

Effects of Occupational Exposure on the Health of Rag Pickers Due to Fungal Contamination at Waste Dumping Sites in Gwalior (India)

Harandra K. Sharma^{*1}, Khursheed Ahmad Wani², Aakash Ahmad Bhat¹

1) School of Studies in Environmental Science, Jiwaji University, Gwalior (M.P.) India

2) Department of Environmental Science, ITM University, Gwalior (M.P.) India

Corresponding author: drhksharmagwl@gmail.com

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ABSTRACT

We investigated fungal contamination near different waste dumping sites and assessed the health risk factors of rag pickers associated with collection of waste in Gwalior during the year 2014-15. Petri plates were exposed at waste dumping sites and were transferred to the laboratory, analysis and identification was mainly carried out by culturing the fungal colonies by following standard procedures. A pretested questionnaire was used to evaluate the health problems among the rag pickers. Results indicated that all the dumping sites are contaminated with different types of fungal pathogens like *Alternaria alternate*, *Aspergillus flavus*, *A. fumigates*, *A. niger*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. Our study reported higher incidence of musculoskeletal and respiratory diseases among rag pickers. There is also strong need for carrying out similar assessment studies for other cities too. This will entail generation of more precise site specific information regarding fungal species and associated health risk factor.

Key words: Fungal Contamination, Waste, Health, Rag Pickers, Gwalior

INTRODUCTION

Municipal waste is the habitat for various microorganisms, wherein their range of occurrence and concentration depends on various factors such as type and location of the facility, the season and the time. Further, the type of dumping waste site may also decide the potential and survival of many microorganisms that may cause many diseases [1]. Rapid urbanization of many cities in India has led to the growth of populations near dumping grounds and may exacerbate the risks. In addition to respiratory tract infections, it has been suggested that associated enteric microorganisms common in dumping ground may produce intestinal tract infections [2].

Fungi are distributed everywhere and are dependent on various environmental parameters like rainfall, moisture, temperature, wind and geographical location. It has been reported that airborne fungi may cause asthma, rhinitis and dermatitis besides they are considered as a source of plant and animal pathogens [3, 4, 5, 6]. Due to their importance to the human health, agriculture and food spoilage more emphasis by the scientific community to study the airborne fungi has been given over the years [7, 8, 9].

A large number of fungi have been isolated by various researchers from self-heating material and were used in decomposing of municipal solid waste at elevated temperature that usually resulted from microbial thermogenesis [10]. Fungi capable of

producing extracellular enzymes responsible for degradation of cellulose are known, some of them being highly cellulolytic, which include species of *Aspergillus*, *Trichoderma* and *Sclerotium* and these species are also being considered for commercially exploitation [11].

The exposure to bioaerosols may cause a number of undesirable effects, such as gastrointestinal, dermatologic, respiratory and allergy problems. More specifically, exposure to bioaerosols deriving from waste processing [12] may pose health risks to workers working in composting plants and in collection, transport and segregation of urban solid waste [13, 14].

There is lack of data on effect of occupational exposure of waste handlers / rag picker in different regions of India. Although a large chunk of population in India is managing and handling waste that is obtained from households, shops, industries, agricultural sources and exposed daily to different types of diseases due to pathogenic microorganisms present in the waste. In Gwalior Madhya Pradesh no systematic work has been carried out so far on this respect. This aim of the current investigation is to document the different species of fungi present in the waste generated from different sources in Gwalior and to know the diseases / risk factor of waste handlers/rag pickers due to fungal exposure.

METHODOLOGY

Study area

The present study was done on microbial contamination at different waste dumping sites of Gwalior city. Gwalior a historic city located on the periphery of Madhya Pradesh has geographical position at 26° 12' north and 78° 18' east. Gwalior a popular city in the country has a population of 2032036 with a density of 446/km² [15]. The main source of waste in Gwalior city is domestic waste, vegetable market waste, paper waste, industrial waste, fruit market waste, meat market waste, Hotel/restaurant waste, market waste, building material waste [16].

Gwalior has a sub-tropical climate with hot summers from late March to early July, the humid monsoon season from late June to early October and a cool dry winter from early November to late February. Under **Koppen's climate classification** the city has a humid sub-tropical climate. The highest recorded temperature was 53°C and the lowest was -1°C. The average temperature in the month of May and June is 33-35°C and receives a rainfall of about 970 mm which is concentrated during monsoons that starts at the end of June. A unique feature of the climate of the Gwalior district is the presence of the three specific seasons i.e., monsoon, winter and summer [17].

Sampling sites

Bada, Kampoo, Lashkar, Morar and Golaykamandir were selected for the present study (Fig 1). The sampling was carried out separately at every site in triplicate to represent the whole area during the year 2014-15. These sites are usually used as waste dumping /storage sites and a large number of rag pickers and waste handlers are visiting regularly to these sites. Further these sites are situated in commercially important locations in the Gwalior city.

Exposure of Petri plates

The methodology adopted for the present study includes field survey at five different waste dumping sites of Gwalior, generally petriplates were exposed at waste dumping sites and were transferred to the laboratory, analysis and identification was mainly carried out by culturing the fungal colonies by following various procedures and guidelines provided by [18,19].

The collected samples were collected and transported to the laboratory as soon as possible and the process of analysis was done the same day or the next. Most of the sampling was done between 9AM-12PM. The samples were stored in refrigerator in case the sampling was done in the evening or the analysis was not possible on the same day.

Enumeration of Colony Forming Unit (CFU)

Colony Forming Unit (CFU) was mainly enumerated after the colony counting of fungal colonies by taking into account the following equation described by Omeliansky.

$$N = 5a \times 10^4 (bt)^{-1}$$

Where N=microbial CFU/m³ of indoor air; a=number of colonies per Petri dish; b=dish surface (cm²); t=exposure time (min).

Identification of Fungi

Fungal colonies were identified based on its morphological characters. However, further identification to the genus level was done by microscopic examination. For this, a small piece of the material was taken from the colony with the help of a sterile needle. This was mounted on a slide with a drop of Lacto-phenol, a cover slip was placed over it and gently tapped. The prepared slide was observed under a binocular microscope and identified, making use of standard manuals. The colony morphological features were recorded using a stereo binocular microscope.

Evaluation of health hazards:

The microorganism pathogenicity and the role they play in deteriorating the health of waste handlers was accessed by collecting information through questionnaire and personal investigation.

Statistical analysis:

The mean, percentage and CFU were calculated with the help of computer programme MS Excel, 2010.

RESULTS

Temperature and humidity

Temperature, moisture and wind speed are the most important factors that influence the growth of fungi in both indoor and outdoor environments. Usually fungal growth is favored at water activity of 0.95–0.99, while 0.65–0.90 and 0.88–0.99 are reported to be required for the growth of xerophilic fungi and yeasts [20]. The temperature in buildings of about 20–250 °C, promotes the growth of mesophilic fungi. However, the temperature below optimum level slows down the growth of fungi. Wind speed also plays a great role in disperse of fungal spores.

Meteorological parameters

The sampling was carried out during the summer season by collecting samples at five different sites, the minimum temperature of about 39°C occurred during sampling first in the month of April and the maximum temperature was found during the month of June. Sampling first and second was mainly carried out at the humidity (%) of 44 respectively and third sampling was mainly carried out at 29 %. Sampling first and second was carried out at a wind

speed of 2 km/h, while third sampling third was carried out at a wind speed of 1 km/h (Table 1).

Fungal count

The colonial count of fungi was carried out after the incubation of exposed plates at a temperature of 27 °C for 5 to 7 days. The highest colonial count of fungi during the month of April has been recorded at site III of Bada, which was 34 (no of colonies / plate), while the lowest fungal count at site I of Golay ka mandir, which was 8 (no of colonies/ plate). The highest fungal count during the month may has been recorded at site I of Golaykamandir, which was 33 (no of colonies/plate), while the lowest fungal count has been recorded at site III of Lashkar, which was 12 (no of colonies/plate). The highest fungal count during the month of June was recorded at site III and site I of Bada and Golaykamandir respectively, which was 31 (no of colonies), while the lowest fungal count has been recorded at site I of Lashkar, which was 15 (no of colonies/plate) (Table 2).

Fungal contamination

The result indicate that the highest fungal contamination (CFU/m³) during the month of April has been recorded at dumping site Bada at site III, which was 1782.40 CFU/m³ that represented a percentage of 26.4 % of the total colony forming unit, while the lowest fungal CFU/m³ has been recorded a at dumping site Golaykamandir at site I, which was 419 CFU/m³ representing a percentage of 7.9 % of the total colony forming unit (Table 3, 4). The highest fungal CFU/m³ during the month of May has been recorded at dumping site Golaykamandir at site I, which was 1729.98 CFU/m³, that represented a percentage of 25 % of total CFU/m³, while the lowest fungal CFU/m³ has been recorded at dumping site

Morar at site I, which was 786.35 CFU/m³ representing a percentage of 11.3 % of total CFU/m³ (Table 3, 4).

The results also indicated that there were little fluctuations in fungal contamination during different months between the sub sites of Bada and Kampoo respectively, which was mainly due to equal and even distribution of the waste quantity and little changes in meteorological parameters like temperature, humidity and wind speed at these sites, as the sampling was carried in same season. However, changes were observed in fungal contamination during different months among the sub sites of Lashkar, Morar and GolayKaMandir respectively, which mainly occurred due to uneven equal waste amount and uneven distribution of organic waste at the different sub sites. The different species that were identified at various sampling sites include *Alternaria alternate*, *Aspergillus flavus*, *A. fumigates*, *A. niger*, *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* (Table 5).

Health hazards of fungi

The results of the present study collected through a simple and standard questionnaire investigated that the workers and rag pickers handling the waste mainly suffer from allergic respiratory diseases, gastro intestinal diseases, infectious diseases, musculoskeletal diseases and injuries (Fig 2).

Table 1: Average meteorological measurements during sampling

Sampling	Month	Temperature (°C)	Moisture (%)	Wind speed (km/h)
First	April	39	44	2
Second	May	40	44	2
Third	June	47	29	1

Table 2: Colony count of fungi at different sampling sites during summer

No	Dumping site	Site I (colonies/pl)			Site II (colonies/pl)			Site III (colonies/pl)		
		April	May	June	April	May	June	April	May	June
1	Bada	25	26	23	30	27	25	34	32	31
2	Kampoo	29	29	27	25	22	23	31	20	25
3	Lashkar	19	30	28	12	29	23	14	12	22
4	Morar	20	15	15	29	15	22	24	13	24
5	Golaykamandir	8	33	31	22	31	28	25	20	22

Table 3: Colony Forming Units of fungi at different sites during summer

Dumping site	Site I (CFU/m ³)			Site II (CFU/m ³)			Site III (CFU/m ³)		
	April	May	June	April	May	June	April	May	June
Bada	1205.7	1316.01	1205.74	1310.59	1415.44	1310.59	1625.1	1677.55	1625.13
Kampoo	1520.28	1520.28	1415.44	1310.77	1153.32	1205.74	1625.13	1048.47	1310.59
Lashkar	996.05	1572.71	1463.86	629.08	1520.28	1205.74	733.93	629.08	1153..23
Morar	1046.47	786.35	786.35	1520.28	786.35	1153.23	1310.77	681.51	1258.16
Golaykamandir	419	1729.98	1625.13	1153.32	1625.13	1463.83	1310.77	1048.47	1153.23

Table 4: Area wise distribution of Fungi at different sites during summer

Sampling site		Site I (CFU/m ³)	Distribution (%)	Site II (CFU/m ³)	Distribution (%)	Site III (CFU/m ³)	Distribution (%)
Bada	April	1310.59	24.8	1572.71	25.4	1782.40	26.4
	May	1316.01	19.0	1415.44	21.8	1677.55	33.0
	June	1205.74	18.6	1310.59	20.7	1625.13	25.0
Kampoo	April	1520.28	28.7	1310.71	21.2	1625.13	24.0
	May	1520.28	22.0	1153.32	17.7	1048.47	20.6
	June	1415.44	21.8	1205.74	19.0	1310.59	21.2
Lashkar	April	996.05	18.8	629.08	10.2	733.93	10.8
	May	1572.71	22.7	1520.28	23.4	629.08	12.4
	June	1463.86	22.5	1205.74	19.0	1153.23	17.7
Morar	April	1045.47	19.8	1520.28	24.6	1310.77	19.4
	May	786.35	11.3	786.35	12.1	681.51	13.4
	June	786.35	12.1	1153.23	18.2	1258.16	19.4
Golaykamandir	April	419	7.9	1153.32	18.6	1310.77	19.4
	May	1729.98	25.0	1625.13	25.0	1048.47	20.6
	June	1625.13	25.0	1463.83	23.1	1153.23	17.7

Table 5: Types of fungal species isolated from different sampling sites

Fungal species	Bada	Kampoo	Lashkar	Morar	Golaykamandir
<i>Alternaria alternata</i>	+	+	+	+	+
<i>Aspergillus flavus</i>	+	+	+	+	+
<i>A. fumigatus</i>	+	+	+	+	+
<i>A. niger</i>	+	+	+	+	+
<i>Cladosporium</i>	+	+	+	-	+
<i>Fusarium</i>	+	+	+	+	+
<i>Mucor</i>	+	-	+	+	-
<i>Penicillium</i>	-	-	+	+	+
<i>Rhizopus</i>	+	+	-	-	+

DISCUSSION

There is a general consensus among the scientific community that fungi prefer waste sites as they provoke food from the waste by releasing cellulolytic enzymes. Cellulolytic enzymes play an important role in nature's biodegradation processes where plant lingo cellulosic materials are efficiently degraded by cellulolytic fungi. Fungi are known agents of decomposition of organic matter in general and of cellulosic substrate. This study clearly represents that *Aspergillus*, *Cladosporium*, *fusarium*, *Alterneria*, *Penicillium*, *Rhizopus* and *Mucor* showed their presence at the waste dumping sites (Table 5). More moderate cellulose producers were also recorded among other fungi. Gautam *et al.* [21] have been reported that to screen the highest cellulolytic ability of fungi *Aspergillus fumigatus* and very low cellulase activity showed by *Humicola sp.*, *Torula sp.*, these fungi isolated from municipal solid waste. In this study, *Aspergillus sp.*, *Penicillium sp.* and *fusarium*, which is the most extensively studied cellulase producer, isolated from waste. Reese and Levinson [22] reported that a few studies have been conducted earlier with *Aspergillus niger* and *Trichoderma sp.* to investigate their cellulolytic ability.

The fungi are omnipotent in nature and are present at the waste dumping sites due to their ability to invade places that are enough unhygienic and prepativate on different substances that have enough moisture content

in suitable environmental conditions for the fungal growth. They also help in biodegradation process as most of the fungi secrete cellulolytic enzymes. Knowledge of species and density of outdoor airborne fungi in a given environment is important in analyzing contamination and the identification and management of different allergic diseases, this study was therefore conducted at different waste dumping sites, where huge amount of domestic municipal solid waste acts as a breeding ground for these air borne fungi. In the present study nine genus of fungi including *Aspergillus*, *Cladosporium*, *fusarium*, *Alterneria*, *Penicillium*, *Rhizopus* and *Mucor* were identified in outdoor environment at five waste dumping. *Aspergillus*, *Alternaria*, *Cladosporium* and *Penicillium* were the most prevalent and appeared to be the most common genera in almost all sites.

The results of the study mainly investigated the distribution of fungal colonies over various dumping sites in Gwalior, during the summer season, and it was observed that the fungal contamination at the waste dumping sites is quite high, most of the dumping sites were highly dominated by the fungal species like *Aspergillus*, *Cladosporium*, *fusarium*, *Alterneria*, *Penicillium*, *Rhizopus* and *Mucor*. *Penicillium spp.* was found in abundance during third sampling in the month of June. The high percentage of organic waste at the waste dumping site at Bada may be the cause of abundance of fungal species.

The presence of *Aspergillus* spp. and *Rhizopus* spp. At the study site may cause allergic reactions among the rag pickers in Gwalior. A toxin "ochratoxin A" released by different species of *Aspergillus* is not only carcinogenic to humans but is weakly mutagenic due to initiation of oxidative DNA damage. Aflatoxins produced by different species of *Aspergillus* are lethal and it is well known fact that aflatoxin cause acute hepatic necrosis, that may later result in cirrhosis or carcinoma of the liver. *Aspergillus flavus* found at the waste dumping sites of Gwalior is the agent of aspergillosis, that has ability to cause infarction by invading the arteries of the lung and brain [23]. On the other hand, *Rhizopus* may cause rhinocerebral, thoracic, gastrointestinal, and cutaneous problems to exposed human population while as ergot alkaloid is produced by *Rhizopus oryzae* which is also toxic to humans [24]. Further, the species of *Penicillium* may colonize the lungs to create more constant allergenic stimuli that may result in lung damage by chronic obstruction of the airways with viscid mucus [25].

Biological air quality assessment is essential since it is related to the human health, food industry and animals and plants pathogenicity. In the last decade, interest among researchers has increased in investigating the outdoor and indoor airborne fungi due to their adverse effects on human health (6, 8).

Moisture, nutrients and temperature are the most important factors that influence the growth of fungi on building materials [26]. The requirement for moisture depends on the fungal genus or species. Usually fungal growth is favored at aw of 0.95–0.99, while 0.65–0.90 and 0.88–0.99 is required for the growth of xerophilic fungi and yeasts [27]. Nutrients in house dust and water favor fungal growth on building materials. Fiber glass, galvanized steel gathered with dust or lubricant oil residues, allows the growth of fungi (28, 29). The temperature below optimum level slows down the growth of fungi. pH range of 5–6.5 in building materials allows the best growth of most of the fungi [29, 30]. Sufficient light and oxygen are also critical for the growth of fungi in indoor environments [31, 32]. Separate collections of organic and non organic household waste is a common practice in many countries [9,8]. This often involves indoor storage of organic waste, including fruits, vegetables and food remain in apartment buildings in highly populated areas before it is taken for disposal. As a result, decomposition of organic waste may begin inside the waste bin and may act as a source of fungal spores [33].

The presence of different fungal species in the waste may cause different types of diseases. Inhalation or ingestion is a principal route of exposure to fungal propagules. Products of mold growth or microbial volatile break down products may contribute to symptoms of illness or discomfort independently on

exposure to fungal biomass [34]. The role of indoor fungi in irritative disorders i.e. primarily non-infective diseases such as allergy and asthma, has long been recognized.

The presence of mold in waste dumping sites can cause sinusitis due to inflammation of para nasal sinuses and damp concrete floors will enhance the threat of irritated stuffy or running nose and may cause itching, burning or irritated eyes. The association between nasal polyps and skin reactivity to *Candida albicans* in patients exposed to indoor pollution has been reported and contact with air borne fungal spores will result persistent cough in childrens[35].

An allergen exposure increased the chances of allergic sensitization and was a risk factor for an early asthma onset as well as enhanced disease severity (36). Exposure to a variety of fungi such as *Aspergillus* spp. and *Fusarium* spp. may result in serious respiratory infections in immune compromised persons (37, 38, 39, 40). People with impaired immune system who spend most of their time in indoor environments contaminated by fungi may develop serious fungal infections [41, 42]. Chronic obstructive pulmonary disease, asthma, cystic fibrosis are disorders among persons potentially infected with *Aspergillus* [43]. In cystic fibrosis or asthma patients, *Aspergillus* spp. can develop allergic broncho pulmonary aspergillosis, invasive or semi-invasive pulmonary aspergillosis and pulmonary aspergilloma [44].

A large part of waste in Gwalior is recycled by rag pickers as Gwalior city does not have any proper waste management system. The rag pickers are those who forage the waste dumpsites searching for the hidden treasure in the waste and convert waste into wealth. Apparently poor healthy young men are involved in this business which serves as a source of livelihood for them. This type of occupation has several health risk factors. The rag pickers do not have sufficient personal protective equipments during recycling of the waste in Gwalior and are not usually well protected and they go about with shabby clothes and worn-out shoes or slippers.

The tragedy is that rag pickers are unaware about the health risk factors, of this profession. The fungal strains isolated from the waste are causative agents of different types of diseases. There is not a proper mechanism of waste separation at source in Gwalior city. The government has never educated people of Gwalior to separate waste at source and the households were not provided dust bins to separate the waste. It was observed that the heaps of the waste remain at the dumping sites uncovered for weeks and that is enough for fungal strains to reproduce and cause diseases not only to the workers but to the entire residents of the area. The study has not investigated the bacterial species present at these sites which is a limitation to

arrive on some solid conclusions. However, the detailed studies must be carried out to identify the bacterial strains and clinical investigations of the rag pickers may prove very fruitful to make further recommendations.

Arising out of limitations related to present study some uncertainties would always remain, however, waste a resource at wrong place in wrong time should be managed based on the responses of most groups of persons (rag pickers in present study), who are most often neglected when such strategies are formed out in air conditioned offices and they are invited. Under the circumstances, exposure studies for population at risk need to be investigated in general, and examine long term effects of the reduction or increase of exposure to a pollutant in the waste. Our present analysis, in particular points out that rag pickers happen to be the most sensitive group.

ETHICAL ISSUES

The participants were informed about the purpose of the study and due permission was taken from every participant and signatures were taken from each participant.

CONFLICT OF INTEREST

NIL

AUTHORS' CONTRIBUTIONS

Every author participated equally for the present study during each stage of the present work

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