EDGEWOOD
RESEARCH, DEVELOPMENT & ENGINEERING CENTER
U.S. ARMY CHEMICAL AND BIOLOGICAL DEFENSE COMMAND

ERDEC-TR-367

PROGRAM FOR THE ASSESSMENT OF BACKGROUND BIOAEROSOLS IN THE INDUSTRIAL CITY OF PUNE, INDIA

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Aberdeen Proving Ground, MD 21010-5423

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13. ABSTRACT *(Maximum 200 words)*

The Biological Systems Integration Team, Research and Technology Directorate, U.S. Army Edgewood Research, Development and Engineering Center (ERDEC), has many missions related to defense against biological aerosols. One of these is the establishment of sites inside and outside the U.S. to measure and characterize bioaerosols and to accumulate a reference database on biological backgrounds in the atmosphere. Our most completely instrumented site is at ERDEC, Aberdeen Proving Ground, MD. Other measurements are being carried out at Mace Head, Ireland, and we are working to establish a collaborative program to access data from many sites in Great Britain. A recent bonus was the application of the "Rupee Fund" to our requirements, giving us a proposed bioaerosol monitoring site in Pune, India, at no cost to ERDEC. The Rupee Fund was set up by the U.S. State Department years ago when it could not recover monies intended for a failed project in India. Instead, Indian scientists were asked to propose research projects against these funds. This report describes how the Rupee Fund is expected to provide a valuable biomonitoring site for 3-5 years, without cost, and with the author serving as the principal investigator.

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PREFACE

The work described in this report was authorized under Project No. 622384/ACB2, Aerobiology. This work was started in June 1995 and completed in June 1996.

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1. INTRODUCTION

1.1 The "Rupee Fund"

Many years ago, millions of US dollars were sent to India to pay for a project that could not be completed. Because Indian rupees cannot be taken from the country, the US dollars could not be withdrawn from India. It was decided to earmark this money for use by universities and research establishments throughout India. Researchers would apply for funding from this "Rupee Fund" by submitting research proposals to the US State Department, through its embassy in New Delhi, and to a panel of Indian educational officials at the national level. The proposal format is similar to that presented in this report. Since ERDEC scientists can serve as technical monitors for projects carried out that are pertinent to their own research, such research can be performed at no cost to ERDEC and employing cheap technical labor rates prevailing in India. A priority among biological defense programs at ERDEC is the establishment of stations to measure bioaerosol backgrounds worldwide. Thus the Rupee Fund is a true asset in this research.

1.2 About Pune City and Its University

The city of Pune is described as the "Queen of Deccan." It is situated 192 km south of Bombay. It is a city of great historical and cultural importance and is equally well known as a great seat of learning. There are numerous centers of historical and cultural significance in and around Pune.

The University of Pune was established in the year 1949 on a 411 acre campus of grass and woodlands. It is a teaching and affiliating university with 38 post graduate departments, 170 affiliated colleges, and 80 recognized institutes. The university also has important national facilities on its campus relevant to the research described in this report, such as animal tissue culture, bioinformatics and advanced computing.

Interdisciplinary schools in natural and social sciences have been established at the initiative of the University Grants Commission. The jurisdiction of the university at present covers three districts of the state of Maharashtra, namely Pune, Ahmednagar and Nasik.

The University's Vice-Chancellor maintains his office in a building (Figure 1) which was built as the governor's house during the British occupation of India. The grounds are extensively gardened (Figure 2), although conditions in Pune city are often dirty and unkempt. The Department of Physics building is shown in Figure 3. It is also set on landscaped grounds (Figure 4

The University's Biophysics Section, which will perform the research discussed in this report, is well-equipped. For example, a thermoluminescence set-up with its data acquisition system is shown in (Figure 5), a biopotential measurement system is shown in Figure 6, and a Perkin-Elmer spectrofluorometer is shown in Figure 7. Also available are a Hitachi absorption spectrometer (Figure 8), a modern microscope with camera attachment (Figure 9), and a microelectrode system for additional biopotential measurements (Figure 10).

The Biophysics Section is already involved in the collection and analysis of particles in the air at several different locations in Pune. These measurements will continue as part of the new research program. Examples of two of these particle collection stations are shown in Figures 11 and 12.

1.3 Philosophy of the Project

Pune is one of the fastest developing cities in India, which presents an ideal model for many kinds of studies. One finds an interesting mixture of local beliefs and modern ingredients associated with rapid industrial development. Within the last two decades there has been a fast industrial growth which has resulted in a rapid increase in population from 0.5 million to 2-3 million.

There was also an alarming increase in vehicular traffic. This has put tremendous pressure on facilities such as sanitation and water supply, and it has also polluted the environment. Efforts are therefore being made to understand the changing environment in order to take appropriate measures. Various organizations have been carrying out studies related to the quality of water, air pollution, waste product management, and noise pollution.

However, no studies regarding particulate matter and biobackgrounds have been carried out so far. The present project aims at performing such studies. The results obtained are expected to go a long way toward ensuring a healthy environment for Pune city. This study will also provide guidelines for other cities in India and also for cities in other developing countries.

2. THE RESEARCH PROGRAM

2.1 Definition of the Problem and Objectives

The present program is aimed at assessing the biobackgrounds of Pune city. The city is located at (18.31 N, 73.55 E) in India and has a population of about 3 million. This is one of the industrial cities and has a high vehicular density. The climatic conditions are moderate and temperature fluctuations are in the range of 7 C - 37 C throughout the year. The humidity is about 60-70% in summer and increases to 97% in the rainy season.

These conditions seem favorable for the growth of pathogens, and in recent years there has been an increase in the occurance of airborne diseases such as viral fever. In this respect it is most appropriate to carry out investigations related to the biobackgrounds. The plan of this study includes collection of air samples at heights of 50 to 100 feet from the ground level, with the help of an air sample collector. The samples will be collected on agar plates and will be cultured in the laboratories. The pathogens will be isolated, characterized and identified using routine techniques.

Thus the objectives of this research are to collect, isolate and characterize airborne pathogens in Pune city.

Figure 1. Vice-Chancellor's Office

2.2 Existing State of Knowledge

The presence of particulate matter in urban environments is becoming hazardous and has resulted in many human casualties (Ref.1) such as during the December 1952 London Smog episode. The particulate matter in the environment is caused by vehicle exhausts, power stations and also through anthropogenic emissions (Ref.2). In urban areas, road transport is a dominant factor and

Figure 2. Part of the University Garden.

Figure 3. Department of Physics Building.

contributes as high as 94% of the total particulate matter. Now the particulate matter comprises very small particles of about 1 um diameter. Apart from chemical and physical particulate matter, biological matter is also found to exist in the atmosphere. Studies carried out so far indicate the presence of bacterial aerosols in dental clinics (Ref.2). Literature scans for the years 1993-1996 reveal that there are very few studies which have been related to the bio backgrounds. This gives us a scope to initiate efforts to obtain a better understanding of living matter suspended in an urban environment.

Figure 4. Landscaping in Front of the Physics Building.

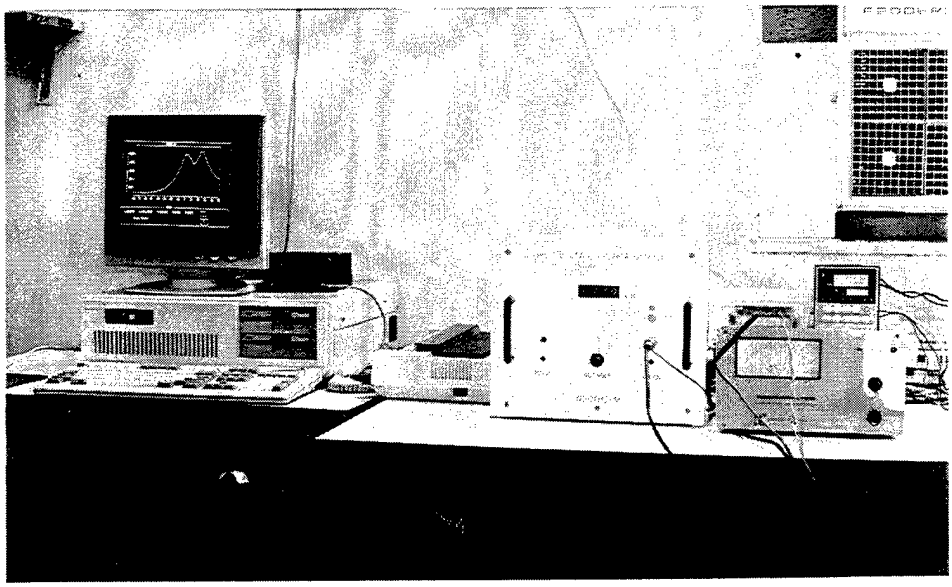

Figure 5. Thermoluminescence Set-Up with Data Acquisition.

2.3 Importance of the Research

The international and national states of knowledge on the subject are such that various studies have been carried out to estimate the particulate matter in the atmosphere and to characterize the species in relation to their size distribution. However, fewer studies are reported about bio backgrounds which exist in an urban environment. In India, practically no such studies have been carried out so far. Growing industrialization

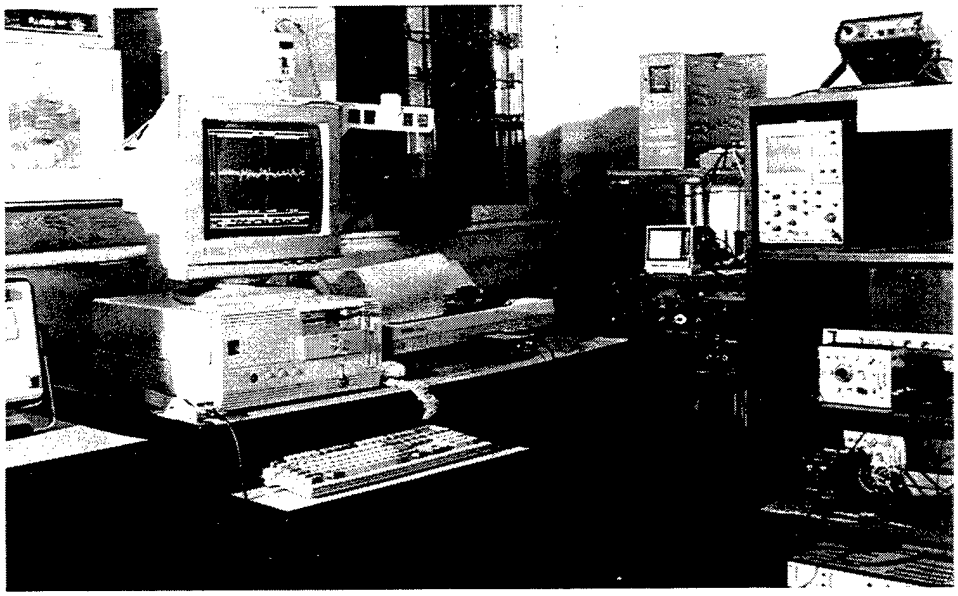

Figure 6. Biopotential Measurement System.

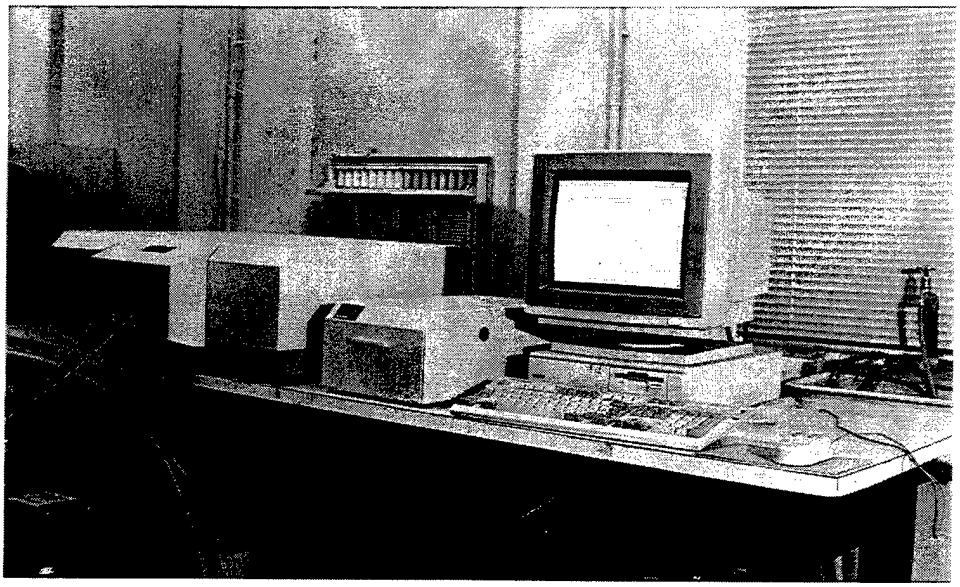

Figure 7. Perkin-Elmer Spectrofluorometer.

is polluting an urban environment which may have hazardous effects on human population in the near future. Therefore, it is very much essential that we have a proper understanding of this polluted environment, so that proper precautions can be taken to control further degradation. Pune city being in its early stages of industrial development, information obtained through these studies could be useful for similar situations elsewhere in the world.

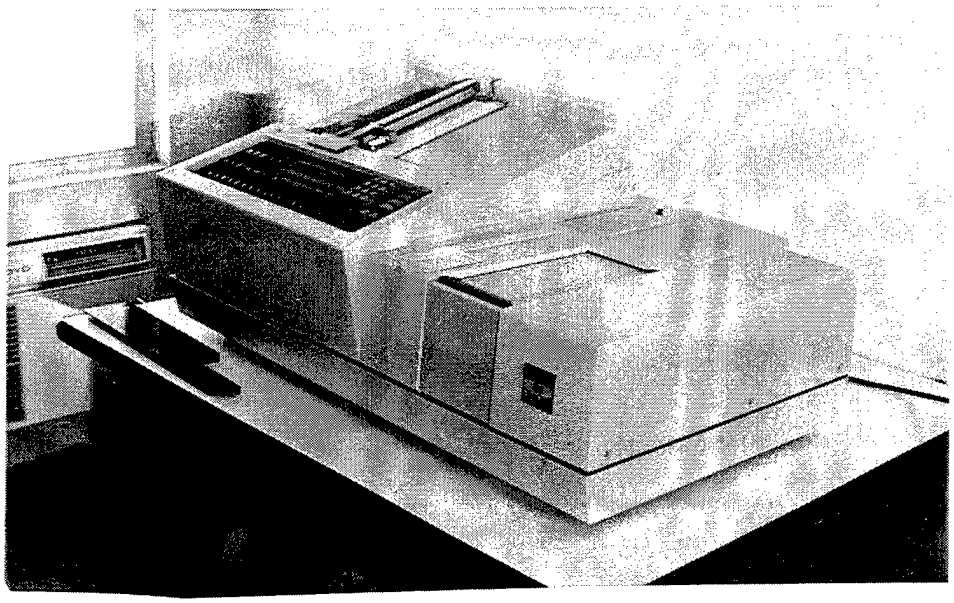

Figure 8. Hitachi Absorption Spectrometer.

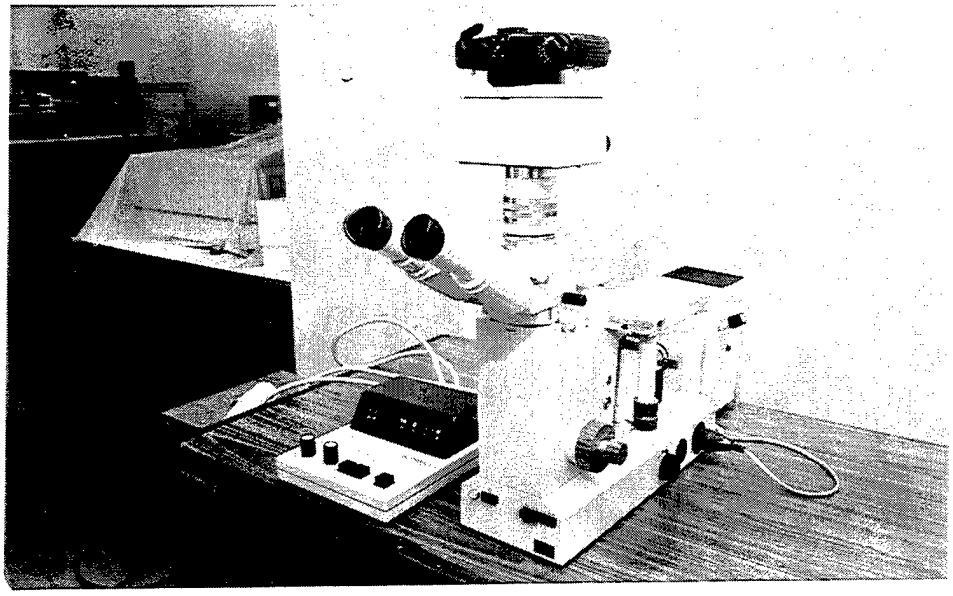

Figure 9. Modern Microscope with Camera Attachment.

This information would be useful for getting an early warning for a possible epidemic and also to take measures for strengthening the immunity of the population likely to be affected.

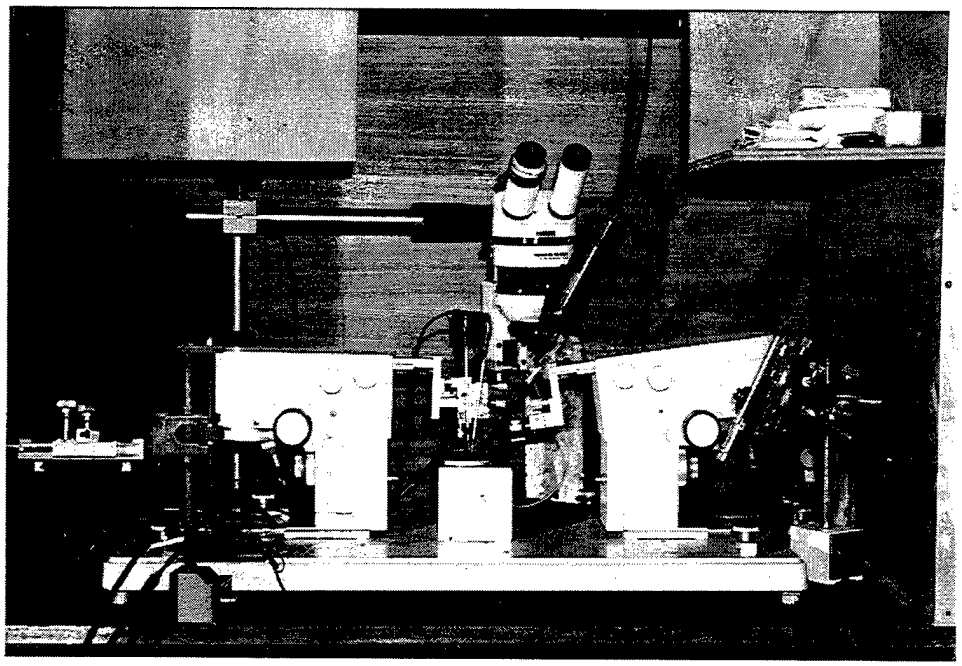

Figure 10. Microelectrode System for Biopotential Measurements.

Figure 11. Particle Collector Site in Pune City; Particles up to 10um Diameter Can Be Collected.

2.4 Expertise Available

The group assigned to this project will include Dr. P.B. Vidyasagar, who is a Reader in Biophysics and is the coordinator of the School of Basic Medical Sciences. He has 18 years of teaching and research experience, having guided 5 students for the Ph.D. and 18 students for the M.Phil. degree. He has supervised schemes such as culturing of dorsal root ganglion cells and effects of lead on algae Spyrogyra. He has vast experience in the interdisciplinary areas. He has published extensively in national and international journals and has attended several conferences and workshops in India and abroad. He is a member of the Institute of Physics, UK and Indian Biophysical Society, India, He has been conducting studies such as the effect of lead pollution on gas station workers and also sound level measurement in Pune city.

Dr. Bhushan Patwardhan has an M.Sc., Ph.D. (Biochemistry), expertise in physico-chemical methods of analysis, microbiological techniques and environmental health. He has worked in the area of natural product drug discovery, complimentary medicine and presently he is working on a project on air pollution due to vehicular emissions. Dr. Patwardhan has established an interdisciplinary School of Health Sciences which undertakes various studies and projects on community health. In the present project on biomaterials, his expertise in the field of particulate air pollution will be useful in collecting biomaterials from different areas of Pune city. He also has interactions with a research group at Pune studying bioaerosols.

Figure 12. Another Particle Collector Site in Pune City.

2.5 Research Methodology

The preliminary survey conducted in Pune city in relationship to the distribution of particulate matter will be used to make the selection of sites for sample collection. Information available regarding the health status of the residents in relation to the respiratory and cardiovascular diseases would also be used for this purpose. Samples such as suspended bacteria, pollen grain, fungi, etc. in selected areas will be collected from ground level to a height of 200 feet. From the samples collected, the size distribution of the particulate matter will be studied.

Identification of biological and nonbiological matter will be carried out using routine culturing techniques. Isolation and identification of the biological matter will be carried out using staining techniques in combination with fluorescence and phase contrast microscopy. Biochemical identification tests would also be used if required. Measurement of parameters such as wind direction, wind velocity, temperature, relative humidity, etc., will be carried out and their relation with the biobackgrounds would be studied. These studies will be carried out around the year to study the seasonal variations. A health status survey in the area of interest would be carried out. Efforts will be made to identify the sources of the observed biobackgrounds.

2.6 Organization and Scheduling

The work elements will be organized and carried out as follows:

- procurement of necessary equipment and appointment of staff;
- selection of work areas and sample collection;
- culturing of specific living matter and isolation of the same;
- identification and characterization;
- monitoring of the levels of identified living matter throughout the year;
- measurement of parameters such as wind velocity, temperature, humidity, etc., and their correlation with the biobackgrounds;
- monitoring of levels of the living matter along with the status survey of the residents in that area;
- source identification and remedies.

Statement of the nature and extent of collaboration involved, including equipment/material to be provided by the US collaborator to the Indian researchers: No equipment will be provided by the US collaborator to the Indian side. The nature of the collaboration includes use of data analysis facilities and advice of the US experts in the studies to be undertaken. But this agreement does not preclude the loan or other provision of an instrument such as an Aerodynamic Particle Sizer (APS) to the Indian side if US funds should become available, given that an APS would greatly enhance the gathering of data within this project.

Time Schedule based on the work elements identified above:

First Year:
- procurement of material, equipment and appointment of staff;
- selection of areas and collection of samples; size distribution analysis;
- culturing of specific living matter; isolation of the same;
- identification and characterization.

Second Year:
- monitoring of the levels of identified living matter throughout the year;
- correlation with parameters such as wind velocity, temperature, humidity, etc.

Third Year:
- monitoring of levels along with the status survey of the residents in that area;
- source identification and remedies.

Duration: Normally a project is approved for 3 years; it can be extended up to 2 more years.

Personnel: One technical person with B.Sc. and experience at M.Sc. level; one scientific person with M.Sc. and two years' research experience. 36 man months divided evenly between the two.

3. DISCUSSION AND CONCLUSIONS

As described above, this contractural effort appears to meet all necessary requirements to provide ERDEC with valuable and continuing data base on bioaerosol backgrounds in an important area of the world. India's atmospheric conditions are unique and constantly-changing. Weather in the Pune region originates in the Middle East, and thus atmospheric monitoring might take on added importance in future conflicts. We conclude that the Rupee Fund is

gifting ERDEC with an important link in its efforts to establish a worldwide network of monitoring sites to gain insights into bioaerosol backgrounds.

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