

# AN ANALYTICAL STUDY OF THE AIR QUALITY, MICROBIAL INDEX AND METAL-MICROBIAL INTERACTIONS USING LEAF AS AN ASSESSMENT TOOL

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**Abstract:** Suspended particulate matter (SPM) refers to the very fine solid or liquid aerosol particles suspended in the atmospheric air. Sometimes, in certain areas, due to industrial or combustion activities, there can be a fraction of particulate heavy metal found in the air of that particular place, which can have considerable negative impact on the air quality of that place, and in turn, can have adverse health effects. However, the natural microbial population found on the leaf surfaces can sometimes possess abilities to tolerate the metal content of the deposited SPM and also uptake those metals, which ultimately turn out to be beneficial for both the plant as well as the air quality of that place. In the present study, 10 different places in and around Kolkata were chosen and a common garden plant, *Hibiscus rosasinensis* (China rose), was selected from each place and the SPM deposits on the leaf surfaces were collected. Firstly, the varying SPM deposits on the leaf surfaces were analyzed by weighing followed up by the total dissolved solids and electrical conductance tests. The next part of the study aimed at studying the effects of deposited SPM on the physiology of those respective plants, for which tests like chlorophyll assay, along with estimation of transpiration index and stomatal index were carried out. Also, the categorization of the microbial population into various microbial communities was done experimentally to obtain a clear idea regarding the existence of the types and diversity of microbes of those areas. Finally, more significant aspects like metal ( $Pb^{+2}$ ) tolerance and metal ( $Pb^{+2}$ ) uptake (metal of maximum existence) by the leaf microbial population (culture of maximum existence) of a particular location (Howrah), identified to be a member of the genus *Micrococcus* was studied and quantified, thereupon paving a new vista in the arena of micro-remediation and removal of air pollution issues to a greater elaboration.

**IndexTerms:** Suspended particulate matter, heavy metals, Atomic absorption spectroscopy, micro-remediation, *Micrococcus* sp, Kolkata.

## I. INTRODUCTION

Air pollution seems to be one of the most life-threatening issues and a severe concern of the current century. Rapid increase in modernization, development and technical advancements in all spheres of life in the recent years, have lead to marked deterioration in quality and purity of air [5]. It is quite alarming and disappointing to note that the main cause of the fall is not only limited to Nature's own actions, but majority being a part of anthropological activities.

Suspended particulate matter (SPM) in air refers to all airborne solid and low vapour pressure liquid particles, which involve a complex, multi-phase system consisting of a spectrum of aerodynamic particles ranging from below 0.01  $\mu\text{m}$  to 100  $\mu\text{m}$  or larger. With reference to the data provided by the National Ambient Air Quality Standards (NAAQS), SPM also known as only PM (particulate matter) [13] is categorized into  $PM_{2.5}$  and  $PM_{10}$  depending upon their size [12], which is generally expressed in terms of micrometers ( $\mu\text{M}$ ). SPM can be either present in the atmosphere as a result of direct emissions (Primary SPM) or can be formed in the atmosphere itself as a result of various gaseous transformation reactions (Secondary SPM). A fraction of this SPM, which is contributed by heavy metals vaporized during combustion with equilibrium pressures low enough to form nuclei mode particles yielding particulate matter, has been a prime aspect of our study, considering the fact that long-term exposure to metallic particles may cause impairment of pulmonary surfactant and lung function, resulting in chronic respiratory diseases and several other health hazards [14].

Microorganisms share both a direct and indirect relationship with pollution. Air-borne microorganisms play a dominant role in transforming pollutants especially SPM that reach the environment [17]. This is due to the fact that microorganisms exhibit an extremely wide metabolic diversity, and hence, are able to degrade a very wide variety of chemical compounds.

Nowadays, with growth of industrialization and extraction of natural resources, there has been a considerable increase in the accumulation of heavy metals in the environment [3]. Contamination of ground water, surface water, soil, sediments and air with hazardous heavy metals and toxic chemicals is one of the major threats facing the world. As they cannot be broken down to non-toxic forms, they intervene into food-webs and therefore have long-lasting effects on the ecosystem [7, 18]. However, microorganisms uptake heavy metals actively (bioaccumulation) and/or passively (adsorption). The microbial cell walls, which mainly consist of polysaccharides, lipids and proteins, offer many functional groups that can bind heavy metal ions, and these include carboxylate, hydroxyl, amino and phosphate groups. However, it is very difficult to maintain a healthy population of microorganisms due to heavy metal toxicity and other environmental factors [6].

In this study, the air quality prevalent in 10 different zones of Kolkata was evaluated by estimation of the accumulation of SPM on leaves, its effect on the microbial diversity, and ultimately how these microbes in turn combat against the metals borne with SPM. The reason for carrying out the study forward with leaves lies solely in the definition of SPM, as it is air borne and tends to collect on vegetation [19, 15]. This study mainly takes into consideration the manifold ways in which the internal functionality of a plant such as its transpiration rate, chlorophyll content and its overall physiology is affected due to the deposition of suspended particulate matter on the surface of leaves [1, 8]. Not only does the deposited SPM contribute to the clogging of stomatal pores [9] thereby affecting the normal functioning of plant systems, but also the SPM carries along with itself many traces of heavy metals which does have a different impact on the plant depending on whether those heavy metals are taken up by the microflora dwelling on the leaf surface or they keep on accumulating to affect the plant, in a negative way. Moreover, leaves as the sample helped to improvise the study by metal analysis, their subsequent tolerance by the phylloplane microorganisms, their metal uptake, and to study effect of metals in the plant physiology itself. All these evaluations in a direct or indirect way throw light on the quality of air persisting in that particular location. However, it should be noted that the study has used only one plant system as the model with the impression that the mechanisms revealed herein apply to all, with only degree of the effects varying with location. However, given the quantity or level of air pollution in varied locations, it is inevitable that significant differences may exist in the mechanisms of manipulation.

Hence, for a successful analysis and study, the leaves of *Hibiscus rosasinensis*, commonly called China rose, were collected from each location and further work of SPM evaluation, estimation of the metals present on the leaves, their interaction with the microorganisms present, tolerance index and subsequent uptake were carried forward. With the proceeding of the work,  $Pb^{+2}$  (lead) [4] was found to be the dominant heavy metal among all the four metals ( $Cu^{+2}$ ,  $Fe^{+2}$ ,  $Ni^{+2}$ ,  $Pb^{+2}$ ) studied. Moreover, among all the zones, the maximum concentration of  $Pb^{+2}$  was reported from Howrah and hence, the microbial colony from Howrah showing a dominant growth on the culture plates, was utilized to discern if such a  $Pb^{+2}$  tolerant culture could be an efficient means of metal removal as well.

## II. MATERIALS AND METHODS

### 2.1 Materials

Leaves of *Hibiscus rosasinensis* from 10 different areas of Kolkata and its outskirts were taken, viz., Batanagar, Behala, Bhadreswar, Bowbazar, Dumdum, Garia, Howrah, Jadavpur, Salt Lake and Shyambazar. Nutrient broth and agar media were used to culture the microorganisms. Chemicals like Hydrochloric acid, Nitric acid, Methanol, Cobalt chloride, lead acetate; metal salts ( $Cu^{+2}$ ,  $Fe^{+2}$ ,  $Pb^{+2}$ ,  $Ni^{+2}$ ) were used for the study.

### 2.2 Methodology

#### 2.2.1 Plant under study

*Hibiscus rosasinensis* (China rose) is an evergreen bushy shrub or small tree, growing about 2.5–5 m tall and 1.5–3 m wide, having glossy leaves. Flowers are solitary, bisexual, complete, brilliant red in colour that blooms in summer and autumn.

The main reason for the choice of this particular plant is credited to the fact that its leaves harbour a broad surface area beneficial for deposition of particulate matter on them. Also the fact that the plant is available at ease in almost any area in Kolkata posed a major advantage.

#### 2.2.2 Aspects under study

The levels of pollution in different areas can be estimated by the decrease in transpiration rate, decrease in stomatal count and decrease in the chlorophyll content of leaves (chlorosis), as in a highly polluted area, the amount of particulate matter settled on leaf surfaces will be high enough to cut down the above factors. The microbial community found suspended in the air of different areas can be studied by collecting the leaf wash of plants from the respective areas and analyzing it for any microbial population. The SPM collected on the surface of leaves were also checked for detection of deposited metal, to gain knowledge about any suspended metal dust impurities that might be present in the air. Microorganisms are able to tolerate and uptake heavy metals by chelation and sequestration, resulting either in removal of toxic metal from sensitive sites or conduction of essential metals to their specific cellular destination. This study was carried out at a later stage.

### 2.2.3 Sites chosen for study

Leaf samples were collected from 10 different areas of Kolkata and its outskirts (outdoor) viz., Batanagar, Behala, Bhadreshwar, Bowbazar, Dumdum, Garia, Howrah, Jadavpur, Salt Lake and Shyambazar (**Table 1**). A culture room (indoor) was chosen to be the “control” where the air was quite sterile.

**Table 1:** Description of sites chosen for study

LOCATION	CO-ORDINATES	ACTIVITIES
1. Batanagar	22.5133° N, 88.2230° E	Residential activities, Less traffic, Developing zone
2. Behala	22.4981° N, 88.3108° E	Vehicular movement, Road traffic, Construction, Residential activities
3. Bhadreshwar	22.8276° N, 88.3457° E	Residential activities, Less traffic, Developing zone
4. Bowbazar	2.5668° N, 88.3615° E	High vehicular movement, Road traffic, Jewellery(Au, Ag) making, Residential activities
5. Dumdum	22.6471° N, 88.4317° E	Airport zone, High vehicular movement, Road traffic, Residential activities
6. Garia	22.4629° N, 88.3968° E	Vehicular movement, Residential activities
7. Howrah	22.5958° N, 88.2636° E	Railway zone, Vehicular movement, Road traffic, Commercial activities, Residential activities
8. Jadavpur	2.4940° N, 88.3707° E	University zone, Vehicular movement, Residential activities
9. Salt lake	40.7608° N, 111.8910° W	Residential activities, Less traffic, Software sector
10. Shyambazar	22.5982° N, 88.3687° E	High vehicular movement, Road traffic, Commercial activities, Residential activities

### 2.2.4 Procedures undertaken

#### i. SPM evaluation

For calculating the percentage increase in Suspended particulate matter (SPM), the healthy leaves of *Hibiscus rosasinensis* from different areas were collected and their initial weights were measured. These leaves were washed with sterile distilled water and their weights were again measured after the wash. The difference in the final and the initial weights gave the mean suspended particulate matter on these leaves.

#### ii. Measurement of Total dissolved solids (TDS) and Electrical conductance (EC)

Total dissolved solid is the measure of all organic and inorganic substances dissolved in a given liquid revealing the proportion of different solids. Fresh filter papers were weighed and the leaf wash was passed through them. The residue left on the filter paper was then kept in hot air oven and dried. The dried filter paper having the leaf wash residue was again weighed and the difference in weight of the filter papers gave the measure of the total dissolved solid (TDS). Electrical conductance was performed to measure the solution's ability to conduct electricity i.e., to measure the purity of the sample. Four medium sized leaves from each area were taken and washed in 50 ml of distilled water. Then the lid of the conductivity meter was inserted in the leaf wash and the results were noted in  $\mu\text{S}$  (microsiemens).

#### iii. Estimation of transpiration index of plant under study

3% cobalt chloride solution was taken and poured into a petri dish. Some filter paper strips were then dipped into the cobalt chloride solution and kept for 3-5 minutes until they became pink in colour (when wet). The strips were then removed and were allowed to dry till it turned blue in colour (on drying). The dried strips of cobalt chloride paper were placed on the transpiring surface of the leaf. Glass slides were placed over it and the slides were clipped using binder clips. The time taken by the cobalt chloride paper to change its blue colour to pink was noted on the transpiring surface of the leaf. Similarly, the time for a standard colour change of cobalt chloride paper over a free water-evaporating surface was also taken into account in order to calculate the transpiration index per leaf sample.

**iv. Total stomatal count**

To determine the stomatal index, a thick layer of adhesive was applied on the lower surface of the leaf and was allowed to dry. The adhesive layer was then peeled off after it was completely dried and the impression of the same was then observed under the microscope (40X with 0.45mm field size) in order to obtain the stomatal index.

**v. Chlorophyll assay and Chlorosis estimation**

Methanol extract of 0.5g fresh green leaves and 0.5g dried yellow leaves from each set up was prepared separately and centrifuged at 5000 rpm for 5 minutes at room temperature. Then the supernatants were collected in separate eppendorf tubes and absorbance of chlorophyll content was measured at 652nm and 665 nm using UV-Vis spectrophotometer.

**vi. Phyllosphere Organism isolation and characterization**

- a) **Isolation of organism:** Leaves from different areas were suspended in 10 ml of sterile distilled water separately.  $10^{-1}$  and  $10^{-2}$  dilutions for each sample were prepared in 1:10 ratio in separate tubes of sterile distilled water. 1 ml from each of the dilutions corresponding to each of the samples were added to molten nutrient agar (N.A), homogenized, then poured on petriplates and kept for 48 hours incubation.
- b) **Characterization of the organism:** Bacterial smear was prepared on glass slide and gram staining was performed and observed under the light microscope.

**vii. Metal detection on leaf surface**

Medium sized leaves from each location were taken and washed in 10 ml of deionized water. To this, 10 ml leaf wash, 10 ml 2%  $\text{HNO}_3$  was added and boiled on hot plate in porcelain crucibles for 15 minutes (process known as acid hydrolysis). Then it was filtered and the filtrate was collected in a test tube. A blank solution was prepared by adding 10 ml of 2%  $\text{HNO}_3$  in 10 ml distilled water. Concentrations of metals chosen as standard are listed in **Table 2**. The prepared standard solutions for copper, nickel, iron and lead, the filtrate after acid hydrolysis and the blank solution were subjected to Atomic Absorption Spectroscopy (AAS) for metal detection assay and the data was noted down.

**Formula used:-**  $V_1 \times S_1 = V_2 \times S_2$

**Table 2:** Concentrations prepared as standard for metal detection

Standard	Concentration of metals (in ppm)
1	1
2	2
3	5
4	10
5	15
6	20

**viii. Metal ( $\text{Pb}^{+2}$ ) tolerance of the selected bacterial isolate**

The  $\text{Pb}^{+2}$  tolerance test was performed with the bacterial culture that was found in maxima on the culture plates of Howrah (the location that recorded the maximum concentration of  $\text{Pb}^{+2}$  accumulations). Solutions of different concentration (50 ppm, 100 ppm, 150 ppm, 200 ppm) were prepared by adding 24 hours log phase bacterial culture, nutrient broth and lead acetate [ $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$ ] solution from the original stock solution having concentration of 1000 ppm, as listed in **Table 3**.

Calculation for 1000 ppm lead ( $\text{Pb}^{+2}$ ) stock solution -

Mol weight of  $\text{Pb}^{+2} = 207.2$

Mol weight of  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 = 325.29$

% of  $\text{Pb}^{+2}$  in  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2 = (207.2 / 325.29) \times 100 = 63.697\%$

Therefore, to prepare 1000 ppm  $\text{Pb}^{+2}$  stock 1.57 grams of  $\text{Pb}(\text{C}_2\text{H}_3\text{O}_2)_2$  was dissolved in 1000ml of deionised water.

**Table 3:** Concentration of metal (Pb<sup>2+</sup>) for metal tolerance and metal uptake test

Concentration (ppm)	Amount of metal solution added (ml)	Amount of nutrient broth added (ml)	Amount of inoculum added (ml)
Control	-	9	1
50	0.5	8.5	1
100	1	8	1
150	1.5	7.5	1
200	2	7	1

The tubes were then kept in shaker and absorbance was measured at 580 nm for four consecutive days.

#### ix. Metal (Pb<sup>2+</sup>) uptake by the selected bacterial isolate

Metal uptake capacity for the selected isolate was checked at 100ppm since LD50 was noted at the said concentration. Three tubes were taken out of which the first one was control, consisted of 9ml NB and 1ml of culture inoculum. Second was the test with 8ml of NB, 1ml of 100ppm lead acetate solution and 1 ml of inoculum and the third tube was considered as blank consisted of 10ml of NB. All the three tubes were centrifuged at 5000rpm for 10 minutes and the supernatant was collected in a separate tube and subjected to acid hydrolysis followed by Atomic Absorption Spectroscopy (AAS). The pellet formed was resuspended in 2ml sterile water and again centrifuged at 5000 rpm for 10 minutes. The supernatant and pellet was collected separately and acid hydrolysis was done to perform AAS.

#### x. Characterization of the selected bacterial isolate

After confirmation of the fact that the selected culture was tolerant to Pb<sup>2+</sup> and had the ability to uptake or remove metal, it was further subjected to microscopic characterization and various biochemical assays like gram staining, catalase, oxidase, bacitracin susceptibility test in order to characterize the culture.

#### xi. Statistical Analysis

Data are represented as mean value  $\pm$  SD. The experiments were performed in triplicate. To find the significance of the difference, *P* values of the different experimental groups were calculated by one-way ANOVA using Instat3 statistical software package. The *p* value \* <0.05 and \*\* < 0.01 are considered significant and \*\*\* <0.001 considered as highly significant. n.s is non-significant.

### III. RESULTS AND DISCUSSION

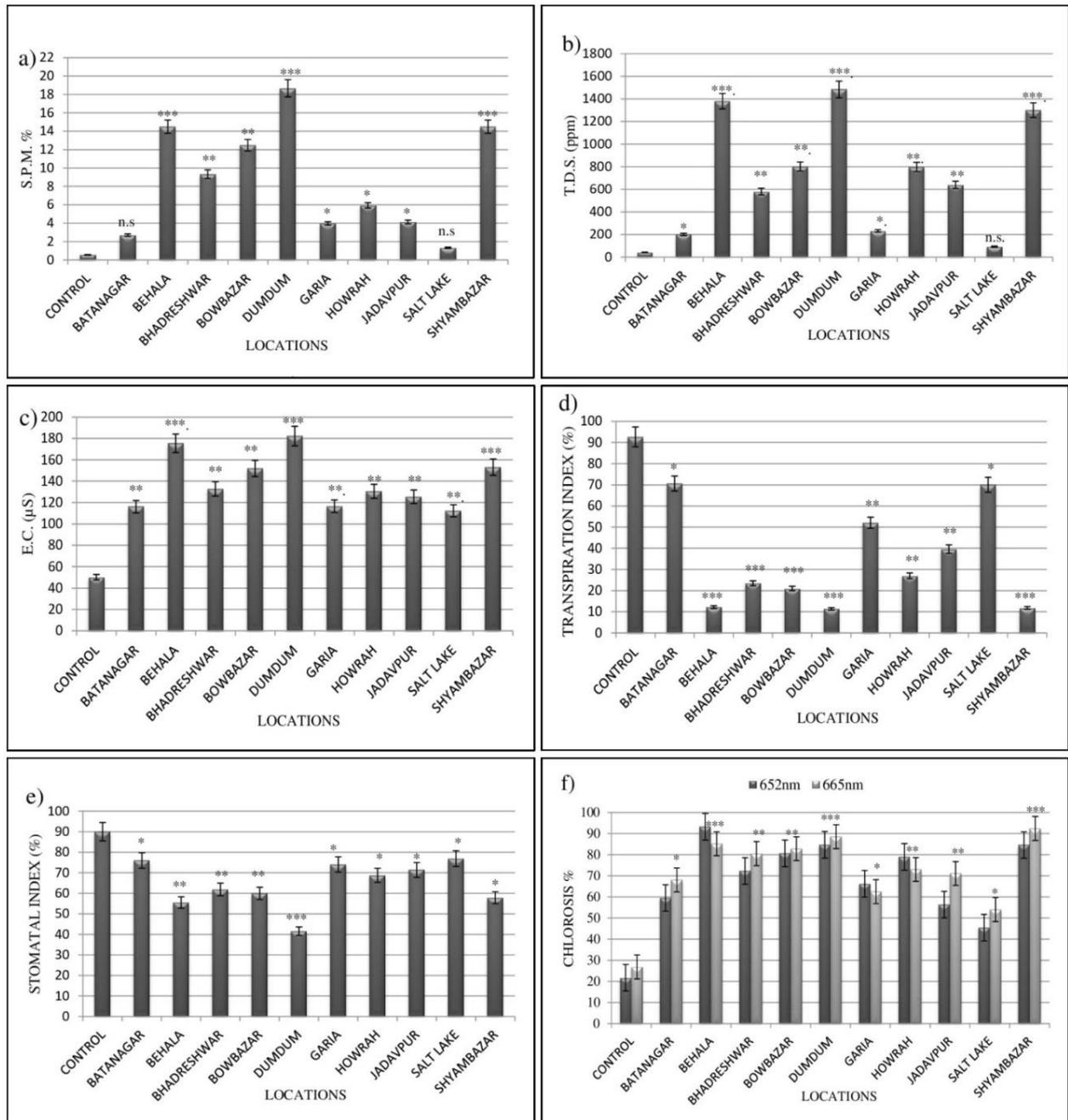
#### 3.1. Effects of SPM on different physiological parameters that affect plant survivability and in turn reflect the air quality of the zone concerned

SPM plays a crucial role in the regulation and maintenance of air pollution in the city. Depositions of SPM of different size, shape and chemistry is responsible for the determination of air quality index. It was thus essential to conduct a thorough evaluation of the parameters that are affected by SPM, all of which together affects the manifold physiological functions of leaves, thereby accounting for the outdoor air index. Hence, the SPM of the chosen locations were measured by weighing the amount of particulate matter that deposited on the leaves (size 5x4 cm<sup>2</sup>) collected from the outdoor environment of each location. It was found evidently that Dumdum had the highest concentration of SPM (18.67%) whereas Salt Lake recorded the least value (1.33%). Other zones such as Batanagar, Behala, Bhadrashwar, Bowbazar, Garia, Howrah, Jadavpur and Shyambazar recorded SPM levels of 2.7, 14.5, 9.33, 12.48, 3.97, 5.94, 4.13 and 14.5 % respectively (**Fig.1a**). This result clearly established the fact that the pollution index in Dumdum was greater than in any other location, and hence, the number of health hazards in Dumdum is expected to be more prominent with respect to air borne diseases. Further, on conducting the measurement of the Total Dissolved Solids (TDS) deposited on the leaf surface by filtration method followed by the measurement of the electrical conductance (EC) [11] of the leaf samples from all 10 places, the purity level of air in the concerned areas were determined (**Fig. 1 b & c**). Results revealed that Dumdum had a very poor quality of air, as it exhibited a significant elevation in the amount of TDS (1484 ppm), and a high conductance of electricity (182.2  $\mu$ S) ensured that it was significantly polluted.

Two major factors that is dependent on SPM, significantly the transpiration index (TI) [1] and stomatal index (SI) [9], were also noted. It is well-known that upon rising of the SPM level, the TI (**Fig. 1d**) and the SI (**Fig. 1e**) are destined to downswing on account of the fact that SPM causes disruption of the normal transpiration system of the plant body, thereby bringing about adverse effects on plant health. The transpiration assay conducted by the cobalt-chloride method and the measurement of stomata suggests that places with greater SPM has very correctly shown comparatively lesser values of the above parameters. Taken together, it was noted that Dumdum with the maximum extent of SPM level had exorbitant levels of TDS and EC along with declined values of TI and SI.

### 3.2. Chlorophyll content is altered by SPM interference

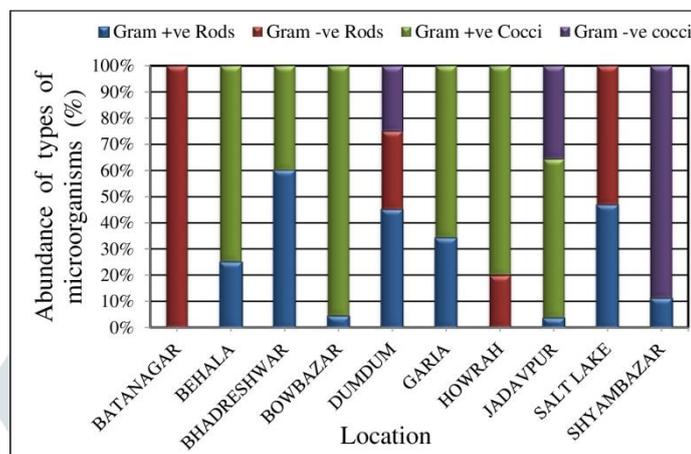
Chlorophyll is the most important class of pigments involved in photosynthesis in higher plants. Chlorophyll occurs in several distinct forms, most significantly chlorophyll *a* and *b* are the major types found in higher plants. Leaf is the site of photosynthesis, where chlorophyll is considered to be the most essential component [8]. Hence, it was necessary to study the effect of SPM on the chlorophyll content of leaves. The chlorophyll content of both a fresh green leaf and a dried yellow leaf isolated from each location was observed by preparation of the methanol extract of the leaf wash followed by spectrophotometric analyses at 652nm (for chlorophyll *b*) and 665 nm (for chlorophyll *a*) ranges and a comparison of the two leaves brought forth the effect of SPM on chlorophyll denoted by the percentage of chlorosis. Results (Fig. 1f) revealed a high amount of chlorosis in Behala, Dumdum, Shyambazar and Bowbazar. A number of factors may be held responsible for this yellowing of leaves, among which deficiency of nutrients like iron, magnesium, zinc, nitrogen and/or proteins is a major one which can be directly or indirectly attributed to the cause of SPM.



**Figure 1:** *Hibiscus rosasinensis* leaves were collected from 10 different localities of Kolkata and subjected to measurement of different parameters in order to determine the effect of air pollution on the different physiological functions of a leaf. a) The suspended particulate matter (SPM) content of leaves collected from different areas under study was measured by weighing. b) Total dissolved solid (TDS) content of leaf wash. c) Electrical conductance (EC) of leaf wash. d) Transpiration index of leaves. e) Stomatal index of leaves. f) Estimation of the chlorophyll content of leaves before and after it has been acted upon by SPM. The experiments were repeated three times, yielding similar results, and data are expressed as mean+SD. p value \* <0.05 and \*\* < 0.01 are considered significant and \*\*\* <0.001 considered as highly significant. n.s is non-significant.

### 3.3. The microbial consortia on the phylloplane is affected by the deposited SPM

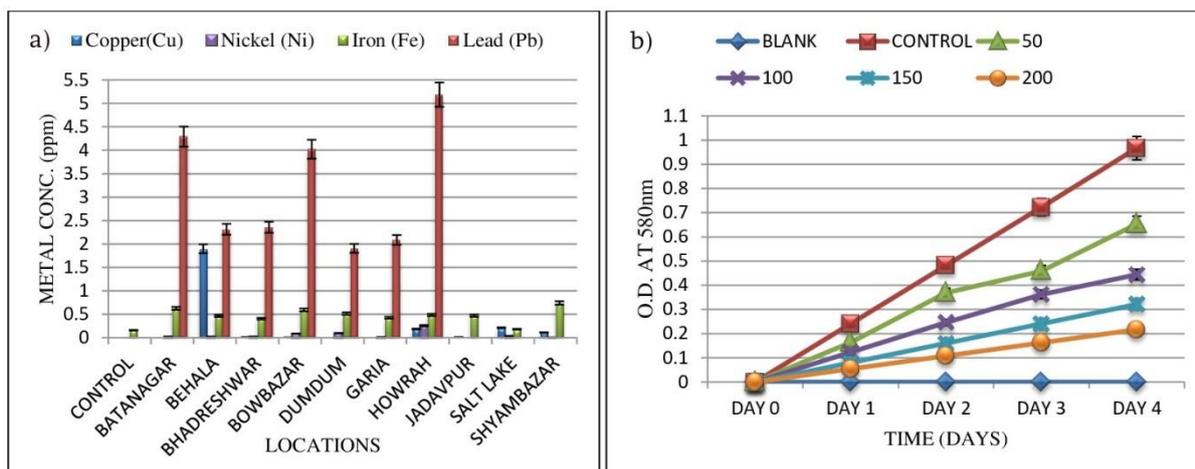
SPM is known to produce strong effects on the microbial population. However, air-borne microbes possess advance body-anatomy and biological attributes that help them combat against air pollution. They are, in fact, known to render the environment safe by carrying out beneficial functions such as biodegradation, biosorption, biofiltration, biosequestration [10] etc. Isolation and the further characterization of the microbial communities obtained from the plates of different places present a wide variety and extensive range of microbial diversity on the confined phylloplane. However, it should be carefully noted that the count and variety of microbes is dependent on the location from where the leaf has been isolated [16], which in turn, is dependent upon the air quality of that location indeed. An area-wise distribution of bacteria has been depicted in **Fig. 2** as obtained under basic laboratory conditions.



**Figure 2:** An area wise distribution of the microbial consortia that dominated the phylloplane was drawn in order to get an idea of the micro-creatures that dwelt within a range of non-polluted to a highly polluted area. Careful isolation followed by characterization of the microbial communities obtained from the different locations presents a wide variety and extensive range of microbial diversity on the confined phylloplane.

### 3.4. Heavy metal accumulation on leaf surface and their efficient uptake by the selected isolate

Anthropogenic activities lead to substantial release of toxic heavy metals into the environment. Heavy metal pollution is generating hype in recent years causing severe environmental pollution and threatening human lives. Hence, a study in order to bring out a co-relation between the heavy metals deposited on a leaf surface and their adverse effects on the air quality and more significantly on the microbial world was highly mandatory. Among those well-known, the most striking ones found dominating the leaf surface of the chosen locations were Copper ( $\text{Cu}^{+2}$ ), Nickel ( $\text{Ni}^{+2}$ ), Iron ( $\text{Fe}^{+2}$ ) and Lead ( $\text{Pb}^{+2}$ ). The results clearly indicated that the air of Behala (1.898 ppm) and Shyambazar (0.741 ppm) had the highest concentration of  $\text{Cu}^{+2}$  and  $\text{Fe}^{+2}$  respectively, whereas, Howrah had both  $\text{Ni}^{+2}$  (0.257 ppm) and  $\text{Pb}^{+2}$  (5.186 ppm) in maximum (**Fig. 3a**). The dominant metal was indeed  $\text{Pb}^{+2}$ , that was obtained maximally in the zone of Howrah. Since  $\text{Pb}^{+2}$  was essentially found to be highest in concentration among the rest, further experiments were conducted taking  $\text{Pb}^{+2}$  as the signature metal. Interestingly, the studies of metal tolerance and uptake were followed up using the microbial culture (the culture of maximum existence on the nutrient agar plate of Howrah and hence the culture of interest) to check if it had any  $\text{Pb}^{+2}$  remediation abilities. The culture upon characterizing had shown a gram positive nature. The growth and tolerance of the culture was noted for 4 successive days by growing it at different concentrations of  $\text{Pb}^{+2}$  including 50, 100, 150 and 200 ppm (**Fig. 3b**) compared with that of culture-medium devoid of  $\text{Pb}^{+2}$ . The LD50 of the microbial culture was estimated to be at 100 ppm concentration. Further, studies done to evaluate the concentration of metal uptaken and residual metal [20], exhibited that the culture at a concentration of 100 ppm could absorb nearly 51.444 ppm  $\text{Pb}^{+2}$  (**Table 4a**). The species was identified as *Micrococcus* sp. on the basis of its morphological and biochemical profile [2] (**Table 4b**). Hence, with such excellent results that it had exhibited, it is being considered that the culture could be a ray of hope for excellent micro-remediation technologies in the near future.



**Figure 3:** a) Determination of metal concentration of the different areas under study: On studying the metal concentration range obtained in the various locations, it was noted that the dominant metal was Pb<sup>2+</sup>, that accounted maximum in the zone of Howrah. b) Metal (Pb<sup>2+</sup>) tolerance by the culture of interest: A culture of maximum existence from Howrah was checked for Pb<sup>2+</sup> tolerance. The growth and tolerance of the culture under study was noted for 4 successive days by growing it at different concentrations of Pb<sup>2+</sup> compared with that of culture medium devoid of Pb<sup>2+</sup>. The LD50 of the microbial culture was estimated to be at 100 ppm concentration.

**Table 4:** a) Metal (Pb<sup>2+</sup>) uptake by the culture of interest: Studies done to evaluate the concentration of metal uptaken and residual metal exhibited that the culture, at a concentration of 100 ppm, could absorb nearly 51.444 ppm Pb<sup>2+</sup>. b) Characterization and identification of the culture of interest: The morphological and biochemical analyses revealed the selected bacterial isolate as *Micrococcus* sp.

SAMPLES	RESIDUAL METAL (ppm)
Blank	0
1 <sup>st</sup> supernatant (control)	0.208
1 <sup>st</sup> supernatant (100 ppm Pb <sup>2+</sup> )	42.020
2 <sup>nd</sup> supernatant (control)	0.279
2 <sup>nd</sup> supernatant (100 ppm Pb <sup>2+</sup> )	6.536
TOTAL RESIDUAL METAL = 42.020 + 6.536 = 48.556 ppm	
TOTAL ADSORBED METAL = 100 - 48.556 = 51.444 ppm	

NAME OF TEST/ CHARACTERISTICS	RESULTS
Gram staining	+
Shape	Cocci
Arrangement	Single, pairs, tetrads
Catalase	+
Oxidase	+
Bacitracin susceptibility test	Sensitive
CONCLUSION: The bacteria is concluded to be a member of the genus <i>Micrococcus</i> .	

**IV. CONCLUSION**

The significant increase in particulate pollution and profound deterioration of the air quality in the past few years, attributed to rapid industrialization, population growth and associated increase in energy demands, pose serious threat to global public health. The results of the present study clearly established the fact that the pollution index in Dumdum (SPM = 18.67%, TDS = 1484 ppm, EC = 182.2 μS) was greater than in any other location, and hence, the number of health hazards in Dumdum is expected to be more prominent with respect to air borne diseases. The effects of the deposited SPM on the physiology of the respective plants were studied by estimating the transpiration index (T.I.), stomatal index (S.I.) and the chlorophyll content, and all the three parameters were found to be inversely related with the amount of deposited SPM on leaves. Study of heavy metal contamination of the air of the areas revealed the presence of different heavy metals (Cu<sup>2+</sup>, Ni<sup>2+</sup>, Fe<sup>2+</sup>, Pb<sup>2+</sup>) on the leaves. The existence of lead (Pb<sup>2+</sup>) was found to be dominant; Howrah showing the highest concentration of Pb<sup>2+</sup>. The Pb<sup>2+</sup> tolerance and Pb<sup>2+</sup> uptake by a bacterial culture (culture of maximum existence) from the leaf wash of Howrah was studied and quantified. This Pb<sup>2+</sup> tolerant microorganism, which is identified to be a member of the genus *Micrococcus*, could be a potential agent for an effective, efficient, and economical method for bioremediation in a Pb<sup>2+</sup> contaminated environment without disturbing the target environment.

**V. ACKNOWLEDGMENT**

The authors are thankful to the Principal and Management of St. Xavier's College (Autonomous), Kolkata for providing all necessary facilities and financial requirements to carry out the work. The authors are extremely grateful to the H.O.D. (Post-graduate Department of Microbiology, St. Xavier's College, Kolkata) and all the faculties concerned and involved herein for their constant guidance, assistance, suggestions and constructive criticism which was indeed needed for the success of the project.

## VI. CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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