Arabitol and Mannitol as Tracer for Fungal Contribution to Size-Differentiated Particulate Matter of Rural Atmospheric Aerosols

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***Abstract*—In this study we used biomarkers for analyzing the impact of fungal spore in ambient aerosols collected from rural area of eastern central Indian region during winter 2011.Mannitol and arabitol were used as tracer to estimate the impact of fungal activity in the ambient aerosols in our study site. We present the first estimates for the impact of fungal spores to the ambient aerosols mass loading in the eastern central India. This biomarker are quantified in two particle size fraction i.e. $PM_{2.5–10}$ (Coarse) and PM_1 (Submicron). Mannitol is dominant over arabitol in both two size fraction during winter period. The mass concentrations of these two biomarkers were higher in coarse size fraction as compared to submicron sizes. Strong correlation was found between the mass concentration of mannitol and arabitol in coarse size fraction only, whereas other size fractions were moderately or poorly correlated to each other. These showed that the sources for mannitol and arabitol in coarse size fractionwere similar.**

***Index Terms*—PM, biomarkers, mannitol, arabitol, spearman correlation.**

I. Introduction

The ambient aerosol (Particulate matter or PM) is considered to be one of the most significant issues due to their many adverse effects towards the human health [1], [2]. PMs are directly originated from sources or form by the interaction of primary pollutant with meteorological parameter like relative humidity, temperature, environmental pressure and rain fall etc. Aerosols are classified as organic and inorganic aerosols [3]. Carbonaceous material participates as a major constituent (70-90 %) of biomass burning aerosols [4]. Approximately 30-75% of the carbonaceous matter represents the water-soluble organic carbon (WSOC) [5], [6]. The major sources of biomass burnings are uses of wood and coal for cooking and heating activities, and burning of rice straws in agricultural lands after harvesting period [3], [7].

Pulmonary irritation, infection and allergies are some common symptoms which are originated from the biologically active PMs. Particularly, airborne fungi can cause extensive collection of unfavorable response like trigger for asthma attack in humans depending on the type and quantity present. Airborne particles that initiate from living organisms, plants, cell parts, pollen, bacterial and fungal spores are termed bioaerosols or biologically active aerosols [8]. Aerosols containing biological components can have a significant effect on human health by causing primarily irritation, infection and allergies [9]. Recent studies have clearly demonstrated that feasible micro-organisms present in the atmosphere can contribute to atmospheric chemistry through degradation processes as well as chemical change due to the release or desorption of molecules from microbiological entities [10]. Biological particles contributed an average of 39% of the organic carbon mass in particles with aerodynamic diameters less than 10 μm [11], [12]. Sugar alcohols such as mannitol and arabitol are tracers for biogenic activity associated in aerosols because these carbonaceous materials originated from metabolic activity of fungi, bacteria, small vertebrates and other living system [8]. Mannitol and arabitol are frequently used as a tracer for fungal metabolic activity present aerosols [12]-[14]. Mannitol and arabitol are the biomarker for primary biological PMs and soil re-suspension that contains biological materials together with fungi and bacteria [12]. Mannitol and arabitol have significant contribution in $PM_{2.5-10}$ and $PM_{2.5}$ aerosols [13]. More recent estimations suggest that fungal spores accounted for ~60% of coarse atmospheric organic carbon and for ~39% of the coarse aerosol mass as was measured in Vienna during spring and summer of 2005 [14]. Sugar alcohols are mainly originated from the microorganisms [12]. Feasible fungal spores are present in smoke from outlying biomass fires [15], [16]. Therefore, the main goal of this study is to investigate the influence of fungal metabolic activity using mannitol and arabitol as biomarkers to size-differentiated aerosols collected from eastern central India during winter of 2011. The concern is that even a rural and religious area, which is believed to be environmentally clean, is under the influence of unfavorable response to humans due to fungal metabolic activities. This study will give some clues on how to manage the air quality issue and fill the gap in this research area.

II. Experimental Section

Atmospheric PMs were collected from rural site of Chhattisgarh, India. Rajim sampling site (20˚ 59′ N 81˚ 55 E, Fig. 1), located in Chhattisgarh province, is believed to be a famous religious place due the association of three river i.e. Mahanadi, Pairee and Sodbhadra, and is known as *Triveni Sangam*. The sampling site is situated at an altitude of 281 m above from the sea level. This study mainly influence by the biomass burning activities, soil re-suspension and frequent agricultural activity that promoting the growth of microorganism like fungal spores.

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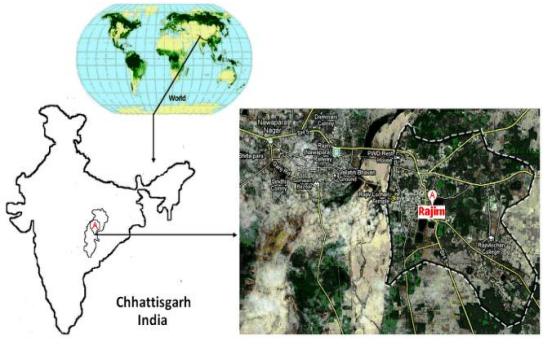

Fig. 1. Study site in eastern central India (Rajim, Chhattisgarh).

The collection of size-differentiated PMs was done at the top of a double-storied building at height of ~15 meters above the ground level using eight-stage cascade impactor sampler (Modal TE 20-800, USA) with an average flow rate of 28.3 L min^{-1}. The initial and final flow rate was checked by dry gas meter (Model 12393959, Invensys (TM) Purchased by Thermo Fisher Scientific). The cut-off diameter of the poles apart stage of eight-stage cascade sampler are; stage 0: 10-9.0 µm, stage 1: 9.0-5.8 µm, stage 2: 5.8-4.4 µm, stage 3: 4.4-2.5 µm, stage 4: 2.5-2.1 µm, stage 5: 2.1-1.0 µm stage 6: 1.0-0.7 µm and stage 7: 0.7-0.4 µm. Aerosol samples were collected for twenty four hrs, incessantly in the month of October and November 2011. Aerosol particle were collected on pre-treated 80 mm glass fiber filters.

The mass concentration of size-differentiated PMs was determined by the gravimetric analysis. The filters were placed in desiccators for twenty four hrs before and after the sampling to take out the absorbed water and weighed, after taking the filters out of the desiccators, using an analytical mass balance (Sartorius, Model CP225D) with a reading precision of 10 µg. Each of the filters including backup filter was analyzed gravimetrically by taking proper care in order to avoid minute divergence in mass measurements. Two of the most important factors to consider mass concentration measurement are variation of weight with temperature (T) and relative humidity (RH). Thus, the filters were first habituated for twenty four hrs at 20 ℃ and 35% RH. All weight measurements were repeated three times to make sure consistency and readings were established when the difference was not beyond from 5 µg. To ensure the quality of data, field blank samples were also collected during the study period. The gravimetric mass of aerosols (µg) was calculated by subtracting the weight of the filter after sampling from that of the previous sampling and the concentration (µg m^{-3}) was determined by dividing the aerosol mass by total volume of air sampled (m^3). After the gravimetric analysis, the sampled filters and field blanks were placed in clean polyethylene (PE) bottles. All samples were kept at -20 ℃ until chemical analysis in order to inhibit fungal growth. The mass concentration of the sampled filters obtained was corrected for field blank values.

Two sugar alcohol frequently used as tracer for fungal metabolic activity in aerosols are chemically analyzed in $PM_{2.5-10}$ and PM_1 aerosols. Organic compounds were extracted from the filter using 10.0 mL of deionized water (>18.2 MU cm^{-1}, Analytical System Model D11901, Barnstead) and a cellulose acetate syringe membrane (C020A025A, 25 mmf, pore size 0.2 mm, Advantec) in a PE bottle continuously and agitated for 90 min on a shaker (TS-500, Yihder). Extraction was performed in an unlit refrigerator at 4 ℃ to prevent the decomposition of extracted organic compound (Tsai *et al.*, 2008). Extracted samples were then stored at -20 ℃. Blank filters were treated in the same way for observe contamination to correct background concentrations. 100 mg of each compound was dissolved in deionized water and the final volume adjusted to 100 mL for a 100 mg L^{-1} stock solution. These solutions were stored at -18 ℃ until used to make standard solutions and these, in turn, were stored at 4 ℃ to prevent degradation during analysis. Sugar alcohols were determined using a Dionex ICS-2500 IC equipped with pulsed amperometric detection (PAD), a GP50 gradient pump coupled to a Teflon injection valve with 400 mL sample loop, a CarboPac MA1 guard column (50 mm length × 4 mm I.D.) and anion-exchange analytical column (250 mm length × 4 mm I.D.), a Dionex ED50 electrochemical detector with a gold working electrode and a pH electrode as reference. Dissolution of carbonate into NaOH can hinder the separation and to reduce the potential for this, exposure of NaOH solutions to atmospheric CO_2 was minimized. Method detection limit (MDLs) for mannitol and arabitol varied from 18.8 mg L^{-1} to 4.76 mg L^{-1}.

III. Result and Dicussion

TABLE I: Mass Concentrations and Statistical Parameter of Biomarkers during Winter Period (2011)

Biomarkers	Mean (ng/m^3)	[a]SD	Range (ng/m^3)	[b]CV
Mannitol				
$PM_{2.5-10}$	1682.2	147.0	1453-1883	0.09
PM_1	716.6	112.2	534-838	0.16
Arabitol				
$PM_{2.5-10}$	265.9	101.2	114.1-389	0.38
PM_1	101.3	66.7	11-250	0.66
[a]Standard deviation; [b]Coefficient of varience ([a]SD/Mean)				

Atmospheric conditions are dependent on the size distribution and chemical composition of aerosols. According to the recent studies, mannitol and arabitol are used as biomarker of fungal spores (Bauer *et al.*, 2008a). The productions of arabitol and mannitol during winter are stronger than expected. Some fungal spores in smoke cause allergic reactions and activate asthma attack [17]. Fig. 2 and Table 1 shows the mean mass concentration and twenty four hrs variation in mass concentration of biomarker in $PM_{2.5-10}$ and PM_1 aerosols, respectively. Significant variation in the twenty four hrs average concentration of mannitol and arabitol was found during the study period.

Daily average concentrations of these two biomarkers were showed same pattern of spikes. The spikes of coarse peaks were dominant over the submicron spikes. This shows that soil re-suspension is major source of these biomarkers as compared to biomass burning for this sampling area. Airborne fungal spores contribute potentially to the organic carbon of the atmospheric PM, largely in the coarse aerosol[18]. Feasible fungal spores are present in smoke from remote biomass burning [15]. Submicron aerosols were mainly originated from the combustion sources [3]. In Rajim sampling site, biomass i.e., rice straw, wood and cow-

dung cake are used for cooking and heating in home especially during winter period [3]. The concentration of mannitol ranged from 1453 to 1883 ng m^{-3} and 534 to 838 ng m^{-3}, whereas for arabitol it was varied between 114.1 and 389 ng m^{-3}, and 11 and 250 ng m^{-3} in $PM_{2.5-10}$ and PM_1, respectively. The mean mass concentration of mannitol and arabitol was higher in coarse particle and this was mainly due to the agglomeration of fungal spores and soil re-suspension during the study period. Airborne fungal spores contributed potentially to the organic carbon of the atmospheric PMs, mainly in the coarse aerosol as compared to fine aerosol during winter and this was might be due to the agglomeration of fungal spores [16], [19]. The total concentration of mannitol in both size fractions is high as compared to arabitol. This might be due to the low decomposition rate of vegetative detritus during winter period. One more step is needed for the formation of arabitol as compared to mannitol which is less likely to be found during stable period i.e., winter period. Thermal and photolytic decomposition phenomena during winter period is less due to the low concentration of oxidizing species like OH^-, NO_2^-, SO_2 and other organic free radical [9], [20], [21]. Considerable mass concentration of mannitol (716.6 ng m^{-3}) and arabitol (101.3 ng m^{-3}) were also found in submicron aerosols due to smoke originated from biomass burning activities during winter. The concentrations of mannitol (21.9 ng m^{-3}) and arabitol (8.3 ng m^{-3}) were also higher during winter in Israel during 2008 and 2009

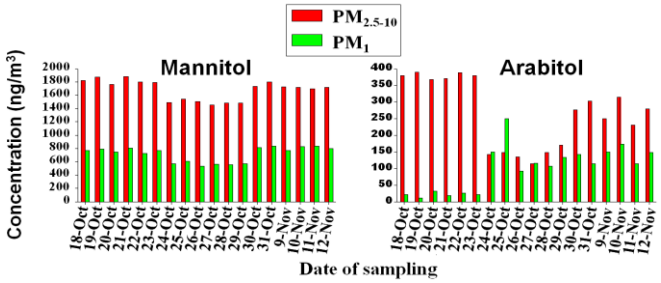

Fig. 2. Daily average concentration of mannitol and arabitol in $PM_{2.5-10}$and PM_1.

TABLE II: BIOMARKERS AND THEIR CORRELATIONS IN THEIR DIFFERENT PARTICLE SIZES $PM_{2.5-10}$ (COARSE) AND PM_1 (SUBMICRON) WERE EXAMINED USING SPEARMAN CORRELATION

Biomarker	Mannitol in coarse	Mannitol in submicron	Arabitol in coarse	Arabitol in submicron
Mannitol in coarse		**0.58***	**0.90****	-0.60**
Mannitol in submicron	**0.58***		**0.54***	-0.04
Arabitol in coarse	**0.90****	**0.54***		-0.61**
Arabitol in submicron	-0.60**	-0.04	-0.61**	
*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).				

Spearman correlation was chosen for explaining the relationship between the biomarkers in size-differentiated aerosols. The result of correlation analysis is given in Table 2. Mannitol and arabitol are highly correlated (r_{sp}= 0.90) in coarse aerosol and showed their origination from similar kind of sources i.e., re-suspension of biologically active soil. These biomarkers might be originated from metabolic activity of fungal spores present in the atmospheric aerosols [22]. Mannitol found in submicron aerosol was moderately correlated with that found in coarse aerosol. Arabitol in submicron aerosol was negatively correlated with mannitol found in coarse and submicron aerosols and suggested their different emission sources. Biomarkers associated with submicron aerosol are typically originated from combustion of microbial active biomass [15], whereas, biomarkers associated with coarse aerosol are originated from microbial active soil re-suspension [23], [24].

IV. CONCLUSION

Mannitol and arabitol were quantified in two size fractions i.e., coarse and submicron to estimate the impact of fungal spore to aerosol mass loading during winter of 2011 in rural study site of eastern central India. The results of this study suggested that the biological particles may significantly contribute to the mass loading in atmospheric aerosols. Mannitol (1682.2 ng m^{-3}) and arabitol (265.9 ng m^{-3}) were showed higher concentration in coarse particles and indicated that biologically active soil re-suspension and fungal spore agglomeration was common source in this study site. Significant concentration of mannitol (716.6 ng m^{-3}) and arabitol (101.3 ng m^{-3}) were found in submicron particles, which was mainly due to the biomass burning activities for cooking and heating purposes in and around the study area. Strong correlation (r_{sp} = 0.90) was found between mannitol and arabitol in coarse aerosol and indicated similar sources of origin.

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