Research Paper

Volume : 5 | Issue : 4 | April 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

A Study on Isolation And Identification of Airborne Bacteria At Three Different Sewage Treatment Plant Locations in Visakhapatnam City, A.p

Environmental Science

KEYWORDS : bioaerosols, bacteria, sewage treatment plant.

M.Ch.Shravanthi	Department of Environmental Sciences, Andhra University
N. Ravi Kumar	Department of Environmental Sciences, Andhra University
T. Byragi Reddy	Department of Environmental Sciences, Andhra University

ABSTRACT

The article presents monitoring and evaluating the levels of Bioaerosols (airborne bacteria) concentrations as an indicator of microbial air pollution in and around the sewage treatment plants in Visakhapatnam city (A.P).The air samples were collected using the N-6 stage Anderson's sampler from different sites associated with various phases of sewage treatment processes. The concentrations were evaluated from three sewage treatment units, the largest STP located at I Town with handling capacity of 38 MLD followed by Appughar STP and Arilova STP of 25 MLD & 13MLD capacity. The concentrations of total mesophilic bacteria at I Town, Appughar & Arilova STP were 20279.98 CFU/m3,10775.47CFU/m3 and 4669.69 CFU/m3 respectively. In conclusion, the airborne bacterial concentrations were quite high at I Town STP location compared to Arilova and Appughar STP's. The potentially pathogenic infectious bacteria detected were: Bacillus sps, Staphylococcus aureus, Escherichia coli, Streptococcus, Micrococcus sps, Pseudomonas aeruginosa.

INTRODUCTION

Bioaerosols are defined as collection of aerosolized biological particles (e.g. microbes, by-products of living organisms) capable of eliciting diseases that may be infectious, allergic, or toxigenic with the conditions being acute or chronic. Bioaerosol monitoring is a rapidly emerging area of industrial hygiene. Bioaerosols generated from different sources often pose a threat to human beings. In many cases the direct working environment of humans and the harmful factors it contains, may appear to be a place of great health risk. A particular source of microbiological air pollution in municipal facilities is primarily sewage treatment plants. Workers involved in gathering or processing wastes in sewage treatment plant face the potential risk of being exposed to hazardous factors. The number and composition of the air micro flora in the premises of sewage treatment plants depends on the area of sewage exposed to atmospheric factors, the type and degree of sewage contamination, means of sewage management, atmospheric and climatic conditions, mixing and transportation of sewage, gathering and lengthy storing of unstable biomass, as well as elevated temperature is conducive to bioaerosols formation.

Impaction, filtration, and impingement are three common sampling techniques used to separate and collect bioaerosols. In the current sampling impaction method is adopted using Andersons N-6 stage sampler. This study was conducted aiming to determine the bacterial concentrations in the air of the three selective locations of municipal sewage treatment plants in Visakhapatnam city, identify the bacterial genera, to determine the degree of microbiological load of the air and to draw attention of the authorities to the potential negative health effects of workers due to airborne bacteria & suggest precautionary measures to be taken during operations.

Sources of Bioaerosols in Sewage Treatment Plants

Sewage treatment plants are considered potential sources of airborne pathogenic microorganisms. Sewage water consists of many pathogens such as viruses, bacteria, fungi, protozoa, etc. which originate from domestic activities, commercial, and other sectors. Bio aerosols are generated at different stages of wastewater treatment process, particularly in process that containing moving mechanisms and performed aeration of wastewater [1]. Bioaerosol particles (bacteria and fungi) exist naturally in the air and their survival depends on several environmental factors such as radiation ultraviolet, temperature, humidity and pressure, the type of microorganisms, and some pollutant availability in the atmosphere [2]. Important properties characterizing bioaerosols are size, viability, infectivity, allergenicity, toxicity, and pharmacological activity. Due to the health and welfare effects of bioaerosols, obtaining exposure limits is crucial to public safety. Bioaerosols can produce a wide range of health effects. The potential hazard caused by bioaerosols is related to pathogenicity of specific microorganisms, environmental conditions, exposure pathway, and immunologic response of the host [3]. In STP's, the highest emissions of bioaerosols occurs in pretreatment and primary clarifiers units and those containing moving mechanical equipment for wastewater aeration hence, they can easily enter the lung and cause infections in immuno-compromised individuals and allergic reaction [4].

The sewer workers are exposed to droplet aerosols that may contain a large variety of infectious, immunotoxic and allergenic biohazards. An endotoxin could be a cause of airways and intestinal inflammation and work-related symptoms (diarrhea, fatigue, nose irritation) in sewer workers [5]. It has been demonstrated that there exists a significant relationship between exposure to rod-shaped bacteria and the occurrence of fatigue and headache in sewage treatment workers [6]. The workers of sewage treatment plants could be exposed to changeable quantities of airborne bacteria which may vary depending upon type and capacity of the facility, performed activities and weather conditions. In these people's working environment, the source of the biological factors detrimental to their health is most often: human and animal excretion, sewage, wastes, soil, plant and animal products, and dust. These factors are transmitted through the aerial droplet and aerial-dust route, the skin and mucous membranes, and rarely through the digestive tract [7, 8 & 9]. This triggers allergies, infections, diseases and epidemics.

MATERIALS AND METHODS

Air samples were drawn from three different sewage treatment plants (I Town, Appughar & Arilova) located at Visakhapatnam (a city located in Andhra Pradesh).Four different site locations were selected at each STP to assess the level of microbial load occurring throughout the treatment process. The samples of air were collected using the N-6 stage Anderson's sampler for 10 minutes with a calibrated flow rate of 28.3 l/m using selective & differential agar media placed on six stages. Air sampling was conducted in November 2015 at the air temperature 31-32°C and

in between 11A.M-1.PM (operational hours). The samples were taken upwind, 145 cm above the ground, close to the sewage treatment installations (0.5–1.0 m). Altogether, air samples were taken at the following sites: Inlet to a sewer duct, Aeration tank, clarifier and Outlet. Air samples are collected in order to determine the concentrations of airborne bacteria.

Microbiological Examination of the Air

A petri dish containing an appropriate type of agar is positioned under each stage. These agar media's were prepared in the laboratory and transferred under sterile condition to STP's. After sample collection, the culture media were placed in zip kips and transferred to the laboratory in cool box. To identify bacterial isolates, the plates were placed in an incubator at temperature of 35°±0.5 °C for 24–48 hrs. Then, colonies on each plate were counted and reported as colony-forming unit (CFU/m^3), while the bacterial genera were identified according to Bergey's manual and bio-chemical tests. Additionally, during the course of study, at every station the air temperature (°C), relative humidity (%) were measured.

RESULTS

Bacterial colonies which grew on particular grounds were counted and the results are tabulated as the number of cells or cell aggregates capable of developing in the form of colonies (CFU i.e. Colony Forming Units) present in 1 m^3 of air. In order to specify in detail the degree of atmospheric air pollution in the following sewage treatment plants, identification of isolated microorganisms was conducted. The mean temperature ranged from 31^{0}C – 32^{0}C & relative humidity varied between 72% -74%.The concentrations of gram-negative bacteria at initial phases (screening & aeration) of sewage treatment plants were comparatively higher than those found at the final phases (secondary sedimentation, sludge dewatering and outlet). Among the Gram positive bacteria, *Staphylococcus spp. Streptococcus and Micrococcus spp*, showed the widest emission in Station-1&2 (ITown & Appughar STP's) and *Bacillus spp*, was dominant in Satation-3 (Arilova STP).Four genera of Gram-negative bacteria belonging to *Enterobacter spp, Pseudomonas spp*, *Bacillus spp*, *Klebsiella* were isolated. The concentrations of total mesophilic bacteria at station-1 was 20279.98 131 CFU/m^3 (I Town) recording 4788.49 CFU/m^3, 8446.38 CFU/m^3, 4370.98 CFU/m^3, 2674.131 CFU/m^3 near Inlet, Aeration, Secondary clarifier and Outlet. At station-2 (Appughar), the total airborne bacterial concentrations showed 10775.47 CFU/m^3 (Inlet-3851.54 CFU/m^3, Aeration tank-4236.7 CFU/m^3, Secondary clarifier-1609 CFU/m^3& Oulet-1077.69 CFU/m^3) respectively. At station-3 (Arilova), the total airborne bacterial concentrations showed 4669.69 CFU/m^3 (Inlet-1398.71 CFU/m^3, Aeration tank-1772.08 CFU/m^3, Secondary clarifier-567.14 CFU/m^3 & Oulet-931.76 CFU/m^3) respectively. The results showed that the aeration tank with concentrations of 8446.38 CFU/m^3.had the greatest effect on emission of bacterial bioaerosols. Sampling results for the airborne bacterial concentrations for the selected sampling stations is presented in Table 1, 2 and 3.

Table 1 Airborne Bacterial Concentrations as CFU/m^3 at Sewage Treatment Plant – 1 Town (38 MLD)

S.no	Type Of Media Used	Inlet	Aeration Tank	Secondary Clarifier	Outlet
1	Phenyl Ethyl Alcohol Agar	236.74	116.60	130.74	61.84
2	Endo agar	102.8	28.26	14.13	3.53
3	Blood agar	1710.24	3643.10	1363.95	789.22
4	Pseudomonas Agar	1243.81	1886.92	2321.55	1176.45
5	SS Agar	42.40	17.66	7.06	35.33
6	Eosin Methylene Blue Agar	106.22	89.54	7.06	21.201
7	Mannitol Salt Agar	646.64	621.90	116.60	42.40
8	Tryptic Soy Agar	699.64	2042.40	409.89	544.16

Temperature-32^{0}C, Relative Humidity-74%

Table 2 Airborne Bacterial Concentrations as CFU/m^3 Sewage Treatment Plant -Appughar (25 MLD)

S.No	Type Of Media	Inlet	Aeration Tank	Secondary Clarifier	Outlet
1	Phenyl Ethyl Alcohol Agar	519.43	886.92	639.57	325.08
2	Endo Agar	28.26	38.86	17.66	3.53
3	Blood agar	236.74	155.47	70.67	286.21
4	Pseudomonas Agar	371.02	1802.12	618.37	208.48
5	SS Agar	14.13	42.40	3.53	7.06
6	Eosin Methylene Blue Agar	42.40	49.46	12.40	7.06
7	Mannitol Salt Agar	561.83	187.27	35.33	42.40
8	Tryptic Soy Agar	2077.73	1074.20	212.01	197.87

Temperature-31^{0}C, Relative Humidity-72%

Table 3 Airborne Bacterial Concenterations as CFU/m^3 at Sewage Treatment Plant-Arilova (13MLD)

S.No	Type Of Media	Inlet	Aeration Tank	Secondary Clarifier	Outlet
1	Phenyl Ethyl Alcohol Agar	498.23	756.18	116.60	42.40
2	Endo Agar	56.53	130.74	12.40	31.80
3	Blood agar	621.90	279.15	137.80	119.07
4	Pseudomonas Agar	102.47	84.80	42.40	95.40
5	SS Agar	10.06	12.40	0.00	0.00
6	Eosin Methylene Blue Agar	7.06	127.20	10.60	28.26
7	Mannitol Salt Agar	35.33	332.15	226.14	314.48
8	Tryptic Soy Agar	67.13	49.46	21.20	300.35

Temperature-31^{0}C, Relative Humidity-74%

DISCUSSION & CONCLUSION

Microbiological analysis of the bacterial

aerosols revealed a higher number of organisms. A high emission of microbes into the free air has been observed near the aeration tanks during the sewage treatment process. At stations such as inlet, primary sedimentation tank/clarifier & sewage aeration tanks, a high level microbial air load by bacterial isolates was noted with most of them being pathogenic. While analyzing levels of microbiological load in the air of a particular area depending on climatic conditions, a greater number of microbes was usually observed in the months of lower temperatures (winter) and stronger winds. On the basis of the results of the research study conducted in the premises of the STP's of Visakhapatnam city, it can be noted that the air at many of the stations is highly polluted. As far as indicator bacteria are concerned, the place of the highest emission was near the inlet sewer duct and aeration tanks. Nearly, the presence of coliforms bacteria was identified at many

Volume : 5 | Issue : 4 | April 2016 • ISSN No 2277 - 8179 | IF : 3.508 | IC Value : 69.48

stations, which confirms that they are good indicators of sewage aerosols emissions into the air. The higher concentrations of bacterial aerosols were observed the pretreatment stage. Multiple meteorological factors can also impact the viability of microbes found within the aerosols. If one takes into consideration the fact that the workers and employees on the premises of such surrounding of the treatment units without any protective measures against the impact of the polluted air, run a relatively high risk of falling ill with dangerous diseases. The composition and concentration of airborne Gram positive and Gram-negative bacteria were given stress in the current study because higher level of these micro-organisms in the air can be detrimental to human health. In order to prevent negative effects of such facilities on the surroundings, areas of protective zones around sewage treatment plants should be defined and properly developed.

REFERENCES:

[1] Pascual L, Pérez-Luz S et al (2003) Bioaerosol emission from wastewater treatment plants. Aerobiologia 19(3–4):261–270.

[2] Cox CS, Wathes CM (2010) Bioaerosols handbook, crc press.

[3] Burkowska A, Kalwasińska A et al (2012) Airborne mesophilic bacteria at the Ciechocinek health resort. Pol J Environ Stud 21(2):307–312 Carducci A, Tozzi E et al (2000) Assessing airborne biological hazard from urban wastewater treatment.Water Res 34(4):1173–1178.

[4] Recer GM, Browne ML et al (2001) Ambient air levels of Aspergillus fumigatus and thermophilic actinomycetes in a residential neighborhood near a yard-waste composting facility. Aerobiologia 17(2):99–108.

[5] Rylander R: Health Effects Among Workers In Sewage Treatment Plants. *Occup Environ Med* 1999, 56, 354-357.

[6] Melbostad E, Eduard W, Skogstad A, Sandven P, Lassen J, Søstrand P, Heldal K: Exposure To Bacterial Aerosols And Work-Related Symptoms In Sewage Workers. *Am J Ind Med* 1994, 25, 59-63.

[7] Dutkiewicz J., Górny R.L. Biological factors haz- ardous to human health: classification and criteria of expo- sure assessment. Med Pr. 53, (1), 29, 2002 [In Polish].

[8] Butarewicz A., Kowaluk-Krupaa. Microbiological Air Contamination On The Sewage Storage Of City Waste Area In Augustów. Ochr Pow Probl Odpad. **38**, (5), 167, 2004 [In Polish].

[9] Kruczalak K., Olańczuk-Neyman K. Microorganisms In The Air Over Wastewater treatment plants. Pol. J. Environ. Stud. 13, (5), 537, 2004.

[10] Williams ST, Sharpe ME, Holt JG (Eds): *Bergey's Manual of Systematic Bacteriology. Vol. 4*. Williams & Wilkins, Baltimore 1989.

[11] Douwes J., Mannetje A., Heederik D. Work-Relat- Ed Symptoms In Sewage Treatment Workers. Ann Agric Environ Med. 8, 39, 2001.