Asian J. Exp. Sci., Vol. 22, No. 3, 2008; 245-254

Assessment of Air-borne Bacteria of Urban Grain-market Area

Karuna S. Verma[1] and Apurva K. Pathak[2]*

1. Aeroallergens and Immunology Laboratory,
Department of Biological Sciences, Rani Durgavati University,
Jabalpur-482001 (M.P.); India
2. Department of Pathology & Microbiology,
Modern Dental College & Research Center,
Indore (M.P.)-453112, India.

Abstract : Generations of dust consisting bio-particle are common phenomenon during the post harvesting processing and handling of farm commodities. These organic dusts are responsible for generation of many respiratory disorders. The present study was done to identify, enumerate and differentiate the respirable and non-respirable fraction of gram-negative and total type of bacteria based on seasonality and other environment factors. A model has also been prepared by using Stepwise Linear Regression Analysis in order to predict the amount of bacteria with relation to environment factors.

Key words : Air-borne bacteria, Respiratory disorder, Environmental factors, Prediction.

Introduction

More people are involved worldwide in agriculture than in any other work related activity (Jacobs, 1994). The agricultural workforce tends to differ from most other occupations in that it has a larger proportion of the workforce older than 65 years and younger than 16 years (Merchant and Reynolds, 2000). Particulate matter is emitted into the air from various pollution sources such as industrial activities, vehicles and agricultural processes. Agricultural operations are one of the most important sources of airborne organic dust and bioaerosols （aerosols or particulate matter of microbial, plant or animal origin that is often used synonymously with organic dust) (Douwes *et al*., 2002). Microorganisms are important components of dusts since they occur naturally in such materials as manure, silage and compost and can colonize in other farm materials when conditions are favourable for growth, e.g., during storage of moist grain, hay and straw (Eduard, 1997). Although microorganisms need to be viable for pathogenic effects to occur allergic responses (which are much more widespread) can be triggered by both viable (culturable and non-culturable but viable) and non-viable cells (Kenny *et al*., 1998). Most of the microorganisms present are mainly noninfectious (Dutkiewicz, 1992) but inhalation of non-infectious microorganisms and their constituents (bacteria, actinomycetes, fungal spores, algae, plant cells, insects and mites their fragments, endotoxin from gram-negative bacteria, mycotoxins and glucans from fungi.) can cause inflammation of the respiratory system while antigens and allergens may activate the immune system and cause allergic and immunotoxic effects (Eduard, 1997). Theoretically, bioaerosols can be launched from point, linear or area sources (Pillai and Ricke, 2002). The quantity and quality of these air borne microorganism is dependant upon various factors like the sources, the inhabitants and the environment (Frobisher *et al.*, 1974)

* **Corresponding author :** Dr. Apurva K. Pathak, Deptt. of Pathology and Microbiology, Modern Dental College & Research Center, Indore (M.P.)-453112, India; E-mail: *pathak.apurva@gmail.com*

The degree of dysfunction in exposed persons depends on the biological potency and concentration of exposure as well as on individual susceptibility. Airborne contaminants frequently occur in concentrations and compositions that challenge the defense mechanisms of the lung. This may be of particular importance in the case of susceptible workers and minors, whose exposure by the virtue of family-type operations is difficult to avoid. Epidemiological and clinical studies have contributed to the identification of associations between respiratory disorders and agricultural exposures. Chronic bronchitis, asthma, hypersensitivity pneumonitis, organic dust toxic syndrome and chronic airflow limitation have been found to occur in agricultural workers, (Zejda and Dosman, 1993) whereas; gram-negative bacteria alone are responsible for mucous membrane irritation; extrinsic allergic alveolitis; organic dust toxic syndrome; bronchitis; asthma & infection. (Lacy and Dutkiewicz, 1994). There are many different diseases and conditions that can be found in agricultural workers. Since the average human inhales about 10 m^3 of air per day inhalation is the predominant route resulting in adverse health effects (Pillai and Ricke, 2002). Nevertheless this does not mean that the respiratory tract is the sole route of exposure. Other pathways of exposure include the conjunctiva, while ingestion of microbes from surfaces (e.g. hands, food, etc) contaminated by deposition may also provide the necessary dose of infection. According to Lurie the infectious dose is a single organism in the right place (Lurie *et al*., 1950).

The transportation and forecasting of air borne microorganism is of prime importance to epidemiologist in order to access the chances of outbreak of disease in community environment and to evaluate the degree of pathogenicity of these organism. In this present study, not only the enumeration of air borne bacteria has been done but also a model has been prepared to establish the relation between the bacteria and its ambient environment.

Material and Methods

Jabalpur (Latitude: 23.2; Longitude: 79.95; Altitude: 391.) is the third biggest city of Madhya Pradesh. The city of Jabalpur is set in a most attractive stretch of country. The metropolis itself 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 hilly tracts in and around Jabalpur are covered by luxuriant vegetations. The climate of Jabalpur is overall pleasant and salubrious, has a year-round tropical climate generally characterized by warm days and cooler evenings.

Sampling site

The Grain market of Jabalpur is one of the crowdies place situated at the centre of the city. A large amount of commodities from different region were transported to sell. In order to evaluate the bacterial aerosols generation in course of grain handling this site has been selected. Area sampling should always be carried out near the potential sources of bioaerosols such as air supply systems, machinery and at or near the workers' position (Attwood, 1985). Duplicate samples in time and place must always be taken (Attwood, 1985).

The aero-bacteriological sampling has been done within the premises of grain market (*Krishi Upaj Mandi*), in duplicate and fortnightly in order to cover all the major season. The metrological data were collected from weather station Jabalpur. Apart from temperature and humidity five other metrological parameter were also recorded in order to analyze their effect on airborne bacterial population

Isolation from air

The Andersen two-stage viable (microbial) particle sampler (2-STG) has been developed for monitoring bioaerosols. It is a multi-orifice, cascade impactor with 400 holes per stage, drawing air at a flow rate of 28.3 L min^{-1}. This sampling rate is comparative to the breathing rate of a person going about their normal work. However worker respiration rates will rise with increased metabolic rate. In women respiration rates are lower (Collins, 2003). The different stages separate the airborne particles in size fractions. The stages have 50% cut-off diameters of 0.6 to 7 ìm (depending on the orifice used) and the impaction holes are arranged in a regular pattern that facilitates counting of colonies (Eduard and Heederik, 1998). As the air velocity increases across the different impacting surfaces the smaller particles get deposited resulting in the upper stages collecting the larger particles while the lower stages collect the smaller particles (Pillai and Ricke, 2002).The organisms get deposited because the air is forced to make a 90 degree turn to pass around the edge of the plate. This allows the smaller particles to pass around the growth medium while the larger particles cannot make the turn and impact on the surface (Collins, 2003). Dividing bioaerosols into size categories is important for epidemiological research (Bartley *et al.,* 1994).

Since the culture methods have been used widely for the measurements of airborne microorganisms in the work environment (Eduard, 1997). 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; 1966) at one meter height from the ground extramurally within the premises of grain market. The sampler was operated for two minutes at the site. For enumeration and identification of total viable type of bacterial population present in air, the TGYE medium plate kept on upper stage of the sampler, whereas for enumeration and isolation of gram- negative enteric bacteria, the EMB media plate were 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 human being.

Isolation from sources

This sampling was done in order to identify the presence of the bacteria in decayed materials and debris of market area and compare with those were present in air. Water samples were collected from associated sewage system of grain market, 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 was 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 ^{o}C were poured over inoculums by Pour plate technique (Krieg, 1981). Then plates were incubated at 37 ± 2 ^{0}C for 24 to 48 hrs.

Air contamination standards

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 can be calculated from the formula:

$$B = \frac{1000N}{RT} bcp^{-m}$$

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. (Collee *et al.,* 1999). 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 production of specific metabolites (Eduard and Heederik, 1998). After gram staining of bacteria, a study by Krahmer *et al.* (1998) divided the results into four categories as: actinomycetes, gram positive rods, gram negative rods, and gram positive cocci. In this study identification of isolates was done by using standard methods and manual (Jones and Sackin, 1980; Krieg and Halt, 1984; Collee *et al*.,1999; and Baron *et al*.,1994).

Statistical analysis

The number of samples collected will influence the precision of the exposure estimate and the associated confidence limits (Grantham, 2001). Monitoring agricultural working environments can be difficult and time consuming, which can lead to a small number of samples being collected (*Nieuwenhuijsen et al*., 1998). In order to analyze the effect of various environmental factors on the prevalence of airborne bacterial population and degree of its effectiveness with other environmental factors, multiple correlation coefficients and multiple regressions analysis was done (Aegerter *et al*., 2003; Allard, 1998; Box and Tiao, 1975; and Box and Jenkins, 1976).All the data were presented in the form of table and figures.

Results

The Grain Market area characterized by wide activity of grains handling creates dust at ambient atmosphere. 80 colonial morpho-types of isolates with 2974 bcp m^{-3} on TGYE and 22 bcp m^{-3} on EMB were recorded (Figure: 1); filamentous bacteria accounts 45% of total types of isolates. Filamentous bacteria belonging to the groups of

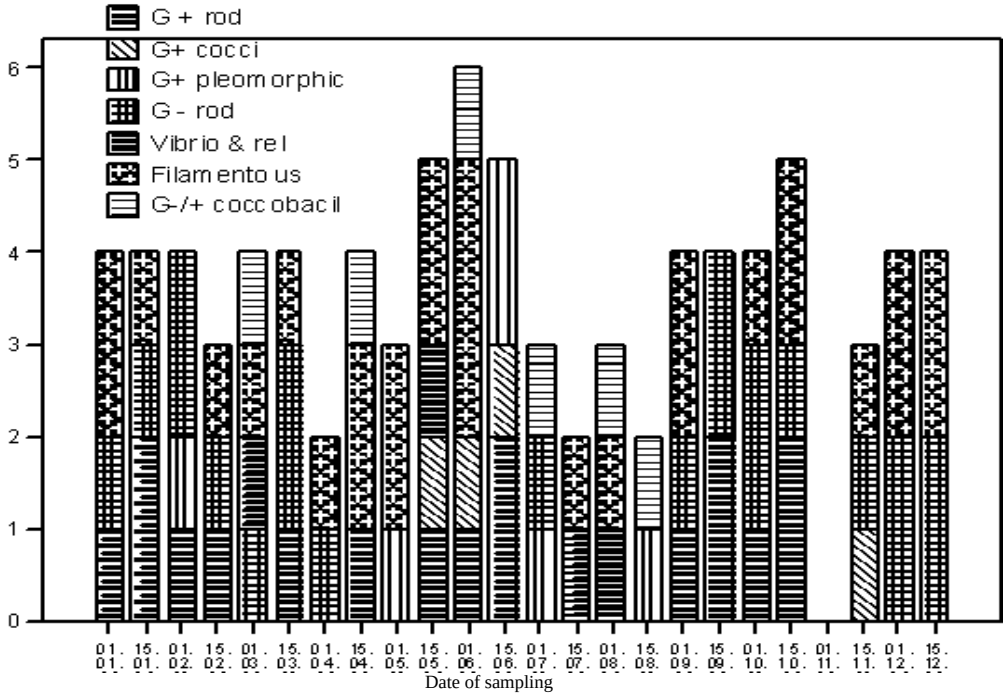

Fig. 1 : Types of bacteria isolated from Grain market

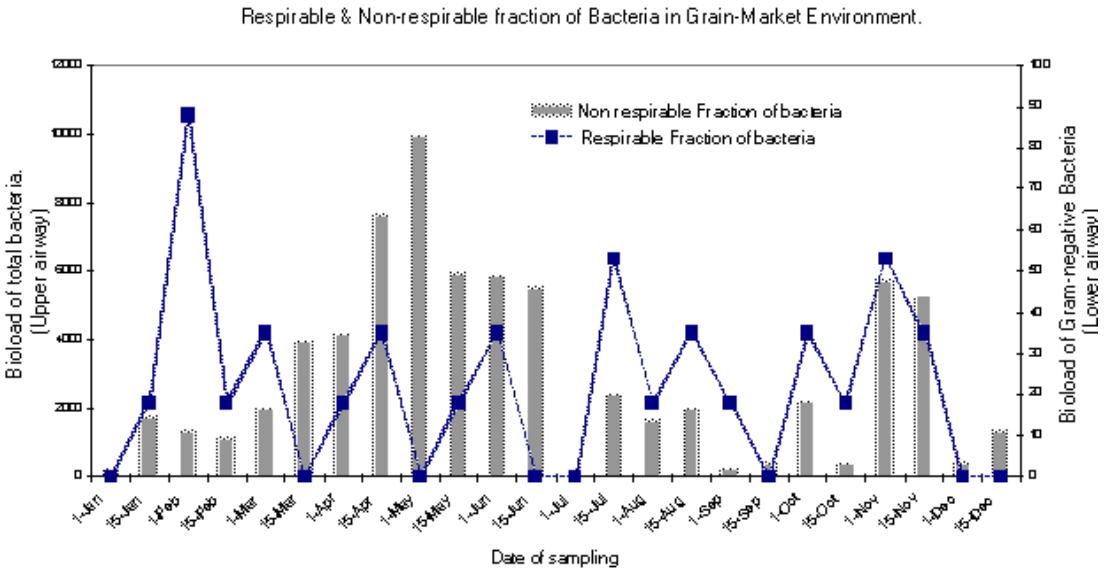

Fig. 2 : Respirable and Non-respirable Bioload of Bacteria

Actinomycetes are dominant (45%), followed by gram-negative bacteria (27%) and gram-positive rod (15%), rest 13% were belonging to the other groups, atmospheric bioload of family *Enterobacteriaceae* was recorded 29 bcp m^{-3} for this atmosphere. Species of the genera, *Enterobacter* and *Erwinia* were predominant among *Enterobacteriaceae*. The respirable fraction of gram-negative bacteria account less than one percent (0.70%) of total bacteria recorded (Fig. 2).

During winter season, the concentration of Gram-negative bacteria is highest (36%) of total type of species reported, compare to monsoon (26%) and summer (19%). Whereas, the bioload of total type of bacteria in summer is 6×10^3 bcp m^{-3}, in winter 2×10^3 bcp m^{-3} & in monsoon 1.7×10^3 bcp m^{-3} is reported. The fraction of *Enterobacteriaceae* is highest in winter (45%), followed by summer (29%) and Monsoon (26%).

Among gram negative bacteria *Acinetobacter* spp and *Pseudomonads* were reportedly predominant. The *Erwinia herbicola* reported dominant among *Enterobacteriaceae.* Apart from these, some other gram-negative bacteria i.e. *Flavimonas oryzihabitans* & *Vibrio diazotrophicus* also reported during sampling.

The average bioload of total viable bacteria, total respirable bacteria and total Gram Negative bacteria is 2974, 22 and 29 respectively; the Standard deviation of 2699, 22 and 32 implies the range 275 to 5673,zero to 44, and zero to 61 respectively accounted in humidity ranging from 33 to 84 and temperature ranging from 19 to 31ºC. (Table: 1). Correlation of bio-load of total viable bacteria with humidity is negatively strong (-0.654) and with temperature is positively strong (0.653), linear and direct despite of the fact that the correlation between temperature and humidity is week (-0.406). There is no correlation established between environmental factors and gram-negative bacteria of this environment. (Table: 2).

About 42.7% of the variance is accounted for by the model I and for model II it is 60.7%, 68.44 unit decrease in bacterial bio load by increasing one unit in humidity when only humidity is taken into account for the forecasting and when both temperature and

humidity is taken into account 48.72 unit decreases and 200.5 unit increases in bacterial bio load by increasing one unit of humidity and temperature respectively, and the constant is 6975 shows the bio-load at any humidity condition and 848 when both the factors where considered (Table: 3). The sample Coefficient of Determination after adjusting for the degree of freedom lost in the process of estimating the regression parameter for model I is 0.401 and for model II is 0.569. The higher value of Model II shows that both humidity and temperature is important predicator of bio load for this atmosphere than the humidity alone. By using Stepwise Linear Regression, the model prepared to enumerate total bacterial type Bio load for this atmosphere is:

- Model I (R:0.654): Bioload in Grain Market = 6975.062 + (-68.436) Averagte Humidity
- Model II (R:0.779): Bioload in Grain Market =848.327 + (-48.719) Averagte Humidity + (200.502) Average Temperature

From the ANOVA table (Table: 4) under degree of freedom column V1=1, V2=22 and Fov is 16.408(Sig. 0.001) for the model I and Fov is 16.197(Sig. 0.000) for the model II, using the significance level of 0.05, implies that critical value or Fcv = F0.001 is 4.30 from the F distribution table. Thus, we can reject Ho in favour of Ha. This means that the linear regression model that has been estimated is not a mere theoretical construct indeed it does exist and is substantially significant. Se for

Table 1 : Descriptive Statistics

Variables	Mean	Std. Deviation	N
Humidity (Avg.)	58.458	25.7783	24
Temperature (Avg.)	24.808	6.2412	24
Bioload of total viable bacteria	2974.417	2699.1003	24
Bioload of total respirable bacteria	22.083	22.1417	24
Total Gram Negative bacteria	29.458	31.525	24

Table 2 : Correlations

Variables⇒ ⇓	Bioload of total viable bacteria			Bioload of total respirable bacteria			Total Gram Negative bacteria		
	Correlation	Significance (2-tailed)	df	Correlation	Significance (2-tailed)	df	Correlation	Significance (2-tailed)	df
Humidity (Avg.)	-0.654	0.001	22	0.086	0.689	22	0.152	0.478	22
Temperature (Avg.)	0.653	0.001	22	0.064	0.766	22	0.096	0.654	22
Bioload of total viable bacteria	1	.	0	0.086	0.689	22	0.036	0.868	22
Bioload of total respirable bacteria	0.086	0.689	22	1	.	0	0.849	0	22
Total Gram Negative bacteria	0.036	0.868	22	0.849	0	22	1	.	0

a Cells contain zero-order (Pearson) correlations.

Table 3 : Model Summary (Dependent Variable: Bioload of total viable bacteria)

	Model	
	Model I	**Model II**
R	0.654 (Predictors: (Constant), Hum. Avg.)	0.779 (Predictors: (Constant), Hum. Avg., Temp.Avg.)
R Square	0.427	0.607
Std. Error of the Estimate	2088.675	1771.485

Table 4 : ANOVA (Dependent Variable: Bioload of total viable bacteria)

Model		Sum of Squares	df	Mean Square	F	Sig.
I	Regression	71581879.34	1	71581879.34	16.408	.001(a)
	Residual	95976398.5	22	4362563.568		
	Total	167558277.8	23			
II	Regression	101656939	2	50828469.5	16.197	.000(b)
	Residual	65901338.83	21	3138158.992		
	Total	167558277.8	23			

a *Predictors: (Constant), Humidity (Avg.);* ***b*** *Predictors: (Constant), Humidity (Avg.), Temperature (Avg.)*

Table 5 : Coefficients (Dependent Variable : Bioload of total viable bacteria)

		Model I		Model II		
		(Constant)	Hum. Avg.	(Constant)	Hum. Avg.	Temp.Avg.
Unstandardized Coefficients	B	6975.062	-68.436	848.327	-48.719	200.502
	Std. Error	1075.737	16.895	2179.262	15.681	64.767
t-test		6.484	-4.051	0.389	-3.107	3.096
Significant		0	0.001	0.701	0.005	0.005

model I is 2089, for model II is 1771; bio load could vary by in model I is± 2089, and in model, II is ± 1771 and about the estimated regression equation for each value of average humidity and average humidity and temperature respectively. (Table: 5).

Discussion

The European Community described respirable dust as having a median diameter of 4.0 ìm (ISO, 1995) and they are hazardous when deposited in the gas-exchange area of the lung and penetrate to the level of the terminal bronchioles and the alveoli (the gas-exchange area) (Kirkhorn and Garry, 2000). Agricultural operations are one of the most important sources of airborne organic dust and bioaerosols. Bioaerosols are defined by Douwes *et al.* (2003) as aerosols or particulate matter of microbial, plant or animal origin that is often used synonymously with organic dust. Bioaerosols of different and complex composition are produced from soil and crops during different agricultural operations and occur in diverse environments and can be a vehicle for the dissemination of human and animal pathogens (Pillai and Ricke, 2002). Workers are exposed to microorganisms from laden aerosols emitted from grains, cotton, hay, jute, and tobacco (Adel Hameed and Khodr, 2001).Bioaerosol particles range in size from <0.01 to 100 µm with up to 40% in the respirable range (Kirkhorn and Garry, 2000).

There is no exposure standard for bioaerosols in any other country of the world that is probably due to the fact that, the information about the potential impact about bioaerosols is still developing. Dose-response relationships are often not been well described or understood for bioaerosols (Douwes *et al*., 2002). It is hard to identify a point where exposure to bioaerosols becomes a health hazard (Umbrell, 2003) and this has made it very difficult to implement any exposure limits.

Dust exposure is often used as the proxy exposure limit (Douwes *et al*., 2002). A threshold limit was proposed in the past of 10^5 cfu of total microorganisms per 1 m^3 of air (Dutkiewicz, 1992) and 10^3-10^4 has also been suggested as high (Eduard and Heederik, 1998), but among farmers exposure to 10^6-10^8 spores per cubic meter have shown no-effect (Eduard and Heederik, 1998). Total cfu of both bacteria and fungi at a level of 10^3 cfu m^{-3} of air has also been suggested (Scarpino and Quinn, 1998), and used as an indictor of possible human health concerns (Gibbs *et al*., 2004). Umbrell (2003) has suggested that it is unlikely that there will be any exposure standards in the future, as it is just too hard to determine what levels of exposure are likely to produce consistent and measurable negative health effects. 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, is the TLV standard set up in India. The OEL of 5-10 x 10^3 cfu m-3 for total microorganisms, 1 x 10^3 cfu m^{-3} for Gram-positive bacteria, was suggested in a Scandinavian study (Dutkiewicz, 1997). During this survey the bioload of total bacteria type was recorded in the month of May was 9931m^{-3} is exceeding the TLV for any atmosphere. The respirable fraction of gram-negative bacteria account less than one percent (0.70%) of total bacteria sampled. The bacteria which are able to impact on upper respiratory tract is higher than which invades lower airways. It is probably due to fact that the dust concentrations are higher in this environment.

Shrivastava in 1992 reported highest cfu of total type of bacteria in monsoon followed by winter and summer and of *Actinomycetes* in summer followed by monsoon and winter in market area of Jabalpur (Shrivastava,1992). 10^4-10^6 cfu m^{-3} was noted during wheat harvesting season, similar to the month of summer. In summer highest concentration of gram positive bacteria and filamentous bacteria were reported is similar to the previous workers findings (Adel Hameed and Khodr, 2001). Dutkiewicz, *et al.* (2004) reported 45.5 – 98.3 cfu m^{-3} respirable fraction of airborne microflora (Dutkiewicz, *et al*.,2004). The respirable fraction of gram negative bacteria alone was recorded highest (88 bcpm^{-3}) in the month of February, but average occurrence is reported highest (20.14 bcpm^{-3}) in winter.

The gram negative bacteria *Acinetobacter* spp, Pseudomonads and *Erwinia herbicola* reported as dominant species, many other were also reported both as dominant species and *Erwinia herbicola* among Enterobacteriaceae in agro-environment. (Adel Hameed and Khoder, 2001; Dutkiewicz, *et al.*, 2004; Rylander and Jacobs, 1994).

Most of the bacteria recovered from grain market environments proven respiratory allergens. This high level of endotoxin is potential risk to the workers of grain market and inhabitant of that area. In the light of the fact, most of the bacteria were adhere to the surface of the dust indicated by higher recovery of bacteria on the upper plate of two stages Andersen sampler.

References

Adel Hameed A. A. and Khodr M. I. (2001): Suspended particulates and bioaerosols emitted from an agricultural non-point source. *Journal of Environmental Monitoring* (JME) **3**, 206-209.

Aegerter B. J., Nuñez J. J., and Davis R. M. (2003): Environmental factors affecting rose downy mildew and development of a forecasting model for a nursery production system. *Plant Dis*. **87**,732-738.

Allard R. (1998): Use of time series analysis in infectious disease surveillance. *Bull. World Health Organ*. **76**, pp. 327-33.

Andersen A. A. (1958): A new sampler for collection, seezing & enumeration of viable airborne bacteria. *J. Bacterial.* **76,** 471 - 484.

Andersen A. A. (1966): A sampler for respiratory health hazards assessment. *Am. Ind Hyg. Assoc. J.* **27**, 260 - 265.

Attwood P. (1985): Quantification of Airborne Organic Dust and its Biologically Active Constituents in the Working Environment. *Wageningen*, Agricultural University, the Netherlands.

Baron E. Jo., Peterson L. R. and Fenigold S. M. (1994): Diagnostic Microbiology. (9th edn.), (Mosby Publication. USA).

Bartley D. L., Chen C-C, Song R., and Fischbach T. J. (1994): Respirable Aerosol Sampler Performance Testing." *American Industrial Hygiene Association Journal* **55,**(1036-1046).

Box G.E.P., and Jenkins G.M. (1976) Time series Analysis, Forecasting and control, (Holden – Day, San Francisco)**.** 575 pp.

Box G.E.P. and Tiao G.C. (1975): Intervention analysis with applications to economic and environmental problems, *Journal of the American Statistical Association*, **70**, pp.70-79.

Collee J.G., Fraser A.G., Marmion B.P. and Simmons (1999): A. Practical Medical Microbiology.(**14th** Eds). (Charchil Livingstone. London).

Collins R. (2003): Culturable Air Sampling for Fungal Contaminants. *The Synergist*. January: 27-29.

Douwes J., Pearce N. and Heederik D. (2002): Does environmental endotoxin exposure prevent asthma?. *Thorax* **57**(**1**): 86-90.

Dutkiewicz J. (1992):Bacteria and their Products as Occupational Allergens. *Pneumonologia I Alergologia Polska,* **60, suppl. 2**: 14-21.

Dutkiewicz J. (1997): Bacteria and fungi in organic dust as potential health hazard. *Annals of Agricultural Environmental Medicine***, 4**: 11-16.

Dutkiewicz J., Krysinka- Traczyk E., Skovska. C, Prazmo Z., Sitkowska J., and Cholewa G., (2004): Exposure to airborne microorganisms, dust and endotoxin during flax scotching on farms. *Ann. Agric. Environ. Med* . **11**: 309–317.

Eduard W. (1997): Exposure to non-infectious microorganisms and endotoxins in agriculture. *Annals of Agricultural Environmental Medicine*. **4**: 179-186.

Eduard W. and Heederik D. (1998): Methods for Quantitative Assessment of Airborne Levels of Noninfectious Microorganisms in Highly Contaminated Work Environments. *American Industrial Hygiene Association Journal*. **59**: 113-127.

Frobisher M., Hinsdill R.D., Crabtree K.T., and Goodheart C.R. (1974): Fundamentals of Microbiology. (W.B. Saunders Co. Japan).

Gibbs S. G., Green C. F., Tarwater P. M. and Scarpino P. V. (2004): Airborne Antibiotic Resistant and Nonresistant Bacteria and Fungi Recovered from Two Swine Herd Confined Animal Feeding Operations. *Journal of Occupational and Environmental Hygiene*. **Nov.**: 699-706.

Grantham D. (2001): Simplified Monitoring Strategies. Tullamarine, *Australian Institute of Occupational Hygienists* (AIOH).

ISO. (1995): ISO-7708:1995. Air quality- Particle size fraction definition for health related sampling. Geneva. *International Organisation for Standardisation* (ISO).

Jacobs R. R. (1994): Risk Environments. Organic Dusts. R. Rylander, and R. R. Jacobs, Boca Raton, (Lewis Publishers).

Jones D. and Sackin M. J. (1980): Numerical methods in the classification & identification of bacteria with special reference to the *Enterobacteriaceae*. In: M. Goodfellow & R. G. Board (Eds.). Microbiological classification & Identification, (Academic Press Inc. London). pp. 73-106.

Kenny L. C., Stancliffe J. D., Crook B., Stagg S., Griffiths W. D., Stewart L. W. and Futter S. J. (1998): The adaptation of existing personal inhalable aerosol samplers for bioaerosol sampling. *American Industrial Hygiene Association Journal*. **59**: 831-841.

Kirkhorn S. R. and Garry V. F. (2000): Agricultural lung diseases. *Environmental Health Perspectives*. **108** (Supplement 4): 705-712.

Krahmer M., Fox K., Fox A., Saraf A. and Larsson L. (1998): Total and viable Airborne Bacterial Load in Two Different Agricultural Environments

Using Gas Chromatography-Tandem Mass Spectrometry and Culture: A Prototype Study. *American Industrial Hygiene Association Journal*. **59**: 524-531.

Krieg N. R. and Halt G. H. (1984): In.: Bergey's Manuals of Systematic Bacteriology. Vol. **I** (Edn.) (Baltimore,William& Wilkins Co).

Krieg N. R. (1981): Enrichment & Isolation. In: P. Gerhasdt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W.A. Wood, N. R. Krieg and G. B. Phillips (Eds). Manual of Methods for General Bacteriology, *Am. Soc. Microbiol.* Washington, D. C.

Lacy J. and Dutkiewicz J. (1994): Bioaerosols and Occupational lung disease. *J. Aerosol. Sci*. **25**(8): 1371-1404.

Lurie M. B., Heppleston A.G., Abramson S. and Swartz I.B. (1950): An evaluation of the method of quantitative air-borne infection and its use in the study of the pathogenesis of tuberculosis. *Am. Rev. Tuberc.* **61** :765-797.

Merchant J. and Reynolds S. (2000). Work in agriculture. Epidemiology of Work Related Diseases. (C. McDonald. London, BMJ Books).

Nieuwenhuijsen M., Kruize H. and Schenker M. B. (1998): Exposure to Dusts and Its Particle Size Distribution in California Agriculture. *American Industrial Hygiene Association Journal*. **58**: 34-38.

Pillai S. D. and Ricke S. C. (2002): Bioaerosols from municipal and animal wastes: background and contemporary issues. *Canadian Journal of Microbiology.* **48**(8): 681-696.

Rylander R. and Jacobs R. R. (1994): Organic Dusts: Exposure, Effects, and Prevention. (CRC Press).

Scarpino P. V. and Quinn H. (1998): Bioaerosol distribution patterns adjacent to two swine-growing-finishing confinement units in the American Midwest. *J. Aerosol Sci*. **29**: S553-S554.

Shrivastava K. (1992): Composition & Significance of Microbial spores in the air at Jabalpur. Ph.D. Thesis. Department of Post Graduate Studies & Research in Biological Sciences.R.D.V.V. Jabalpur.

Umbrell C. (2003): Mould- Creating a Scientific Consensus on a Hot Topic. *The Synergist*. April: 35-38.

Zejda J. E. and Dosman J. A. (1993): Respiratory disorders in agriculture. *Tubercle and Lung Disease*. **74**: 74-86.