Iran. J. Environ. Health. Sci. Eng., 2009, Vol. 6, No. 3, pp. 187-194

AERO-BACTERIOLOGICAL STUDY OF VEGETABLES MARKET AT JABALPUR

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Received 18 April 2009; revised 7 July 2009; accepted 2 August 2009

ABSTRACT
Urban and rural vegetable markets of India characterized by mass activity with a little sanitary measure are responsible for generation of higher quantity of aerosols containing biotic and abiotic components. The aerosols generated in due course of mechanical disturbance, contain many organic compounds enable to sensitize vital respiratory organs of local inhabitants. Inhalation of airborne microorganisms can expose workers to risks from infection, toxicosis and allergy. The presence of non-spore forming gram-negative bacteria in air due to the mechanical areosolization indicates higher rate of dissemination of pollutants in these occupation associated areas. The present aero-bacteriological investigation included enumeration, identification and numerical analysis of different types of culturable airborne bacteria with inhalable fraction of gram-negative bacteria in the vegetable market of the city of Jabalpur, in order to measure the degree of aerobiopollution for this environment. The aero-bacteriological sampling has been done fortnightly for a period of one year. Samples were cultured based on standard methods. The survey revealed that in this type of atmosphere, environmental factors were responsible for the persistence of airborne bacteria with variable effects. The major contributors for aerosol generation were other mechanical activities, since this site is classified under human activity-enriched and highly trafficked site. The bioload of this atmosphere was recorded as high as 2.9×10^3 bacterial carrying particles per cubic meter during winter, dominated by both inhalable and non inhalable fractions of gram-negative bacteria. In summer, soil-borne bacteria were reported dominant in the air. High humidity and low temperature were the major factors for dissemination and distribution of gram-negative bacilli. A regression model with upto 43% variance was prepared in order to predict the bioload for this atmosphere in relation to meteorological parameters.

Key Word: Aerosolization, Aero-bacteriological investigation, Gram-negative bacteria, Vegetable market

INTRODUCTION
Vegetable (greengrocer) market is characterized as a human activity-enriched site and also a highly trafficked site. People in these areas are actively engaged in handling of different vegetables originated from different localities, exposed to large quantities of organic dust, which constitutes not only the vegetable debris, but also a variety of aerosolized microorganism. The aerosolized spore-forming gram-positive bacteria are able to survive in air for a long duration. Gram-negative non-spore forming bacteria can also survive in air upto 390 minutes as a half-life time recorded previously by the workers (Dinter & Muller, 1988). The situation becomes worse when these microorganisms are able to multiply in these aerosols (Dimmick *et al.*, 1979).

Enterobacters are the good indicators of water pollution; also, the presence of enterobacters in the aerosols of the area under study represents the unhygienic practices and conditions. Infectious microorganisms must be viable to cause infections, but infectious as well as non-infectious microorganisms may pose other health hazards even if they are dead and disintegrated. Inhalation of noninfectious microorganisms and their constituents can cause inflammation of the respiratory system, while antigens and allergens may activate the immune system and cause allergic and immunotoxic effects (Malmberg, 1990 and 1991; Rylander, 1994). Previous study has reported high levels of potentially hazardous bacteria, fungi and other allergenic and / or

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immuno-toxic agents in the atmosphere of vegetables market (Verma and Sheore, 1989). It was found that culture techniques did not provide an adequate description of the bacterial burdens of air (i.e., less than 10% of the aerosolized bacteria were capable of forming visible colonies), (Heidelberg *et al*., 1997), but these techniques are always a method of choice only because nonculture-based approaches and culture provide complementary but independent measurements of airborne biopollution (Krahmer *et al.*, 1998).

This comprehensive study has been made in order to evaluate the quantity and quality of potentially hazardous culturable bacteria of viable types represented in the air of vegetable market with special reference to family enterobacteriaceae, and to find the inhalable and non-inhalable amounts of bacteria for this environment. The effects of environmental factors on the total airborne bacterial bioload were also analyzed by using correlation analysis and a regression model for the purpose of prediction was prepared.

MATERIALS AND METHODS

Jabalpur (Latitude: 23.2; Longitude: 79.95; Altitude: 391.) is the third biggest city of Madhya Pradesh in India. The city of Jabalpur stands on a rocky stretch of land about 9.6 km from the river Narmada and 20.8 km from the marble rocks of Bheraghat. Jabalpur is one of the central districts of Madhya Pradesh.The city consists of long narrow plains running northeast and southwest, and is shut in all sides by highlands farming an offshoot from the great valley of the Narmada. The climate of Jabalpur is overally pleasant and salubrious except the later part of the summer season. The year may be divided into three main seasons viz. the summer season (from middle of March to middle of June), the monsoon season (from middle of June to the end of September), and the winter season (from October to middle of March).

Sampling site

The vegetable market of Jabalpur is one of the crowded places situated at the center of the city. Large amounts of fresh vegetables from different regions are transported to the market. In order to evaluate the bacterial aerosols generation in course of green grocery handling this site has been selected. The aero- bacteriological sampling has been done fortnightly for a period of one year in order to cover all major seasons. The metrological data were collected from weather station of Jabalpur. Apart from temperature and humidity, five other metrological parameters were also recorded in order to analyze their effects on airborne bacterial population.

Isolation of bacteria from air

Culture methods have been used widely for the measurements of airborne microorganisms in the work environment (Eduard, 1996). For this study air sampling was done on Tryptone Glucose Yeast Extract (TGYE) Agar Medium (Hi Media) and Eosin Methylene Blue (EMB) Agar Medium (Hi Media) with the help of modified two stages Andersen Sampler (Andersen, 1958 and 1966) at one meter height from the ground extramurally within the premises of vegetable market. The sampler was operated for two minutes at the site, with interval of two weeks over the period of one year. For enumeration and identification of total viable types of bacterial population present in air, the TGYE medium plate was kept on upper stage of the sampler, whereas for enumeration and isolation of gram- negative enteric bacteria, the EMB media plate was kept on lower stage of the sampler to find out the inhalable amount which are able to deposited on lower airway of respiratory system of the people.

Isolation from sources

Sampling was also done in order to identify the presence of the bacteria in the sewage, vegetables and debris of market area and compare with those present in the air. Water samples were collected by holding the glass stopper, sterile bottle near its base in the hand and plugging it (necked downward below the surface) and transporting to the laboratory in an icebox to avoid unpredictable changes in physiochemical as well as bacteriological characteristics. The top soil of debris and marketing vegetables were sampled in sterile polythene bags and airtighted. Processing of samples was done by serial dilution technique (10^{-2} to 10^{-4}) to get only a few cells per mL. One mL of inoculums from each dilution was poured onto sterilized Petri plates of respective media (TGYE & EMB) at 45 ^oC by using Pour plate technique (Krieg, 1981). Then plates were incubated at 37 ± 2 ^oC for 24 to 48 hs.

Air contamination standard

The level of bacterial contamination of air is usually expressed in terms of number of bacteria-carrying particles per m^3 (bcp/m^3) or the bioload (B). B is calculated from the following equation:

$$B=\frac{1000N}{RT} \quad (1)$$

Where cfu is the colony-forming unit counted on the sample plate. Where N is the number of colonies counted on the sample plate, T is the duration of the test in min, and R is the air-sampling rate in liters/min. The threshold value (TLV) for bioload is 50 cfu/m^3 (WHO).

Identification of Isolates

Bacteria can be identified by morphology, gram-staining, growth on specific substances and under special conditions, and also production of specific metabolites (Eduard and Heederik, 1998). In this study, identification of isolates (of sources and air) was done by using standard methods and manuals; carbon source utilization profiles were prepared in order to establish source and sink relation (Jones and Sackin, 1980; Krieg and Holt, 1984; Baron *et al.,* 1994; Collee *et al.*, 1999).

Statistical analysis

In the present study, Pearson's correlation coefficient and multiple stepwise linear regression procedure was used to estimate the impact and degree of effectiveness of meteorological factors (average temperature, average humidity) on airborne bacteria concentration (Aegerter, 2003).

RESULTS

From vegetable market environment, 89 types of isolates were identified (Fig. 1). Bioload recorded of total viable bacteria cfu/m^3 was 2159.33 SD ± 1074.055; of inhalable gram-negative bacteria was 37.58 SD ± 28.195 and of total enteric bacteria was 2.50 SD ±1.642. Highest average recorded bioload from vegetable market environment during winter was 2.9 x 10^3 bcp/m^3, in summer was 2.6 x 10^3 bcp/m^3 and in monsoon was 1.7 x10^3 bcp/m^3. The seasonal variability in conjunction with the types of airborne bacteria was also recorded. During summer, the airborne concentration of Actinomycetes and related group were high. During winter, gram-negative rods were dominant whereas in monsoon gram-positive bacilli were dominant variety. Inhalable fraction of total enterobacteriaceae (Fig. 2 & 3) was recorded the highest during winter (50%), comparing to monsoon (28%), and the minimum during the period of summer (20%).

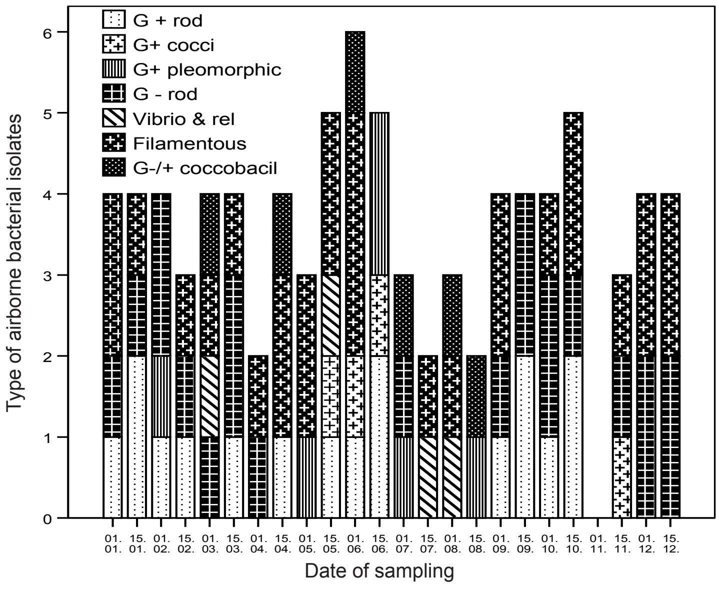

Fig.1: Type of airborne bacterial isolates from vegetable market environment

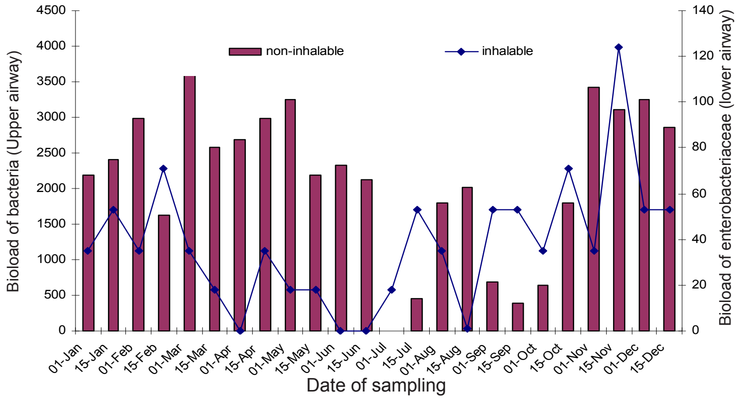

Fig. 2: Inhalable and non-inhalable fraction of viable bacteria at vegetable market environment (cfu/m^3)

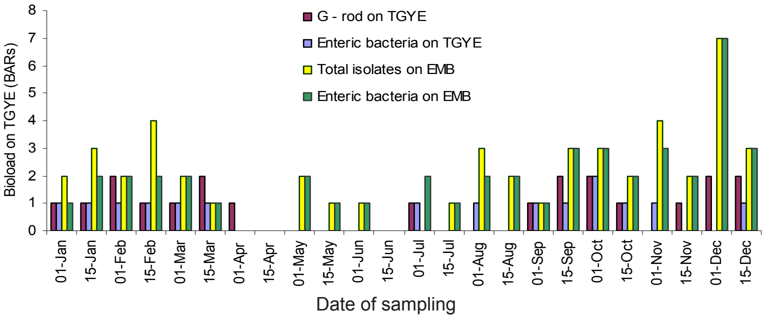

Fig. 3: Gram-negative isolates from vegetable market environment

Filamentous bacteria (33%) were dominant among the total types of viable bacteria, followed by gram-negative rods (25%) and gram-positive bacilli (19%). Species of the genera, *Citrobacter* & *Proteus* were dominant; among the group of enterobacteriaceae, *Citrobacter freundii, Erwinia herbicola* and *Escherichia coli,* along with other gram-negative bacteria i.e. *Chryseomonas luteloa, Vibrio* spp.*, Acinetobacter calcoaceticus, Acinetobacter* spp. and *Pseudomonas* spp. were also reported.

During the study of sources of these microorganisms, the microorganism reported were *Acinetobacter spp.* (*A. calcoaciticus*)*, Citrobacter freundi, Enterobacter spp.* (*gergoviae*), *Proteus spp.* (*mirabilis*)*, Pseudomonas spp.* (*P.mendocina, P. multophila*) and *Serratia spp.* (*plymuthica*) from both the soil and water samples. Whereas *Chryseomonas luteloa, Erwinia harbicola*, *Xenorhabdus sp.* and *Yersinia intermedia* were recovered from the soil of the extramural environment and the species of the genera *Edwersiella, Escherichia* (*E. Coli*)*, Providencia* and *Vibrio* (*V.carchariae, V. diazotrophicus, V. metshnikovii*) were reported from the sewage water sampled.

Coefficient, collinearity and regression analysis showed that the data was linear, consistent, predictable and without outliers. Humidity significantly correlated negatively with total viable culturable bacteria, whereas temperature significantly negatively correlated with viable culturable gram-negative bacteria (Table 1).

Table 1: Correlation coefficient environmental parameters with airborne bacterial isolates

	Dependent variables→ Independent variables↓	Bioload (Total bacteria)	Bioload (Inhalable gram-negative bacteria)	Bioload (Total enteric bacteria)
Pearson correlation	Average humidity	-0.588	0.248	0.437
	Average temperature	-0.024	-0.584	-0.528
Sig. (1-tailed)	Average humidity	0.001	0.122	0.016
	Average temperature	0.456	0.001	0.004

The multiple regression model predicted that, there was 29.82, and 54.1-unit decrease in bacterial bioload accounted for about 43%, 34.1%, and 33.6% of the variance by increasing one unit in humidity and temperature in total types of viable bacteria in vegetable market environments were obtaining. The constant was 5244.9 showing the bioload at any humidity and temperature range. 2.6 unit decrease and 0.01 unit increase in bacterial bioload by increasing one unit in humidity and temperature in total inhalable gram negative bacteria in vegetable market environment were obtained; the constant was 101.59 showing the bioload at any humidity and temperature range. 1.95 unit decrease and 0.3 unit increase in bacterial bioload by increasing one unit in temperature and humidity respectively in total enterobacteriaceae population in vegetable market environment; the constant was 74.89 showing the bioload at any humidity and temperature range. By using multiple regression (Enter), the model prepared for this atmosphere are as follows (hum. = humidity; temp. = temperature; ave = average)**:**

Estimated model of total type of viable bacteria (R^2 43%) =5244.9 - 29.82 x ave.hum. - 54.1 x ave. temp. (2)

Estimated model of total inhalable gram-negative bacteria (R^2 34.1%) = 101.59 + 0 .01 x ave.hum. - 2.6 x ave. temp. (3)

Estimated model of total enterobacteriaceae population (R^2 33.6%) = 74.89 + 0.3 x ave.hum. -1.95 x ave. temp. (4)

From the analysis of variance (ANOVA) (Table 2) under degree of freedom of V1=1, V2=22, the test statistic was the F value of 7.896(Sig.= 0.0028), 5.427 (Sig. = 0.0126) and 5.303 (Sig.= 0.0137) for the models, respectively.Using the significance level of 0.05, implies that critical value (Fcv) was 4.30 from the F distribution table. Thus, we could reject Ho in favour of Ha. This means that the linear regression model that had been estimated was not a mere theoretical construct; indeed, it did exist and was substantially significant. Square Root of Mean Square Error for model was 1045, 24 and 25; bioload could vary by ± 1045, ±24 and ±25 about the estimated regression equation for each value of average humidity and temperature.

Table 2: ANOVA Table; model prepared for airborne bacterial isolates

Analysis of Variance of total types of viable bacteria						
Model term	DF	R2	Sum of squares	Mean square	F-Ratio	Prob level
Intercept	1		1.12E+08	1.12E+08		
Model	2	0.4284	1.14E+07	5682894	7.869	0.0028
Hum. ave.	1	0.4278	1.14E+07	1.14E+07	15.716	0.0007
Temp. ave.	1	0.0825	2189892	2189892	3.032	0.0963
Error	21	0.5716	1.52E+07	722233.3		
Total(Adjusted)	23	1	2.65E+07	1153595		
Analysis of Variance of total inhalable gram negative bacteria						
Model term	DF	R2	Sum of squares	Mean square	F-Ratio	Prob level
Intercept	1		33900.17	33900.17		
Model	2	0.3408	6230.302	3115.151	5.427	0.0126
Hum. ave	1	0.0001	2.507714	2.507714	0.004	0.9479
Temp. ave.	1	0.2794	5108.033	5108.033	8.899	0.0071
Error	21	0.6592	12053.53	573.9777		
Total(Adjusted)	23	1	18283.83	794.9493		
Analysis of Variance of total *enterobacteriaceae*						
Model term	DF	R2	Sum of squares	Mean square	F-Ratio	Prob level
Intercept	1		46816.67	46816.67		
Model	2	0.3356	6513.794	3256.897	5.303	0.0137
Hum. ave	1	0.0591	1146.655	1146.655	1.867	0.1863
Temp. ave	1	0.1458	2829.374	2829.374	4.607	0.0437
Error	21	0.6644	12897.54	614.1685		
Total(Adjusted)	23	1	19411.33	843.971		

DISCUSSION

Extramural airborne bacteriological investigations carried out at the vegetable market in order to analyze the quality and quantity of airborne bacteria and its variations in course of seasonality, revealed that both pathogenic and saprophytic bacterial forms were prevalent in the area of study. The bioload of total viable bacteria, inhalable (respirable) gram-negative bacteria and total enteric bacteria were recorded in the ranges from 1084, 9 and 1 to 3233, 66 and 4 accounted in humidity ranging from 33 to 84 and temperature ranging from 19 to 31^{o}C, respectively. Dutkiewiez *et al*. (2000) reported as high as 10^5 cfu/m^3 in vegetables processing units. Previously, Verma & Sheore (1994) reported the average concentration of viable bacteria in vegetable market atmosphere as $1x10^3$ to $1.3x10^3$ cfu/m^3 in similar sampling site during an aerobiological survey. Increasing human activities and expansion of market area seems to be responsible for generation of the airborne pollutants; this leads to the doubling of bacterial bioload after a decade in same environment.

The gram-negative bacteria accounted less than 2% of total viable culturable bacteria for this atmosphere. From the inhalable amount of viable bacteria, those, which can be deposited on the lower airway of respiratory system of human being, were the highest in the month of November, whereas the viable bacterial bioload was highest in the month of March.

Highest average bioload was recorded in vegetable market environment during the winter, followed by summer and monsoon. Shrivastava (1992) reported highest concentration of airborne bacteria in the month of October followed by July in a similar environment. During the winter season, low temperature and moderate humidity favours the survival of most of the airborne bacteria including gram-negative bacteria. Spraying water over the leafy vegetables is a common practice among the retailers, which is responsible for generation of aerosol. These aerosols stay in air for a longer period during winter due to the absence of desiccation factors, enabling bacteria to survive in air for a comparatively longer duration. The gram-positive bacteria represented during the monsoon in the air, derived from the process of mechanical aerosolization. According to Kelly and Pady (1953) the dry weather favours bacteria to get into the air, the soil borne bacteria of air were greatest in number during spring and autumn, and this finding is similar to the present study. Presence of various types of bacteria in this environment with variations in season, are probably also because of the seasonality of vegetables and variable rate of decomposition of vegetables waste.

During present study, wide varieties of gram-negative bacteria was reported from vegetable market environment. Among *enterobacteriaceae*, *Proteus* spp, *Citrobacter freundii, Erwinia herbicola, Enterobacter spp.* and *Escherichia coli* were reported and other gram-negative bacteria of *Chryseomonas luteloa, Vibrio* spp*., Acinetobacter calcoaceticus, Acinetobacter* spp. and *Pseudomonas* spp. were also reported. The previous researchers while studying similar environment also reported species of *E. coli* (Ercolani, 1979), *Erwinia herbicola* (Dutkiewicz *et al*., 2004), *E. herbicola, Acinetobacter* spp. and *Pseudomonas* spp. (Spiewak *et a*l.1996), *Pseudomonas* spp., *Citrobacter* spp. and *Serratia* spp. (Hilliger, 1991), *Vibrio* spp., *Klebsiella* spp., and *Proteus* spp. (Dutkiewicz, 1978). Presence of vibrio and related genera in the water and soil of this site, are due to the decomposing of vegetables in open ditches and rotten vegetables indicates the poor sanitary measures for that area.

Viability of bacteria has shown too affected by environmental temperature. In a study made by Tham and Zuraimi (2005), the percentages of particles that were viable airborne bacteria at different sizes were all found to be very low at higher temperatures (<1%), which is comparable to the present finding . The survivability pattern shows that humidity had pronounced effect on airborne survival of most of the bacteria, since these airborne bacteria comprises the group of soil-borne actinomycetes, cocci and other gram-positive bacteria have mechanism to resist the desiccation factors. Gram-negative bacteria and members of the group enteric bacteria could only survive in low temperatures and moderate humidity. High humidity and low temperature is a major factor for dissemination and distribution of gram-negative bacilli, especially members of the family enterobacteriaceae for this environment. Lower value of coefficient of determinant illustrate that, though the temperature and humidity governs the distribution, dissemination, and tenacity of airborne bacteria, yet these are not the major factors for generation and aerosolization of airborne microorganisms for this environment. Other factors like dwellers, transporters, services, domestic animals, water spray, and putrefactions are major contributors.

According to National Ambient Air Quality Standards, the annual average of Suspended Particulate Matter (SPM) of residential, rural, & other areas should not be more than 140 µg/m^3 and of Respirable Particulate Matter (RPM) (size less than 10 microns) should not be more than 60 µg/m^3. Since the average dry weight of a bacterium is 0.2 X 10^{-12} g, for this environment the average presence of viable bacteria as SPM accounted nearly 0.44 ng/m^3 seems to be higher and requires that the Municipal Corporation should take strict sanitary measures.

ACKNOWLEDGEMENTS

Authors are greatly thankful to Dr. S.M. Paul Khurana Honorable Vice-Chancellor Rani Durgavati Vishwavidyalaya, Jabalpur, for providing academic and materialistic supports, without which it was hard to perform this work. We also acknowledge the helpful comments and suggestions of the reviewers of IJEHSE.

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