a decrease in the viability of both strains. However, the homo-lactic acid fermentation strains such as *Pediococcus* were more resistant to the effect of sulphur dioxide than the hetero-fermentation strains. SO_2 restricts the growth of acetic acid bacteria during yeast metabolism. It further decreases and delays the cell growth of bacteria such as *Oenococcus oeni*. It also causes a decrease in ATPase activity which might be responsible for the decrease in bacterial viability. (Carreté et al., 2002)

C. Effect of UV-C on fungi

Exposure to UV-C radiation can have various effects on fungi. UV-C radiation has been shown to reduce the mycelial growth of fungi such as Fusarium solani, Macrophomina phaseoli, and Rhizoctonia solani, which are known to cause root rot in sage plants (Braga et al., 2015). UV-C radiation at a wavelength of 222 nm has a potent germicidal effect on vegetative bacterial cells, yeast, and viruses, and is as efficient as 254-nm UV-C radiation (Narita et al., 2020). However, the fungicidal effect of 222-nm UV-C on fungal spores and

hyphae is weaker compared to 254-nm UV-C (Latorre et al., 2012). Some fungal species, such as Cladosporium spp., have shown resistance to UV radiation, which may explain their ubiquity in nature (Sen, 2009). Certain isolates of Metarhizium anisopliae have been found to be resistant to UV radiation, making them potential candidates for commercial use. UV-C treatment resulted in a reduction in fungal growth and aflatoxin content in sesame seeds (Hassan, 2023). The study also found that UV-C LED systems were effective in inactivating fungal conidia on food packaging materials (Belloli et al., 2022). Additionally, UV-C treatment was found to be more efficient in killing spores of Botrytis cinerea in dark and red light conditions compared to white and blue light (P. Zhu et al., 2018). Another study showed that UV-C irradiation could effectively reduce the content of Alternaria mycotoxins in food, with the highest efficiency achieved in methanol and alkaline environments (Logrieco et al., 2009). Furthermore, UV-C treatment was found to eliminate certain fungal spores on the surfaces of fruits and vegetables, reducing the risk of introducing non-native species to the Antarctic environment (Laichmanová & Sedláček, 2019). UV-C exposure has been shown to have an effect on fungal diversity. Studies have demonstrated that UV-C treatment can be used to control fungal proliferation in caves (Pfendler et al., 2019). Additionally, UV-C treatment has been found to reduce the microbial load on the surface of fruits, including mangoes, and modify the structure of bacterial communities (J. Zhu et al., 2021). The inactivation effect of UV light on fungal conidia has also been investigated, with different species showing varying levels of resistance (Hakguder Taze & Unluturk, 2018).

D. Effect of UV-C on bacteria

Studies have demonstrated that UV-C treatment can reduce the microbial load on the surface of fruits, leading to modifications in the structure of bacterial communities (Hakguder Taze & Unluturk, 2018; Vitzilaiou et al., 2021). UV exposure has also been found to induce aggregation in some bacteria, which can impact the UV inactivation kinetics (Fernández-Suárez et al., 2013). Additionally, UV-C treatment of ballast water has been shown to decrease the numbers of viable bacteria and bacterial growth rate, as well as alter the composition of bacterial communities (Petersen et al., 2019). Furthermore, UV-C light has been tested as a method to treat fungal proliferation in caves, resulting in the death of fungal spores and mycelium (Pfendler et al., 2019). Overall, these findings suggest that UV-C exposure can have a significant impact on bacterial diversity in various environments. Studies have shown that UV-C light can reduce the number of bacteria like L. monocytogenes, when

applied for 60 minutes with an intensity of 125 mW/cm2, and when combined with lactic acid, reduces its effect on beef (Brugnini et al., 2021; Tirono, 2023). Furthermore, UV-C exposure has been shown to be an effective disinfectant for a range of bacterial colonies including Bacillus cereus, by 93.9% on various surfaces(Raghuwanshi et al., 2023; She et al., 2020). 222-nm UV-C light has demonstrated a potent germicidal effect on vegetative bacterial cells, bacterial endospores, yeast, and viruses (Narita et al., 2020).

INTRODUCTION

Air pollution is a complex issue that requires intervention from various fields such as science, law, and policy. It is a significant environmental exposure that affects multiple diseases and poses a modifiable health burden. Air pollution consists of various pollutants that can have toxicological impacts on human health, leading to respiratory, neurological, cardiovascular disorders, and other life-threatening diseases. The growth of urban centres, industries, and vehicle populations in recent times has led to serious air quality problems in many countries(You, 2022). The effects of compact urban development on air pollution are complex and depend on pollutant-specific characteristics and emission sources.

Kolkata, one of the oldest cities of India, was founded in 1690 on the eastern bank of the river Hooghly, comprised of the three villages Kalikata, Sutanutee, and Govindpur. Since the end of 19th century, an exponential trend has been observed in the transformation of the city from a rural lifestyle to an urban one. (Antrop, 2004) Population surge due to globalization along with increasing demand for housing led to the necessity of expansion, thus New Town Kolkata (New Town, henceforth) was planned and declared in 1999(HIDCO, 2020). In the case of New Town, the changing land use brought about some ecological changes faced by the people. The conversion of agricultural land into urban land not only brought about economic change but also major environmental changes.

Economic and social development leads to the transformation of an agribusiness-based economy to an industrial urban-based economy. Increase in the levels of industrial SO_2 emissions has been seen in small and medium-sized cities, which shows that urbanization is one of the main driving forces behind emissions, although emissions are more sensitive to industrialization than urbanization, indicating that industrialization remains a key industrial SO_2 pollution contributor (He et al., 2016). Criteria air pollutants are a group of pollutants that are harmful to human health and the environment. They include particulate matter (PM), ozone (O_3), nitrogen dioxide (NO_2), sulphur dioxide (SO_2), carbon monoxide (CO), and lead (Pb). These pollutants are released into the atmosphere from various sources such as emissions from vehicles, industrial processes, and power plants . They can undergo chemical reactions in the atmosphere and be deposited onto the ground, vegetation, and water surfaces. Exposure to criteria air pollutants can lead to a range of health problems including respiratory issues, cardiovascular diseases, and even mental disorders. Additionally, these pollutants can

have negative impacts on soil fertility, crop quality, and marine productivity. To protect human health and the environment, regulatory standards such as the National Ambient Air Quality Standards (NAAQS) have been established to limit the levels of criteria air pollutants in the atmosphere. However, owing to deviation from the said standards has resulted in various changes in the environment, one such, being its impact on the microbial metabolism and concentration.

Total airborne microorganisms when in contact with pollutants; have been seen to increase initially. It however decreases with increasing deterioration of air quality. Maximum microbial load has been observed at moderate pollution. The cumulative effect of meteorological and environmental factors was found to be significant in determining the bioaerosol concentrations.

AIMS AND OBJECTIVES

Air pollution has significant impacts on both the environment and human health. The environment is affected by the release of pollutants into the air, leading to changes in climate, distribution of infectious diseases, and damage to the ecosystems. These pollutants, including ozone, nitrogen dioxide, sulfur dioxide, and particulate matter, are present in the ambient air and can lead to various health issues such as cardiovascular and respiratory diseases. It is crucial to implement strict emission control measures and collaborative efforts to reduce the levels of these pollutants and protect both the environment and human health.

Through the first part of the study we aim at

- Finding a correlation between criteria pollutants and variance in microbial concentrations at sampling sites in New Town
- Studying the variance in microbial load with respect to heavy metal concentrations found using elemental analysis done from road side dust collected from the sampling sites

For the second part of the study, the metabolism of selected strains of fungi and bacteria were studied with the objective of

- Finding the most resilient fungi or bacteria under stressed condition
- Studying the changes in levels of different enzymes produced under stress and non-stress conditions
- Studying the changes in enzyme production levels depending on the growth media of the microbes

CHAPTER 1: SAMPLING LOCATION

1.1. Introduction

Kolkata is one of the oldest cities of India, founded in 1690 on the eastern bank of the river Hooghly, comprised of the three villages Kalikata, Sutanutee, and Govindpur. Until 1793, the city followed a linear pattern of development along the left bank of the Hoogly River and later extended to the right bank after 1793(Roy, 2011). Kolkata's growth and architecture were influenced by England, especially in the areas of politics, economy, society, and culture(Chakraverty & Raychaudhuri, 2000). Over the years, Kolkata underwent rapid formal and functional changes due to migration, urban industrial development, and economic opportunities, leading to environmental degradation. (Banerjee, 2017)

Since the end of 19th century, an exponential trend has been observed in the transformation from a rural lifestyle to an urban one. (Antrop, 2004) Population surge due to globalization along with increasing demand for housing led to the necessity of expansion, thus New Town Kolkata (New Town, henceforth) was planned and declared in 1999(HIDCO, 2020). Even though the initial stages of urbanization cause little to no negative impact on the environment, it has been observed that pollution emissions rise through an area's industrialization and decrease in the post-industrial stage(Peng & Bao, 2006).

In the case of New Town, the changing land use brought about some ecological changes faced by the people. The conversion of agricultural land into urban land not only brought about economic change but also major environmental changes. This includes reduced agriculture and waterbodies. It was found that from 1990 to 2016: 13 km^2 of greenery was lost; 9.3 km^2 of open land was converted to agricultural land and open fields/parks; 1.4 km^2 of aquaculture ponds was converted to tree cover, and 1.45 km^2 of waterbodies were filled up. Due to this change in ecological pattern, the land surface temperature of the area has shown an increase of 0.94ºC (Karmakar, 2022).

1.2. History

New Town Planning Area, constituting of the former Rajarhat Development Authority comprises 45 Mouzas under the South and North 24 Parganas. It was followed by the formation of New Town Kolkata Development Authority in 2007, in order to facilitate provisional civic services. The period from 2007 to 2010 saw a major transformation in the areas of infrastructural facilities over various parts of New Town comprising both residential and commercial spaces. Due to its rapid growth and development, it was considered the fastest-growing planned city of Bengal. In the period from 2011-2016, New Town went on

to become a self-sustainable city with assets like community halls, parks, finance centers, water treatment plants, schools, colleges, market places, generating necessary revenue. (HIDCO, 2020) On being approved by the High Powered Steering Committee, after a thorough evaluation by a Panel of Experts, the plan for New Town Kolkata was provided to the Govt. of India. It was shortlisted by fast track mode K-15016/157/2015-SC-I (Vol. II), on 25/05/2016. (NKGSCCL, 2019)

In 2017, New Town was declared as a Green Smart City under the Smart City Mission which is an urban redesigning program by the Government of India keeping in mind the aim to develop numerous citizen friendly and sustainable smart cities all across the country (NKGSCCL, 2019).

1.3. Geography

Areas of Rajarhat, North 24 Parganas, and Bhangar, South 24 Parganas, came together to form the current area of New Town. For better governance and easier accessibility, the total area is divided into three divisions, Action Area 1, Action Area 2, and Action Area 3. The Government of West Bengal had acquired this area and had started development in a planned manner. The area previously consisted of agricultural land, shallow land, and water bodies before the New Town Development Area Acquisition Act was passed. The goal of this development was to evolve this area as a satellite town and business district adjacent to the metropolis of Kolkata (WBHIDCO, 2012).

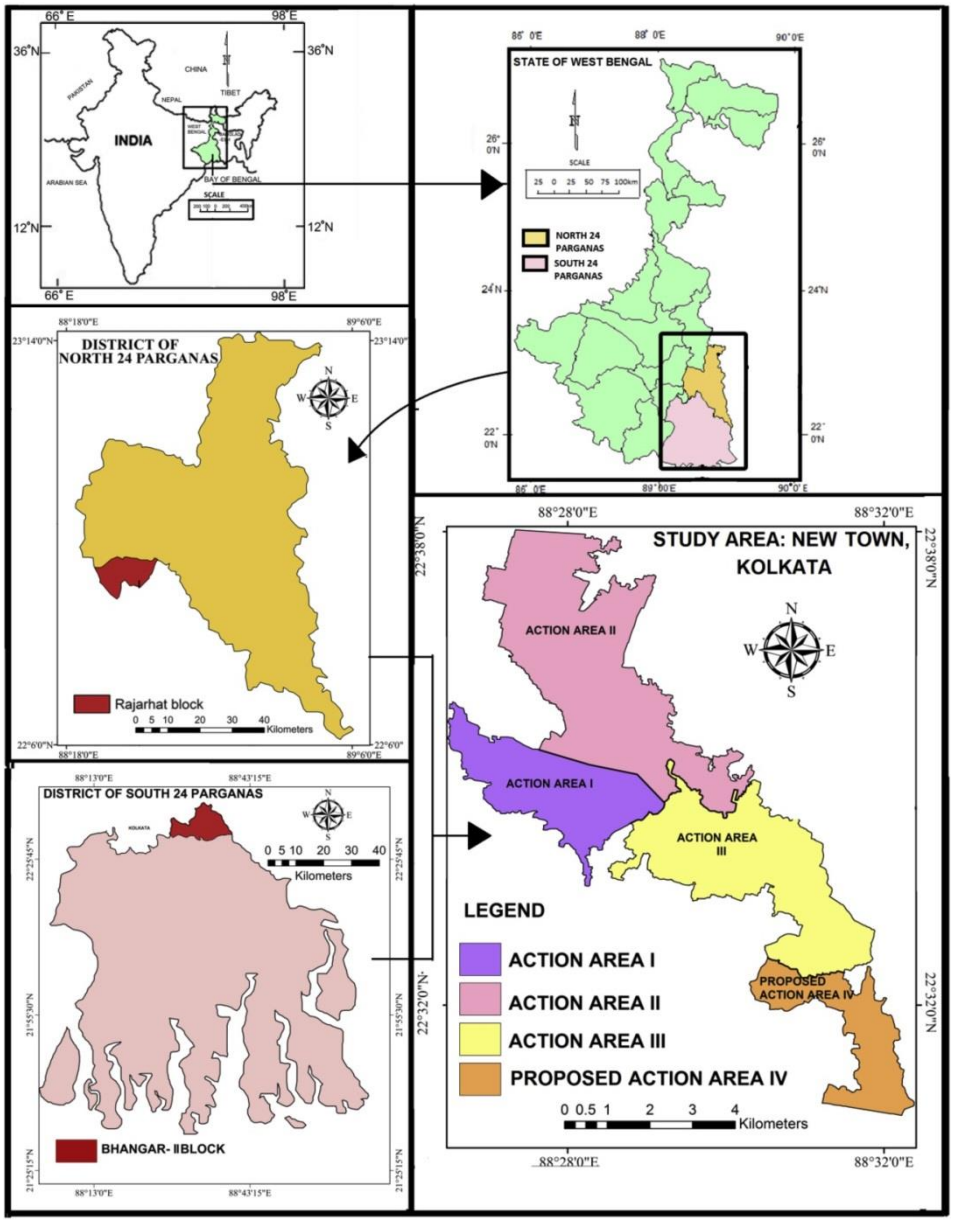


Figure 1: Geographical map of New Town, Source:(Mitra & Banerji, 2018)

1.4. Demography

The carrying capacity of New Town, as per design and planning is 1.5 million comprising of a residential population of 1 million and a floating population of 0.5 million. (WBHIDCO, 2012). Currently, the residential population of the urban space, according to the electoral roll of Rajarhat New Town Assembly constituency, is 0.29 million. (Chief Electoral Officer, West Bengal, 2022). Along with that, the Managing Director of WBHIDCO suggested the average presence of 0.1 million floating population. (Sen, 2022)

The city space including both residential and industrial areas are evolving gradually causing a rise in the residential and floating population. New Town, being a smart city has been

developed in such a way that the distance to work for residential areas has been taken into consideration. The geographical position of New Town, attracts a floating working population from Kolkata, Bidhannagar, Howrah, and various other places. It is expected that with the increase in the level of economic activities, the floating population is expected to show a considerable increase. Gradually, this urban center is developing as not only a hub for service industries (IT & ITES, Fintech, etc.) but also as an education hub and healthcare hub. Besides, New Town offers destinations for amusement and natural tourism (e.g., Eco Park, Aquatica). Hence, New Town attracts a considerably high floating population on weekends and other holidays. Educational institutions and hospitals in this urban space also contribute to the floating population on a regular basis. New Town receives a floating population not only from neighboring cities but also from various other states. The situation of Netaji Subhas Chandra Bose International Airport is close to the area of New Town, Making it easily accessible.

1.5. Sampling Sites

The sampling sites for monitoring of bioaerosols and other pollutants, within New Town, Kolkata, were selected based on the possibility of higher concentration of particulate matter, population density, traffic density, and accessibility of the locations. Total of 12 sampling sites were selected which were representative of the planned urban township. Some locations had deliberately been left out either due to relatively sparse populations or due to the difficulty or threats posed in accessing those locations. The sampling sites are as follows:

- Siddha Galaxia (Residential complex)
- City Center (Shopping mall)
- Eco Urban Village (Picnic spot)
- Mothers Wax Museum (Recreational area)
- Water Treatment Plant
- Rail Vihar Gate 1 (Residential complex)
- Aquatica (Amusement park)
- UEM-Xavier's (University)
- Arts Acre (Arts exhibition center)
- Hatisala (Industrial area)
- Akankha More (Major traffic intersection)

- New Town More (Major traffic intersection)

A map of sampling locations was created using QGIS v3.22.16-Białowieża (QGIS Geographic Information System).

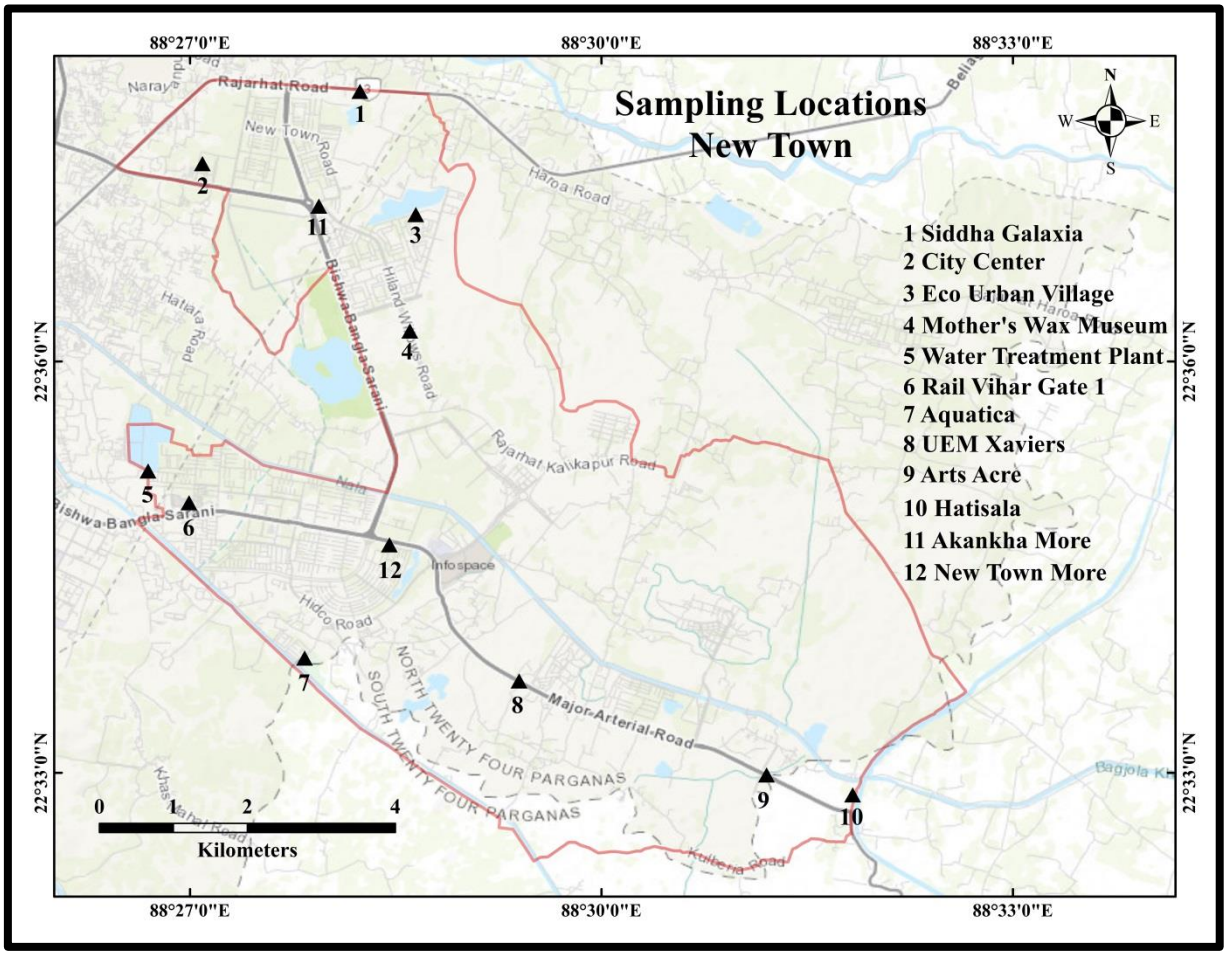


Figure 2: Map of sampling sites at New Town, Kolkata

CHAPTER 2: STATUS OF AIR POLLUTANTS

2.1. Introduction

Economic and social development leads to the transformation of an agribusiness-based economy to an industrial urban-based economy. The introduction of more convenient modes of transportation, the development of metropolitan cities, and more advanced infrastructural communications led to the transformation into urban landscapes (Antrop, 2004). This transformation can be linked with industrialization, modernization, and urbanization all of which have been causes of major concern around the world, each of which has been linked with environmental pollution. It has been seen that the degradation of environmental quality can be directly associated with industrial growth. This environmental degradation, in turn, has a substantial impact on natural systems and affects the environment, thus influencing human activities(Azam & Khan, 2016). Increase in the levels of industrial SO_2 emissions has been seen in small and medium-sized cities, which shows that urbanization is one of the main driving forces behind emissions, although emissions are more sensitive to industrialization than urbanization, indicating that industrialization remains a key industrial SO_2 pollution contributor (He et al., 2016).

At present, climate change owing to air pollution is major threat to the population of rapidly growing cities. Developing nations like India, which are switching from predominantly rural-based populations to urban, have to face critical challenges of sustainable development.

Major indoor and outdoor air pollutants in urban areas can be primary or secondary air pollutants. Primary air pollutants are those which are released directly into the atmosphere includimg particulate matter $PM_{2.5}$, PM_{10}, SO_x, NO_x, CO, ammonia, and dust particles while the secondary air pollutants derived from other sources ozone, smog, Peroxyacyl nitrates (PANs), etc. The usage of low-quality fuel in Indian cities causes SO2 emissions (Zou et al., 2007). Vehicular emissions have been identified as the prevalent source of high NO_2 concentrations (95%) followed by industrial activities and fuel combustion.

Include what are criteria pollutants.

As per the Clean Air Act, EPA sets National Ambient Air Quality Standards (NAAQS) for six common and widespread outdoor ambient air pollutants which poses adverse health and environmental effects, including sulfur dioxide (SO2), nitrogen dioxide (NO2), ozone (O3), particulate matter (PM), carbon monoxide (CO) and lead (Pb). These pollutants are also known as criteria air pollutants(US EPA, 2014). The NAAQS sets the allowable concentrations of these pollutants in ambient (outdoor) air, exceeding these limits poses risk to both human health and the environment.

2.2. Methodology

Status of ambient air pollutants was collected from the manual station of West Bengal Pollution Control Board, at Rajarhat, 24 Parganas (N), which provides location wise air quality data. Prior to January 2023, $PM_{2.5}$ was not measured in these stations.

2.3. Results

Month	SOx Concentration ($\mu g/m^3$)	NOx Concentration ($\mu g/m^3$)	PM_{10} Concentration ($\mu g/m^3$)	$PM_{2.5}$ Concentration ($\mu g/m^3$)
September 2022	3.38±0.02%	44.00±0.056%	69.88±0.12%	-
October 2022	6.44±0.03%	45.89±0.03%	91.22±0.13%	-
November 2022	6.38±0.026%	42.00±0.04%	96.13±0.12%	-
December 2022	8.11±0.03%	44.00±0.04%	111.22±0.16%	-
January 2023	5.33±0.005%	29.44±0.03%	142.33±0.19%	62.88±0.09%
February 2023	5.00±0.005%	27.75±0.008%	100.00±0.18%	45.5±0.08%
March 2023	3.00±0.007%	27.89±0.026%	75.89±0.23%	36.22±0.11%

Table 1: Concentrations of criteria pollutants

As per the National Ambient Air Quality standards, 2019, (*CPCB | Central Pollution Control*

Board, 2019) the permissible limit of SO_2 in the air of residential areas is 50 µg/m^3 , of NO_2 is 40 µg/m^3 , PM_{10} is 60 µg/m^3 , and that of $PM_{2.5}$ is 40 µg/m^3 .

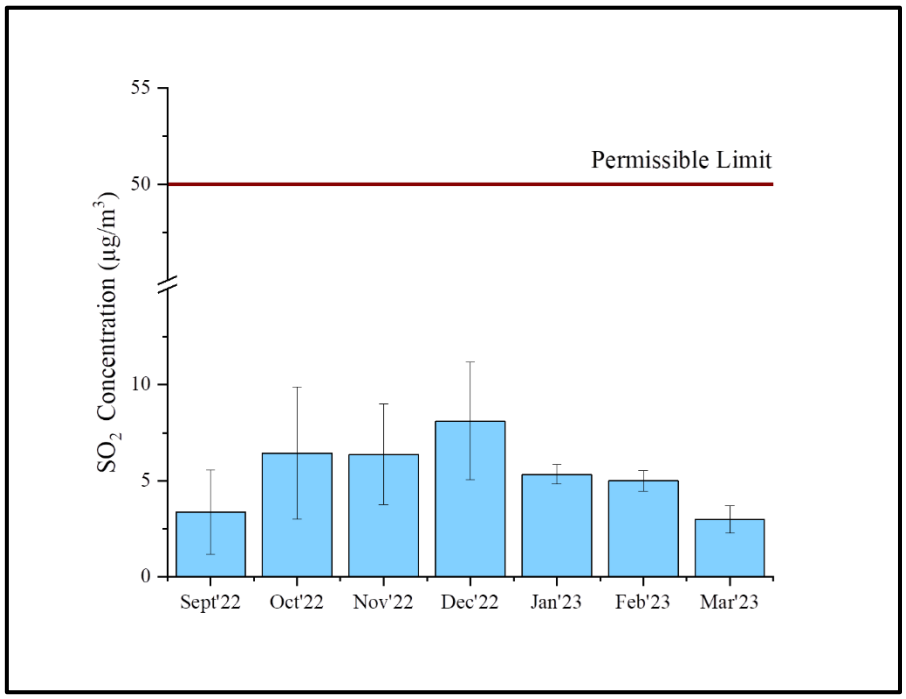


Figure 3: Seasonal variation of SO_2 concentration of New Town

It was observed from the data collected that the SO_2 concentration throughout the months were far lower than the permissible limit indicating that there was no threat of respirable diseases caused by SO_2 alone.

However in higher concentrations of SO2 even in the case of short-term exposures can cause harm to the respiratory system in human, causing difficulty in breathing (US EPA, 2016a).

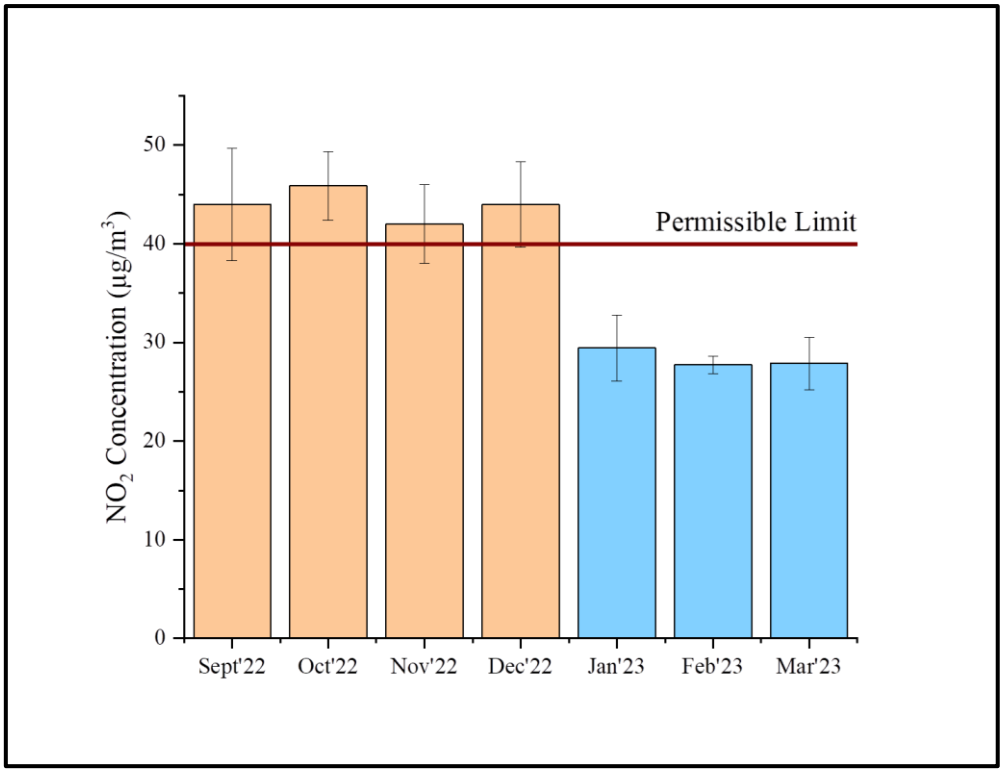


Figure 4: Seasonal variation of NO_2 concentration of New Town

The NO_2 level at the sampling locations was higher than the permissible limit as per NAAQ standards for 57% of the total sampling period. Elevated levels of NO_2 in air could have been as a result of the surrounding vehicular pollution or construction activities. Such high level of NO_2 in air when in contact with the other volatile compounds in air leads to the formation of smog. Clinically, NO_2 irritates the nasopharyngeal region increases susceptibility to respiratory infections (US EPA, 2016b). Additionally, NOx is a potent and selective vasodilator in pulmonary arterial hypertension (Ahmad et al., 2018).

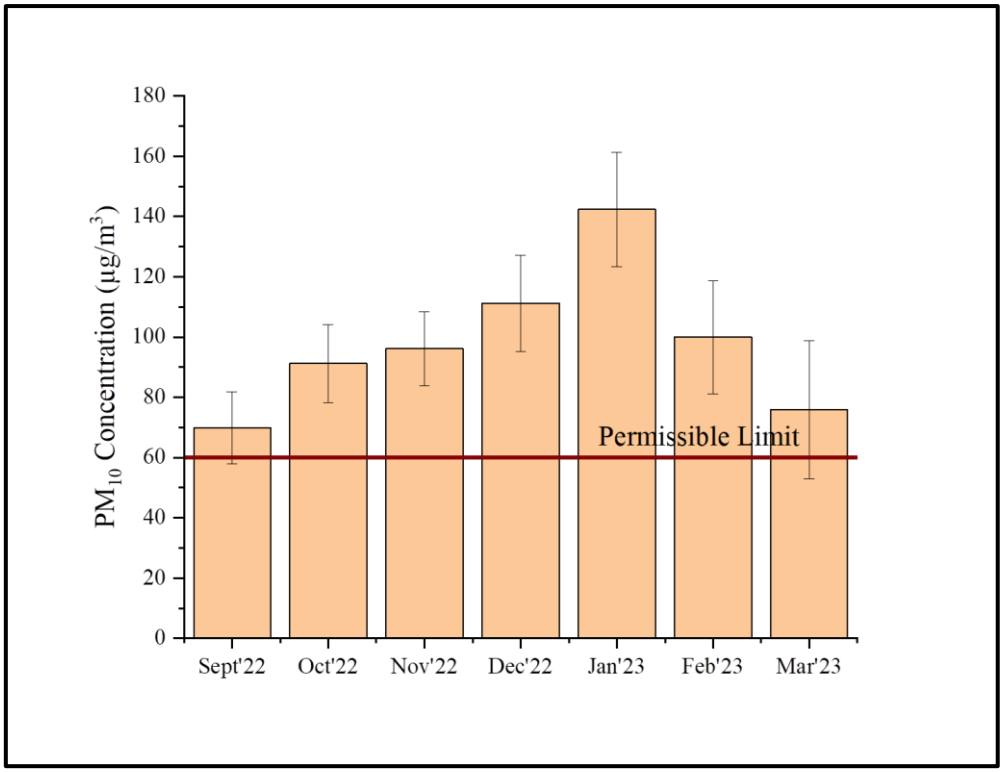


Figure 5: Seasonal variation of PM_{10} concentration of New Town

The concentrations of PM_{10} at the sampling sites have been consistently higher than the permissible limit. PM_{10} is mainly composed of organic and elemental carbon, metals or elements like silicon, magnesium, iron, ions like sulphates, nitrates, ammonium etc. PM_{10} can be generated by physical abrasion processes of crushing or grinding of surfaces. Considering the fact that the sampling location as a whole is still going through major constructional changes, it can be assumed that these construction sites are causing the elevated levels of this pollutant. PM_{10} can deposit in the bronchi and lungs and can pose as a threat for health hazards like respiratory illness, visibility impairment and reduction, aggravate existing heart and lung diseases.

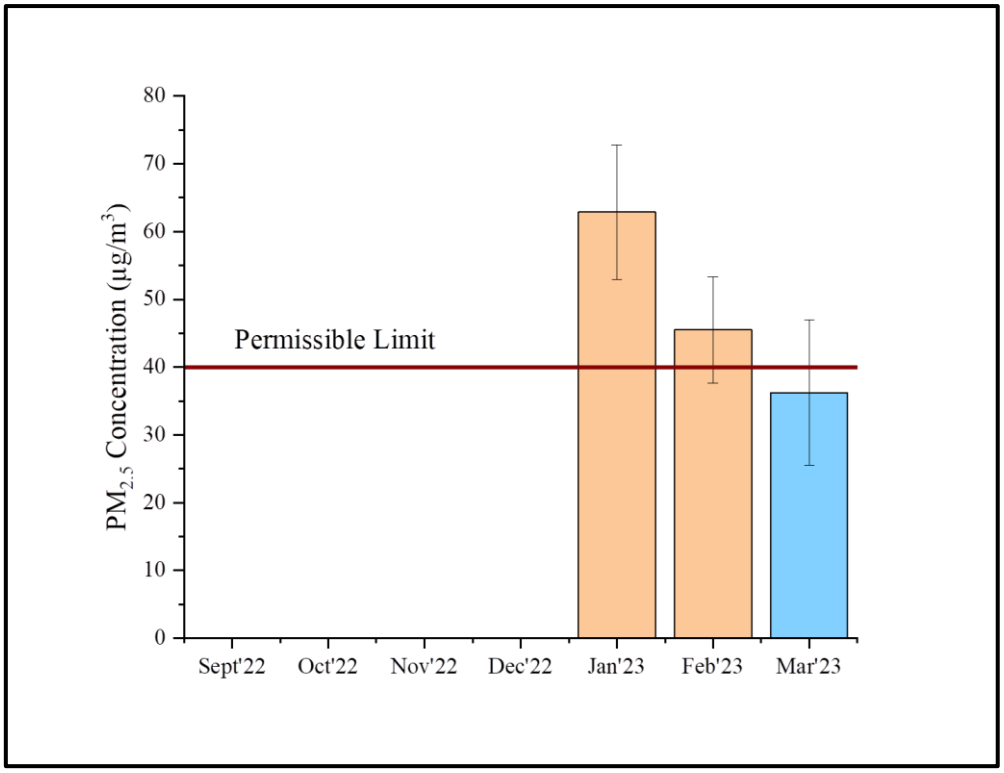


Figure 6: Seasonal variation of $PM_{2.5}$ concentration of New Town

$PM_{2.5}$, are composed mainly of carbonaceous materials, inorganic compounds trace metal compounds. Due to their small diameter, they penetrate into the alveolar regions and into the bloodstream to affect the cardiovascular system. There has been no record of $PM_{2.5}$ measurement at the sampling sites prior to 2023. The data obtained post December 2022 shows that the concentration of $PM_{2.5}$ has crossed the permissible limit 66% times.

2.4. Conclusion

From the data obtained from the West Bengal Pollution Control Board, it was observed that the level of air pollutants, namely SO_2, NO_2, PM_{10} and $PM_{2.5}$ have been above the standard permissible limit in almost 75% times as per the National Ambient Air Quality standards, except for the level of SO_2. PM_{10} level has shown the maximum deviation from the standard limit throughout the sampling tenure. The combined air pollutants has shown an increase from September 2022 and reached its peak in December 2022, there after a decrease in the concentration of air pollutants has been observed. The source of these air pollutants could be the ongoing construction works, coal ovens in the roadside eateries combined with stubble burning in the winter months, leading to the increase in pollutants.

The study was conducted through three seasons, autumn, winter and spring. The pre winter

season shows a gradual increase in the level of pollutants, with a 90.94% increase in SO_2 concentration, 4.29% increase in NO_2 concentration and 30.55% increase in PM_{10} concentration from September to October. The average concentration for SO_2 and PM_{10} shows a further increase of 24.52% and 47.86% respectively, through the winter months of November, December and January. The concentration of NO_2 however decreased drastically in January resulting in a 16.47% decrease in average concentration. However, in the following spring season, the concentration of SO_2 decreased by 40.5%, NO_2 decreased by 24.24% and PM_{10} decreased by 30.63% in the months of February and March.

CHAPTER 3: SEASONAL VARIATION OF BIOAEROSOLS

3.1. Introduction

Bioaerosol is defined as an airborne collection of biological material which is comprised of cells and cellular fragments of bacteria, viruses, fungal spores fungal hyphae, and by-products of microbial metabolism. Pollen grains and other biological materials can also be airborne as bio aerosols. (Stetzenbach, 2009) Even though bioaerosol particles are a small fraction of all aerosol particles in our surroundings, their impact can be critical. They are a means for the transmission of various diseases, can cause allergic reactions, and have effects on the global climate, ecology, and biodiversity. The presence of these bio aerosols has negative effects on respiratory and general health. (Löndahl, 2014)

Studies conducted to observe the concentration of bioaerosols, mainly fungi and bacteria, under various climatic conditions, show seasonal variations depending on a number of factors such as soil moisture, temperature, pH, dissolved organic carbon content and nutrient levels.

Microbial organisms that are dependant on other host plants or animals through commensalism or posses a saprophytic relationship are linked to the life cycle of other organisms of higher order to grow and multiply, this is what, in turn, causes them to be synchronized with seasonal changes and other factors on which the plant life cycle depends, e.g. plant growth and decay, causing a seasonal variation in the microbes. Even the mixing of air masses, in case of extreme atmospheric events, causes biological variability across various areas. (Núñez et al., 2021)`

Bacteria levels exhibited a significantly greater seasonal variation, while fungal levels did not, probably due to lower fungal concentrations occurrence as a result of lower relative humidity values in some regions. Bacterial levels were also observed to be dependent on the number of people present in a room. A general trend has shown a significantly greater concentration of bacteria and fungi in the autumn and post-monsoon seasons, in the countries of South Asia, while minimum concentrations were observed in the winter months of December and January. (P. Kumar et al., 2021; Mentese et al., 2012; Núñez et al., 2021)

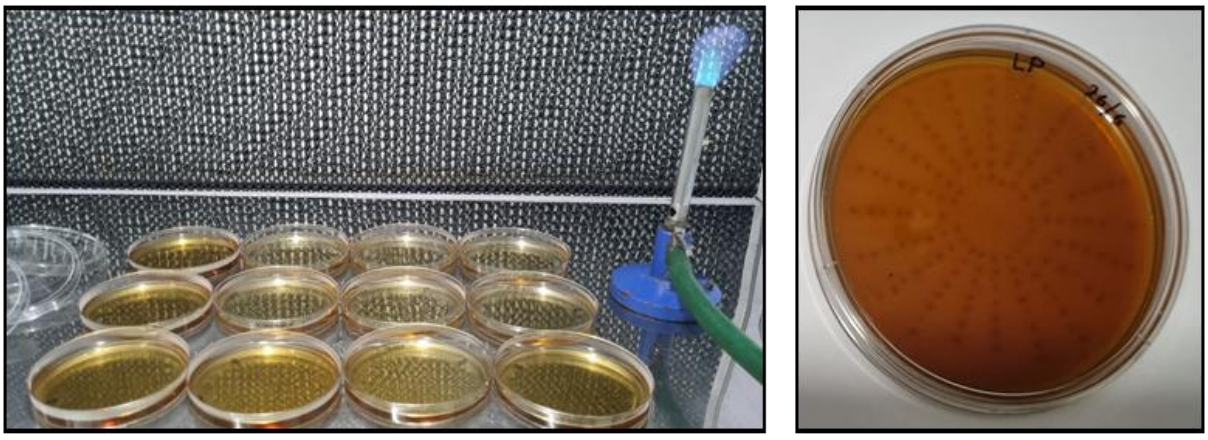
3.2. Apparatus Required

- Andersen’s two-stage cascade impactor
- Tarson disposable petri dish (90mm)

Figure 7: Andersen's two stage cascade impactor

3.3. Sampling

Sampling was conducted at each of the 12 location points for 15 minutes each, using Andersen's two-stage cascade impactor. Air having a flow rate of 28.3 lpm (1CFM) was drawn in through the sampler, and multiple jets of air guided airborne particles to be imprinted onto the surface of Nutrient Agar and Sabouraud Dextrose Agar petri plates. Upon sampling, the fungi and bacteria were allowed to grow under optimum temperatures of 27ºC and 32ºC respectively for 3-4 days till they were ready to be identified morphologically.



(a) (b)

Figure 8: a) Sabouraud Dextrose Agar petri plates,
b) Impression of airborne particles on Nutrient Agar petri plate

3.4. Results

	Fungal Load (cfu/m^3)	Bacterial Load (cfu/m^3)
September 2022	80	180
October 2022	54	168
November 2022	59	143
December 2022	52	107
January 2023	57	134
February 2023	68	133
March 2023	74	158

Table 2: Seasonal variation of microbial load at New Town

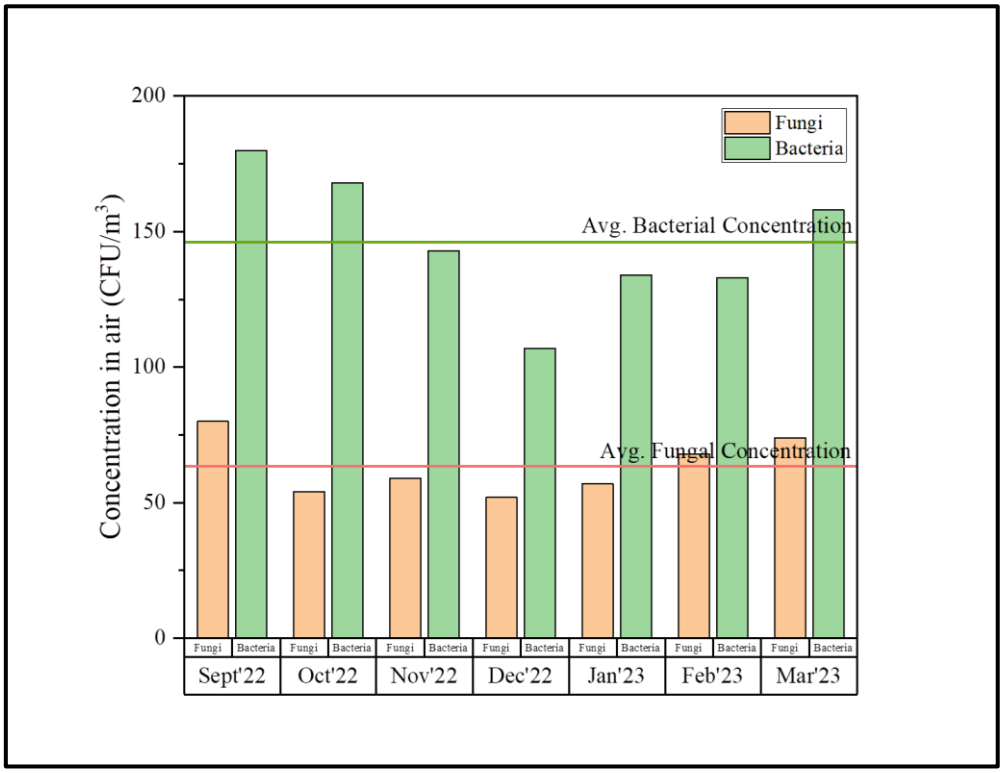


Figure 9: Seasonal variation of Fungi and Bacteria

The overall concentration of bacterial colonies is around 131% more than that of the fungal colonies. The highest concentration of bacterial colony was observed in September 2022, followed by October 2022 and March 2023. Least bacterial colonies were observed during the winter months, mainly December 2022. The overall growth of fungal colonies compared to the bacterial colonies is significantly lower. The colonies of fungi follow a seasonal

variation trend, similar to that of the bacteria with highest concentrations in the months of September followed by March 2023 and February 2023. Least concentration of fungi colony has been observed in December 2022 and October 2022.

3.5. Conclusion

The microbial colonies have shown a general trend of sparse growth in the winter months of November and December and have shown relatively more growth in the post winter or post monsoon season. The diversity of these colonies are dependent on factors like temperature and relative humidity. During the winter months, the temperature goes down up to 15ºC which is much lower than the optimum growth temperature for bacteria and fungi. In the monsoon and post monsoon months of September and October, the temperature varies from 26º-33ºC which is ideal for the growth of both fungi and bacteria. Greater concentrations of bacteria and fungi in the months of September and October could also be caused as a result of greater relative humidity than in the winter months. Fungi colonies has shown relatively lesser variation compared to that of bacteria, hence it can be said that bacterial colonies are impacted more due to the atmospheric changes caused due to seasonal variation, than the colonies of fungi.

CHAPTER 4: CORRELATION BETWEEN BIOAEROSOL DIVERSITY AND CONCENTRATION OF AIR POLLUTANTS

4.1. Introduction:

Studies conducted on bacterial and fungal diversity associated with $PM_{2.5}$ in suburban areas showed an association of the microbial species on seasonal variation and levels of air pollution. It was seen that the species richness and diversity of bacterial communities portrayed a downtrend with the aggravation of air pollution as well as haze levels. It is caused due to the scattering of light by the presence of bioaerosols resulting in a clouded appearance and poor visibility.

The bioaerosols showed an enhancement when exposed to pollutants like SO_2 and NO_2 during morning and evening hours. However, when they were sampled at mid-day, they showed a negative trend indicating that their reaction to air pollutants is influenced by temperature as well. Very low temperatures and events like snowfall have been seen to improve air quality by reducing levels of total airborne microbes.

Total airborne microorganisms when in contact with pollutants, have been seen to increase initially. It however decreases with increasing deterioration of air quality. Maximum microbial load has been observed at moderate pollution. The joint effect of meteorological and environmental factors was found to be an important factor in determining the bioaerosol concentrations.

The characteristics of bioaerosols including their individual characteristics, and when correlated with the environmental factors, have the capacity to impact not only human health, but also influence the dynamics of atmosphere.

4.2. Effect of pollutants on bioaerosols:

The concentrations of airborne microorganisms have been seen to be impacted directly by the concentration of air pollutants as well. When exposed to air pollutants, the concentration of bioaerosols initially increased, on further exposure to pollutants, the concentration was seen to decrease. The highest concentration was seen at a moderate pollution level. It was observed in a study conducted in Beijing where species diversity of bacterial colonies decreased when the pollution levels increased. They also observed an increase in the species richness depending on the seasonal parameter such as temperature and humidity, showing maximum diversity in spring. Various strains of fungi have shown a decrease in the radial growth of the colonies. This could have been caused as a result of mutated mycelial growth. SO_2 affecting the mycelial growth inhibits acid phosphate, inactivating the sulphydial enzyme groups in microbes. This phenomenon is responsible for the antifungal effect of SO_2 on

pathogenic fungi. The spore diameter of fungi has shown an increase in exposure to pollutants; however, the spore germination capacity and length of the germ-tube length were significantly reduced

Bacteria when grouped on the basis of nutrition, growth parameters showed an overall decrease with marked suppression of neutrophilic bacteria compared to acidophilic species when exposed to the pollutants. SO_2 decreases and delays the cell growth of bacteria. It also causes a decrease in ATPase activity which might be responsible for the decrease in bacterial viability.

4.3. Results

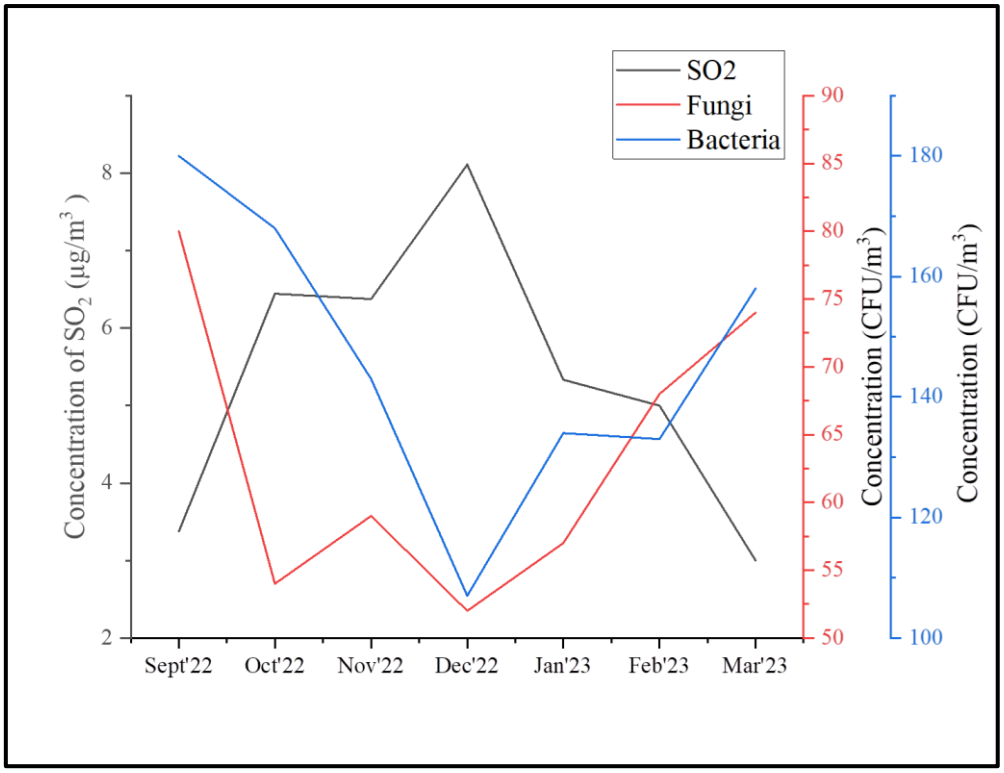


Figure 10: Variation of microbial concentration with respect to SO_2 levels

From Figure 8, we can observe that with the increase in concentration of SO_2, the diversity and concentration of fungi and bacteria both decrease.

	A(X)	B(Y)	C(Y)	D(Y)
Long Name		SO2	Fungi	Bacteria
Units				
Comments	Pearson Correlations	Pearson Correlations	Pearson Correlations	Pearson Correlations
F(x)=				
1	SO2	1	-0.84301	-0.55825
2	Fungi	-0.84301	1	0.60351
3	Bacteria	-0.55825	0.60351	1

Figure 11: Pearson's Correlation between SO_2 and microbial concentration

Pearson's correlation coefficients for the variables were studied and it was found that at $p<0.05$, the concentration of fungi showed a strong negative correlation (-0.84301) while the concentration of bacteria showed a moderately lesser negative correlation (-0.55825). It can thus be observed that the bacterial population has been relatively less impacted by its exposure to SO_2, than the fungal population.

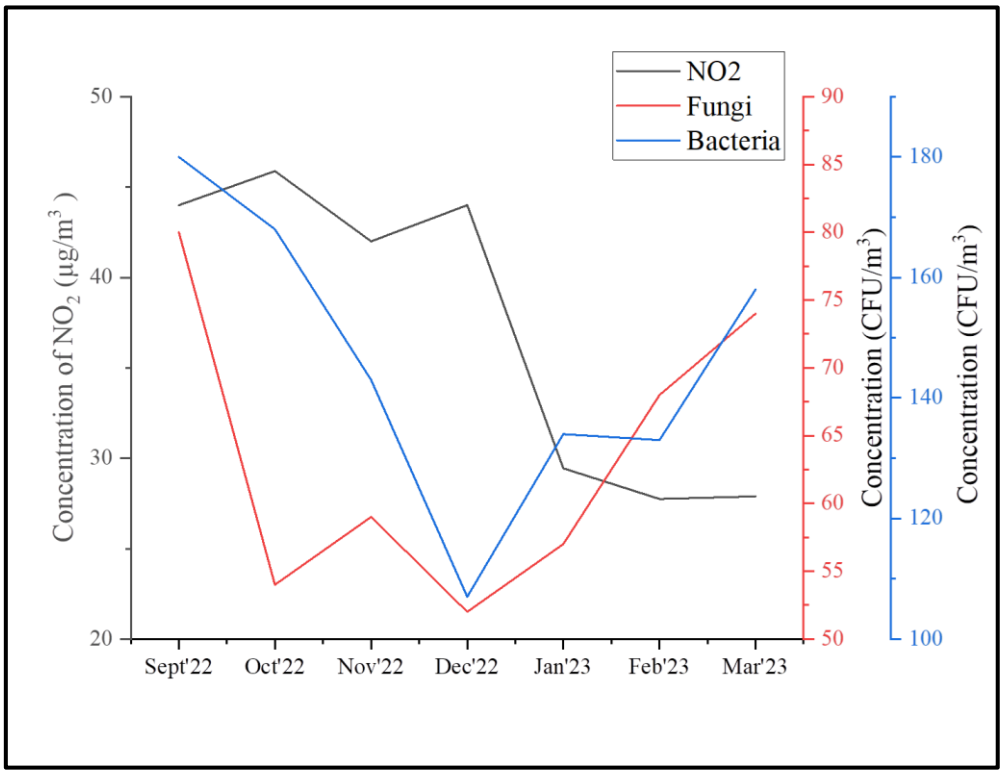


Figure 12: Variation of microbial concentration with respect to NO_2 levels

	A(X)	B(Y)	C(Y)	D(Y)
Long Name		NO2	Fungi	Bacteria
Units				
Comments	Pearson Correlations	Pearson Correlations	Pearson Correlations	Pearson Correlations
F(x)=				
1	NO2	1	-0.30083	0.1287
2	Fungi	-0.30083	1	0.60351
3	Bacteria	0.1287	0.60351	1

Figure 13: Pearson's Correlation between NO_2 and microbial concentration

Pearson's correlation coefficients for the variables were studied and it was found that the concentration of fungi has shown a moderately weak negative correlation (-0.30083) while the concentration of bacteria has shown a very weak positive correlation (0.1287). It can thus be observed that NO_2 impacted the fungal population negatively while it supported the growth of the bacterial population at certain exposure levels.

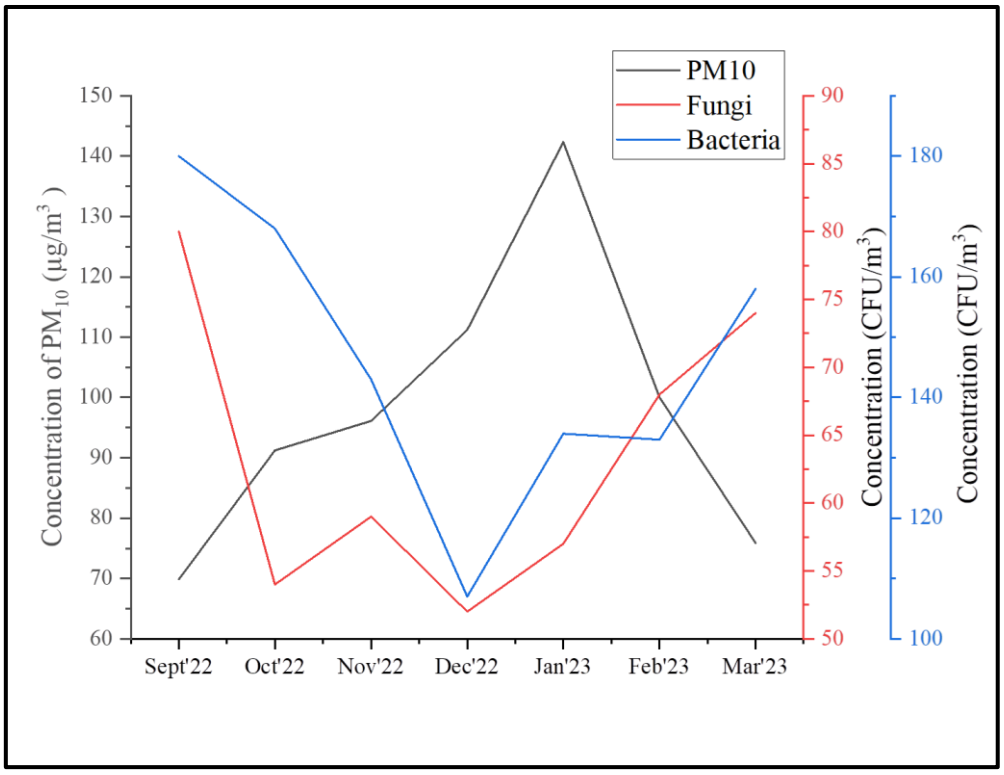


Figure 14: Variation of microbial concentration with respect to PM_{10} levels

	A(X)	B(Y)	C(Y)	D(Y)
Long Name		PM10	Fungi	Bacteria
Units				
Comments	Pearson Correlations	Pearson Correlations	Pearson Correlations	Pearson Correlations
F(x)=				
1	PM10	1	-0.76117	-0.7234
2	Fungi	-0.76117	1	0.60351
3	Bacteria	-0.7234	0.60351	1

Figure 15: Pearson's Correlation between PM_{10} and microbial concentration

Pearson's correlation coefficients for the variables were studied and it was found that the concentration of both fungi and bacteria has shown strong negative correlation to the concentration of PM_{10}. While fungi has shown a negative correlation of -0.76117, bacteria has shown a slightly less correlation of -0.7234. It can thus be observed that PM_{10} hinders the growth of both bacterial and fungal communities.

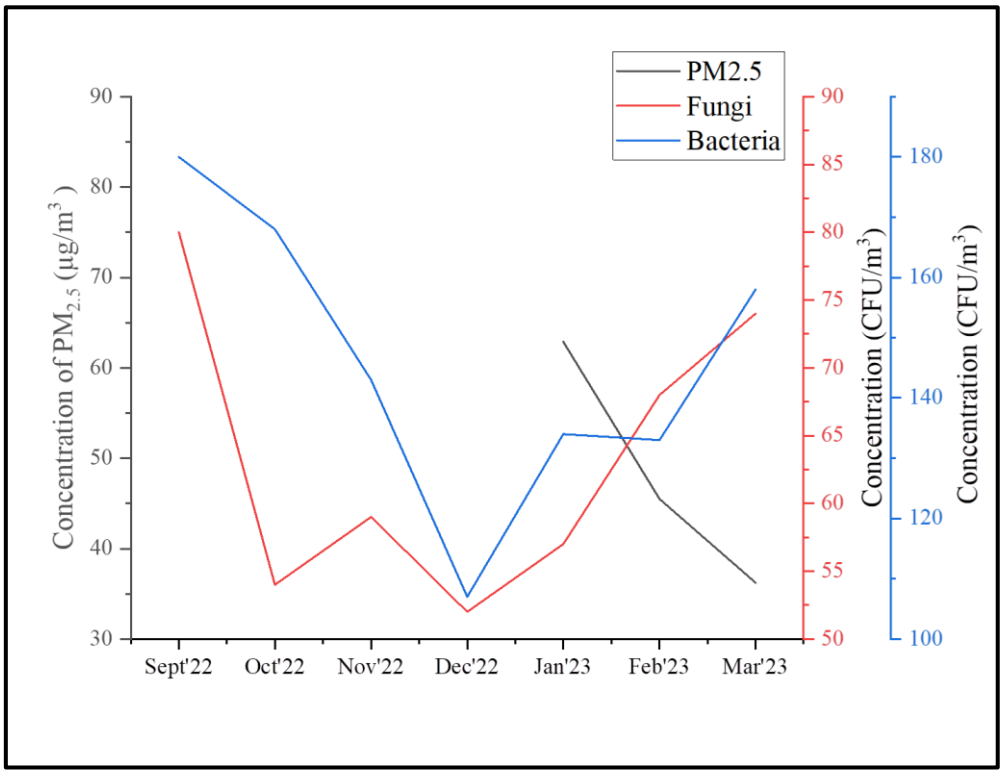


Figure 16: Variation of microbial concentration with respect to $PM_{2.5}$ levels

	A(X)	B(Y)	C(Y)	D(Y)
Long Name		PM2.5	Fungi	Bacteria
Units				
Comments	Pearson Correlations	Pearson Correlations	Pearson Correlations	Pearson Correlations
F(x)=				
1	PM2.5	1	-0.99465	-0.8099
2	Fungi	-0.99465	1	0.60351
3	Bacteria	-0.8099	0.60351	1

Figure 17: Pearson's Correlation between $PM_{2.5}$ and microbial concentration

Pearson's correlation coefficients for the variables were studied at $p<0.05$ and it was found that the concentration of both fungi and bacteria has shown negative correlation to the concentration of $PM_{2.5}$. While fungi has shown a very strong negative correlation of -0.99465, bacteria has shown a moderately lesser correlation of -0.8099. It can thus be observed that $PM_{2.5}$ strongly hinders the growth of both bacterial and fungal communities.

4.4. Conclusion

It has been observed from the individual relations between the pollutants and bio aerosols that in most cases, the growth of the bio aerosols are negatively impacted by the increase in presence of criteria pollutants such as SO_2, NO_2, PM_{10} and $PM_{2.5}$, in air. However we have also observed that despite the presence of the air pollutants, both fungal and bacterial communities have shown considerable seasonal variations and have not been eliminated completely in any season. It was also seen that pollutants such as SO2 were within the permissible limit, as per CPCB guidelines, and thus could not have been solely responsible for the negative impact on the microbes. It can be concluded from these observations that the concentration of pollutants in air might not be responsible for the individual variation in growth of microbes. The pollutants while coexisting in atmosphere might be responsible for the variation in concentration of bio aerosols. Further, it can be stated that the pollutants existing in the concentrations as observed are responsible for the positive growth of the microbes. This growth trend might decrease for higher concentrations of pollutants such as SO_2. The overall concentration might also increase in presence of lesser concentrations of pollutants that are exceeding the CPCB guidelines such as NO_2, PM_{10} and $PM_{2.5}$.

CHAPTER 5: ELEMENTAL ANALYSIS OF ROADSIDE DUST

5.1. Introduction

Elemental analysis of roadside dust helps in assessing the pollution levels in urban areas and identifying the sources of contamination. This information is crucial for implementing effective pollution control measures. It helps in identification of contamination sites and allows for the evaluation of health risks associated with exposure to heavy metals and other toxic elements present in the dust. This helps to understand the potential impact on human health, and in taking appropriate preventive measures. Additionally, elemental analysis of roadside dust provides insights into the contribution of vehicular emissions to environmental pollution. This information can be used to develop strategies for reducing emissions and improving air quality in urban areas. Overall, elemental analysis of roadside dust plays a vital role in understanding and mitigating the environmental and health risks associated with urban pollution.

5.2. Apparatus required

- Duster
- Ziplock bag
- Sieves and mechanical sieve shaker
- Mortar and prestle
- Horiba Scientific X-Ray analytical microscope (XGT-7200)

5.3. Methodology

5.3.1.Sampling

Dust was collected from the road side of sampling locations using duster and then accumulated in ziplock bags. The collected dust was then sieved using 2.36mm sieve to get rid of contaminants such as leaf, sticks, paint particles. The seieved dust was then grinded finely using mortar and prestel to attain a fine and even distribution of the elements in dust. It was then further sieved through 300µm sieve before being prepared for micro-edxrf.

5.3.2.Analysis

5.3.2.1. Energy Dispersive X-Ray Fluoresence Spectroscopy (EDXRF)

Micro-EDXRF is a technique used for the identification and analysis of elements in various

materials. It involves the use of X-ray radiation to excite the atoms in a sample, causing them to emit characteristic X-ray fluorescence. This fluorescence is then detected and analyzed to determine the elemental composition of the sample.

5.3.2.2. Acquisition Data

X-Ray tube volume: 50 KV

Preset Time: 300 s

Max Display Number: 25 point

XGT: 1.2 mm

X-Ray Filter: None

Acquistision Time: 151 s

Acquisition Rate: 36760 cps

Figure 18: a) Collected roadside dust being crushed using mortar and prestle
b) Mechanical sieve shaker

Figure 19: Horiba Scientific X-Ray analytical microscope (XGT-7200)

5.4. Results

Calculation of crustal enrichment factor was done to identify contribution of non crustal sources in collected sample dust, based on average upper continental crust composition as per (Rudnick & Gao, 2003).

Location	Elemental Composition (ppm)			
	Ca	Cr	Fe	Zn
Siddha Galaxia	197633.3	0	113566.7	1000
City Center	103633.3	700	89733.33	933.3333
Eco Urban Village	71866.67	0	113533.3	300
Mother's Wax Museum	96033.33	500	147966.7	800
Water Treatment Plant	121333.3	666.6667	125033.3	766.6667
Rail Vihar Gate 1	129066.7	1866.667	143566.7	1133.333
Aquatica	88266.67	0	64100	400
UEM-Xavier's	88933.33	400	88966.67	433.3333
Arts Acre	66833.33	0	144300	400
Hatishala	74233.33	600	83933.33	300

Akankha More	75800	0	114266.7	2400

Table 3: Elemental composition of road side dust collected from sampling sites

Crustal Composition (ppm)	25658	92	45349	67

Table 4: Composition of reference element, Fe

Location	**Enrichment Factor**			
	Ca	Cr	Fe	Zn
Siddha Galaxia	3.075773	0	1	5.959942
City Center	2.041224	3.845246	1	7.040052
Eco Urban Village	1.118791	0	1	1.788508
Mother's Wax Museum	1.147105	1.665659	1	3.659477
Water Treatment Plant	1.715139	2.628227	1	4.150245
Rail Vihar Gate 1	1.588932	6.409041	1	5.343145
Aquatica	2.433793	0	1	4.223718
UEM-Xavier's	1.766779	2.216218	1	3.296763
Arts Acre	0.8186	0	1	1.876232
Hatishala	1.563181	3.523682	1	2.419244
Akankha More	1.172451	0	1	14.21624
New Town More	1.365938	1.558425	1	6.182009

Table 5: Crustal enrichment factor of the road dust collected from sampling sites

5.5. Conclusion

Crustal enrichment factor for each of the elements at each location was studied and a general trend of Zn>Cr>Ca>Rb>Ti>Mg>Fe>Mn was found. The metals having highest concentration and having EF>2 at least of two locations were selected for further study. Highest enrichment factor was observed for Akankha More> Rail Vihar Gate 1> City Center> New Town More and lowest enrichment factor was found for Arts Acre and Eco Urban Village.

It can be seen that maximum amount of heavy metal have been found at locations having highest traffic inflow. Akankha More and New Town More are two of the largest traffic intersections at New Town. City Center is one of the largest shopping complex of New Town housing over 242 stalls and having an average footfall of 14k on weekdays and 30k on

weekends (*City Centre Newtown, Rajarhat , Kolkata – Shopping Centres Association of India*, 2009). Having this amount of inflow indicates a large number of vehicles passing through that area. The sources of this higher level of Zn and Cr is thus expected to be from traffic emissions. The higher level of Ca in the road dust is mainly from roadside cooking and burning of coal in small eateries present along the streets of New Town. Other than that, since New Town is an area that is currently undergoing constructional development, heavy metal contamination in roadside dust could also have been caused due to the presence of construction dust. On the other hand, Eco Urban Village being an open urban space having grassed area of over 45k sqft, curated to be a picnic spot has comparatively less traffic inflow and thus, less heavy metal contamination.

CHAPTER 6: CORRELATION BETWEEN BIOAEROSOL DIVERSITY AND CONCENTRATION OF ELEMENTS

6.1. Introduction:

Bacteria have been interacting with heavy metals since as early as the time of their evolution. Studies have been conducted on bacterial interactions with heavy metals in extreme environments where the main objective of the study was to observe and assess bacterial resistance, tolerarance, metabolism, and adaptation to exposure of heavy metals. In general, these interactions are directly based on the biological role of the heavy metal species in the microorganisms.While some heavy metals are necessary as essential cofactors for protein activity or to stabilize protein conformations, most heavy metals usually tend to be toxic at higher concentrations. Under these exposures to extreme environments, a major advantage is possessed by those organisms that have the capability to adopt resistance mechanisms in order to withstand these toxic effects caused due to high level of concentrations of heavy metals. ("Bacteria," 2001)

Heavy metals have a deleterious effect on fungi, affecting them at the cellular, biochemical, and molecular levels, leading to mutations and population changes.(Priyadarshini et al., 2021) Fungi have developed various tolerance mechanisms to cope with metal toxicity, including biosorption, bioaccumulation, biotransformation, and efflux of metal ions. (Jyoti et al., 2021) Fungi can also immobilize metals in less toxic forms by forming metal nanoparticles. Arbuscular mycorrhizal (AM) fungi are predominantly biotrophs who in exchange for carbon compounds, these symbiotic fungi, improves the uptake of a highly immobile element in soil, phosphorus (Dhalaria et al., 2020). In certain circumstances, the have also been seen to increase the uptake of nitrogen and micro nutrients, and thus benefitting the plant. In general, AM fungi have a beneficial impact on plant health and its resistance to factors of stress. (Vandenkoomhuyse, 2001)

6.2. Effect of selected heavy metals on microbes

A pot experiment was comducted to determine the effect of Arbuscular Mycorrhizal Fungi (AMF) on the antioxidant system of sorghum under Cr toxicity. It was found that AMF decreased Cr accumulation and oxidative stress while improving antioxidant activities. It effects the structure and composition of bacterial communities in the rhizosphere of plants, AMF decreased Cr concentration in the rhizosphere soil and increased soil carbon input, promoting the growth of beneficial bacteria. AMF under Cr exposure enhanced antioxidant response and improved plant growth. (Hu et al., 2020; P. Kumar, 2021; Wei et al., 2023)

While the effect of Zn on fungi and bacteria was studied, a study found that Zn pollution

triggered adaptive Zn tolerance in populations of Suilloid ectomycorrhizal fungi. It was found that Zn-resistant genotypes had lower Zn concentrations than sensitive isolates at low Zn levels, but at high Zn levels, the differential Zn accumulation pattern between resistant and sensitive isolates became more prominent. In case of ectomycorrhizal fungi and bacteria on the growth of pine seedlings, it was found that the shoot:root ratio was higher in plants inoculated with *Hebeloma crustuliniforme* and bacteria compared to control seedlings under Zn stress condition. On examination of biosorption of Zn ions by fungal mycelia it was found that *Rhizopus arrhizus* exhibited the highest capacity for Zn biosorption. (Colpaert et al., 2005; Dahm et al., 1998; Darugar et al., 2017)

6.3. Data analysis

The 12 sampling locations were roughly grouped into three groups based on their geographical location-

- Siddha Galaxia, City Center, Akankha More, Eco Urban Village and Mothers Wax Museum in the North West.
- Water Treatment Plant, Rail Vihar Gate 1, Aquatica, UEM-Xavier's and New Town More in the South East.
- Arts Acre and Hatisala in the South West.

The geospatial variation in enrichment factor of heavy metals along with concentration of micoorganisms in each of the locations was represented through ArcGIS mapping.

6.4. Results

The enrichment factors were divided into the following types on the basis of their levels:

EF<2: shortage of nominal enrichment

2≤EF≤5: reasonable enrichment

5≤EF≤10: significant enrichment.

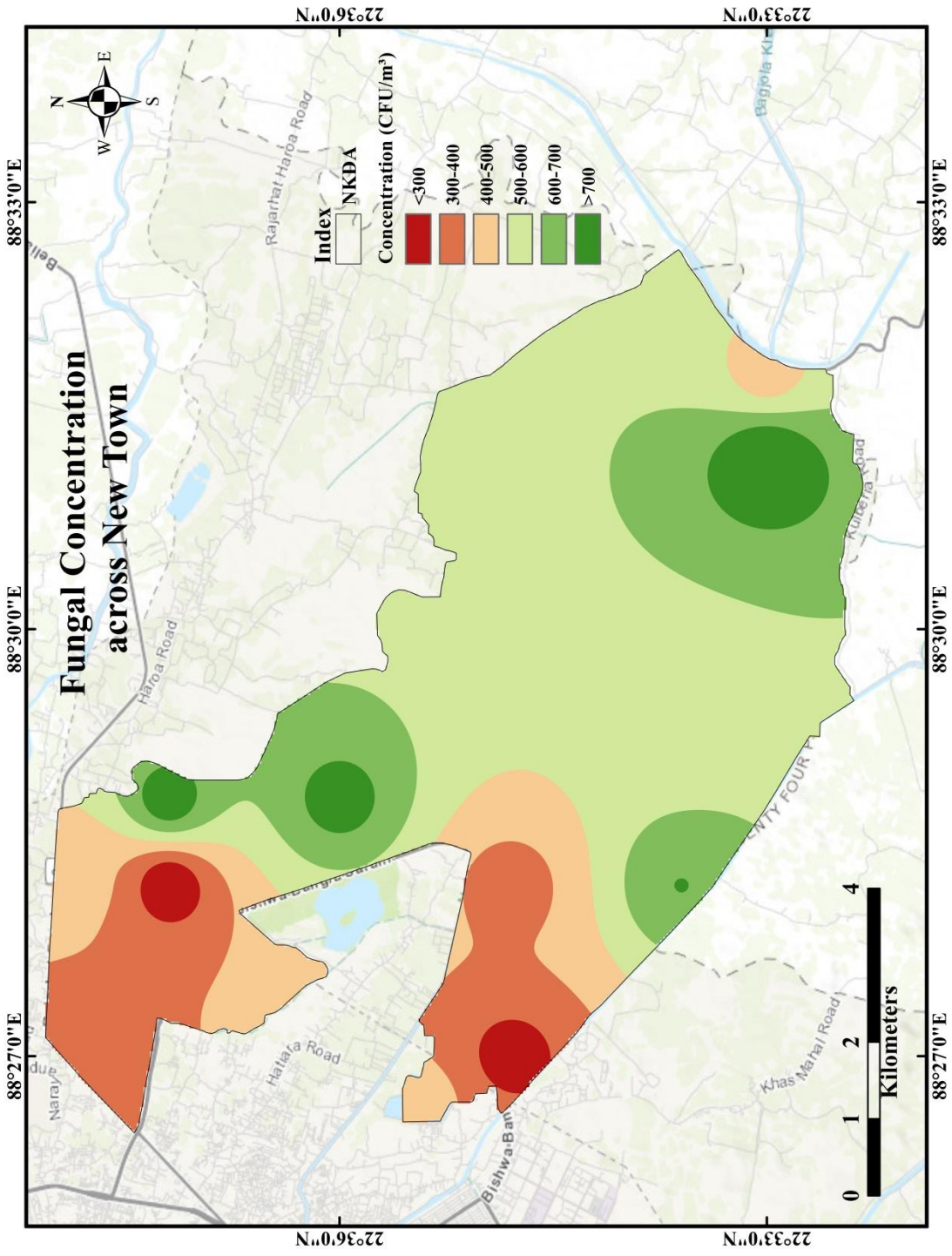


Figure 20: Spatial diversity of fungal concentration across New Town

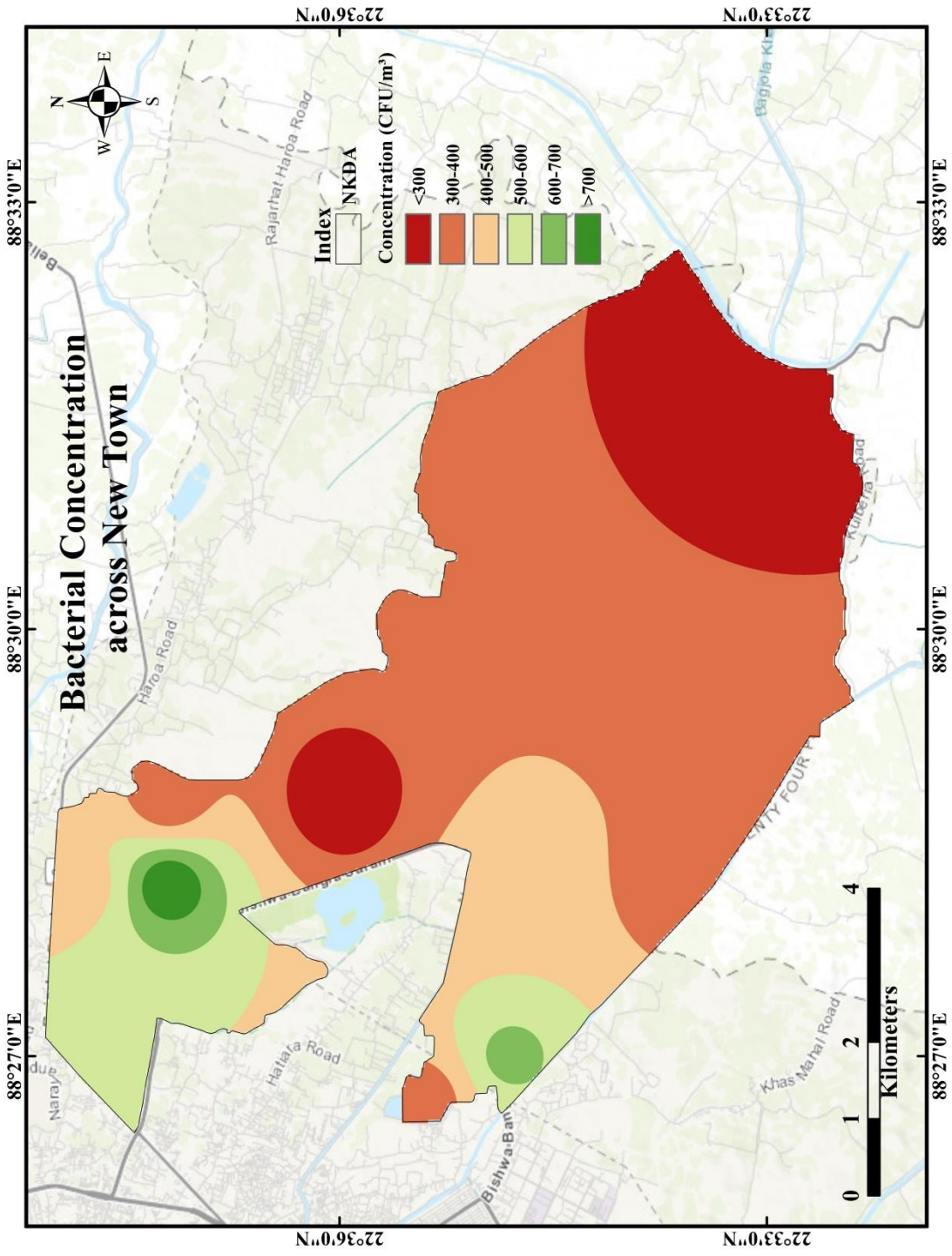


Figure 21: Spatial diversity of bacterial concentration across New Town

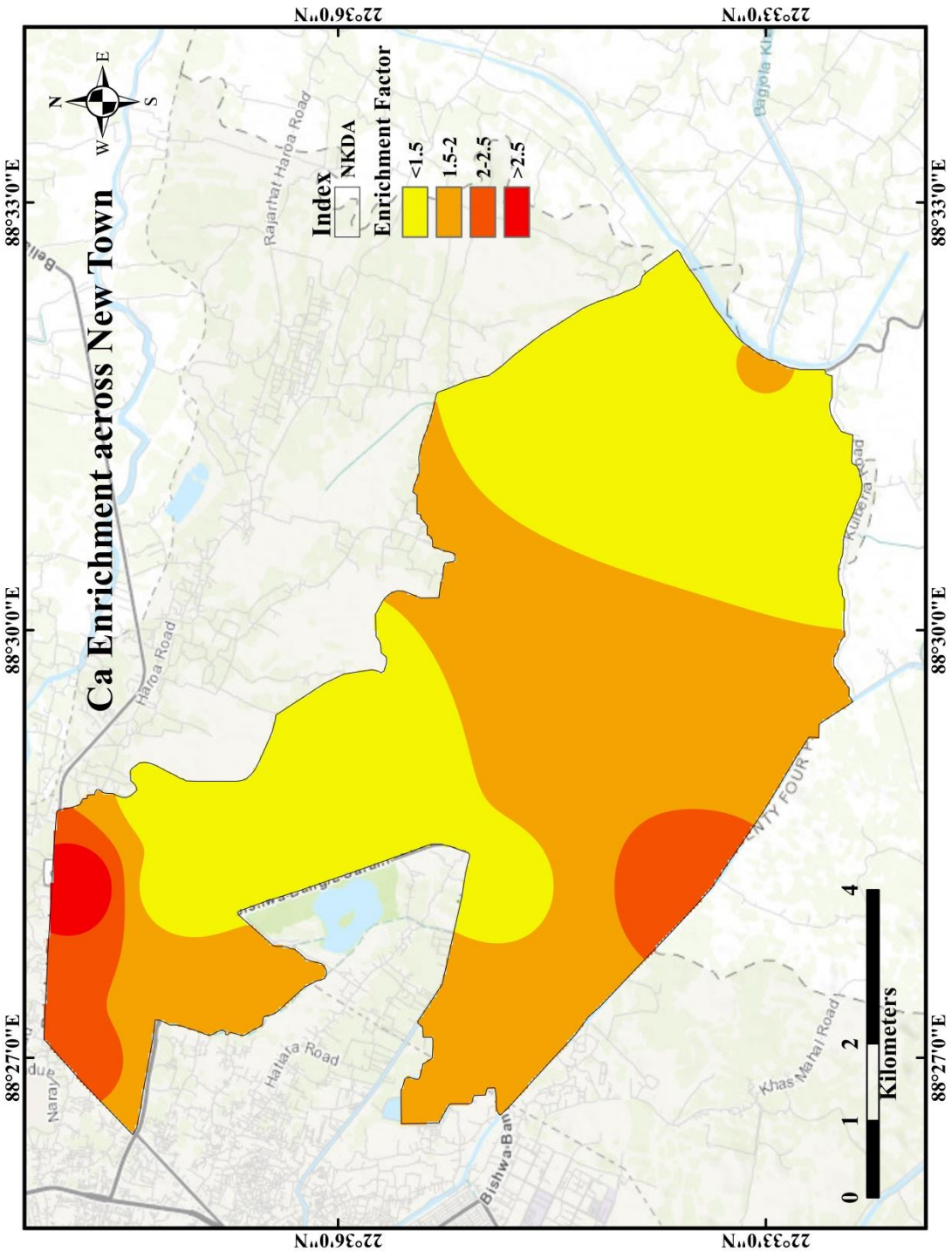


Figure 22: Ca enriched areas across New Town

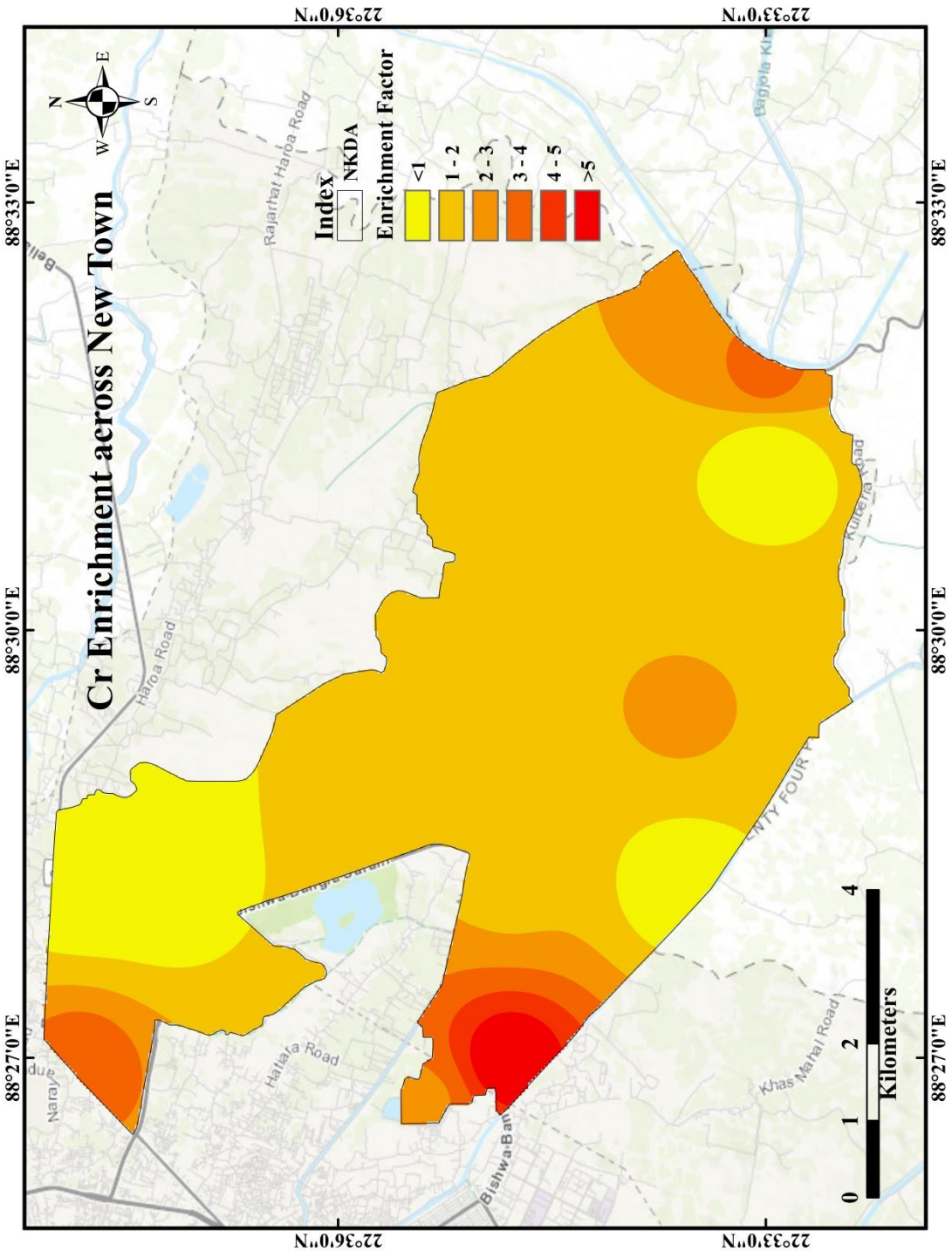


Figure 23: Cr enriched areas across New Town

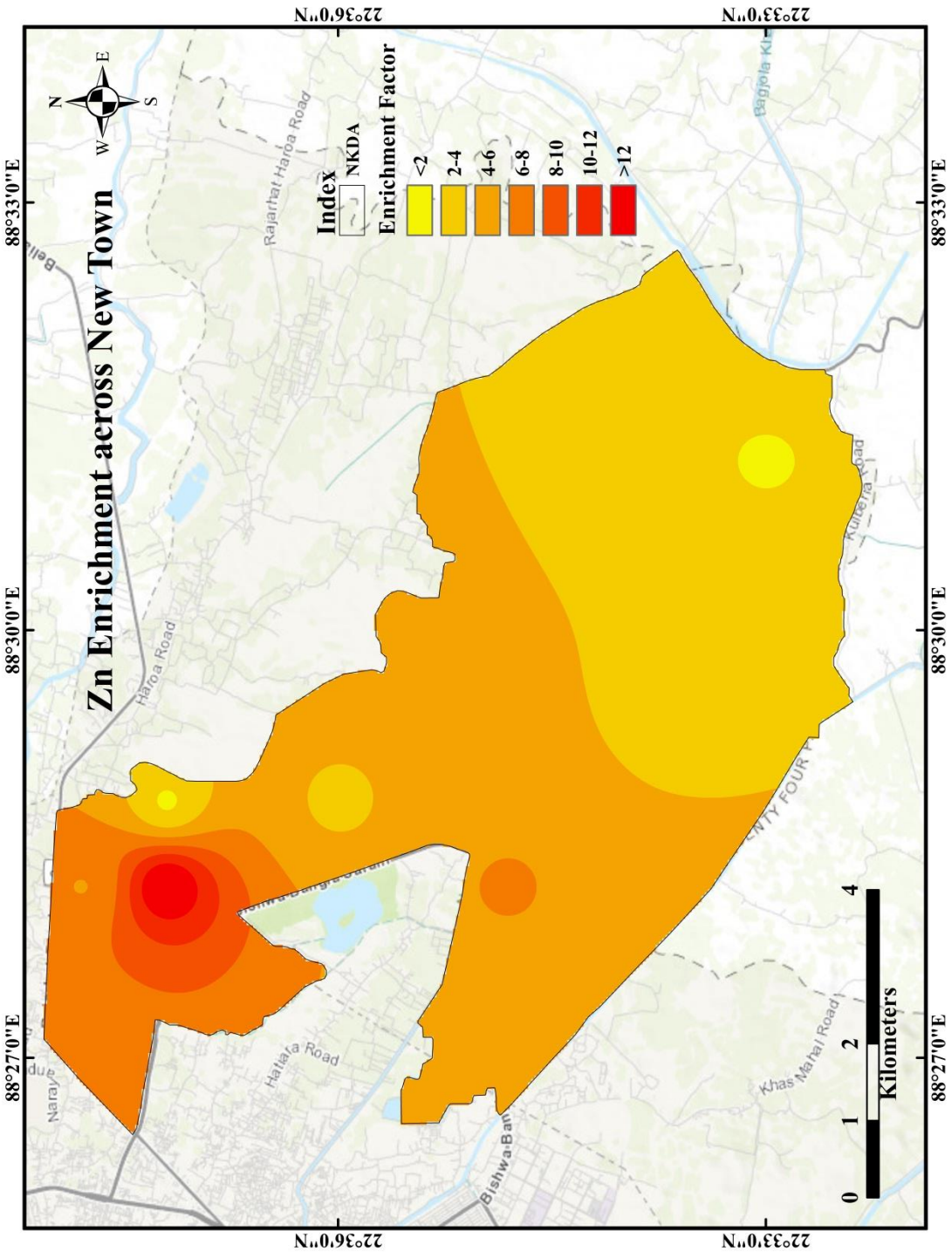


Figure 24: Zn enriched areas across New Town

6.5. Conclusion

The northern and central area of New Town has shown significant crustal Ca enrichment. Simultaneously an increased concentration of fungal colony was found in that area which could have been caused since calcium signalling plays a crucial role in various processes, like growth, development, reproduction, stress tolerance, and virulence in fungi (Roy et al., 2021). The growth of bacterial community, however was dominated in the area with Ca enrichment. Cr enrichment has shown no significant correlation with the concentration of either bacteria or fungi in the area. However, areas with maximum Cr enrichment (EF≥5) has shown a marked decrease in the fungal concentration and moderate increase in the localized bacterial concentration. Zn enrichment (EF≥5) was observed over large areas of New Town. Increase in Zn enrichment was seen to promote bacterial growth since Bacteria can immobilize zinc, through adsorption by bacterial cell walls and sedimentation reactions with phosphate or other anions produced through bacterial metabolism (Pal et al., 2017). In case of fungi, the concentration showed an increase till the enrichment factor reached a threshold limit of EF>10, beyond which the concentration of fungi was seen to decrease as a result of influence on the nutrient acquisition and metal tolerance of fungi.

CHAPTER 7: INVITRO STUDY OF SO_2 AND UV-C RADIATION

7.1. Introduction

Enzymes in microorganisms play a critical role in various biological processes which includes digestion, DNA replication, nerve transmission, and muscular function (Somavarapu et al., 2021). Microbial enzymes are particularly interesting due to their better selectivity and wide range of applications in industries such as agriculture, pharmaceuticals, and biofuels (S. K. Singh et al., 2021). They are also used in food industry for enhancing taste, texture, and aiding in fermentation (Vachher et al., 2021). Microbial enzymes have proven to be effective in bioremediation, as they can degrade or detoxify complex and toxic environmental contaminants (R. S. Singh et al., 2019). These enzymes, such as hydrolases, oxidoreductases, and peroxidases, have been applied to both organic and inorganic pollutants (Sonali & Arora, 2020). In the field of biopharmaceuticals, microbial enzymes offer advantages such as ease of isolation, high consistency, and economic feasibility. They are increasingly being used for the treatment of various diseases, either alone or in combination with other procedures. The use of microbial enzymes in industrial processes has also increased due to their catalytic activity and stability . They have diverse applications in the food, pharmaceutical, and biotechnological industries . Microbes are considered as potential sources of enzymes for industrial applications, such as lipases, cellulases, chitinases, and amylases.

7.2. Enzymes

7.2.1.Catalase

Catalase is an important enzyme in fungi and bacteria. It plays a crucial role in the decomposition of hydrogen peroxide into water and oxygen, making it valuable in various industries such as textile, food, and pharmaceuticals (Santoso et al., 2016). In hydrocarbon-oxidizing bacteria, a decrease in catalase activity is observed during the destruction of petroleum products, indicating its involvement in the initial stage of oxidation .(Gogoleva et al., 2012) Yeasts and fungi predominantly possess heme-containing catalases, and their structure and biochemistry have been extensively studied. (Daum et al., 1998) Fungal catalase has also been utilized for hydrogen peroxide neutralization in growth media for the detection of microorganisms, providing a method for detecting their presence.(Besenmatter et al., 2013) Additionally, catalase incorporation into enumeration media enhances the colony-forming abilities of airborne bacteria, particularly at lower relative humidities. (Marthi et al., 1991) Overall, catalase plays a significant role in the defense against oxidative stress and has

practical applications in various fields.

7.2.2.Protease

Proteases play an important role in fungi and bacteria. Fungi produce acidic, neutral, and alkaline proteases, while bacteria produce only alkaline and neutral proteases (Salwan & Sharma, 2019). Owing to their substrate specificity, wide diversity, catalytic activity and stability, fungal proteases are most ideal for biotechnological applications and are has high industrial demand (Patil et al., 2015). Fungal proteases can easily withstand the harsh conditions in industrial processes and have easy and cost-effective downstream processing and recovery (V. Kumar et al., 2019). Microbial proteases, including those from fungi and bacteria, are widely used in various industries such as food, detergent, pharmaceutical, leather, and waste treatment (Jisha et al., 2013; Karthik et al., 2014). They are environmentally friendly and have commercial importance . Bacterial proteases also have the ability to hydrolyze protein peptide bonds and can act as exotoxins, destroying extracellular structures . Overall, proteases from fungi and bacteria have significant industrial applications and are essential for various biotechnological processes.

7.2.3.RNAse

In fungi, RNAse is involved in gene silencing through RNA interference (RNAi), which shapes many biological processes including pathogenicity.(Shaffer et al., 2022) In bacteria, RNAse is crucial for the establishment of symbiotic interactions with fungi. For example, the bacterial type VI secretion system is potentially involved in symbiosis establishment between endohyphal bacteria and fungal hosts.(Sesma, 2016) Additionally, RNAse is important for the growth and development of bacteria in co-culture with fungi, as shown by gene expression studies.(Gaffar et al., 2019) Furthermore, RNAse is involved in the interaction between arbuscular mycorrhizal (AM) fungi and bacteria, which can stimulate plant growth and inhibit fungal plant pathogens.(Gaffar et al., 2019) Overall, RNAse plays a significant role in regulating gene expression, symbiosis establishment, and pathogenicity in fungi and bacteria.
In bacteria, RNase P is a key enzyme that recognizes and cleaves precursor tRNAs accurately. It interacts with the T- and acceptor-stems of the precursor tRNA, as well as the 5'-leader sequence and the 3'-terminal CCA. The protein subunit of RNase P also affects substrate recognition and the substrates' range that can be used by RNase P. Even though the

protein subunit sequence is not highly conserved among bacteria, different proteins can functionally reconstitute the RNase P holoenzyme.(Burnett et al., 2020)

7.3. Apparatus Required

- Eppendorf cold centrifuge (5810 R)
- Perkin Elmer (Lambda 25 UV/VIS) spectrophotometer
- Eppendorf centrifuge Minispin
- Leica DM 750 microscope with ICC50 HD camera
- UV-C Chamber with 625 $\mu W/cm^2$ intensity lamp
- Labtech LSI-3016R shaking incubator with orbital shaker
- Borosil desiccator 840 cc
- Sterilized Tarson disposable petri plates
- Glass test tubes, conical flasks
- Eppendorf falcon tubes 15ml, Centrifuge tubes 2ml
- Water bath
- Icebox

7.4. Methodology

7.4.1. Identification & Isolation

Upon sampling, the fungi and bacteria were allowed to grow under optimum temperatures of 27ºC and 32ºC respectively for 3-4 days till they were ready to be identified morphologically. Slides of fungi were prepared using Lactophenol Cotton Blue dye and were studied using Leica DM 750 microscope. Bacteria were identified using Gram Staining (Tripathi & Sapra, 2023). Four strains of bacteria and fungi were selected, purified on agar slants, and incubated under optimum conditions. Strains of fungi selected for further study were *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus parasiticus*, and *Penicillium citrinum*. Bacteria identified using gram staining were labelled as *NTA1, NTA2, NTA3*, and *NTA4*.

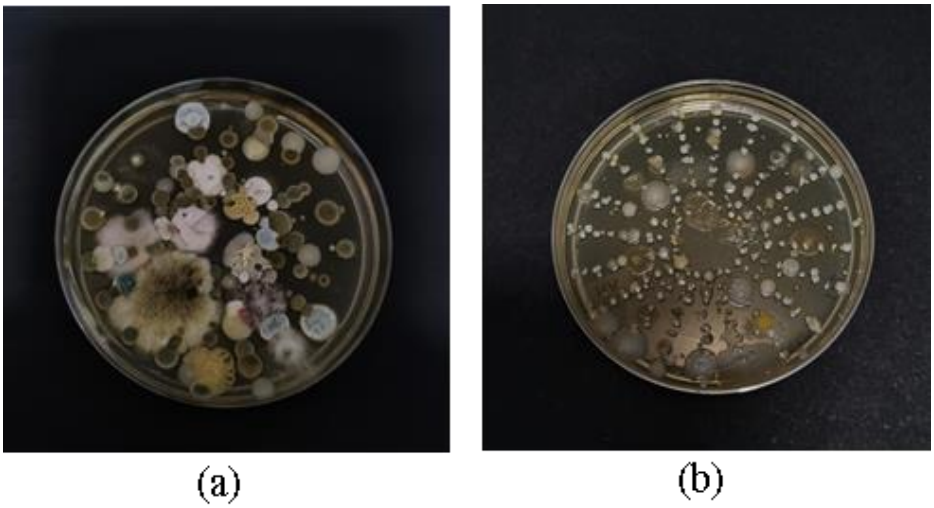


Figure 25: a) Fungal colonies on Sabouraud dextrose agar plate, b) Bacterial colonies on Nutrient agar plate

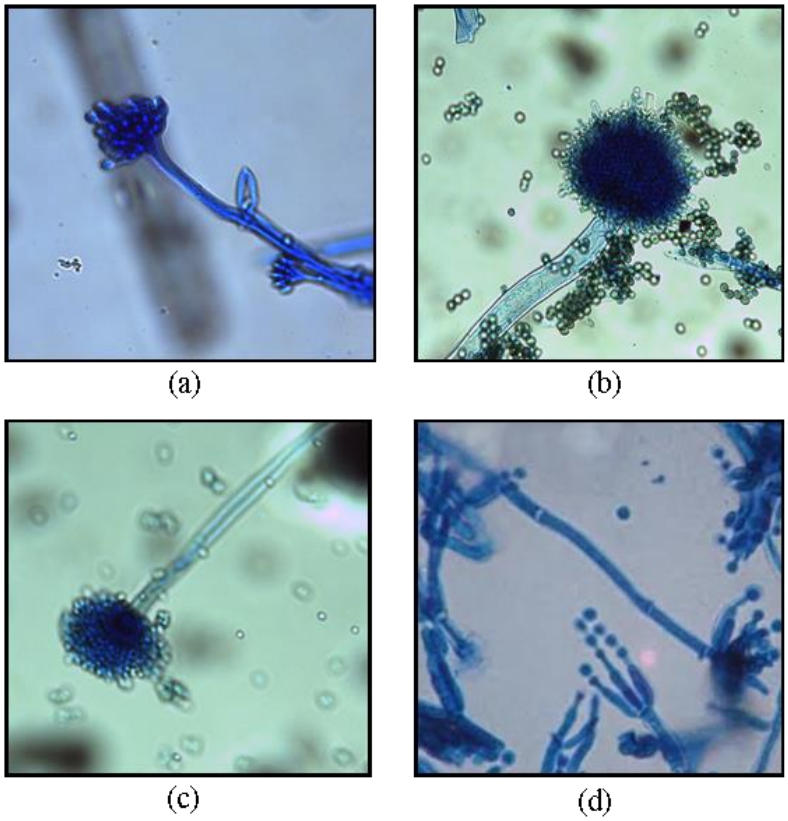


Figure 26: Isolates of a) *Aspergillus flavus*,b) *Aspergillus fumigatus*, c) *Aspergillus parasiticus*, and d) *Penicillium citrinum* viewed under microscope at 40x magnification

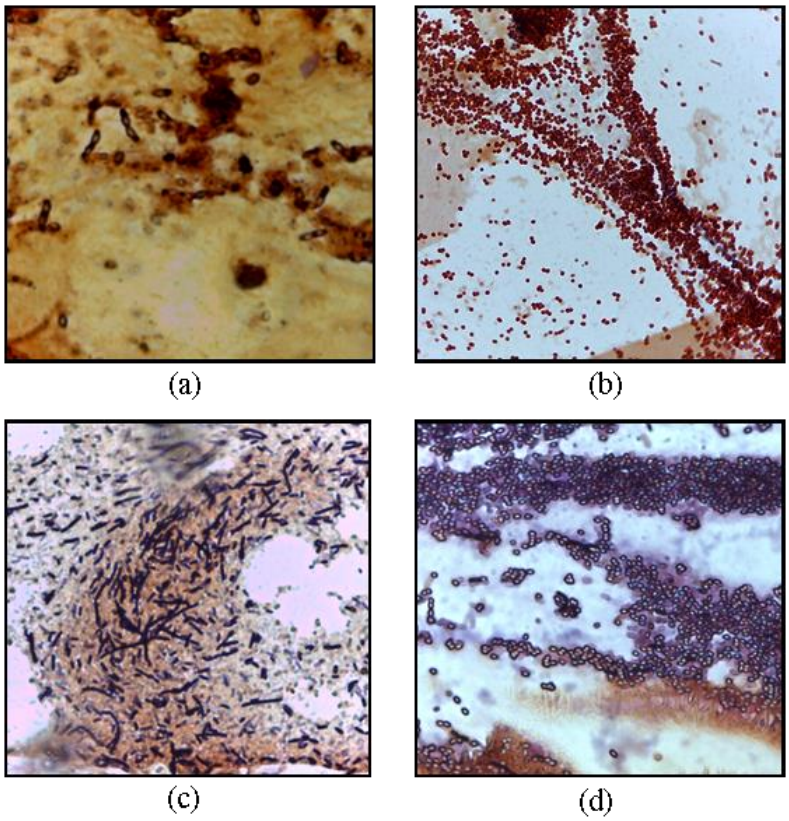


Figure 27: Isolates of a) *NTA1*,b) *NTA2*, c) *NTA3*, and d) *NTA4* viewed under microscope at 40x magnification

7.4.2.Preparation of growth media

7.4.2.1. Peptone media-specific broth

Peptone media-specific broth was prepared dissolving 5g/l glucose, 7.5g/l peptone, 5g/l $MgSO_4.7H_2O$, 5g/l KH_2PO_4, and 0.1g/l $FeSO_4.7H_2O$ in distilled water. The media was then maintained at pH 7.0. (Alnahdi, 2012)

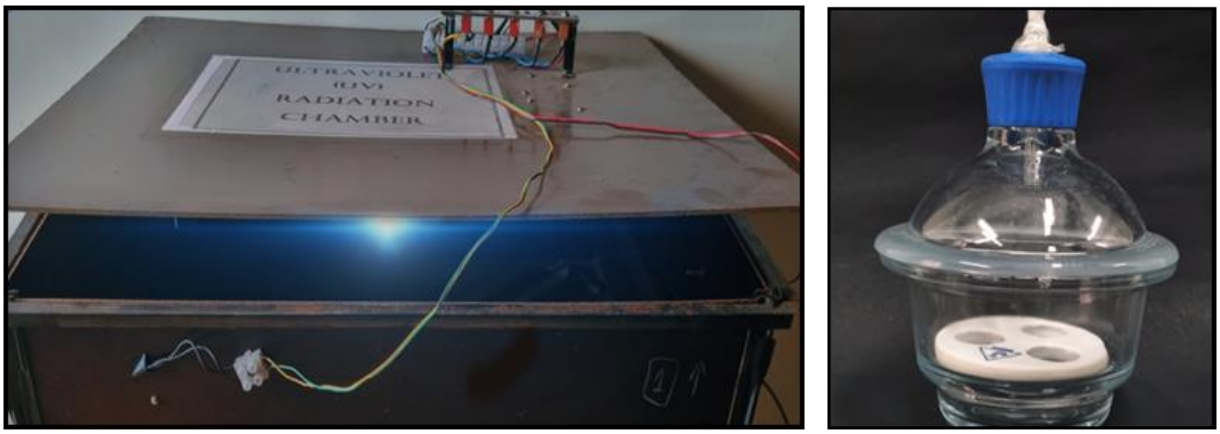
7.4.2.2. Beef Extract media-specific broth

50l of the beef extract broth was prepared by dissolving 2.5g glucose, 0.25g beef extract, 0.1g yeast extract, 0.025g MgSO4, 0.005g CaCl2, 0.1g KNO3 and 0.25g soybean meal in distilled water. The broth was maintained at pH 6.2. (Gomes et al., 1998)

7.4.3.Application of stress & extract preparation

Stress was applied to each pure culture in the form of 25ppm SO_2 applied in a glass jar and

625 µW/cm^2 UV-C, in a UV-Chamber. The cultures were then inoculated in peptone media-specific broth and beef extract media-specific broth, followed by being incubated at 27-30ºC at 145 rpm for 48 hours (Henzler & Schedel, 1991). The cultures were then filtered using Whatman filter paper and the filtrate was used for conducting further assays.



(a) (b)

Figure 28: a) UV-C chamber, b) SO_2 application jar

Figure 29: Cultures after being incubated in peptone media specific broth and beef extract media specific broth

Figure 30: Cultures filtered using Whatman filter paper

7.4.4.Assays

7.4.4.1. Catalase Assay

The 0.6ml of filtrate extracted was added to 1.9ml of 50mM phosphate buffer containing 0.19mM H_2O_2. The catalase activity was quantified by measuring the decrease in absorbance at 240nm. The amount of enzyme that degrades 1µM H_2O_2 per minute was defined as one enzyme unit.

7.4.4.2. Protease Assay

200µl of the crude extract was added to a mixture of 500µl of casein in 59mM phosphate buffer. The mixture was then placed in a water bath at 40°C for 20 minutes. Following this, the reaction was terminated by the addition of 1 ml of 10% (w/v) trichloroacetic acid (TCA) and was kept at room temperature for 15 minutes. The sample was then centrifuged at 10000 rpm for 5 minutes. The supernatant thus obtained was mixed with 2.5 ml of 0.4M Na_2CO_3 and 1 ml of 3-fold diluted Follin Ciocalteus phenol reagent. The resulting solution was incubated at room temperature in the dark for 30 minutes and absorbance of the blue color developed was measured at 660 nm against a tyrosine standard. 1 unit of enzyme activity was calculated as equal to the amount of enzyme that releases 1µg of tyrosine per minute. (Alnahdi, 2012)

(a) (b)

Figure 31: a) Centrifugation at 10000 rpm, b) Segregation of supernatant and deposition

7.4.4.3. RNase Assay

0.5ml of the crude filtrate was added to a mixture of 6 mg of yeast RNA which was incubated

in 0.25 M acetate buffer so that the solution has a total volume of 2ml. The samples were then placed in a water bath at 50°C for an hour. The reaction was then stopped by rapid chilling in an ice bath following which, 5ml of ice-cold ethanol was added and maintained at -10°C for 20 minutes. The samples were then centrifuged at 2000g for 10 minutes, causing the non-hydrolyzed RNA to precipitate. The supernatant was measured at 260nm. 1 unit of enzyme activity was calculated as the amount of enzyme necessary to produce 1mM of 260nm absorbing substance. (Gomes et al., 1998)

(a) (b)

Figure 32: a) Samples placed in water bath at 50°C, b) Rapid chilling in ice bath

(a) (b)

Figure 33: a) Addition of ice cold ethanol, b) Centrifugation at 2000g

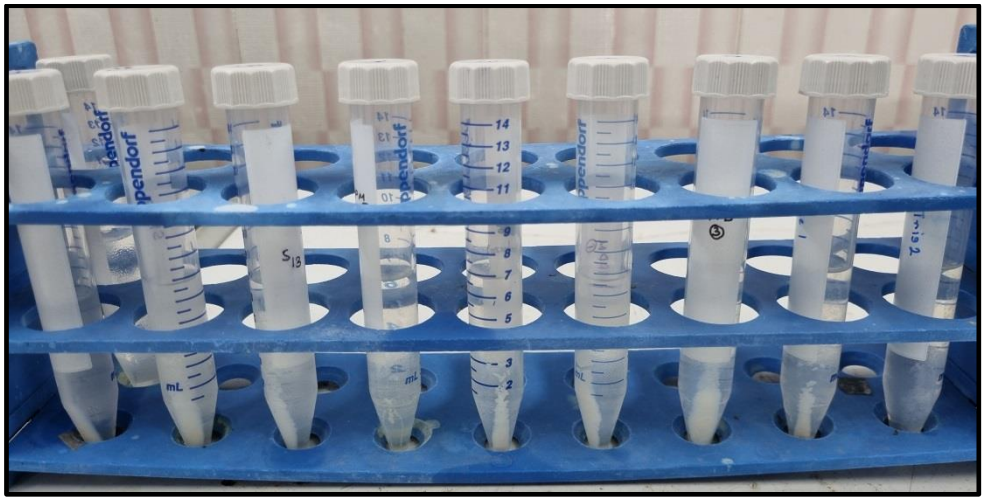


Figure 34: Precipitation of non-hydrolyzed RNA

7.4.5.Analysis

Statistical analysis was conducted using Origin Pro v10.0.0.154 (OriginLab Corporation, Northampton, MA, USA) and SPSS Statistics v29.0.1.0 (IBM Corporation, New York, USA).

7.5. Results

7.5.1.Comparison of total enzyme produced by microbial strains under stressed conditions

Significant amount of enzymes were produced by both bacterial and fungal isolates. Total enzyme produced by each strain of fungi, namely *Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus,* and *Penicillium citrinum.* and bacteria such as *NTA1, NTA2, NTA3,* and *NTA4* were calculated for both SO_2 and UV-C stress. They were then compared to study which stress is causing greater impact on the microbial growth and enzyme producing capacity.

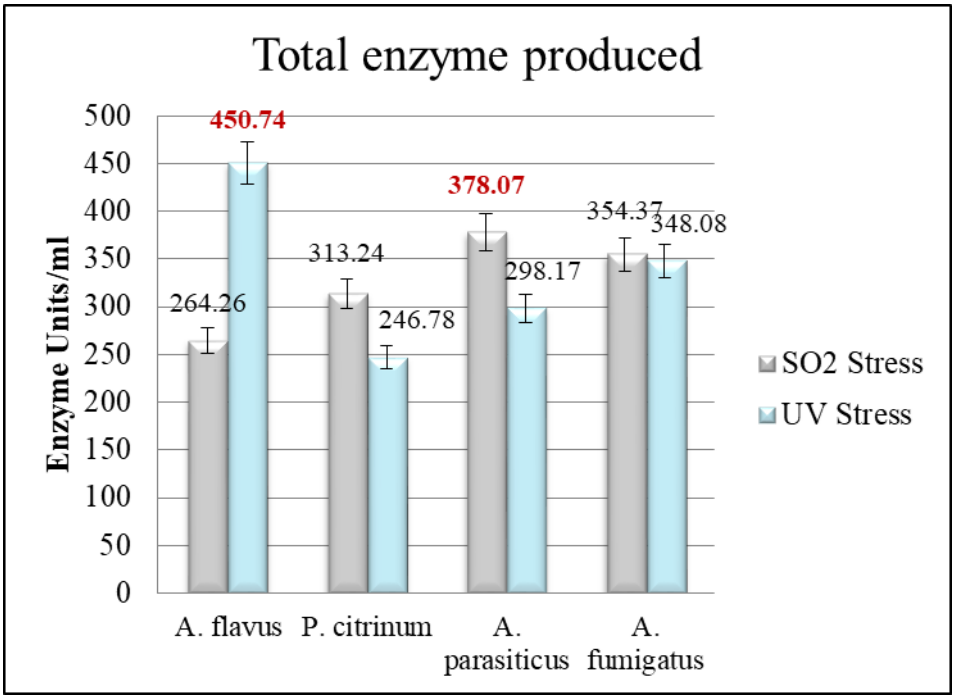


Figure 35: Total enzyme produced by fungi under stressed conditions

In case of fungi, greater amount of enzyme has been produced by organisms under SO_2 stress, except for the case of *A.flavus*. In case of *A. flavus*, a much greater amount of enzyme was produced (450.74 EU/ml) which is 70.56% greater than the amount of enzyme produced under SO_2 stress.

Among the four strains of fungi studied, maximum amount of enzyme produced under UV-C exposure was found in *A.flavus* while the lowest amount of enzyme was produced by *P. citrinum* (246.78 EU/ml).

In case of the fungi strains under SO_2 exposure, highest amount of enzyme was produced by *A.parasiticus* (378.07 EU/ml) while lowest amount of enzyme was produced by *A.flavus* (264.26 EU/ml).

The total enzyme produced under UV-C exposure (1343.77 EU/ml) is 2.5% greater than that produced by total enzyme produced by the fungi under SO_2 exposure (1309.94 EU/ml).

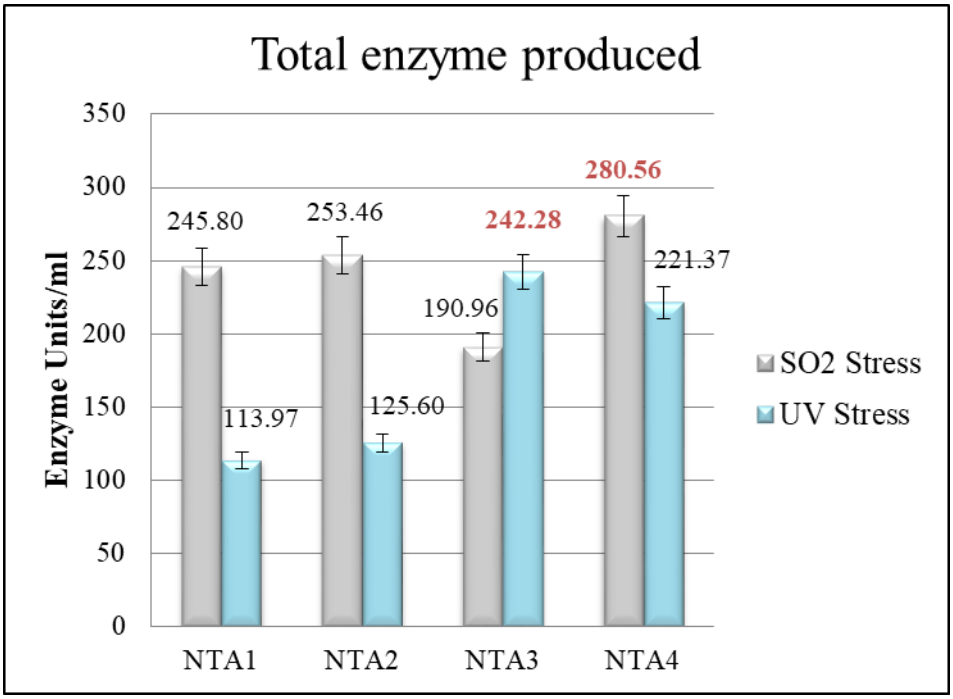


Figure 36: Total enzyme produced by bacteria under stressed conditions

In case of bacteria, greater amount of enzyme has been produced by organisms under SO_2 stress, except for the case of *NTA3*. In case of *NTA3*, a slightly greater amount of enzyme was produced (242.28 EU/ml) under UV-C strain which is 21.18% greater than the amount of enzyme produced under SO_2 stress.

Among the four strains of bacteria studied, maximum amount of enzyme produced under SO_2 exposure was found in *NTA4* (280.56 EU/ml) while the lowest amount of enzyme was produced by *NTA3* (190.96 EU/ml).

In case of the bacterial strains under UV-C exposure, highest amount of enzyme was produced by *NTA3* while lowest amount of enzyme was produced by *NTA1* (113.97 EU/ml).

The total enzyme produced under SO_2 exposure (970.78 EU/ml) is 27.56% greater than that produced by total enzyme produced by the bacteria under UV-C exposure (703.22 EU/ml).

7.5.2.Contribution of each organism towards individual enzyme production

Three enzymes have been selected based on their categorical classification, abundance in wide range of individual and industrial applications, namely catalse under class oxidoreductase that primarily dismutates hydrogen peroxide sourced from superoxides in mitochondria into water and dioxygen and RNase, protease under class hydrolase that

catalyze the hydrolysis of various bonds.

Under the exposure of SO_2 and UV-C stress, the enzyme production capacity of each organism was studied. The most abundantly produced enzyme under stress condition was identified and contribution of each organism towards the enzyme produced was observed.

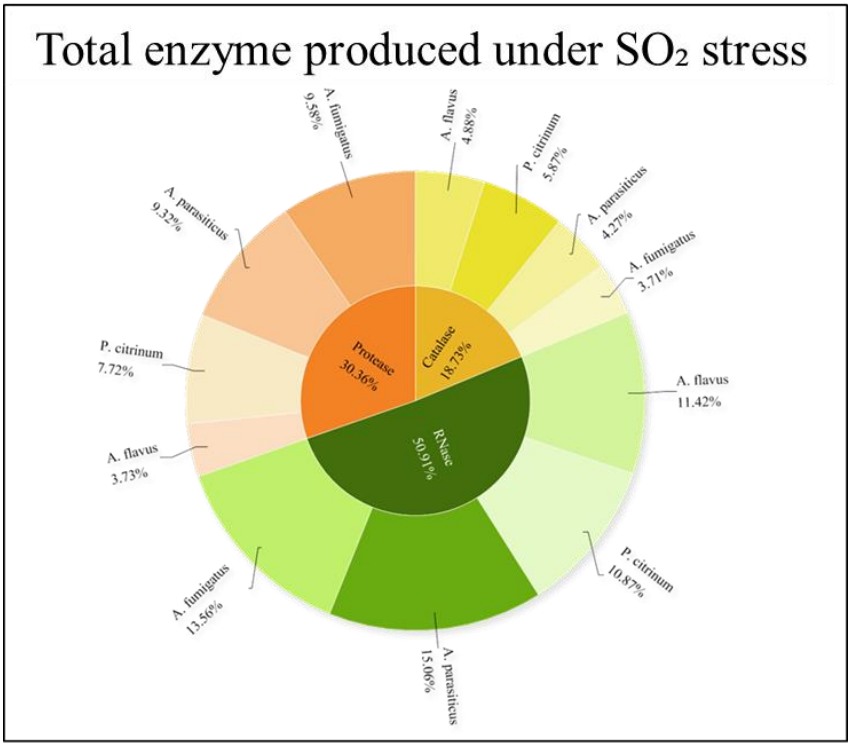


Figure 37: Contribution of each fungi towards total enzymes secreted under SO_2 stress

Under the exposure of SO_2 stress, fungi strains have cumulatively produced 1309.94 Enzyme Units/ml of which, RNase, contributes to 50.91%. *A.parasiticus* (198.776 EU/ml) has contributed majorly to the production of RNase, followed by *A.fumigatus* (178.87 EU/ml). 30.36% of total enzyme, that is 400.578 EU/ml was contributed by protease. Maximum amount of protease was produced by *A.fumigatus* (126.45 EU/ml) followed by *A.parasiticus* (122.96 EU/ml). Least amount of catalase (237.67 EU/ml) was produced by fungi under SO_2 stress of which maximum enzyme was produced by *P.citrinum* (68.01 EU/ml) followed by *A.flavus* (64.27 EU/ml).

Organism	**Enzyme produced (EU/ml)**		
	Catalase	RNase	Protease
A. flavus	64.27651	150.6701	49.31733

P. citrinum	68.01689	143.3752	101.8447
A. parasiticus	56.33361	198.7761	122.96
A. fumigatus	49.04391	178.8695	126.4567

Table 6: Enzyme Units produced by each fungal strain under SO_2 stress

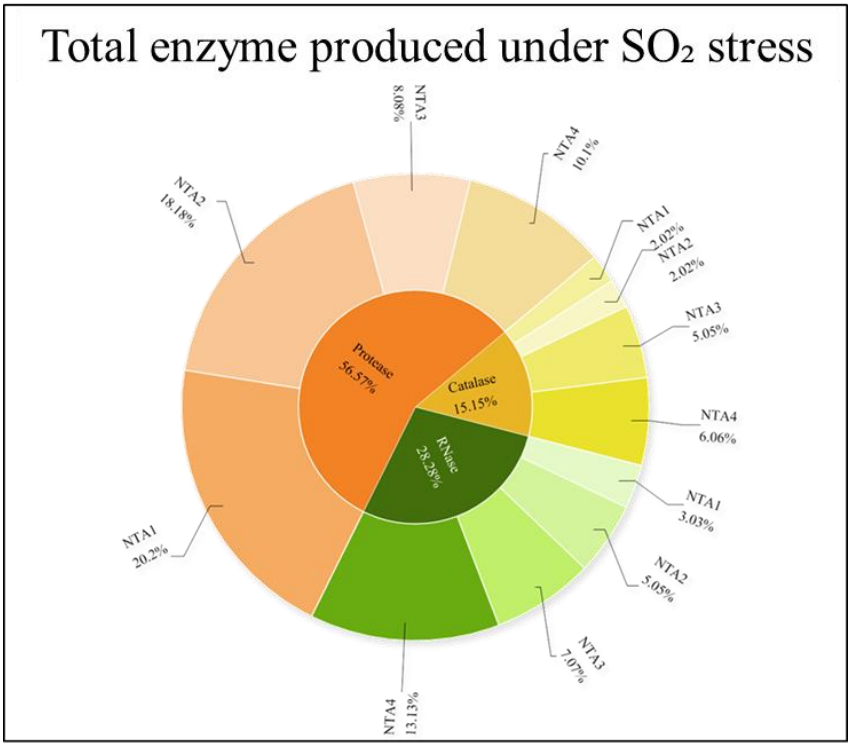


Figure 38: Contribution of each bacteria towards total enzymes secreted under SO_2 stress

Under the exposure of SO_2 stress, bacterial strains have cumulatively produced 970.7681 Enzyme Units/ml of which, protease, contributes to 56.57%. *NTA1* (194.922 EU/ml) has contributed majorly to the production of protease, followed by *NTA2* (179.082 EU/ml). 28.28% of total enzyme that is 271.6261 EU/ml was contributed by RNase. Maximum amount of RNase was produced by *NTA4* (122.714 EU/ml) followed by *NTA3* (65.59 EU/ml). Least amount of catalase (148.54 EU/ml) was produced by bacteria under SO_2 stress of which maximum enzyme was produced by *NTA4* (58.41 EU/ml) followed by *NTA3* (48.1958 EU/ml).

Organism	**Enzyme produced (EU/ml)**		
	Catalase	RNase	Protease
NTA1	20.52987	30.34475	194.922

NTA2	21.40227	52.97441	179.082
NTA3	48.1958	65.59292	77.167
NTA4	58.41205	122.714	99.431

Table 7: Enzyme Units produced by each bacterial strain under SO_2 stress

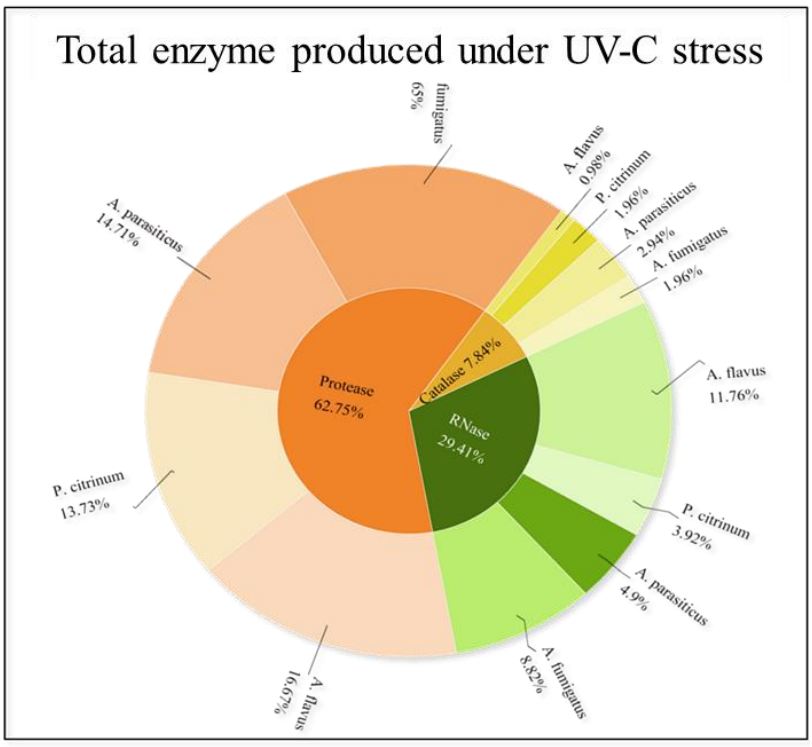


Figure 39: Contribution of each fungi towards total enzymes secreted under UV-C stress

Under the exposure of UV-C stress, fungal strains have cumulatively produced 1299.5 Enzyme Units/ml of which, protease, contributes to 62.75%. *A. fumigatus* (230.2775 EU/ml) has contributed majorly to the production of protease, followed by *A. flavus* (227.209 EU/ml). 29.41% of total enzyme that is 2381.835 EU/ml was contributed by RNase. Maximum amount of RNase was produced by *A, flavus* (151.172 EU/ml) followed by *A. fumigatus* (110.514 EU/ml). Least amount of catalase (88.7134 EU/ml) was produced by fungi under UV-C stress of which maximum enzyme was produced by *A. parasiticus* (36.45 EU/ml) followed by *P. citrinum* (22.8885 EU/ml).

Organism	**Enzyme produced (EU/ml)**		
	Catalase	RNase	Protease
A. flavus	9.507003	151.1724	227.209

P. citrinum	22.88855	53.6776	175.701
A. parasiticus	36.45164	66.47123	195.767
A. fumigatus	19.86619	110.5139	230.2775

Table 8: Enzyme Units produced by each fungal strain under UV-C stress

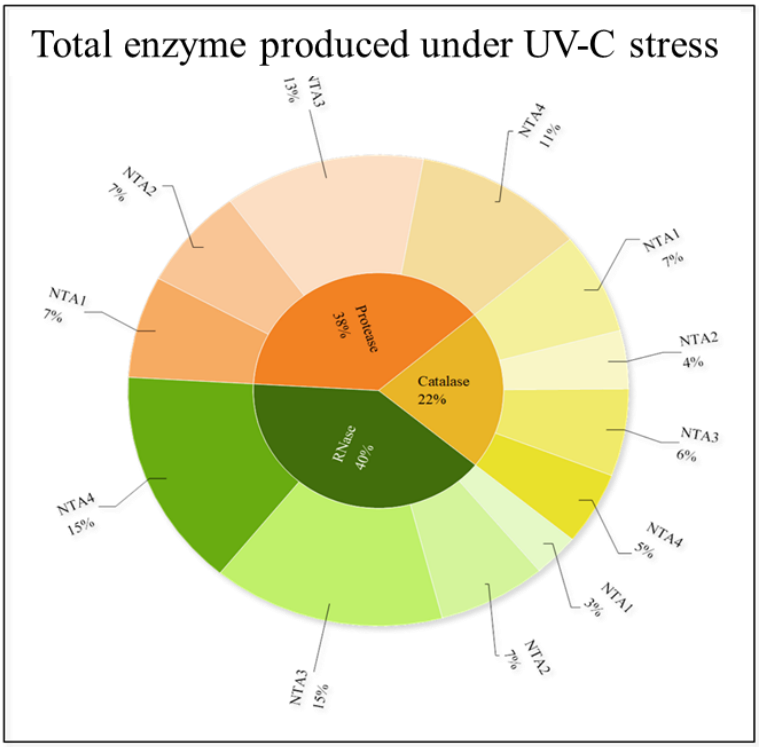


Figure 40: Contribution of each bacteria towards total enzymes secreted under UV-C stress

Under the exposure of UV-C stress, bacterial strains have cumulatively produced 729.6357 Enzyme Units/ml of which, RNase, contributes to 40%. *NTA3* (109.42 EU/ml) has contributed majorly to the production of protease, closely followed by *NTA4* (108.0065 EU/ml). 38% of total enzyme that is 272.964 EU/ml was contributed by protease. Maximum amount of protease was produced by *NTA3* (93.745 EU/ml) followed by *NTA4* (78.828 EU/ml). Least amount of catalase (165.1547 EU/ml) was produced by bacteria under UV-C stress of which maximum enzyme was produced by *NTA1* (49.08 EU/ml) followed by *NTA3* (43.846 EU/ml).

Organism	**Enzyme produced (EU/ml)**		
	Catalase	RNase	Protease
NTA1	49.08334	20.25956	52.572

NTA2	32.5712	53.82785	47.819
NTA3	43.84691	109.4231	93.745
NTA4	39.65322	108.0065	78.828

Table 9: Enzyme Units produced by each bacterial strain under UV-C stress

7.5.3.Preference of growth media broth by each microbial strain

Enzyme production by bio aerosols is not solely dependent on any individual factor such as temperature, humidity, wind speed, solar illumination or pollutants, alone. All these factors along with the substrate in which the fungal and bacterial strains are inoculated, together is responsible for the total amount of enzyme produced. For this study two growth medias, namely peptone broth and beef broth have been selected and the preference of growth media under stressed condition has been studied against non-stressed condition.

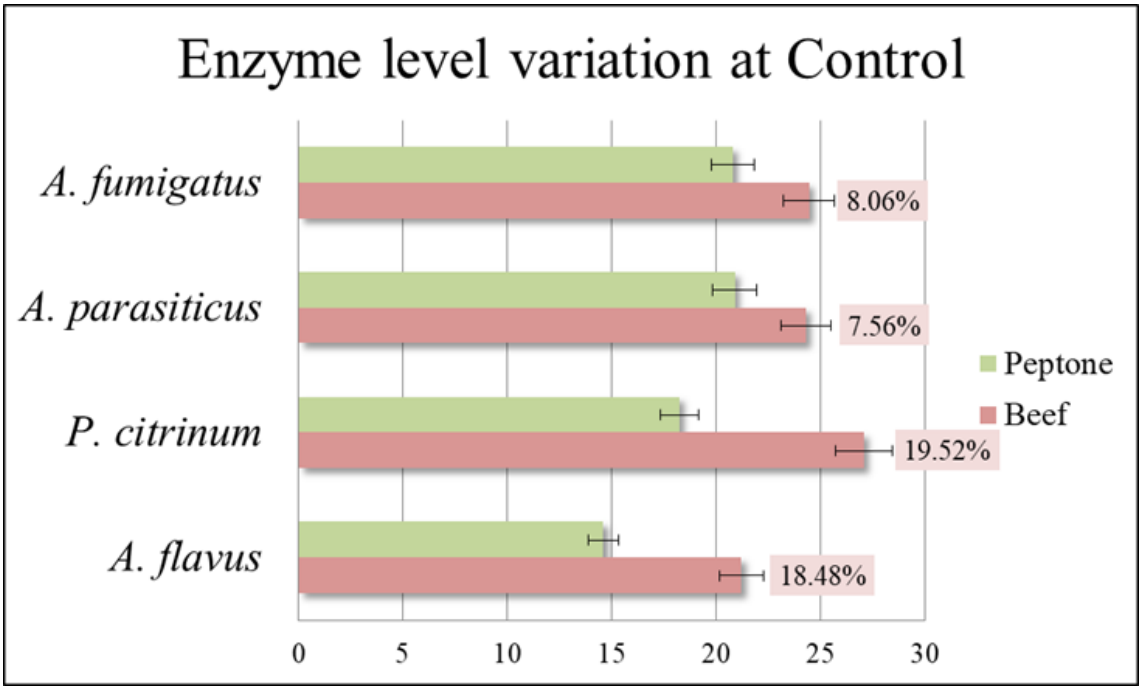


Figure 41: Enzyme level variation in fungal strains depending on the growth media at control condition

Total enzyme secreted by each fungi, growing in each of the growth media broths was studied. It was observed that under control conditions, all of the fungal strains have shown more enzyme production when incubated in beef extract media specific broth compared to when incubated in peptone media specific broth. *P. citrinum* has shown a maximum variation

in enzyme production with 19.52% more enzymes produced in case of beef broth than in case of peptone broth. The average enzyme produced by the fungal strains growing in peptone broth is 74.53 EU/ml, whereas that produced by the same strains when grown in beef broth is 97.06 EU/ml. An average of 13.13% preference towards beef extract media specific broth was observed in the fungi growing in control conditions.

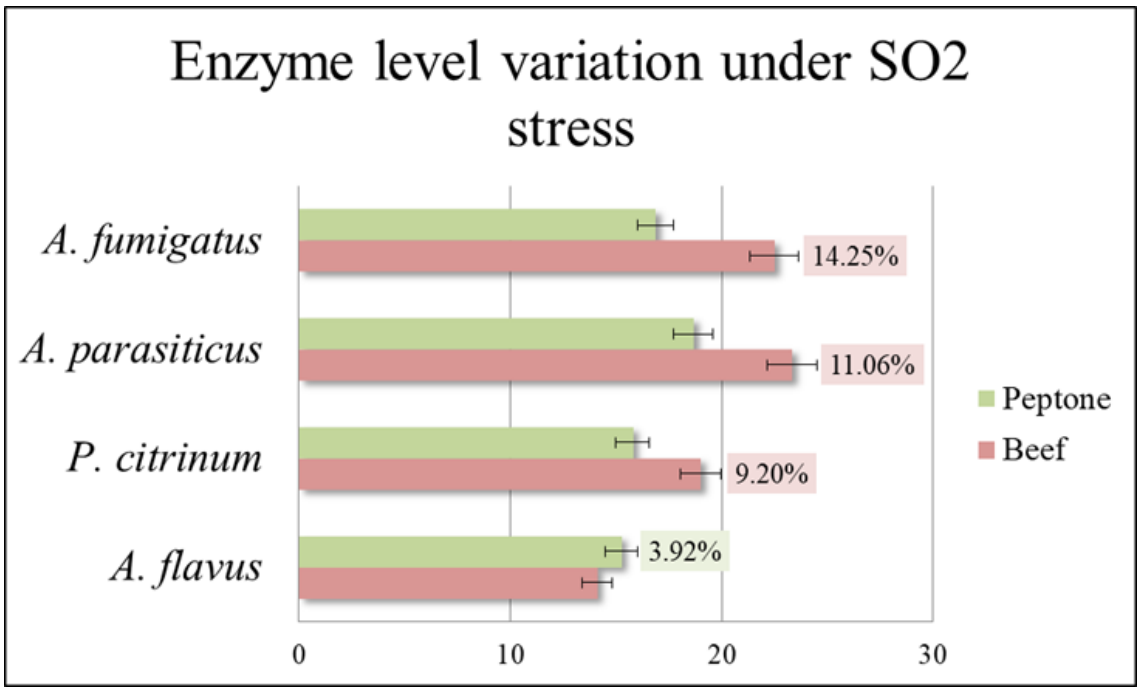


Figure 42: Enzyme level variation in fungal strains depending on the growth media under SO_2 stress condition

On application of 25ppm SO_2 stress, total enzyme secreted by each fungi, growing in each of the growth media broths was studied. It was observed that under this stressed condition, three out of the four fungal strains have shown more enzyme production when incubated in beef extract media specific broth compared to when incubated in peptone media specific broth. *A. fumigatus* has shown a maximum variation in enzyme production with 14.25% more enzymes produced in case of beef broth than in case of peptone broth. However, in case of *A.flavus*, 3.92% more enzyme was produced in peptone specific media broth than beef broth. The average enzyme produced by the fungal strains under SO_2 stress growing in peptone broth is 66.62EU/ml, whereas that produced by the same strains when grown in beef broth is 78.92 EU/ml. An average of 8.45% preference towards beef extract media specific broth was observed in the fungi growing in SO_2 stress conditions.

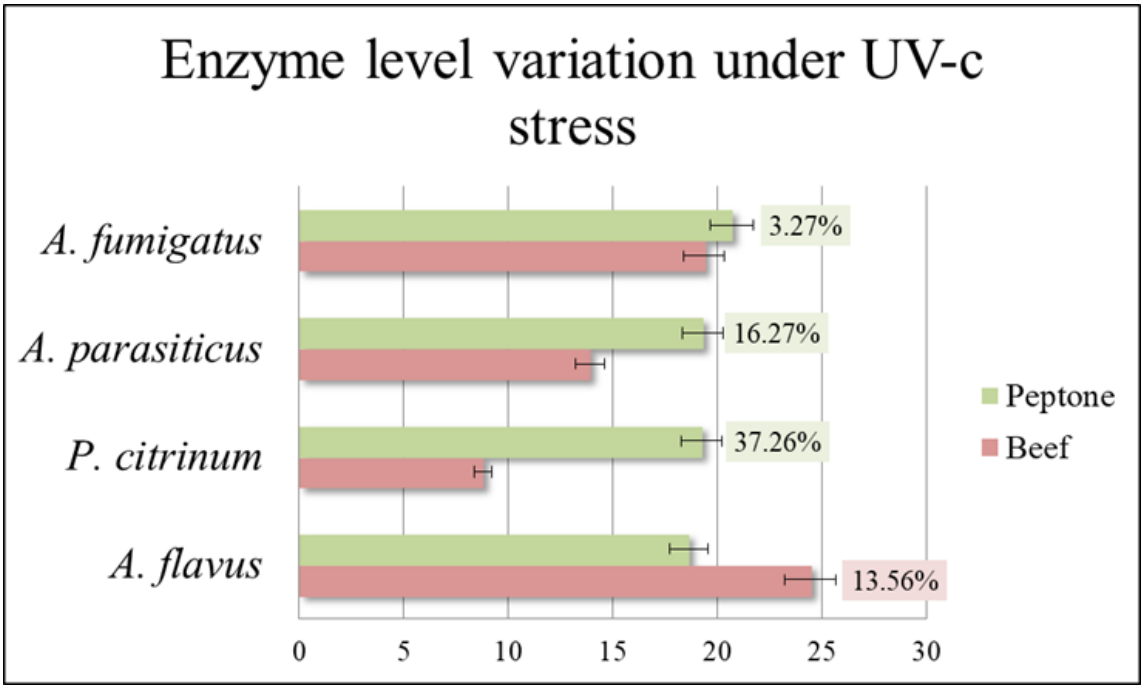


Figure 43: Enzyme level variation in fungal strains depending on the growth media under UV-C stress condition

On application of 625 µW/cm^2 UV-C stress, total enzyme secreted by each fungi, growing in each of the growth media broths was studied. It was observed that under this stressed condition, three out of the four fungal strains have shown more enzyme production when incubated in peptone media specific broth compared to when incubated in beef extract media specific broth. *P.citrinum* has shown a maximum variation in enzyme production with 37.26% more enzymes produced in case of peptone broth than in case of beef broth. However, in case of *A.flavus*, 13.56% more enzymes were produced in beef extract media specific broth than peptone broth. The average enzyme produced by the fungal strains under UV-C stress growing in peptone broth is 77.848 EU/ml, whereas that produced by the same strains when grown in beef broth is 66.54 EU/ml. An average of 7.83% preference towards peptone media specific broth was observed in the fungi growing in UV-C stress conditions.

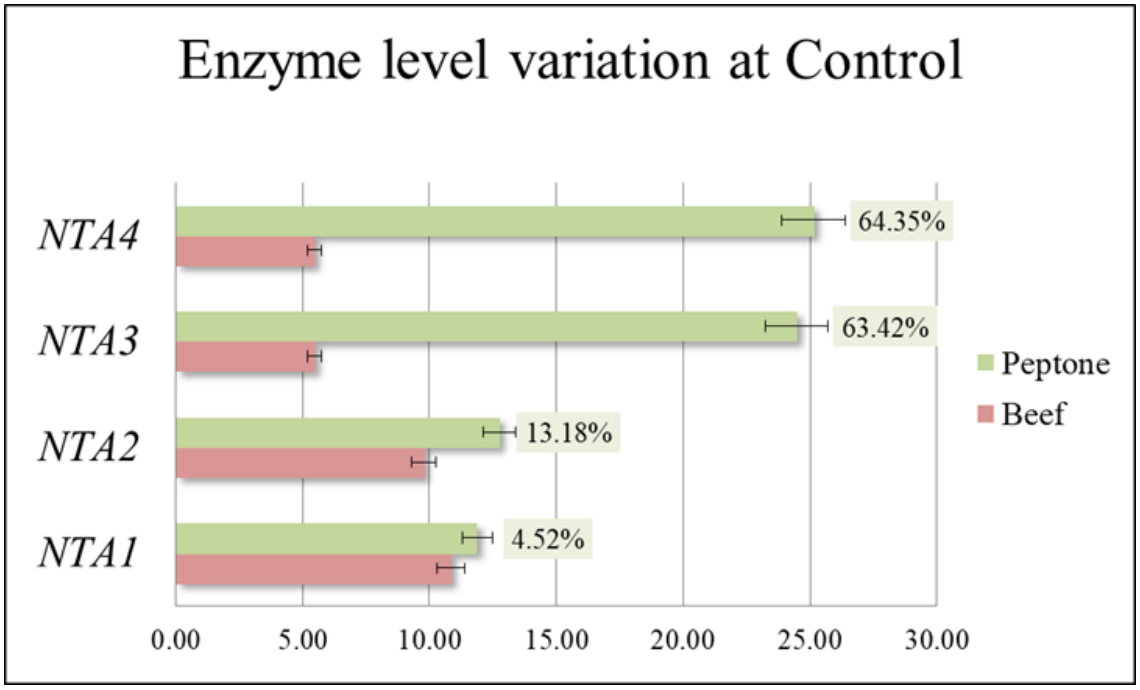


Figure 44: Enzyme level variation in bacterial strains depending on the growth media at control condition

Total enzyme secreted by each bacteria, growing in each of the growth media broths was studied. It was observed that under control conditions, all of the bacterial strains have shown more enzyme production when incubated in peptone media specific broth compared to when incubated in beef extract media specific broth. *NTA4* has shown a maximum variation in enzyme production with 64.35% more enzymes produced in case of peptone media broth than in case of beef broth. The average enzyme produced by the bacterial strains growing in peptone broth is 74.25 EU/ml, whereas that produced by the same strains when grown in beef broth is 31.56 EU/ml. An average of 40.35% preference towards peptone media specific broth was observed in the fungi growing in control conditions.

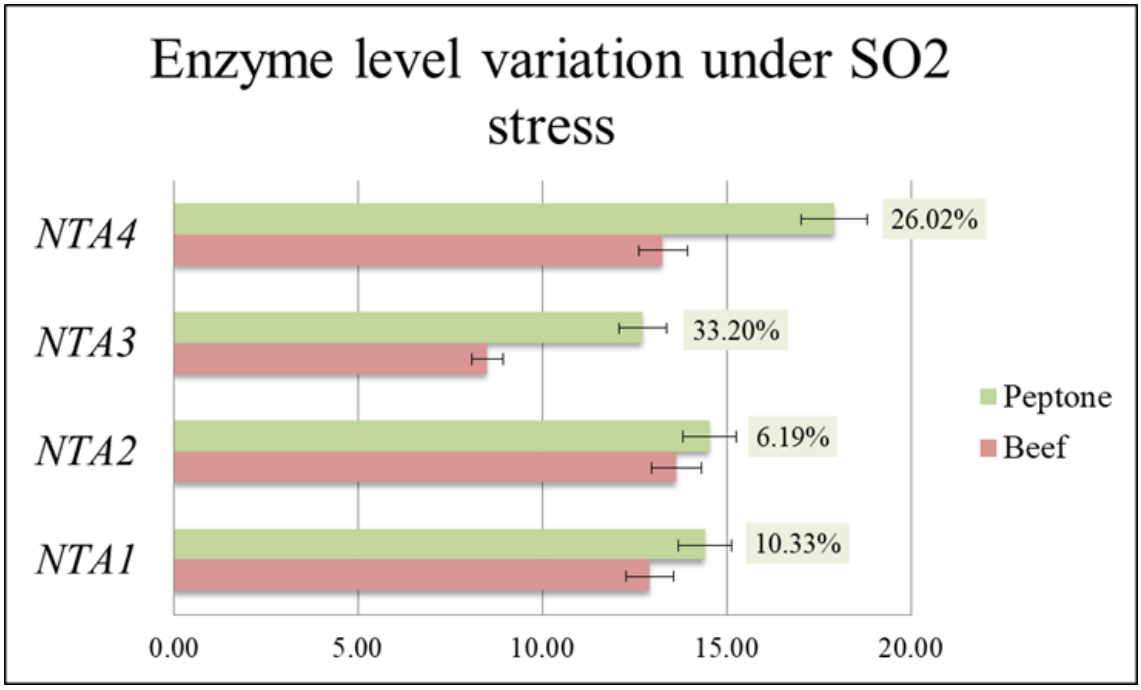


Figure 45: Enzyme level variation in bacterial strains depending on the growth media under SO_2 stress condition

On application of 25ppm SO_2 stress, total enzyme secreted by each bacteria, growing in each of the growth media broths was studied. It was observed that under this stressed conditions, all of the bacterial strains have shown more enzyme production when incubated in peptone media specific broth compared to when incubated in beef extract media specific broth. *NTA3* has shown a maximum variation in enzyme production with 33.20% more enzymes produced in case of peptone media broth than in case of beef broth. The average enzyme produced by the bacterial strains growing in peptone broth is 59.96 EU/ml, whereas that produced by the same strains when grown in beef broth is 48.29 EU/ml. An average of 10.45% preference towards peptone media specific broth was observed in the bacteria growing in SO_2 stress conditions.

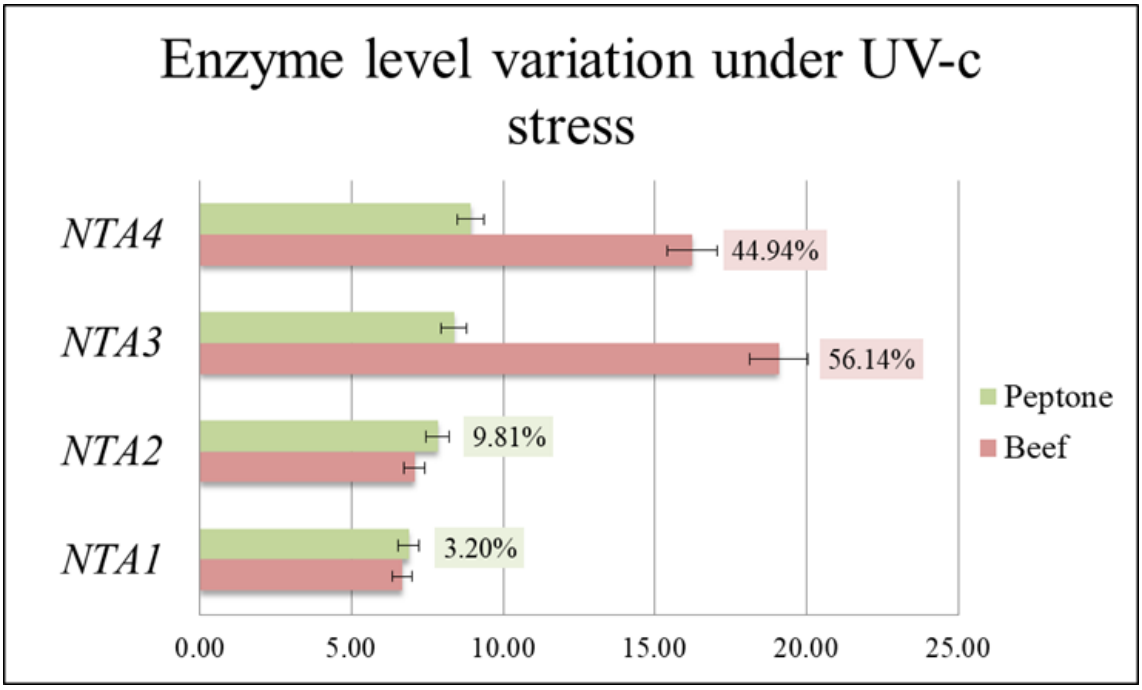


Figure 46: Enzyme level variation in bacterial strains depending on the growth media under UV-C stress condition

On application of 625 µW/cm^2 UV-C stress, total enzyme secreted by each bacteria, growing in each of the growth media broths was studied. It was observed that under this stress condition, the bacterial strains have shown equal preferences in enzyme production when incubated in peptone media specific broth and in beef extract media specific broth. *NTA4* and *NTA3* have shown greater preference for beef extract specific media broth. *NTA3* has shown a maximum variation in enzyme production with 56.14% more enzymes produced in case of beef media broth than in case of peptone broth. The average enzyme produced by the bacterial strains growing in peptone broth is 32.027 EU/ml, whereas that produced by the same strains when grown in beef broth is 49.04 EU/ml. An average of 20.99% preference towards beef extract media specific broth was observed in the bacteria growing in UV-C stress conditions.

7.5.4.Hourly variation of enzyme trend for each organism

The enzyme produced by each strain of bacteria and fungi under stressed condition were studied for 3 hour, 6 hour and 12 hour stress exposure. The variation in enzyme levels was studied with reference to the enzyme produced under no stress (condition) and the organism with most adaptability to pollutants was identified.

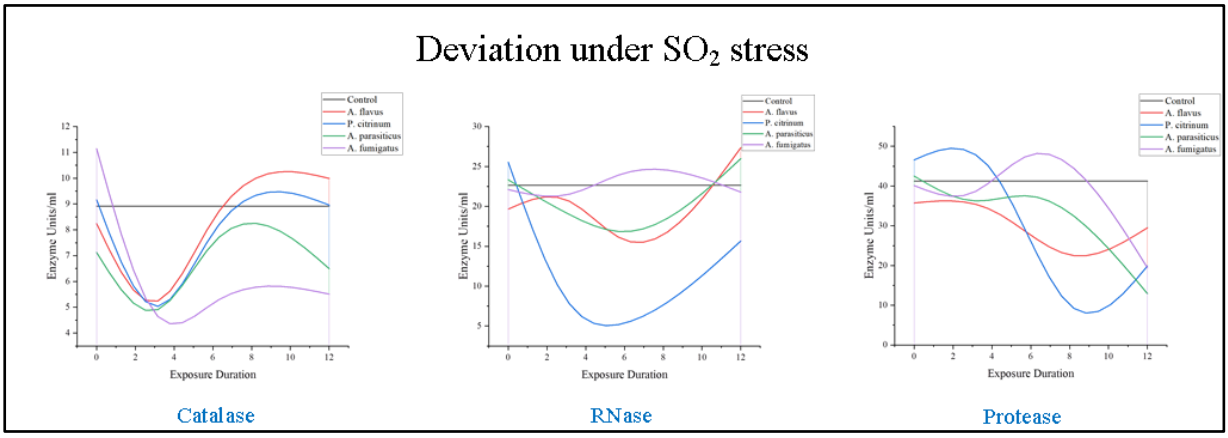


Figure 47: Deviation in enzyme produced by fungal strains under SO_2 stress

On application of SO_2 stress, maximum change in enzyme level with respect to control condition was observed in the production of protease enzyme by *P. citrinum*. However least variation in production of protease was observed in *A. fumigatus*. In case of production of RNase, least deviation was observed in *A. fumigatus* while *P. citrinum* showed maximum change. The production of catalase showed comparatively less variation with *A. fumigatus* having maximum change and *A.flavus* having least variation in enzyme level. It is thus observed that catalase is the most stable enzyme that is produced by fungi under application of SO_2 stress, where as, *A.fumigatus* is most adaptable to the stressed condition having least variation in levels of enzyme produced.

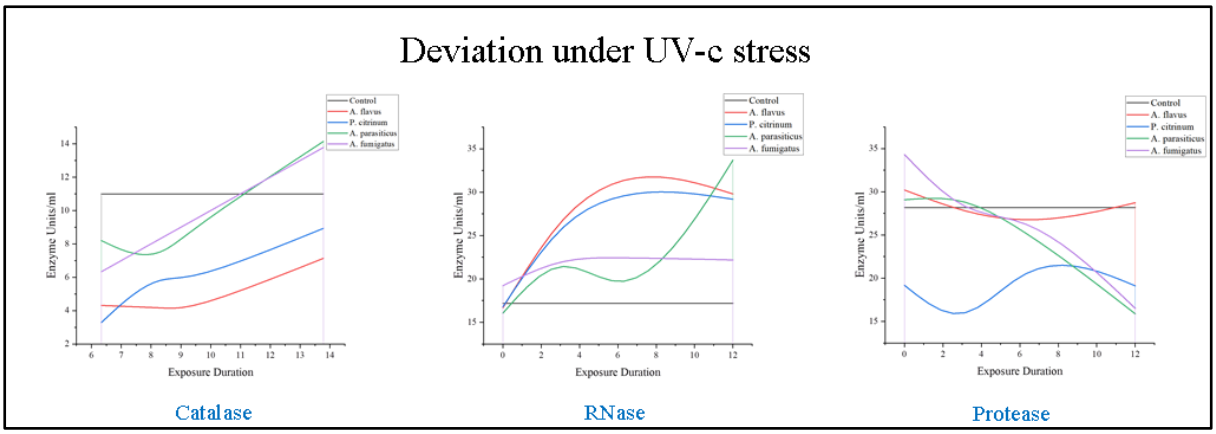


Figure 48: Deviation in enzyme produced by fungal strains under UV-C stress

On application of UV-C stress, maximum change in enzyme level with respect to control condition was observed in the production of RNase enzyme by *A. flavus* and least variation in production of the same was observed in *A. fumigatus*. In case of production of protease, least deviation was observed in *A. flavus* while *P. citrinum* showed maximum change. The production of catalase showed less variation when compared to other enzymes, with *A. fumigatus* having minimum change and *A.flavus* having highest variation in enzyme level. It is thus observed that catalase is the most stable enzyme that is produced by fungi under application of UV-C stress, where as, *A.fumigatus* is most adaptable to the stressed condition having least variation in levels of enzyme produced.

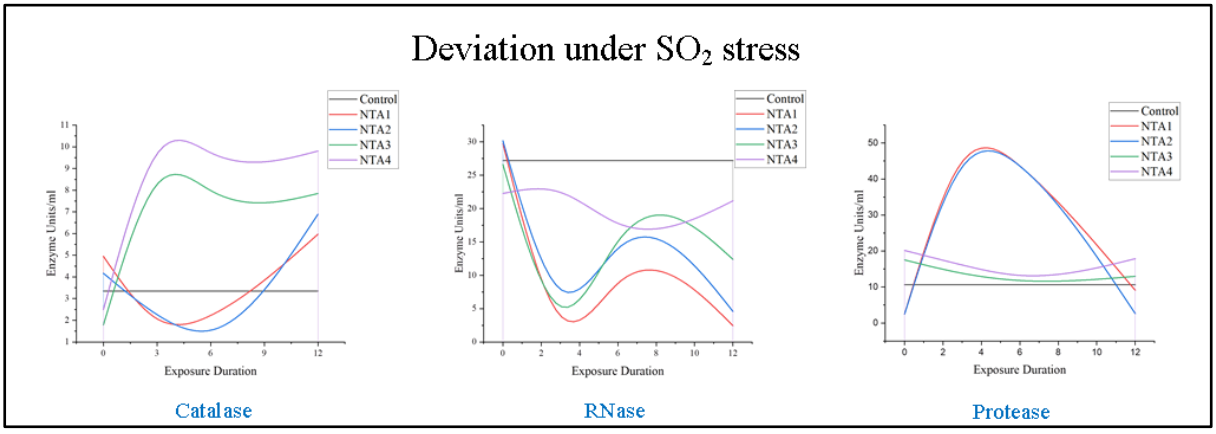


Figure 49: Deviation in enzyme produced by bacterial strains under SO_2 stress

On application of SO_2 stress in bacteria, maximum change in enzyme level with respect to control condition was observed in the production of protease enzyme by *NTA1*. However

least variation in production of protease was observed in *NTA3*. In case of production of RNase, least deviation was observed in *NTA4* while *NTA1* showed maximum change. The production of catalase showed least variation with *NTA4* having maximum change and *NTA1* having least variation in enzyme level. It is thus observed that catalase is the most stable enzyme that is produced by bacteria under application of SO_2 stress, where as, *NTA1* was affected most due to exposure of stress. However no conclusive result could be reached regarding which bacteria showed maximum adaptability to stress.

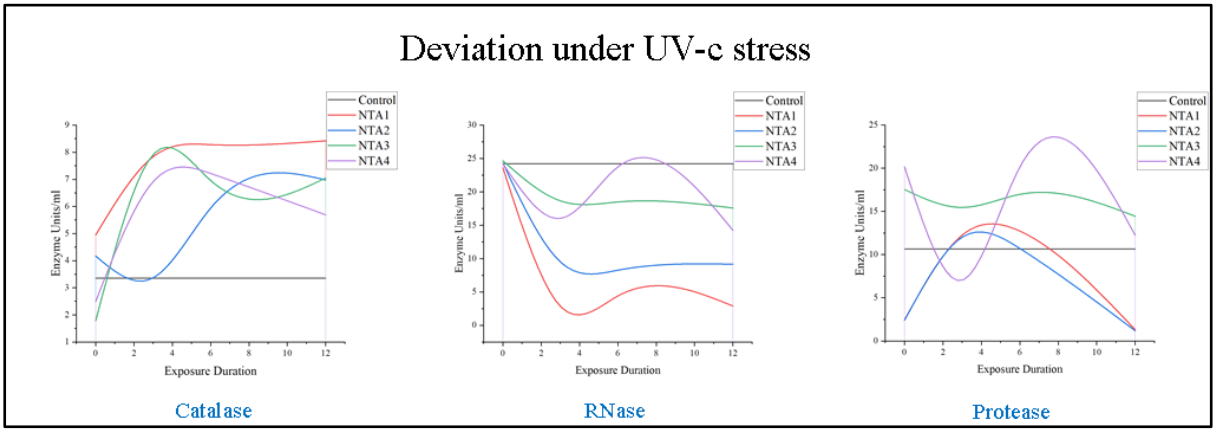


Figure 50: Deviation in enzyme produced by bacterial strains under UV-C stress

On application of UV-C stress, maximum change in enzyme level with respect to control condition was observed in the production of RNase enzyme by *NTA1* whereas least variation in production of RNase was observed in *NTA4*. In case of production of protease, least deviation was observed in *NTA1* while *NTA3* showed maximum change. The production of catalase showed overall least variation with *NTA1* having maximum change and *NTA2* having least variation in enzyme level. It is thus observed that catalase is the most stable enzyme that is produced by bacteria under application of UV-C stress, where as, *NTA1* was affected most due to exposure of stress. However in this case as well, no conclusive result could be reached regarding which bacteria showed maximum adaptability to stress.

7.6. Conclusion

The application of stress on fungal and bacterial cultures have impacts at various levels on their enzyme production capabilities and thus indicating changes in their metabolism.

On being exposed to 25ppm SO_2, fungal strains have produced greater amount of enzymes compared to that under exposure of 625µW/cm^2 UV-C, of which maximum enzyme

produced was RNAse, followed by protease and catalase. While under exposure of UV-C, maximum amount of protease was produced, catalase remained to be the least secreted enzyme. However when the variation of enzyme levels were studied in terms of 3 hour, 6 hour and 12 hour exposure, the levels of catalse were found to be most consistent through all stress period while maximum fluctuation in levels was observed in protease indicating that production of catalase by fungi is least affected by the level of pollutants in atmosphere. A general preference of growth media was observed in case of the fungal strains when exposed to stress with more enzyme being produced in beef extract media specific broth in case of control and SO_2 stress, while the preference shifted to peptone media specific broth in case of UV-C stress.

In case of bacterial strains, when exposed to stress, greater amount of enzyme was produced under SO_2 stess than under UV-C stress. The enzyme protease was secreted the most under SO_2 stress which showed greastest variations when studied under hourly stress. In case of UV-C stress, identical behaviour was shown by RNase enzyme. Even under UV-C stress, catalase was found to be the enzyme that has been secreted in least quantity but was found to be most stable under hourly stress variation. The bacterial strains have portrayed a greater preference towards peptone media specific broth in case of control and SO_2 stress while in case of UV-C stress, more enzyme was produced by the strains growing in beef extract media specific broth.

Studying all the above conditions, it was observed that the preference towards growth media in case of both fungi and bacteria remained the same in case of SO_2 stress, as that in the non stress condition while it changed in case of application of UV-C stress. Catalase through produced in least quantity in all the cases was found to be the most stable enzyme under various stress exposure conditions. Lastly, *A. fumigatus* was found to be the most resilient specie of microorganism under all conditions of stress exposure.

CHAPTER 8: DISCUSSION

Air pollution is a major problem that has toxicological impacts on human health and the environment. It is caused by both human interventions and natural phenomena, and it consists of solid particles and gases in the air. Sources of pollution range from small units like cigarettes to large emissions from motor engines and industrial activities (Harkat et al., 2022). From the data obtained from the West Bengal Pollution Control Board, it was observed that the level of air pollutants like SO_2, NO_2, PM_{10} and $PM_{2.5}$ have been, in most cases, above the standard permissible limit as per the National Ambient Air Quality standards. While PM_{10} level has shown a maximum deviation from the standard limit throughout the sampling tenure. The study conducted through three seasons, autumn, winter and spring showed a gradual increase in the level of pollutants in autumn, with a 90.94% increase in SO_2 concentration, 4.29% increase in NO_2 concentration and 30.55% increase in PM_{10}. The average concentration for SO_2 and PM_{10} shows a further increase of 24.52% and 47.86% respectively, through the winter months. The concentration of NO_2 however decreased drastically in January resulting in a 16.47% decrease in average concentration. However, in the following spring season, the concentration of pollutants in air showed a decrease through the months of February and March. The combined air pollutants have reached its peak in December 2022 thereby decreasing again. Since New Town is a developing township that is going through its implementation stage, major source of these air pollutants is construction works and vehicular pollution. Stubble burning and burning of coal oven in road side eateries are major cause of concern that adds on to the sources of pollutants in air("New Town Stubble Burning Leads to Spike in Pollution," 2020). New Town hosts a fair share of moving population a large number of whom are construction workers and migrant workers who are likely to set up fire on the roadside to keep themselves warm through the winter months, this could be the cause of hike in pollution in December.

The microbial colonies have shown a general trend of sparse growth in the winter months of November and December and have shown relatively more growth in the post winter or post monsoon season. The diversity of these colonies is dependent on factors like temperature and relative humidity. During the winter months, the temperature goes down up to 15ºC which is much lower than the optimum growth temperature for bacteria and fungi. In the monsoon and post monsoon months of September and October, the temperature varies from 26º-33ºC which is ideal for the growth of both fungi and bacteria. It was observed that despite the presence of the air pollutants, both fungal and bacterial communities have shown considerable seasonal variations and have not been eliminated completely in any season. It

was also seen that pollutants such as SO_2 were within the permissible limit, as per CPCB guidelines, and thus could not have been solely responsible for the negative impact on the microbes. The pollutants while coexisting in atmosphere might be responsible for the variation in concentration of bio aerosols. The level of pollution along with atmospheric changes such as temperature and humidity are together responsible for the changes in fungal and bacterial concentration in air.

The correlation of presence of heavy metals in the road dust and their impact on the microbial concentration was also studied through this project. Crustal enrichment factor for each of the elements at each location was studied and a general trend of Zn>Cr>Ca>Rb>Ti>Mg>Fe>Mn was found. The metals having highest concentration and having EF>2 at a minimum of two locations were selected for further study. Highest enrichment factor was observed for Akankha More> Rail Vihar Gate 1> City Center> New Town More and lowest enrichment factor was found for Arts Acre and Eco Urban Village.

It can be seen that maximum amount of heavy metal have been found at locations having highest traffic inflow. Akankha More and New Town More are two of the largest traffic intersections at New Town. The sources of this higher level of Zn and Cr are thus expected to be from traffic emissions. The northern and central area of New Town has shown significant crustal Ca enrichment. Simultaneously an increased concentration of fungal colony was found in that area which could have been caused due to enhancement of growth, development, and virulence in fungi (Roy et al., 2021). The growth of bacterial community however was dominated in the area with Ca enrichment. The higher level of Ca in the road dust is mainly from roadside cooking and burning of coal in small eateries present along the streets of New Town and not from biological sources. Other than that, since New Town is an area that is currently undergoing constructional development, heavy metal contamination in roadside dust could also have been caused due to the presence of construction dust. However owing to the fact that during the sampling tenure of pre-winter, winter and winter, with wind flowing from the North, through the eastern parts of Indo-Gangetic plains, the origin of the Ca in road dust could not have been from organic waste disposal systems at Mollar Bheri or Dhapa (Mallik et al., 2014; Sengupta, 2019). Significant amount of Cr and Zn enrichment was also found from the collected road dust which inhibited the growth of fungi while benefitting the growth of bacteria in some areas. The sources of these heavy metals were predominantly vehicular traffic (Karar et al., 2006) and presence of small industries along the border of NKDA. It was observed from the figures that the variation in the fungal and

bacterial concentrations were non dependant on the presence of any individual heavy metal but on the cumulative presence of pollutants along with the meteorological factors.

For the in-vitro study of the nature of the micro-organisms, four strains each of fungi and bacteria were isolated from the samples collected at New Town. The isolates were then exposed to criteria pollutants such as SO_2 and UV-C stress. Strains of fungi selected for further study were *Aspergillus flavus, Aspergillus fumigatus, Aspergillus parasiticus*, and *Penicillium citrinum*. Bacteria identified using gram staining were labelled as *NTA1, NTA2, NTA3*, and *NTA4*.

The application of stress on fungal and bacterial cultures has impacts at various levels on their enzyme production capabilities and thus indicating changes in their metabolism.

On being exposed to 25ppm SO_2, fungal strains have produced greater amount of enzymes compared to that under exposure of 625μW/cm^2 UV-C, of which maximum enzyme produced was RNAse, followed by protease and catalase. While under exposure of UV-C, maximum amount of protease was produced, catalase remained to be the least secreted enzyme. However when the variation of enzyme levels were studied in terms of 3 hour, 6 hour and 12 hour exposure, the levels of catalase were found to be most consistent through all stress period. In case of bacterial strains, when exposed to stress, greater amount of enzyme was produced under SO_2 stress than under UV-C stress.The enzyme protease was secreted the most under SO_2 stress which showed greatest variations when studied under hourly stress. In case of UV-C stress, identical behaviour was shown by RNase enzyme. Even under UV-C stress, catalase was found to be the enzyme that has been secreted in least quantity but was found to be most stable under hourly stress variation. Studying all the above conditions, it was observed that the preference towards growth media in case of both fungi and bacteria remained the same in case of SO_2 stress, as that in the non-stress condition while it changed in case of application of UV-C stress. Catalase through produced in least quantity in all the cases was found to be the most stable enzyme under various stress exposure conditions. Lastly, of all fungi and bacteria, *A. fumigatus* was found to be the most resilient specie of microorganism under all conditions of stress exposure.

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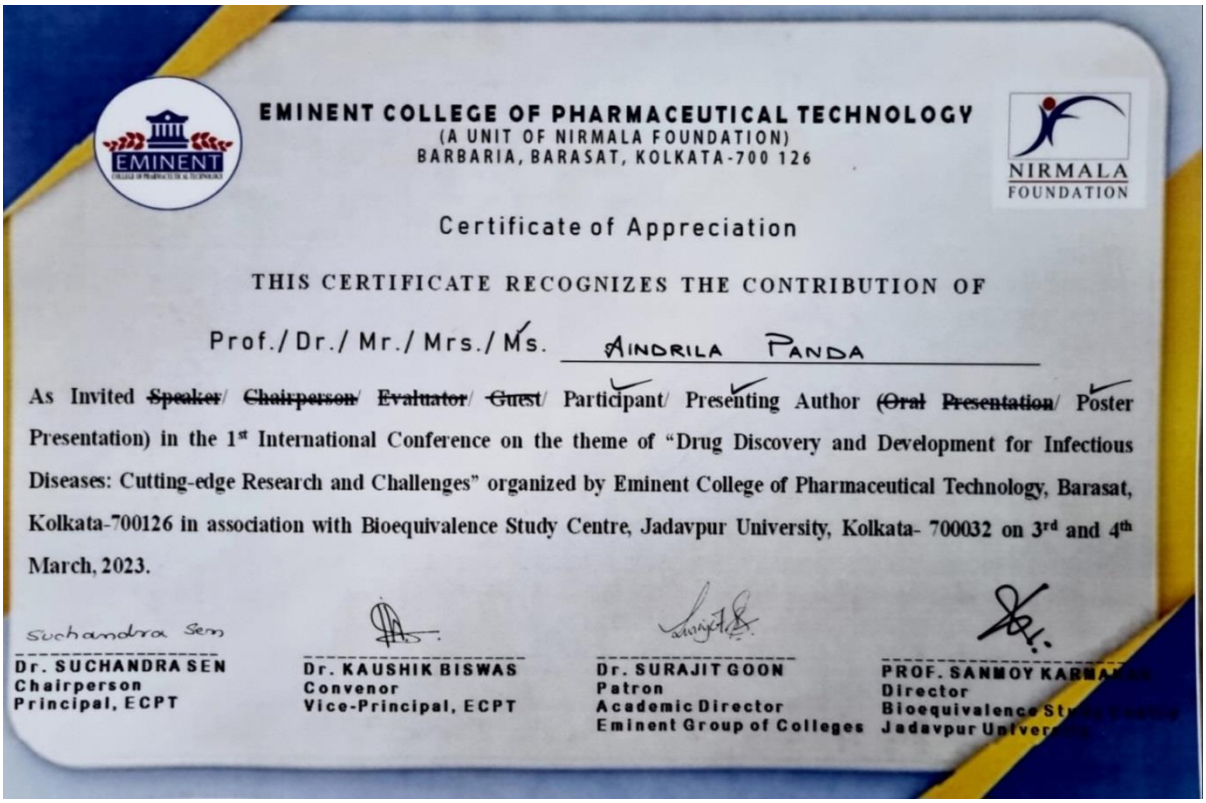
COMMUNICATIONS

Two Day International Seminar
on
Sustainable Development:
From the Perspective of Nature and Nurture
Organized by
Department of Geography, School of Sciences
Netaji Subhas Open University
in collaboration with
Byanjanbarna Foundation
Date: 10th- 11th February, 2023 Time: 10:30A.M.-5:30 P.M.
Venue: Subhas Chandra Sabhaghar, NSOU, Salt Lake, Kolkata-700064, India

This is to certify that ...Aindrila Panda...
of..Jadavpur University.....participated/presented paper titled
..Changes in Microbial Enzyme Production Due To Ambient Air Pollution.....
.....in the above mentioned seminar.

Dr. Biraj Kanti Mondal
Assistant Professor
Dept.of Geography,School of Sciences
Netaji Subhas Open University
and Secretary,Seminar Organizing Committee

Dr. Debabrata Biswas
Secretary
Byanjanbarna Foundation



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Certificate of Appreciation

THIS CERTIFICATE RECOGNIZES THE CONTRIBUTION OF

Prof./Dr./Mr./Mrs./Ms. AINDRILA PANDA

As Invited ~~Speaker~~/ ~~Chairperson~~/ ~~Evaluator~~/ ~~Guest~~/ Participant/ Presenting Author (~~Oral~~ ~~Presentation~~/ Poster Presentation) in the 1st International Conference on the theme of "Drug Discovery and Development for Infectious Diseases: Cutting-edge Research and Challenges" organized by Eminent College of Pharmaceutical Technology, Barasat, Kolkata-700126 in association with Bioequivalence Study Centre, Jadavpur University, Kolkata- 700032 on 3rd and 4th March, 2023.

Suchandra Sen
Dr. SUCHANDRA SEN
Chairperson
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