

## Assessment of Outdoor Bioaerosols In Megacity Istanbul-Turkey and Its Relation with Tobacco Smoke

Asli Baysal<sup>1\*</sup>, Gul Sirin Ustabasi<sup>1</sup>

<sup>1</sup> T.C. Istanbul Aydin University, Health Services Vocational School of Higher Education, 34295 Sefakoy Kucukcekmece, Istanbul, Turkey

E-Mail: [aslibaysal@aydin.edu.tr](mailto:aslibaysal@aydin.edu.tr), [gulustabasi@aydin.edu.tr](mailto:gulustabasi@aydin.edu.tr)

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**Abstract:** Biological substances in the aerosols have important impacts on public health and include bacteria, fungi, etc. that they can be toxigenic, allergenic and/or infectious. These substances can be originated from either natural sources such as evaporated sea spray, windborne pollen, dust, and other geothermal eruptions or from anthropogenic sources, like various industrial and man-made processes. One of the usual and daily man-made processes is tobacco smoke. To investigate the outdoor microbial activity and effect of tobacco smoke on outdoor air, culturable microbial activity, and diversity were measured in smoking and non-smoking outdoor sites. Outdoor air samples collected with manual aerosol samplers in Florya, Istanbul (Turkey) during three months in the winter of 2015-16. Average microorganism concentrations were varied 33-200 CFU/m<sup>3</sup> and 2-120 CFU/m<sup>3</sup> for smoking area and non-smoking area, respectively. The most dominant species were *bacillus*, *staphylococcus* and mold.

**Keywords:** Pollution, Urban air, Microbial characterization, Istanbul, Bioaerosols

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### INTRODUCTION

Outdoor air contains particles; organic or inorganic chemicals and biological substances (bioaerosols) that influence on the environmental health<sup>[1,2]</sup>. Microorganisms (bacteria, fungi, and virus etc.) are an important component of bioaerosols; their concentration varies at different times and sites. As well as cell and spore fragments of the microorganisms can be important sources of allergens, toxins and/or infections<sup>[3,4]</sup>. Microbial activity depends on various physical, chemical and biological factors, among which the nutrient condition in the environment is especially important because it reflects the microbial contribution to the energy flow and nutrient cycling within an ecosystem. Some bioaerosols come from natural sources such as evaporated sea spray, windborne pollen, dust, or geothermal eruptions. Other bioaerosols come from anthropogenic sources like heating and household cooking, agricultural field burning, diesel-fueled engine combustion, and various industrial and man-made processes. Because microbial activity depends on above mentioned factors in the environment, the abundance of microbial activity in bioaerosols is also affected by the season, region and meteorological factors. However, studies on microbial activity in bioaerosols have been very limited<sup>[5,6]</sup>.

Tobacco smoke which is also an indicator for environmental smoke (ETS) or secondhand smoke (SHS) is one of the daily man made processes. It has a complex mixture of thousands of compounds including particulate matter emitted by the combustion of tobacco products and from smoke exhaled by smokers<sup>[7]</sup>. It contains up to 50 chemicals recognized as known and probable human or animal carcinogens, many toxic and irritant agents<sup>[8]</sup>. Other chemicals in the smoke may be present in the tobacco itself, surviving the combustion during the smoking. Tobacco leaves contain compounds that microorganisms naturally colonize and it is rich in both bacteria and fungi that has been known for decades. Exposure to environmental tobacco smoke has also important public health implications; its brief or short exposures may generate significant adverse effect on the human health, like respiratory, cardiac, and even carcinogenic<sup>[1,9-11]</sup>. Its exposure has been commonly investigated in various indoor studies; however, its impact on outdoor bioaerosols has been rarely investigated.

The aim of the study is to investigate outdoor microbial activity (culturable microbial concentration and diversity) and effect of environmental tobacco smoke on bioaerosols comparing with smoking and non-smoking outdoors. Air samples were microbiologically characterized.

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\*Corresponding E-mail: [aslibaysal@aydin.edu.tr](mailto:aslibaysal@aydin.edu.tr)

## EXPERIMENTAL

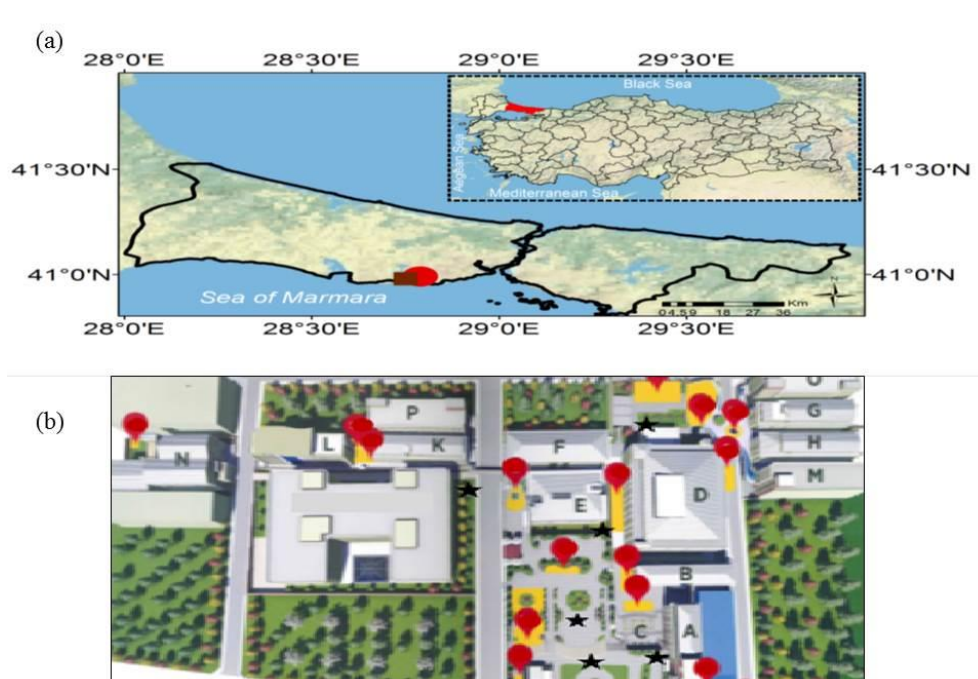
### Study sites

The sampling sites were at the outdoor of the Istanbul Aydin University, Florya, Istanbul, Turkey ( $40^{\circ} 59' 29''\text{N}$ ,  $28^{\circ} 47' 50''\text{E}$ ) and were shown in Figure 1. Sites were high-traffic density and located between main roads and near Istanbul Ataturk Airport which had one of the highest densities as an international airport in the world at the sampling time. Istanbul is a megacity with over 15 million inhabitants, responsible for 40% of Turkey's industrial activities and there are over three million vehicles on the road [12-16]. It has a unique geographical location between Europe and Asia, its climate is Mediterranean, characterized by warm/dry summers and cold/wet winters and its air quality is affected by both anthropogenic and natural sources, like dust storms, sea spray and forest fires.

### Sample collection and characterization of outdoor microorganisms

The study was carried out between November 2015 and February 2016. The culturable microorganism analysis was performed using MAS-100 Eco Airsampler (Merck) device for collecting the outdoor air samples on nutrient medium (blood agar). Duration of sampling stage was 5 minutes and sampling flow rate of 100 L/min. The concentrations of microorganisms were indicated as CFU/m<sup>3</sup> (=colony forming units per m<sup>3</sup> air). The outdoor sites divided two categories which are smoking and non-smoking. Sites were shown in Fig 1. Sampling was done in three days a week, and three times a day, during three months (December, January, and February). Average values were taken for measurements.

For the quantitative analysis, the samples were incubated on nutrient medium (blood agar). Afterwards, samples were kept in a humid oven for 24 h at 37°C. Once the colonies were obtained, they were stored in a 4°C room until analysis. The identification of outdoor microorganisms was conducted with regard to surface structure and growth pattern of the colonies by using a Gram staining method.



**Figure 1.** (a) The locations of smoking and non-smoking sample collection area (red circle) and Florya meteorology station (brown square) over Istanbul. (b) The inset shows the location of Istanbul in Turkey. Smoking (balloon point) and non-smoking (star point) areas in Istanbul Aydin University Florya campus, Istanbul, Turkey.

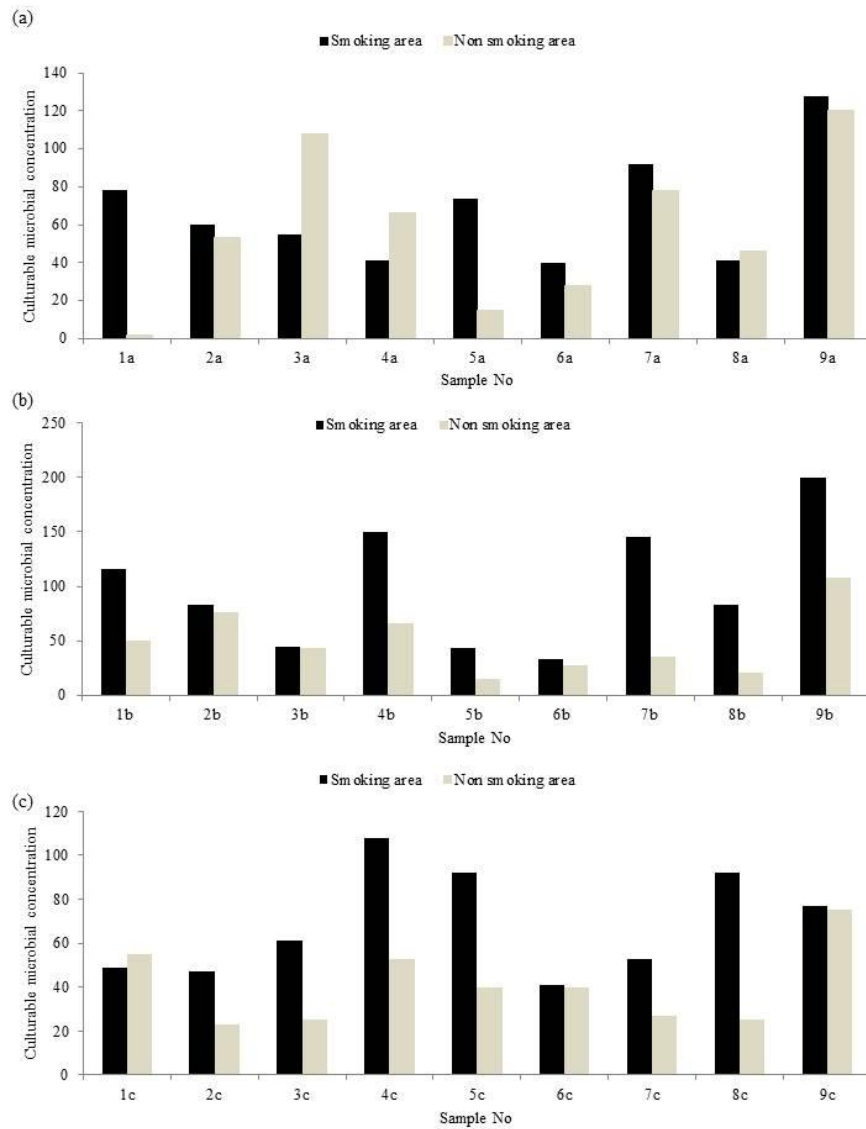
## RESULTS AND DISCUSSION

To understand the outdoor microorganism activity and effect of environmental smoke on outdoor air, culturable microorganism concentration and diversity were analyzed and results compared with non-

smoking area in campus, Istanbul Aydin University, Istanbul-Turkey. As can be seen in Figure 2, average microorganism concentrations were measured  $78.78 \pm 40.90$  CFU/m<sup>3</sup> (33-200 CFU/m<sup>3</sup>) and  $48.96 \pm 30.24$  CFU/m<sup>3</sup> (2-120 CFU/m<sup>3</sup>) for smoking area and non-smoking area, respectively. According to the non-parametric “Wilcoxon signed rank-test”, there is significant difference between the means of each sample of total microorganism in smoking and non-smoking sample area found by the both sampling area at 95% confidence level ( $P > 0.05$ ). Despite the fact that sampling area is outdoors, the total microorganism concentration is significantly affected by the environmental tobacco smoke. In the winter there had not significant difference hourly temperature, however natural (day light etc.) or anthropogenic (traffic, human population etc.) sources can be effective on the culturable microorganisms. To determine these effects on the microbial activity, sample collection had done three times a day which are morning, midday and afternoon, and the results statistically compared. In non-smoking sampling points the average culturable microorganism concentration was found  $57.3 \pm 40.1$  CFU/m<sup>3</sup>,  $68.9 \pm 29.8$  CFU/m<sup>3</sup>, and  $40.3 \pm 17.8$  CFU/m<sup>3</sup> in the morning, midday and afternoon, respectively. There has a significant correlation between morning and midday for non-smoking area results (significance 0.656 at the 0.05 level; 1-tailed). Whereas in smoking sampling points the average culturable microorganism concentration was found  $67.7 \pm 29.1$  CFU/m<sup>3</sup>,  $99.8 \pm 57.1$  CFU/m<sup>3</sup>, and  $68.9 \pm 24.1$  CFU/m<sup>3</sup> in the morning, midday and afternoon, respectively. The significant correlation was found between midday and afternoon for smoking area (significance 0.596 at the 0.05 level; 1-tailed). Also the results showed that the culturable microbial activity increased in the midday both in smoking and non-smoking area.

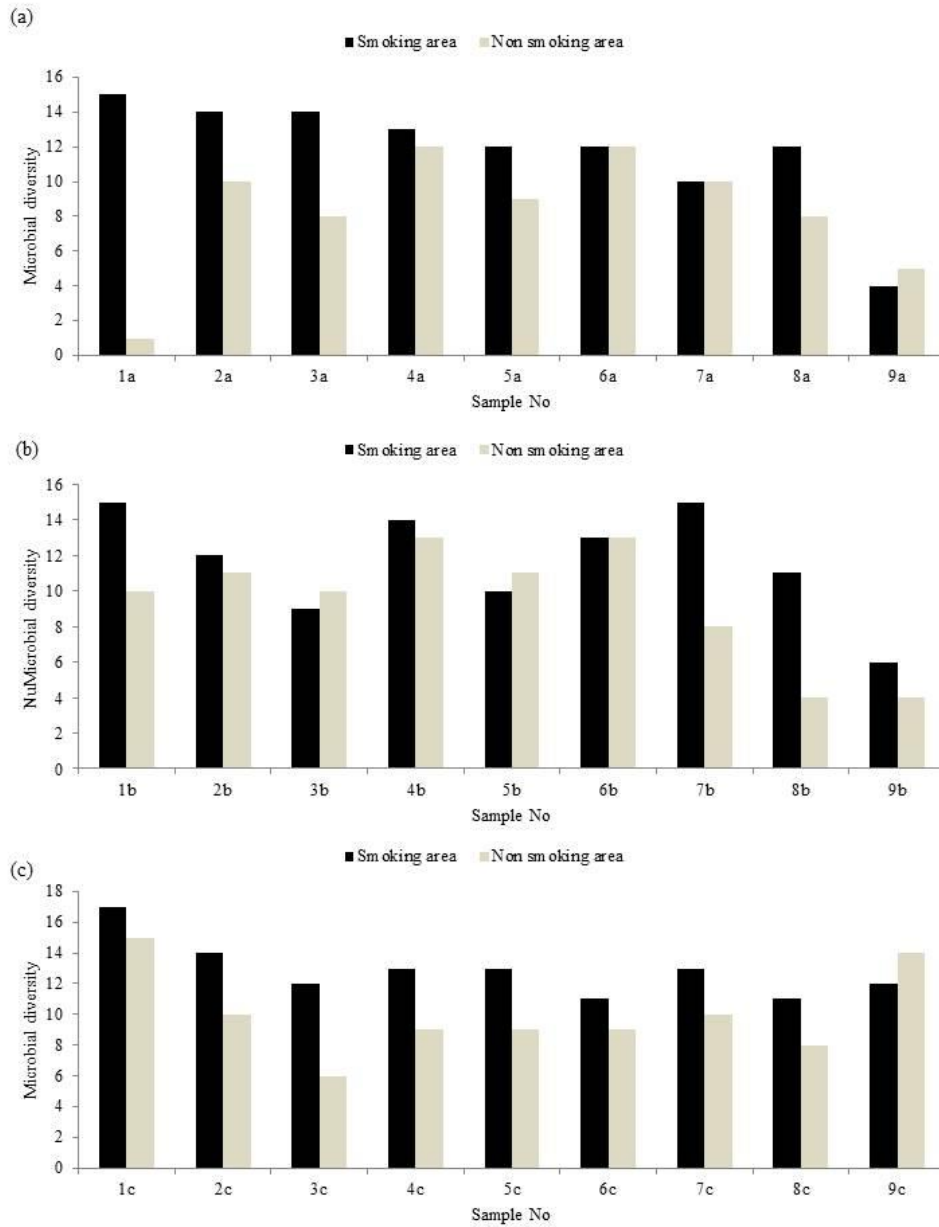
Additionally, microorganism diversity was investigated in smoking and non-smoking area, and diversity was found  $12.1 \pm 2.7$  and  $9.2 \pm 3.2$  in smoking and non-smoking area, respectively.

Significant difference was found according to the non-parametric Wilcoxin signed rank-test (in Figure 3). The correlation was found between morning and midday for smoking area results (significance 0.590 at the 0.05 level; 1-tailed), and between morning and afternoon for non-smoking area results (significance 0.707 at the 0.05 level; 1-tailed), also there is a slight correlation between morning and midday for non-smoking area (significance 0.453 at the 0.05 level; 1-tailed).



**Figure 2.** Variation of culturable microbial concentration in smoking and non-smoking area at university campus, Istanbul-Turkey according to sampling time. (a) morning, (b) midday, (c) afternoon.

There is a need for baseline information about the general characteristics of the bioaerosols to support many applications related to public health and international security <sup>[17]</sup>. To understand the general characteristics of the microorganisms, collected samples were identified and results are shown in Table 1. The percentage of species identified in the level of  $19.4 \pm 14.7$ ,  $11.8 \pm 12.0$ ,  $3.3 \pm 5.5$ ,  $4.9 \pm 7.7$ ,  $38.2 \pm 18.9$  and  $22.5 \pm 19.1$  as mold, *coccus*, *diplococcus*, *streptococcus*, *staphylococcus*, and *bacillus* in smoking sampling area. Whereas the percentage of the species accounted  $23.0 \pm 25.7$ ,  $10.2 \pm 12.7$ ,  $2.9 \pm 5.7$ ,  $1.2 \pm 3.4$ ,  $33.9 \pm 28.9$ ,  $28.8 \pm 21.9$  as mold, *coccus*, *diplococcus*, *streptococcus*, *staphylococcus*, and *bacillus* in non-smoking sampling area. While there has high standard deviations, the dominant species were *staphylococcus*, *bacillus*, and mold not only in smoking but also in non-smoking area. Although high microbial concentration was found in midday, the high microbial variations were obtained in morning and afternoon.



**Figure 3.** Variation of microbial diversity in smoking and non-smoking area at university campus, Istanbul-Turkey according to sampling time. (a) morning, (b) midday, (c) afternoon.

**Table 1.** Percentage of the species of microorganisms collected in sampling areas.

Day	Microorganism type	Smoking area			Non-smoking area		
		Morning(a)	Midday(b)	Afternoon(c)	Morning(a)	Midday(b)	Afternoon(c)
1	mold	20	38	0	100	30	38
	coccus	9	0	0	0	0	10
	diplococcus	18	0	0	0	0	10
	streptococcus		0	0	0	0	0
	staphylococcus	9	15	50	0	35	10
	bacillus	44	46	50	0	35	32
2	mold	28	33	46	44	25	60
	coccus	5	0	8	11	0	0
	diplococcus	0	0	0	0	0	0
	streptococcus	0	22	8	0	0	0
	staphylococcus	56	11	23	22	25	0
	bacillus	11	33	15	22	50	40

3	mold	27	0	0	0	29	0
	coccus	18	0	0	20	0	0
	diplococcus	0	0	14	20	0	0
	streptococcus	0	0	14	0	0	0
	staphylococcus	55	60	29	0	29	50
	bacillus	0	40	43	60	43	50
4	mold	9	17	9	0	9	0
	coccus	9	25	0	25	0	25
	diplococcus	0	8	9	0	0	13
	streptococcus	0	0	9	0	0	13
	staphylococcus	64	17	45	38	27	13
	bacillus	18	33	27	38	64	38
5	mold	28	18	18	15	18	14
	coccus	16	18	28	34	27	36
	diplococcus	0	0	0	0	0	0
	streptococcus	0	18	8	0	0	0
	staphylococcus	56	28	38	17	37	25
	bacillus	0	18	8	34	18	25
6	mold	0	45	33	30	22	25
	coccus	22	9	0	0	0	0
	diplococcus	11	0	11	12	12	0
	streptococcus	0	0	0	0	0	0
	staphylococcus	44	27	33	46	22	38
	bacillus	22	18	22	12	44	38
7	mold	20	22	10	11	0	68
	coccus	0	22	0	11	20	16
	diplococcus	8	0	0	11	0	0
	streptococcus	20	0	0	11	0	0
	staphylococcus	32	34	10	44	0	16
	bacillus	20	22	80	11	80	0
8	mold	9	0	0	64	0	0
	coccus	9	17	12	0	0	33
	diplococcus	9	0	0	0	0	0
	streptococcus	0	20	0	0	0	0
	staphylococcus	53	63	80	18	100	67
	bacillus	20	0	8	18	0	0
9	mold	25	25	43	0	0	18
	coccus	50	25	16	0	0	8
	diplococcus	0	0	0	0	0	0
	streptococcus	0	0	8	0	0	8
	staphylococcus	25	50	25	100	100	38
	bacillus	0	0	8	0	0	28

## CONCLUSION

To the best of our knowledge, it is the first attempt in the assessment of the bioaerosols in the outdoor environment of the megacity Istanbul-Turkey, as well as an investigation of the effect of environmental tobacco smoke on bioaerosols in the outdoor environment in the literature. According to the parametric as well as non-parametric tests, there was a difference between the means of the microorganism concentration between in smoking and non-smoking samples as well as mean values the number of microorganism type of collected samples found by the both sampling area at 95% confidence level ( $P > 0.05$ ). While there were some limitations in this study, environmental tobacco smoke affected the outdoor microbial concentration and diversity. According to the results obtained in the study at first sight, there was connection between the temperature and microbial activity, it needs extra effort to reveal the statement. While the most dominant microbiologic species were *bacillus*, *staphylococcus* and mold both in smoking and non-smoking area, their percentage were greater in smoking area than non-smoking area and these species are mostly known as sources of allergens, toxins and/or infections. This study is a contribution to understand a global problem of environmental tobacco smoking associated microorganisms and factors affecting their survivability on outdoor air quality.

## **ACKNOWLEDGEMENT**

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