Indian Journal of
AIR POLLUTION CONTROL

VOL. VII No. 1 March 2007

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Published by
Indian Association for Air Pollution Control
(Delhi Chapter)
C/o Envirotech Instruments Pvt. Ltd.

Indian Journal of Air Pollution Control Vol. VII No. I March, 2007 pp 6-15

A Study of the Impact of *Yagya* on Indoor Microbial Environments

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Abstract

It is universally believed that airborne microorganisms such as thermo-philic action-mycetes, fungi, protozoa bacteria and several pathogens are responsible for infectious or allergic disorders such as asthma and humidifier fever. As people spend approximately 90% of the time indoors, the indoor atmospheric microbes pose a serious threat to the exposed people. A review of the ancient Indian literature reveals that treatment of atmosphere with the *Yagya* fumes resulted in reducing the atmospheric bacterio-statics. To confirm the same, several experiments by performing *Yagya* were conducted indoors during 2004 - 2005at a place where the external and internal factors were controlled. The sample taken on a day before the actual performance of the *Yagya* was the background for the quantities of bacteria, fungi, pathogen and total micro-flora, while the sampling done on the subsequent days of *Yagya* were the experimental observations for comparative studies. The results of all the experiments together revealed that there was reduction in the colony counts of all micro-flora and further that the reduction in the microbe level was significant even on the 2nd and 3rd day after *Yagya*, and continued even up-till seven days after *Yagya*.

Key words: Air micro-flora; Bacteria; Fungi; Pathogen; *Yagya*

1. Introduction

In the last several years, a growing body of scientific evidence has indicated that air within homes and other buildings can be more seriously polluted than outdoors considering even the largest and most industrialized cities. Since most of the people spend approximately 90 percent of their time indoors, therefore, for many people the risk to their health may be greater due to exposure to air pollution indoors than outdoors. It is believed that airborne microorganisms, such as thermo-philic action-mycetes, fungi, protozoa [Finnegan et al, 1987], bacteria and several other pathogens, are responsible for infectious or allergic disorders such as asthma [Burge et al, 1985; Burrel, 1991] and humidifier fever [Edwards, 1980] in exposed people, and also that airborne contaminants cause symptoms of sick building syndrome such as dermatological, ocular and ear problems among exposed office workers in mechanically ventilated buildings [Bholah and Subratty, 2001].

Yagya, a Vedic procedure of burning some herbs in fire along with some rituals, is known for purification of atmospheric pollution through removal of foul odour, lowering of harmful gas levels and removal of harmful microbes [Joshi, 1998]. Consequences of carrying out *Yagya* in the internal environment have not been scientifically studied and recorded. Some scientific theoretical analysis of *Yagya* (Agnihotra) was done in the past by Prakash [1937], and by Mondak, Ming Lai, Bhujbal, and Mutalikdesai, [personal communication] but it has been without any experimental backup.

In order to confirm the claims made in Vedas, several preliminary indoor experiments by performing *Yagya* have been conducted so as to study the effect on the air Microflora. In the following the analysis of these experiments is described.

2. Materials and Methods

2.1 Plan of the Study

The present study was conducted indoors at the M.S.Apartments, K.G. Marg, Delhi. In the study several experiments were conducted during the months of June 2004, January 2005, February 2005 and April 2005. In these experiments the atmosphere was treated with *Yagya*, a Vedic process described by Dr Sharma Acharya [personal communication]. The presence of air microflora in the atmosphere was measured a day before, during, and after the experiments to make a comparative study of the effect of *Yagya* on the presence of air microflora. In these experiments it was strictly observed that the variability factors like the number of persons attending, atmospheric variables, and inputs used like wood, herbs (Havan Samigri), Ghee etc., were kept constant.

2.2 Measurement of the Airborne Micro-organisms

Microorganisms are generally well dispersed and are capable of living and growing in a particular environment. The small size of the micro-organisms results in very intimate contact with the environmental particulates [Spendlove, 1975] offering a very high surface area to volume ratio for the environmental factors to act on the organisms, which are particularly sensitive to changes in the levels of temperature, light, pH, organic and inorganic nutrients, carbon dioxide, oxygen, water etc., [Flannigian et al, 1991, Bovallius et al, 1978], with the extreme levels having more effect on microorganisms than they do on higher plants and animals which are in some ways isolated from the environment.

Through the act of *Yagya*, where a number of herbs are burnt, an environment is created where the microorganisms, particularly the disease causing pathogens, are annihilated. Measurement of airborne microorganisms thus allows the assessment of the exposure to the indoor microbial pollution.

2.3 Methodology of Sampling

The ambient air was sampled by using the *'Gravity Settle Method'*. Although this method does not give very accurate assessment of the microbes in the atmosphere, it can still give good comparative results. A set of four petri-dishes pre-treated with i) Nutrient Agar(NA), ii) Potato Dextrose Agar (PDA), iii) Total Count Agar (TCA) and iv) Mac Conkey Agar (MCA) were exposed at a time to capture and cultivate the bacteria, fungi, total microflora and pathogens respectively on them. Four samples were taken in a day and mean plot of the microflora was drawn to see their variability during the day. In each of the experiments, sampling was done a day before the experiment, then on the day of experiment, which was performed early in the morning, and then till three days after *Yagya*, till three days to see the effect. In another experiment sampling was continued for 7 days after *Yagya* to study the effect over a longer period.

The experiments being conducted indoors, external variations like wind, rainfall vegetation etc. did not affect. The temperature range & humidity was observed to be almost constant during the days of experiments. The petri-dishes treated with NA, PDA and TCA were exposed in the center of the room at a height of 0.6 m from the ground for a period of one minute and the dish treated with MCA was exposed for 5 minutes. All the doors and windows were closed while taking the sample so as to let the microflora settle down freely without any disturbance from the air currents.

2.4 Microbiological Analysis

The samples were taken on the same day to the lab of Central Pollution Control Board (CPCB), Delhi, where they were kept in an incubator for 5 days at a uniform temperature of 30° centigrade. Thereafter the dishes were taken out and the colonies of the microflora developed were counted. In the absence of identification system, it was not possible to identify the microbes individually hence the total counts have been used as the data for analysis. The parameters measured were bacteria, fungi, total microflora(TMF) and pathogens.

2.5 Schedule of Experiments

Four indoor experiments have been considered for study. In all the 4 experiments, background sampling was done a day prior to the main experiment, and sampling was continued during the day of experiment (*Yagya*) and continuously till three days after. In the 4th experiment the sampling was continued till 7 days after *Yagya* to study the long term effect. The days of the experiments were as follows:

	Dates	**Microflora sampled**	**Total Samples**
1	26.6.04 - 30.6.04	All Four	20
2	22.1.05 – 26.1.05	All Four	20
3	19.2.05 – 23.2.05	All Four	20
4	8.4.05 – 16.4.05	All Four	27

3. Data Analysis

The day of *Yagya* has been termed as 'During' and the days after have been termed as Day1, Day2 and Day3. In order to see the effect of *Yagya* on air microflora, 'pre-post' design has been developed where the before (*Yagya*) readings are compared with those during (*Yagya*) and after (*Yagya*) readings in pairs separately for all the four types of microflora. The null hypothesis which is tested as:

H_o: "There is no difference in air microflora in the pre and post measurements" against

H1: "There is difference in air microflora in the pre and post measurements".

The idea is that if the treatment of atmosphere with the '*Yagya*' had no effect, the average difference between the measurements of microflora is due to random causes and not due to the *Yagya*. In this case the null hypothesis is accepted. On the other hand, if the (*Yagya*) treatment did have an effect, the average microflora counts are not equal and the null hypothesis is rejected.

The following types of analysis of colony counts for the various microflora have been performed on the data so generated:

1. The descriptive summary statistics for all the four sets of experiments has been worked out and is given in Table 1;
2. All the microbial counts have been studied for their percentage change so as to see the comparable effect of *Yagya* on them;
3. Paired T-test procedure has been performed to test the null hypothesis; and
4. Lastly the average microflora counts have been plotted in the form of line charts, for all the four types of *air microflora* to see their comparative path of growth / decay.

Table 1: The Summary of Colony Counts for the Various Microflora (All the four experiments together) and number of samples N=20

	Back Ground (D-1)		Day of Exp. (Do)		One Day After (D1)		Two Days After (D2)		Three Days After (D3)	
	Mean	Std Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Bacteria	38	37.32	29	14.84	25	33.73	19	16.85	13	9.60
Fungi	16	14.88	6	3.88	8	7.41	3	3.13	3	2.28
TMF	30	29.07	24	17.71	22	32.96	16	15.09	12	11.56
Pathogen	14	16.50	4	2.09	4	3.50	3	3.08	2	2.19

The descriptive Table 1 displays the mean, and standard deviation of the counts of all the 4 experiments considered together. Although the sampling in the experiment conducted in April 2005 was continued for 7 days after *Yagya*, for the purpose of comparative calculations in the summary descriptive statistics given in Table 1, only first 5 days sampling data was considered. It can be seen that the average count of colonies of Microflora have decreased on the day of experiment and continued to do so till two days after as compared to the background count. The variability of counts has also more or less decreased over the period of 5 days, for air microflora .

To see the percentage change in the air microflora that occurred on account of *Yagya*, the mean value of the four sets of samples taken in one day has been taken as the representative count for that particular microflora for that day and has been used for comparison. The four sets of experiments conducted in June,'04, January '05, February '05 and April '05 have been termed as experiment nos. 1, 2, 3 & 4 respectively in the Tables 2 - 5.

In each set of experiment, the comparison of average colony counts of each type of microflora, viz. Bacteria, Fungi, TMF and Pathogens on the Day of *Yagya* (D0), 1 day after *Yagya* (D1), two days after *Yagya* (D2), and three days after *Yagya* (D3) has been made with respect to the background (D-1) counts. The percentage change of each count has been shown in the immediate next column. The reduction in counts with respect to the background has been shown as negative and vice-versa.

Table 2: Percentage Change in Bacteria Counts (Average Colony Counts)

Set of Experiment	Back Gr. BG	During *Yagya* D0	% Ch during *Yagya*	1 Day After D1	% Ch I day after	2 Days After D2	%Ch 2 days after	3 Days After D3	%Ch 3 days after
1	12	9	-25	6	-50	5	-58	1	-92
2	10	27	170	3	-70	4	-60	6	-40
3	43	43	0	75	74	35	-19	16	-63
4	90	37	-59	16	-82	32	-64	25	-72

Table 3: Percentage Change in Fungi Counts

Set of Experiment	Back Gr. BG	During *Yagya* Do	% Ch during *Yagya*	1 Day After D1	% Ch I day after	2 Days After D2	%Ch 2 days after	3 Days After D3	%Ch 3 days after
1	3	4	33	3	0	1	-67	0	-100
2	3	2	-33	1	-67	1	-67	2	-33
3	24	8	-67	18	-25	5	-79	4	-83
4	32	10	-69	8	-75	7	-78	5	-84

Table 4: Percentage Change in TMF Counts

Set of Experiment	Back Gr. BG	During *Yagya* Do	% Ch during *Yagya*	1 Day After D1	% Ch I day after	2 Days After D2	%Ch 2 days after	3 Days After D3	%Ch 3 days after
1	12	7	-42	4	-67	5	-58	1	-92
2	14	19	36	4	-71	5	-64	5	-64
3	35	43	23	71	103	31	-11	25	-29
4	69	33	-52	12	-83	27	-61	18	-74

Table 5: Percentage Change in Pathogen Counts

Set of Experiment	Back Gr. BG	During *Yagya* Do	% Ch during *Yagya*	1 Day After D1	% Ch I day after	2 Days After D2	%Ch 2 days after	3 Days After D3	%Ch 3 days after
1	3	2	-33	2	-33	1	-67	0	-100
2	3	3	0	1	-67	0	-100	1	-67
3	38	7	-82	9	-76	7	-82	5	-87
4	14	4	-71	3	-79	4	-71	1	-93

In all the four Tables 2 - 5, it is seen that there is a reduction in the counts of microbes one day after, two days after and even three days after the experiment. However on the day of experiment some of the microbes have increased in some cases, which is as per expectations since microbes are known to grow with heat and nutrition.

In order to study the duration effect of *Yagya* on atmospheric microbes, the 4th experiment was conducted till 7 days after *Yagya*, and the results are given in Table 6. It is seen from this Table that there is significant decrease in counts with respect to the background even 7 days after the experiment showing the effect of *Yagya* continuing even after 7 days of performing *Yayga*.

Table 6: Percentage Change in microflora Counts during April 2005 Experiment

Microflora	Back Gr. BG	During *Yagya* Do	% Ch during *Yagya*	1 Day After D1	% Ch I day after	2 Days After D2	%Ch 2 days after	3 Days After D3	%Ch 3 days after
Bacteria	90	37	-59	16	-82	32	-64	25	-72
Fungi	32	10	-69	8	-75	7	-78	5	-84
TMF	69	33	-52	12	-83	27	-61	18	-74
Pathogen	14	4	-71	3	-79	4	-71	1	-93

Contd.

Microflora	Back Gr. BG	4 Day After D4	% Ch 4 day after	5 Day After D5	% Ch 5 day after	6 Days After D6	%Ch 6 days after	7 Days After D7	%Ch 7 days after
Bacteria	90	9	-90	12	-87	10	-89	7	-92
Fungi	32	3	-91	6	-81	4	-88	4	-88
TMF	69	10	-86	13	-81	11	-84	9	-87
Pathogen	14	1	-93	2	-86	2	-86	3	-79

The treatment of the atmosphere with *Yagya* has led to the reduction of microbes. In order to see whether the reduction is statistically significant, the consolidated data of the four experiments was subjected to Kolmogrov-Spectrum Test to check for the normalcy of the data. Large significance values (>.05) in all the cases except on Day 2 indicate that the observed distribution corresponds to the theoretical distribution. Then the data (mean Values) for colony counts on the background day were compared with the colony counts on the day of *Yagya*, 1 day after, 2 days after, and 3 days after respectively. This was done to see if the microbial colony counts were similar to the background i.e. whether there was any effect of treating the atmosphere with *Yagya* on the microbial population. For this purpose paired sample T - Test was applied.

The pairs formed were: BackGround with the day of *Yagya*, BackGround with 1 day after, BackGround with 2 days after, and BackGround with 3 days after. The results of the paired samples statistics are given in Table 7, while the T-Test results are given in Table 8.

From the results in Table 8, it may be seen that the significance value / probability for Pair 1 and Pair 2 is higher than .05 (p>.05) and hence the null hypothesis is accepted. For Pair 3 and Pair 4, the hypothesis is rejected. This implies that *Yagya* had non-significant impact on the microbe counts on the day of experiment as well as 1 day after. However, the impact is significant (p<.05) on the 2nd day and 3rd day after *Yagya*. Hence it is seen that the impact of *Yagya* in reduction of air microflora is significantly more on 2nd day and third day after *Yagya*. This establishes the fact that the effect of *Yagya* continues for a long time even after the physical process has been completed. These results are the combined effect of all the four experiments

In order to see the effect of *Yagya* in the case of an individual experiment, the results of experiment 4, conducted in April 2005, were analyzed. In this case sampling was continued till seven days after *Yagya* to study the effect of *Yagya* over a longer period. The data given in Table 6 was subjected to Kolmogrov-Spectrum test for checking the normalcy of data. It was seen that in all the cases the significant values were much higher than 0.05, meaning thereby that the distribution of the data was normal and we can safely apply Paired T-Test procedure for all the Microflora combined. The results of this analysis are shown in Table 9.

Table 7: Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	BackG	25.31	16	25.224	6.306
	During	16.13	16	15.227	3.807
Pair 2	BackG	25.31	16	25.224	6.306
	day1	14.75	16	23.317	5.829
Pair 3	BackG	25.31	16	25.224	6.306
	day2	10.63	16	12.559	3.140
Pair 4	BackG	25.31	16	25.224	6.306
	day3	7.44	16	8.579	2.145

Table 8: Paired Samples Test

		Paired Differences		t	df	Sig. (2-tailed)
		Mean	Std. Deviation			
Pair 1	BackG - During	9.188	18.203	2.019	15	0.062
Pair 2	BackG - day1	10.563	27.193	1.554	15	0.141
Pair 3	BackG - day2	14.688	16.410	3.580	15	0.003
Pair 4	BackG - day3	17.875	18.654	3.833	15	0.002

Table 9: Paired Samples Test

		Paired Differences		t	df	Sig. (2-tailed)
		Mean	Std. Deviation			
Pair 1	BackGr – During	30.083	58.767	1.773	11	0.104
Pair 2	BackGr - day1	41.333	61.256	2.337	11	0.039
Pair 3	BackGr - day2	33.750	63.861	1.831	11	0.094
Pair 4	BackGr - day3	38.500	56.354	2.367	11	0.037
Pair 5	BackGr - day4	45.083	60.614	2.577	11	0.026
Pair 6	BackGr - day5	42.833	62.562	2.372	11	0.037
Pair 7	BackGr - day6	44.417	63.345	2.429	11	0.033
Pair 8	BackGr - day7	45.250	60.327	2.598	11	0.025

It is seen from Table 9 that except on the day of experiment and 2 days after, the reduction in Microflora as compared to the background on all the other days was significant (sig. <.05).

4. Graphical Representation

The line charts for each experiment have been shown separately in Figs. 1 – 4 to see the behaviour of microflora in individual experiments.

4.1 Aggregate Bacterial Count

Fig. 1 represents the mean bacterial counts for all the experiments. It is seen from here that in general bacterial counts compared to the background have come down on the day of experiment and are also reduced or have remained constant one day, two days and even 3 days after the experiment of performing *Yagya*. Almost similar picture appears for other microflora also. The exceptions have been seen for one case each in February 2005 and January 2005 experiments when bacterial counts have increased.

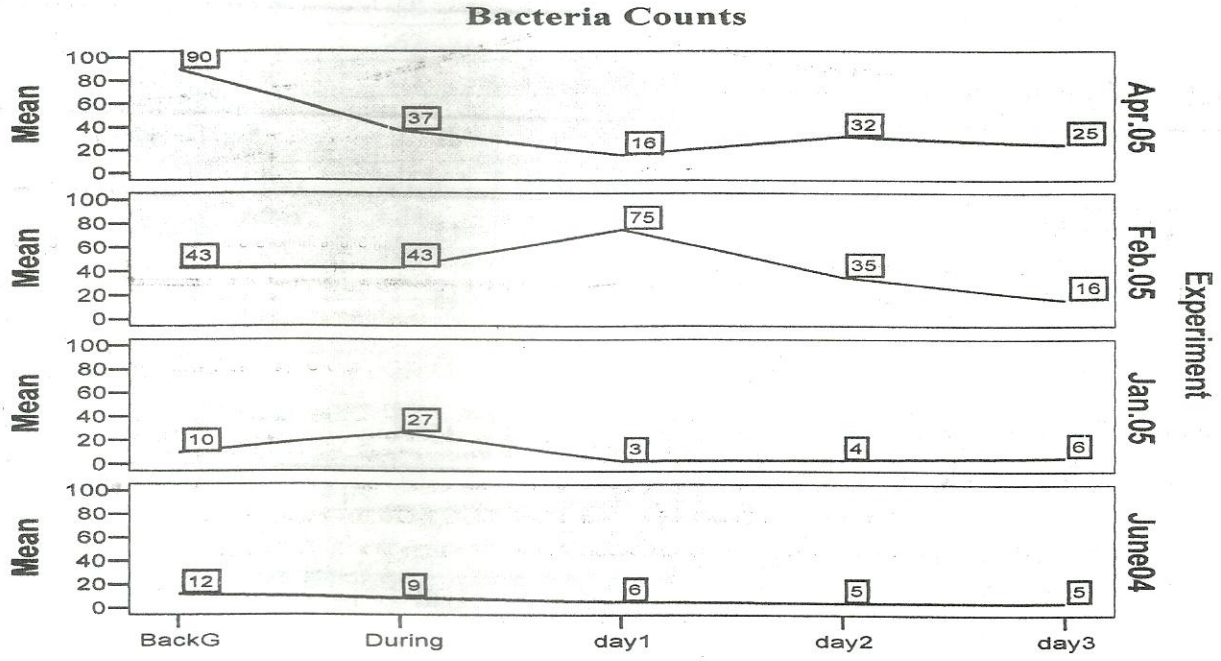

Fig. 1: Bacterial Counts (Mean) for all the experiments before, during and after the *Yagya*.

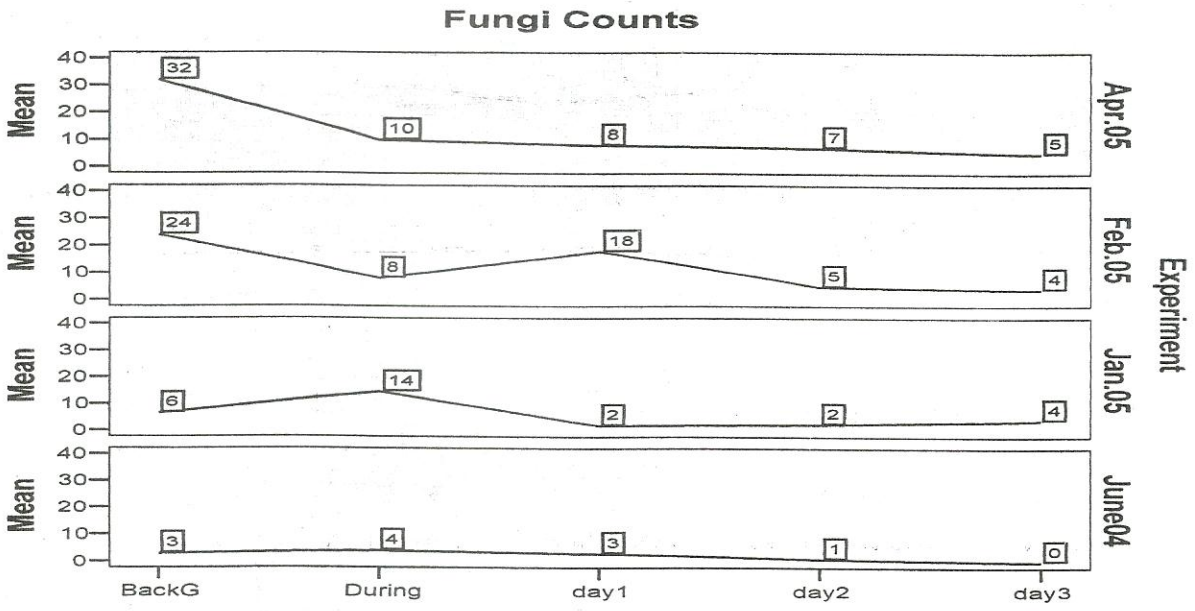

Fig. 2: Fungi Counts for all the experiments before, during and after the *Yagya*.

A similar picture of reduction in microbes is seen in Figures 2 to 4 where excepting one or two cases, the microflora have reduced after *Yagya* and have remained less than the back ground count.

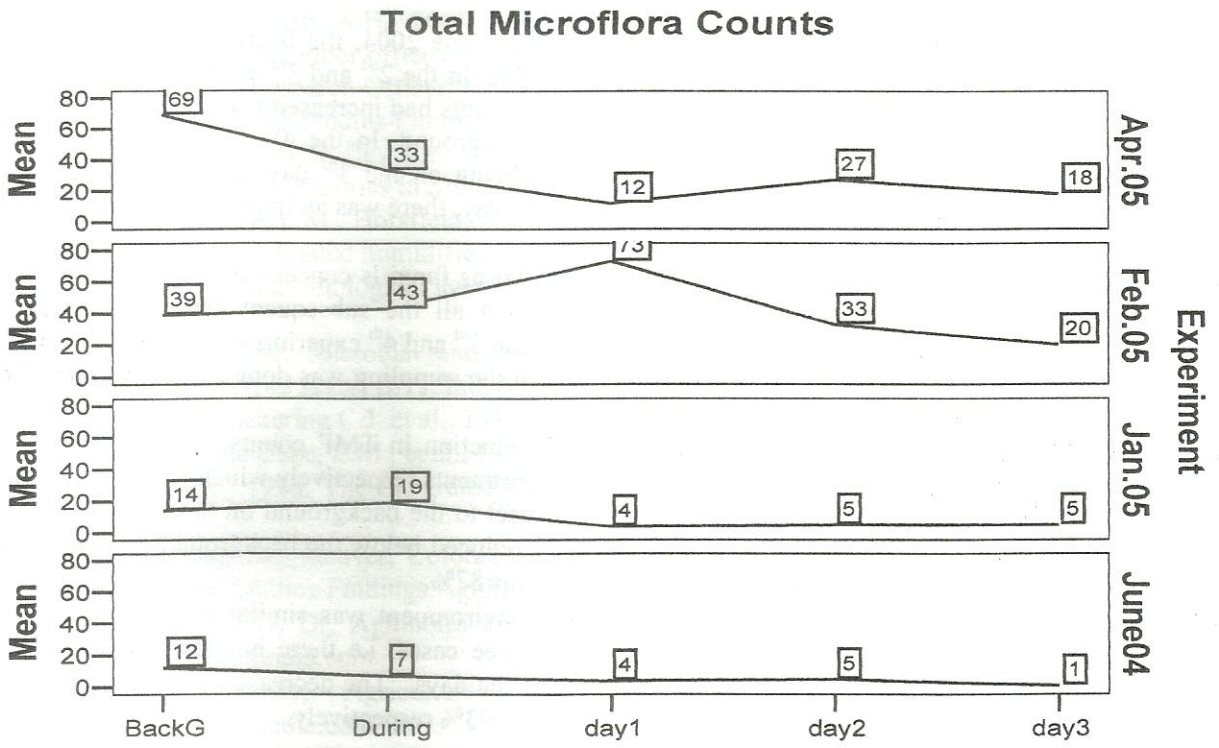

Fig. 3: Microflora Counts for all the experiments before, during and after the *Yagya*.

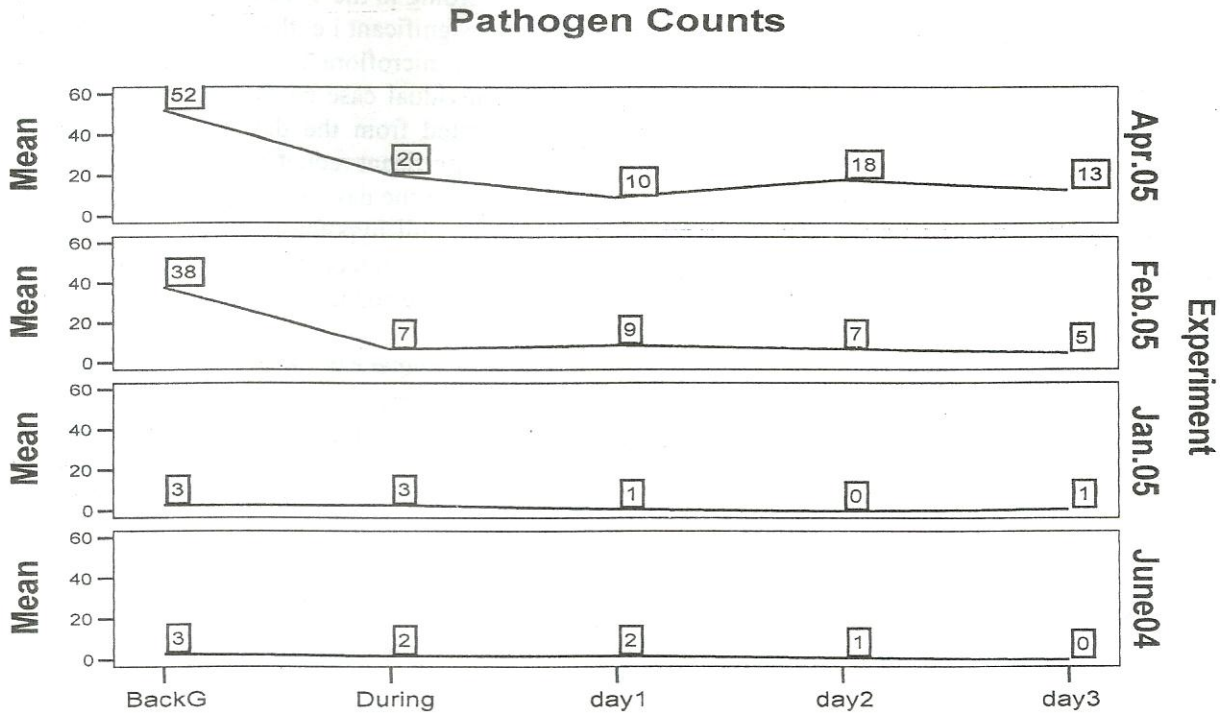

Fig. 4: Pathogen Counts for all the experiments before, during and after the *Yagya*.

5. Results and Discussion

5.1 Extent of Change

All the parameters have been studied for their percentage change to see the effect and its direction i.e. whether it has become better or worse.

- **Bacteria**: In the first set of experiments conducted in June 2004, the bacteria counts had reduced gradually and had become almost negligible 3 days after. In the 2nd and 3rd experiments conducted in January and February 2005 respectively, however, the counts had increased once but the decrease was significant (about 40% and 63%) compared to the background. In the 4th experiment conducted in April 2005, the decrease is on all days with 72 % being on the 3rd day. The 4th experiment was continued till 7 days after and it was seen that on the 7th day, there was an impressive reduction of 93% in bacteria counts compared to the background.
- **Fungi:** The experiments have been very effective as far as fungi is concerned. The counts have been lower than the background in all the cases on almost all the subsequent days. In the first two experiments the reduction was 100% and 33% and in the 3rd and 4th experiments, it was 83% and 84 % on the 3rd day after *Yagya*. In the 4th experiment when the sampling was done till 7 days after *Yagya*, the reduction was 88% on the 7th day.
- **Total Microflora (TMF):** There has been overall reduction in TMF counts. On day3 there was a reduction of 92%, 64%, 29% and 74% in the four experiments respectively which is quite satisfactory. However, there was some increase in TMF with respect to the background on the day of experiment and on day1 and day2 cases and it was subsequently reduced below the background counts. In the 4th experiment the reduction continued till day7 when it was 87%.
- **Pathogen:** The picture of Pathogens in the indoor environment was similar to the fungi, with the overall trend remaining the same as in the above three cases, i.e there has been reduction in the pathogen colony counts in almost all the cases on all the days. The decrease on the day3 in the four experiments has been recorded as 100%, 67%, 87% and 93% respectively.

5.2 Paired T-Test:

The analysis of the combined results of all the four experiments has revealed the effectiveness of *Yagya* on the various air microflora on the day of *Yagya* and the days after. It is seen that the reduction of microflora is not significant on the day of *Yagya* and one day after since according to the T-Test for the pairs Background – During *Yagya* and Background and one day after, counts are significant i.e. the probability attached to the T-statistic is significant ($p>0.05$). However, the reduction in the microflora counts is significant on the 2nd and 3rd day after *Yagya* as the probability is <0.05. The individual case study for the microflora in the experiment number 4 shows that the effect of *Yagya* has stated from the day of *Yagya* itself and the effectiveness has increased over the period of 8 days. There is significant reduction of microflora on day one after *Yagya*, and on days 3,4,5,6 & 7 after *Yagya* ($p<0.05$). Only on the day of *Yagya* and two days after, the significance value is > 0.05 but < 1, thereby implying that the null hypothesis is rejected at 0.1 level of significance, whereas, for the remaining days the null hypothesis is rejected at 0.05 level of significance. This implies that in statistical terms *Yagya* has been effective in the reduction of air microflora even till 7 days after it had been performed.

The reason behind reduction of various Microflora after *Yagya* can be attributed to the fact that all the fatty substances used in *Yagya* are combinations of fatty acids, which volatilise easily. The combustion of glycerol portion gives acetone bodies, pyruvic aldehyde and glyoxal etc. [Bovallius et al, 1978; Prakash, 1973]. The hydrocarbons produced in the reactions further undergo slow combustion and as a result methyl and ethyl alcohols, formaldehyde, acetaldehyde, formic acid and acetic acids are formed. The reactions keep on taking place in the atmosphere for a long time even after the process of *Yagya* is physically complete.

6. Conclusion

In view of the results above it can be concluded that the results of the study are indicative that *Yagya* has a significant impact in the reduction of air microflora in the indoor environment. The identification of the

microbes can reveal the type of microbes before and after *Yagya*, however, this could not be done in the present case on account of non-availability of required facilities.

References

1. Bholah R., and Subratty A.H., 2001: Airborne fungal colony forming units and symptoms of sick building syndrome in indoor office environment in Mauritius, Poll Res. 2P (4) : 495 – 499.
2. Bhujbal, B.G. Dr., Research Officer, Maharashtra State Grape Growers' Assoc. Poona India, Agnihotra and Grapes, Agnihotra Studies-Findings,Agnihotraindia.com
3. Bovallius A, Bucht B.,Roffey R.& Anas P., 1978:Three year investigation of the natural airborne bacterial flora of four localities in Sweden, Appl. Envir. Microbiol
4. Burge, P.S., Finnegan, M., Horsfield, N.,Emery, D., Austwick, P., et al,1985: Occupational asthma in factory with a contaminated humidifier, Thorax 40, 248 – 254.
5. Burrel, R., 1991: Microbiological agents as health risks in indoor air, Envir. Health Perspec, 95, 29 – 34.
6. Edwards, J.H., 1980: Microbial and immunological investigations and remedial actions after an outbreak of humidifier fever, Br. J. Ind. Med. 37, 55-62.
7. Finnegan M. J., Pickering C.J. et al., 1987: Amobae and humidifier fever. Clin. Allergy, 17, 235 – 242.
8. Flannigian, B., Mc Cabe, E.M., et al., 1991: J. Appl. Bacteriol. Suppl. 70, 61 – 73.
9. Joshi, Rajni, Dr., 1998: The Integrated Science of *Yagya*, Yug Nirman Yojana, Gayatri Tapobhumi, Mathura.
10. Ming Lai, Tung, Dr., Denver, Colorado Agnihotra Ash and Water Soluble Phosphates , Karnatak, India, Agnihotra Studies-Findings,Agnihotraindia.com
11. Mondkar, Arvind D. Dr, Agnihotra and Microbes, A Laboratory Experience , Agnihotra Studies-Findings,Agnihotraindia.com
12. Mutalikdesai, Abhay, Agricultural experience with vanilla plants, Agnihotra Studies-Findings,Agnihotraindia.com
13. Prakash, Satya, Dr (1937): Agnihotra – A study from the chemical stand point, Sarva Deshik Arya Pratinidhi Sabha, Delhi.
14. Sharma Achaya, Shri Ram Pt: Procedure of *Yagya*, Yug Nirman Yojana , Mathura
15. Spendlove, J.C., (1975) Penetration of structures by microbial aerosols. Dev. Ind. Microbiol 16,427 – 436.