threshing etc. Thus, the agricultural practices can pollute the air with plant pathogens.

10) A long term study of the micro-organisms in their particular environment concerned, is absolutely necessary for their effective control.
PART - II

INTRAMURAL MICROBIAL AIR-POLLUTION
III. BIO-DETERIORATION ASPECT

Intramural microbial pollution of air and its relevance to storage diseases of food grains
REVIEW OF LITERATURE

The contamination of indoor environment with the presence of microbial population and other contaminants is certainly a major health problem. Microbial pollutants are present inside the buildings such as homes, schools, colleges, hospitals, farms, caves and industries, in fact all the closed spaces.

It was Pasteur (1861) who showed that the bacterial pollutants are present in the air, and a decade later Lister (1871) realised the significance of clean air in the operation theatre. He devised a carbolic acid spray which became a basic part of his new antiseptic surgical methods.

From the hygienic point of view, Miquel (1883) studied the microbial content of hospital air in the crowded wards in Paris. Rostrup (1909), Windslow and Browne (1914), Flensborg Samsoe-Jensen (1949), Nilsbey (1949), Wallac et al. (1950), and Swaebly and Christensen (1952) also studied the microbial content of indoor environment. Lurie et al. (1957) isolated some dermatophytes from the indoor environment of caves. Williams (1958) carried out some preliminary survey of bacterial pollutants of caves in South Wales (U.K.).

It has been recognised for sometime that microbial pollutants and other particulates can be generated by indoor activities involving cooking, home heating and air conditioning etc. Wide spread use of aerosol sprays can also disseminate biological materials. It was shown that when the air of a room was examined, flakes of keratin are found in abundance and they are often have
attached with bio-particles and the air of a room contains four times more bio-particles than the outdoor air (Lewis, 1971). Therefore, the house dust is more allergenic than the road dust; but under certain circumstances indoor concentration of bio-particles may actually exceed the outdoor concentration (Dorothy, 1977). The source of these microbes was a matter of speculation. They must enter the boundary layer either from interface of floors, walls, roofs, window-sills with the wind current from outdoor air.

Recent investigation into indoor microbial content of air, has been carried out by Marjut (1977) in the working environment of a mill and a veneer factory of Kuopio city, Central Finland. It is reported that high concentration of bio-particles in the veneer factory seems to be caused by unclean raw materials despite of the presence and movement of people working in the factory.

Pohjola et al. (1977) reported that the concentration of *Penicillium* and *Aspergillus* species in the damping room of a garbage disposal plant in Turku was 6,000 times greater as compared to outdoor air.

The composition of the microbial pollutants of the glass house air had received little attention. The importance of mildew on roses caused by the air-borne fungal bio-pollutants, *Sphaerotheca pannosa* (Wallr) Lev. var. *rosae* Woron, has not been always recognised. Commercially, roses are cultivated in green houses for garden planting of which the flowers are intended to be sold on the national and international market. But most problems with mildew arise with green roses and expected that outdoor wind
speeds are strongly related to the high concentration of mildew bio-pollutants rather than the presence and movement of working people in the glass house (Finking, 1977).

In buildings, where the animals are housed the microbial air pollution is usually high, owing to the presence of bedding, fodder and dried faeces and to contamination from the animal coats. The organisms in the air are not diluted and dispersed, as in outdoor air, so that their numbers are continuously rising. They survive longer in humid atmosphere away from sunlight. The number of bio-pollutants will depend on the number of animals housed, the ventilation and the amount of disturbances of dust (Hawker et al., 1960).

Anthrax, a bacterial disease of animals, caused by air borne bio-pollutants *Bacillus anthracis* provided the fatal infection of the lungs. Similarly other air-borne animal diseases including Mammalion, Aspergilosis, Facial eczema and other fungal diseases of domestic animals have been given greater attention to Veterinary Pathologists (Brook, 1966).

Studies in connection with the indoor air-pollution conducted in an open cattle shed in the Government Dairy farm near Vishakhapatnam, did not observe the significant occurrence of the air-borne bio-pollutants in high concentration, because of the possibility of free movement of air considered to be diluted the pollutant concentrations (Sreeramulu, 1961).

Most of the aspects of intramural microbial pollution of air in relation to human, plant and animal diseases have been discussed. However, a very little attention has been paid to the
bio-deterioration of stores, equipment and materials, library books, paintings of Ajanta and Ellora caves in which the substrate, organisms and environment interact. It is the ecological aspect of bio-deterioration where 'Aerobiology' is concerned (Rajan et al., 1952; Allexander, 1945; Hueck, 1965; Tilak and Kulkarni, 1972; Tilak, 1974; Tilak and Vishwe, 1975; Sinha, 1976; Tilak and Chakre, 1977).

Why not the microbial content of huge storage houses, ship-basements etc. be examined where millions of tonnes of grains and other food stuff are stored, where lot of bio-deterioration occurs?

The losses of stored food grain have been assessed by a number of workers and agencies, important of these are Cyone (1945) 3.0 %; FAO (1946) 10 %; and Expert Committee (1967) 6.2 %. Johnson (1948) has estimated that about 1 % of the grain crops of the world is lost due to the deterioration of fungi.

India loses 10 million tonnes of food grains every year because of rats, insects, mites, microorganisms and due to inadequate and poor facilities for food grain storage. These storage losses constitute a major problem in developing countries like India where cereals constitute a staple food (Venkata Subramanian, 1976).

Insects are a major cause of loss in stored grain, they not only consume the materials but also contaminate them with insect fragments, insect scales, eggs and faeces. Today several hundred different species of insects are associated in one way or other with stored grain but fortunately only a few cause serious damage to cereals, when stored even good conditions (Raghwan, 1978).
More than fifty species of fungi and considerable number of species of bacteria have been isolated from seeds. Bacteria do not normally appear to be involved in the deterioration of stored grains, because they require free water to grow and seeds are seldom to store under conditions where free water is available (Christensen, 1957; Saharan, 1970).

Although fungi are a major cause of spoilage in stored grain, probably rank second only to insects, was described more than 60 years ago. Only recently the problem has become recognised as one of the some importance (Duvel, 1909; Shanahan, 1910; Semumick and Gilman, 1944; Christensen, 1955; Tuite and Christensen, 1955, 1957; Papavizas and Christensen, 1958, Wallace and Sinha, 1962; Sinha et al., 1971, 1975, 1976; Raghwan, 1978).

Different workers have often different explanations for the deterioration of stored food grains, important of these; Haris and Kundson (1948) considered it as due to insect fragments. Venkata Rao et al. (1959) pointed out it as due to uric acid. Linko and Sogn (1960) reported as due to glutamic acid. Christensen (1965, 1966, 1969), Fanse and Christensen (1966) determined the influence of moisture, temperature and prolonged period of storage are responsible for the deterioration of grains. Wallace and Sinha (1962, 1975, 1976) stressed the importance of bio-pollutants due to imported seeds considered for such damages. Semumick and Cunnington (1954-1976) felt that the ecological factors are the main cause of stored grain spoilage as they directly determined the growth and activities of bio-pollutants.
Thus, several biotic and variable abiotic factors are responsible for the deterioration of food grains during storage.

Several factors are involved in different combinations in regard to the deterioration of stored grains. Of these, the Warehouse itself often provides a congenial environment for the growth of saprophytes and parasitic forms, in addition to the insect activities and other agents. Climatic conditions like temperature, humidity etc. prevailing in different regions also bring about various kinds of deterioration in the stored commodities (Mukherjee et al., 1968; Agrawal, 1976; Yadav and Kailasanathan, 1977).

Inside the Warehouse the bio-pollutants are generally settled on the stored bags, grains and sometimes deposited on the inner walls of the Warehouse. During activities such as handling, cleaning, storing and aerosol spraying, the settled bio-particles disturbed and dispersed in the Warehouse environment. Besides, the opened doors, windows and ventilation also permit wind currents from outside and help to disperse the deposited bio-pollutants. Most of the bio-pollutants responsible for stored grain deterioration are transported through wind currents. Thus, the bio-pollutants is a major constituent of Warehouse environment.

In India, however, it has never been considered their content in the air cause a serious loss to stored food grains inside the Warehouse. Such investigation would be helpful in determining the composition and the percentage of bio-pollutants inside the Warehouse air, their seasonal variation and its possible role in storage diseases. It was with this idea, the present investigation was undertaken.
MATERIALS AND METHODS

Air Sampler (Plate V):

The Rotorod sampler is fully described by Perkin (1957). The device relies upon the high efficiency, with which small airborne particles are deposited on narrow cylinders, oriented at right angles to high velocity winds (Gregory, 1957). The sampler consists of a small, constant speed, battery operated motor is used to whirl thin-sticky-coated brass rods, about its axis at a constant high speed. It has been developed into a cheap and portable and high efficiency sampler. It is well fitted for using in the field and relatively independent of external wind speed.

Collecting arms of the model are made up of 0.159 cm (1/16 inch) square section brass rods slightly bent inwards. The vertical arms are 6 cm long and 4 cm from the axis.

According to Gregory (1951), the width should give more than 60 to 70% efficiency of deposition for 20 μ diameter spores at wind speeds above 4 m.p.h. (2 mm/sec). The model employees D.C. controlled speed motors of the type used for record players. With the rods in position the motor gives 2300 r.p.m.

Sampling rate: The sampling rate is the volume swept by the collecting surface per unit time. The dimensions selected make this:

\[ 2 \text{ (arms)} \times 0.159 \text{ cm} \times 6 \text{ cm} \times 8 \times 2300 \times 10^{-3} \]
\[ = 48.0 \times 10^{-3} \times 2300 \text{ litres/min.} \]
\[ = \text{approximately 110 litres/min.} \]
**Sampling surface:**

Since the sampler is originally intended for direct observation of Uredospores on rods, no mounting is necessary. The use of glycerine-gelatine or petroleum jelly has been recommended now.

The Rotorod sampler has been used for collecting a wide variety of air-borne bio-particles. After the jelly or vaseline sets the edges of cellotape are trimmed back to the width of the rods with sharp razor blade (the alternative would be to apply the transparent cellotape trim and then coated with adhesive).

**Collection efficiency:**

The American model has been tested for efficiency and it gave 85% efficiency. Wind speed has little effect on the efficiency, unless it is close to the linear velocity of collector. High winds, however, did increase drag on and load on the motor. The Rotorod sampler tested by Carter (1960) gave 60 to 90% efficiency. The efficiency of the present model is 85%.

**Location of the Warehouse Building:**

The Maharashtra State Warehousing Corporation of Aurangabad is situated in Osmanpura, which is about 2 km towards east from the Railway Station. There are three main storage buildings and the storage capacity of each Warehouse is about 775 metric tonnes.

The Warehouse is a common storage type, not equipped with any special ventilation or temperature control features. It has generally been considered the main Warehouse buildings of Aurangabad city where greater quantity of cereals are stored. The
cereals viz., sorghum, wheat, bajra and rice were collected by the corporation from five districts of Aurangabad and kept about 6 to 12 months under safe storage.

The food grains from different regions of Maharashtra, and occasionally from different parts of India were also kept under storage. After storing and cleaning the food grains were distributed in different parts of Aurangabad district for human consumption.

Studies in the microbial pollution of air inside the warehouse was carried out from the month of April 1976 to March 1978, continuously for two years.

The Rotorod sampler was operated near the stored bags and run twice daily for 30 minutes in the morning (10.30 hours to 11.00 hours) and 30 minutes in the evening (17.00 hours to 17.30 hours).

After sampling the air the cello tape was cut into 4 equal parts, of 1.5 cm length before adhesive was applied, and mounted with a 22 mm square cover glass with suitable mountant like glycerine jelly.

The strips were scanned under binocular microscope of different magnifications and high power objectives.

The numbers thus obtained were estimated to the total number and percentage contribution of the different bio-pollutants per cubic meter of air of the trap surface.

The important bio-pollutants were studied in detail.
TABLE I: Physical characteristics of the Warehouse environment
(From 1 April 1976 to 31 March 1977).

<table>
<thead>
<tr>
<th>Month</th>
<th>Average air temperature (°C)</th>
<th>Relative humidity (%)</th>
<th>Stored grain temperature (°C)</th>
<th>Stored grain moisture (%)</th>
</tr>
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<tbody>
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<td>24</td>
<td>34.50</td>
<td>13</td>
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<td>E 27</td>
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M* - Morning;  E* - Evening.
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<th>Month</th>
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<th>Relative humidity (%)</th>
<th>Stored grain temperature (°C)</th>
<th>Stored grain moisture (%)</th>
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M* - Morning;  E* - Evening.
Fig. I - Meteorological data of the Warehouse environment from 1 April 1976 to 31 March 1977.
Fig. II - Meteorological data of the Warehouse environment from 1 April 1977 to 31 March 1978.
Fig. III - Principal Bio-pollutants of the Warehouse environment from 1 April 1976 to 31 March 1977.
PRINCIPAL BIO-POLLUTANTS OF THE WAREHOUSE ENVIRONMENT DURING 1ST APR. 1976 TO 31ST MAR. 1977

MONTHLY TOTAL No. OF BIO-POLLUTANTS / m³ OF AIR

RHIZOPUS

PENICILLIUM

ASPERGILLUS

CLADOSPORIUM

HELMINTHOSPORIUM

ALTERNARIA

CURVULARIA

MORNING
EVENING
MONTHLY

APR MAY JUN JUL AUG SEPT OCT NOV DEC JAN FEB MAR
Fig. IV - Principal fungal Bio-pollutants of the Warehouse environment from 1 April 1977 to 31 March 1978.
Fig. V - Insect and insect parts in the Warehouse environment from 1 April 1976 to 31 March 1977.
Fig. VI - Insect and insect parts in the Warehouse environment from 1 April 1977 to 31 March 1978.
Fig. VII - Microbial pollutants of the Warehouse environment and its relation to grain shortages.
MICROBIAL POLLUTANTS OF WAREHOUSE AIR AND ITS RELATION TO GRAIN SHORTAGES
(FROM 1ST APRIL 1976 TO 31ST MARCH 1978)

TOTAL CONTRIBUTION OF MICROBIAL POLLUTANTS (%)

GRAIN SHORTAGES, %

Scale = 0.5 mm = 1%
RESULTS

I. PHYSICAL FACTORS (Fig. I and II):

The rate of deterioration of stored food grains is mainly dependent on air temperature, relative humidity of the warehouse environment, grain temperature and the moisture content of the stored food grains.

Air Temperature:

The air temperature was one of the most important intramural environmental factors which affected the nature of survival of bio-pollutants inside the warehouse air. They varied in the concentrations according to their temperature requirement.

During the period of investigation (1976-78) into the microbial survey of air pollution inside the warehouse environment. The maximum average air temperature was reached up to $35^\circ C$, while the minimum air temperature was up to $23^\circ C$. The average air temperature was in between $25^\circ C$ to $33^\circ C$ during wet period, while in winter and dry conditions it was about $23^\circ C$ to $31^\circ C$ and $31^\circ C$ to $35^\circ C$ respectively. The maximum($35^\circ C$) and minimum($24^\circ C$) air temperature was greater during 1976-77 as compared to those of 1977-78 (maximum $33^\circ C$; minimum $23^\circ C$). The air temperature recorded in the evening was slightly higher throughout the period of investigation than those of temperature recorded in the morning (Table I and II).

Relative Humidity:

The maximum relative humidity was reached up to $86\%$ in the month of July while in the month of March and April the lowest
average air humidity was 24%. In wet seasons of 1976-78 the relative humidity was in between 76 to 86% whereas during winter and summer seasons it was about 29 to 65% and 24 to 40% respectively. The relative humidity recorded in the evening was less as compared to those of morning (Table I and II).

**Grain Temperature:**

The stored grain temperature measured by the ordinary thermometer, inserting inside the stored bags, found that the stored grain temperature was slightly higher than the air temperature of the Warehouse environment (Table I and II).

**Grain Moisture:**

The grain moisture was recorded monthly, by the use of Toshniwal Moisture Balance. The maximum moisture content (16%) of the stored grains was found in the month of October 1976 and February 1978. The minimum moisture content of the stored grains was 12% during 1976-77 and 10% during 1977-78. (Table I and II).

II. **BIO-POLLUTANTS (Fig. III to VI):**

**Alternaria Nees (Plate VI A):**

a) **Microscopical identification:** Conidia dark, crossed with vertical septa, variously shaped, obclavate to elliptical or ovoid, frequently borne acropetally in long chains, often borne singly with an apical, simple or branched appendage of different sizes, belonging to different species.

b) **Incidence in the air:** The spores of *Alternaria* occurred throughout the period of investigation. They comprised 3.92%
during 1976-77 and 3.31% during 1977-78 to the total microbial content of the Warehouse air. The maximum concentration (195/m$^3$) was recorded in the morning during the month of July 1976 while the lowest concentration (35/m$^3$) was noted in the month of February evening 1977 (Table III and IV).

During 1977-78 the highest number of *Alternaria* spores (180/m$^3$) was noted in the month of May morning 1977 and March morning 1978. The minimum concentration (20/m$^3$) was found to be in the evening during January 1978 (Fig. IV).

The concentration (2355/m$^3$) was greater during 1977-78 as compared to those of (2170/m$^3$) 1976-77.

c) **Effect of meteorological factors:** The concentration was correlated with the meteorological factors like air temperature, air humidity, grain temperature and the moisture content of the stored grain of the Warehouse environment.

The maximum number (195/m$^3$) appeared in the month of July 1976 was corresponded to the 27°C average air temperature and 84% relative humidity, while the maximum number (180/m$^3$) recorded in the month of May 1977 and March 1978 was correlated to the 33°C and 32°C average air temperature and 39% and 25% relative humidity respectively (Table I and II).

The daily maximum concentration (20/m$^3$) occurred on 12 July 1976 and 9 September 1977 (30/m$^3$) coincided with the 26°C air temperature and 80 to 82% relative humidity. The survival of bio-pollutants inside the Warehouse air was also contingent on other factors such as grain temperature and grain moisture. The maximum concentration (195/m$^3$) appeared in the month of July 1976
was correlated with the grain temperature between the range of 26° to 26.50°C and 13% moisture content of the stored grains. Similarly, when the grain temperature was in between 31.50° to 33.50°C and 12% grain moisture coincided with maximum concentration (180/m³) recorded in the month of May 1977. In March evening 1978, when the grain moisture was in between the range of 31.50° to 32.50°C, associated with 12% moisture content of the stored grains also coincided with the maximum concentration (180/m³).

Machacek et al. (1951) reported that 69 and 62% of wheat and oats respectively were infected with Alternaria species.

Wallace and Sinha (1962, 1975a, 1975b) stated that Alternaria was one of the dominant field fungi that was common and abundant on the imported seeds. They further stated that a rise and fall in temperature had more effect on the frequency of occurrence of Alternaria species, which was highest in the summer months.

Lutey and Christensen (1963) found that a rise in moisture content and temperature of the seed, caused a decrease in occurrence of Alternaria species under laboratory conditions.

Winger and Smith (1970) have reported and enumerated the genera which act as deteriorating agents.

Tilak and Kulkarni (1972), while studying the air-spore outside and inside the caves, have reported Alternaria 4.14% and 1.14% from the total microbial content of air inside and outside the caves respectively. The concentration was correlated with the low temperature and high humid conditions.

Tilak and Vishwe (1975) studied the microbial content of air inside the library, Marathwada University, Aurangabad. It was
reported to be one of the most important biodeteriorating agents which contributed 4.25% to the total microbial content of air. It was predominantly present during wet period when the temperature was 25.50°C and the relative humidity 65%.

The occurrence and abundance of *Alternaria* type of bio-pollutant is more during the humid days and is in conformity with the reports of earlier workers.

**Aspergillus** Link ex Fries (Plate VI B):

a) **Microscopical identification:** Conidia single celled, globose, variously coloured when in mass, catenate, 4-6 x 3.8-4 u in size, produced basipetally, rarely trapped along with the conidiophores and vasicle; conidiophores upright, simple, terminating in a globose or clavate, swelling, bearing phialides at the apex or radiating from the entire surface.

b) **Incidence in the air:** The maximum concentration (455/m³) during 1976-77 was encountered in September morning 1976 and (320/m³) in August morning 1977, during the period of 1977-78 investigation. In the total microbial content of air, these bio-pollutants contributed 6.15% in 1976-77 and 2.30% in 1977-78 (Table III and IV).

It was also noticed from the observations that the bio-pollutants were completely absent from the Warehouse air in the month of September evening 1976, February and June morning 1977 and from December 1977 to March 1978, except February morning 1978. It was found to be abundant and continuously present in the wet season only (Figs. XIII and IV).
The concentration (3405/m³) was greater during 1976-77 as compared to those (1710/m³) of 1977-78.

c) Effect of meteorological factors: The peak concentration (455/m³) was recorded in September 1976, when the average air temperature was in between 27° to 28°C and the relative humidity 80 to 82%. The temperature 26° to 27°C and the relative humidity 84 to 85% coincided with the maximum concentration (320/m³) in August 1977. High numbers (60/m³) were encountered on 6 September morning 1976 and (50/m³) on 18 August morning 1977 where the daily average air temperature was in between 25° to 26.50°C and the relative humidity 80 to 85%.

The maximum concentration recorded during the period of investigation correlated with the grain temperature and percentage of moisture content of the stored grains. The maximum concentration (455/m³) observed in September 1976 coincided with the 27.50° to 29°C grain temperature and 14% grain moisture. Similarly, in August 1977 when the grain temperature was 27°C to 27.50°C and 14% moisture content, was correlated with the highest concentration (320/m³) recorded.

Tilak and Vishwe (1975) reported 2.57% concentration of Aspergillus during wet period.

Marjut (1977) in Finland reported that the Aspergillus was occasionally common in the working environment of a mill and a veneer factory.

Cladosporium Link (Plate VI C):

a) Microscopical identification: Conidia dark, one or two celled, variable in shape and size, ovoid to cylindrical.
irregular, few typically lemon shaped, 4.28 x 2-4.7 u; conidiophores dark, branched, variably near apex of middle portion, clustered or singed.

b) **Incidence in the air:** The highest concentration (910/m$^3$) was recorded in the month of August evening 1976 and lowest (30/m$^3$) in the month of February evening 1977. It was also recorded from the observation that the bio-pollutants were completely absent in the month of October evening, November morning 1976 and March evening 1977 (Figs. III and IV).

During the period of 1976-77 the concentration (5195/m$^3$) was greater as compared (4385/m$^3$) to 1977-78 period of investigation.

During 1977-78, the concentration increased after June, reached peak (1115/m$^3$) in August evening 1977, started decreasing in September, again increased from November to January with some fluctuation in their numbers. It was also recorded from the observation that the bio-pollutants were completely absent in the months of June evening, September evening and October morning 1977, and in February evening 1978. They contributed 9.38% during 1976-77 and 6.13% during 1977-78 to the total microbial content of air (Table III and IV).

c) **Effect of meteorological factors:** The maximum concentration (910/m$^3$) recorded in the month of August evening 1976, was associated with the 28°C air temperature and 84% relative humidity. Similarly, in the month of August 1977 when the air temperature was 27°C and relative humidity 84%, was correlated with the maximum concentration (1115/m$^3$).

The daily maximum concentration (72/m$^3$) recorded on 16 August
evening 1976 and on 4 August evening 1977 (60/m³) correlated with the 28.5°C temperature and 84% relative humidity.

The maximum concentration was correlated with the grain temperature and stored grain moisture. The highest concentration (910/m³), recorded in the month of August evening 1976, coincided with 27°C grain temperature and 14% grain moisture. Similarly, in the month of August evening 1977 when the grain temperature was 27.50°C and 14% moisture content, correlated with the maximum concentration (1115/m³) recorded in the month.

Tilak and Kulkarni (1972) observed their concentration 60.42% inside and 6.40% outside the caves.

Tilak and Vishwe (1975) recorded 2.46% bio-pollutants from the total catches. The maximum percentage contribution of *Cladosporium* recorded on two days when relative humidity was fairly high, associated with strong wind currents.

Marjut (1977) in Finland reported, *Cladosporium* as the most dominant genus in the mill.

**Curvularia** Boed. (Plate VI D):

a) **Microscopical identification:** Conidia dark, end cells lighter, 3-6 celled, more or less fusiform, typically bent or curved with one or two of the central enlarged cells. Two types of spores were found; one 22-34 x 7.8-17.5 μ and another 33.8-47 x 9.35-21 μ in size.

b) **Incidence in the air:** The bio-pollutants were present throughout the period of investigation. They occurred in high concentration reaching highest (215/m³) in June evening 1976 and
(220/m$^3$) in June morning 1977. The lowest concentration (20/m$^3$) was noted in February evening 1977 and (30/m$^3$) in March evening 1978. (Figs. III and IV).

The concentration (2565/m$^3$) was greater during 1977-78 as compared (2280/m$^3$) to 1976-77. They contributed 4.12 % during 1976-77 and 3.62 % during 1977-78.

c) Effect of meteorological factors: The highest concentration (215/m$^3$) recorded in June evening 1976 coincided with the 33°C average air temperature and 40 % relative humidity. The maximum concentration (220/m$^3$) recorded in the month of June morning 1977 attributed 30°C average air temperature associated with 76 % relative humidity.

The daily maximum number (25/m$^3$) recorded on 25 June evening 1976 and on 29 June 1977 was corresponded to the 30°C and 32°C air temperature associated with 80 % and 75 % relative humidity respectively.

The maximum concentration was correlated with the grain temperature and stored grain moisture. The peak concentration (215/m$^3$) observed in the month of June evening 1976 correlated with the 33°C grain temperature and 12 % of moisture content of the stored grains. Similarly, the grain temperature of 33°C associated with 12 % grain moisture was correlated with the highest concentration in the month of June evening 1977.

Tilak and Srinivasulu (1967) recorded Curvularia from the air-spores, which showed the highest concentration (40.17 %).

Tilak and Kulkarni (1972) recorded the concentration of Curvularia 5.60 % both outside and inside the caves.
Helminthosporium Link (Plate VI E):

a) **Microscopical identification:** Conidia dark, more than 3 cells, cylindrical or ellipsoid, sometimes slightly curved or bent, ends rounded. Spores of various types and sizes were studied under this group.

b) **Incidence in the air:** The bio-pollutants were present throughout the years from April 1976 to March 1978. The highest concentration (255/m$^3$) was recorded in June 1976 and (315/m$^3$) in June morning 1977. The lowest concentration (50/m$^3$) was observed in February evening 1977, while in December evening 1977 and February morning 1978 the concentration (40/m$^3$) was low (Figs. III and IV).

The percentage contribution was 5.34 % during 1976-77 and 4.83 % during 1977-78 (Table III and IV).

c) **Effect of meteorological factors:** The maximum number (255/m$^3$) appeared in the month of June morning 1976 was corresponded to the 33°C average air temperature and 40 % relative humidity. The maximum number (315/m$^3$) was recorded in the month of June morning 1977 when the air temperature was 30°C and relative humidity 76 %.

The peak concentration (255/m$^3$) observed in the month of June morning 1976 was correlated with 30.50°C grain temperature associated with 10 % water content of the stored grains. Similarly, the highest concentration (315/m$^3$) recorded in June 1977 was associated with the 30.50°C and 10 % water content of the stored grains.

Wallace and Sinha (1962) reported that the Helminthosporium
species were usually associated with cereal grains and classified as "field fungi".

Tilak and Vishwe (1975) recorded spore types of Helminthosporium and were transported from outside sources through the wind currents.

**Penicillium Link (Plate VI F):**

a) **Microscopical identification:** Conidia hyaline or brightly coloured mass, single-celled, mostly globose or ovoid, 0.7-1.68 μm in size.

b) **Incidence in the air:** The bio-pollutants mainly occurred from June to December with high concentration. The maximum concentration appeared in August and September 1976 reaching highest peak (955/m³) in August evening 1976 and (470/m³) in July morning 1977. They were absent from the month of April evening to June evening 1976, and from April to June 1977 (Figs. III to VI).

The percentage contribution recorded in 1976–77 was 7.51% and 2.86 during 1977-78.

C) **Effect of meteorological factors:** The peak concentration (955/m³) recorded in the month of August evening 1976 was correlated with the 28°C average air temperature associated with 84% relative humidity. In July morning 1977 the highest concentration (470/m³) was observed when the average air temperature was 29°C and relative humidity 86%.

The maximum concentration (955/m³) observed in the month of August evening 1976 when the grain temperature was 27°C associated
and the 14% moisture content, when the stored grain temperature was 29°C and 12% moisture content of the grains, associated with the maximum concentration (470/m³) observed in July 1977.

Wallace and Sinha (1965) reported that the seeds in hot spots were predominantly infected by storage fungi among which *Penicillium* species were the most abundant, even in relatively dry grains.

Tilak and Vishwe (1975) have examined the humid walls and indicated the presence of *Penicillium* species.

Wallace *et al.* (1976) reported different species of *Penicillium* as the predominant storage fungi.

Marjut (1977) recorded *Penicillium* spores which were the most frequent genus in the Veneer factory.

Subrahmanyan (1978) reported the *Penicillium anersonii* Stolk fungal spores generally present in October and March while isolating thermophilic fungi from dust accumulated on rarely used library books where the existence of elevated temperature was not detectable.

The present reports agreed with the earlier workers.

**Rhizopus Ehrenb. (Plate VI G):**

a) **Microscopical identification:** The bio-pollutants are unequal, irregular, round or oval, angular, striate, 9-11 x 7.4-8 µ in size.

b) **Incidence in the air:** The *Rhizopus* was found to be abundant and continuously present in the wet seasons of both the years of the investigation. The maximum indoor atmospheric
concentration (4425/m$^3$) of warehouse air was recorded in September evening 1976 and (2120/m$^3$) August evening 1977 (Figs. III to VI).

Their contribution to the total microbial pollutants was 26.67% during 1976-77 and 10.88% during 1977-78. The concentration (12,870/m$^3$) was greater during 1976-77 as compared to those (7670/m$^3$) of 1977-78 period of investigation.

c) Effect of meteorological factors: The peak concentration (4425/m$^3$) of *Rhizopus* type of bio-pollutants noted in the month of September evening 1976, was correlated with 28°C average air temperature and 80% relative humidity. Similarly, the average air temperature of 27°C and the relative humidity 84% was corresponded with the maximum concentration (2120/m$^3$) observed in August evening 1977.

All these records show that they belong to "wet-spore" group, recorded the most predominant bio-deteriorating agent throughout the period of investigation.

The grain temperature of 27°C and grain moisture 14% were corresponded with the maximum number (4425/m$^3$) of *Rhizopus* spores in the month of September evening 1976. The highest number (2120/m$^3$) noted in the month of August evening 1977 when the grain temperature was 27.50°C and grain moisture 14%.

Tilak and Kulkarni (1975) recorded *Rhizopus* spores while studying the air-spora of sugarcane fields at Aurangabad. The spores were recorded throughout the rainy season (June to October) and belong to "wet-spora" group.

Tilak and Vishwe (1975) reported 1.14% from the total air-spora inside the library.
Wallace and Sinha (1976) reported the maximum percentage of *Rhizopus* (10%) on white wheat seeds which was originated from Australia.

Marjut (1977) reported that the *Rhizopus* was the most frequent fungus in the Veneer factory in Finland.

**Insects and its heterogenous group:**

Insect scales were recorded throughout the year. The maximum concentration (765/m³) was noted in the month of July morning 1976 and (550/m³) in August morning 1977. The low concentration (20/m³) was encountered in April morning 1976 while they were completely absent from the warehouse air in the month of April morning 1977. In total microbial pollutants the insect scales contributed 10.96% during 1976-77 and 6.04% during 1977-78 (Table III and IV).

Insects, insect parts, and their eggs were recorded throughout the years if not on every occasion. The maximum population (35/m³) of microscopic insects in the air was observed in September morning 1976 and (75/m³) September evening 1977. The insects were completely absent from April evening to June morning and in February 1976. The maximum population (35/m³) of insect eggs was noted during January morning and September evening (15/m³) 1977 (Figs. VII and VIII).

The insect population was constituted 0.35% during 1976-77 and 0.36% during 1977-78 while the contribution of insect eggs was 0.38% during 1976-77 and 0.08% during 1977-78 (Table III and IV).

The peak concentration of this heterogenous group of insects
was observed during rainy season when the average air temperature was in between 25° to 27°C and the relative humidity 80 to 85% during 1976-77, while the average air temperature was in between 26° to 30°C and relative humidity 80 to 85% during 1977-78 corresponded with the high concentration.

Grain temperature and grain moisture were in between 26° to 31°C and 13 to 16% respectively during 1976-77, and 27 to 32°C temperature and 10 to 14% moisture content of the stored grains during 1977-78 correlated with the maximum concentration of the heterogenous group of insects.

In India, through some of the workers interested in the study of air-borne allergens (Kalra and Dumbrey, 1957; Choubal and Deodikar, 1964; Sreeramulu and Ramlingam, 1966) reported their incidence in the air.

The incidence of insects, insect scales, insect eggs and insect fragments in Warehouse air followed a seasonal trend. It is evident from the result of the earlier workers in England (Freeman, 1938; Hamilton, 1959), India (Kalra and Dumbrey, 1957; Choubal and Deodikar, 1964; Reddi, 1970) and Australia (Rees, 1964) followed the seasonal trend in their occurrence in the air.
## TABLE III: Intramural microbial pollutants of the Warehouse environment (From 1 April 1976 to 31 March 1977).

<table>
<thead>
<tr>
<th>Month</th>
<th>Bio-pollutant types</th>
<th>Total No. of Bio-pollutants/m³ of air</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Altamaria</td>
<td>Aspergillus</td>
</tr>
<tr>
<td>April</td>
<td>M 100</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>E 130</td>
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<td></td>
<td>E 85</td>
<td>180</td>
</tr>
<tr>
<td>July</td>
<td>M 195</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>E 180</td>
<td>270</td>
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<tr>
<td>August</td>
<td>M 65</td>
<td>200</td>
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<tr>
<td></td>
<td>E 85</td>
<td>160</td>
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<tr>
<td>September</td>
<td>115</td>
<td>455</td>
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<tr>
<td></td>
<td>E 115</td>
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<tr>
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<td>M 105</td>
<td>50</td>
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<tr>
<td></td>
<td>E 80</td>
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<td>November</td>
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<td>E 50</td>
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<tr>
<td></td>
<td>E 65</td>
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<tr>
<td>February</td>
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<td>55</td>
</tr>
<tr>
<td></td>
<td>E 35</td>
<td>-</td>
</tr>
<tr>
<td>March</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>1055</td>
<td>1520</td>
</tr>
<tr>
<td>contri-</td>
<td>E 1115</td>
<td>1885</td>
</tr>
</tbody>
</table>

Total No. of bio-pollutants/m³ of air | 2170 | 3405 | 5195 | 2280 | 2955 | 4160 | 12,870 | 195 | 6075 | 70 | 210 |

% contribution to the total microbial content of air | 3.92 | 6.15 | 9.38 | 4.12 | 5.34 | 7.51 | 26.67 | 0.35 | 16.98 | 0.12 | 0.38 |

* Whole Mount.
<table>
<thead>
<tr>
<th>Month</th>
<th>Alternaria</th>
<th>Aspergillus</th>
<th>Cladosporium</th>
<th>Curvularia</th>
<th>Helminthosporium</th>
<th>Penicillium</th>
<th>Rhizopus</th>
<th>Insects (106)*</th>
<th>Insect scales</th>
<th>Insect parts</th>
<th>Insect eggs</th>
<th>Total No. of Bio-pollutants/m³ of air</th>
</tr>
</thead>
<tbody>
<tr>
<td>April</td>
<td>165</td>
<td>130</td>
<td>85</td>
<td>70</td>
<td>130</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>-</td>
<td>5</td>
<td>1220</td>
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<td>May</td>
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<td>45</td>
<td>120</td>
<td>250</td>
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<td>-</td>
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</tr>
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<td>120</td>
<td>160</td>
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<td>70</td>
<td>45</td>
<td>285</td>
<td>1270</td>
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<td>360</td>
<td>10</td>
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<td>175</td>
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<td>65</td>
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<td>280</td>
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<td>310</td>
<td>75</td>
<td>190</td>
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<td>60</td>
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<td>-</td>
<td>-</td>
<td>855</td>
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<td>March</td>
<td>180</td>
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<td>125</td>
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<td>-</td>
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<td>5</td>
<td>80</td>
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<tr>
<td>Total contribution</td>
<td>1370</td>
<td>625</td>
<td>1640</td>
<td>1495</td>
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<td>100</td>
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<td>Total No. of Bio-pollutants/m³ of air</td>
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<td>1710</td>
<td>4335</td>
<td>2565</td>
<td>3400</td>
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<td>7670</td>
<td>260</td>
<td>260</td>
<td>165</td>
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<tr>
<td>% contribution to the total microbial content of air</td>
<td>3.31</td>
<td>2.30</td>
<td>6.13</td>
<td>3.62</td>
<td>4.83</td>
<td>2.86</td>
<td>10.88</td>
<td>0.36</td>
<td>6.04</td>
<td>0.23</td>
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<td></td>
</tr>
</tbody>
</table>

* Whole Mount.
**TABLE V: Microbial pollutants of the Warehouse environment and its relation to grain losses**

<table>
<thead>
<tr>
<th>Month</th>
<th>1 April 1976 to 31 March 1977</th>
<th></th>
<th>1 April 1977 to 31 March 1978</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total contribution of the microbial pollutants (%)</td>
<td>Monthly reports of godown shortages (%)</td>
<td>Total contribution of the microbial pollutants (%)</td>
<td>Monthly reports of godown shortages (%)</td>
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<td>3.87</td>
<td>1.67</td>
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<td>0.98</td>
</tr>
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<td>4.69</td>
<td>1.12</td>
<td>4.53</td>
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</tr>
<tr>
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<td>0.90</td>
<td>4.42</td>
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<td>21.84</td>
<td>1.81</td>
<td>20.12</td>
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<tr>
<td>August</td>
<td>13.21</td>
<td>2.81</td>
<td>24.55</td>
<td>3.54</td>
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<td>September</td>
<td>24.87</td>
<td>3.70</td>
<td>12.20</td>
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<td>October</td>
<td>8.26</td>
<td>4.14</td>
<td>7.54</td>
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</tr>
<tr>
<td>November</td>
<td>4.83</td>
<td>3.54</td>
<td>5.59</td>
<td>1.85</td>
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<td>0.26</td>
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<td>1.51</td>
<td>0.42</td>
<td>3.56</td>
<td>1.75</td>
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</table>

* The data is provided by the Superintendent, Maharashtra State Warehousing Corporation, Aurangabad.
DISCUSSION

The viability of the microbial pollutants inside the Warehouse air and its role in grain spoilage is an important consideration of the present investigation. The major bio-pollutants showed variation both in quality and quantity in different seasons of the year and different times during the day.

_Ehizorus_ species was the only bio-pollutant from the group of Phycomycetes and one of the commonest and dominant genus contributed highest percentage throughout the period of investigation.

The occurrence and abundance of _Ehizorus_ species was more during humid days (June-October) when the average air temperature was 25° to 33°C and the relative humidity in between 80 to 86 %. It exhibited an evening pattern in the forenoon hours. The rapid changes in air humidity inside the Warehouse air resulting in the hygroscopic movements of conidiophores leading to the detachment of the spores.

It is more or less definite as has been emphasized by Gregory (1961) that these spores are blown from some saprophytic sources. Stored grain is a very good source as it is the best natural medium for the growth of this bio-pollutant.

_Ehizorus_ species the commonly occurring bio-pollutant on breads, mature fruits, vegetables, decaying organic plants and animal matters where they grow saprophytically in this area and may be transported from the outside through the wind currents. Thus, the occurrence of _Ehizorus_ in the season is due to suitable conditions prevailing in the season.
The other common bio-pollutants in the Warehouse atmosphere were those of Aspergillus, Cladosporium and Penicillium species. The seasonal and monthly observations revealed that the number of these bio-pollutants in the Warehouse air was comparatively more during the months of July to September. During wet season the spore concentration was high because due to suitable high humidity and low temperature favoured in the season. The samples of the gunny bags, stored grains, humid walls after critical examination indicated the presence of these, also served as a source of microbial pollutants of the Warehouse air.

Alternaria, Curvularia and Helminthosporium species usually were present inside the Warehouse air throughout the period of investigation. The occurrence of these bio-pollutants was in abundance inside the Warehouse air during the months of September to November and February to April.

It was justified by the fact that these forms colonize the standing crop plants and when they have been harvested, they at the time of threshing the crop plants, disseminate their spores in the atmosphere, carrying their number in the wind and served the source of microbial content of Warehouse air.

These bio-pollutants were abundant and the presence of high numbers of these also coincided with the handling of the cereals because the settled bio-particles disturbed by the activities such as cleaning, aerosol spraying and handling of the cereals during storage.

In general, the bio-pollutants occurred mostly during wet season when the average ambient air temperature was in the range
of 25\(^\circ\) to 30\(^\circ\)C and relative humidity 80 to 90 \%. While during summer when the air temperature was in the range of 30\(^\circ\) to 35\(^\circ\)C and relative humidity in between 24 to 40 \% the microbial pollutants decreased in quantity. This was due to high temperature inside the Warehouse air.

From the above observations, however, it is evident that low temperature associated with high humid conditions increases the percentage of bio-deteriorating agents, while increases in temperature and less humid conditions effectively reduces the concentration of these.

Insects are one of the biggest sources of damage to stored food-grains, together with microbial pollutants they share a substantial quantity of food grains which we produce.

The category of insect parts, insect scales and fragments of various sizes and shapes and even complete insects, and the significance of the variations in the concentration of these inside the Warehouse air is also considered in relation to bio-deterioration of stored food grains.

Insects and insect eggs were particularly sensitive to very high temperature. High humidity and low temperature during rainy season perhaps favour them to make and lay eggs during winter season respectively. It seems that the rainy season is the favourable period to grow and increase their population in the Warehouse air.

The concentration of eggs in air was comparatively very low because of their attachment to the stored grains and were
difficult to blow in the air. The samples of the humid walls
after critical examination indicated the presence of insect eggs
also served as a source of microbial pollutants of the indoor air.

It was also observed that the maximum concentration of
insects, insect scales, eggs and insect fragments in Warehouse
air in the month of December, January and February, corresponded
with the maximum growth of certain crops like sugarcane, cotton,
bajra, jawar and wheat in this area.

The wind velocity was high during these months probably
responsible to transport the insects and its heterogenous groups
from outside, mostly during the morning time of the days. Some
of the variations in the monthly total catches probably due to
variation in the weather conditions and its effect on the insect
fauna.

Stored grains are also subjected to serious deterioration
due to the presence of excessive moisture on the grains or due to
absorption of moisture if the atmosphere surrounding the grains
is too humid. This explained during the rainy season. According
to Johnson (1948) at the critical moisture levels for different
grains (14 % moisture in equilibrium with 74 to 75 % relative
humidity) the fungal spores germinate on the seeds. Mathur (1952)
mentioned that moisture increases the rate of respiration of the
grain and unless the excessive moisture is removed quickly,
deterioration cannot be controlled. It has been stated that the
spoilage of grains at water content levels above 15 % is largely
due to micro-flora normally found on the grains (Mathur, 1952).

The grain temperature in relation to bio-deterioration is
also considered as an important factor (Christensen, 1957). The high temperature may actually kill the bio-pollutants. This probably explains the common observation that the grain temperature which has been dried to a particular water content (Mathur, 1952).

Recently it has been shown by Christensen (1978) that soyabees of 14.0 to 14.3 % moisture content at 5° to 8°C temperature have been stored for several years without any invasion by storage fungi; but when kept at 30°, soyabees of 14.0 to 14.3 % moisture would be invaded by storage fungi within a few weeks and extensive damage would develop within a few months. Similarly maize of 18 to 19 % moisture content can be kept for months at 5°C without damage from storage fungi.

Ingold (1971) stated "The spore discharge is naturally affected by temperature but the effect is conditioned by previous treatment".

In this study grain moisture seems to be preconditioning factor while grain temperature is an important factor at the actual time of spore discharge. Therefore, a combination of low temperature and low moisture content of stored grains is, of course, preferable for long term storage. For the major function of aeration of stored grains is to bring about a uniform and moderately low temperature throughout the stored grains. The uniform temperature reduces or transfers of moisture from one place to another in the grains. The moderately low (5° to 10°C if possible) temperature reduces the rate of growth of storage life, if the grains have a high enough moisture control to permit them to grow.
Though the maximum concentration appeared from the months of June to September, but the maximum storage losses recorded in the month of October (Fig. VII).

Therefore, it can be concluded that the microbial deterioration of food grains during storage naturally affected by microbial pollutants of the Warehouse air but the effect is delay type (in terms of losses).

In India, the present conditions of the Warehouse buildings, although they are designed to have a low thermal conductivity, allow the entry of heat, water vapour, air along with insects and other bio-pollutants from outside.

Studies on the infestation of wheat by *Sitophilus oryzae* Linn. under different ecological conditions indicated that air tight conditions of storage is superior to ventilated conditions and that the pest is unable to breed under air tight conditions irrespective of the moisture content of the wheat. Under ventilated conditions the pest is also unable to breed if the moisture content of wheat is 9 percent or less. The pest fails to breed under air tight conditions as the egg stage gets killed. Full grown larval and adult stages of *Tribolium castaneum* Herbst. were affected under air tight conditions which suggests great possibilities to control of the pest in wheat by restoring to air tight means (Wealth of India - Raw Materials, 1976).

The microbial pollutants have a capacity to cause serious grain losses if the weather conditions and Warehouse conditions and other factors are congenial in the particular season.
Therefore, the ecological studies on microbial pollutants, their sources, release, dispersion and their possible role in food grain deterioration are absolutely necessary to control the survival of bio-pollutants inside the Warehouse environment.
SUMMARY

1) The Intramural microbial pollution of air in relation to bio-
deterioration of food grains during storage was investigated
at the Maharashtra State Warehousing Corporation, Osmanpura,
Aurangabad.

2) The investigation was conducted by the use of Perkin's (1957)
Rotorod sampler for a period of 2 years (1976-78).

3) The correlation between the occurrence of the principal air-
borne bio-pollutants, their seasonal and monthly variation,
the effect of weather and the losses of stored grains inside
the warehouse was studied in detail.

4) The important bio-pollutants showed variations in quality and
quantity in different seasons of the year and different times
during the day.

5) Rhizopus species the only myco-organic pollutant from the
group of Phycomycetes and one of the commonest and dominant
genus which contributed highest percentage during humid days.

6) The other common bio-pollutants in the warehouse environment
were those of Aspergillus species, Cladosporium species and
Penicillium species. The concentration of these bio-pollutants
was high due to high humidity and low temperature during the
wet season.

7) The gunny bags, grains, humid walls indicated the presence of
these bio-pollutants served as sources of the microbial
pollutants inside the warehouse air.

8) The bio-pollutants such as Alternaria, Curvularia and
Helminthosporium species were present throughout the period of investigation. The concentration was also more during humid days. The maximum concentration of these was also coincided with the standing crop and during harvesting.

9) The low temperature associated with high humid conditions increased the percentage of the bio-deteriorating agents, while high temperature and less humid conditions effectively reduces the concentration of these.

10) The spore discharge is naturally affected by the grain moisture while grain temperature was important at the actual time of spore discharge.

11) The population of the insects and its heterogenous group was greater during high humid conditions and sensitive to very high temperature.

12) The rainy season was the favourable period to grow actively and increase their population in the Warehouse air.

13) The concentration of eggs in air was comparatively low as their attachment to the stored grains which become difficult to blow in the air.

14) The humid walls indicated the presence of insect eggs as a source of microbial pollutants of the indoor air.

15) The maximum population of insects and insect scales during winter season corresponded with the maximum growth of certain crops like sugarcane, cotton, bajra and wheat of this area.

16) The high wind velocity during December, January and February, corresponded with the high concentration of the bio-pollutants.
Therefore, it can be concluded that microbial deterioration of the food grains during storage naturally affected by microbial pollutants of the Warehouse air but the effect is delay type and the microbial pollutants have a capacity to cause serious grain losses, if the weather and Warehouse conditions are congenial in the humid season.

17) The monthly report of Warehouse shortages (losses) in the month of October was more. The maximum godown shortage of stored food grains was higher in the month of October, estimated for the months of August, September and October corresponded with the maximum concentration of the bio-pollutants.

18) A knowledge about the survival of the microbial pollutants inside the Warehouse environment is indespensible to control the bio-pollutants.