



HOKKAIDO UNIVERSITY

Title	Hydroxy fatty acids as fingerprint biomarkers for the atmospheric transport of soil microorganisms and terrestrial higher plant metabolites
Author(s)	Tyagi, Poonam
Degree Grantor	北海道大学
Degree Name	博士(環境科学)
Dissertation Number	甲第12415号
Issue Date	2016-09-26
DOI	https://doi.org/10.14943/doctoral.k12415
Doc URL	https://hdl.handle.net/2115/63819
Type	doctoral thesis
File Information	Tyagi_Poonam.pdf



2016

Hydroxy fatty acids as fingerprint biomarkers for
the atmospheric transport of soil microorganisms
and terrestrial higher plant metabolites

(土壌微生物と陸上高等植物代謝物質の大気輸送バ
イオマーカーとしてのヒドロキシ脂肪酸)

Doctoral Dissertation

Division of Earth System Science

Graduate School of Environmental Science

Hokkaido University, Japan

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Acknowledgments

This degree is one of the most important and valuable things I have ever done in my 30 years of life. Never before have I been trusted my abilities and strengths that I have been in the last three years. For that I am thankful to God and proud of myself for having got this far. There are several people who knowingly and unknowingly helped in the successful completion of this doctoral thesis and deserve gratitude. If I unintentionally omit any names, I seek their forgiveness.

With profound sense of devotion, I express my most sincere thanks to my supervisor **Prof. Kimitaka Kawamura** for his guidance and help. He smilingly and patiently remained caring during my research period. Despite being so busy, he used to review my dissertation, research articles and give his valuable suggestions to improve it. I would also like to thank my seniors, colleagues and technical staff at my laboratory for their friendly support during my research work.

I also want to give credit to Prof. **Osamu Seki** and Prof. **Yuzo Miyazaki** for guidance at the initial phase of my research work and their kind comments on my research activities.

A very big thank you goes to **Ministry of Education, Culture, Sports, Science and Technology** (MEXT) Japan for providing me PhD scholarship and **Institute of Low Temperature Sciences** (ILTS) for giving me financial support to attend national and international conferences.

I am also thankful to all my friends at Hokkaido University and my home town (Delhi) who facilitated me in the achievement of my aims and also made my stay very comfortable.

I am also gratified to my husband, **Dr. Srinivas Bikkina**, for his unique and unmatched support and love. Without him, this degree would have not been possible. He had been a great motivation and guide during my PhD work.

A very important and heartfelt thank goes to my **Mummy and Papa and mother and father in laws** who have always loved me no matter what, believed in me, supported me in many ways. They are always proud of me and it does matter to me a lot. I owe everything to my **family**.

Abstract

Lipid biomarkers have long been employed as a powerful tool to assess the relative significance of soil microorganisms, epicuticular plant waxes and also, to some extent, for characterizing microbial communities in different environmental archives. Among the various lipid class compounds, hydroxy fatty acids (FAs) are one such proxy due to their ubiquitous occurrence as an essential membrane component of soil microorganisms and higher plant waxes. In particular, the positional β -isomers of hydroxy FAs having carbon chain length from C₁₀ to C₁₈ are specific to endotoxin/lipopolysaccharides (LPS) of Gram-negative bacteria (GNB). The long-term exposure to airborne endotoxin can cause severe allergies and infections in human beings. Apart from β -, other positional isomers of hydroxy FAs such as α - and ω -short-chain homologues have the potential to be used as tracers of soil microorganisms. However, they are also abundant in microalgae, cyanobacteria, sea grasses, and plant waxes. Moreover, these hydroxy FAs can also act as intermediate products of photochemical as well as microbial oxidation of long-chain monocarboxylic acids to dicarboxylic acids.

Hydroxy FAs from soil microbes and plants can be emitted into the atmosphere due to the wind abrasion during dust events and soil-dust re-suspension during biomass burning (forest fires/agricultural burning). Once in the atmosphere, these hydroxy FAs can be transported to distant places and, thus, can have severe impacts on climate, human health and downwind ecosystems. The major objectives of my thesis are to better understand the source regions, atmospheric transport and deposition of soil- and plant-associated bacteria, and also, higher plant metabolites in the East Asian outflow (Asian dust) to the North Pacific. To accomplish these targets, hydroxy FAs were measured in different atmospheric samples including aerosol (urban, coastal, marine), snow (fresh and snow pit) and rain water samples collected from the East Asian outflow.

Results obtained from my thesis contribute to fill-up the gap between the sources of hydroxy FAs and their deposition into fresh water and marine sediments. A clear seasonal trend in the atmospheric abundances of hydroxy FAs over marine (Jeju and Chichijima) islands is noteworthy with spring/winter maxima and summer/autumn minima. Furthermore, these seasonal cycles are consistent with the prevailing meteorology. Air mass back-trajectories and other soil microbial tracers (such as trehalose) indicate the influence of Asian dust outbreaks originating from Mongolian and Gobi Deserts and also from China loess

plateau during winter and spring. To examine the influence of open biomass burning on soil microbes, hydroxy FAs have also been measured in wheat residue combustion derived aerosols over Mt. Tai, North East China. These analyses have revealed significant emissions of β -hydroxy FAs (tracers of GNB) and endotoxin in high biomass burning events. It has been also found that these hydroxy FAs can be removed from the atmosphere efficiently by snow and rain as compared to aerosols.

The overall results obtained through my study clearly document the long-range atmospheric transport of hydroxy FAs, which are tracers of soil microbes (bacteria, fungi, protozoa) and higher plant waxes, from the Asian continent to the North Pacific. The Asian dust outbreaks in spring can lead to increase in atmospheric abundances of hydroxy FAs and, thereby, resulting in the occurrence of health impacts in downwind regions due to the exposure to these airborne particles. This chemical marker based approach (culture independent technique) is a suitable tool for the rapid and comparative analysis of bacterial dry mass and their endotoxin in the various environmental samples which excludes the need of biological recovery and microbial cultivation.

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Chapter 1

Introduction

Chapter 1 provides a brief introduction of aerosols with major emphasis on hydroxy FAs as potential tracers of soil microbes and plant metabolites. Present chapter discusses their environmental significance, long-range aeolian transport to marine environment and deposition to the surface waters and hence, its pivotal role in carbon exports to sediments. A review of previous research work carried out over East Asia and North Pacific is presented in this chapter. Furthermore, this chapter also includes the rationale and objectives of my thesis work.

1.1 Aerosols-Climatic significance

Aerosols, a suspension of solid particles and/or liquid droplets in ambient air, play a crucial role in governing the chemical composition of Earth's atmosphere [Stocker *et al.*, 2013], affecting the radiation budget of this planet via direct (scattering and/or absorbing incoming or outgoing solar radiation; [Andreae and Ramanathan, 2013; Gustafsson and Ramanathan, 2016] and indirect effects (*i.e.*, influencing cloud droplets and CCN nuclei abundances and thereby modifying the microphysical properties of clouds) [Riipinen *et al.*, 2011; Rosenfeld *et al.*, 2014], visibility and causing acute health effects [Lelieveld *et al.*, 2013; Lelieveld *et al.*, 2015]. More importantly, regional emissions of anthropogenic and natural aerosols from South and East Asia has been proposed as a major cause for accelerated climate change (*e.g.*, glacier retreat, increase in premature deaths and droughts, monsoon breaks) over these regions [Huebert *et al.*, 2003; Ramanathan *et al.*, 2007; Xu *et al.*, 2009; Huang *et al.*, 2014]. Most of these predictions carry a large uncertainty due to the fact that lack of consideration of chemical composition of ambient aerosols and their complex multiphase heterogeneous chemistry in the atmosphere in current climate models [Penner *et al.*, 1998; Kaufman *et al.*, 2002].

1.2 Aeolian transport of microorganisms and higher plant metabolites

Ambient aerosols contain a significant fraction of organic matter, which originate mainly from biogenic and anthropogenic sources [Kroll *et al.*, 2011]. Bioaerosols (*i.e.*, aerosols from biogenic sources) are ubiquitous in nature and are one such component of airborne organic matter. These bioaerosols include microorganisms, spores, and fragments of plants, pollen and small insects as well as their fragments. The concentrations of bioaerosols account for ~3-11% of fine particulate aerosol mass [Simoneit and Mazurek, 1989; Polymenakou, 2012]. Bauer *et al.* [2002] have documented that average mass concentration bacterial aerosols contribute to 0.01% of organic carbon in cloud water, snow, rain and aerosol samples. The airborne soil- and plant-derived organic matter, which extended up to an altitude of 5 km, has been detected in the springtime dust-laden aerosols from the East Asian outflow to the North Pacific [Simoneit *et al.*, 2004]. Atmospheric transport acts as a conveyor of pathogenic/beneficial microbes with potential impacts on downwind oceanic ecosystems and public health [Griffin, 2007 and references therein]. Desert dust storms play an important and most efficient transportation mechanism of biogenic particles (such as soil

microbes, plant metabolites) over the downwind ocean regions, and thus, influence the marine microbial population in the surface waters [Griffin and Kellogg, 2004; Kellogg and Griffin, 2006].

The largest sources of dust in East Asia are the Gobi, Taklamakan and Badain Juran Deserts. The dust storms in East Asia operate only on a seasonal basis with the majority of atmospheric transport occur during spring (March-May) season and impacting the remote areas viz., the French Alps, the Arctic and the North Pacific [Arimoto, 2001; Grousset *et al.*, 2003; Serno *et al.*, 2014]. However, significant amount of dust can be generated from these arid regions in China and Mongolia, transported to long distances and, thereby, get dispersed in the Northern hemisphere [Uematsu *et al.*, 2003; Chin *et al.*, 2004]. Annually, ~4,000,000 tons of Asian dust particles fall on the North Pacific including Japanese Islands [Yamaguchi *et al.*, 2014]. It has also been documented that Asian dust takes around 7 to 9 days to cross the Pacific Ocean and can also impact the air quality in the Caribbean and the Americas [Uno *et al.*, 2009].

Asian dust is also an efficient carrier of bacterial cells and, thus, contributes to their diversity over the downwind ecosystems [Yamaguchi *et al.*, 2012]. The high altitude intercontinental transport of microbial biomass associated with soil dust particles have also been observed from Asia to North America [Creamean *et al.*, 2013; Smith *et al.*, 2013]. In addition to inorganic particles, clouds of desert dust can carry microorganisms and their fragments as well other biogenic particles [Griffin, 2007]. Therefore, atmospheric transport of soil microbes and terrestrial plant lipids is important to study in the East Asian outflow to the North Pacific. Airborne soil microbes can impact human health in regional and downwind ecosystems as well, play an important role in the geographical distribution of pathogenic and non-pathogenic species *via* long-range atmospheric transport.

Different analytical approaches that include both culture-dependent (*e.g.*, genetic based studies) and -independent (*e.g.*, chemical marker based) techniques have been used in the literature to characterize the soil microbes and higher plant metabolites in the dust-laden aerosols over East Asia [Wakeham *et al.*, 2003; Yamaguchi *et al.*, 2012] (Table 1.1). With the rise of genetic approaches, it is now possible to simultaneously count and identify the genus level of airborne soil microorganisms. However, these methods are expensive and, therefore, can only be used for limited number of samples. Generally, less than 1% of microbial community is culturable in any environment [Tong and Lighthart, 1999]. These culture-

dependent studies show low reproducibility of stressed airborne bacteria. Furthermore, microscopic based examination is a widely used approach for assessing the both culturable and non-culturable airborne microbes. However, this approach provides a little information on toxic and allergenic components of microbes [Douwes *et al.*, 2003]. On the contrary to culture dependent techniques, biomarker (*aka.* chemical marker/proxy) based methods become a good alternative for studying microbial contributions in ambient aerosols and pelagic sediments [Kawamura and Gagosian, 1987; Kawamura, 1995; Simoneit *et al.*, 2004]. The fatty acids (<C₂₀ homologues) are constituent cell membrane lipids of airborne soil bacteria and, therefore, can be used as specific indicators for the estimation of microbial biomass. These chemical marker based quantification of soil microbes can easily be applied for large number of samples (thus, increasing throughput) using gas chromatography/mass spectrometry (GC/MS) technique.

Table 1.1. Analytical techniques used for the identification and quantification of bioaerosols.

Methods	Advantages	Disadvantages
Culture dependent	Colony forming units counts and identification via substrate utilization; low-cost assays	<1.0% of the bacterial community is culturable in low stress conditions, recovery is critical, reproducibility issues
Culture independent Microscopy; staining	Oldest tools	Many organisms cannot be identified, prone to error, require expertise, time-consuming
immunological identification	Species- and strain-specific, identifications are possible	false-positive counts, >10 ⁴ cells per sample are required for useful data
Identification via nucleic acid amplification and/or hybridization	detect organisms in the community that occur in very low numbers	cost of cloning kits, the required expertise in sequence interpretation,
direct counts; and fatty-acid analysis	address viable biomass due to rapid phospholipid turnover	costs of analysis, overlapping profiles, biomass to cell count conversion, and compositional shift from variance in growth conditions
Biomarkers	<i>Specific to microbes, slow degradation over time, Lipopolysaccharides/bacterial biomass can be estimated, sensitive</i>	<i>Characterization of bacterial groups is difficult, marker to biomass conversions</i>

1.3 Hydroxy FAs as biomarker lipids of soil microbes and plant metabolites

Lipids (long-chain carboxylic acids) are important for the energy storage and cell membrane structure in the living organisms to complete their needs and functions [Christie, 2003]. As a result, these lipids can be utilized as biomarkers; compounds directly associated with certain ecological processes or set of organisms in a particular ecosystem. Several studies have documented the occurrence of land derived lipid compounds in sediments and attributed their sources as higher plants and soil microorganisms [Volkman *et al.*, 1980; Kawamura and Gagosian, 1988; Kawamura, 1995; Kawamura *et al.*, 2003; Wakeham *et al.*, 2003]. In subsequent studies, lipids were examined in the surface micro-layer of the oceans [Wakeham *et al.*, 2003; Cochran *et al.*, 2016]. All these studies have demonstrated the usefulness of these lipid class compounds (as biomarkers) as well as the bulk organic matter in order to understand terrestrial carbon sources and cycling of organic matter in sediments. A biomarker is, therefore, defined as a chemical compound derived from structure/constituent component that is unique to a particular microbial group or community [Lee *et al.*, 2004]. Hence, biomarker can be employed for characterizing and quantifying a specific microbial group or community instead of an individual microbial species. Biomarkers can also be used to estimate the microbial biomass and allergens using conversion factors for health assessment [Paba *et al.*, 2013].

Lipid biomarkers include various organic compounds such as alkanes, aldehydes, alcohols and fatty acids and their functional isomers. Among these, hydroxy fatty acids (FAs) have been proposed as bacterial and higher plant tracers in various environmental studies [Zelles, 1999; Wakeham *et al.*, 2003; Lee *et al.*, 2004; Cochran *et al.*, 2016]. Different positional isomers of hydroxy FAs are present in soil microorganisms [Wakeham *et al.*, 2003]. These microorganisms synthesize several monomeric chemical structures of hydroxy FAs that are not found elsewhere in nature [Szponar and Larsson, 2001]. Especially β -hydroxy FAs (or 3-hydroxy FAs) from C_{10} to C_{18} are usually found in the endotoxin (lipopolysaccharides/LPS) of Gram-negative bacteria (GNB). These β -hydroxy FAs are the integral component of lipid A region of the LPS and responsible for their endotoxin potency of GNB. Although this lipid A region is well conserved, however, considerable variability occur in terms of length and chemical composition between different species of GNB [Larsson, 1994]. Fig. 1.1 depicts the typical structure of GNB, their endotoxin and chemical structure of β -hydroxy FAs. Since β -hydroxy FAs degrade very slowly in the atmosphere and,

hence, these can be employed as potential biomarkers for quantification of endotoxin/GNB in the environmental samples.

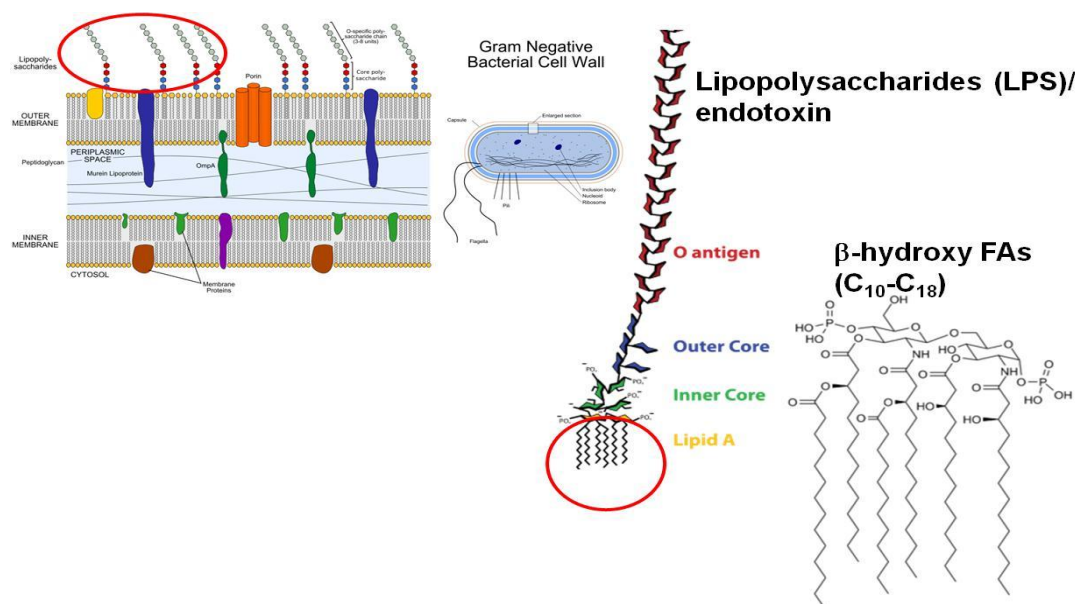


Fig.1.1. Schematic diagrams of Gram-negative bacteria cell envelopes, endotoxin/lipopolysaccharides and saturated carbon chains of β -hydroxy FAs [Williams, 2001].

Ever since their first identification in old lacustrine sediments by Eglinton *et al.* [1968], hydroxy FAs have received much attention as specific markers of terrigenous microbial inputs into sediments [Perry *et al.*, 1979; Kawamura and Ishiwatari, 1984b; Wakeham, 1999], soils [Zelles, 1999 and references therein], dust [Saraf *et al.*, 1997; Milton *et al.*, 2000] and continental [Lee *et al.*, 2004] as well as in marine aerosols [Kawamura, 1995]. Along with β -hydroxy FAs, other positional isomers (*e.g.*, α - and ω -) of hydroxy FAs have also been used to trace the contribution of soil microorganisms (*e.g.*, bacteria, fungi, algae) and higher plants in the sediments [Cranwell, 1981] and marine dissolved organic matter [Wakeham, 1999]. Abrasion of the leaf surfaces or re-suspension of the dust due to wind induced mechanical shear, both contribute to the atmospheric abundances of hydroxy FAs [Rogge *et al.*, 1993a]. Therefore, α -, β - and ω -hydroxy FAs can be used as tracers of soil microbes and plant metabolites in the airborne particulate matter.

In the atmosphere, aliphatic short-chain α - and β -hydroxy FAs (typically C_{10} - C_{20}) originate mainly from the structural constituents of many soil microorganisms (bacteria, fungi,

yeasts and protozoa; Table 1.2) [Ratledge and Wilkinson, 1988]. On the other hand, long-chain α -, β - and ω -hydroxy FAs (from C₁₆ to C₃₄) are abundant in micro-algae/algal detritus [Cranwell, 1981], cyanobacteria [Matsumoto and Nagashima, 1984; Matsumoto *et al.*, 1984], sea-grasses [Volkman *et al.*, 1999], and plant epicuticular waxes [Simoneit, 1989; Rogge *et al.*, 1993b]. Moreover, these α -, β - and ω -hydroxy FAs can also act as intermediates in the photochemical oxidation pathways, by which long-chain fatty acids are degraded [Volkman *et al.*, 1998; Wakeham, 1999].

Table 1.2. Probable sources of hydroxy fatty acids (FAs) in the environmental samples.

Compound class	C-number		Probable sources
	Range	C _{max}	
α -OH FAs	C ₁₃ -C ₁₉	C ₁₆ , C ₁₈	Biopolymers of fungi, soil bacteria, protozoa, , α -oxidation/photochemical oxidation of HMW* FAs,
	C ₂₀ -C ₃₄	C ₂₄ , C ₂₂	Higher plants, microalgae
β -OH FAs	C ₉ -C ₂₀	C ₁₂ , C ₁₆ , C ₁₈	Bacterial cell wall lipids, cyanobacteria, β -oxidation of HMW* FAs, fungi, yeasts
	C ₁₀ -C ₁₈	C ₁₆ , C ₁₂	Gram-negative bacteria (GNB), methanogenic bacteria, soil bacteria
ω -OH FAs	C ₈ -C ₁₉	C ₁₆ , C ₁₂	Microbial metabolites/biosynthesis, cutin, photochemical oxidation of HMW* FAs
	C ₂₀ -C ₃₂	C ₂₄ , C ₂₂	Cell wall of algae, Suberin, higher plant seeds

HMW* FAs=high molecular weight fatty acids

1.4 Environmental significance of hydroxy FAs

The environmental significance of hydroxy FAs in aerosols include both health and climate effects.

1.4.1. Health impacts: Airborne endotoxin (fever causing agents) of soil microbes have been extensively studied for their health related issues in different occupational settings [Park *et al.*, 2001; Deacon *et al.*, 2009; Schlosser *et al.*, 2009]. This endotoxin is commonly found in the outer layer of GNB and can activate the immune system independent on the viability of the bacteria. Since β -hydroxy FAs of LPS is responsible for their endotoxin activity of GNB, their occurrence in the ambient air causes acute and chronic respiratory disorders including infection diseases, acute toxic effects, allergies and asthma like health problems [Paba *et al.*, 2013]. Therefore, these β -hydroxy FAs can be used to assess the endotoxin concentrations in aerosols during their long-range atmospheric transport.

1.4.2. Climatic impacts: Airborne bacteria can act as ice nuclei (IN) and cloud condensation nuclei (CCN). In particular, Gram-negative bacteria are well studied (especially *Pseudomonas sp.*, *Erwinia sp.*, *Xanthomonas sp.*, and *Sphingomonas sp.*) ice nucleating microbes and their nucleating activity is associated with their membrane bound lipids [Bowers *et al.*, 2009]. Since hydroxy FAs are the integral components of the membrane bound lipids of GNB and, therefore, can play a crucial role in controlling ice and cloud condensation nucleating activity in the atmosphere [Maki and Willoughby, 1978]. Climate related impacts of hydroxy FAs can be classified into two groups: (1) direct and, (2) indirect effects. Direct effects include the formation of film forming compounds by the fatty acid salts on humid aerosols. This film acts as the barrier and can inhibit the transport of gases and free radicals (*aka.* barrier effect) as a result of which reduces the evaporation of water from the aerosol surfaces. The scavenging efficiency of particles by larger cloud and rain droplets will be reduced due to the barrier effects of organic films [Mochida *et al.*, 2002]. Indirect effects are caused due to the composition of hydroxy FAs in the cell membrane of GNB, which can affect the nucleating activity of these bacteria in the atmosphere [Maki and Willoughby, 1978].

1.5 Quantification of endotoxin/Gram-negative bacteria dry mass

Although several studies have reported acute and chronic health effects related to the exposure of indoor and outdoor high endotoxin [Rylander, 2006], there is lack of standard international protocol for sampling and analyzing airborne endotoxin. Limulus Amebocyte Lysate (LAL) assay is the most commonly used analytical method to determine endotoxin levels in various environmental samples. Indeed this method is very sensitive, however, LAL assay can encounter contamination from various organic matter and other biological active components (such as β -glucans from molds and Gram-positive bacteria) [Pomorska *et al.*, 2007]. Other shortcomings are that this method detects only about one third of the active water soluble endotoxin and, that the remainder is present inside the fragments of dust particles/bacterial cells goes undetected [Paba *et al.*, 2013]. Thus, estimation of biologically active endotoxin requires more specific, reproducible and quantitative measurement technique.

Gas chromatography/mass spectrometry (GC/MS) can be used for quantitative detection of β -hydroxy FAs in lipid A, which have been used as chemical markers of the

presence of LPS/endotoxin. Numerous studies have employed GC/MS technique for the quantification of β -hydroxy FAs in various environmental samples such as settled dust [Saraf *et al.*, 1997], aerosol [Hines *et al.*, 2003; Lee *et al.*, 2004] and sediments [Wakeham, 1999; Zhang *et al.*, 2014]. The determination β -hydroxy FAs from carbon number 10 to 18 as LPS markers instead of LAL can be useful when determining endotoxin in samples rich in Gram-positive bacteria and molds. The GC/MS based quantification of β -hydroxy FAs can provide information about the bacteria (*i.e.*, sources of LPS) as the relative distribution of β -hydroxy FAs differs among species of Gram-negative bacteria.

1.6 Rationale

The western North Pacific is an ideal site to study the impact of Asian outflow on the marine atmospheric boundary layer (MABL). Based on the measurement of various optical and chemical properties of aerosols during winter (December-February) and spring (March-May), earlier studies have documented the downwind transport of airborne pollutants from East Asia to the North Pacific [Kawamura, 1995; Mochida *et al.*, 2003a; Chen *et al.*, 2013; Boreddy and Kawamura, 2015; Verma *et al.*, 2015]. These observations include the aerosol samples collected from the neritic and pelagic islands in the North Pacific that unfolded the seasonal occurrence of East Asian outflow.

The long term observations over the Pacific Ocean reveal significant amount of mineral dust transport from East Asia during spring season [Huebert *et al.*, 2003; Simoneit *et al.*, 2004] and, therefore, occurrence of soil microbes and higher plant waxes in the MABL. However, no attempt has been made to determine the atmospheric abundances of hydroxy FAs (tracers of soil microbes and plant waxes), their sources, and molecular distributions in the East Asian outflow to the North Pacific. Moreover, it is not clear whether seasonality of the East Asian outflow could be efficient enough to deliver the soil microbes and higher plant waxes to the North Pacific in a contemporary climate change scenario. Although paleoclimatic studies have used these compounds to investigate the contribution of terrestrial lipids to deep sea sediments over the recent past [Kawamura, 1995; Wakeham, 1999; Kawamura *et al.*, 2003; Wakeham *et al.*, 2003; Volkman, 2006], not a single study has been conducted to assess the seasonality and sources of hydroxy FAs (tracers of bacteria and higher plant metabolites) in marine aerosols.

Chapter 1: Introduction

Overall, present day anthropogenic and natural changes in climate and global biogeochemistry alter the transport pattern and/or chemical characteristics of soil microbes and higher plant metabolites entering the ocean from the atmosphere. Thus, it becomes crucial to re-establish more specific, reproducible and quantitative analytical methods for assessing the soil-borne microbes in ambient aerosols. Therefore, a systematic study has been undertaken to study the impact of continental outflow from East Asia on the atmospheric abundances of hydroxy FAs (tracers of soil microbes and plant waxes), their sources, transport pattern and seasonality over the North Pacific.

1.7 Work Plan

With the above rationale, year round collection of total suspended particulate matter (TSP) samples from Gosan, Jeju Island during 2001-2002 and Chichijima during 1990-1993 and 2001-2003 to observe the seasonal trends of hydroxy FAs and identify their source specific molecular distributions during long-range transport. To assess the relative significance of oceanic microbial sources to atmospheric hydroxy FAs over the western Pacific, TSP samples were collected during autumn season (representing pristine oceanic influence) along the longitudinal cruise tracks (spanning between 40 °N, 120 °E and -40 °S, 135 °E). The biomass combustion also injects significant amount of paved agricultural dust and, thus, may contribute to atmospheric hydroxy FA. Therefore, TSP samples were collected from Mt. Tai during summer, a period in which the sampling site is influenced by the biomass burning emissions (BBEs). In addition, fresh snow samples in winter from Sapporo, northern Japan and snow pit samples in spring from Mt. Tateyama (central Japan) were collected to understand the cloud related processes of soil microbes and plant waxes over East Asia. All these continental and marine aerosols, snow and snow pit samples were investigated for the atmospheric abundances of hydroxy FAs using GC/MS.

1.8 Objectives

- 1) To assess the spatial, temporal variability and seasonal variability (if any) and molecular distributions of α -, β - and ω -hydroxy FAs in the East Asian outflow to the North Pacific.
- 2) To assess the relative significance of atmospheric abundances of hydroxy FAs from anthropogenic (*e.g.*, biomass burning emissions) *vis-à-vis* natural sources (dust-laden aerosols over Gosan, Chichijima, cruise samples).
- 3) Comparison of the relative abundances and molecular distributions of hydroxy FAs between fresh snow and snow pack samples with those of ambient aerosols from East Asia to ascertain the link between aerosol seeding and their cloud chemistry driven by microorganisms.
- 4) To assess the endotoxin concentrations and GNB dry mass in aerosols, snow and snow pit samples from East Asia.

Chapter 2

Experimental Methods

Chapter 2 presents a brief description about the sampling locations, aerosol collection and analytical procedures developed and adopted for the analysis of hydroxy FAs and other supporting chemical constituents (*e.g.* total carbon, water-soluble organic carbon, sugars and related compounds and water soluble ionic constituents) used in this study.

2.1 Aerosol, snow and snowpack collection

Hydroxy FAs (microbial and terrestrial lipid biomarkers) were assayed in continental aerosols (Mt. Tai, North China Plain), snow (Sapporo, northern Japan), and snowpack (Mt. Tateyama, central Japan) samples collected from East Asia and as well as in marine aerosols collected during a cruise and over the adjacent and remote islands (Gosan and Chichijima, respectively) in the western North Pacific (Fig. 2.1). These samples were examined to ascertain the influence of regional sources of soil microbes and terrestrial lipid waxes of plants over East Asia and their transport to the North Pacific in the East Asian outflow.

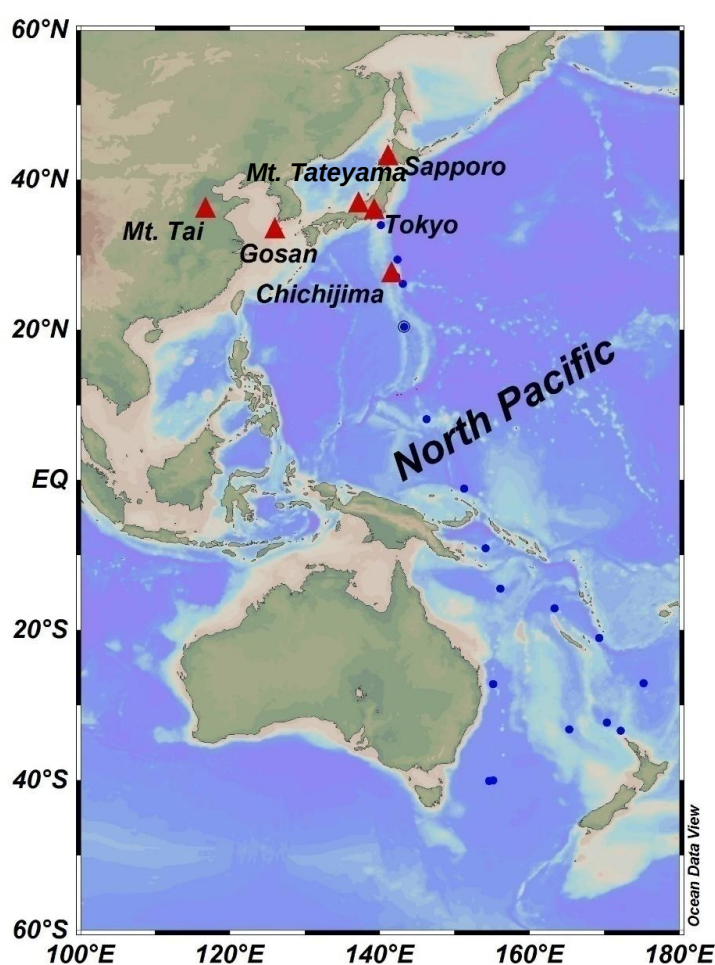


Fig. 2.1. A map showing is geographical locations of all the sampling sites.

For this thesis, bulk aerosol samples (total suspended particles; TSP) were collected from Chichijima ($N=69$ in April1990-November 1993; $N=94$ in January 2001-January 2003), Gosan, Jeju Island ($N=48$; April2001-March 2002), western Pacific cruise ($N=19$; August-October 1992), Mt. Tai ($N=23$; June 2006). All these aerosol samples were collected on pre-

combusted (at 450 °C for 6 h) quartz fiber filter (20 x 25 cm², PALLFLEX[®]™ 2500QAT-UP) using a high volume air sampler, which is operated with a nominal flow rate of 1.0 m³ min⁻¹. The sampling duration varies from a few hours to 2 days for Mt. Tai and cruise samples, while it ranged from a week to biweekly for island based collection of TSP. The filter samples including field blanks (collected by placing with quartz filter and without running the pump, unlike aerosol samples) were placed individually in a cleaned glass bottle (150 ml) with a Teflon-lined screw cap and transported to the laboratory in Sapporo. After collection, samples were stored at -20 °C until analysis of chemical constituents (*e.g.*, hydroxy FAs).

A total of eleven fresh snow samples were collected from the rooftop of the Institute of Low Temperature Science (ILTS) building, Hokkaido University in Sapporo during intensive snowfall periods (January-March) in 2010 and 2011. The detailed description about snow collection and analytical protocol of lipid fraction analyses is similar to that described in *Yamamoto et al.* [2011]. To avoid the contribution of any possible impurities from the dry deposition of aerosols, 1-2 cm of surface snow cover were removed prior to sample collection. Thereafter, snow samples were collected into a cleaned glass jar (8 L) by using a stainless steel shovel. In each glass jar, mercuric chloride (HgCl₂) was added before sampling to prevent microbial activity. Soon after the collection, glass jars were tightened with a Teflon-lined screw cap and stored at -20 °C until analysis.

The accumulated snow samples from two snow pits (width: ca. 8 m, depth: ca. 6 m), which were dug at the Murodo-Daira site (36.58 °N, 137.36 °E, 2450 m above mean sea level), were collected on the western plateau of Mt. Tateyama (3015 m above mean sea level), Toyama prefecture, central Japan. Mt. Tateyama faces the Sea of Japan and is located on the outflow pathway of the Asian dust transport. On April 18, 2009 and April 17, 2011, two snow pit holes were dug to the depth of 6.0 and 6.5 m, respectively, and snowpack samples were collected using a pre-cleaned shovel to avoid possible contamination. It has been found that dust-containing snow layers in the snow pits, which showed a light yellow-brown color and were later characterized by high Ca²⁺ concentrations (tracer of dust), suggesting a long-range transport of Asian dust to the sampling site [*Mochizuki et al.*, 2015]. Previous studies [*Osada et al.*, 2004; *Maki et al.*, 2011; *Tanaka et al.*, 2011; *Kawamura et al.*, 2012] also suggested that snow samples collected from the same site were influenced by Asian dust. Six and seven snow samples including dust layers were collected from the snow pits in 2009 and 2011, respectively, and placed in a pre-cleaned glass jar (8 L), to which HgCl₂ was added prior to

sample collection to prevent any microbial activity. The thickness of snow layer collected was approximately 10 cm in 2009 and 5-20 cm in 2011 [Mochizuki *et al.*, 2015].

2.2 Sample extraction

All the samples including aerosol, snow, snowpack and rain water samples were processed and extracted for the total hydroxy FAs (LPS bound and free) according to an extraction procedure initially developed by Kawamura *et al.* [2003] and later modified by Yamamoto *et al.* [2011].

2.2.1. Aerosol filters extraction

Each filter aliquot (1/8-1/4 fraction of filter) was saponified with 10 ml of 0.1 M KOH/methanol using DIONEX ASE200 at 100 °C and 1000 psi for 5 minutes followed by subsequent ultrasonication with dichloromethane (DCM) (10 ml x 3). In the absence of ASE instrument (technical failure); filter samples were saponified with 10 ml of 0.1 M KOH/methanol under reflux (80 °C for 2 h) and extraction with DCM. After each extraction step, the extracts were combined and reduced to 1 ml using rotary evaporation. Then, the extract was divided into neutral and alkaline fractions. The neutral fraction was separated by adding 10 ml water, and extract with 10 ml hexane/DCM (10:1) for three times. The alkaline fraction was first acidified with 1 ml of 6 M HCl and carboxylic acids were extracted with DCM. The carboxylic acids were derivatized with 0.5 ml of 14% BF₃/methanol (SUPELCO™) at 80 °C for 1 h to form their methyl ester analogues, referred as fatty acid methyl esters (FAMES). 10 ml of water and hexane/DCM (10:1) were added to FAMES and water layer was removed by shaking (to remove excess derivatizing agent and hydrofluoric acid). The FAMES were further separated to monocarboxylic acid, dicarboxylic acid and hydroxy acid methyl ester fractions using a silica gel (deactivated with 1% H₂O) column chromatography. The solvent layer is transferred to another flask, concentrated and divided it into three fractions on a 4 cm silica gel column (A1: 3.8 ml hexane/DCM (1:2), A2: 2.2 ml DCM/ethyl acetate (98:2), A3: 3.5 ml DCM/methanol (95:5)). Until injection onto the GC/MS system, the sample extracts were stored at -20 °C. The procedure of extraction of aerosol filters for hydroxy FAs is summarized in Fig. 2.2.

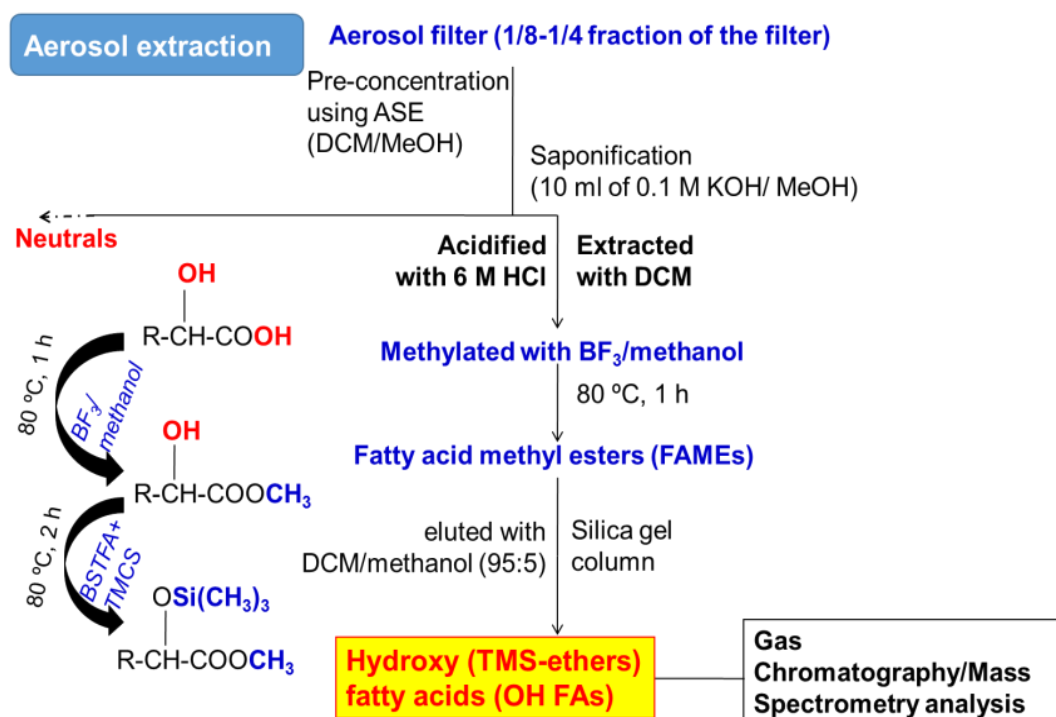


Fig. 2.2. Schematic representation of the procedure for the aerosol filters extraction.

2.2.2. Snow/snowpack extraction

Prior to the extraction, 10 ml 6 M HCl is added to an aliquot (0.2-0.8 L) of melt water from snow and snowpack samples, followed by extraction with DCM/ethyl acetate (2:1) for three times (first 70 ml, second 40 ml, third 40 ml solvent). All these extracts were combined in a 200 ml flask and concentrated to ~200 μl using a rotary evaporator. This concentrate was transferred into a pre-combusted 1.5 ml glass vial and evaporated to dryness by using N_2 gas blow down. The dried extract in vial was saponified with 1.0 M KOH in methanol at 80 °C for 2 h. After the saponification, procedure of hydroxy FAs extraction is similar to aerosol samples. Snapshot of the procedure to analyze snow and snowpack samples is presented in Fig. 2.3.

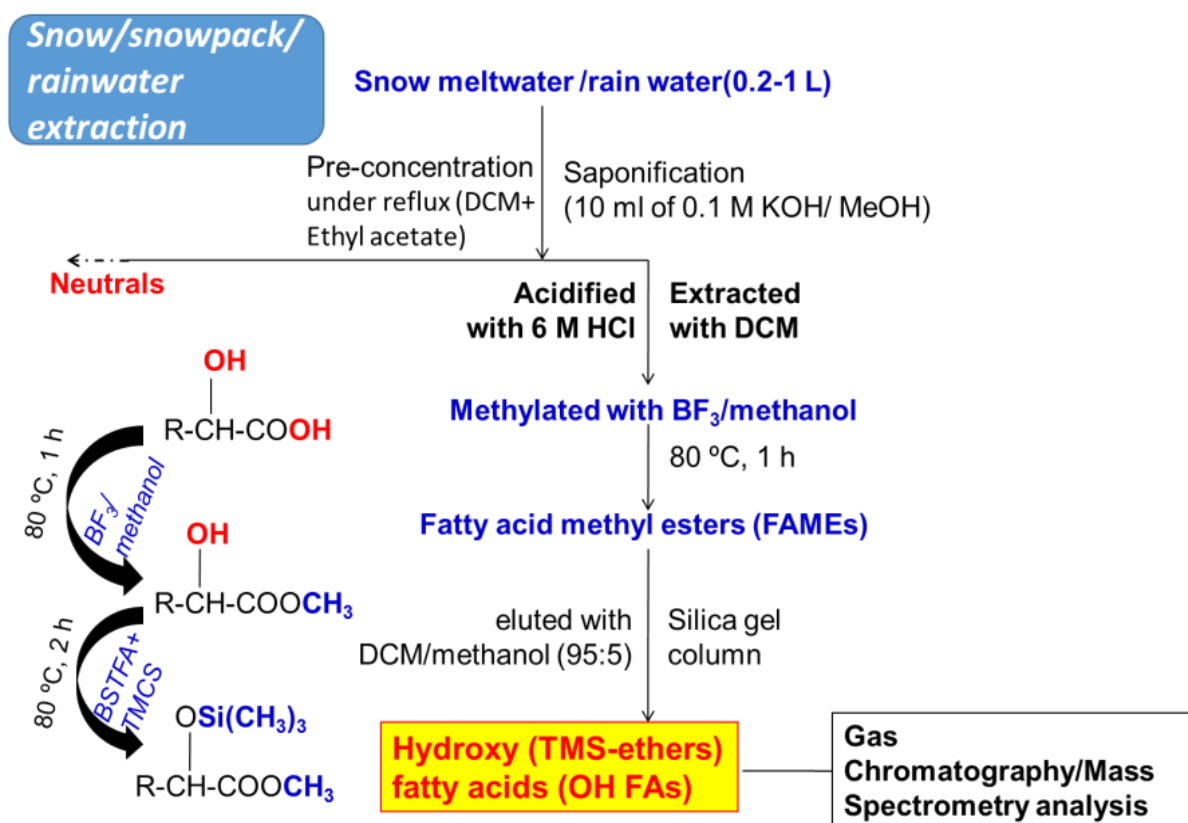


Fig. 2.3. Schematic representation of the procedure for the aerosol filters extraction.

2.3 Identification and quantification of hydroxy FAs by Gas chromatography/mass spectrometry (GC/MS)

Prior to GC/MS injection, the hydroxy FA fraction was derivatized with N,O-bis-(trimethylsilyl) trifluoroacetamide (BSTFA) (SUPELCOTM Analytical) at 80 °C for 1 h to convert hydroxyl groups to trimethylsilyl (TMS) ethers. After the reaction, 50 μL of n-hexane solution containing 1.43 $\text{ng } \mu\text{L}^{-1}$ of internal standard (C_{13} n-alkane/tridecane, Wako) were added to dilute the derivatives.

Compound identification and quantification was conducted using a mass spectrometer (Hewlett-Packard Model 5975 C inert XL EI/CI mass-selective detector, MSD with Triple-Axis Detector) interfaced with a gas chromatograph (Agilent Technologies, Model 7890 GC). For compound fragmentation, the MS was operated in the electron impact mode (70 eV). Compound separation was accomplished using a split/splitless injector and HP-5 fused silica column (Agilent 19091J-202; 325°C; 19 m long x 0.2 μm internal diameter, 0.5 μm film thickness). Fig. 2.4 represents the basic principle and working procedure of GC/MS.

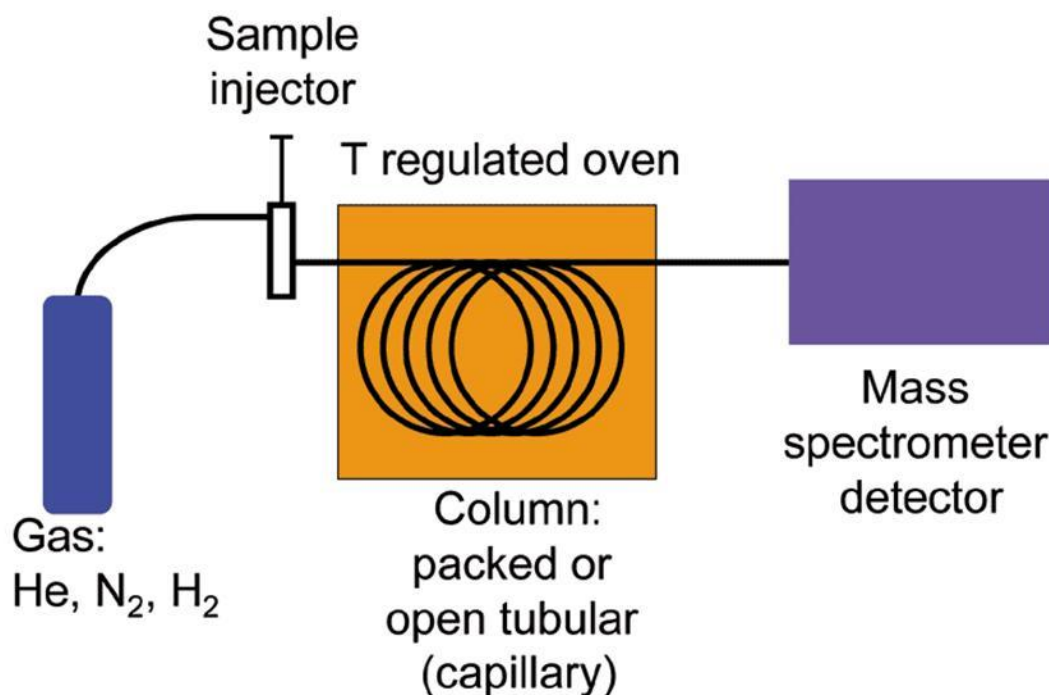


Fig. 2.4. Working principle of gas chromatography/mass spectrometry (GC/MS).

For the quantification of hydroxy FAs, the GC oven temperature was programmed from 50 °C (2 min) to 120 °C (15 °C min⁻¹), then to 305 °C (15 min) at 5 °C min⁻¹. Helium was used as a carrier gas at a flow rate of 1 ml min⁻¹. Data were acquired and processed with the Chemstation software. Identification of hydroxy FAs was done by comparing retention time and mass spectra with authentic TMS derivatives of *n*-C₁₂ and *n*-C₁₆ α -hydroxy FAs; *n*-C₁₂, *n*-C₁₄, *n*-C₁₅, and *n*-C₁₆ β -hydroxy FAs and *n*-C₁₆, *n*-C₂₀ and *n*-C₂₂ ω -hydroxy FAs, which were also used as external standards (Fig. 2.5).

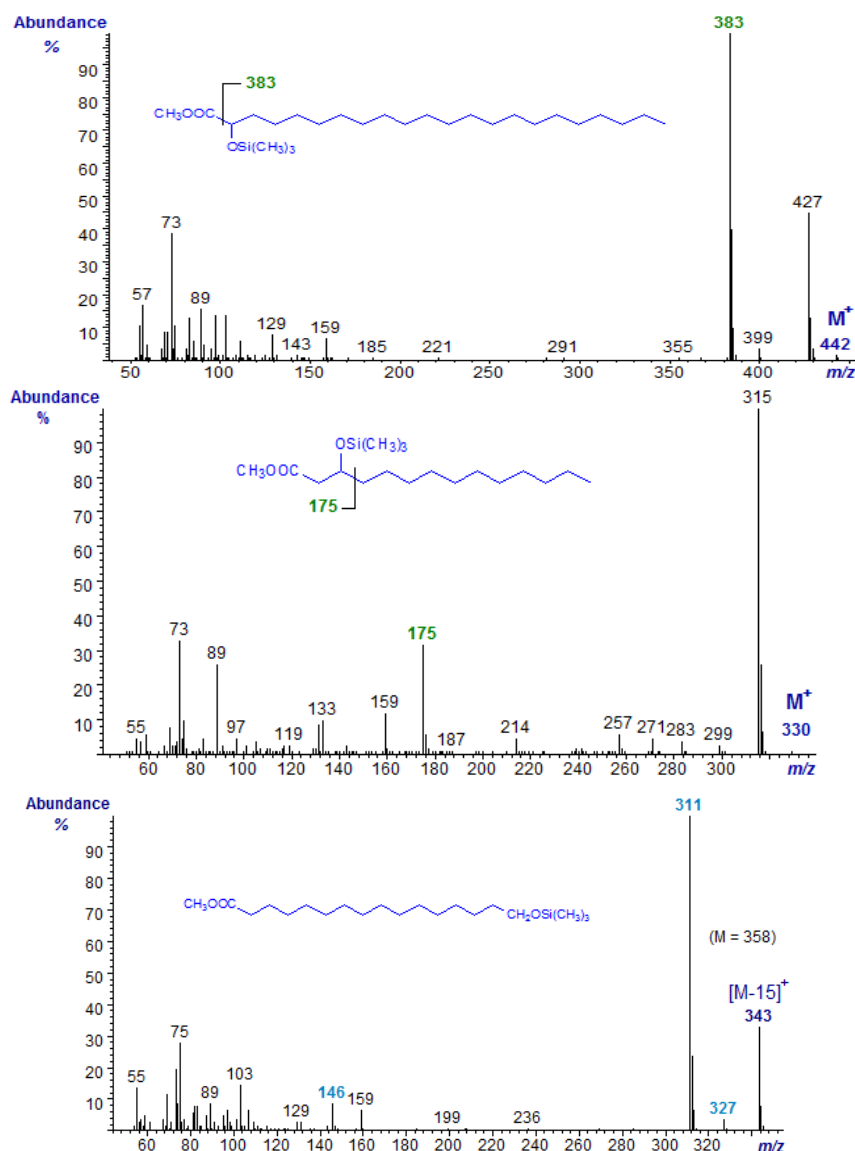


Fig.2.5. Mass spectra and fragmentation patterns of α -hydroxydocosanoic acid (C_{22} - α -hydroxy FAs), β -hydroxypalmitic acid (C_{16} - β -hydroxy FAs) and ω -hydroxypalmitic acid (C_{16} - ω -hydroxy FAs) authentic standards.

2.4 Quality assurance

Lab and field blanks were analyzed before the real sample to check the recovery of hydroxy FAs. The blanks were extracted in the same manner as real samples were analyzed. 50 μ L of free C_{12} , n - C_{14} , n - C_{15} , and n - C_{16} β -hydroxy FAs, and n - C_{16} , n - C_{20} and n - C_{22} ω -hydroxy FAs authentic standards in aqueous solution were spiked on the pre-combusted filters and extracted following the same procedure as shown in Fig. 2.2. The recoveries of authentic hydroxy FAs standards are summarized in Table 2.1. For the identification of

hydroxy FAs in real samples, authentic hydroxy FA standards were derivatized to TMS derivatives, diluted with 50 μL of *n*-hexane solution containing 1.43 ng μL^{-1} of internal standard and injected onto GC/MS. Their retention time and mass spectra were compared with hydroxy FAs detected in the real aerosol and wet precipitation samples. Reproducibility test was conducted using three different parts of the same aerosol sample KOS 111 which was collected from the Gosan site, Jeju Island using a high volume sampler. The analytical errors in the triplicate analysis were <3% for C_8 to C_{16} , C_{21} and C_{23} to C_{28} ; <5% for C_{20} and C_{22} and <22% for C_{17} and C_{18} homologues of ω -hydroxy FAs [Kariya, 2007]. No target compounds were detected in procedural field and lab blank filters.

Table 2.1. Recovery of authentic hydroxy fatty acids (FAs).

Standards	Concentrations (ng μL^{-1})	Recovery (%)
β-Hydroxy FAs		
C_{12}	50	86
C_{14}	104	75
C_{15}	52	69
C_{16}	100	73
ω-Hydroxy FAs		
C_{16}	51	78
C_{20}	50	75
C_{22}	51	73

2.5 Estimation of endotoxin and bacterial dry mass

Endotoxin is outer membrane components of GNB and made up of lipopolysaccharides (LPS). β -Hydroxy FAs from C_{10} to C_{18} are present in the lipid A region of LPS and responsible for the endotoxic activity as caused by intact LPS of the GNB. Lipid A from different GNB displays heterogeneity due to the presence and nature (type and chain

length) of the β -hydroxy FAs [Paba *et al.*, 2013]. Previous studies attempted to quantify the airborne endotoxin using the atmospheric concentrations of β -hydroxy FAs measured in aerosol, dust and snow samples [Hines *et al.*, 2003; Lee *et al.*, 2004]. Atmospheric concentrations of endotoxin in aerosol samples can be estimated using mathematical expression as given below:

$$\text{Endotoxin (LPS, ng m}^{-3}\text{)} = [(\sum \beta\text{-(C}_{10}\text{-C}_{18}\text{)-hydroxy FAs; nmol m}^{-3}\text{)/4}] \times 8000 \dots \dots \text{Eq (1)}$$

In the equation (1), 8000 is assumed as an average molecular weight of environmental LPS [Mielniczuk *et al.*, 1993; Pomorska *et al.*, 2007] and β -hydroxy FAs are the total of both LPS-bound and free hydroxy FAs. Endotoxin concentrations in aerosol samples were reported in ng m^{-3} . In snow, and snowpack samples, endotoxin concentrations were reported in ng kg^{-1} . To convert to Endotoxin Units (EU), the value in nanograms (ng) was multiplied by 10 [Pomorska *et al.*, 2007] and final endotoxin concentrations were reported in EU m^{-3} .

Mass loading of airborne Gram-negative bacteria (GNB) was estimated by using the approach initially suggested by Balkwill *et al.* [1988] and later on by Lee *et al.* [2004]. A chemical marker to bacterial mass conversion factor of 15 nmol of β -hydroxy FAs from C_{10} to C_{18} per mg dry cell weight was used for estimating the dry cell weight of GNB. Hence, the sum of mass concentrations of β -hydroxy FAs (in nmol m^{-3}) was converted into equivalent dry cell weight of GNB (*i.e.*, in mg m^{-3}) by normalizing with 15. Estimated dry cell weight of GNB was reported in ng m^{-3} in the aerosol samples and in ng kg^{-1} in snow, snowpack and rain water samples.

2.6 Certified Reference Material (CRM)

To better assess the source regions for hydroxy FAs in particulate matter, the China loess (CJ-1) and simulated Asian dust (CJ-2) were analyzed similar to other aerosol samples prepared by National Research Center for Environmental Analysis and Measurement, China, and National Institute for Environmental Studies (NIES), Japan. The CJ-1 sample was collected in Malan loess horizon (1.8-2.5 m deep) near Huining of Gansu Province, and this sample had a particle size range of 100-400 μm (mode diameter $\sim 49 \mu\text{m}$). The CJ-2 sample was collected as surface loess (0-6 cm deep) from the southeast part of the Tengger Desert in the Ningxia Hui autonomous region of China with a particle size of $<100 \mu\text{m}$ (mode diameter $\sim 26 \mu\text{m}$). Detailed description on chemical composition of CJ-1 and CJ-2 samples is given in Nishikawa *et al.* [2000].

2.7 Air mass back-trajectory and cluster analysis by HYSPLIT model

The air mass back-trajectories (AMBTs) analyses provide information on potential source regions of hydroxy FAs in atmospheric samples. For this study, 7-10 days back-trajectories were downloaded either from the Air Resource Laboratory (NOAA) website (<http://ready.arl.noaa.gov/hypub-bin/trajsrc.pl>) or computed by using offline computer based hybrid single particle lagrangian integrated trajectory model (HYSPLIT, version-4 [Draxler and Rolph, 2009; Draxler et al., 2012]. Archived meteorological data sets (Reanalysis global, 1948-present) from the NOAA air resources laboratory were used in both online and offline mode. Back-trajectories were computed at air mass arrival heights of 100, 500 and 1000 m above ground level to get the better picture of local as well regional atmospheric transport.

Furthermore, to identify the potential sources, transport pathways and percentage contribution of source regions to atmospheric hydroxy FAs over all the sampling sites in East Asian outflow, cluster analysis was performed using PC-based HYSPLIT model [Draxler and Rolph, 2013 and references therein]. The principle behind the cluster analysis was to minimize the spread within the cluster and maximize the variability in between them using k means clustering algorithm in which horizontal moving speed and direction was used principal parameters [Hartigan and Wong, 1979]. Three to four clusters were identified which provide information on the potential source regions of hydroxy FAs. Moreover, fire count data were overlapped with the mean trajectory path of these clusters. All the red dots in cluster analysis represent the fire spots in the source regions (downloaded from <https://earthdata.nasa.gov/data/near-real-time-data/firms/active-fire-data#tab-content-6>). This is to ascertain the influence of biomass combustion in the source regions on the emission and transport pathways of bacteria and plant waxes over sampling sites.

Chapter 3

Long Term (Decadal Scale) Observations of Hydroxy Fatty Acids as Microbial Tracers in Ambient Aerosols over Chichijima: Sources and Transport Pathways over the North Pacific

Chapter 3 presents the results of hydroxy FAs in TSP from the Chichijima Island in the western North Pacific. In addition, causes for the observed relative difference in the abundances and molecular distributions among seasons (Winter/Spring vs. Summer/Autumn) have been discussed in brief.

3.1 Introduction

A wide range of hydroxy fatty acids (FAs) are found in lipid fractions isolated from a variety of organisms [Downing, 1961] including bacteria [Wilkinson, 1988], algae [Blokker *et al.*, 1999] and higher plants [Molina *et al.*, 2006; Pollard *et al.*, 2008]. Several studies have been carried out to assess the abundances of hydroxy FAs in environmental samples such as lacustrine and marine sediments [Eglinton *et al.*, 1968; Kawamura and Ishiwatari, 1981; 1982; Cardoso and Eglinton, 1983; Kawamura and Ishiwatari, 1984b] and lake waters [Kawamura *et al.*, 1987] as well as marine aerosols [Kawamura, 1995]. Despite having their wide spread occurrence, unfortunately these compounds have received little attention from atmospheric chemists. In general, hydroxy FAs can be categorized into different classes on the basis of the number and position of the hydroxyl (OH) group. In the literature, the position of OH group in hydroxy FAs reported so far, are mainly limited to α -, β -, C₉, (ω -1)-, and ω -positions. Among these, aliphatic α -, β - and ω -mono-hydroxy FAs have been used as tracers of soil microorganisms [Kawamura, 1995], bacteria [Lee *et al.*, 2004] and terrestrial plants [Kawamura *et al.*, 2003] in ambient aerosols, respectively. Likewise, hydroxy FAs have been used as geochemical tracers to evaluate microalgae [Gelin *et al.*, 1997], bacterial and terrestrial plant contributions to sediments [Cardoso and Eglinton, 1983].

Short-chain β -hydroxy FAs (C₁₀–C₁₈) are important structural constituents of lipopolysaccharides (LPS) and lipid A, especially in the cell wall of Gram-negative bacteria (GNB) [Paba *et al.*, 2013]. These β -hydroxy FAs are essentially associated with bacterial endotoxin activity [Wilkinson, 1988]. Studies have been performed for the quantification and characterization of endotoxin and GNB in ambient aerosols by using β -hydroxy FAs as biomarkers [Laitinen *et al.*, 2001; Hines *et al.*, 2003; Lee *et al.*, 2004]. Similarly, long-chain aliphatic β -hydroxy FAs have also been reported as intermediates in the β -oxidation of monocarboxylic acids by microorganisms in the old lacustrine sediments from the English Lake district [Eglinton *et al.*, 1968].

It has been suggested that ω -hydroxy FAs (C₁₁–C₂₈) are the pivotal structural components of cell wall of plants [Molina *et al.*, 2006; Pollard *et al.*, 2008] and green algae [Blokker *et al.*, 1999]. A possible bacterial source was also identified by Skerratt *et al.* [1992] who found C₂₆, C₂₈ and C₃₀ (ω -1)-hydroxy FAs in methane utilizing bacteria. Recent scientific work has suggested that some microalgae can be a potential source of mid-chain hydroxy FAs (C₃₀–C₃₄) in marine environments [Gelin *et al.*, 1997]. Thus, these compounds

can also be used as biomarkers for soil microorganisms and higher plant metabolites in ambient aerosols. Rather few studies exist in the literature that focuses on tracing the pathways of atmospheric hydroxy FAs in remote marine aerosols [Kawamura, 1995]. However such studies are, in particular, useful in understanding the changes in atmospheric circulation over the geological past and to assess the long-range atmospheric transport of microorganisms along with the continentally derived particulate matter.

Several studies have documented the impact of Asian outflow to the western North Pacific during winter and spring in delivering the airborne particulates and trace gases. However, to the best of our knowledge, no single study exists in the literature for assessing the long-range atmospheric transport of soil microbes in the continental outflow from Asia to the open ocean waters of the North Pacific. In this regard, we aim to trace the presence of soil microbes in aerosols collected from the Asian outflow in the western North Pacific using certain biomarkers. The present study was carried out over remote marine island, Chichijima, located in the western North Pacific to observe the changes in the long-range atmospheric transport of bacterial tracers to the ocean.

In this chapter, an attempt has been made to use hydroxy FAs as a proxy to understand the linkage between the terrestrial components and those in the oceans and pelagic sediments. In a previous study, Kawamura *et al.* [2003] have documented the chemical compositions of marine aerosols collected from a remote island, Chichijima during 1990-1993 and 2001-2003. These samples were investigated for the atmospheric abundances of hydroxy FAs in order to assess the long-range transport of terrestrial microbes in the East Asian outflow as per the analytical procedures described in chapter 2.

3.2 Aerosol collection and prevailing meteorology

Aerosol samples were collected on a biweekly basis from April 1990 to November 1993 and January 2001 to January 2003 at the Ogasawara downrange station of the Japan Aerospace Exploration Agency (JAXA, elevation: 254 m above mean sea level) in Chichijima (27°04 'N; 142°13 'E) in the western North Pacific (Fig. 3.1). For this study, bulk aerosol (TSP) samples ($N=69$ and $N=94$) were collected using a high volume air sampler and quartz filter with a nominal flow rate of $1.0 \text{ m}^3 \text{ min}^{-1}$ [Kawamura *et al.*, 2003]. In general, the collection period for each aerosol sample varied in between 4 and 6 days. The air sampler was setup on the top (5 m above the ground) of the base of the parabola antenna for the satellite tracking. During the study period, the relative humidity changed from 60-90% (av.

~78%). In general, the sampling site is characterized by prevailing westerlies in winter and spring, whereas trade winds dominate in summer and autumn seasons during both the sampling periods.

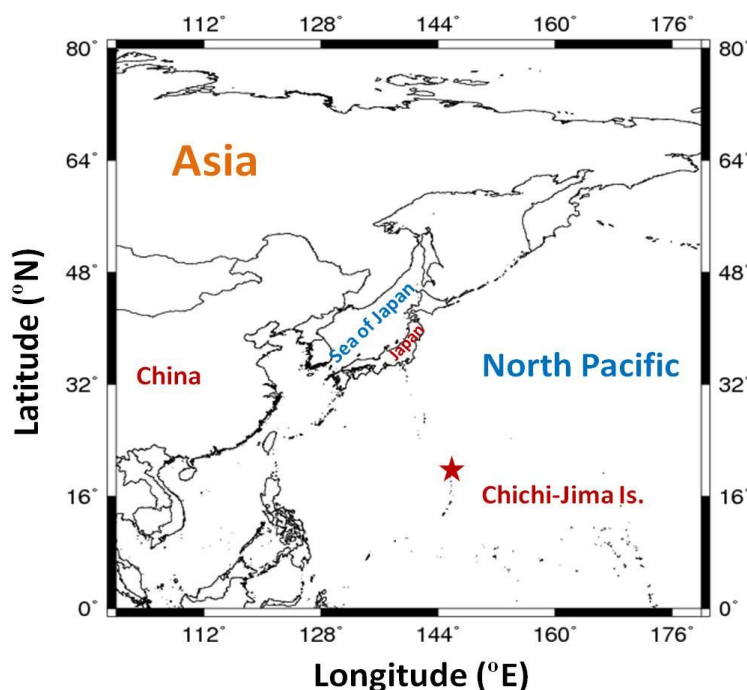


Fig.3.1. Geographical location of the sampling site (Chichijima; 27°04 'N; 142°13 'E) used for the collection of aerosols from the western North Pacific.

3.3 Results and Discussions

3.3.1. Air mass back-trajectory analysis

To assess the sources that contribute to aerosol composition over Chichijima, 7-day isentropic air mass backward trajectories (AMBTs) were computed for the sampling days using HYSPLIT (Hybrid Single Particle Lagrangian Integrated Trajectory Model, version 4) and NCEP-reanalysis archived meteorological datasets from NOAA air resources laboratory. Fig. 3.2 depicts the AMBT clusters for the sampling days, grouped according to seasons, over Chichijima during 1990-1993 and 2000-2003. It is implicit from Fig. 3.2 that the generalized pattern of back-trajectories shows a seasonal shift in the wind regimes with the impact of continental outflow over the sampling site during winter and spring in comparison with the dominance of maritime air masses that prevail in summer and autumn. However, occasional shift in wind regimes is noteworthy for the air masses that are also coming from continents due to reverse shifting in air circulation during summer and autumn.

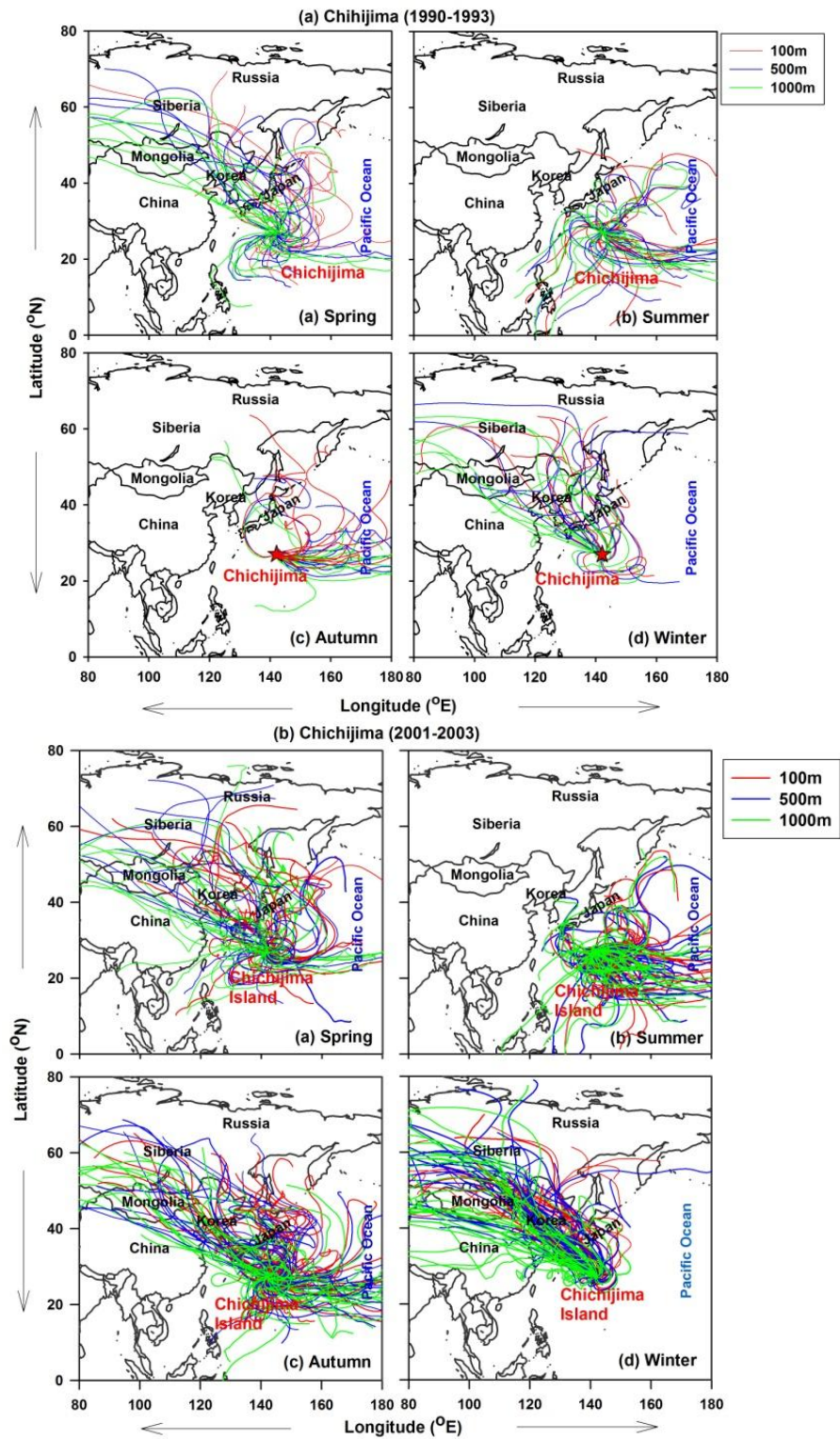


Fig.3.2. Typical 7-day isentropic air mass back-trajectories (AMBTs) during the four seasons (winter: December-January, spring: March-May, summer: June-August, autumn: September-November) for the sampling days (a) 1990-1993 and (b) 2001-2003.

Chichijima is a suitable site to investigate the mineral dust transport from East Asia along with the polluted air masses from China [Mochida *et al.*, 2003a; Yamamoto *et al.*, 2011; Chen *et al.*, 2013]. Mineral dust transport from East Asia is very common during late winter and spring [Chun, 2001; Lin, 2001; Murayama *et al.*, 2001] and originate in arid and semi-arid regions in northern China, Mongolia, and Central Asia under high surface wind conditions [Duce *et al.*, 1980; Uematsu *et al.*, 1983]. In addition to dust transport, previous studies reported on terrestrial lipid compounds [Kawamura *et al.*, 2003], dicarboxylic acids [Mochida *et al.*, 2003a], levoglucosan and saccharides [Chen *et al.*, 2013] in marine aerosols collected from Chichijima. These studies have focused mainly on changes in the atmospheric abundances and molecular compositions with respect to the seasons [Gagosian and Peltzer, 1986]. However, no such attempts have been performed to understand the seasonal patterns of bacterial transport by continental outflows to the North Pacific.

Bacteria in marine ecosystem are significantly and positively correlated with phytoplankton biomass [Bird and Kalff, 1984; Linley and Newell, 1984; Cho and Azam, 1990; Li *et al.*, 2004]. Airborne bacteria mainly contribute to insoluble organic aerosols and their concentrations found to be lower in marine atmospheric boundary layer (MABL) than those over continents [Harrison *et al.*, 2005; Prospero *et al.*, 2005; Griffin *et al.*, 2006]. Subsequent studies have shown that concentrations of culturable bacteria in marine air are closely related to dust concentrations, suggesting their sources to be dust plumes in non-biologically active regions [Prospero *et al.*, 2005; Griffin *et al.*, 2006]. So far, there is no study reporting the total bacterial concentration in remote marine air. In this study, an attempt has been made to assess the bacterial mass over Chichijima on the basis of hydroxy FAs.

3.3.2. Mass concentrations

A brief statistical summary regarding range, mean and standard error of the concentrations of measured hydroxy FAs are presented in Table 3.1 for 1990-1993 and Table 3.2 for 2001-2003 sampling periods. The α -hydroxy FAs were only observed for 2001-2003 dataset but not in the 1990-1993. It is noteworthy that all the even carbon numbered β -hydroxy FAs show high concentrations than odd carbon homologues and follow the similar hierarchy, $C_{12} > C_{10} > C_8 > C_{16} > C_{18} > C_{14}$ for the 1990-1993 dataset and $C_{12} > C_{10} \geq C_{16} > C_{18} > C_{14}$ for 2001-2003 dataset. The total mass concentrations of β -hydroxy FAs varied from 11 to 850 pg m^{-3} during 1990-1993 and from 0.00 to 4408 pg m^{-3} during 2001-2003. The observed significant increase ($p < 0.05$; $t\text{-score} = 4.3$; $df = 144$) in β -hydroxy FAs from 1990-1993 to

2001-2003 datasets suggest the increased contribution from soil microbes from East Asia during latter sampling period.

Table 3.1. Statistical summary (Min-Max, average $\pm$ standard deviation, and median) of mass concentrations of β - and ω -hydroxy FAs (C_8 - C_{31} and C_{11} - C_{28} , respectively) in marine aerosols (in pg m^{-3}) collected from Chichijima during 1990-1993.

Carbon number	β -Hydroxy FAs			ω -Hydroxy FAs		
	Min - Max	Av. $\pm$ S.D.	Median	Min - Max	Av. $\pm$ S.D.	Median
C_8	7.41 - 81.4	36.6 ± 4.1	31.4			
C_9	5.8 - 63.7	29.1 ± 2.3	26			
C_{10}	6.2 - 308	44.2 ± 5.8	30			
C_{11}	3.0 - 29.6	12.1 ± 0.8	11.7	0.00 - 30.4	7 ± 1.4	4.2
C_{12}	2.95 - 557	46 ± 12	16.3	0.00 - 575	62 ± 18	1.1
C_{13}	0.8 - 22.1	6.6 ± 0.7	5.8	0.00 - 331	10.4 ± 5.85	0.71
C_{14}	3.0 - 98.5	21 ± 2	17	0.72 - 1168	74 ± 25	6.7
C_{15}	0.07 - 19.4	6.1 ± 0.8	5.3			
C_{16}	4.4 - 140	32.1 ± 4	18	0.34 - 2418	174 ± 45	23.8
C_{17}	0.5 - 50.9	5.6 ± 1.7	3.4			
C_{18}	2.4 - 94.5	22 ± 3	11.5	0.00 - 93	11.4 ± 3	1.76
C_{19}	0.3 - 16.6	5.4 ± 1.3	4.1			
C_{20}	1.03 - 56.6	11.3 ± 1.6	56.6	0.00 - 139	14 ± 3	3
C_{21}	1.23 - 18.5	5.2 ± 1.6	3.3			
C_{22}	0.3 - 40.3	7.4 ± 1.6	4.7	0.00 - 843	82 ± 27	9
C_{23}	0.9 - 15.4	4.7 ± 1.7	2.7			
C_{24}	0.53 - 23.8	6.3 ± 1.6	3.9	0.00 - 2497	89 ± 40.8	8
C_{25}	1.55 - 5.7	4.2 ± 1.3	5.3			
C_{26}	2.6 - 8.1	5.1 ± 1.0	5.5	0.00 - 199	22.8 ± 9.5	1.6

C ₂₇	1.62 - 4.6	3.1 ± 1.5	3.1			
C ₂₈	3.3 - 6.3	5.1 ± 0.9	5.5	0.00 - 62.6	6.7 ± 1.8	0
C ₃₀	0.00 - 2.9	2.9	2.9			
C ₃₁	0.00 - 1.5	1.5	1.5			

The dominance of even carbon numbered ω -hydroxy FAs (C₁₆ > C₂₂ > C₂₄ > C₁₂ > C₁₄) is also clearly apparent from the concentration data during both the sampling periods (1990-1993: C₁₆ > C₂₄ > C₂₂ > C₁₄ > C₁₂ and 2001-2003: C₁₆ > C₂₂ > C₂₄ > C₁₂ > C₁₄). In this study, the sum of mass concentrations of measured ω -hydroxy FAs varied from 0.03-2500 pg m⁻³ during 1990-1993 and 0.00-5020 pg m⁻³ during 2001-2003. The apparent increase of total ω -hydroxy FAs mass ($p < 0.1$; $df = 152$) over Chichijima from 1990-1993 to 2001-2003 could be due to either increased atmospheric transport of soil microbes (either associated with high wind speed or source strength of the emissions) in the East Asian outflow.

Table 3.2. Statistical summary (Min-Max, mean $\pm$ standard error, and median) of mass concentrations of α -, β - and ω -hydroxy FAs (pg m⁻³) in marine aerosols collected from Chichijima during 2001-2003.

Compounds	Mean $\pm$ SE	Range (Median)	Mean $\pm$ SE	Range (Median)	Mean $\pm$ SE	Range (Median)
	α -Hydroxy FAs		β -Hydroxy FAs		ω -Hydroxy FAs	
C ₈					10.4 $\pm$ 1.66	1.28-46.3 (7.09)
C ₉			45.3 $\pm$ 4.16	2-134 (40)	13. $\pm$ 1.48	1.49-68.2 (11.6)
C ₁₀			101 $\pm$ 15.3	2-1000 (70.8)	8.11 $\pm$ 0.96	0.53-43.1 (6.16)
C ₁₁			36.3 $\pm$ 4.46	2.1-277 (27.1)	6.81 $\pm$ 0.68	1.36-30.2 (5.33)
C ₁₂			145 $\pm$ 36.1	0-1553 (38.6)	50.1 $\pm$ 7.3	0.69-359 (31.5)
C ₁₃	5.00 $\pm$ 3.03	1.98-8.03 (5.00)	29.4 $\pm$ 5.27	0.8-320 (18.9)	5.02 $\pm$ 0.49	0.31-25 (4.68)
C ₁₄	1.05 $\pm$ 0.00	1.05-1.05 (1.05)	61.9 $\pm$ 7.98	7.2-489 (42.5)	36.2 $\pm$ 5.18	0.31-298 (21.7)
C ₁₅	3.31 $\pm$ 1.12	2.19-4.43 (3.31)	21.4 $\pm$ 2.94	0.8-186 (16)	6.41 $\pm$ 0.73	0.40-37 (4.16)
C ₁₆	8.38 $\pm$ 1.25	0.87-63.4 (5.89)	99 $\pm$ 11.4	5.5-581 (69.6)	124 $\pm$ 19.8	0.75-1150 (59)

Chapter 3. Hydroxy FAs over Chichijima

C ₁₇	1.34±0.14	0.52-3.00 (1.20)	13.5±1.65	0.8-102 (9.5)	8.62±1.26	0.23-53.3 (5.03)
C ₁₈	5.35±1.07	0.58-52 (2.23)	77.7±9.80	2.4-464 (49.3)	16.1±2.13	0.40-98.3 (9.54)
C ₁₉	2.09±0.88	0.00-6.62 (1.03)	14.6±2.12	0.8-92 (10.1)	7.84±1.12	0.18-37.1 (4.34)
C ₂₀	2.72±0.60	0.56-18.2 (1.32)	49.4±5.99	5.4-241 (33)	21.1±3.11	0.27-177 (10.6)
C ₂₁	2.76±0.44	0.51-12.7 (1.45)	10.5±5.71	2.7-27.4 (5.9)	7.02±1.3	0.31-55.7 (3.57)
C ₂₂	6.16±0.77	0.64-39.4 (3.97)	16.4±5.27	3.7-35 (12.4)	74.5±13.1	0.22-791 (37.6)
C ₂₃	4.55±0.69	0.71-36.3 (2.69)	10.1±3	7.1-13.1 (10.1)	8.81±1.13	0.17-47.3 (5.64)
C ₂₄	15.4±2.05	1.23-123 (10.3)	21.1±6.92	14.2-28 (21.1)	62.8±11.7	0.79-735 (34.2)
C ₂₅	3.85±0.52	0.51-26 (2.22)	5.6±0.11	5.4-5.67 (5.6)	9.27±1.2	0.61-48.1 (6.77)
C ₂₆	4.27±0.65	0.62-30.7 (2.47)	11.1±0.87	10.3-12 (11.1)	27.3±5.33	0.42-340 (13.6)
C ₂₇	2.40±0.35	0.53-16.5 (1.47)	4.1±1.88	2.2-5.96 (4.1)	8.41±1.09	1.08-45.4 (5.53)
C ₂₈	3.25±0.45	0.55-21.2 (2.11)	15.6±4.08	11.5-19.6 (15.6)	30.3±6.83	0.87-407 (14.5)
C ₂₉	2.79±0.35	0.00-14.1 (1.88)	6.6±0.00	6.6-6.58 (6.6)	7.58±1.14	0.43-45.4 (5.8)
C ₃₀	2.80±0.40	0.61-19.1 (2.18)	17.8±0.00	17.8-17.8 (17.8)	11.9±2.14	0.81-92.7 (7.53)
C ₃₁	2.73±0.40	0.00-14.2 (2.09)	5.8±0.00	5.8-5.8 (5.8)	5.72±1.8	0.61-19.1 (3.6)
C ₃₂	2.79±0.29	0.66-8.45 (1.93)	10.1±0.00	10.1-10.1 (10.1)	10.3±3.14	2.27-27.6 (7.51)
C ₃₃	5.25±1.14	1.55-10.2 (4.64)				
C ₃₄	4.11±0.94	1.28-8.97 (3.32)				
Total	59±7.3	0.00-379 (36.9)	529±68	0.00-4408 (376)	453±70.7	0.00-5020 (228)

As mentioned earlier, not many studies exist in the literature that documents the atmospheric mass concentration of ω -hydroxy FAs over the open oceans. However, *Kawamura* [1995] reported the presence of ω -hydroxy FAs (C₁₂-C₃₀) in one marine aerosol sample (collected during August 1-3, 1989) whose concentration was $\sim 900 \text{ pg m}^{-3}$. It is noteworthy that atmospheric concentrations of ω -hydroxy FAs (C₁₁-C₂₈) in summer from this study (av. $450 \pm 250 \text{ pg m}^{-3}$ for 1990-1993 dataset) are somewhat consistent with that reported in *Kawamura* [1995]. In the remote marine aerosol samples from the western North Pacific,

ω -hydroxy FAs (av. C_{11} - C_{28} ; $480 \pm 120 \text{ pg m}^{-3}$) are more abundant than β -hydroxy FAs (C_8 - C_{31}) ($230 \pm 200 \text{ pg m}^{-3}$), being consistent with the previous study by Kawamura [1995].

3.3.3. Seasonal variability

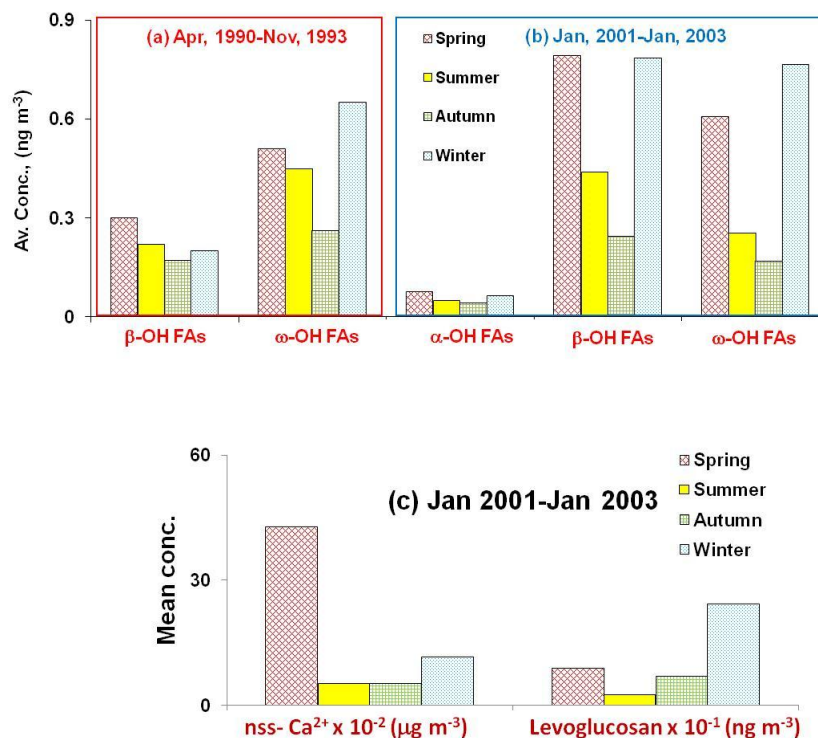


Fig.3.3. Seasonal variability of mean atmospheric concentrations of hydroxy FAs and chemical markers in TSP collected over Chichijima during (a) 1990-1993 and (b and c) 2001-2003, respectively.

A clear seasonality was observed for the total mass concentration of α -, β - and ω -hydroxy FAs over Chichijima during 1990-1993 and 2001-2003. Relatively high concentrations of hydroxy FAs over Chichijima during winter and spring clearly indicate the impact of East Asian outflow on remote oceanic regions of the North Pacific. The α -hydroxy FAs were not found in the TSP analyzed during 1990-1993 and only detected in marine aerosols collected during 2001-2003. The seasonally averaged total mass concentration of α -hydroxy FAs were higher in winter/spring than those observed in summer/autumn over Chichijima during 2001-2003 (Fig. 3.3b). A similar trend is noteworthy for β -hydroxy FAs, whose concentrations were found to be highest in spring followed winter and/or summer, while atmospheric abundances were lowest during autumn over Chichijima (Fig. 3.3a and b).

In spring, Chichijima receives mineral dust through the long-range transport from source regions in East Asia (*viz.*, Chinese loess sediments, desert dust from Mongolia). Over Chichijima, the impact of dust transport from East Asia can be traced based on the atmospheric mass concentrations of water-soluble non-sea salt calcium (nss-Ca^{2+}). Several studies have used nss-Ca^{2+} to assess the contribution of mineral dust during the long-range atmospheric transport [Boreddy and Kawamura, 2015]. A comparison of seasonal variability of β -hydroxy FAs (this study) with nss-Ca^{2+} concentrations (Fig. 3.3c) over Chichijima during 2001-2003 [Boreddy and Kawamura, 2015], reveals their source as “*soil microbes associated with the mineral dust transport from East Asia*”. Therefore, higher atmospheric abundances of β -hydroxy FAs in spring (Fig. 3.3b) indicate the aeolian transport of soil microbes to the North Pacific.

Interestingly, the mean mass concentrations of total ω -hydroxy FAs over Chichijima were found to be highest in winter followed by spring and summer, while lowest in autumn during both the sampling periods (1990-1993 and 2001-2003). A remarkable similarity in the seasonal trend of ω -hydroxy FAs (Fig. 3.3a and 3.3b) with levoglucosan (a specific smoke tracer from biomass burning, Fig. 3.3c; [Simoneit *et al.*, 1999; Verma *et al.*, 2015]), indicate the predominance of biogenic/biomass burning emissions in the East Asian outflow to the North Pacific during winter followed by spring. Therefore, observed high atmospheric abundances of ω -hydroxy FAs (Fig. 3.3b) over Chichijima in winter/spring emphasize the source significance in the East Asian outflow.

3.3.4. Molecular distribution

The molecular distributions of atmospheric α -, β - and ω -hydroxy FAs over Chichijima are characterized by the even-to-odd carbon predominance. It is noteworthy that the molecular distributions of α -hydroxy FAs over Chichijima during 2001-2003 are characterized by the predominance of C_{24} (α -hydroxytetracosanoic acid) especially in winter and spring. However, relative dominance of C_{16} (α -hydroxyhexadecanoic acid), C_{18} (α -hydroxyoctadecanoic acid) and C_{20} (α -hydroxyeicosanoic acid) hydroxy FAs are also noteworthy in summer and autumn over Chichijima. Although Chichijima is being influenced by the East Asian outflow during winter and spring, the mass concentrations of α -hydroxy FAs do not vary significantly among seasons (Fig. 3.4). This observation indicates that these α -hydroxy FAs are not source specific and, perhaps, originate from the photochemical

oxidation and/or other chemical reactions (*e.g.*, hydroxylation of plant waxes) involving other high molecular weight (HMW) fatty acids, which have both terrigenous (*e.g.*, lipids from the higher plant waxes) and marine sources (*e.g.*, algae, sea grasses; [De Leeuw *et al.*, 1995; Volkman *et al.*, 1998; Otto and Simpson, 2006]) during transport. Moreover, α -oxidation of HMW fatty acids during transport could be a source of long-chain ($>C_{20}$) α -hydroxy FAs [Cranwell, 1981]. Therefore, predominance of α -(C_{21} - C_{34})-hydroxy FAs over Chichijima during winter and spring seasons could be due to their origin from fatty acid metabolism during long-range atmospheric transport.

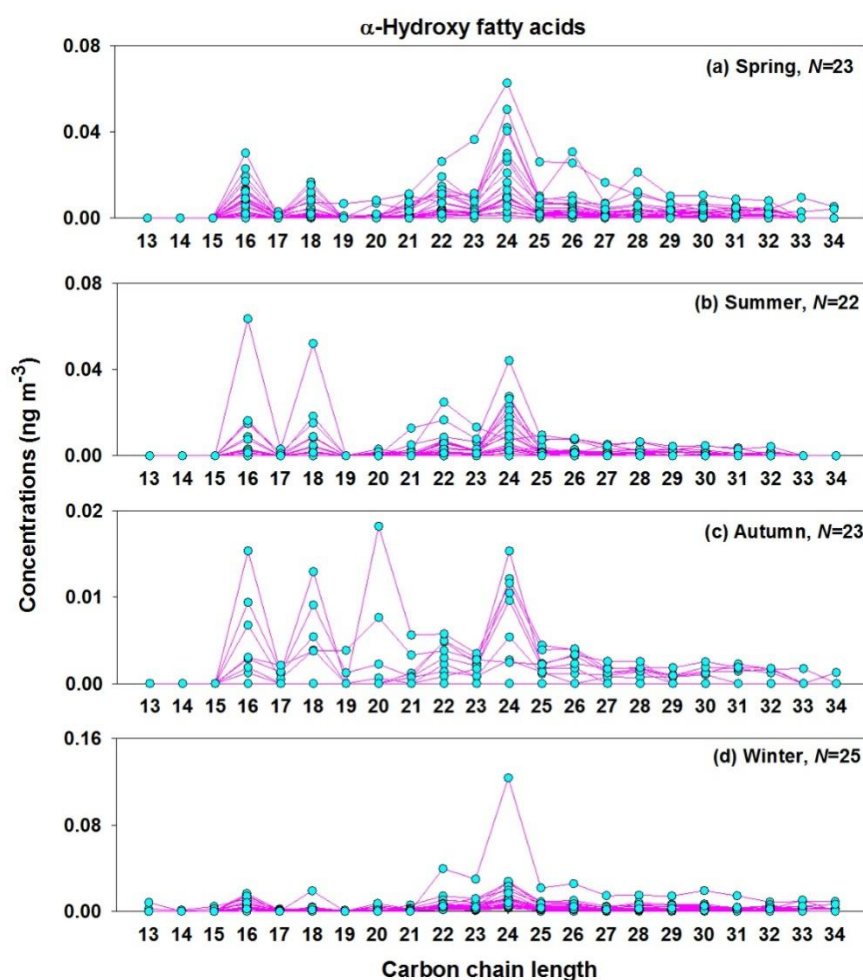


Fig.3.4. Seasonal variability of molecular distributions of α -hydroxy FAs (C_{13} - C_{34}) in marine aerosols over Chichijima during 2001-2003.

Previous studies found that α -(C_{22} - C_{30})-hydroxy FAs in green microalgae (genus *Choricystis*) from freshwaters [Volkman *et al.*, 1998; Zhang *et al.*, 2014]. Likewise, Mochida *et al.* [2003b] documented high concentrations of low molecular weight (LMW; C_{14} - C_{19}) saturated fatty acids in marine aerosols collected over the North Pacific in summer/autumn than those found in winter/spring as a result of increased sea spray emission during former

seasons. Thus, it is likely that emission of dissolved organic matter along with sea salts from the ocean surface could be a potential source of α -hydroxy FAs over Chichijima during summer and autumn.

A comparison of molecular distributions of β -hydroxy FAs in aerosols collected during 1990-1993 and 2001-2003 over Chichijima is presented in Fig. 3.5. This comparison reveals common source and transport pattern of these microbial hydroxy FAs (GNB), which are responsible for the observed even carbon predominance in the molecular distributions of β -isomers. In a previous study, *Wilkinson* [1988] suggested the outer membrane of GNB comprised of LPS, which contributes primarily for the atmospheric abundances of even carbon numbered β -hydroxy FAs (C_{10} - C_{18}). Moreover, the molecular distributions of β -hydroxy FAs in spring and winter are characterized by the C_{12} (β -hydroxydodecanoic acid) predominance in both the sampling periods (1990-1993 and 2001-2003). This feature is also noteworthy for springtime aerosols collected over Gosan (Jeju Island) during 2001-2002, which is very close to continental sources in East Asia (*see more detailed discussion in Chapter 4*). Moreover, the AMBTs for the sampling days over Chichijima during winter and spring clearly showed the impact of East Asian outflow to the North Pacific. Therefore, observed molecular distribution of β -hydroxy FAs over Chichijima indicate the source specific contribution of soil microbes in East Asia (*e.g.*, Mongolian Desert dust and Chinese loess sediments).

In contrast to winter/spring, the molecular distributions of β -hydroxy FAs over Chichijima during summer and autumn, showed the predominance of C_{16} (β -hydroxyhexadecanoic acid) $> C_{18}$ (β -hydroxyoctadecanoic acid) and/or C_{10} (β -hydroxydecanoic acid) $> C_{14}$ (β -hydroxymyristic acid) and/or C_{20} (β -hydroxydodecanoic acid). The AMBTs reveals the influence of oceanic sources during summer and autumn. The observed variations in the molecular distributions of β -hydroxy FAs between winter/spring and summer/autumn might be due to differences in the contributing source type (*i.e.* soil bacteria and/or marine dissolved organic matter, respectively).

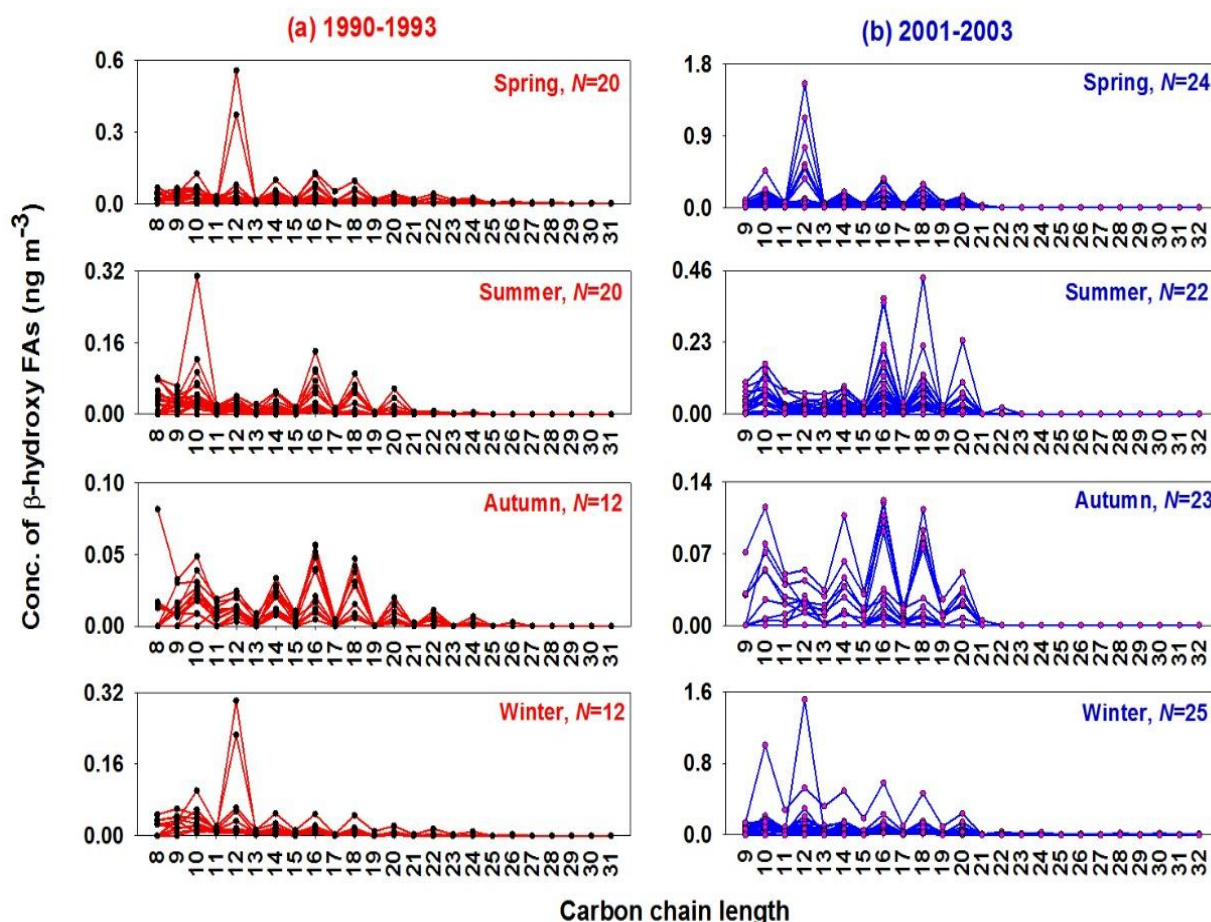


Fig.3.5. Seasonal variability of molecular distributions of β -hydroxy FAs (C_8 - C_{32}) in marine aerosols collected over Chichijima during 1990-1993 and 2001-2003.

Dissolved or particulate organic matter from the productive open ocean waters of the North Pacific could be a source of atmospheric hydroxy FAs over Chichijima. Wakeham *et al.* [2003] suggested the predominance of C_{16}/C_{14} β -hydroxy FAs in the marine cyanobacteria (e.g., *Trichodesmium*; a blue green alga). The surface waters of the North Pacific in summer/autumn is characterized by the wide spread occurrence of *Trichodesmium* blooms [Wilson, 2003]. Based on the analyses of stable carbon isotopes of aerosols from the productive ocean waters of the North Pacific in summer, Miyazaki *et al.* [2011] documented the contribution of marine derived organic matter is as high as $\sim 88 \pm 12\%$. Since easterlies dominate over Chichijima during summer and autumn, the MABL has weak (or no) influence from the East Asian outflow. Therefore, observed molecular distributions of β -hydroxy FAs with C_{16} predominance over Chichijima (Fig. 3.5) is attributed to their contribution from oceanic source (organic matter emitted from the ocean surface).

Unlike β -hydroxy FAs, the molecular distributions of ω -hydroxy FAs over Chichijima between the two sampling periods (1990-1993 and 2001-2003) for all seasons showed predominance of C_{16} , C_{22} , C_{24} and C_{14} ω -hydroxy FAs (Fig. 3.6). However, it is noteworthy that concentrations of ω -hydroxy FAs in spring/winter over Chichijima are higher than those found in summer/autumn (except for summer seasons during 1990-1993). Despite change in AMBTs, similar molecular distribution pattern indicate the contribution of the higher plant waxes to ω -hydroxy FAs concentrations in winter/spring, while local-island based vegetation/ocean derive organic matter is the major source in summer/autumn. Even-to-odd carbon preference in the molecular distributions of ω -hydroxy FAs (Fig. 3.6) is an indicative of biogenic sources from lipid residues of microflora (bacteria, algae, fungi, etc.) and epicuticular waxes of vascular plants [Simoneit, 1989; Rogge *et al.*, 1993a]. The even carbon predominance of ω -hydroxy FAs is implicit during both the sampling periods at Chichijima (Fig. 3.6). Furthermore, ω -hydroxy FAs with odd C numbers are insignificant and were not detectable in most of the samples except for C_{13} (ω -hydroxytridecanoic acid), whose relative abundance is on average $\sim 1\%$.

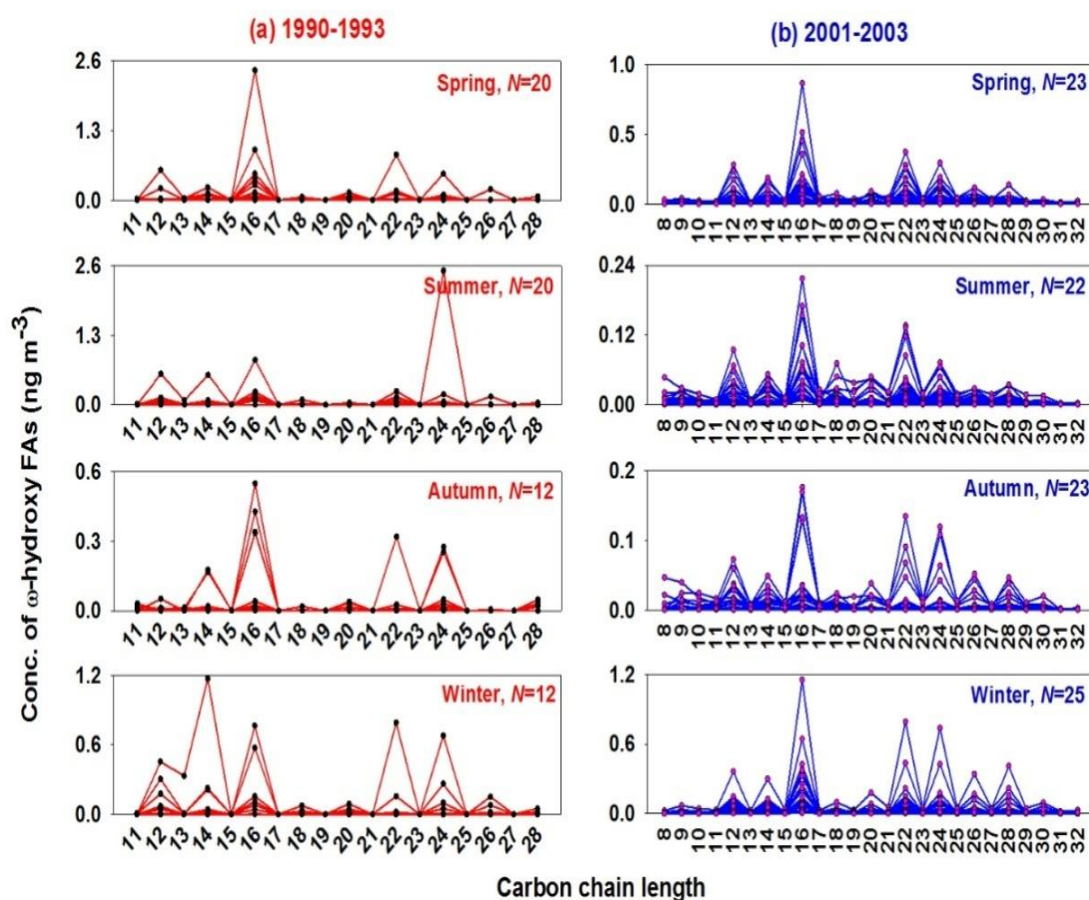


Fig.3.6. Seasonal variability of molecular distributions of ω -hydroxy FAs in marine aerosols collected over Chichijima during 1990-1993 and 2001-2003.

Similar to this study from Chichijima, *Kawamura* [1995] has also documented the even carbon number predominance of ω -hydroxy FAs (C_{12} - C_{30}) with a peak of C_{16} or C_{22} in marine aerosols from the North Pacific. Relatively high concentrations of ω - C_{16} - C_{28} -hydroxy FAs in marine aerosols are due to their omnipresence in cutin and suberin of terrestrial plants [*Graca and Santos*, 2006; *Molina et al.*, 2006; *Pollard et al.*, 2008]. In contrast, the LMW ω -hydroxy FAs (C_{11} - C_{14}) are probably produced by ω -oxidation of corresponding monocarboxylic acids present in soil particles. These ω -hydroxy FAs are probably associated with soil dust particles and have also been reported to be present in terrestrial higher plants [*Eglinton et al.*, 1968] and in lacustrine and marine sediments [*Cardoso and Eglinton*, 1983; *Kawamura and Ishiwatari*, 1984a;b]. Overall, we found higher abundances of ω -hydroxy FAs than β -hydroxy FAs in marine aerosol samples during the study period. This could be due to the reason that microbes prefer the pathway of ω -oxidation of fatty acids and other lipids present in soils [*Kawamura*, 1995]. It is very likely that these hydroxy FAs are transported to the open ocean during long-range atmospheric transport along with soil particles during the study period

3.3.5. Relative abundances

The classification of β -hydroxy FAs on the basis of carbon number could provide crucial information regarding the GNB species. *Saraf et al.* [1997] documented the contributions of individual β -hydroxy FAs vary among different species and thus, these fatty acids can be used to identify the GNB community. Thereafter, subsequent studies have documented that C_{10} - C_{18} β -hydroxy FAs (here after referred as GNB-specific β -hydroxy FAs) can be used as bacterial biomarkers in aerosols [*Wilkinson*, 1988; *Hines et al.*, 2003; *Lee et al.*, 2004]. Based on the preliminary analyses, the relative abundances of GNB-specific β -hydroxy FAs in their total mass concentration (ΣC_8 - C_{31} β -hydroxy FAs) are found to be high over Chichijima in both the sampling periods (1990-1993~76 $\pm$ 14%; 2001-2003~85 $\pm$ 10%). This observation clearly emphasizes the applicability of β -hydroxy FAs as a biomarker for assessing their atmospheric abundances of GNB in marine aerosols. However, occurrence of relatively low atmospheric abundances of other β -hydroxy FAs (*i.e.*, $< C_{10}$ and $> C_{20}$, which are not specific to GNB) over Chichijima could be due to the intense photochemical oxidation during long-range transport of terrestrial plant metabolites to the MABL.

On a decadal scale, no change in relative abundances of α -, β - and ω -hydroxy FAs was observed based on the comparison of two datasets (1990-1993 and 2001-2003). Furthermore, β -hydroxy FAs (~50%) dominate the relative abundances of total hydroxy FAs followed by ω -hydroxy FA (~40%) with minor contribution from α -hydroxy FAs (Fig. 3.7). However, significant increase (~13%; t-score = 4.5, df = 143; p < 0.01) with regard to the relative abundance of GNB-specific β -hydroxy FAs in their mass concentration (*i.e.*, ΣC_8-C_{32} β -hydroxy FAs, referred here as f_{GNB}) in TSP over Chichijima was observed from 1990-1993 to 2001-2003 (Fig. 3.8). This observation indicates the increased contribution of soil microbes or marine phytoplankton derived organic matter over Chichijima, on a decadal time series sampling.

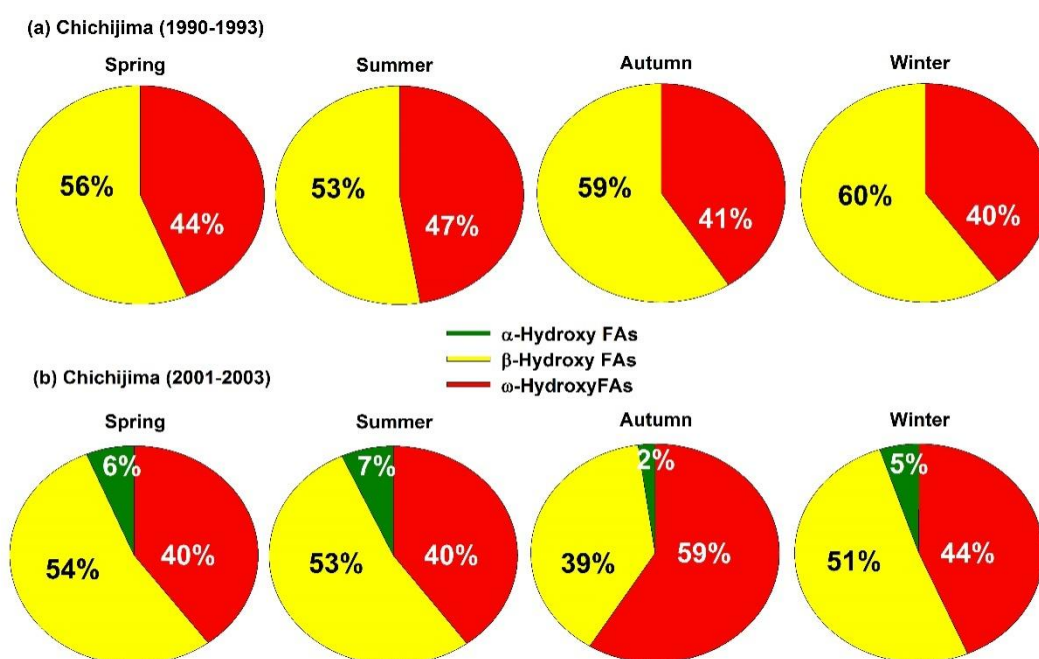


Fig.3.7. Relative abundances of α -, β - and ω -hydroxy FAs in their total mass concentration in TSP collected over Chichijima during 1990-1993 and 2001-2003.

Interestingly, AMBTs in both sampling periods (1990-1993 and 2001-2003) showed the transport of air masses from similar dust source regions in East Asia during winter and spring and, hence, the contribution of soil microbes in the East Asian outflow from similar source regions. Surprisingly, increase in relative abundances of GNB-specific β -hydroxy FAs (*i.e.*, $C_{10}-C_{18}$ β -hydroxy FAs) is not only observed for winter and spring but also for summer and autumn, during which mostly oceanic sources contribute to atmospheric hydroxy FAs. Therefore, increased mobilization of soil microbes in the East Asian outflow on a decadal

scale emphasizes the need for further studies focusing on their role in microbial biodiversity (e.g., increase in grazing pressure) and marine community structure. However, it is not clear at this moment on the actual cause of observed relative increase in GNB-specific β -hydroxy FAs over Chichijima during summer and autumn.

Trichodesmium blooms usually occur in the surface waters of the western North Pacific (WNP) during early summer after having the influence of Asian dust events during spring [Wilson, 2003]. For instance, Bishop *et al.* [2002] documented that marine particulate organic carbon fluxes in the surface ocean are almost doubled over a two week period after the impact of Asian Dust (e.g., from Gobi Desert) outbreaks in spring 2001. In a recent study, Shiozaki *et al.* [2015] also observed wide spread occurrence of *Trichodesmium* blooms from summer to autumn in the temperate oceanic waters of northwestern North Pacific (i.e., spanning between 38-40°N and 141-143°E). In this context, Yuan and Zhang [2006] observed a strong correlation between Asian dust events and high primary productivity in the western North Pacific.

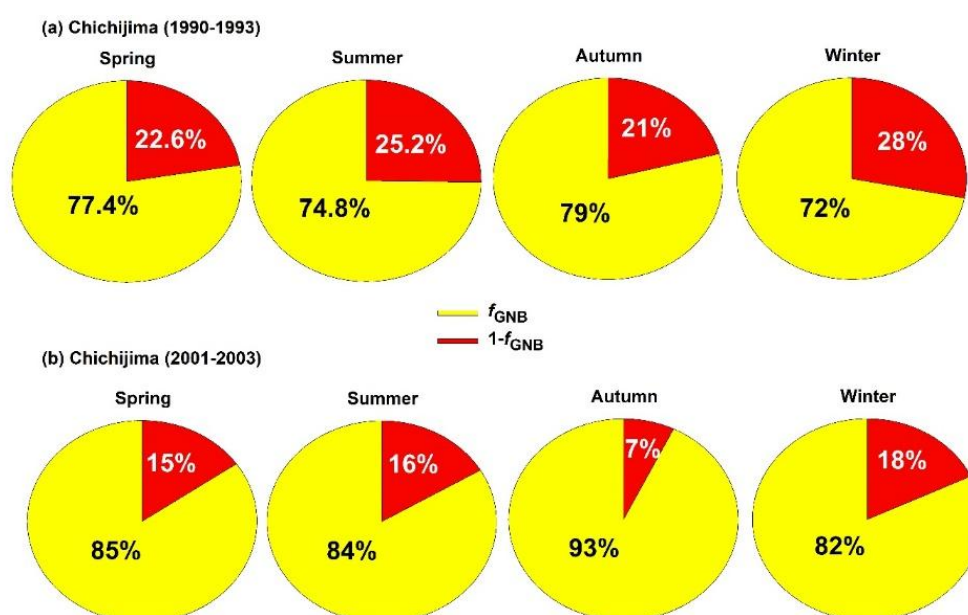


Fig.3.8. Fractional abundances of GNB-specific β -hydroxy FAs (f_{GNB}) in their total mass concentrations ($\Sigma\text{C}_8\text{-C}_{31}\beta$ -hydroxy FAs) in TSP collected from Chichijima during 1990-1993 and 2001-2003.

Another study by Tan *et al.* [2013] based on the long term analysis of impact of Asian dust on the downwind oceanic regions of the North Pacific showed a strong coupling between chlorophyll a concentration in the surface waters and the overhead dust load in the MABL. All these observations highlight a potential role of mineral dust transport in the East Asian

outflow to the North Pacific during spring season followed by a significant increase in phytoplankton biomass (*e.g.*, *Trichodesmium* blooms) in early summer to autumn. Wakeham *et al.* [2003] suggested that C₁₀-C₁₈ β -hydroxy FAs are structural parts of LPS from the marine GNB species (*viz.*, *Trichodesmium sp.*). Therefore, observed increase in contribution of GNB-specific β -hydroxy FAs in the East Asian outflow to the North Pacific during spring for the 2001-2003 dataset is also accompanied by consequent sea-to-air emission of marine derived organic matter during summer months.

A comparison of data from both sampling periods (*i.e.*, 1990-1993 and 2001-2003) over Chichijima reveal that the relative abundances of LMW ω -hydroxy FAs dominate their total mass concentrations (*i.e.*, Σ C₁₁-C₃₁ ω -hydroxy FAs). This observation indicates the perennial influence of marine derived organic matter over Chichijima. Since C₁₆ ω -hydroxy FAs are abundant in the terrestrial vegetation [Eglinton *et al.*, 1968] and also originate from the dissolved or particulate organic matter emitted from the ocean [Wakeham, 1999]. However, the relative influence of continental outflow (*e.g.*, contribution from higher plant waxes) is more over Chichijima during spring and winter than summer and autumn, as inferred based on the relative decrease in concentrations of C₁₆ ω -hydroxy FAs during latter seasons. Similar to GNB-specific β -hydroxy FAs, HMW ω -hydroxy FAs from C₂₀-C₃₀ (tracer for higher plant waxes) in the East Asian outflow also showed significant increase in relative abundances to their total mass concentration of ω -hydroxy FAs (Fig. 3.9). This observation indicates the relative increase in contribution of terrestrial higher plant waxes in the East Asian outflow to the North Pacific, when compared between 1990-1993 and 2001-2003 datasets.

Surprisingly, such a relative increase in contribution of HMW ω -hydroxy FAs are also noteworthy for summer period between 1990-1993 and 2001-2003 datasets. However, no change in relative abundances of HMW ω -hydroxy FAs was observed in autumn season over Chichijima when compared between 1990-1993 and 2001-2003 datasets. Air masses during summer and autumn over Chichijima originated mostly from the remote North Pacific. Therefore, continental source contribution to HMW ω -hydroxy FAs cannot explain their observed increase in relative abundances over Chichijima during summer. If local-island based vegetation contributes to such increase in relative abundances of HMW ω -hydroxy FAs during summer over Chichijima, the similar pattern could also influence during autumn

season. Hence, additional factors must be influencing their emissions over Chichijima during summer.

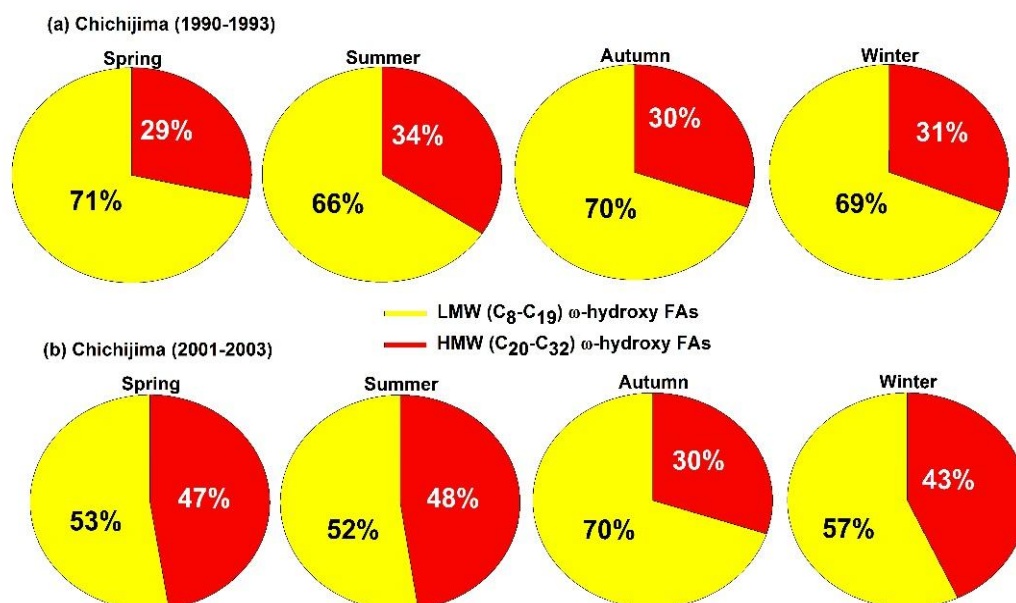


Fig.3.9. Relative abundances of low and high molecular weight (LMW and HMW) ω -hydroxy FAs in their total mass concentration in TSP from Chichijima during 1990-1993 and 2001-2003.

In general, saturated long-chain HMW fatty acids (*e.g.*, C₂₄, C₂₆ and C₂₈) mostly originate from the epicuticular waxes of higher plants [Waterson and Canuel, 2008], while LMW fatty acids originate from the soil microbes and marine- (*i.e.* algae/phytoplankton) derived organic matter [Wakeham, 1999; Wakeham *et al.*, 2003]. Therefore, contribution from phytoplankton derived organic matter in summer cannot explain the observed increase in relative abundances of HMW ω -hydroxy FAs. Mochida *et al.* [2003b] documented that sea-to-air emission of fatty acids increase towards summer and then decreased towards winter. Under this scenario, recently deposited particles from the impact of East Asian outflow on the surface waters could be emitted back to the MABL through bubble bursting mechanism during summer months. Perhaps, this could be the reason behind lack of such increase in case of relative abundances during autumn season over Chichijima (Fig. 3.9).

3.3.6. Cluster analysis-Source apportionment

To assess the contribution of various source regions to atmospheric hydroxy FAs over Chichijima, cluster analysis was performed (Fig. 3.10) by combing the endpoint files of AMBTs using PC-based HYSPLIT model. Details of cluster analysis are given in chapter 2.

In this study, four and three clusters can best represent the meteorological characteristics of the transport pathways over Chichijima during 1990-1993 and 2001-2003, respectively. Furthermore, MODIS derived fire count data were superimposed along with the mean trajectory paths of each identified clusters on the geographic map of East Asia containing the sampling location (Chichijima) to evaluate the effects of biomass burning on the atmospheric abundances and transport of hydroxy FAs over Chichijima (Fig. 3.10). Fire count data is not available for the TSP collected during 1990-1993. In both the sampling periods (1990-1993 and 2001-2003), the clustering of AMBTs reveal that aerosol composition (and, hence, hydroxy FAs) over Chichijima is predominantly influenced by the continental air masses from Siberia, Mongolia and up to some extent from northern China in winter.

Based on the analyses of anhydrosugars in ambient aerosols collected over the Okinawa Island in the North Pacific, *Zhu et al.* [2015] found the impact of biomass burning emissions (BBEs) is maximum in East Asian outflow in winter. Likewise, the transport of biomass burning derived saccharides from Siberia and Mongolia were detected in remote marine aerosols from Chichijima Island in the western North Pacific [*Chen et al.*, 2013]. Previous studies documented that BBEs also a source of hydroxy FAs [*Otto and Simpson*, 2006]. Therefore, observed molecular distributions of hydroxy FAs over Chichijima during winter might have contribution from BBEs in the North China Plain and mineral dust from China, Mongolia and Siberia. The cluster analysis during spring and autumn showed mixed source contribution of continental and oceanic air masses with an increased impact of East Asian outflow during former season. In both the sampling periods (1990-1993 and 2001-2003), the cluster analysis revealed transport from similar source regions (*i.e.*, Siberia, Mongolia and North China Plain). However, slight variation in the contribution of aerosols over Chichijima from these source regions is noteworthy between 1990-1993 and 2001-2003.

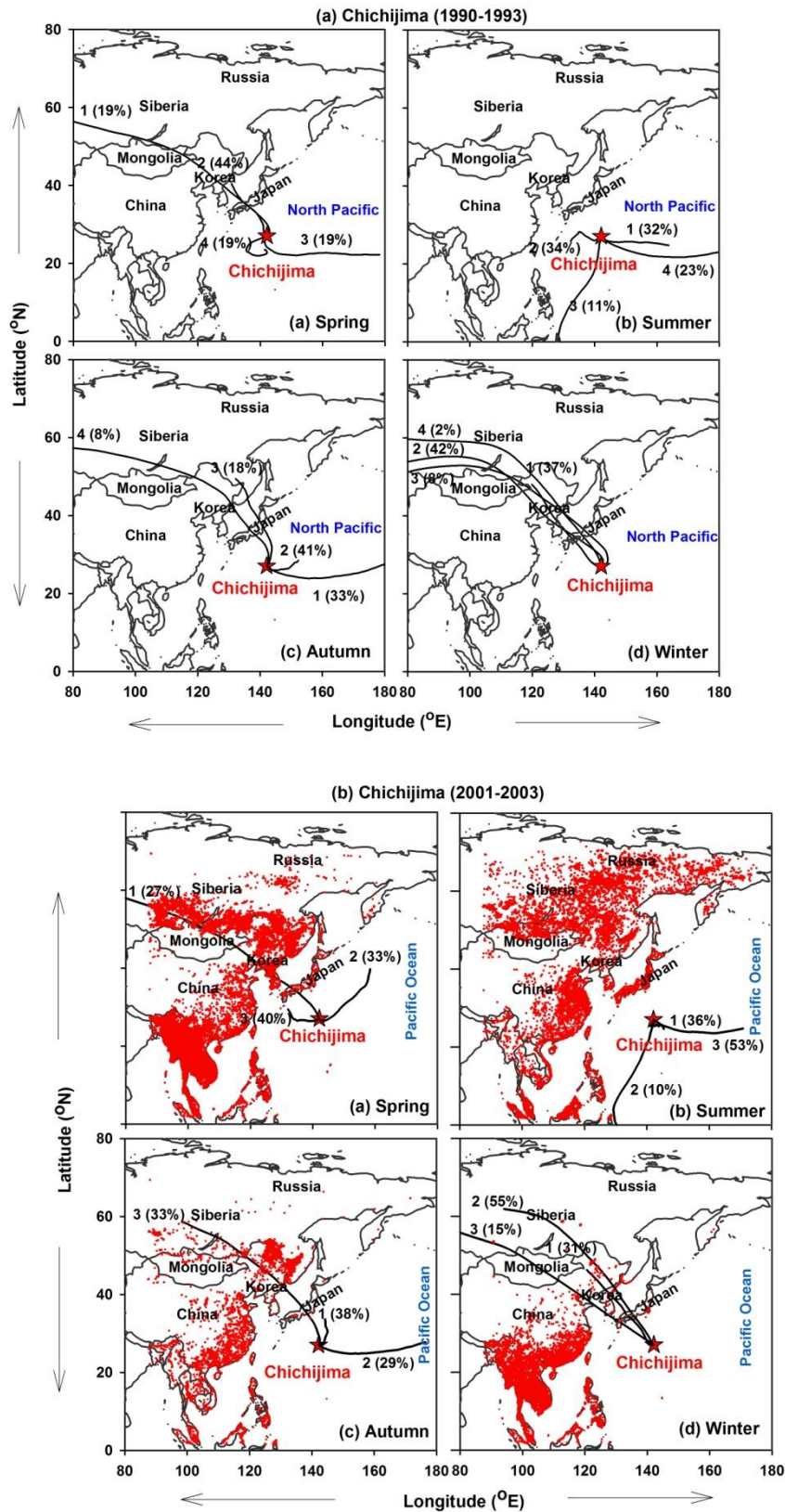


Fig.3.10. Mean trajectory paths of air mass back-trajectories for the 4 clusters during (a) 1990-1993 and (b) 2001-2003 over Chichijima, North Pacific.

Tan et al. [2012] documented the impact of Mongolian desert dust and Chinese loess sediments on the Yellow Sea, South China Sea and the remote North Pacific. Likewise, several studies have documented the influence of Siberian BBEs on the MABL of the North Pacific [Ding et al., 2013; Verma et al., 2015]. Based on the fire count data from East Asia together with cluster analysis, we found that biomass burning derived aerosols from Siberia mixed with dust particles from Mongolia and China in spring season before reaching Chichijima. Therefore, observed β - and ω -hydroxy FAs over Chichijima during spring and autumn seasons has contribution of soil microbes associated with Mongolian Desert dust and higher plant waxes from Siberia. In contrast, oceanic air masses dominate the aerosol composition (here, hydroxy FAs) over Chichijima during summer. Observed molecular distributions of hydroxy FAs in summer, therefore, attributed to contribution from either oceanic source or from the local vegetation. Interestingly, atmospheric transport of hydroxy FAs over Chichijima during autumn season is consistent with that observed in spring with major contribution from oceanic air masses from North Pacific (Fig. 3.10). In a recent study, *Tsuda et al.* [2015] documented the occurrence of phytoplankton blooms in seas around Japan. Therefore, marine derived organic matter emitted along with sea spray could contribute to atmospheric hydroxy FAs over Chichijima during autumn season.

3.3.7. Endotoxin and GNB-dry mass

Endotoxin/lipopolysaccharides (LPS) present in the membranes of Gram-negative bacteria (GNB), is responsible for the generalized inflammations in humans caused by the exposure to these compounds [Maitra et al., 1986]. The most common route of exposure to airborne endotoxin (also known as fever causing agents) is inhalation, which has been associated with a variety of clinical symptoms, including respiratory disorders [Mielniczuk et al., 1993 and references therein]. Previous studies suggested that β -hydroxy FAs, as determined by GC/MS, can be used as molecular tracers for the presence/absence of airborne endotoxin [Maitra et al., 1986; Saraf et al., 1997; Pomorska et al., 2007; Paba et al., 2013]. Lipid A region of endotoxin consists of a large portion of β -hydroxy FAs from carbon number 10 to 18 with even carbon dominance [Paba et al., 2013]. These fatty acids in lipid A can be quantified from both biologically-active and inactive endotoxin [Laitinen et al., 2001].

At Chichijima, estimation of endotoxin and dry cell mass of GNB was done in aerosols sampled by using a marker to microbial mass conversion factors on the basis of β -(C₁₀-C₁₈)-hydroxy FAs (Table 3.3). The endotoxin concentrations showed clear spring/winter

maxima (60 ± 12 EU m⁻³ and 49.6 ± 11.3 EU m⁻³, respectively) and summer/autumn minima (47.5 ± 10 EU m⁻³ and 34.5 ± 5.4 EU m⁻³, respectively), following the seasonality of β -hydroxy FAs during the sampling period 2001-2003. However, no clear seasonality was observed for the sampling period 1990-1993 (spring: 19.6 ± 3.8 EU m⁻³; summer: 14.3 ± 2.9 EU m⁻³; autumn: 10.4 ± 1.62 EU m⁻³ and winter: 15 ± 3.4 EU m⁻³). The concentrations of endotoxin at Chichijima are much lower than the proposed occupational endotoxin exposure limit ~ 90 EU m⁻³ (DECOS, 2010) in both the periods. The GNB dry mass, assessed based on β -hydroxy FAs, at Chichijima is also higher in spring/winter than summer/autumn for 2001-2003 but seasonality was not clear for the 1990-1993 sampling period. Several studies have documented the significance of long-range atmospheric transport of soil microorganisms [Griffin, 2007; Lee *et al.*, 2007 and references therein] during dust events. Our results clearly show the higher abundances of dry mass of GNB in spring and winter followed by autumn and summer.

Table 3.3. Endotoxin and GNB dry mass concentrations in TSP collected from Chichijima during 1990-1993 and 2001-2003.

Sampling site (year)	Bacterial components	Spring	Summer	Autumn	Winter
		Av $\pm$ SE	Av $\pm$ SE	Av $\pm$ SE	Av $\pm$ SE
Chichijima (1990-1993)	Endotoxin (EU m ⁻³)	19.6 $\pm$ 3.8	14.3 $\pm$ 2.9	10.4 $\pm$ 1.62	15 $\pm$ 3.4
	Dry cell wt of GNB (ng m ⁻³)	65.5 $\pm$ 12.7	47.5 $\pm$ 10	34.5 $\pm$ 5.4	49.6 $\pm$ 11.3
Chichijima (2001-2003)	Endotoxin (EU m ⁻³)	60 $\pm$ 12	30 $\pm$ 5.6	17.4 $\pm$ 4.7	58.3 $\pm$ 14
	Dry cell wt of GNB (ng m ⁻³)	200 $\pm$ 39	99.7 $\pm$ 18.7	58.1 $\pm$ 15.9	194 $\pm$ 46.7

Significant increase was observed in endotoxin and dry mass GNB in TSP over Chichijima from 1990-1993 to 2001-2003 is due to the enhancement in the relative abundance of C₁₀ to C₁₈ β -hydroxy FAs in their mass concentration (see Fig. 3.8). This observation indicates the increased contribution of soil microbes or marine phytoplankton derived organic matter over Chichijima, on a decadal time series sampling. This could be due to the increased anthropogenic activities, availability of more arid regions and changes in atmospheric circulations globally. AMBTs also suggest the transport of these endotoxin

molecules along dust from the Asian continent (Russia, Mongolia and China) in spring and winter. These endotoxin molecules are associated with Gram-negative coliform bacteria of *Enterobacteriaceae* family [Pomorska *et al.*, 2007] and thus, chances of human infections can increase due to the decadal changes in their atmospheric transport to the North Pacific.

3.4 Conclusions

To the best of our knowledge, the results obtained in this study is the first of its kind in applying a biomarker approach to determine the long-term contributions and transport of hydroxy FAs of Gram-negative bacteria and terrestrial plants to remote marine aerosols. However, our study is in continuation to that documented for marine aerosols (in only one sample) by Kawamura [1995], who re-emphasizes the significance of these compounds as biomarkers for bacterial transport through aeolian pathway. In the present study, TSP samples were used to determine β - and ω -hydroxy FAs collected in Chichijima over the two sampling periods (1990-1993 and 2001-2003). Substantial seasonal differences were observed in the carbon number predominance of β - and ω -hydroxy FAs. These biomarkers are primarily associated with terrestrial plants and soil microorganisms; but our study shows that continents and oceans equally contribute to the microbial biomarkers in marine aerosols.

The observed similarity in relative abundances of β -hydroxy FAs in marine aerosols indicates the presence of GNB in both air masses transported from continents and originated in the western North Pacific. Thus, our study characterizes the source regions of β - and ω -hydroxy FAs. Our results show that ω -hydroxy FAs are mainly contributed by terrestrial plants and soil microbes whereas β -hydroxy FAs are ubiquitous in environment owing to their bacterial sources in both polluted continental and pristine oceanic air masses. Carbon number of β -hydroxy FAs was also found variable among different seasons, which can be potentially used as specific tracer for the particular bacterial species. Thus, our study demonstrates the usage of β - and ω -hydroxy FAs in marine aerosol samples as a potential proxy to trace the GNB species and terrestrial plant metabolites and also their sources (continental vs. marine) regions.

Moreover, both continental and oceanic air masses contribute equally to the relative abundances of β -hydroxy FAs, which clearly indicate that both polluted (continents) and pristine (ocean) ecosystems are having microorganisms. The β -hydroxy FAs (from C₁₀ to C₁₈) have been used for assessing the atmospheric abundances of endotoxin and the GNB dry

mass for the continental aerosols [*Hines et al.*, 2003; *Lee et al.*, 2004]. However, GNB are also abundant in the seawater and their cell membrane comprise of long-chain fatty acids with OH groups located at β - and ω -positions [*Giovannoni*, 2000]. Likewise, the analysis of lipid biomarkers in the ultra-filtered seawater samples from the equatorial and North Pacific revealed the presence of C_{12} - C_{20} fatty acids and C_{10} - C_{18} β -hydroxy FAs, which are the constituents of marine bacterial membrane [*Wakeham et al.*, 2003]. Another study by *Mochida et al.* [2002] had documented an increase in atmospheric abundances of saturated fatty acids (C_{14} - C_{20}) during a summer cruise conducted in the North Pacific, for which the surface waters are characterized by high primary productivity. Since bacterial cells contain β - and ω -hydroxy FAs, they can be co-emitted with sea spray from the productive ocean surfaces to the air like fatty acids.

Chapter 4

Atmospheric Transport of Soil Microorganisms and Higher Plant Waxes in the East Asian Outflow to the North Pacific Rim Using Hydroxy Fatty Acids: Year Round Observations at Gosan, Jeju Island

Chapter 4 presents the results of hydroxy FAs in TSP from the Gosan, Jeju Island in the western North Pacific. A comparison of results of Gosan and Chichijima in terms of seasonality of molecular distributions was made to better constrain the source specific signatures of soil microbes and higher plant waxes.

4.1 Introduction

Endotoxin/lipopolysaccharides (LPS) of Gram-negative bacteria (GNB) are widely studied as airborne infectious species [Pomorska *et al.*, 2007; Paba *et al.*, 2013]. These LPS are the components of the outer cell membrane that is important for the viability of GNB. Endotoxin can be released from the surface of GNB to the atmosphere and cause severe Gram-negative infections and generalized inflammations [Rietschel *et al.*, 1994]. The endotoxin in LPS contains lipid A, which is made up of β -hydroxy fatty acids (FAs). These β -hydroxy FAs (in particular from C₁₀ to C₁₈) are responsible for the toxic effects of LPS and, together with other components, they regulate essential functions of GNB [Maitra *et al.*, 1986; Paba *et al.*, 2013].

Ever since their first identification in lacustrine sediments by Eglinton *et al.* [1968], β -hydroxy FAs have received much attention as specific markers of terrigenous microbial inputs into sediments [Perry *et al.*, 1979; Kawamura and Ishiwatari, 1984b; Wakeham, 1999], soils [Zelles, 1999 and references therein], dust [Saraf *et al.*, 1997; Milton *et al.*, 2000] and continental [Lee *et al.*, 2004] aerosols. Along with β -hydroxy FAs, α - and ω -isomers have also been used to trace the contribution of soil microorganisms (*e.g.*, bacteria, fungi, algae) and higher plants in sediments [Cranwell, 1981] and marine dissolved organic matter [Wakeham, 1999]. Abrasion of the leaf surface lipids and re-suspension of soil particles due to wind induced mechanical shear both contribute to the atmospheric abundances of hydroxy FAs [Rogge *et al.*, 1993a]. Therefore, α -, β - and ω -hydroxy FAs can be used as tracers of soil microbes and plant metabolites in the airborne particulate matter.

In the atmosphere, aliphatic short-chain α - and β -hydroxy FAs (typically C₁₀-C₂₀) originate mainly from the structural constituents of many soil microorganisms (bacteria, fungi, yeasts, and protozoa) [Ratledge and Wilkinson, 1988]. On the other hand, long-chain α -, β - and ω -hydroxy FAs (from C₁₆ to C₃₄) are abundant in micro-algae/algal detritus [Cranwell, 1981], cyanobacteria [Matsumoto and Nagashima, 1984; Matsumoto *et al.*, 1984], sea-grasses [Volkman *et al.*, 1999], and plant epicuticular waxes [Simoneit, 1989; Rogge *et al.*, 1993a]. Moreover, these α -, β - and ω -hydroxy FAs can also act as intermediates in the photochemical oxidation pathways, by which long-chain fatty acids are degraded [Volkman *et al.*, 1998; Wakeham, 1999].

The impact of continental outflow from East Asia significantly influences the chemical composition of atmospheric aerosols over the western North Pacific during winter and spring. To assess the seasonality of continental outflow, previous studies setup a long-term aerosol characterization station at the Gosan site, Jeju Island (South Korea). These aerosol samples have been studied for various inorganic and organic tracer compounds that unfolded the seasonal occurrence of East Asian outflow to the western North Pacific [Kawamura *et al.*, 2004; Fu *et al.*, 2012]. However, no attempt has been made to determine the molecular distributions of hydroxy FAs. To assess the long-range atmospheric transport of bacteria and plant metabolites, we have collected aerosol samples at Gosan site during an ACE-Asia (Aerosol Characterization Experiment) campaign [Huebert *et al.*, 2003].

Air- and soil-borne microorganisms can be pathogenic to humans and can cause severe allergies over the wide areas as a result of the atmospheric transport. Several studies have been conducted to measure the dust-laden bacteria and their health impacts [Griffin, 2007 and references therein]. However, atmospheric researchers face difficulties in the identification and quantification of these pathogenic bacteria during their long-range atmospheric transport. To solve this, hydroxy FAs, specifically β -isomers, can serve as the markers for bacteria and plant metabolites during their atmospheric transport over the Pacific. These fatty acids can also be used in the quantification of endotoxin associated with GNB.

In the present study, hydroxy FAs were determined in marine aerosols collected over Gosan, Jeju Island, to trace bacteria and plant metabolites during their long-range atmospheric transport. Further, seasonal and temporal variations were assessed in the concentrations of hydroxy FAs to see the relative influence of continental vs. oceanic sources. To characterize the sources and source regions, molecular distribution of hydroxy FAs was assessed and source apportionment of marine aerosols (carriers for GNB) was performed. In addition, endotoxin and GNB dry mass were quantified from β -hydroxy FAs in marine aerosols using microbial mass conversion factors to assess the potential pathogenic effects of these airborne allergens during the atmospheric transport.

4.2 Experimental methods

4.2.1. Site description and sample collection

Gosan site is located at the top of a 71 m cliff on the western edge of Jeju Island, South Korea, facing the Asian continent. This site is not seriously affected by human activity

because major residential areas are far away from the site. Jeju Island (33.36 °N, 126.53°E; area of 1845 km²; population of 0.55 million) is surrounded by Mainland China, Korea and Kyushu Island of Japan and located at the boundary of the Yellow Sea and East China Sea (Fig. 4.1). Furthermore, the meteorology of Gosan is dominated by westerly winds from winter (December-February) to spring (March-May), majorly coming from the Asian continent. On the other hand, winds mostly blew from the Pacific Ocean over Gosan during summer (June-August) and autumn (September-November). Owing to its remote and unpolluted location as well as of its location on the pathway of atmospheric transport of particulate matter from the Asian continent to the North Pacific, Gosan has been used as a super site for the study of chemical compositions of air masses in the representative background of Northeast Asia during the ACE-Asia campaign [Huebert *et al.*, 2003].

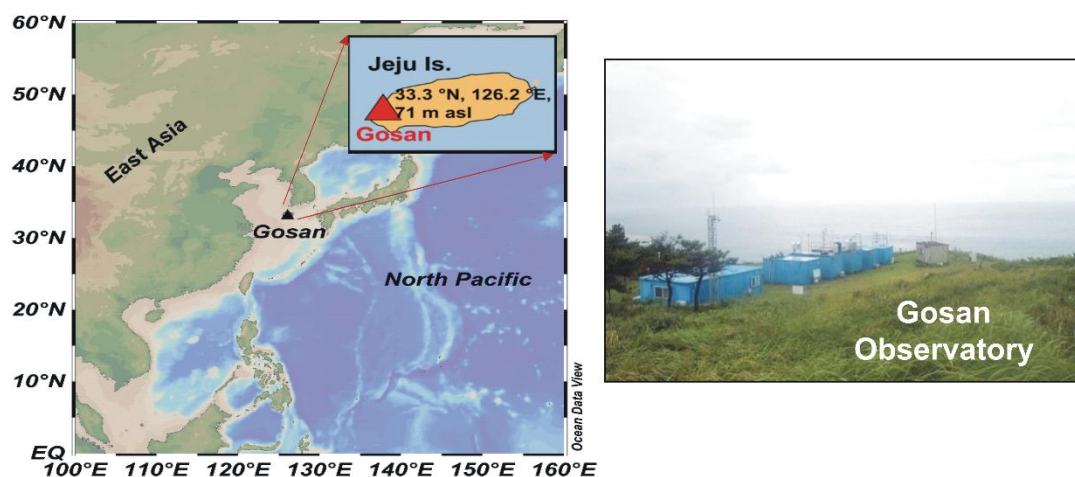


Fig.4.1. Geographical map, showing the location of sampling site (Gosan) in Jeju Island, located in the downwind of East Asian outflow.

Gosan receives mineral dust in winter/spring from the arid areas such as the Gobi and Taklamakan deserts in China, Mongolia and Russia [Duce *et al.*, 1980]. Asian dust serves as a carrier for soil microorganisms in East Asia to the North Pacific. Several studies have been carried out over Gosan to understand the sources, transport, and transformation processes of mineral dust and anthropogenic aerosols in the East Asian outflow to the North Pacific [Kawamura *et al.*, 2004; Wang *et al.*, 2009a; Yamamoto *et al.*, 2013; Kundu and Kawamura, 2014]. However, only few studies focus on the atmospheric transport of soil microbes and terrestrial lipid components from East Asia to the North Pacific [Kawamura *et al.*, 2003].

Aerosol samples were collected at Gosan site on a daily/few days basis from April 2001 to March 2002 as a part of ACE-Asia campaign [Kawamura *et al.*, 2004]. Pre-combusted (450 °C, 6 h) quartz fiber filters (PALLFLEX[®] 2500QAT-UP, 20 cm x 25 cm) were used to collect aerosol samples by using high volume air sampler (Kimoto AS-810, flow rate 50 m³ h⁻¹) installed on the top of the tower (~15 m above the ground) during April 2001 and then, after May 2001, sampler was moved down to the rooftop of a trailer house (~3 m above the ground). Filters were kept in a clean glass jar with a Teflon-lined screw cap before and after sampling. Precautions were taken to avoid contamination while collecting aerosol and blank filter samples. After sampling, all the filters were stored in a dark cold room at -20 °C until analysis.

Hydroxy FAs were extracted, identified and quantified in aerosol and CRM samples as described in chapter 2.

4.3 Results and Discussion

4.3.1. MODIS aerosol products and back-trajectory analyses

The Moderate Resolution Imaging Spectro-radiometer (MODIS) provides a large-scale regional view of the aerosol during the ACE-Asia time period. MODIS aerosol products were employed to analyze the seasonal variability of aerosols over East Asia. In this study, MODIS/Terra monthly global products (MOD08_M3) of aerosol optical depth (AOD) at 550 nm (1° to 1° spatial resolution) were used (Fig. 4.2). Detailed descriptions of the MODIS products, retrieval algorithms, accuracy and validation over East Asia have been described in previous studies [Chu *et al.*, 2005; Remer *et al.*, 2005].

In Fig. 4.2, violet to blue colors indicate clearer air and low AOD values (0-0.4) increasing through color scale to dark red represents higher pollutant levels and higher AOD (0.6-1). During spring and winter, AOD values are very high in the northern and southeast China. This could be due to the severe dust events mixed with urban/industrial pollutants observed in spring and winter. In addition, biomass burning from Southeast Asia is responsible for emitting high levels of soot and organic compounds to the atmosphere during winter and early summer. Strong convective currents generated during the Asian dust and biomass burning seasons can uplift the pollutants and biogenic particles (including soil microorganisms) and help in their atmospheric transport to the long distances reaching as far as the America and beyond.

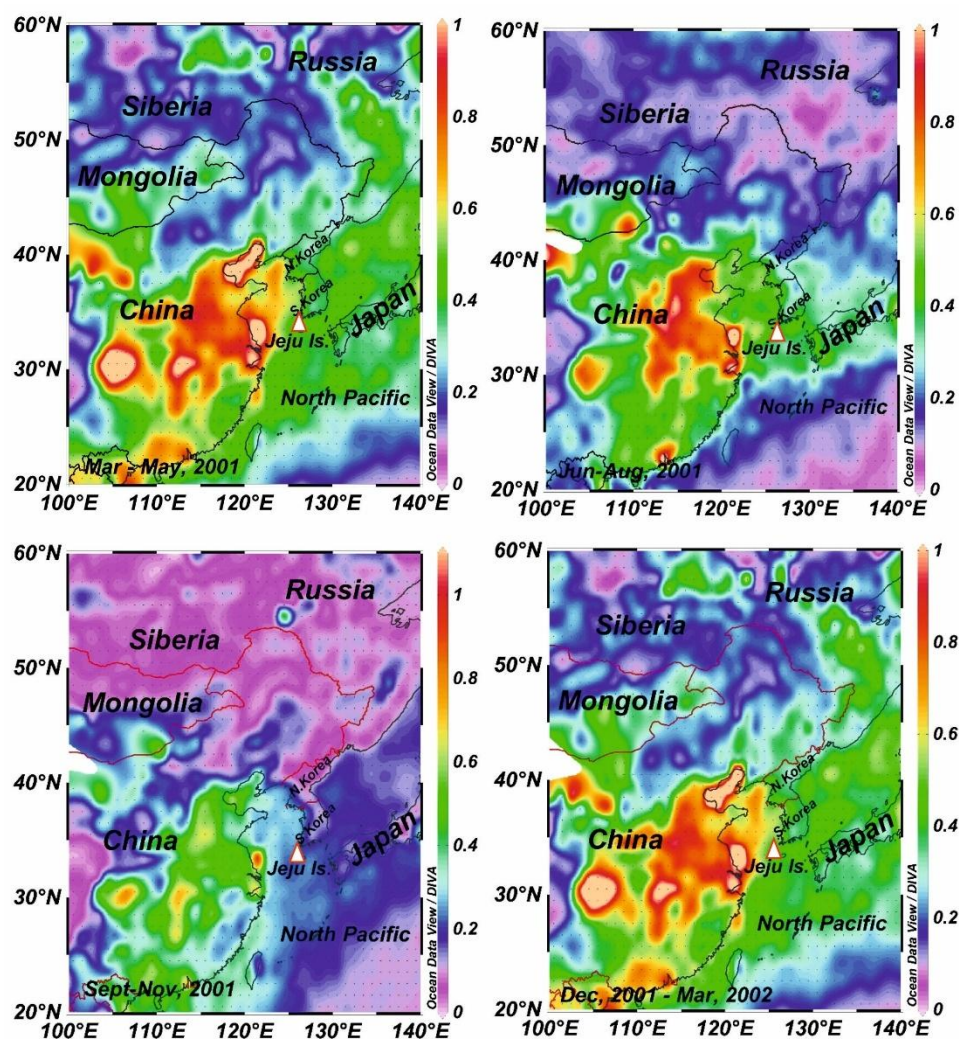


Fig.4.2. Map, showing the sampling site (Jeju Island) along with aerosol optical depth derived from MODIS satellite (AOD) during April 2001-March 2002.

Air mass back-trajectories (AMBTs) provide information on potential source regions of hydroxy FAs in TSP at Gosan. For this study, 7-day isentropic AMBTs were computed at air mass arrival heights of 100, 500 and 1000 m above ground level (Fig. 4.3) using a hybrid single particle lagrangian integrated trajectory model (HYSPLIT, version-4; [Draxler and Rolph, 2009; Draxler et al., 2012]) and using archived meteorological data sets from the NOAA air resources laboratory. AMBTs for winter and spring samples showed their origin from Siberia, Mongolia and up to some extent from China. Large spread in AMBT cluster (Fig. 4.3) for the sampling days in spring than those in winter, suggests more heterogeneity or temporal shifts in the contributing source regions during spring. On the other hand, AMBTs in summer mostly originated from the western North Pacific due to prevailing westerlies. Since autumn is a transition period between summer and winter, AMBTs showed mixed

origin (*i.e.*, from oceanic and continental air masses) over Gosan. Overall, AMBT analyses clearly suggest that Gosan is heavily influenced by the East Asian outflow during winter (December-February) and spring (March-May).

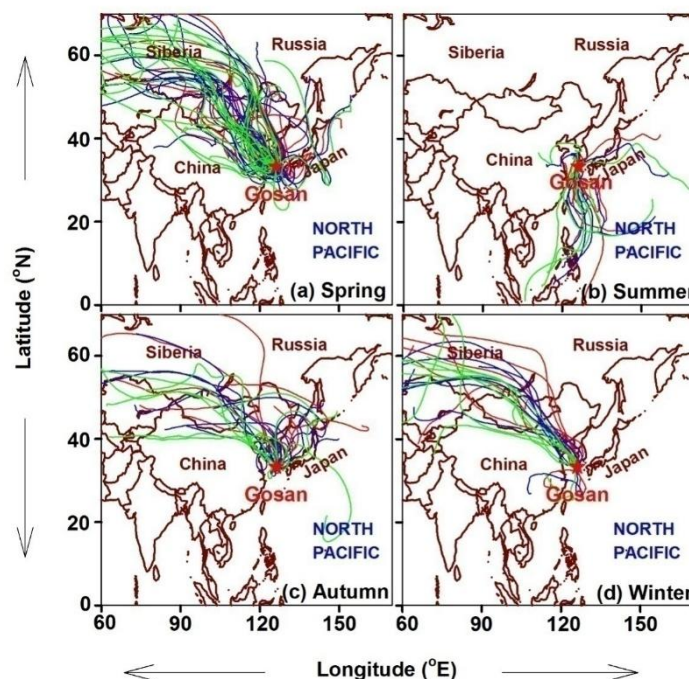


Fig.4.3. 7-day isentropic air mass back-trajectories computed at arrival heights of 100, 500 and 1000 m (correspond to red, blue and green, respectively) using HYSPLIT model for the sampling days (April 2001-March 2002) over Gosan, Jeju Island.

4.3.2. Seasonal and temporal variability

Mass concentrations of hydroxy FAs over Gosan showed pronounced temporal (Table 4.1) and seasonal variability (Fig. 4.4). In particular, total mass concentrations of β -(C₇-C₂₀)- and ω -(C₇-C₃₄)-hydroxy FAs were higher in spring (av. 3.7 and 20.5 ng m⁻³, respectively) and winter (1.9 and 13 ng m⁻³, respectively) than those in summer and autumn. This is attributed to their enhanced atmospheric transport of soil- and plant-associated microbes, and higher plant waxes from the Asian continent (Fig. 4.4). Interestingly, total mass concentrations of α -(C₂₁-C₃₄)-hydroxy FAs were found to be higher in winter (0.86 ng m⁻³) than spring and autumn (0.34 and 0.41 ng m⁻³, respectively; Fig. 4.4). These α -hydroxy FAs, unlike β - and ω -hydroxy FAs, have mixed sources such as crop-residue combustion, epicuticular waxes and also from the α -oxidation of monocarboxylic acids during their atmospheric transport [Fulco, 1967; Volkman, 2006]. For instance, high concentrations of α -hydroxy FAs were also found in fresh snow collected in Sapporo, northern Japan and in

aerosols influenced by the intense biomass burning emissions over Mt. Tai in the North China Plain. As α -hydroxy FAs do not possess any specific sources, they cannot be employed as tracers for plant and microbial metabolites in environmental samples.

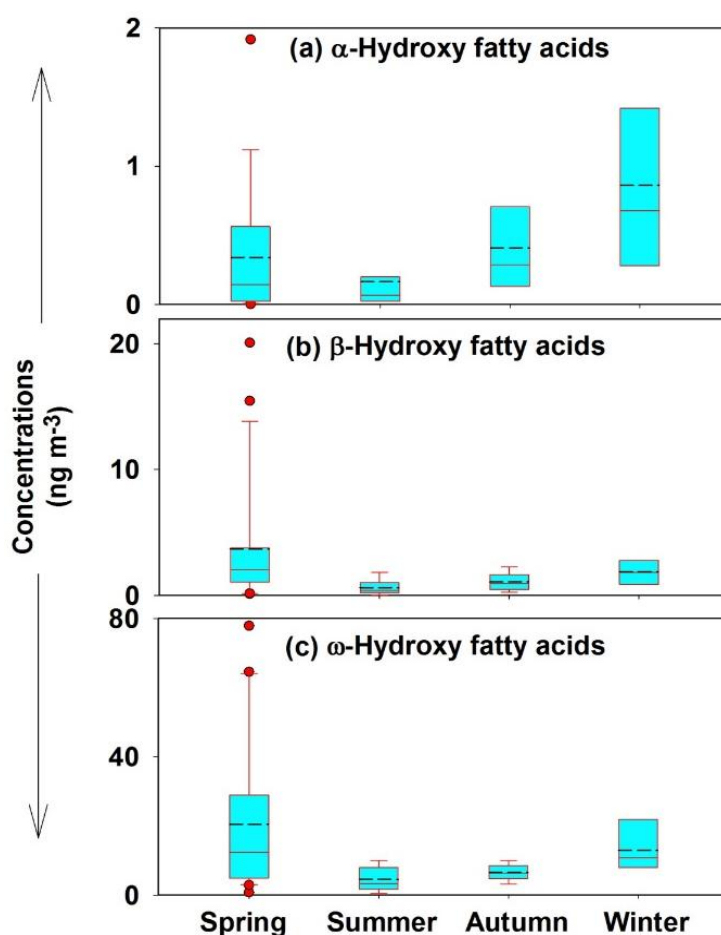


Fig.4.4. Box plots, showing the seasonal variability of mean atmospheric concentrations of measured α -, β - and ω -hydroxy fatty acids in TSP samples collected over Gosan, Jeju Island during April, 2001-March, 2002. The lower and upper edges of the boxes represent 25 and 75 percentile data, respectively. Likewise, the bottom and top whiskers correspond to 5 and 95 percentile data, respectively. The solid and dotted lines in each box refer to median and mean concentrations, respectively.

Table 4.1. Statistical summary of concentrations of α -, β - and ω -hydroxy fatty acids (FAs) in ng m^{-3} in TSP samples collected over Gosan, Jeju Island during April, 2001-March, 2002.

Compounds	Av. $\pm$ SE	Range (Median)	Av. $\pm$ SE	Range (Median)	Av. $\pm$ SE	Range (Median)
	α -Hydroxy FAs(C_{21} - C_{34})		β -Hydroxy FAs(C_7 - C_{20})		ω -Hydroxy FAs(C_7 - C_{32})	
C_7			0.09 $\pm$ 0.01	0.01-0.21 (0.09)	0.28 $\pm$ 0.11	0.02-1.04 (0.18)
C_8			0.21 $\pm$ 0.03	0.02-0.88 (0.19)	0.17 $\pm$ 0.06	0.00-2.63 (0.1)
C_9			0.18 $\pm$ 0.02	0.02-0.61 (0.16)	0.18 $\pm$ 0.02	0.01-0.50 (0.15)
C_{10}			0.29 $\pm$ 0.06	0.01-2.26 (0.18)	0.15 $\pm$ 0.02	0.01-0.54 (0.12)
C_{11}			0.1 $\pm$ 0.01	0.00-0.41 (0.07)	0.23 $\pm$ 0.03	0.01-0.71 (0.15)
C_{12}			0.86 $\pm$ 0.4	0.00-15.6 (0.12)	0.6 $\pm$ 0.07	0.01-2.67 (0.48)
C_{13}			0.07 $\pm$ 0.01	0.00-0.25 (0.05)	0.1 $\pm$ 0.01	0.00-0.33 (0.09)
C_{14}			0.16 $\pm$ 0.03	0.01-0.84 (0.12)	0.46 $\pm$ 0.05	0.04-1.8 (0.37)
C_{15}			0.06 $\pm$ 0.01	0.00-0.18 (0.04)	0.21 $\pm$ 0.02	0.02-0.68 (0.19)
C_{16}			0.17 $\pm$ 0.02	0.01-0.65 (0.14)	3.59 $\pm$ 0.53	0.06-17.1 (2.62)
C_{17}			0.04 $\pm$ 0.01	0.00-0.15 (0.03)	0.22 $\pm$ 0.06	0.00-2.38 (0.15)
C_{18}			0.12 $\pm$ 0.02	0.00-0.50 (0.09)	0.71 $\pm$ 0.31	0.01-13.6 (0.3)
C_{19}			0.03 $\pm$ 0.00	0.00-0.11 (0.02)	0.17 $\pm$ 0.02	0.00-0.62 (0.14)
C_{20}			0.07 $\pm$ 0.01	0.01-0.27 (0.05)	0.63 $\pm$ 0.14	0.01-4.46 (0.33)
C_{21}	0.04 $\pm$ 0.01	0.00-0.10 (0.03)			0.41 $\pm$ 0.1	0.03-3.39 (0.19)
C_{22}	0.07 $\pm$ 0.01	0.01-0.24 (0.07)			2.83 $\pm$ 0.95	0.02-37 (0.79)
C_{23}	0.04 $\pm$ 0.01	0.00-0.16 (0.03)			0.19 $\pm$ 0.03	0.01-1.01 (0.12)
C_{24}	0.08 $\pm$ 0.01	0.00-0.27 (0.05)			1.97 $\pm$ 0.62	0.02-25.2 (0.75)
C_{25}	0.04 $\pm$ 0.01	0.00-0.13 (0.04)			0.19 $\pm$ 0.03	0.00-0.98 (0.13)
C_{26}	0.06 $\pm$ 0.01	0.00-0.24 (0.04)			0.81 $\pm$ 0.16	0.02-4.36 (0.44)
C_{27}	0.04 $\pm$ 0.01	0.00-0.12 (0.03)			0.14 $\pm$ 0.02	0.00-0.55 (0.11)
C_{28}	0.06 $\pm$ 0.01	0.00-0.21 (0.05)			0.42 $\pm$ 0.07	0.01-1.74 (0.23)
C_{29}	0.04 $\pm$ 0.01	0.00-0.10 (0.02)			0.11 $\pm$ 0.02	0.00-0.35 (0.08)
C_{30}	0.07 $\pm$ 0.01	0.01-0.22 (0.05)			0.15 $\pm$ 0.02	0.02-0.45 (0.12)
C_{31}	0.02 $\pm$ 0.00	0.00-0.06 (0.02)			0.1 $\pm$ 0.05	0.06-0.15 (0.1)
C_{32}	0.05 $\pm$ 0.01	0.00-0.16 (0.04)			0.12 $\pm$ 0.03	0.05-0.23 (0.09)
C_{33}	0.01 $\pm$ 0.00	0.00-0.03 (0.01)				
C_{34}	0.03 $\pm$ 0.01	0.00-0.07 (0.03)			0.07 $\pm$ 0.00	0.00-0.07 (0.07)
Total	0.41 $\pm$ 0.09	0.00-1.92 (0.2)	2.29 $\pm$ 0.53	0.04-20.1 (1.6)	13.6 $\pm$ 2.4	0.4-77.9 (8.5)

Overall, similar seasonal pattern is also reflected in the total mass of hydroxy FAs, which showed the highest average concentration in spring (24.2 ng m^{-3}) followed by winter (15.5 ng m^{-3}), autumn (7.9 ng m^{-3}) and summer (5.3 ng m^{-3}). Moreover, these results are consistent with the seasonal trend documented for remote marine aerosols from Chichijima Island in the western North Pacific during 1990-1993 and 2001-2003 (*see chapter 3*). However, concentrations of hydroxy FAs over Chichijima are lower than those observed over Gosan. Higher concentrations over Gosan is most likely due to the proximity of continental sources (biogenic/anthropogenic emissions), whereas the dilution effect is more significant during long-range transport of aerosols over Chichijima.

The seasonal trends of hydroxy FAs over Gosan typically reflect the variability in the contributing source regions and their emissions in the East Asian outflow over the North Pacific. This seasonal variability can be better understood by examining the pollution rose diagrams (generated using “*Openair package*” [Carslaw and Ropkins, 2012; Carslaw, 2015]), where concentrations of hydroxy FAs are combined with meteorological parameters (Fig. 4.5). Over Gosan, winds blowing from NE and/or NW are responsible for the observed high concentrations of β - and ω -hydroxy FAs during winter and spring. Combining this information with AMBT analyses, we infer that atmospheric transport of aerosols from Northeast Asia (northern China, Mongolia and Siberia) mostly contributes to hydroxy FAs in TSP collected at Gosan in winter and spring.

Despite the lower concentrations, a similar pattern is noteworthy for β - and ω -hydroxy FAs over Gosan during autumn (Fig. 4.5). A sudden change in prevailing winds from NW and/or NE to SW over Gosan (*i.e.*, transport mostly from the South/East China Sea) during summer months could explain the observed lowest concentrations of β - and ω -hydroxy FAs. The pollution rose diagrams of α -hydroxy FAs indicate a notably high concentration in winter during which Gosan is influenced by the long-range atmospheric transport of aerosols from NE and/or NW China. However, their concentrations remain low over Gosan for other seasons, perhaps; due to their mixed sources (*see the above discussion*).

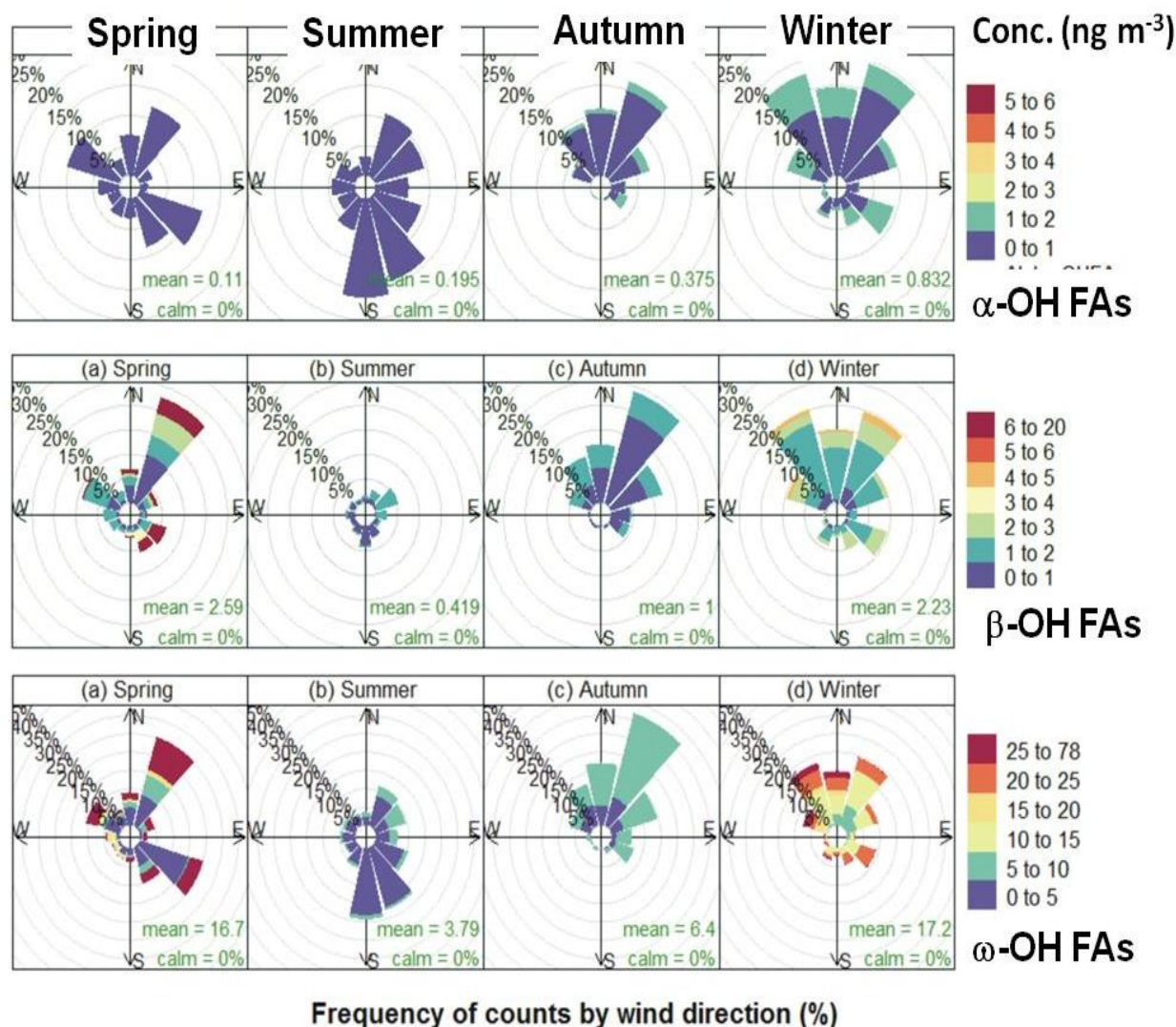


Fig.4.5. Pollution rose diagram (source: ‘OpenAir package’), showing the seasonal variability of α -, β - and ω -hydroxy FAs (OH FAs) in TSP collected at Gosan during April 2001– March 2002.

A comparison of seasonal variability between hydroxy FAs and other chemical tracers in TSP [Kawamura *et al.*, 2004; Fu *et al.*, 2012] is also made to further support the arguments related to their contribution from microbial lipids associated with mineral dust transport in the East Asian outflow (Fig. 4.6). Several studies have used water-soluble non-sea-salt Ca^{2+} (nss- Ca^{2+}) in ambient aerosols to trace the transport of mineral dust [Arimoto *et al.*, 2004; Srinivas and Sarin, 2012]. Likewise, methanesulfonic acid (MSA) in the East Asian outflow originates from biomass burning/biogenic emissions over the North Pacific [Boreddy and Kawamura, 2015]. Likewise, trehalose is a tracer for fungal spores associated with soil organic matter and has been observed over Chichijima during winter and spring [Chen *et al.*,

2013]. Therefore, concurrent increases in soil organic matter (source of GNB-specific β -hydroxy FAs) and biogenic emissions (ω - and α -hydroxy FAs) in the East Asian outflow during winter and spring are responsible for the observed seasonal variability of hydroxy FAs over Gosan.

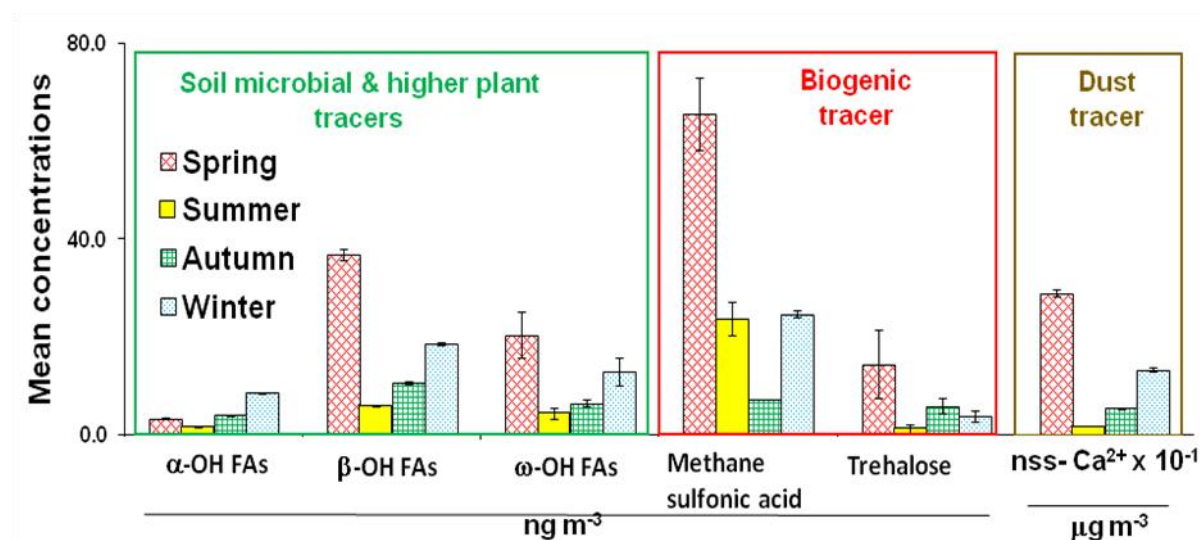


Fig.4.6. Seasonal variability of mean concentrations of water-soluble non-sea-salt (nss) - Ca^{2+} , methanesulfonic acid (MSA), trehalose and hydroxy FAs (α -, β -, and ω -isomers) in TSP collected at Gosan during April 2001-March 2002. The data of trehalose are from Fu et al. [2012].

4.3.3. Molecular distributions

The molecular distributions of α -, β - and ω -hydroxy FAs over Gosan are characterized by even-to-odd carbon predominance. A notable feature that pertains to these molecular distributions is the occurrence of only high (HMW: C_{21} - C_{34} ; Fig. 4.7) and low (LMW: C_7 - C_{20} ; Fig. 4.8) molecular weight α - and β -hydroxy FAs, respectively. However, we have detected both LMW and HMW ω -hydroxy FAs (Fig. 4.9) during the study period (April, 2001-March, 2002).

Interestingly, molecular distributions of α -hydroxy FAs, which are characterized by a predominance of C_{22} (α -hydroxydocosanoic acid) and C_{24} (α -hydroxytetracosanoic acid), exhibited a similar pattern for all the seasons. This could be associated with their origin, that is, photochemical oxidation and/or chemical reactions (hydrolysis of epicuticular leaf waxes) involved with biogenic fatty acids, which have contribution from both terrigenous (e.g., epicuticular waxes of higher plants [Kawamura et al., 2003]) and marine sources (e.g., algae,

sea grasses [Volkman *et al.*, 1980; De Leeuw *et al.*, 1995]). Cranwell [1981] suggested that HMW α -hydroxy FAs (*i.e.*, $>C_{20}$) are derived as the by-products of microbial mediated fatty acid metabolism through α -oxidation. Thus, abundances of C_{21} - C_{34} α -hydroxy FAs at Gosan during winter and spring seasons indicate their origin from photochemical or microbial oxidation of higher plant waxes during long-range atmospheric transport. Likewise, α -hydroxy FAs from C_{22} to C_{30} were detected in green microalgae from genus *Choricystis* and those from C_{26} to C_{30} were identified in class *Eustigmatophyceae* [Volkman *et al.*, 1998; Zhang *et al.*, 2014]. Therefore, transport of marine derived algal decomposed products, emitted from the ocean surface, could explain the atmospheric abundances of α -hydroxy FAs during summer and autumn (for which AMBTs showed marine origin).

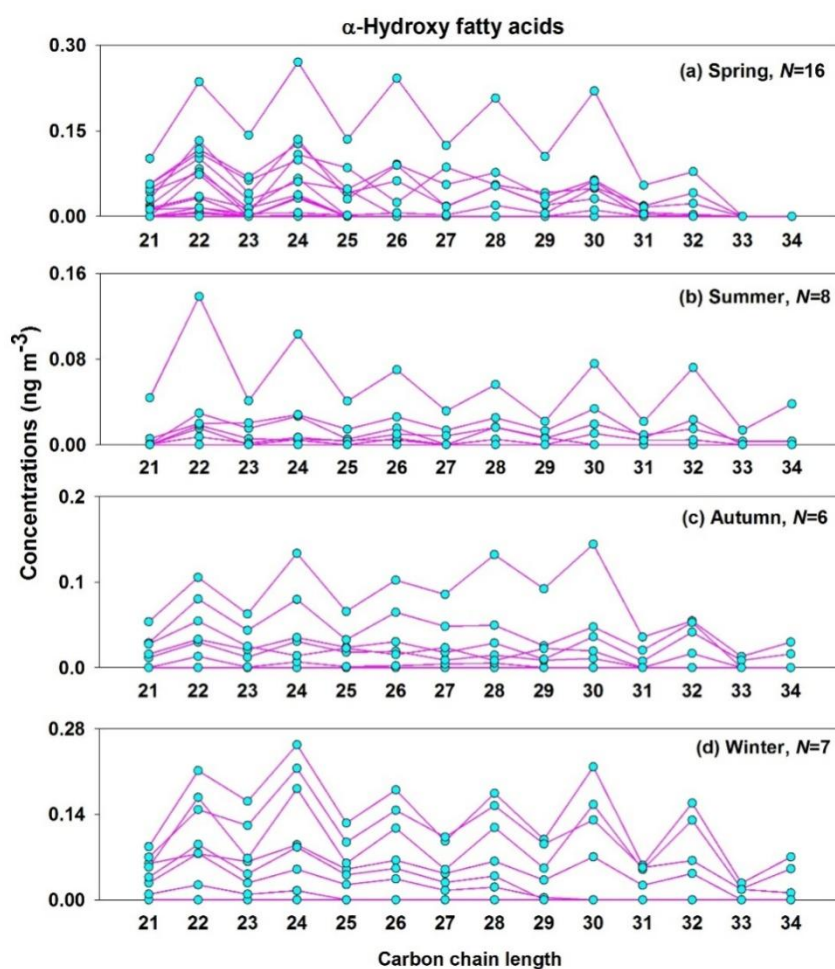


Fig.4.7. Molecular distributions of α -hydroxy FAs (C_{21} - C_{34}) in aerosols collected over Gosan during 2001-2002.

A comparison of molecular distributions of β -hydroxy FAs over Gosan with those from Chichijima reveals their common source and transport pattern (Fig. 4.8). A remarkable similarity in the molecular distributions of β -hydroxy FAs was observed in spring between Gosan and Chichijima, with a characteristic C_{12} predominance. A closer look at the measurements of β -hydroxy FAs indicates that TSP samples collected in April at Gosan showed relatively high concentrations of C_{12} β -hydroxy FA (β -hydroxydodecanoic acid). These samples are significantly influenced by the dust outbreaks (also referred as Kosa events) in East Asia, as inferred from higher abundances of Ca^{2+} (a proxy for mineral dust) in TSP samples. *Maki et al.* [2014] documented large amounts of *Cyanobacteria* (Gram-negative bacteria) during Kosa events which can be transported from the Sea of Japan and the continental area of China to the North Pacific. Likewise, molecular distribution of β -hydroxy FAs showed similar temporal trends in summer and autumn at both the receptor sites, characterized by the predominance of C_{16} (β -hydroxypalmitic acid) followed by C_{10} (β -hydroxydecanoic acid) or C_{18} β -hydroxy FA (β -hydroxystearic acid).

The observed shifts in the molecular distribution of β -hydroxy FAs between spring and summer/autumn are mostly due to differences in the contributing source type (*i.e.*, soil bacteria and/or marine dissolved organic matter, respectively). *Wakeham et al.* [2003] documented that dissolved or particulate organic matter from *Trichodesmium sp.* (marine Gram-negative cyanobacteria) is characterized by the predominance of C_{14} (β -hydroxymyristic acid) or C_{16} β -hydroxy FA. In a previous study, *Wilson* [2003] documented wide spread and long lasting occurrence (covering more than 350,000 km² and lasting for a period of four months) of *Trichodesmium* blooms in a subtropical gyre of the North Pacific during late summer. Since easterly wind regimes dominate Chichijima and Gosan during summer and autumn, observed molecular distributions with C_{16} (β -hydroxypalmitic acid) predominance in marine aerosols could be attributed to their contribution from marine derived organic matter. *Kundu and Kawamura* [2014] suggested that marine derived organic matter from the surface water of the western North Pacific could influence the atmospheric aerosols over Gosan, as inferred by the increased atmospheric abundances of azelaic acid (a photochemical oxidation product of marine biogenic unsaturated fatty acids such as oleic acid) and methanesulfonic acid (a photochemical oxidation product of oceanic dimethyl sulphide).

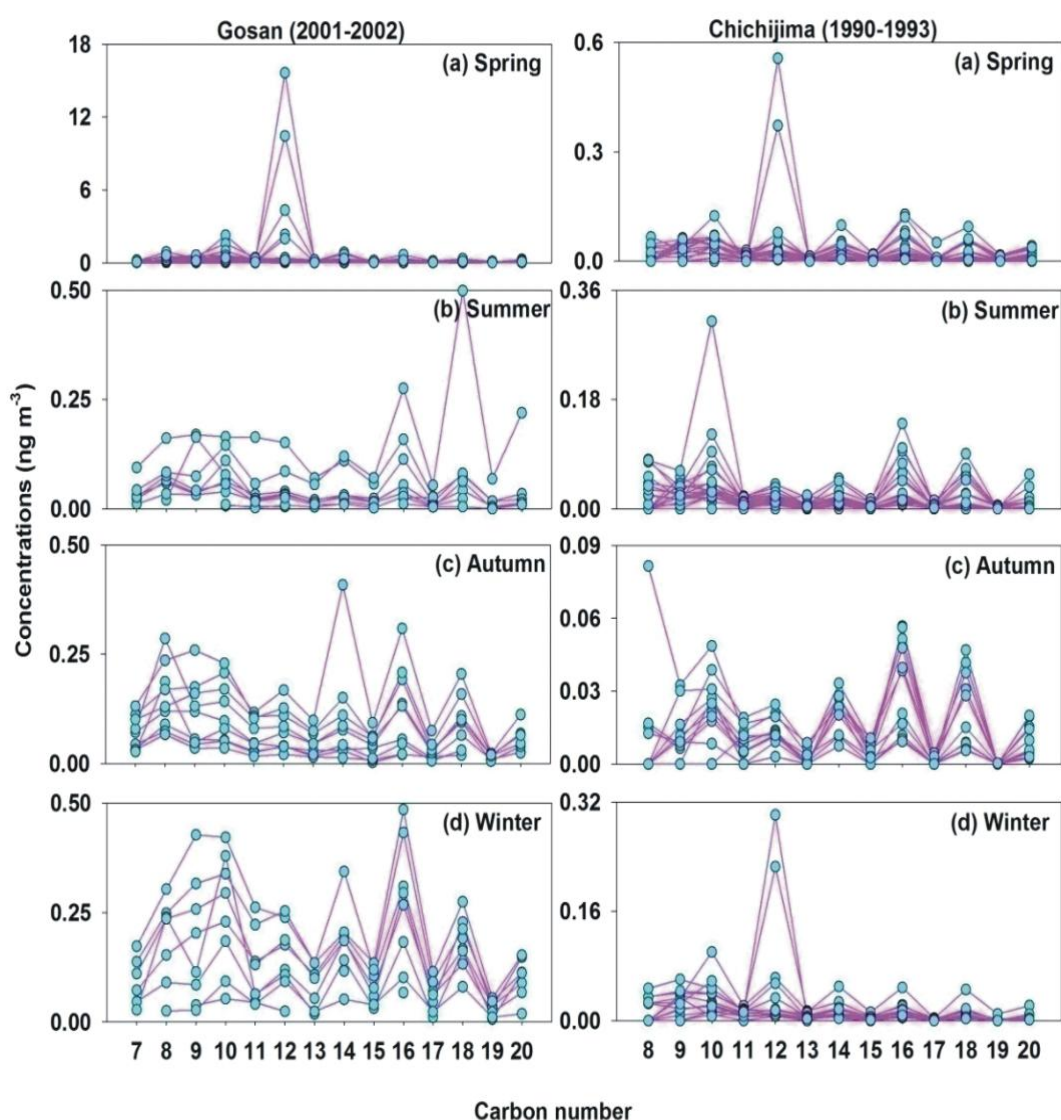


Fig.4.8. Molecular distributions of β -hydroxy FAs in aerosols collected over Gosan during 2001- 2002 and Chichijima during 1990-1993

On the other hand, a significant mismatch is noteworthy with regard to molecular distributions of β -hydroxy FAs in winter over Gosan and Chichijima. However, it is implicit from Fig. 4.8 that molecular distributions over Chichijima in winter are consistent with those in spring. Since Gosan is much closer to the continent (East Asia) than Chichijima, the impact of local anthropogenic/natural sources (such as urban dust/loess deposits, respectively) from China (inferred based on dominant air mass origin; Fig. 4.3) over Gosan could be a likely factor for the observed discrepancies between the two receptor sites. The similar molecular distributions of β -hydroxy FAs between winter and spring over Chichijima with increased atmospheric abundances during latter season could be due to their common source of soil microbes (also supported by the similar AMBT cluster; Fig. 4.3) and enhanced

mobilization of mineral dust in spring. Also, the similarity in the distribution of β -hydroxy FAs between Gosan and Chichijima during spring with relatively high concentrations for the former sampling site could be explained by the proximity of Gosan site to the Asian continent.

Higher concentrations of ω -hydroxy FAs were observed at Gosan than those from Chichijima (Fig. 4.9), which could be attributed to the proximity of the former sampling site to continental sources (*e.g.*, terrestrial higher plant emissions). At Gosan, highest concentrations of ω -hydroxy FAs were observed in spring followed by winter and autumn. However, the concentrations were found to be lowest in summer over Gosan when the air mass transport is mainly from the western North Pacific. At Chichijima, abundances of ω -hydroxy FAs in summer are comparable to those of spring followed by winter and autumn. A remarkable similarity in the predominance of C_{16} ω -hydroxy FA (16-hydroxypalmitic acid) is noteworthy for all seasons over Gosan, perhaps linked to its proximity to continental sources of higher plants.

The molecular distributions of ω -hydroxy FAs over both Gosan and Chichijima in spring and winter are characterized by the predominance of C_{16} and/or C_{22} (22-hydroxydocosanoic acid) followed by C_{24} (24-hydroxytetracosanoic acid), suggesting their common source contribution from land derived higher plants. However, significant differences were observed between Gosan and Chichijima with respect to the predominance of ω -hydroxy FAs in summer (C_{16} and C_{24} , respectively). The shift in the carbon chain length of ω -hydroxy FAs at Chichijima in summer cannot be explained by the impact of East Asian outflow. However, the local sources (island based emissions from vascular plants, although minor) could contribute for such shifts in the molecular distribution of ω -hydroxy FAs.

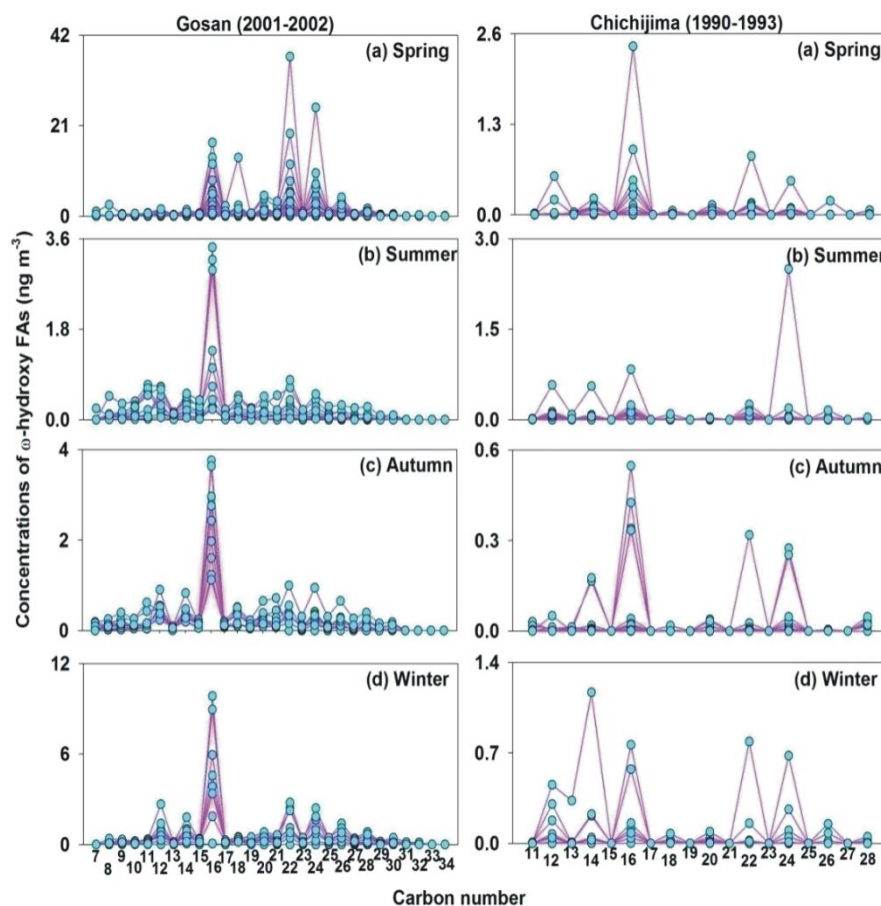


Fig.4.9. Molecular distributions of ω -hydroxy FAs in aerosols collected over Gosan during 2001-2002 and Chichijima during 1990-1993.

4.3.4 Cluster analysis: source apportionment

AMBTs were clustered according to season in order to ascertain the relative contribution of various geographical sources to atmospheric abundances of hydroxy FAs over Gosan (Fig. 4.10). Furthermore, MODIS derived fire count data were superimposed on mean trajectory pathways of each identified clusters to evaluate the effects of biomass burning on the atmospheric abundances and transport of hydroxy FAs at Gosan (Fig. 4.10). In spring, localized air masses within Korea contribute to 40% of aerosol composition at Gosan, while the transport from Siberia and Mongolia accounts for 52% (cluster 2 and 3) and 7% (cluster 4), respectively. Recent studies documented the impact of Mongolian desert dust on the Yellow Sea and South China through long-range atmospheric transport [Tan *et al.*, 2012]. Likewise, several studies have documented the influence of long-range atmospheric transport of Siberian biomass burning emissions to the North Pacific [Ding *et al.*, 2013; Verma *et al.*,

2015]. Based on the analyses of ^{14}C and biogenic organic tracers in aerosols collected from Sapporo (Japan), Pavuluri *et al.* [2013] documented the influence of biomass burning emissions in Siberia through long-range atmospheric transport. Therefore, hydroxy FAs over Gosan during spring season may have a contribution from both Mongolian desert dust and higher plant waxes from Siberia as part of the East Asian outflow to the North Pacific.

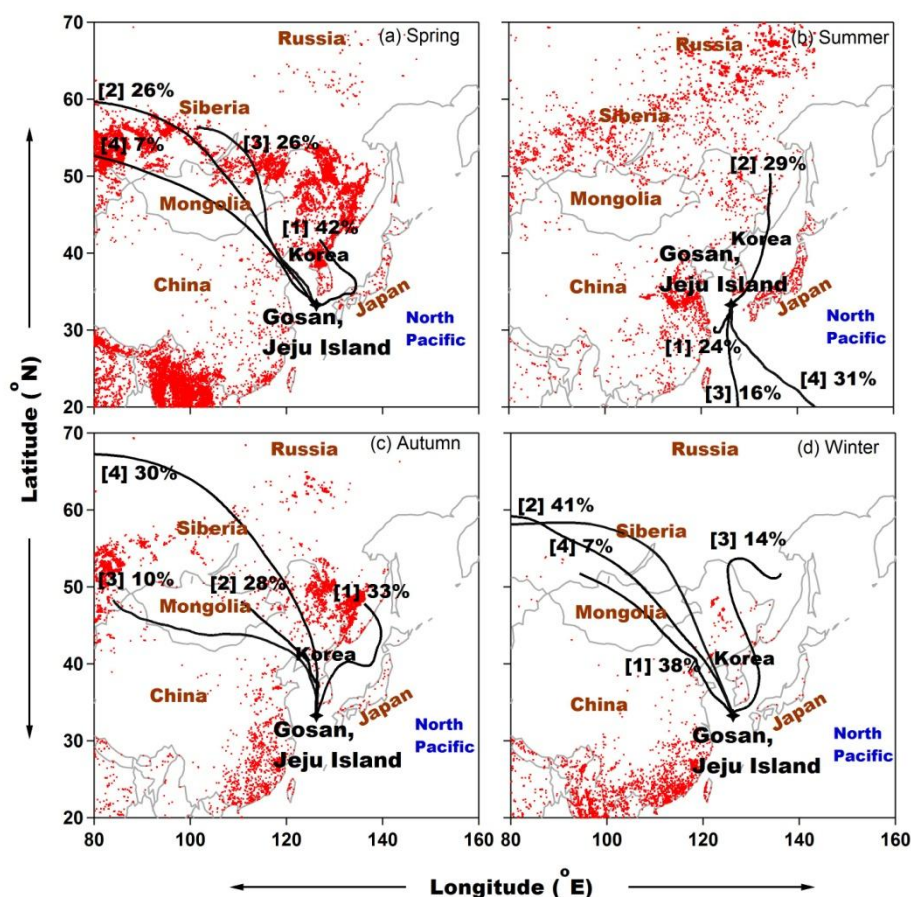


Fig.4.10. Mean trajectory paths of air mass back-trajectories for the four clusters at 100 m above ground level during April 2001-March 2002 over Gosan, Jeju Island.

Although no change was observed in mean trajectory paths of four clusters for winter and spring, relative source contribution to the sampling site (Gosan) varied significantly. Air mass transport from China and Korea in winter over Gosan contributed 38% and 14%, respectively, while transport from Siberia and Mongolia together accounted for ~41% and 7%, respectively. Zhu *et al.* [2015] documented that biomass burning emissions in China could influence the organic aerosol composition over the North Pacific during winter. Similar molecular distributions of β -hydroxy FAs in Gosan winter aerosols with two CRM samples (CJ-1 and CJ-2) indicate an important contribution of soil microbes from Chinese loess.

Therefore, transport of Chinese loess and biomass burning emissions could contribute significantly to atmospheric hydroxy FAs over Gosan during winter. Likewise, biomass burning-derived saccharides from Siberia and Mongolia were detected in remote marine aerosols from Chichijima in the western North Pacific [Chen *et al.*, 2013]. Hence, Siberian forest fires could contribute to hydroxy FAs over Gosan in winter *via* atmospheric transport. Interestingly, major source regions in autumn are somewhat similar to those in winter with minor contribution from oceanic air masses (cluster 1) from the Sea of Japan accounting for 30%. Recently Tsuda *et al.* [2015] documented the occurrence of phytoplankton blooms in the seas around the Japanese Islands. Therefore, marine derived organic matter emitted with sea spray could contribute to ambient hydroxy FAs over Gosan in autumn.

On the other hand, oceanic air masses transported across the Sea of Japan, North Pacific, Yellow Sea, and East China Sea over Gosan in summer account for 29% (cluster 2), 31% (cluster 4), 16% (cluster 3) and 24% (cluster 1), respectively (Fig. 4.10). Several studies have suggested that marine cyanobacteria and dissolved organic matter in the surface water mostly contain short-chain homologues of β - and ω -hydroxy FAs [Wakeham *et al.*, 2003]. In addition, phytoplankton blooms occur during late spring to early summer in the Sea of Japan [Kwak *et al.*, 2013], Yellow Sea and East China Sea [Yamaguchi *et al.*, 2012], and North Pacific [Miyazaki *et al.*, 2011]. Therefore, emission of sea spray containing marine algal excreta, dead phytoplankton and dissolved organic matter from the Sea of Japan, North Pacific, Yellow Sea, and East China Sea could be a major source of hydroxy FAs over Gosan during summer months.

4.3.5. Provenance of β -hydroxy FAs as bacterial tracers

As mentioned earlier, Gobi desert of Mongolia is a potential source region for hydroxy FAs measured in spring aerosols collected over Gosan. Seasonally averaged concentrations of β -hydroxy FAs were highest in spring (especially, March-April). These hydroxy FAs are specific markers for GNB and other soil microorganisms in environmental samples. β -Hydroxy FAs from C-number 10 to 18 are abundant in GNB cell membranes and can be employed as chemical markers (soil- and plant-associated bacteria) in atmospheric samples such as snow and marine aerosols. In this study, higher abundances of β -hydroxy FAs are in accordance with Asian dust events as characterized by high aerosol mass concentrations in spring [Fu *et al.*, 2012].

Dust events 1 and 2 were observed during 11-13 and 24-25 April 2001 having aerosol mass concentrations of $288 \mu\text{g m}^{-3}$ and $241 \mu\text{g m}^{-3}$, respectively. Dust event 3 was recorded during 21-23 March, 2002 with an average aerosol concentration of $883 \mu\text{g m}^{-3}$. These dust events were also characterized by the higher atmospheric abundances of nss-Ca^{2+} (a tracer of soil dust), trehalose (a tracer of soil microbes) and stable carbon isotopic ratios ($\delta^{13}\text{C}$) of total carbon in aerosol samples collected from Gosan [Kawamura *et al.*, 2004; Fu *et al.*, 2012]. During these dust events, atmospheric abundances of β -hydroxy FAs were higher and, hence, can be contributed by soil microorganisms residing over/in dust particles. To distinguish the source region for these dust events, we grouped the air mass back-trajectories into two clusters (Fig. 4.11) by using the HYSPLIT model. It is noteworthy that each of the three dust events is originating from the Mongolian Desert.

In addition, predominance of LMW and similar molecular distributions of β -(C_7 - C_{20}) ($\text{C}_8 > \text{C}_{10} > \text{C}_{12} \approx \text{C}_{14}$) were observed for the three Asian dust events having an origin from the Mongolian desert (Fig. 4.11). Furthermore, no similarity was found in the molecular distributions of β -hydroxy FAs between these three dust events and CJ-1 (Chinese loess) and CJ-2 (Asian mineral dust, China) samples. This result excludes a likely contribution of soil microbes (or GNB) from Chinese loess deposits. However, molecular distributions of β -hydroxy FAs in wintertime aerosols over Gosan match with CJ-1 and CJ-2, suggesting their source contributions from Chinese loess. Similarly, halotolerant bacteria into the free atmosphere were detected above the ground, Dunhuang City, China, [Maki *et al.*, 2008] where the long-range atmospheric transport of desert dust is frequently observed in winter. Therefore, observed molecular distributions of LMW β -hydroxy FAs in spring can be explained by the significant contribution of soil microbes from the Mongolian Gobi desert rather than Chinese loess.

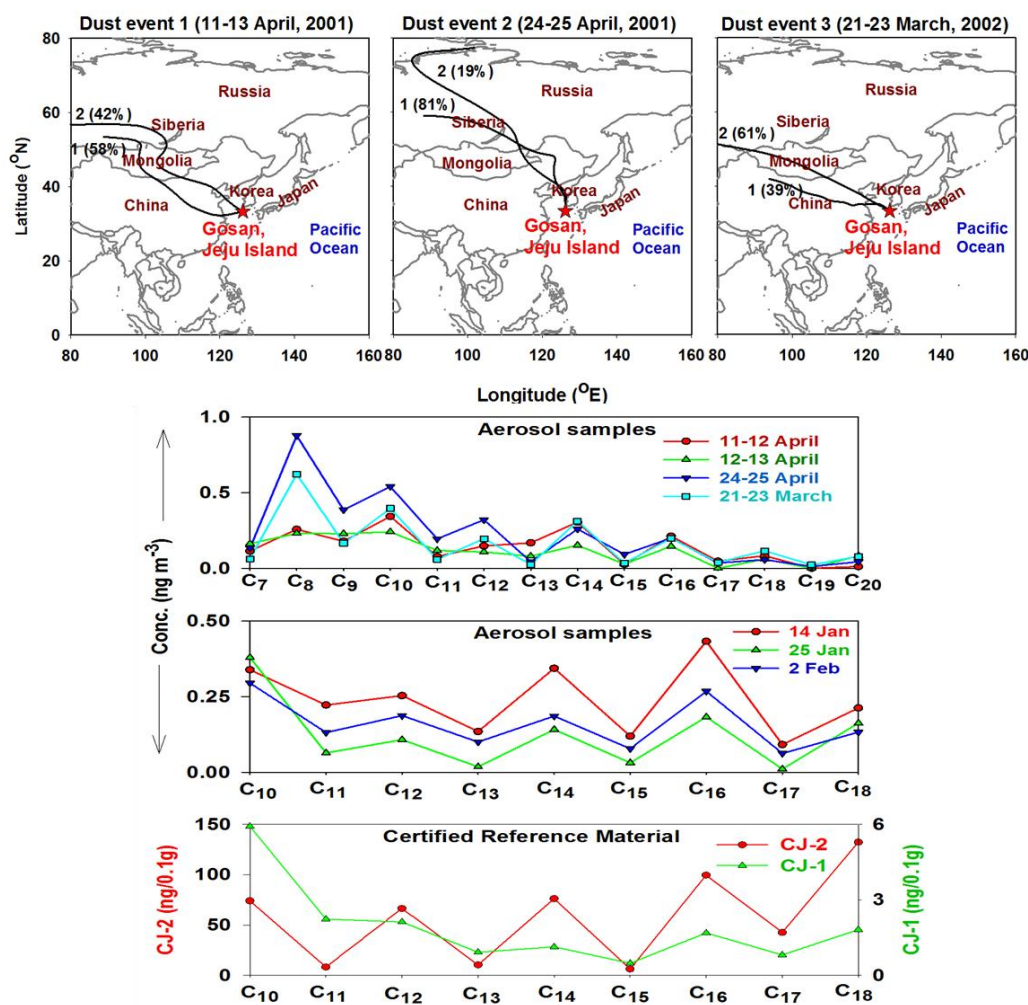


Fig. 4.11. Mean air mass back trajectory pathways of identified clusters for three specific dust events and molecular distributions of β -hydroxy FAs during three dust events.

4.3.6. Relative significance of continental vs. marine sources

Waterson and Canuel [2008] have used concentration ratio of terrigenous to aquatic fatty acids, a concept that was initially introduced by Meyers [1997], to examine the relative contribution of continental (higher plant waxes/soil microbes) vs. oceanic sources (algal/phytoplankton derived organic matter) to sediments. Similar approach was used based on hydroxy FAs over Gosan to deconvolute the relative significance of soil microbes *vis-à-vis* higher plant waxes in the East Asian outflow. In general, long-chain homologues of hydroxy FAs (*e.g.*, C₂₄, C₂₆, and C₂₈) are mostly from the epicuticular waxes of higher plants [Waterson and Canuel, 2008], while short-chain homologues from the soil microbes and

marine (*i.e.*, algae/phytoplankton) derived organic matter [Wakeham, 1999; Wakeham *et al.*, 2003] dominate the atmospheric abundances of hydroxy FAs. The concentration ratio of sum of C_{24} , C_{26} , and C_{28} hydroxy FAs to sum of C_{12} , C_{14} , and C_{16} hydroxy FAs; which can mathematically be represented as follows.

$$R_{OHFAs} = \left[\frac{C_{24} + C_{26} + C_{28}}{C_{12} + C_{14} + C_{16}} \right]_{OHFAs}$$

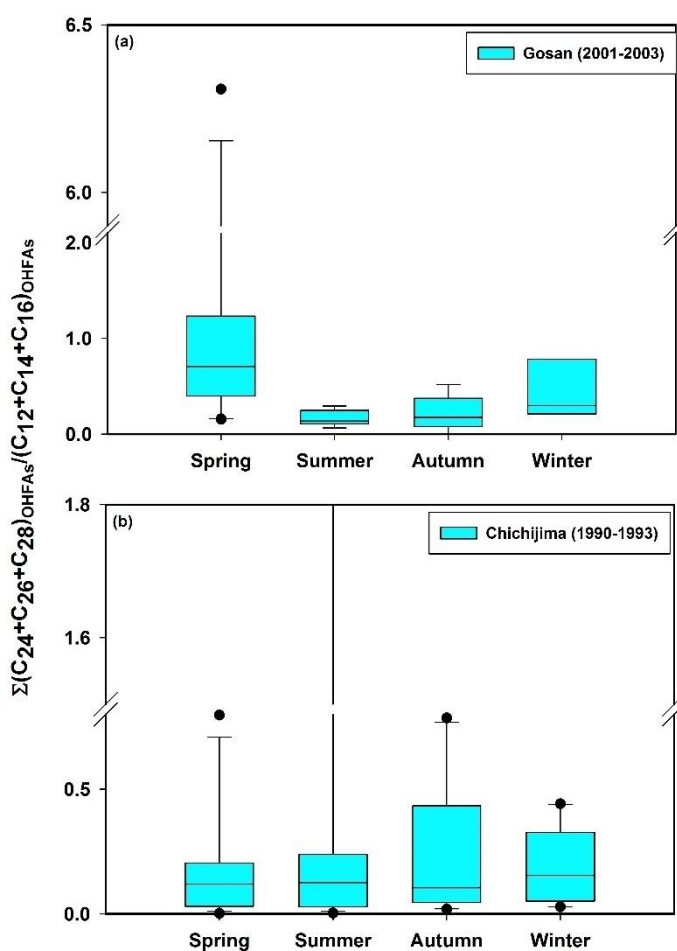


Fig.4.12. Box-whisker plots, showing the concentration ratio (R_{OHFAs}) of sum of C_{24} , C_{26} , and C_{28} hydroxy FAs to sum of C_{12} , C_{14} , and C_{16} hydroxy FAs at (a) Gosan and (b) Chichijima.

R_{OHFAs} follows a seasonal trend at Gosan with higher ratios in spring (median: 0.57) and winter (0.30) and lower ratios in summer (0.16) and autumn (0.17) (Fig. 4.12a). A comparison of R_{OHFAs} from Gosan with those from Chichijima (Fig. 4.12b) indicates a predominance of continental sources over Gosan site, while marine sources mostly contribute

to atmospheric hydroxy FAs at Chichijima site. No such seasonality is apparent for R_{OHFAs} at Chichijima because it is a pelagic oceanic island, where marine derived organic matter dominates hydroxy FAs in summer/autumn aerosols. Although influenced by the continental pollutants, oceanic sources overwhelm the contribution of the East Asian outflow even in spring and winter. On the other hand, R_{OHFAs} at Gosan in spring and winter indicate the dominant emissions from vascular plants (*e.g.*, lipid waxes) over soil microbes in the East Asian outflow.

4.3.7. Endotoxin levels and GNB dry mass

Endotoxin/lipopolysaccharides (LPS) present in the membranes of GNB, are responsible for the generalized inflammations in humans caused by the exposure to these compounds [Maitra *et al.*, 1986]. Lipid A region of endotoxin consists of a large portion of β -hydroxy FAs from carbon number 10 to 18 with even carbon dominance [Paba *et al.*, 2013]. These fatty acids in lipid A can be quantified from both biologically-active and inactive endotoxin [Laitinen *et al.*, 2001]. Hence, β -hydroxy FAs certainly have a potential as chemical markers for endotoxin in atmospheric particulate matter. At Gosan, endotoxin concentrations and GNB dry cell mass were estimated in aerosols sampled at Gosan by using a marker to microbial mass conversion factors on the basis of β -hydroxy FAs (C_{10} - C_{18}).

The endotoxin concentrations clearly showed spring/winter maxima (279 ± 98 EU m^{-3} and 114 ± 19.8 EU m^{-3} , respectively) and summer/autumn minima (35.5 ± 12 EU m^{-3} and 58.4 ± 13.9 EU m^{-3} , respectively). The concentrations of endotoxin (116 ± 10 EU m^{-3}) at Gosan site are much lower than those observed in vegetable storage houses in Germany assayed by both the techniques, LAL assay and β -hydroxy FAs [Laitinen *et al.*, 2001]. This could be due to higher abundances of GNB in the indoor aerosols collected from poorly ventilated vegetable storage houses than ambient aerosols collected at Gosan. However, endotoxin concentrations at Gosan (this study) are much higher than those reported in the urban and coastal aerosols as well as indoor dust samples collected from Israel [Yona *et al.*, 2014] and aircrafts in the USA [Hines *et al.*, 2003]. These results suggest that Jeju Island is heavily influenced by the continental outflow from East Asia, where re-suspended mineral dust and plant metabolites contribute significantly to ambient aerosols.

Yona *et al.* [2014] suggested that levels of endotoxin emitted from oceanic sources (*e.g.*, marine algae) and transported to urban and coastal aerosols are lower than those derived

from terrestrial sources. Similarly, *Hines et al.* [2003] measured the endotoxin concentrations for the ambient indoor samples collected inside the aircrafts and found that the air qualities are very clean compared to the outdoor air. Our results from Gosan are comparable to the endotoxin levels in aerosols collected at Science Museum (122 EU m^{-3}) in Hong Kong, which has higher population density and higher traffic load than those at other studied sites in Hong Kong [*Lee et al.*, 2004].

The estimated endotoxin levels at Gosan were considerably higher than the proposed occupational endotoxin exposure limit $\sim 90 \text{ EU m}^{-3}$ (DECOS, 2010) in spring and winter. AMBTs suggest the transport of endotoxin molecules along the dust from the Asian continent (Russia, Mongolia and China) in spring. Heinrich et al. [2003] documented high concentrations of endotoxin of LPS in $\text{PM}_{2.5-10}$ aerosols. This finding supports our observations that endotoxin is associated with dust, which can be transported long distances over the North Pacific. In summer and autumn, concentrations are well under the short- and long-term airborne exposure limit set by DECOS, 2010 [*Paba et al.*, 2013]. However, endotoxin molecules can be contributed from marine microbes in summer and, to a lesser extent, from the continental atmospheric transport in autumn.

The GNB dry mass, assessed based on β -hydroxy FAs at Gosan, is also higher in spring/winter ($928 \pm 326 \text{ ng m}^{-3}$ and $380 \pm 40.7 \text{ ng m}^{-3}$, respectively) than summer/autumn ($118 \pm 26.2 \text{ ng m}^{-3}$ and $194 \pm 30.5 \text{ ng m}^{-3}$, respectively). Several studies have documented the significance of long-range atmospheric transport of soil microorganisms [*Griffin*, 2007; *Lee et al.*, 2007 and references therein] during dust events. Our results clearly show higher abundances of dry mass of GNB in spring and winter than autumn and summer. The estimated dry mass loadings in winter at Gosan are comparable with those of *Lee et al.* [2004] at Hong Kong Science Museum ($313 \pm 1.3 \text{ ng m}^{-3}$). Likewise, estimated GNB dry masses at Gosan in autumn/summer are comparable to those reported at Hong Kong University ($182 \pm 1.3 \text{ ng m}^{-3}$).

Airborne bacteria are an integral component of atmospheric particulate matter and their abundance and composition are shaped by seasonal shifts in atmospheric conditions and also by the characteristics of the local terrestrial environment. Our study uses the chemical markers to determine soil- and plant-associated bacteria and endotoxin in aerosols, which can be useful for monitoring their health and climatic impacts. This study suggests that soil microbes and plant pathogens are the major contributors to the biodiversity over the

downwind oceanic regions influenced by the continental sources. Also, β -hydroxy FAs from C₁₂ to C₁₈ are associated with Gram-negative coliform bacteria of *Enterobacteriaceae* family, which are the source of airborne endotoxin [Pomorska *et al.*, 2007]. Thus, these bacteria could be pathogenic and infectious to the exposed population in the coastal areas.

4.3.8. Conceptual model: aeolian transport of soil microbes & higher plant metabolites

Overall, comparison of long-term datasets on hydroxy FAs from Gosan (this study) and Chichijima reveals similar seasonal changes in the composition of organic aerosols over the North Pacific during winter and spring (Fig. 4.13). Moreover, the concentrations of β - and ω -hydroxy FAs over Gosan are higher than those from Chichijima during the East Asian outflow season, which occurs in winter and spring. This observation together with their similar molecular distributions of β -hydroxy FAs reveals their sources of soil microbes as Chinese loess deposits in winter and mineral dust from the Mongolian Desert. On the other hand, biomass burning/biogenic emissions are the major sources of ω -hydroxy FAs in the continental outflow from East Asia. The relative decrease in mass concentrations of hydroxy FAs over Chichijima further indicates a significant terrigenous contribution of soil microbes and epicuticular waxes in the East Asian outflow to pelagic (deep sea) sediments. Therefore, this study re-establishes the significance of atmospheric hydroxy FAs as a potential paleoclimatic tracer for assessing the terrestrial contribution (*i.e.*, soil microbes and plant metabolites) with regard to contemporary changes in the climate of the Anthropocene.

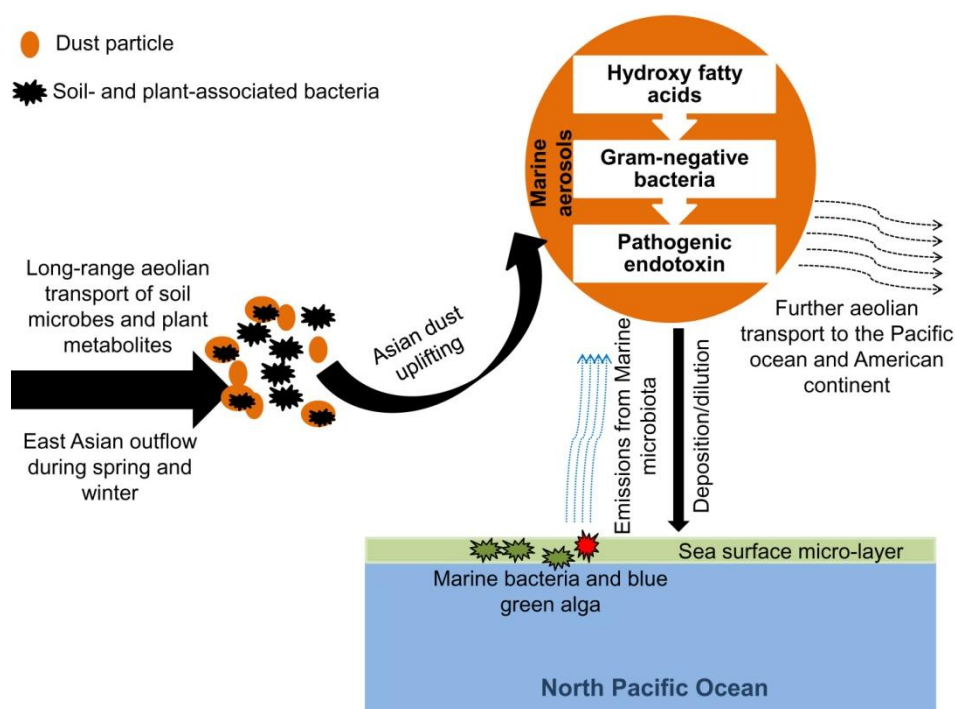


Fig.4.13. Conceptual model, showing the aeolian transport of soil microbes and plant metabolites in the East Asian outflow to the North Pacific, based on the comparison of molecular distributions and seasonal variability of α -, β - and ω -hydroxy FAs in aerosols over Gosan, Jeju Island (2001-2002) and Chichijima (1990-1993).

4.4. Summary and Conclusions

Atmospheric transport of soil microorganisms and higher plant waxes in East Asia significantly influences the aerosol composition over the North Pacific. This study investigates, for the first time, the year round atmospheric abundances of α -, β - and ω -hydroxy fatty acids (FAs), tracers of soil microorganisms (β -isomers) and plant waxes (α - and ω -isomers), in total suspended particles (TSP) collected at Gosan, Jeju Island during April 2001-March 2002. These hydroxy FAs showed a pronounced seasonality with higher concentrations in winter/spring and lower concentrations in summer/autumn. The seasonal variability of β -hydroxy FAs is consistent with other tracers of soil microbes (trehalose), re-suspended dust (nss-Ca^{2+}) and stable carbon isotopic composition ($\delta^{13}\text{C}$) of total carbon. This observation together with back-trajectories over Gosan in spring clearly indicates their source as “Asian dust”. A comparison of molecular distributions of β -hydroxy FAs over Gosan with those in Asian mineral dust standards (CJ-1 and CJ-2) and marine aerosols from a remote

island in the North Pacific (Chichijima) reveal that desert sources in China during winter and arid regions of Mongolia and Far East Russia during spring are the major contributors of soil microbes. Also, contribution from higher plant waxes dominates the East Asian outflow over soil microbes. This study clearly shows that atmospheric transport of mineral dust from East Asia is a carrier of airborne bacteria and higher plant waxes to the North Pacific, where it can influence the microbial diversity and marine community structure in the surface water.

A comparison of molecular distribution of β -hydroxy FAs in TSP with those measured in Asian mineral dust standards (CJ-1 and CJ-2) reveal that desert sources in China during winter and arid regions of Mongolia and Far East Russia during spring are the major contributors of soil microbes over Gosan. Furthermore, molecular distributions of β -hydroxy FAs in springtime aerosols over Gosan are also consistent with those collected from remote island Chichijima in the western North Pacific. The clustering of AMBTs together with characteristic dominance of C_{12} β -hydroxy FAs over Gosan and Chichijima during spring reveals their source as “*Mongolian Desert*”. Also, contribution from higher plant waxes dominates the East Asian outflow over soil microbes. This study clearly shows that bacteria and higher plant waxes can either attach themselves to dust particles or form aggregates with them, and are available to long-range atmospheric transport.

Furthermore, atmospheric abundances of endotoxin and dry cell weight of bacteria in aerosols were estimated using the marker-to-bacterial mass conversion factors. Similar to the concentrations of hydroxy FAs, we found higher values of endotoxin and bacterial mass in winter and spring than in summer and autumn. This seasonal co-variability of endotoxin, bacterial dry mass and trehalose (tracer for soil microbes) clearly shows an atmospheric transport of soil- and plant-associated bacteria that can pose severe health impacts due to the exposure to their toxic metabolites.

Chapter 5

Trans-Hemispheric Distribution of α -, β -, and ω -Hydroxy Fatty Acids in the Marine Aerosols from the Western North Pacific: Sources & Latitudinal Variability

Chapter 5 presents the results of hydroxy FAs in TSP collected over the western Pacific during autumn cruise. The latitudinal variability of hydroxy FAs was discussed in terms of their influence from East Asia for the northern Pacific samples, while those from southern Pacific are influenced by oceanic sources.

5.1 Introduction

Atmospheric transport of mineral dust to the open ocean has a considerable influence on the biogeochemical cycles of dissolved carbon pool through the supply of various inorganic and organic constituents [Schulz *et al.*, 2012; Jickells and Moore, 2015]. Furthermore, atmospheric input is also a major source of terrestrial lipid compounds to the pelagic sediments. Several studies have used the hydroxy fatty acids (FAs) in deep sea sediments to understand the transport and distributions of soil microbes and plant waxes over the recent past [Kawamura, 1995; Zhang *et al.*, 2014; Wakeham *et al.*, 2003]. These hydroxy FAs are the constituent part in lipid fractions of a variety of organisms that include bacteria [Wilkinson, 1988], algae [Blokker *et al.*, 1998] and higher plants [Pollard *et al.*, 2008; Molina *et al.*, 2006]. Several studies have observed the occurrence of hydroxy FAs in lacustrine and marine sediments [Eglinton *et al.*, 1968; Volkman *et al.*, 1980; Cranwell, 1981] and lake waters [Kawamura *et al.*, 1987] as well as marine aerosols [Kawamura, 1995]. However, in the context of climate change scenario with regard to changing wind pattern, sources, and source strengths of soil microbes and plant waxes, there is urgent need to fully understand the use of hydroxy FAs.

The hydroxy FAs are categorized into different classes on the basis of the number and position of the hydroxyl (OH) group. Most commonly reported compounds are the aliphatic α -, β - and ω -mono-hydroxy FAs, which have been employed as tracers of soil microorganisms [Kawamura, 1995], bacteria [Lee *et al.*, 2004] and terrestrial plants [Kawamura *et al.*, 2003] in ambient aerosols, respectively. Likewise, hydroxy FAs have also been used as geochemical tracers to evaluate microalgal [Gelin *et al.*, 1997], bacterial and terrestrial plant contributions to sediments [Cordoso and Eglinton, 1983]. The aliphatic homologues series of β -hydroxy FAs from C₁₀ to C₁₈ are important structural constituents of lipopolysaccharides (LPS) and lipid A, especially in the cell wall of Gram-negative bacteria (GNB) and, hence, associated with bacterial endotoxin activity [Wilkinson, 1988; Paba *et al.*, 2013].

Rather few studies exist in the literature that focuses on tracing the pathways of atmospheric hydroxy FAs in remote marine aerosols [Kawamura, 1995]. However, these study focus mainly on characterizing their sources and transport pattern in the East Asian outflow, occurring during winter and spring. However, summer and autumn season were

poorly characterized in terms of sources of hydroxy FAs, their molecular distributions, and ambient variation in marine aerosols. Such studies are, in particular, useful in understanding the changes in atmospheric circulation over the geological past and to assess the long-range atmospheric transport of microorganisms along with the continentally derived particulate matter. This study investigates the sources of hydroxy FAs in remote marine aerosols collected from the western Pacific during autumn season. The major objectives of this study is to assess the latitudinal variability of atmospheric hydroxy FA during autumn season so as to understand the distributions of microbial lipids (continental vs. oceanic origin) over the remote oceanic regions of the western Pacific.

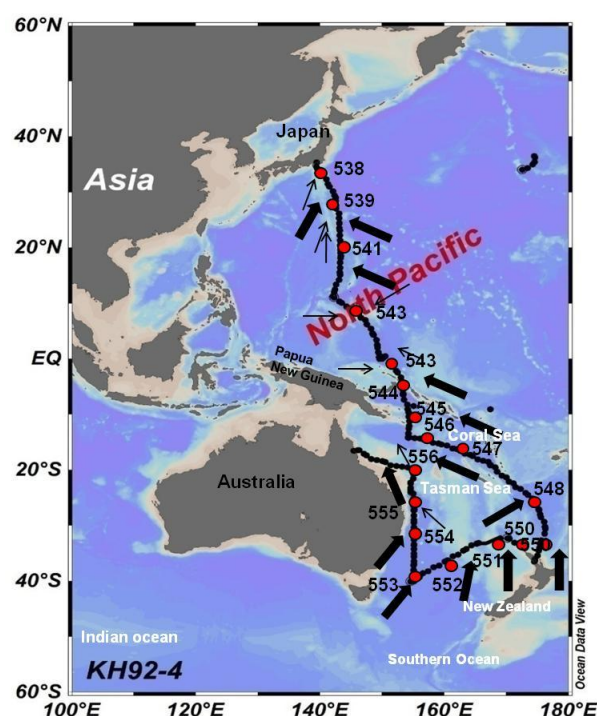


Fig.5.1. Cruise track for KH92-4 started on 16 September 1992 at Tokyo (Japan) and ended on 26 October, 1992 at Cairns (Australia). Illustrated are the aerosol filters identification numbers which were collected continuously during the cruise. Regular and bold arrows indicate the surface winds above and below 7 ms^{-1} , respectively.

5.2 Aerosol sampling

For this study, aerosol samples were collected on a pre-combusted (450°C for 6 h) quartz fiber filter (PALLFLEX[®]™, $20 \times 25 \text{ cm}^2$) during the cruise KH92-4 (16 September-24 October, 1992) in the western Pacific Ocean (Fig. 5.1). The high volume air sampler

(Shibata HVC 1000; flow rate: $1.0 \text{ m}^3 \text{ min}^{-1}$) was setup on the upper deck of the *R/V Hakuho Maru* (~14 m above mean sea level) and each sample was collected for ~24-48 h. To ensure the clean sample collection, sector wind controlled ($\pm 45^\circ$ and $\geq 5 \text{ ms}^{-1}$) sampling system was connected in line with high volume air sampler. All meteorological parameters were obtained from onboard weather station of the ship. The cruise track and surface wind conditions ($>7 \text{ ms}^{-1}$) at the time of sampling are shown in Fig. 5.2. After the sampling, all the filters were stored in a pre-cleaned glass bottles with Teflon lined screw caps at -20°C until analysis. Further details of cruise track and aerosol sampling are given in *Sempere and Kawamura, [2003]*. These TSP samples were analyzed for α -, β - and ω -hydroxy FAs according the analytical methods detailed in chapter 2.

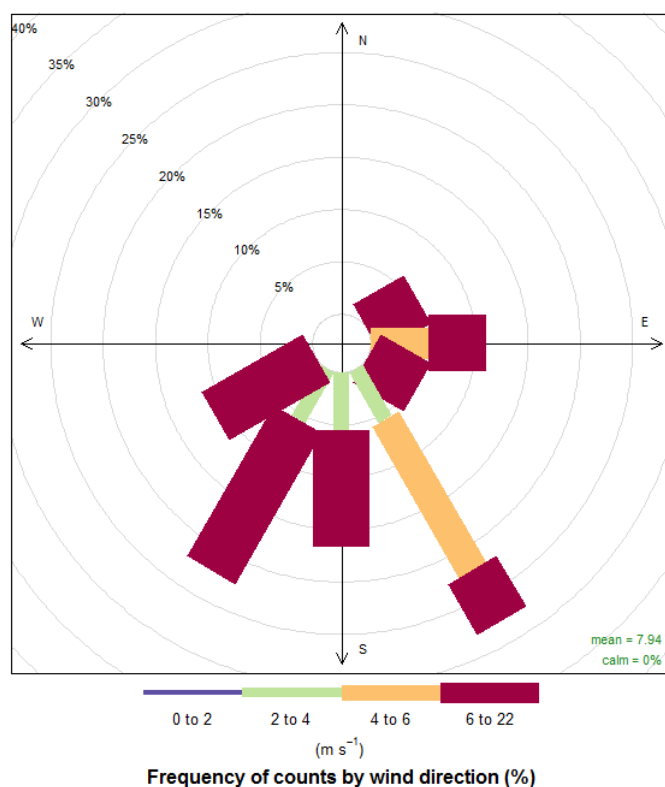


Fig.5.2. Windrose, plotted based on the measured wind speed and wind direction for the sampling days during KH92-4 cruise.

5.3 Results and Discussion

5.3.1. Air mass back trajectory analysis

To assess the contribution of various source regions in East Asia and the Pacific Ocean to the TSP collected along the cruise track, 7-day isentropic air mass back-trajectories (AMBTs) were computed using hybrid single particle lagrangian integrated trajectory model (HYSPLIT, version-4; [Stein *et al.*, 2015]. AMBT analyses show that aerosol samples collected between 40 °N and 20 °N (*i.e.*, QFF538 to 540) had influence from East Asia, while those collected between 0° and –40 °S (*i.e.*, QFF543 to 556) had influence from pristine oceanic sources (Fig. 5.3). Meanwhile, one sample collected between 20 °N and 0° (QFF541) showed influence from Papua New Guinea (Fig. 5.3). These results are consistent with previous findings in the literature, which emphasized mixed contribution of continental and oceanic sources to aerosols over the western Pacific [Kawamura, 1995; Mochida *et al.*, 2002]. Although weak, previous studies have documented the impact of Asian outflow on the chemical composition of aerosols over the western North Pacific (WNP) during autumn season [Verma *et al.*, 2015; Boreddy and Kawamura, 2015]. Therefore, observed mixed influence of continental and oceanic air masses to the TSP collected during KH92-4 is particularly relevant.

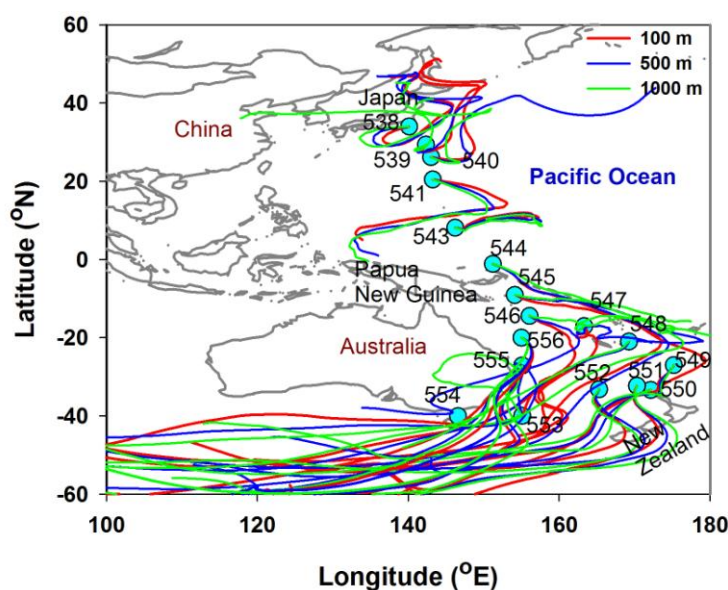


Fig.5.3. 10-day isentropic air mass back trajectories for the sampling days along the cruise track of KH92-4 in the western Pacific.

5.3.2. Latitudinal variability

In this study, a homologues series of β - and ω -hydroxy FAs from C₉ to C₂₀, and C₉ to C₃₁, respectively, were detected during the study period. Interestingly, β -hydroxy FAs were observed in almost all TSP samples collected during the cruise. However, ω -hydroxy FAs

were observed only in 9 samples, among which entire range of compound from C₉ to C₃₁ were detected for three TSP (QFF538, 553, and 555). It has been suggested that high molecular weight (HMW) fatty acids originate mainly from continental sources such as emissions from higher plants, while microbial sources either of continental or oceanic origin contribute to low molecular weight (LMW) fatty acids [Waterson and Canuel, 2008]. Therefore, relative abundances of LMW- (ΣC_9-C_{20}) and HMW- ($\Sigma C_{21}-C_{31}$) ω -hydroxy FAs in their total mass concentration (ΣC_9-C_{31}) were examined to distinguish the relative dominance of continental vs. oceanic sources. These calculations provided a hint regarding the relative dominance of oceanic sources, as inferred from the higher relative abundances of LMW ω -hydroxy FAs (range: 58-90%; av. 90%) during the cruise.

Unlike ω -hydroxy FAs, only LMW β -hydroxy FAs from C₉ to C₂₀ were observed during the KH92-4 cruise. However, this is in contrast to those reported in chapter 3 from a remote island in the WNP (*i.e.*, Chichijima), where both LMW and HMW ω -hydroxy FAs were abundant during autumn season. The observed discrepancy could be explained based on the TSP collected in this study is from the much distant locations in the WNP than Chichijima, thereby having a rather weak influence of atmospheric outflow from East Asia during the cruise. The total mass concentrations of β - and ω -hydroxy FAs exhibited pronounced latitudinal variability with a strong north to south gradient of high to low concentrations for the TSP collected in the MABL of the western Pacific (Fig. 5.4). Since marine aerosols from the WNP showed significant influence from East Asia, the observed high concentrations of β - and ω -hydroxy FAs could have contribution from soil microbes and higher plant waxes, respectively. However, contribution of β - and ω -hydroxy FAs from marine sources overwhelm the continental sources over the Pacific Ocean as the sampling period (autumn) is a transition season of changing winds from easterlies to westerlies.

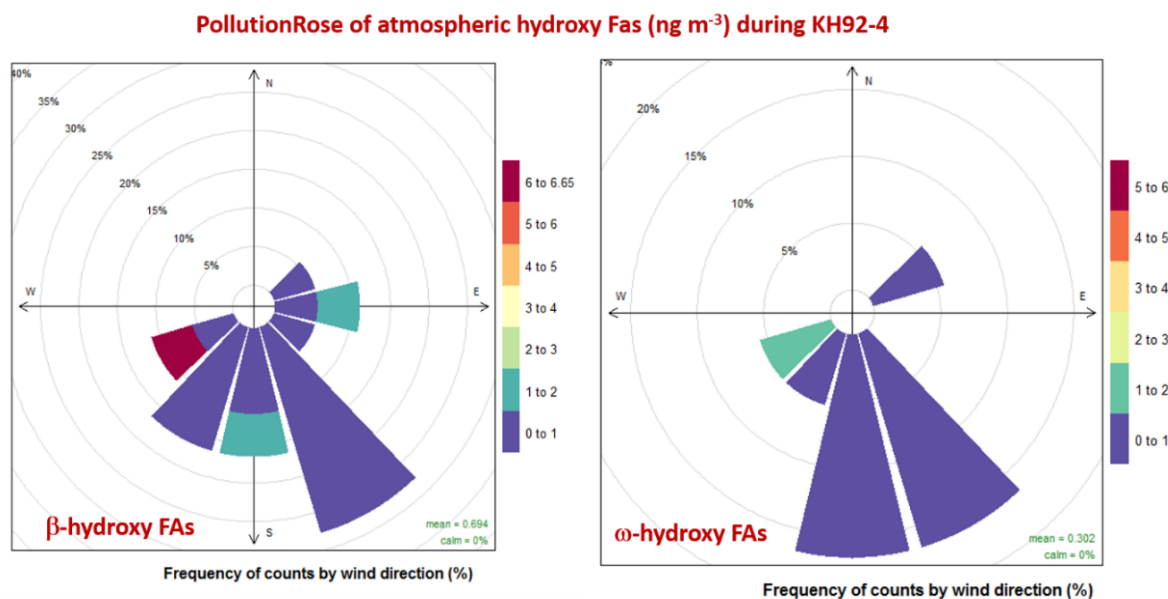


Fig.5.4. Pollution rose, showing the temporal variability in the total mass concentrations of β - and ω -hydroxy FAs according to their changing wind direction.

In order to further understand the relative contribution of oceanic vs. continental sources to β - and ω -hydroxy FAs, the latitudinal variability of azelaic acid (C₉ diacid) concentrations were examined during the study period (Fig. 5.5). Interestingly, azelaic acid also showed similar north to south concentration gradient with higher and lower concentrations, respectively. Azelaic acid, a photochemical oxidation product of biogenic unsaturated fatty acid having a double bond predominantly at C₁₁ position, is emitted from the ocean surface along with sea spray emissions [Tedetti *et al.*, 2007; Zahardis and Petrucci, 2007; Kawamura and Bikkina, 2016]. Therefore, observed latitudinal variability of β -hydroxy FAs and the predominance of LMW ω -hydroxy FAs over the western Pacific could be due to their contribution from the dissolved or microbial degraded particular organic matter emitted from the surface waters along with sea spray.

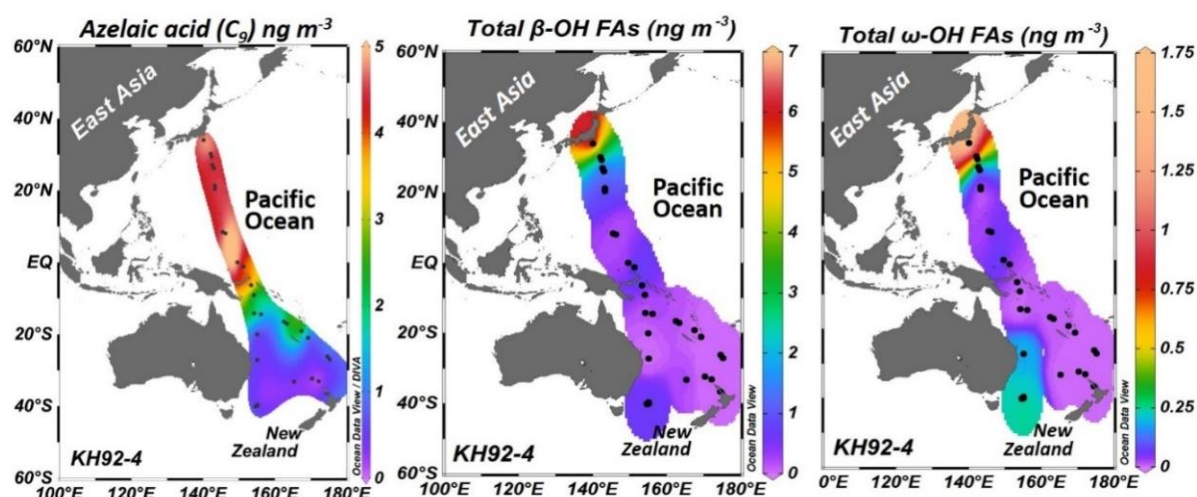


Fig.5.5. Latitudinal variability of azelaic acid (C_9 diacid), total mass concentration of β - and ω -hydroxy FAs during the western Pacific cruise (KH92-4).

Interestingly, the ambient total mass concentrations of β - and ω -hydroxy FAs over the western Pacific during KH92-4 cruise are associated with high wind speeds (Fig. 5.2) and mostly originating from SW or SE (Fig. 5.5). In a previous study, *Mochida et al.* [2002] documented high atmospheric abundances of fatty acids over the Pacific Ocean in summer and autumn seasons, which are attributed to their emissions from the ocean surface along with the sea spray due to prevailing high wind speeds. Since relatively high wind speeds ($>7 \text{ m s}^{-1}$) were observed over the western Pacific, which leads to increased emission of dissolved or particulate organic matter from the surface waters along with sea spray emissions. Likewise, another study by *Kunwar and Kawamura* [2014] documented increased atmospheric abundances of azelaic acid in summer/autumn seasons, assessed based on the year round observations from a remote island (Okinawa) in the WNP. Therefore, high wind speeds over the western Pacific during KH92-4 cruise could transfer ocean derived organic matter to the MABL.

Wakeham et al. [2003] documented the occurrence of C_{10} - C_{18} β -hydroxy FAs in the surface waters as integral part of dissolved organic matter, contributed mainly from the LPS of microbial lipids (marine GNB species). Based on the predominance of C_{16} and C_{22} hydroxy FAs in remote oceanic aerosols and pelagic sediments, *Kawamura* [1995] suggested their contribution from both marine sources and terrestrial sources. Therefore, observed higher wind speeds during the KH92-4 cruise could lead to enhanced emissions of hydroxy

FAs from the surface waters of the western Pacific Ocean. However, as mentioned earlier, the TSP collected between 20 °N and 40 °N are influenced by the transport from East Asia during the cruise. Therefore, TSP collected during KH92-4 cruise had contribution not only from oceanic sources but also from the continental sources (higher plant waxes).

A ratio of sum of concentrations of LMW fatty acids to HMW fatty acids has been proposed by *Waterson and Canuel* [2008] to ascertain the relative dominance of oceanic vs. continental sources to deep sea sediments. Using the similar strategy, a mass ratio of hydroxy FAs (R_{OHFAs}) has been proposed, which is ratio of sum of concentrations of C₁₂, C₁₄ and C₁₆ hydroxy FAs to sum of concentrations of C₂₂, C₂₄ and C₂₆ hydroxy FAs, to examine the relative contribution of microbial sources against higher plant waxes. The underlying principle involves in this approach to decipher the relative significance of microbial sources (*i.e.*, either continental or marine origin) and higher plant waxes are the source specific contribution of LMW and HMW-hydroxy FAs, respectively. In this study, HMW β -hydroxy FAs were not observed during KH92-4 cruise and only observed in three samples for ω -hydroxy FAs. The R_{OHFAs} varied from 2.7 to 10.2, indicating the dominant contribution of hydroxy FAs from microbial sources rather than those from higher plant waxes during the study period.

5.3.3. Molecular distributions

The molecular distributions of atmospheric β -hydroxy FAs over the western Pacific during KH92-4 cruise are depicted in Fig. 5.6. The molecular distribution of β -hydroxy FAs showed odd carbon predominance especially for the measured short-chain homologues following the order, C₉>C₁₀>C₁₁>C₁₂. However, even carbon predominance is noteworthy for the remaining homologues of β -hydroxy FAs. Also, C₁₀ β -hydroxy FA is present in almost all samples collected during the KH92-4 cruise. Interestingly, only C₁₀ and/or C₁₆ β -hydroxy FAs were observed in few samples. Overall, these molecular distributions (*i.e.*, predominance of odd carbon β -hydroxy FAs) from the western Pacific Ocean are contrary to those documented from the Chichijima Island in the WNP, which showed even carbon predominance.

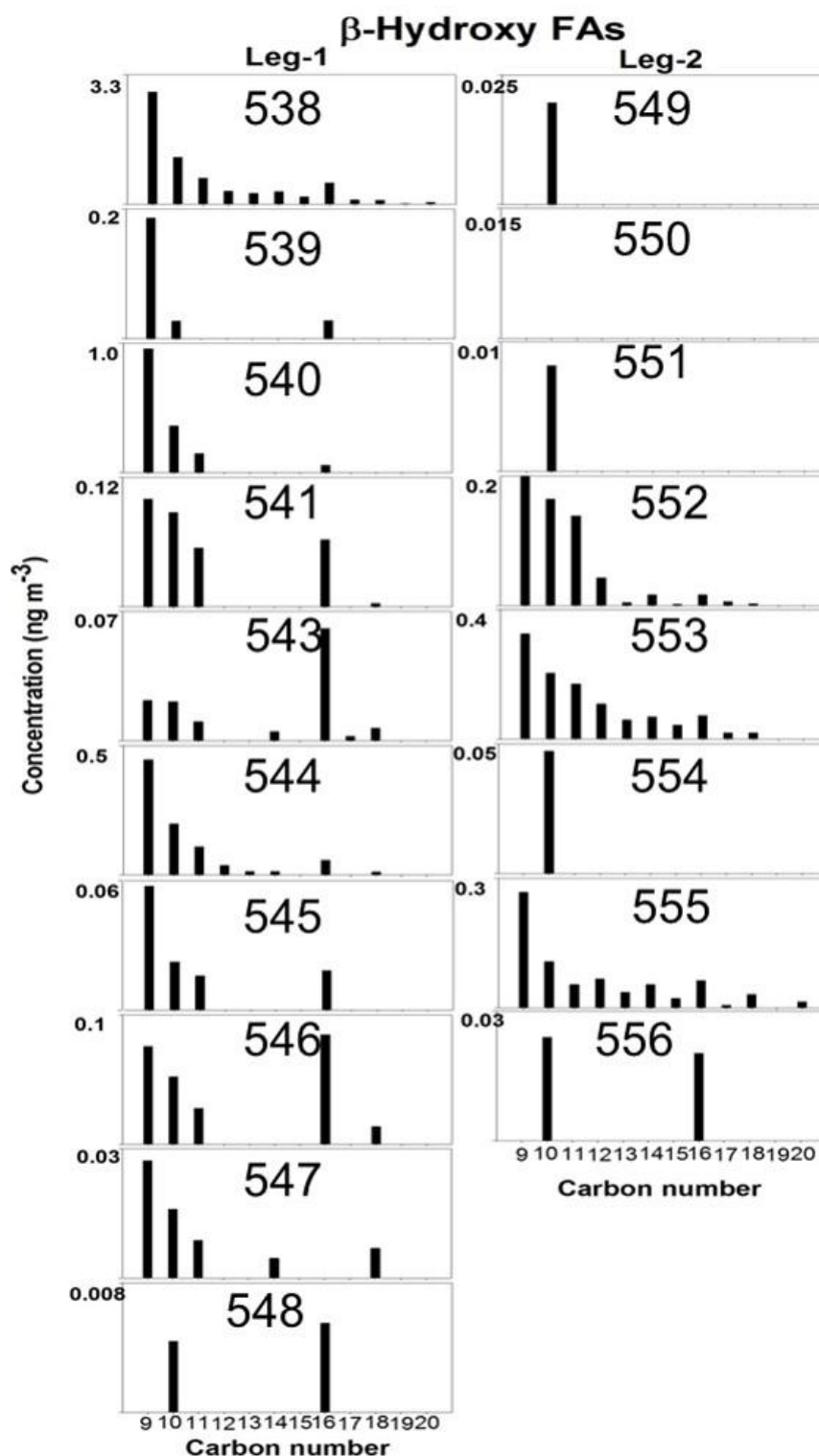


Fig.5.6. Molecular distributions of β -hydroxy FAs according to their carbon chain length number during KH92-4 cruise in the western Pacific.

In a recent study, *Cochran et al.* [2016] observed a sudden change in the molecular distribution of even-to-odd carbon numbered saturated fatty acids in the sea spray aerosols, which are emitted from the surface ocean during a phytoplankton bloom. Their study explained the occurrence of odd carbon number fatty acids based on the chemical degradation (or oxidation) process in fine sea spray aerosols [*Cochran et al.*, 2016]. Furthermore, their study also documented even-to-odd carbon predominance of saturated fatty acids (with a ratio of even-to-odd greater than one) in the sea spray aerosols, sea-surface microlayer and also in the sub-surface waters during a mesocosm experiment. This observation indicates the active role of biological processes in the surface ocean in controlling the atmospheric abundances of saturated fatty acids (here β -hydroxy FAs) in the MABL.

The β -oxidation of saturated fatty acids is normally preferred over α -oxidation pathway, which is the main cause for their existence in the marine organic matter [*Wakeham*, 1999; *Wakeham et al.*, 2003; *Volkman*, 2006]. Therefore, the concentration of C_9 and C_{11} β -hydroxy FAs exceeding C_{10} and C_{12} β -hydroxy FAs, respectively in the MABL during KH92-4 cruise is attributed to the chemical evolution (*i.e.*, through oxidation) of saturated fatty acids emitted from the ocean surface along with sea spray aerosol. Concentrations of azelaic acid (Fig. 5.5), a photochemical oxidation product of biogenic unsaturated fatty acid (oleic acid) which is in turn a marine phytoplankton derived organic matter, in the MABL were also high during the study period. As mentioned earlier, high wind speeds ($>7 \text{ m s}^{-1}$) observed in this study could transfer more efficiently the marine derived organic matter from the surface waters during sea spray formation.

For instance, *O'Dowd et al.* [2015] documented a clear link between fraction of organic matter in sea spray aerosol and phytoplankton biomass (*i.e.*, inferred based on a strong correlation with *Chlorophyll a* and net primary production). Furthermore, it has been suggested that the enriched organic fraction in sea spray aerosols is largely due to the emission of aggregated and decomposed phytoplankton derived organic matter in the surface waters [*O'Dowd et al.*, 2015]. Likewise, the composition of organic mass in sea spray emissions is similar to that of sea water [*Meskhidze and Gantt*, 2010; *Cochran et al.*, 2016]. The surface waters of the WNP in summer/autumn are characterized by the occurrence of *Trichodesmium* blooms [*Wilson*, 2003; *Dandonneau et al.*, 2004]. Based on the measurements of $\delta^{13}\text{C}$ of organic carbon in aerosols over the productive ocean waters of the North Pacific during a summer cruise, *Miyazaki et al.* [2011] suggested that marine derived

organic matter contribution could be as high as $\sim 88 \pm 12\%$. Since easterlies dominate during both summer and autumn seasons over the western Pacific, it has been inferred that hydroxy FA concentrations in the MABL during KH92-4 cruise are mostly influenced by the oceanic microbial sources (Fig. 5.6). Therefore, observed molecular distributions of β -hydroxy FAs is a combination of emission of marine derived organic matter containing saturated FAs (or β -hydroxy FAs) followed by their oxidation degradation in the MABL of the western Pacific.

The molecular distribution of ω -hydroxy FAs showed even-to-odd carbon predominance of C_{16} followed by C_{12} and C_{14} . Interestingly, in one sample (QFF554), concentration of C_{11} ω -hydroxy FAs exceeded to that of C_{16} . It has been suggested that C_{16} and C_{18} ω -hydroxy FAs are usually found in epicuticular plant waxes of cutin, whereas C_{16} - C_{22} ω -hydroxy FAs are observed in suberin [Kolattukudy *et al.*, 1975; Volkman, 2006]. However, long-chain saturated ω -hydroxy FAs also found in sea grasses [Volkman *et al.*, 1980; Volkman, 2006] and in marine aerosols [Kawamura, 1995]. In general, microbial sources (either soil or marine bacteria) contribute to short-chain hydroxy FAs, while plant waxes account for long-chain ($>C_{20}$) hydroxy FAs. Although a homologous series of ω -hydroxy FAs from C_9 - C_{30} were observed, in particular higher abundances are noteworthy for short-chain homologues (C_9 - C_{20}). Therefore, observed molecular distribution of ω -hydroxy FAs also indicate their dominant contribution from marine derived organic matter over the western Pacific Ocean rather than those derived from continental sources.

5.3.4. Relative abundances

For the entire cruise samples, the sum of concentrations of β -hydroxy FAs dominate (61-100%, av. 92%) the total hydroxy FAs mass (ΣC_9 - C_{20} - β -hydroxy FAs + ΣC_9 - C_{30} - ω -hydroxy FAs). A comparison of relative abundances of hydroxy FAs with those from East Asia and remote islands in the WNP (Fig. 5.7) revealed rather disparate picture. The distribution of relative abundances of hydroxy FAs are characterized by the predominance of β -hydroxy FAs over the western Pacific, while those are dominated by ω -hydroxy FAs followed by β - and α -hydroxy FAs over East Asia. The α -hydroxy FAs were not detected in marine aerosols over the western Pacific Ocean (this study). However, α -hydroxy FA were observed over the Chichijima Island in the WNP for the winter and spring samples. Similar to KH92-4 cruise, the relative abundances of β -hydroxy FAs dominate over ω -hydroxy FAs at Chichijima. Since α -hydroxy FAs are formed as intermediate oxidation products during

transport, absence of α -hydroxy FAs over the western Pacific during KH92-4 might reflect the fresh emissions of hydroxy FAs from the oceanic sources. On the other hand, predominance of ω -hydroxy FAs followed by β -hydroxy FA reflects their contribution from higher plant waxes followed by soil microbes over East Asia.

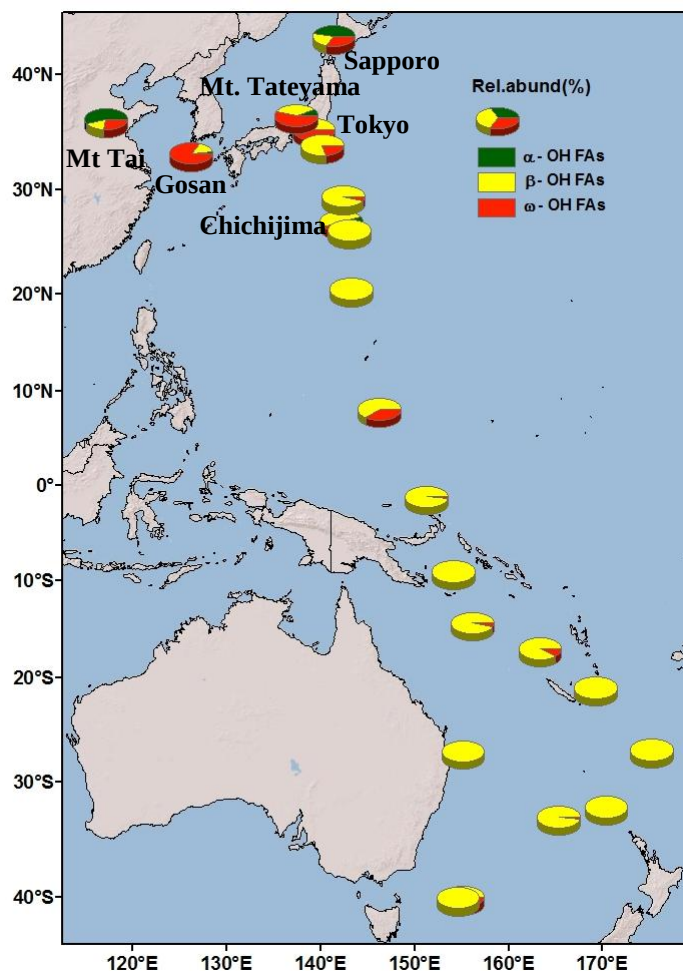


Fig.5.7. Comparison of relative abundances of hydroxy fatty acids (OH FAs) in marine aerosols during KH92-4 cruise in the western Pacific Ocean with marine aerosols from oceanic Islands (Chichijima, Gosan) and continental aerosols, snow and snow pit samples from East Asia.

5.4 Conclusions

Atmospheric input of soil microbes and plant waxes are a major source of terrestrial lipid compounds to the surface ocean and, thus, contributing to deep sea sediments. In this study, occurrence of soil microbes and plant metabolites were investigated in the remote marine aerosols collected during a western Pacific cruise (KH92-4: September-October 1992, spanning between 40 °N and 40 °S), through β - and ω -hydroxy FAs. The total mass concentrations of β - and ω -hydroxy FAs in marine aerosols exhibited a latitudinal variability with a strong north to south gradient of high to low concentrations over the western Pacific Ocean. Furthermore, these hydroxy FAs mostly originate from SW or SE (oceanic origin) and are associated with high wind speeds (*i.e.*, more sea spray emissions). A similar latitudinal variability was also noteworthy for azelaic acid, a photochemical oxidation product of biogenic unsaturated fatty acid emitted from the ocean surface along with sea spray, over the western Pacific. Occurrence of only C₉-C₂₀ β -hydroxy FAs with characteristic odd carbon predominance in the molecular distribution (C₉>C₁₀>C₁₁>C₁₂) for LMW compounds and even carbon predominance for the remaining β -hydroxy FAs indicate their formation from the photochemical oxidation of marine derived organic matter. A comparison of relative abundance of hydroxy FAs between this study and those available from the literature reveal the dominance of β -hydroxy FA (marine microbial origin) over the western Pacific and ω -hydroxy FAs (plant waxes) over East Asia.

Chapter 6

Impact of Biomass Burning on Soil Microorganisms and Plant Metabolites: A View from Molecular Distributions of Atmospheric Hydroxy Fatty Acids over Mt. Tai

Chapter 6 presents the results of hydroxy FAs in TSP collected over Mt. Tai (North China). The concentrations and molecular distributions of hydroxy FAs were discussed in terms of their source strength as well as with respect to high and low biomass burning events.

6.1 Introduction

Field burning of agricultural crop-residue is a traditional practice employed in South and East Asia for many years to clear and fertilize the lands, which emits a significant amount of organic compounds in the atmosphere on a global scale [Andreae and Merlet, 2001]. Several studies have been carried out to ascertain its impact on organic aerosols in terms of various organic compounds (*e.g.* diacids, sugars, and lipids) [Fang *et al.*, 1999; Kawamura *et al.*, 2013; Wang *et al.*, 2009; Wiesenberger *et al.*, 2009]. However, studies focusing on the role of this anthropogenic source (*i.e.*, biomass burning) in terms of soil microbes and higher plant waxes have been limited in the literature [Yang *et al.*, 2012].

The air-borne microorganisms (*e.g.* bacteria, viruses, and fungal spores) and their long-range atmospheric transport have received considerable attention owing to their potential impacts on human health, animals, and plants. In addition, these bioaerosols can act as cloud condensation and ice nuclei [Bauer *et al.*, 2003; Bowers *et al.*, 2009; Despres *et al.*, 2012; Huffman, 2013]. However, knowledge on microbial aerosols is particularly deficient due to the absence of reliable measurement techniques and the limitations in culturing and quantification of microorganisms. Alternatively, the determination of certain chemical markers (*e.g.* hydroxy fatty acids) has been proven as an efficient means to assess the contribution from air-borne Gram-negative bacteria (GNB) and higher plant metabolites [Hines *et al.*, 2003; Lee *et al.*, 2004; Lee *et al.*, 2007].

Hydroxy fatty acids (FAs) can be used as tracers for microorganisms such as bacteria, fungi as well as for algae, and higher plants during their transport *via* air [Tyagi *et al.*, 2015a]. Positional isomers (*i.e.*, α -, β - and ω -) of these hydroxy FAs have been identified in sediments [Kawamura and Ishiwatari, 1982; Parker *et al.*, 1982; Perry *et al.*, 1979; Wakeham, 1999], dust [Fox *et al.*, 1993; Hines *et al.*, 2003; Saraf *et al.*, 1997], lake waters and sediments [Kawamura and Ishiwatari, 1984; Kawamura *et al.*, 1987; Zhang *et al.*, 2014], snow, and continental [Graham *et al.*, 2003; Lee *et al.*, 2004] and marine aerosols [Kawamura, 1995]. Among the three isomers, β -hydroxy FAs (from C₁₀ to C₁₈) have been reported as chemical markers for the GNB as they are abundant in their cell membrane and are also responsible for their toxic effects (*e.g.* respiratory problems, skin infections) [Ratledge and Wilkinson, 1988]. Similarly, short-chain α -hydroxy FAs (typically C₁₂ to C₂₀) are the structural constituents of many soil microorganisms (*e.g.* bacteria, fungi, yeasts, and protozoa) [Ratledge and Wilkinson, 1988]. Long-chain α -, β - and ω -hydroxy FAs (C₁₆ to C₃₂)

are abundant in microalgae, cyanobacteria [Matsumoto and Nagashima, 1984; Matsumoto *et al.*, 1984], sea grasses [Volkman *et al.*, 1999], and plant waxes [Rogge *et al.*, 1993; Simoneit, 1989]. Moreover, these α -, β - and ω -hydroxy FAs can also act as intermediates of photochemical and microbial oxidation of long-chain monocarboxylic acids to dicarboxylic acids [Volkman *et al.*, 1998; Wakeham, 1999].

Concentrations and molecular distributions of hydroxy FAs and their spatiotemporal variability in the atmosphere are, in particular, useful to better assess the long-range transport of soil microbes and plant waxes. The mountain sites and high altitude towers provide a means to assess the sources and transport pathways of atmospheric aerosols. To understand the impacts of biomass burning on soil- and plant-associated microbes (in particular GNB) as well as higher plant waxes, we measured hydroxy FAs in the aerosol samples collected over Mt. Tai, located in the North China Plain (NCP). The objective of this study is to examine the impact of wheat residue burning on the emissions of air-borne soil microorganisms and higher plant waxes over Mt. Tai through the atmospheric abundances and molecular distributions of hydroxy FAs.

6.2 Experimental methods

6.2.1. Sampling site and prevailing meteorology

Aerosol samples (TSP) were collected at the summit of Mt. Tai (36.25° N, 117.10° E; 1534 m above sea level), which is surrounded by flat lands (<200 m in altitude) and an independent peak in Shandong province, central East China (Fig. 6.1). Due to its sufficient elevation, Mt. Tai intercepts the Asian outflow, and the mountain top is mostly covered with rocks and bushes. Since the summit of Mt. Tai intervenes into the free troposphere (*i.e.*, above the planetary boundary layer) during nighttime, perhaps, it could serve as an ideal site to study the atmospheric chemistry [Kanaya *et al.*, 2013; Kawamura *et al.*, 2013] and also to trace the chemical markers of the soil microorganisms and plant metabolites. Moreover, prevailing meteorology dictates the physico-chemical properties of aerosols over Mt. Tai. Generally, winds come from the west or southwest, however, during the periods of 8–10 and 14–15 June, 2006, the northwest to north winds were dominated; delivering cold and dry air masses from Northern China and Siberia over Mt. Tai (Fig. 6.2) [Kanaya *et al.*, 2013].

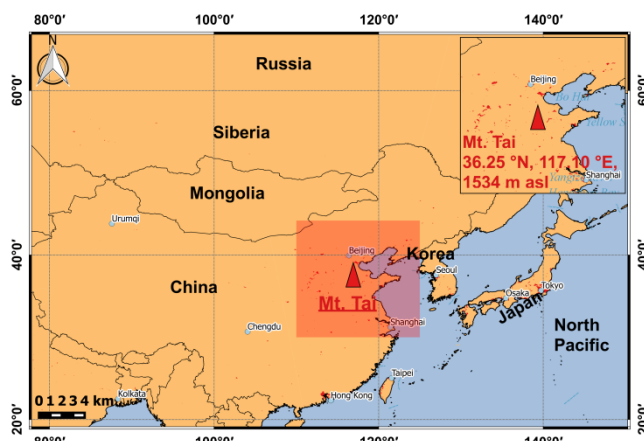


Fig.6.1. A Map is showing the geographical location of Mt. Tai (36.25 °N, 117.10 °E).

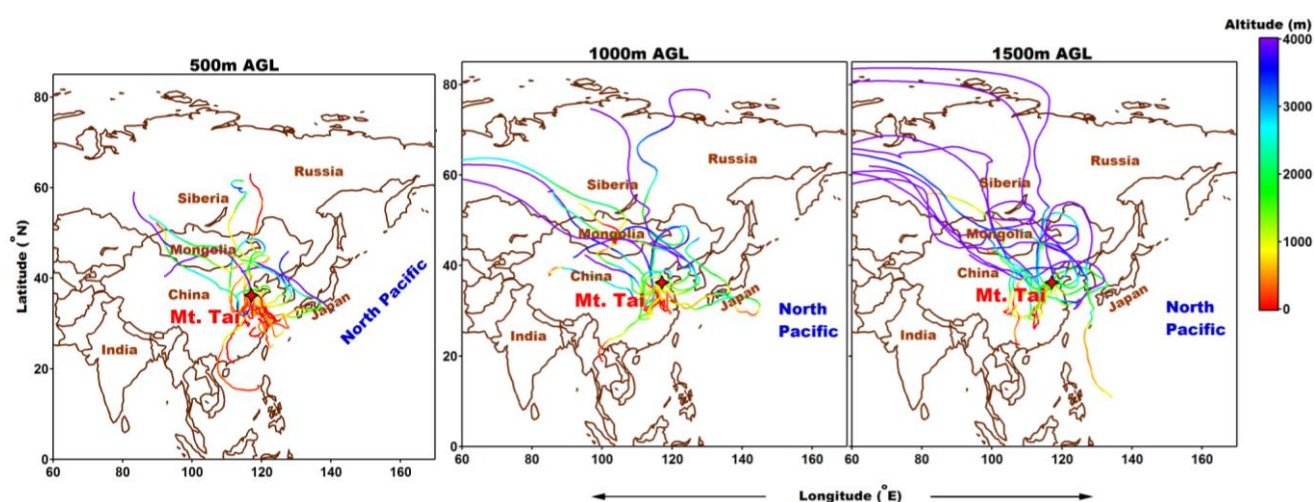


Fig.6.2. Geographical location of sampling site (Mt. Tai, red star indicates the site), together with 3-day isentropic air mass back-trajectories (6–28 June, 2006) at arrival heights of 500, 1000 and 1500 m above the ground level as calculated by the HYSPLIT Model.

6.2.2. Aerosol samples collection and analyses

Total suspended particles (TSP) were collected on a day/night basis from 6 to 28 June 2006 ($N=20$) as part of the Mt. Tai Experiment (MTX2006) campaign. An overview of the aerosol collection and sampling site is given in our previous publications [Kanaya *et al.*, 2013 and references therein]. The TSP samples were collected on pre-combusted (450 °C for 6 h) quartz fiber filters, 20 x 25 cm, PALLFLEX[®] 2500QAT-UP, using a high-volume air sampler (flow rate: 1.0 m³ min⁻¹). The filter samples including three blanks were placed individually in a cleaned glass bottle (150 mL) with a Teflon-lined screw cap and transported to the laboratory in Sapporo. After the collection, aerosol samples were stored at -20 °C until

analysis. More detailed description regarding the meteorological conditions and aerosol collection over Mt. Tai can be seen in our previous publications [Fu *et al.*, 2008; Kanaya *et al.*, 2013; Kawamura *et al.*, 2013].

6.3 Results and Discussion

6.3.1. Fire counts and air mass backward-trajectory analysis

To assess the probable source regions contributing to atmospheric abundances of hydroxy FAs over Mt. Tai, three-day isentropic air mass backward-trajectories (AMBTs) were computed for the sampling days (Fig. 6.2) using the Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT) model, [Draxler and Rolph, 2013 and references therein]. The HYSPLIT model used the archived meteorological datasets (GDAS, from 1940–present) from the National Oceanic and Atmospheric Administration (NOAA) air resources laboratory as an input file. Furthermore, the back-trajectory analyses were combined with fire count data (obtained from <https://earthdata.nasa.gov/data/near-real-time-data/firms>) to identify the contribution of biomass burning activities (specific for our sampling duration) in the NCP and Southeast Asia to the aerosols sampled over Mt. Tai (Fig. 6.3). This is to examine the contribution from various source regions and the influence of biomass burning events (BBEs) on the atmospheric abundances of hydroxy FAs. The AMBT analyses revealed a generalized pattern with the maximum contribution from local sources such as Anhui, Jiangsu, Shandong, Henan and Hebei in the NCP in early June of 2006 (Fig. 6.2). However, occasionally for some sampling days (mostly in the late June), AMBT analyses showed long-range transport from Russia, Siberia, Mongolia, eastern China and southern part of Japan. The TSP samples from Mt. Tai were also analyzed for biomass combustion tracers [Fu *et al.*, 2008].

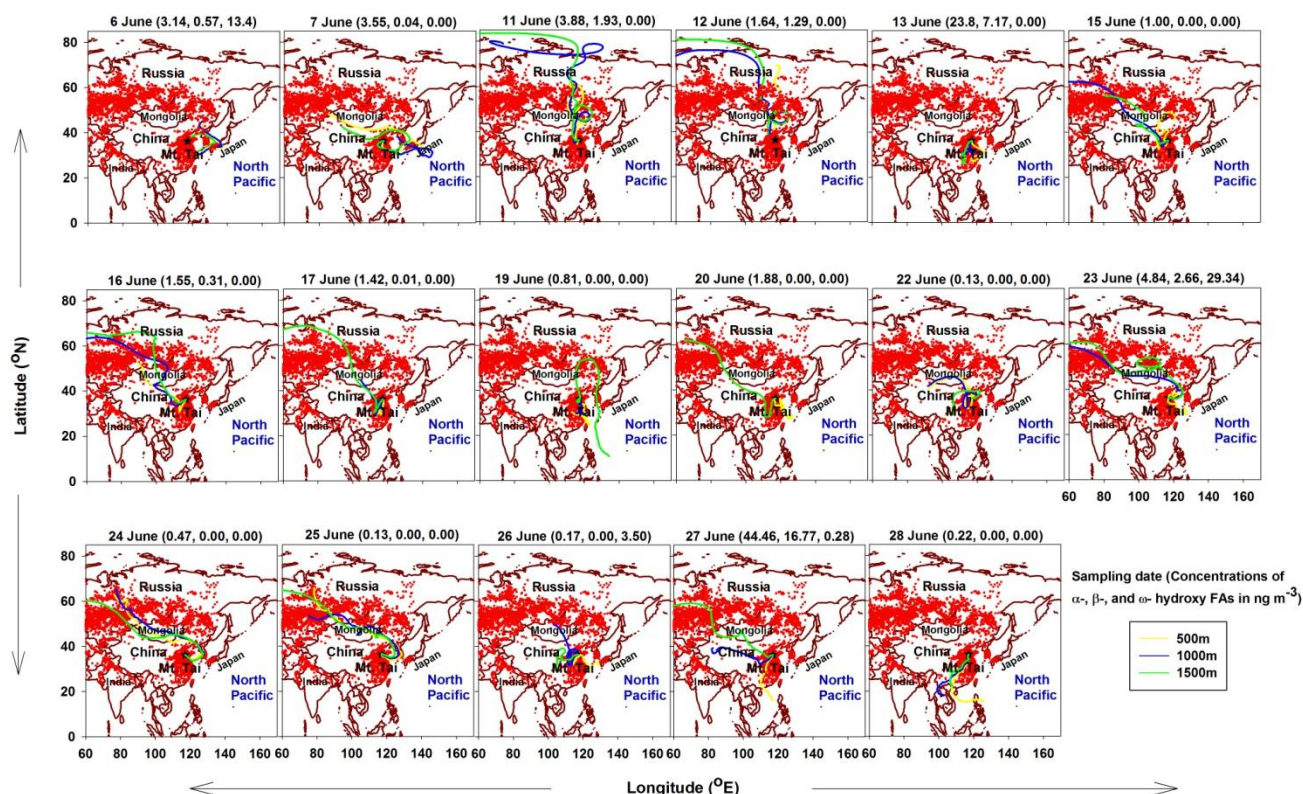


Fig.6.3. Daily variations in air masses starting (HYSPLIT Model) from 6 to 28 June 2006 at arrival heights of 500, 1000 and 1500 m above ground level combined with daily fire spots (NASA EOSDIS website) and temporal concentrations of each hydroxy fatty acids (FAs). Sampling dates along with concentrations of α -, β - and ω -hydroxy FAs (in ng m^{-3}) in parenthesis are shown above the each panel and different colors indicate the different arrival heights of the air mass back-trajectories.

Pollution rose diagrams were used to examine the variability of atmospheric abundances of hydroxy FAs with wind frequency (i.e., percentage of winds blowing from a certain direction for a particular time period compared to total) and wind direction over Mt. Tai. In general, high mass concentrations of total hydroxy FAs over Mt. Tai are mostly associated with the atmospheric transport from South and Southwest. However, a sudden change in prevailing winds from South and Southwest to North was observed during 8–10 and 14–15 June 2006, while Mt. Tai received air masses originated from East China during 22–25 June 2006 (Fig. 6.4). The impact of wheat harvest residue burning emissions in the NCP on Mt. Tai aerosols was documented by previous studies [Fu *et al.*, 2008; Suthawaree *et al.*, 2010; Yamaji *et al.*, 2010]. Consequently, the concentration data of hydroxy FAs were differentiated into two categories as days with high (H-BBEs: 6–7, 11–14 and 27 June 2006) and low biomass burning emissions (L-BBEs: 15–26 and 28 June 2006). This classification was solely based on the relatively high concentrations of levoglucosan [Fu *et al.*, 2008], a

pyrolysis product of cellulose and hemicellulose [Simoneit *et al.*, 1999], during H-BBEs ($>500 \text{ ng m}^{-3}$) compared to those observed during L-BBEs ($<120 \text{ ng m}^{-3}$). To assess a succinct influence of fire events in the NCP on the atmospheric abundances of soil microbes, hydroxy FAs were not assayed for the “clean period” (8–10 and 14–15 June) as explained in Kanaya *et al.* [2013].

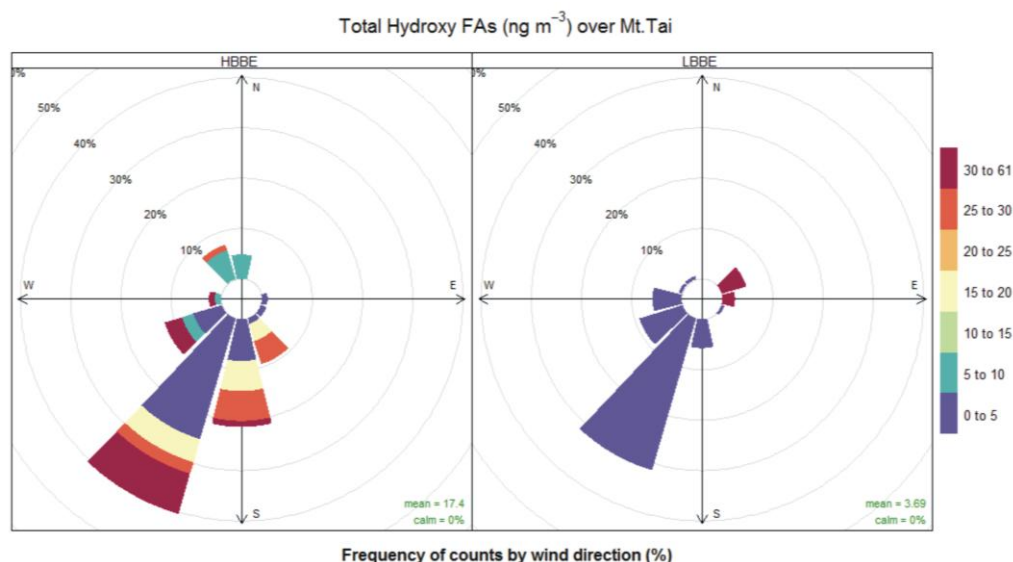


Fig.6.4. Pollution rose diagram for total hydroxy fatty acid mass concentration in ambient aerosols (TSP) collected over Mt. Tai during MTX-2006 campaign.

6.3.2. Molecular distribution

A homologues series of α -(C₉–C₃₂)-hydroxy FAs was detected during both H- and L-BBEs (Fig. 6.5a). However, β - and ω -hydroxy FAs were found in relatively small number of samples ($N=10$ and $N=4$, respectively). In general, higher concentrations of α - and β -hydroxy FAs were detected during H-BBEs (Table 6.1), which might be due to their contribution from open field burning in the NCP (also supported by the AMBTs). It has been suggested that soil- and plant-associated microbiota are the major sources of air-borne hydroxy FAs [Lee *et al.*, 2004; Rogge *et al.*, 1993; Zelles, 1997; Zogg *et al.*, 1997]. Therefore, we attribute the sources of hydroxy FAs over Mt. Tai to the contribution from the soil microbes/plant pathogens associated with fugitive dust/aerosols from the agricultural fields in the NCP. Furthermore, Mt. Tai is located within the NCP and significantly influenced by the emissions of organic aerosols derived from large-scale field burning activities in the NCP as supported by other molecular markers [Kawamura *et al.*, 2013; Fu *et al.*, 2008].

Table 6.1. A summary of α -, β - and ω -hydroxy fatty acids (FAs) measured in aerosols collected over Mt. Tai during high (H-BBEs: 6-14 and 27 June, 2006) and low biomass burning emissions (L-BBEs: 15-26 and 28 June, 2006) in the NCP.

Compounds	H-BBEs ($n=8$; ng m ⁻³)			L-BBEs ($n=12$; ng m ⁻³)		
	Mean $\pm$ SE	Range	Median	Mean $\pm$ SE	Range	Median
α-Hydroxy FAs						
C ₉	0.72 $\pm$ 0.59	0.06-1.89	0.22	0.19 $\pm$ 0.00	0.00-0.19	0.19
C ₁₀	3.60 $\pm$ 3.56	0.04-7.16	3.60	0.002 $\pm$ 0.00	0.00-0.002	0.002
C ₁₁	1.16 $\pm$ 1.01	0.05-3.17	0.24	0.18 $\pm$ 0.00	0.00-0.18	0.18
C ₁₂	1.25 $\pm$ 1.16	0.04-5.87	0.12	0.07 $\pm$ 0.02	0.03-0.10	0.07
C ₁₃	0.38 $\pm$ 0.17	0.15-0.72	0.27	0.17 $\pm$ 0.05	0.07-0.35	0.16
C ₁₄	1.19 $\pm$ 1.07	0.07-8.70	0.25	0.13 $\pm$ 0.04	0.04-0.38	0.09
C ₁₅	0.55 $\pm$ 0.23	0.13-1.90	0.31	0.19 $\pm$ 0.05	0.03-0.36	0.21
C ₁₆	1.27 $\pm$ 0.69	0.02-4.25	0.31	0.09 $\pm$ 0.02	0.01-0.24	0.08
C ₁₇	0.22 $\pm$ 0.09	0.01-0.63	0.12	0.04 $\pm$ 0.01	0.00-0.09	0.04
C ₁₈	0.59 $\pm$ 0.26	0.03-1.69	0.27	0.08 $\pm$ 0.02	0.01-0.23	0.07
C ₁₉	0.23 $\pm$ 0.11	0.03-0.70	0.11	0.03 $\pm$ 0.01	0.00-0.08	0.03
C ₂₀	0.84 $\pm$ 0.43	0.02-2.88	0.25	0.10 $\pm$ 0.03	0.00-0.35	0.07
C ₂₁	0.18 $\pm$ 0.07	0.03-0.53	0.08	0.02 $\pm$ 0.01	0.01-0.05	0.02
C ₂₂	0.61 $\pm$ 0.07	0.02-2.71	0.11	0.05 $\pm$ 0.01	0.00-0.13	0.04
C ₂₃	0.25 $\pm$ 0.11	0.02-0.80	0.10	0.03 $\pm$ 0.01	0.01-0.12	0.02
C ₂₄	0.53 $\pm$ 0.27	0.01-1.98	0.14	0.08 $\pm$ 0.02	0.00-0.22	0.06
C ₂₅	0.21 $\pm$ 0.09	0.02-0.72	0.10	0.05 $\pm$ 0.01	0.02-0.13	0.04
C ₂₆	0.26 $\pm$ 0.10	0.02-0.78	0.13	0.06 $\pm$ 0.01	0.01-0.16	0.06
C ₂₇	0.36 $\pm$ 0.17	0.02-1.19	0.16	0.04 $\pm$ 0.02	0.01-0.13	0.03
C ₂₈	0.31 $\pm$ 0.11	0.07-0.78	0.22	0.09 $\pm$ 0.04	0.01-0.39	0.05
C ₂₉	0.27 $\pm$ 0.11	0.02-0.72	0.23	0.14 $\pm$ 0.08	0.01-0.47	0.07
C ₃₀	0.31 $\pm$ 0.16	0.04-0.93	0.19	0.11 $\pm$ 0.08	0.01-0.52	0.04
C ₃₁	0.08 $\pm$ 0.00	0.00-0.08	0.08	0.06 $\pm$ 0.00	0.00-0.06	0.06
C ₃₂	0.14 $\pm$ 0.00	0.14-0.15	0.14	0.11 $\pm$ 0.00	0.00-0.11	0.11
Subtotal	10.08 $\pm$ 5.50	0.17-44.46	3.35	1.04 $\pm$ 0.38	4.84-12.5	0.68
β-Hydroxy FAs						
C ₉				0.08 $\pm$ 0.00	0.00-0.08	0.08
C ₁₂	0.03 $\pm$ 0.02	0.01-0.05	0.03			
C ₁₃	1.13 $\pm$ 0.00	0.00-1.13	1.13			
C ₁₄	0.09 $\pm$ 0.04	0.04-0.19	0.08	0.04 $\pm$ 0.02	0.01-0.17	0.03
C ₁₅	0.10 $\pm$ 0.05	0.01-0.17	0.13	0.05 $\pm$ 0.00	0.00-0.05	0.05
C ₁₆	0.77 $\pm$ 0.56	0.10-3.02	0.20	0.10 $\pm$ 0.01	0.09-0.12	0.10
C ₁₇	0.46 $\pm$ 0.27	0.11-1.00	0.26	0.12 $\pm$ 0.07	0.05-0.20	0.12

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C ₁₈	1.56±0.87	0.09-4.78	0.48	0.32±0.18	0.13-0.50	0.32
C ₁₉	0.52±0.16	0.36-0.67	0.52	0.09±0.00	0.00-0.09	0.09
C ₂₀	1.33±0.58	0.37-2.71	1.12	0.12±0.00	0.00-0.12	0.12
C ₂₁	0.88±0.41	0.10-1.46	1.09	0.09±0.00	0.00-0.09	0.09
C ₂₂	1.25±0.99	0.26-2.24	1.25	0.17±0.00	0.00-0.17	0.17
C ₂₄	0.16±0.00	0.00-0.16	0.16			
C ₂₈	0.32±0.26	0.05-0.58	0.32			
C ₂₉				0.28±0.00	0.00-0.28	0.28
C ₃₀				0.32±0.00	0.00-0.32	0.32
C ₃₁				0.12±0.00	0.00-0.12	0.12
C ₃₂	0.42±0.00	0.00-0.42	0.42	0.45±0.00	0.00-0.45	0.45
Subtotal	3.47±2.06	0.00-16.8	0.93	0.25±0.22	0.00-2.66	0.00
ω-Hydroxy FAs						
C ₁₂	0.88±0.00	0.00-0.88	0.88	1.65±0.00	0.00-1.65	1.65
C ₁₃				0.96±0.00	0.00-0.96	0.96
C ₁₄	0.57±0.45	0.13-1.02	0.57	1.82±0.00	0.00-1.82	1.82
C ₁₅				0.24±0.00	0.00-0.24	0.24
C ₁₆	0.76±0.01	0.75-0.77	0.76	1.84±0.00	0.00-1.84	1.84
C ₁₇				2.74±0.00	0.00-2.74	2.74
C ₁₈	0.41±0.03	0.38-0.44	0.41	1.78±0.00	0.00-1.78	1.78
C ₁₉	0.56±0.00	0.00-0.56	0.56	2.32±0.00	0.00-2.32	2.32
C ₂₀	0.80±0.75	0.05-1.55	0.80	3.02±0.00	0.00-3.02	3.02
C ₂₁	0.22±0.22	0.00-0.43	0.22	0.63±0.00	0.00-0.63	0.63
C ₂₂	0.94±0.72	0.23-1.66	0.94	1.42±0.00	0.00-1.42	1.42
C ₂₃	0.37±0.26	0.11-0.63	0.37	0.59±0.00	0.00-0.59	0.59
C ₂₄	1.27±0.75	0.52-2.02	1.27	1.45±1.28	0.17-2.73	1.45
C ₂₅	0.58±0.00	0.00-0.58	0.58	1.94±0.00	0.00-1.94	1.94
C ₂₆	1.05±0.05	1.00-1.09	1.05	1.59±1.48	0.11-3.07	1.59
C ₂₇	0.70±0.00	0.00-0.70	0.70	0.83±0.00	0.00-0.83	0.83
C ₂₈	0.67±0.51	0.17-1.18	0.67	1.75±0.00	0.00-1.75	1.75
Subtotal	8.42±4.92	3.50-13.34	8.42	14.8±14.5	0.28-29.3	14.80

SE (Standard Error) = $\sigma/N^{1/2}$, where σ refers to the standard deviation of total samples (N).

The measured β -hydroxy FAs are the structural cell membrane lipid components of soil microbes. The even carbon predominance of β -hydroxy FAs is a source specific indicator for the contribution from soil GNB. Several studies have documented a similar feature that pertains to even carbon number predominance for the soil GNB worldwide. The AMBTs for the sampling days and cluster analysis revealed a dominant contribution from the NCP. Moreover, a lack of significant shift in the molecular distributions of β -hydroxy FAs could be

expected if these are derived *via* in situ formation in the atmosphere or derived from industrial emissions. Further, we observed a significant correlation (Table 6.2) of β -hydroxy FAs with other soil microbe tracers (*e.g.* trehalose, a proxy for fungal spores in soils), indicating an impact of biomass burning emissions and associated atmospheric processes in the NCP.

The molecular distributions of α -hydroxy FAs (Fig. 6.5a) showed even C-number predominance during H-BBEs and followed the sequence as $C_{14} > C_{10} > C_{16} \approx C_{12}$. However, no such pattern in the molecular distributions of α -hydroxy FAs was observed during L-BBEs (Fig. 6.5a). Furthermore, the relative abundance of even C-numbered α -hydroxy FAs to their total mass concentration (*i.e.*, Σ even and odd C) was $\sim 71\%$ during H-BBEs and $\sim 61\%$ during L-BBEs. Moreover, total mass concentrations of α -hydroxy FAs were higher than those of β - and ω -isomers over Mt. Tai in June 2006 (Table 6.1). *González-Pérez et al.* [2004] discussed the effect of biomass burning on soil organic matter. According to their study, lipid fraction of soil organic matter undergoes substantial changes (*i.e.*, translocation of organic matter) as a result of high temperature biomass combustion (*e.g.* forest fires). Interestingly, *Poole et al.* [2010] documented that β -hydroxy FAs and associated endotoxin levels were found to be highest in the atmospheric mineral dust from the agricultural fields. However, their study focuses on the settled agricultural dust from the fields rather than the re-suspended atmospheric mineral dust during fire activities (this study). Therefore, observed predominance of α -hydroxy FAs followed by β - and ω -hydroxy FAs over Mt. Tai could be due to a prevalent change in their distribution pattern of lipid compounds during biomass burning emissions in the NCP.

The molecular distributions of β -hydroxy FAs also exhibited even C-number predominance, among which C_{18} was dominant followed by C_{16} or C_{20} (Fig. 6.5b). These observations were contrary to those reported in the sediments and cell membrane of GNB, where molecular distributions were characterized by the predominance of C_{16} and/or C_{14} [*Hedrick et al.*, 2009; *Zhang et al.*, 2014].

Table 6.2. Pearson correlation coefficient (*r*) among the measured hydroxy fatty acids (FAs) and other chemical compounds in TSP collected over Mt. Tai in the North China Plain during the period of wheat residue burning (6–28 June 2006).

<i>Parameter</i>	^{\$} $\Sigma\alpha$ -Hydroxy FAs	[£] $\Sigma\beta$ -Hydroxy FAs	[#] $\Sigma\omega$ -Hydroxy FAs	Galactosan	Mannosan	Levogluconan	Arabitol	Mannitol	Trehalose
$\Sigma\alpha$ -Hydroxy FAs	1.00								
$\Sigma\beta$ -Hydroxy FAs	0.99	1.00							
$\Sigma\omega$ -Hydroxy FAs	-0.02	0.11	1.00						
Galactosan	0.92	0.63	-0.04	1.00					
Mannosan	0.91	0.57	-0.05	0.95	1.00				
Levogluconan	0.91	0.64	-0.05	0.99	0.98	1.00			
Arabitol	0.80	0.92	-0.03	0.83	0.79	0.83	1.00		
Mannitol	0.80	0.86	-0.05	0.72	0.71	0.72	0.96	1.00	
Trehalose	0.54	[#] 0.71	-0.25	0.69	0.61	0.69	0.76	0.74	1.00

Note: r-critical is the probability of *n* measurements (i.e., total number of samples) of two uncorrelated variables would yield a correlation coefficient comparable to or greater than a particular value [Pugh and Winslow, 1966].

^{\$}for α -hydroxy FAs, *n*=16; r-critical=0.5; Error=5%.

[£]for β -hydroxy FAs, *n*=7; r-critical=0.8; Error=3%; # correspond to r-critical=0.7 with error of 8%.

[#]for ω -hydroxy FAs; correlation coefficient are rather weak due to the limited number of sample with detection (*n*=4).

This could be due to an increased soil temperature of agricultural fields (*i.e.*, up to 300 °C) in the NCP during BBEs. LPS-derived β -hydroxy FAs are sensitive to soil temperature [Zogg *et al.*, 1997], thus, increased ground heating during biomass burning can affect their molecular distributions, emitting lower molecular weight species to gaseous phase. Zogg *et al.* [1997] examined the variability of molecular distribution of β -hydroxy FAs in soil for lower temperatures (5–25 °C) than what is usually observed during BBEs (*i.e.*, as high as 300–1000 °C). Therefore, we attribute the observed shifts in molecular distributions of β -hydroxy FAs to their contribution from disturbed agricultural soils during BBEs in the NCP.

Moreover, at extremely high temperature, thermal evaporation or degradation of hydroxy FAs can occur in soil- and plant-associated bacteria. For example, molecular distributions of long-chain *n*-alkane from rye and maize combustion shifted to their short-chain homologues at 500 °C, maximizing at C₁₈ with the predominance of even C-numbered compounds [Wiesenberg *et al.*, 2009]. Therefore, observed molecular distributions of hydroxy FAs during BBEs could be different than those from the complete absence of biomass combustion. Although the mass concentrations of atmospheric ω -hydroxy FAs were similar to those of both H-BBEs and L-BBEs, we observed significant shifts in the temporal variability of their molecular distributions over Mt. Tai (Fig. 6.5c). Molecular distributions of ω -hydroxy FAs were also characterized by an even C-number predominance with the occurrence of HMW compounds (>C₂₀) during H-BBEs and of LMW compounds (<C₂₀) during L-BBEs (Fig. 6.5c).

The even C-numbered predominance of hydroxy FAs in this study indicates the contribution from soil microbes and vascular plants. It has been suggested that lipids from terrestrial biomass (*i.e.*, either soil organic matter or plant waxes), in particular saturated fatty acids, are characterized by the predominance of even C-numbered compounds [Wiesenberg *et al.*, 2009]. For instance, saturated *n*-fatty acids from C₂₀ to C₃₂ with strong even/odd predominance are specific to epicuticular waxes [Eglinton and Hamilton, 1967; Meyers, 2003], which have been found in soils [Morrison, 1969], lake sediments [Cranwell, 1981] and deep sea sediments [Simoneit, 1977]. Likewise, Volkman *et al.* [1998] documented a significant contribution of even C-numbered saturated hydroxy FAs from microalgal sources in lacustrine and intertidal sediments. The air mass back-trajectory analyses for the sampling periods over Mt. Tai showed an atmospheric transport from the NCP. Because the air masses had a substantial influence from BBEs in the NCP, even/odd carbon predominance of

hydroxy FAs over Mt. Tai indicates a significant contribution from microbial biomass associated with soil organic matter emitted during fire activities.

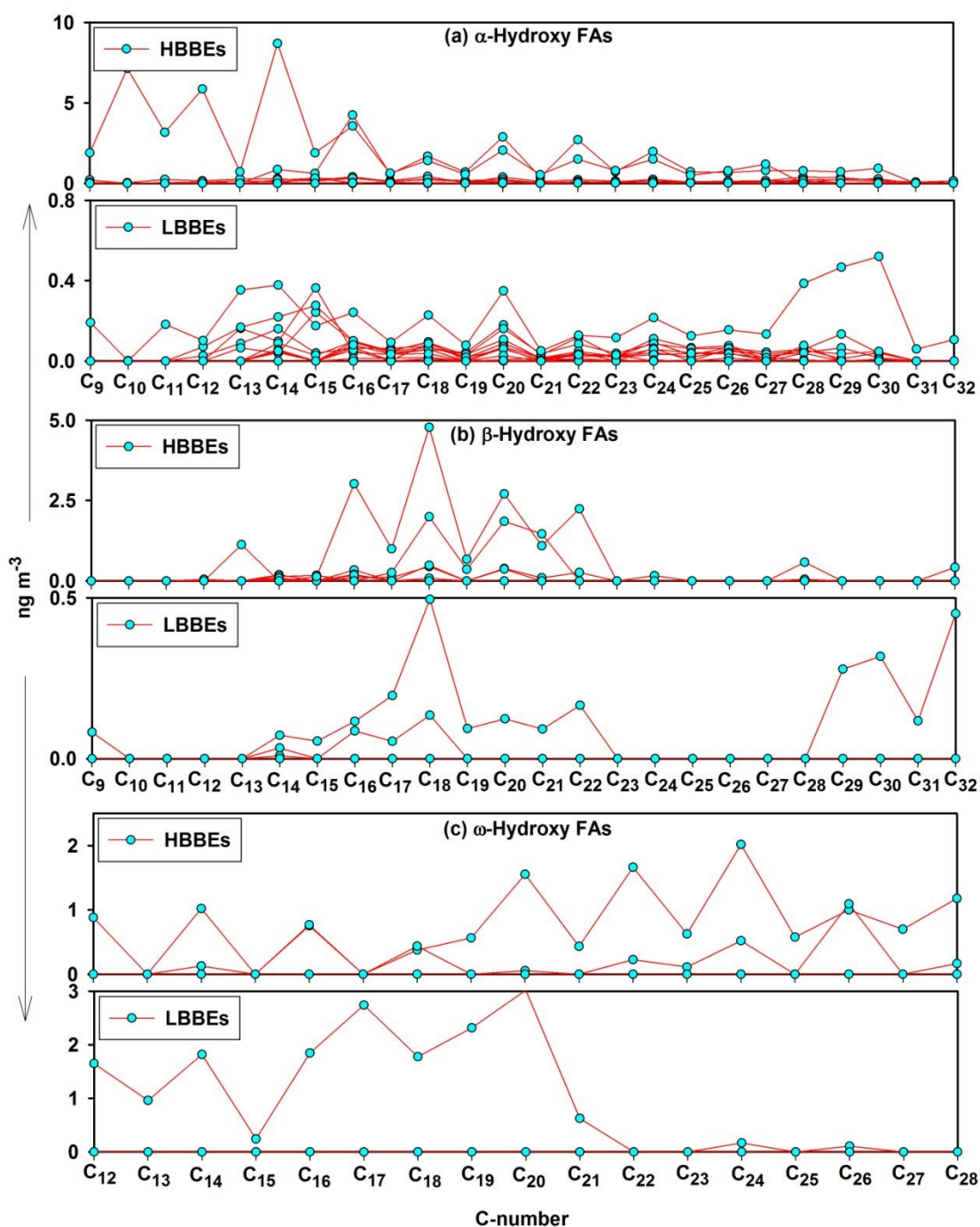


Fig.6.5. Molecular distributions of (a) α -hydroxy FAs (C₉-C₃₂), (b) β -hydroxy FAs (C₉-C₃₂) and (c) ω -hydroxy FAs (C₁₂-C₂₈) during high (H-BBEs; upper panel) and low (L-BBEs; lower panel) biomass burning emissions in summertime aerosols collected over Mt. Tai.

6.3.3. Average temporal concentration of hydroxy FAs

The impact of the emissions of postharvest crop-residue burning in the NCP to TSP collected over Mt. Tai was ascertained through the analyses of various organic markers such as diacids, sugars, and anhydrosugars [Fu *et al.*, 2008; Kawamura *et al.*, 2013]. Therefore, hydroxy FAs over Mt. Tai can have a contribution either from biomass burning or an associated process during biomass combustion (*e.g.* re-suspension of soil-dust during fire activities). This inference was based on the co-variability of atmospheric concentrations of hydroxy FAs (Fig. 6.6) with levoglucosan (a proxy for BBEs), trehalose and mannitol (tracers for soil microbes) [Fu *et al.*, 2008] over Mt. Tai. Soil microbes or plant pathogen can also be transported long distances through synoptic-scale atmospheric circulations. For instance, numerous studies have documented a significance of long-range transport of Siberian forest fire-derived aerosols on the lipid class compounds [Yamamoto *et al.*, 2011], dicarboxylic acids [Aggarwal and Kawamura, 2008; Pavuluri *et al.*, 2013] and soil microbes [Tyagi *et al.*, 2015b]. On a similar way, Fang *et al.* [1999] observed higher input of vascular plant markers (alkanes, alkanols and alkanoic acids) in late September when BBEs were intense in Indonesia. Since Mt. Tai also received a transported air mass from Mongolia and Siberia for some sampling days in late June, it is likely that soil microbes/plant pathogens from these regions are responsible to the measured hydroxy FAs.

The high concentrations of levoglucosan ($400\text{--}5000\text{ ng m}^{-3}$) during the study period indicate a significant contribution from biomass burning activities. In contrast, trehalose and mannitol were found in surface soils of crop fields, being associated with fugitive dust in atmospheric particulate matter [Rogge *et al.*, 2007]. Furthermore, trehalose was also found in aerosols sampled during the wheat straw burning [Wang *et al.*, 2011]. Higher temperatures and convective currents generated during H-BBEs could result in the suspension of fugitive dust and associated microbial biomass from the agricultural fields in the NCP. Therefore, similar temporal trends of hydroxy FAs (present study) with levoglucosan, trehalose and mannitol (Fig. 6.6) revealed that biomass-burning activities could be an indirect source of soil microbes in air-borne particulate matter *via* the injection of soil-dust particles.

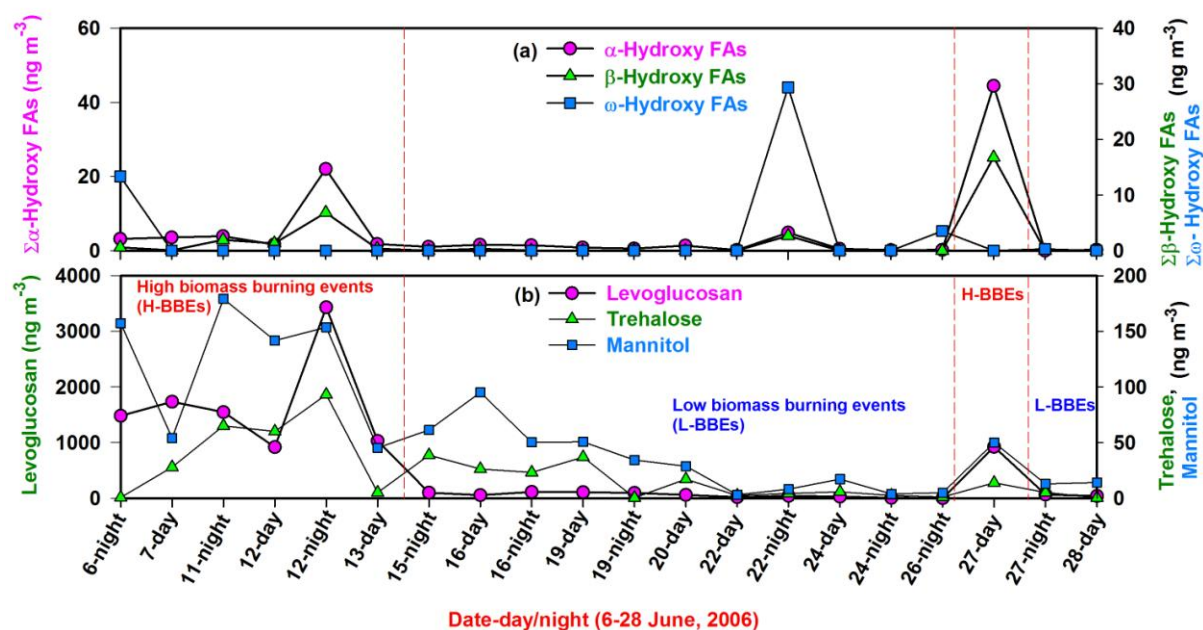


Fig.6.6. Temporal variability of concentrations of (a) α -, β - and ω -hydroxy FAs and (b) levoglucosan, trehalose and mannitol in aerosols collected over Mt. Tai with respect to H-BBEs (6-14 and 27 June) and L-BBEs (15-26 and 28 June) in northern China during the summer of 2006.

In the previous studies, few reports discuss an impact of BBEs on air-borne microorganisms. For example, forest fires lead to a significant reduction in the abundance of the bacterial biomass due to the heat shock generated by fire emissions [Adeniyi, 2010; Neary *et al.*, 1999; Xiang *et al.*, 2014]. In this study, we observed a sporadic decrease in atmospheric abundances of hydroxy FAs after BBEs, as evidenced by the apparently constant ratio of HMW/LMW hydroxy FAs (*i.e.*, confirmed by the unpaired two-tailed *t* test, Table 6.3). Being consistent with our results, higher abundances of HMW fatty acids (C_{20} – C_{30}) were also reported in the same TSP samples from Mt. Tai [Fu *et al.*, 2008], suggesting the dominance of wheat straw burning in early June 2006. Thus, concentrations of hydroxy FA were lower just after the BBEs in the NCP.

Table 6.3. Unpaired two-tailed *t* test results for the measured hydroxy fatty acids along with other biomass burning tracer compounds in TSP samples collected over Mt. Tai during high (H-BBEs; 6-14 June and 27 June, 2006) and low biomass burning events (L-BBEs; 15-26 June and 28 June, 2006) occurred in the NCP.

*Data obtained from Fu et al. 2008.

Parameter	H-BBEs (ng m ⁻³)	L-BBEs (ng m ⁻³)	<i>t</i> score, df, P-value
	Min.-Max. (Mean±SE)		
Σα-hydroxy FAs	2-44 (11±6.1)	0.1-4.8 (1.3±0.4)	2.4, 18, < 0.05
Σβ-hydroxy FAs	0.04-16.7 (4.0±2.3)	0.01-2.7 (1.0±0.8)	0.8, 8, > 0.05
Σω-hydroxy FAs	13.3	0.3-29.3 (15.9±9.2)	not applicable
HMW/LMW- α-hydroxy FAs	0.2-2.5 (0.98±0.28)	0.24-1.14 (0.64±0.08)	1.5, 17, > 0.05
HMW/LMW- β-hydroxy FAs	0.25-0.76 (0.46±0.11)	1.15	not applicable
HMW/LMW- ω-hydroxy FAs	1.59	0.79-1.53	not applicable
*Levogluconan	918-3432 (1580±332)	0.4-111 (55±11)	6.4, 18, < 0.05
*Trehalose	0.6-93.1 (37.9±13.3)	0.4-38.6 (12.6±3.9)	2.3, 18, < 0.05
*Mannitol	45-179 (112±22)	3-95 (30±8)	4.3, 18, < 0.05

6.3.4. Relative abundances

The relative abundances of two classified groups (*i.e.*, LMW C₉–C₁₉ and HMW C₂₀–C₃₀ or C₂₀–C₂₈) of α -, β - and ω -hydroxy FAs to their total mass concentrations were shown in Fig. 6.7. The relative abundances of LMW α - and β -hydroxy FAs (C₉–C₁₉) were higher during L-BBEs (53% and 81%, respectively) than those observed during H-BBEs (49% and 70%, respectively). In particular, molecular distributions of α -hydroxy FAs were characterized by the predominance of LMW species during both L- and H-BBEs (Fig. 6.5a). Although relative abundances of LMW β -hydroxy FAs showed a slight increase from H-BBEs to L-BBEs (similar to α -hydroxy FAs), they dominate in both events. *Almendros et al.* [1988] observed significant differences in the molecular distributions of microbial lipids in soil after fire activities, with a dominant occurrence of LMW fatty acid homologues (*i.e.*, <C₂₀). Therefore, observed predominance of LMW α - and β -hydroxy FAs (C₉–C₁₉) during L-BBEs could be attributed to changes in the molecular distributions of these compounds due to high temperature biomass combustion in the NCP.

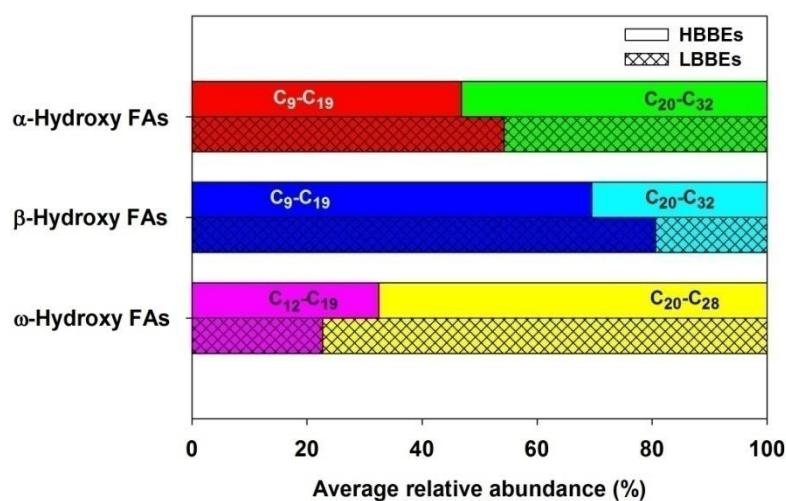


Fig.6.7. Relative abundances of low molecular weight (LMW) α -(C₉–C₁₉), β -(C₉–C₁₉), ω -(C₁₂–C₂₈) hydroxy FAs and high molecular weight (HMW) α -(C₂₀–C₃₂), β -(C₂₀–C₃₂), ω -(C₂₀–C₂₈) hydroxy FAs in the aerosols collected over Mt. Tai during high and low biomass burning emissions (H- and L-BBEs).

LMW β -hydroxy FAs and, to some extent, α -hydroxy FAs are specific to soil microbes [Keinänen *et al.*, 2003; Wilkinson, 1988]. These patterns were also found (*i.e.*, predominance of LMW β -hydroxy FAs in the total mass concentrations: Σ (LMW and HMW)) for remote marine aerosols collected over Chichijima Island in the North Pacific.

Therefore, predominance of these two groups of LMW hydroxy FAs (α - and β -) during L-BBEs could represent their background (or undisturbed) signatures of soil microbes. In addition, a predominance of LMW α - and β -hydroxy FAs during H-BBEs could be caused by the charring process of biomass combustion, which usually occurs at high temperatures. As LMW hydroxy FAs have been assigned to soil microbes and plant pathogens (*i.e.*, in particular GNB) and, hence, can be used as a marker for the residues of charred biomass coming from soil surfaces and plant where microbial activity is high.

One reason for changes in the molecular distribution of hydroxy FAs could be higher temperatures associated with biomass combustion, which affect the soil microbes. For instance, *Williams et al.* [2012] reported a substantial decrease ($\sim 25\%$) in GNB abundances, as estimated from phospholipids fatty acid (PLFA) analyses in soils followed by a forest fire. Similarly, predominance of LMW β -hydroxy FAs was also reported for springtime dust-laden aerosols sampled over the Chichijima Island. Therefore, observed predominance of LMW β -hydroxy FAs over Mt. Tai during H- and L-BBEs could be due to their contribution from source specific soil microbes.

In contrast, the predominance of HMW ω -hydroxy FAs in both events (67% in H-BBEs and 77% in L-BBEs) could represent a significant contribution from higher plant waxes (*e.g.* suberin, cutin; Fig. 6.7). However, LMW ω -hydroxy FAs were in part derived from ω -oxidation of long-chain hydroxy FAs [*Kawamura*, 1995] in soil microbes and might have contributions from higher plants. Overall, distributions of relative abundances in this study were attributed to their common source (*i.e.*, sourced from fugitive dust inputs during H-BBEs/L-BBEs in the NCP) and/or long-range atmospheric transport over Mt. Tai.

Among the measured hydroxy FAs, α -isomers dominate the total concentrations of hydroxy FAs during both H- and L-BBEs ($\sim 68\%$ and 77% , respectively; Fig. 6.8). The relative abundances of β -hydroxy FAs in total hydroxy FAs showed higher values ($\sim 21\%$) during H-BBEs than those ($\sim 2\%$) during L-BBEs. Although ω -hydroxy FAs (C_{12} – C_{28}) were detected only in few aerosol samples ($n=4$), their contribution in H-BBEs ($\sim 11\%$) was lower than those observed in L-BBEs ($\sim 21\%$). These discrepancies can be explained on a basis of sources of hydroxy FAs as discussed in previous sections. The predominance of HMW α -hydroxy FAs (C_{20} – C_{32}) in their total mass concentrations was observed (*i.e.*, 53%) during H-BBEs (Fig. 6.8). This observation suggests the contribution from higher plants and also partly

from α -oxidation of fatty acids [Cranwell, 1981]. In addition α -isomers can also be produced from the photochemical oxidation of HMW FAs during their long-range atmospheric transport. Hence, these cannot be employed as specific tracers for plant waxes in the environmental samples.

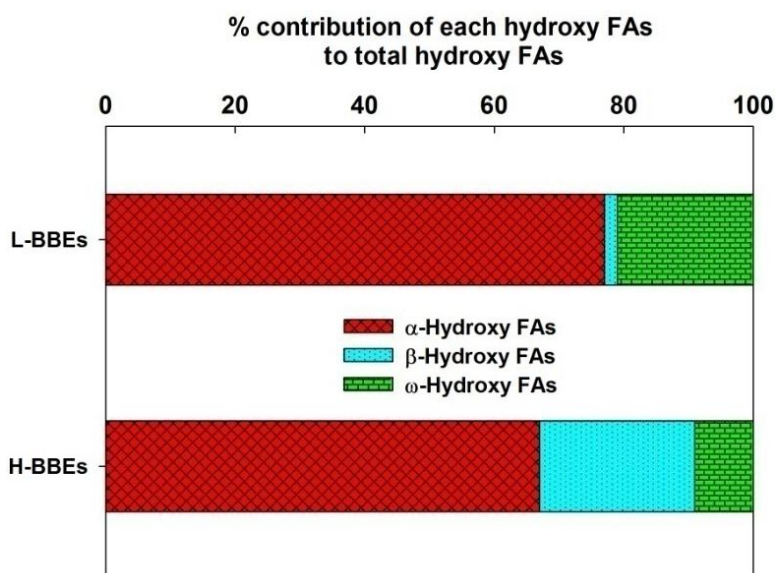


Fig.6.8. Relative contributions (in %) of α -, β - and ω -hydroxy FAs in total hydroxy FAs measured in ambient aerosols collected over Mt. Tai during high (H-BBEs: 6-14 June and 27 June) and low (L-BBEs: 15-26 June and 28 June) biomass burning events in the summer of 2006.

6.3.5. Source apportionment-Multiple linear regression analysis

To assess the potential sources/transport pathways, multiple linear correlation matrices were computed among the measured hydroxy FAs and other chemical species (Table 6.2). It is important to state here that we have detected α -hydroxy FAs in almost all samples ($n=19$), while β - and ω -hydroxy FAs were identified in limited numbers of samples ($n=10$ and $n=4$, respectively). The observed correlations of α - and β -hydroxy FAs with biomass burning-derived sugar compounds (*e.g.* galactosan, mannosan, levoglucosan and trehalose) were quenched by three outliers, in which two sampling dates correspond to H-BBEs (13 and 27 June 2006) and one is from L-BBEs (23 June 2006). Thus, these data points were excluded from the linear regression analyses to get a realistic correlation coefficient.

High concentrations of total α -hydroxy FAs (22 ng m^{-3}), total β -hydroxy FAs (6.8 ng m^{-3}), and sugar compounds (galactosan: 103 ng m^{-3} ; mannosan: 62 ng m^{-3} ; levoglucosan: $3.43 \mu\text{g m}^{-3}$; trehalose: 93 ng m^{-3}) were found on 13 June 2011. Likewise, the TSP samples collected on 27 June 2006 also showed much higher concentrations of sugar compounds (galactosan: 14 ng m^{-3} ; mannosan: 32 ng m^{-3} ; levoglucosan: 923 ng m^{-3} ; trehalose: 14 ng m^{-3}) than those collected before and after this date. Maximum concentration of total α -hydroxy FAs (44 ng m^{-3}) and total β -hydroxy FAs (17 ng m^{-3}) were found on 27 June 2006. The temporal shift in the concentrations of these compounds on 27 June 2006 is rather significant in levoglucosan and α -hydroxy FAs (~ 10 folds). Therefore, concurrent increase in the atmospheric abundances of α -hydroxy FAs and sugar compounds on these specific dates clearly indicates the more pronounced influence of biomass burning activity in the NCP and subsequent transport to the summit of Mt. Tai.

In contrast, a decreasing trend in the mass concentrations of anhydrosugars (*i.e.*, galactosan, mannosan and levoglucosan) was observed and trehalose on 23 June 2006, while atmospheric abundances of $\Sigma\alpha$ - and β -hydroxy FAs increased by a factor of 5 and 10, respectively. The sudden declines in the mass concentrations of sugar compounds are consistent with those of other organic markers measured (*e.g.* dicarboxylic acids on 23 June 2006). A sudden shift in prevailing wind direction was also observed from Southwest to East over Mt. Tai (see Fig. 6.2) during 22–25 June, which was associated with a high-pressure front system located near Japan. Interestingly, for this sampling period, exceptionally high concentrations of total ω -hydroxy FAs (22 ng m^{-3}) were observed; much higher than those of $\Sigma\alpha$ - and β -hydroxy FAs (4.8 and 2.7 ng m^{-3} , respectively; Fig. 6.2). Therefore, these three sampling dates (13, 23 and 27 June 2006) were excluded while examining the provinces of hydroxy FAs over Mt. Tai.

Moderate to strong correlations of α -hydroxy FAs with anhydrosugars (*i.e.*, galactosan, mannosan, and levoglucosan: tracers for BBEs) [Simoneit, 2002], sugar polyols (*e.g.*, arabitol, mannitol), and sugars (*e.g.* trehalose) are noteworthy during the study period. Likewise, good correlations were observed among β -hydroxy FAs (tracers for GNB) [Tyagi *et al.*, 2015a], arabitol, mannitol and trehalose (tracers for fungal spore and soil microbes) [Fu *et al.*, 2008], suggesting their common source. This can be explained by the dust emissions from agricultural fields in the NCP, Mongolia and Siberia.

6.3.6. Potential sources and transport pathways of hydroxy FAs

To identify the potential sources and transport pathways of hydroxy FAs over Mt. Tai, we further performed cluster analysis using PC-based HYSPLIT model [Draxler and Rolph, 2013 and references therein]. The principle behind the cluster analysis was to minimize the spread within the cluster and maximize the variability in between them using k means clustering algorithm in which horizontal moving speed and direction was used principal parameters [Hartigan and Wong, 1979]. Four clusters were identified, which provide information on the potential source regions of hydroxy FAs over Mt. Tai (Fig. 6.9). Moreover, fire count data were superimposed with the mean trajectory path of these clusters. This is to ascertain the influence of biomass combustion in the source regions on the emission and transport pathways of bacteria and plant waxes over Mt. Tai.

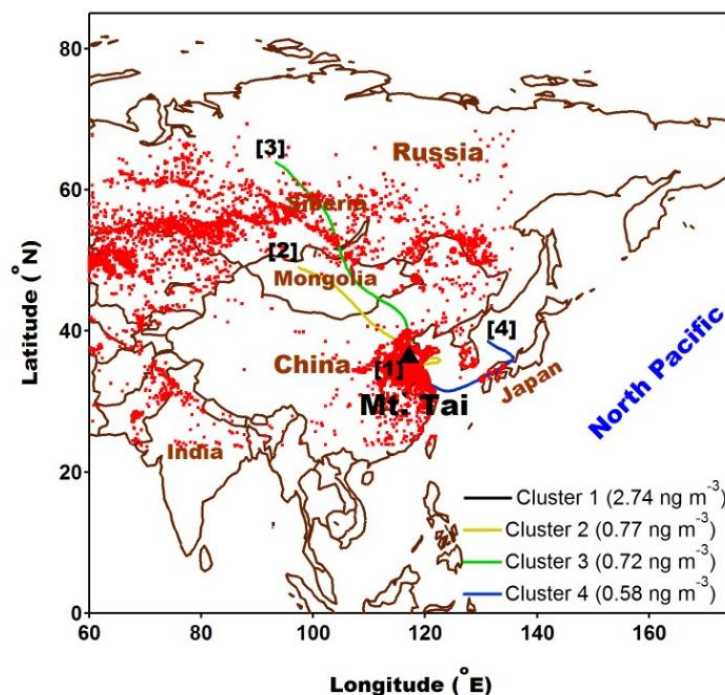


Fig.6.9. Mean horizontal plots of 7-day isentropic air mass back-trajectories at 500 m above ground level were grouped into 4 clusters as suggested by the NOAA HYSPLIT model. The colors black, yellow, green and blue indicate the mean clusters from 1 to 4, respectively. The numbers in parenthesis indicate the concentrations of total ($\alpha + \beta + \omega$) hydroxy FAs (in ng m^{-3}) according to the fraction of trajectories coming from respective source regions. The triangle in black color indicates the location of the sampling site and hot spots (fire spots) distribution as red dots.

The molecular distributions of hydroxy FAs over Mt. Tai can be explained by their contribution from four geographical locations, accounting for 57% (cluster 1: the NCP), 16% (cluster 2: Mongolia), 15% (cluster 3: Siberia) and 12% (cluster 4: maritime) of TSP samples. The average concentration of total hydroxy FAs (*i.e.*, $\Sigma(\alpha+\beta+\omega)$ -hydroxy FAs) multiplied by the percentage of air masses from each cluster (or source), provides a relevant source contribution to Mt. Tai (Fig. 6.9). The maximum percentage contribution (57%, cluster 1) of air masses over Mt. Tai originates from the NCP (conc., $\sim 2.74 \text{ ng m}^{-3}$) that includes regions such as Anhui, Jiangsu, Shandong, Henan and Hebei. These regions are characterized by agricultural waste burning after wheat harvest [Fu *et al.*, 2008]. Since the mean trajectory pathway is intercepted by BBEs in the NCP (Fig. 6.9 and Fig. 6.3), it could be inferred that biomass combustion and its associated process (*e.g.* soil- and plant-associated microorganisms) account for the high percentage contribution of hydroxy FAs over Mt. Tai.

Long-range atmospheric transport of hydroxy FAs could be an important source of hydroxy FAs over Mt. Tai because clusters '2' and '3' showed the contributions from Mongolia and Siberia (av. 0.77 and 0.72 ng m^{-3} of hydroxy FAs, respectively). Arid parts of these regions are the major sources of terrestrial organic matter (lipids) transported to Mt. Tai [Fu *et al.*, 2008] and further to the western North Pacific [Kawamura *et al.*, 2003; Tyagi *et al.*, 2015a; 2015b]. Mongolia (cluster 2) is characterized by arid climate, soil-derived particles, and the associated bacteria. Likewise, cluster 3 corresponds to the transport from Siberia (Russia), accounting for 15% of total hydroxy FAs. Since the mean AMBT for cluster 3 is intercepted by BBEs over Siberia (Fig. 6.9), soil microbes and plant metabolites from this region could partly explain the observed concentrations of hydroxy FAs. Agarwal *et al.* [2010] documented an influence of Siberian BBEs on the chemical composition of organic aerosols over East Asia during summer. Hence, it can be inferred that transport from Siberian BBEs could be an important source of hydroxy FAs in TSP over Mt. Tai during the summer of 2006.

Cluster 4 showed a minimal contribution to measured hydroxy FAs (av. 0.58 ng m^{-3}) by the maritime air masses that are traversing from the Sea of Japan to Mt. Tai. Several studies suggested that cell metabolites from cyanobacteria and marine algae could be a source of hydroxy FAs [Volkman *et al.*, 1998; Wakeham *et al.*, 2003; Wilkinson, 1988] via sea-to-air emission of micro-layer films. Therefore, we infer that the marine-derived contribution could explain, to some extent, the observed atmospheric abundances of hydroxy FAs over Mt. Tai.

6.3.7. Endotoxin levels and dry mass loading of GNB

Endotoxin concentrations and dry mass of GNB were estimated using β -hydroxy FAs. Endotoxin/lipopolysaccharides (LPS), constituents of GNB cell membranes, are potential inflammatory agents, and can lead to adverse health effects on human [Poole *et al.*, 2010]. The airborne endotoxin was quantified in various occupational and industrial environments using an assay with kinetic chromogenic limulus amoebocyte lysate (LAL) [Binding *et al.*, 2004; Lee *et al.*, 2004 and references therein]. However, LAL assay includes potential interferences from β -glucans originating from plants/moulds and is not sensitive enough to some bacteria [Binding *et al.*, 2004; Chow *et al.*, 2015; Lee *et al.*, 2004].

Alternately, some studies estimated the endotoxin levels and bacterial dry mass using β -hydroxy FAs [Lee *et al.*, 2004; Lee *et al.*, 2007]. These fatty acids are constituents of lipid A of LPS, which are responsible for the health effects of airborne endotoxin [Binding *et al.*, 2004; Ratledge and Wilkinson, 1988]. Few studies addressed the health burden of prolonged exposure to endotoxin emitted from the different biomass combustion in homes [Rosati *et al.*, 2005; Semple *et al.*, 2010]. However, any single study has not been performed on airborne endotoxin measurement in open field biomass burning emissions and their atmospheric transport to distant regions.

The influence of biomass combustion on the emission and atmospheric transport of GNB and endotoxin (discussed in section 6.3) to Mt. Tai were reported here. Endotoxin concentrations reported in this study (Table 6.4) are five times higher during H-BBEs (117 EU m^{-3}) compared to those estimated during L-BBEs (22.5 EU m^{-3}). These are consistent with the higher atmospheric abundances of GNB tracers (*i.e.*, β -hydroxy FAs ($\text{C}_{10}\text{--C}_{18}$)) during L-BBEs: 0.42 ng m^{-3} and H-BBEs: 2.15 ng m^{-3}). This could be caused by the increased emissions of GNB associated with soil-dust and plant pathogens during H-BBEs than those occurred during L-BBEs. Endotoxin levels in H-BBEs are slightly higher ($117 \pm 74 \text{ EU m}^{-3}$) than those documented in domestic wood-burning emissions from Nepal and Malawi (43 EU m^{-3} and 40 EU m^{-3} , respectively) [Semple *et al.*, 2010]. However, endotoxin concentrations reported in the wood burning emissions [Semple *et al.*, 2010] are comparable to those observed during L-BBEs ($22.5 \pm 14.6 \text{ EU m}^{-3}$). Surprisingly, Rosati *et al.* [2005] reported that endotoxin concentrations in domestic wood burning emissions from India varied

from moderate to high levels (*i.e.*, 24 EU m⁻³ and 190 EU m⁻³) even for the short-term sampling time (<60 min).

Table 6.4. A statistical summary of the estimated endotoxin and dry mass of Gram-negative bacteria (GNB) in summertime aerosols collected over Mt. Tai during high and low biomass burning emissions (H- and L-BBEs) occurred in the NCP in June, 2006.

Biomass burning emissions	Mean±SE	Range	Median
Endotoxins (EU m ⁻³)			
H-BBEs	117±74	2-549	38
L-BBEs	22.5±14.6	0.5-50.2	16.7
Dry mass of GNB (ng m ⁻³)			
H-BBEs	390±246	7-1830	126
L-BBEs	75±48.8	1.8-167	55.7

The observed variability in the endotoxin concentrations between open biomass burning and indoor biomass combustion could be associated with the differences in the analytical method employed. However, dilution of sampled air masses from the BBEs during transport can partly explain the observed low abundances over Mt. Tai. Being similar to our approach, *Lee et al.* [2004] estimated the endotoxin abundances using hydroxy FAs in PM₁₀ collected at rural and urban environments (68.6±11.5 EU m⁻³ and 123±12.6 EU m⁻³, respectively) in Hong Kong. The endotoxin levels from Mt. Tai during H-BBEs are somewhat consistent with those documented by *Lee et al.* [2004]. A comparison of endotoxin levels over Mt. Tai with those estimated for remote marine aerosols (av. 11±1.21 EU m⁻³ at Chichijima Island), indicate a long-range transport of airborne pathogens from the continent to the open ocean. Further studies are required to examine the effects of these sustained airborne bacterial particles, which can be potentially pathogenic to densely populated cities during indoor and outdoor biomass burning.

The mass loadings of GNB (Table 6.4) during H-BBEs (390 ng m⁻³) are higher than those reported in PM_{2.5-10} aerosols at both rural (45±1.5 ng m⁻³) and urban sites (93±1.4 ng m⁻³) [*Lee et al.*, 2004]. Higher mass loading of GNB in H-BBEs can be linked to the soil-dust emissions during biomass combustion and subsequent long-range atmospheric transport.

Furthermore, GNB dry mass estimates from *Lee et al.* [2004] are comparable to those observed during L-BBEs (75 ng m^{-3}) in our study.

Every year, the NCP faces serious air pollution problem caused by the field burning of agricultural residues (*e.g.* wheat/rice straws) and soil-dust emissions in its rural areas. Studies have reported high abundances of organic compounds in Chinese urban cities [*Fu et al.*, 2008 and references therein] and also in Changdao Island in the East China Sea [*Feng et al.*, 2007]. These high concentrations are due to an increased usage of fossil fuels and wide spread biomass burning combined with soil emissions. The present study shows a significance of hydroxy FAs from wheat straw burning emissions in the NCP. In addition, our study raises the concern for upcoming risk factor of endotoxin emissions from biomass burning, which can affect human health in developing and developed nations *via* long-range atmospheric transport.

6.4 Summary and Conclusions

Biomass burning events (BBEs) in the North China Plain is one of the principal sources of air-borne pollutants in China and also for the neighboring countries. To examine the impact of BBEs on soil bacteria and other higher plant metabolites, their tracer compounds, hydroxy fatty acids (FAs), were measured in the bulk particulate matter (TSP) over Mt. Tai during the period of wheat residue burning in June 2006. Higher inputs of epicuticular waxes and soil microorganisms during high BBEs (H; 6–14 and 27 June) relative to low BBEs (L; 15–26 and 28 June), were characterized by increased concentrations of homologous series of α -(C₉–C₃₂), β -(C₉–C₃₂) and ω -(C₁₂–C₂₈) hydroxy FAs in TSP samples. However, their relative abundances were not significantly different between H-BBEs and L-BBEs, suggesting their common source/transport pathways. We also found high concentrations of trehalose and mannitol (tracers of soil microbes), and levoglucosan (tracer of biomass combustion) during H-BBEs than L-BBEs. These results are consistent with hydroxy FAs, suggesting that they are associated with biomass combustion processes of agricultural wastes as well as re-suspension of mineral dust and plant pathogens. In addition, enhanced concentrations of endotoxin and mass loading of Gram-negative bacteria during H-BBEs ($117 \text{ endotoxin units (EU) m}^{-3}$ and 390 ng m^{-3} , respectively) were noteworthy as compared to those in L-BBEs (22.5 EU m^{-3} and 75 ng m^{-3} , respectively). Back-trajectory analysis and fire spots together with temporal variations of hydroxy FAs revealed an impact of biomass burning on emissions and atmospheric transport of bacteria and plant metabolites.

The present study shows an impact of biomass burning on the atmospheric abundances and molecular distributions of hydroxy FAs, potential tracers for soil microbes, plant pathogens, and higher plant waxes. Higher concentrations of hydroxy FAs were observed during H-BBEs than during L-BBEs. Estimated endotoxin concentrations during H-BBEs are also higher than the health-based occupational guidance limit ($\sim 90 \text{ EU m}^{-3}$) suggested by Dutch expert committee on Occupational Standards (DECOS) 2010 in Netherlands. Furthermore, these results are consistent with other organic tracers from biomass burning (*e.g.*, levoglucosan, mannosan, and galactosan), and soil microbes (*e.g.* trehalose and mannitol). This observation highlights a significance of re-suspended dust from charred agricultural soils during fire emissions, which can act as a principal source of airborne soil microbes. Figure 6.10 summarizes the whole idea, which is addressed in this study. Overall, temporal shifts in the molecular distributions of these tracer compounds are likely associated with high temperatures occurred during agricultural residue combustion. However, the molecular distributions of hydroxy FAs from Mt. Tai are different with respect to C-number predominance than those documented from the remote Island (Chichijima) in the western North Pacific, which also receives soil-borne microbes from the Asian dust.

It can be concluded that any types of biomass burning (outdoor/indoor) can severely influence the emission of soil- and airborne microbes, and increase the risk of exposure to their potent pathogenic particles (endotoxin) regionally as well as globally. Further studies are required on the atmospheric abundances of hydroxy FAs that enhance our understanding on bacterial emissions, atmospheric transport, and their scavenging by particulate matter.

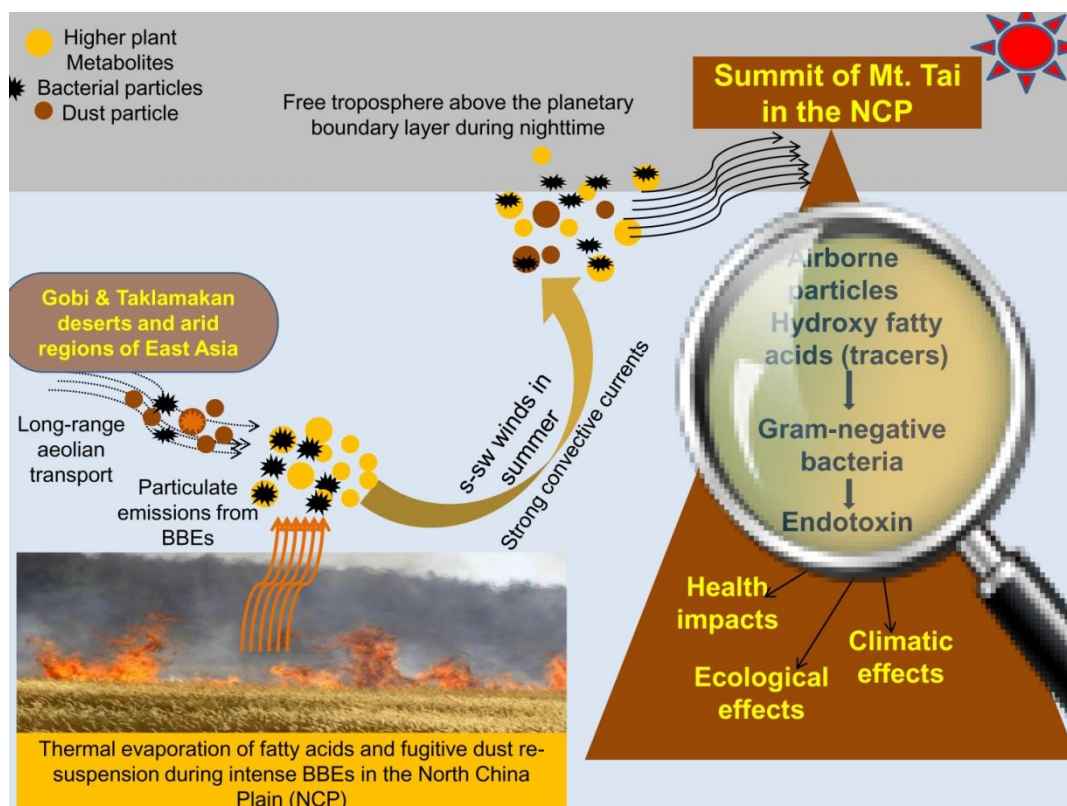


Fig.6.10. Conceptual model is explaining the atmospheric transport of hydroxy fatty acids (FAs) to high mountain sites. Higher abundances of bacterial tracers (hydroxy FAs) in re-suspended dust during biomass burning in the North China Plain (NCP) were observed in the aerosols collected at the summit of Mt. Tai. Intense open field burning of agriculture-residue can influence the microbial abundances and diversity in the atmosphere as well as their long-range transport, which can pose potential health impacts globally.

Chapter 7

Hydroxy Fatty Acids in Fresh Snow Samples from Northern Japan: Long-Range Atmospheric Transport of Gram-Negative Bacteria by Asian Winter Monsoon

Chapter 7 presents the results of hydroxy FAs in fresh snow collected from Sapporo (Japan). The molecular distributions of snow were interpreted in terms of their in-cloud oxidation processes.

7.1 Introduction

Lipid biomarkers from terrigenous plants, algae, fungi and soil microorganisms have been reported extensively in aerosols [Simoneit, 1977; Gagosian *et al.*, 1981; Gagosian *et al.*, 1987; Kawamura, 1995; Conte and Weber, 2002; Kawamura *et al.*, 2003; Simoneit *et al.*, 2004], sediments [Kawamura and Ishiwatari, 1984; Kawamura *et al.*, 1987; Kawamura, 1995; Zhang *et al.*, 2014], ice core [Sankelo *et al.*, 2013] and rain/snow [Kawamura and Kaplan, 1986; Satsumabayashi *et al.*, 2001; Yamamoto *et al.*, 2011]. These studies have utilized fatty acids as a proxy to assess the terrigenous contribution of higher plant waxes to various environmental samples owing to their abundant presence in biopolymers of plants and microorganisms. Similarly, certain hydroxy fatty acids (*e.g.*, C₁₀-C₁₈ β -hydroxy FAs) have been proposed as a tracer to understand the airborne bacterial transport.

These tracer compounds in snow samples may be important to better understand the contribution of plant and pathogenic bacteria to regional vs. long-range atmospheric transport [Hines *et al.*, 2003; Lee *et al.*, 2004; Lee *et al.*, 2007] as their presence in the atmosphere can affect the CCN and ice nuclei activity [Morris *et al.*, 2008]. To the best of our knowledge, this study is the first to report α , β - and ω -hydroxy FAs in snow samples. Snow efficiently scavenges airborne particles including soil microbes and higher plant metabolites in the free boundary layer of troposphere. Since hydroxy FAs from GNB and plants are inert in nature, they do not undergo chemical modification during snow accumulation. Therefore, hydroxy FAs in fresh snow can be used as a tracer to assess the sources and transport pathways of microorganisms and plant metabolites. In this study, hydroxy FAs were determined in fresh snow samples collected from Sapporo, Japan, to evaluate the qualitative contribution from GNB and higher plant metabolites. These results support the hypothesis that measured hydroxy FAs are important tracers to better understand the contribution of microorganisms to the organic matter in snow. More importantly, the possible transformations of these chemical markers were also discussed during long-range atmospheric transport.

7.2 Site description

Sapporo (43.07 °N, 141.36 °E) is the capital of Hokkaido, whose population is 1.9 million (June, 2013). Sapporo receives cold and dry air masses with heavy snowfall during the Asian winter monsoon. The average temperature of Sapporo in winter goes up to ~ 2 °C [Yamamoto *et al.*, 2011]. Snow cover over the ground and fallen leaves of deciduous

plants suppresses the suspension of soil particles during winter whereas the emissions of plant biomarkers from local vegetation are minimal. During winter season, Asian monsoon affects the regional climate, air quality and human health in Japan, delivering anthropogenic aerosols and dust from China and Siberia [Yamamoto *et al.*, 2011]. Several studies have examined the chemical and isotopic composition of ambient aerosols in various types of air masses in Sapporo [Aggarwal and Kawamura, 2008; Pavuluri *et al.*, 2013; Yamamoto *et al.*, 2011] to better understand the impacts of anthropogenic and biogenic contributions from Siberia, North China and surrounding oceans. However, no study is available from Sapporo, which focuses on the transport of microorganisms using organic markers.

In this study, eleven fresh snow samples were collected from the rooftop of the Institute of Low Temperature Science (ILTS) building, Hokkaido University in Sapporo during intensive snow fall periods (January-March) in 2010 and 2011. The detailed description about snow collection and analytical protocol of lipid fraction analyses is similar to that described in Yamamoto *et al.* [2011]. To avoid the contribution of any possible impurities from the dry deposition of aerosols, 1-2 cm of surface snow cover were removed prior to sample collection. Thereafter, snow samples were collected into a cleaned glass jar (8 L) by using a stainless steel shovel. In each glass jar, mercuric chloride (HgCl_2) was added before sampling to prevent microbial activity. Soon after the collection, glass jars were tightened with a Teflon-lined screw cap and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. The detailed description about snow collection and analytical protocol of lipid fraction analyses were given in chapter 2.

7.3 Results and Discussion

7.3.1. Air mass backward trajectory analysis

The air mass back-trajectories (AMBTs) provide a means to qualitatively assess the source regions of airborne pollutants over a receptor site. For this study, 7-day isentropic AMBTs were computed using hybrid single particle lagrangian integrated trajectory (HYSPLIT) model [Draxler and Rolph, 2013 and references therein]. The meteorological parameters (GDAS data sets) from NOAA air resources laboratory were used as an input for the HYSPLIT model. Fig. 7.1 shows the AMBT cluster at arrival heights of 500, 1000 and 1500 m over Sapporo during sampling days of winter 2010 and 2011. In almost all snow sampling periods in Sapporo, the AMBTs show plausible influence of air masses from Russia and Siberia via the long-range atmospheric transport.

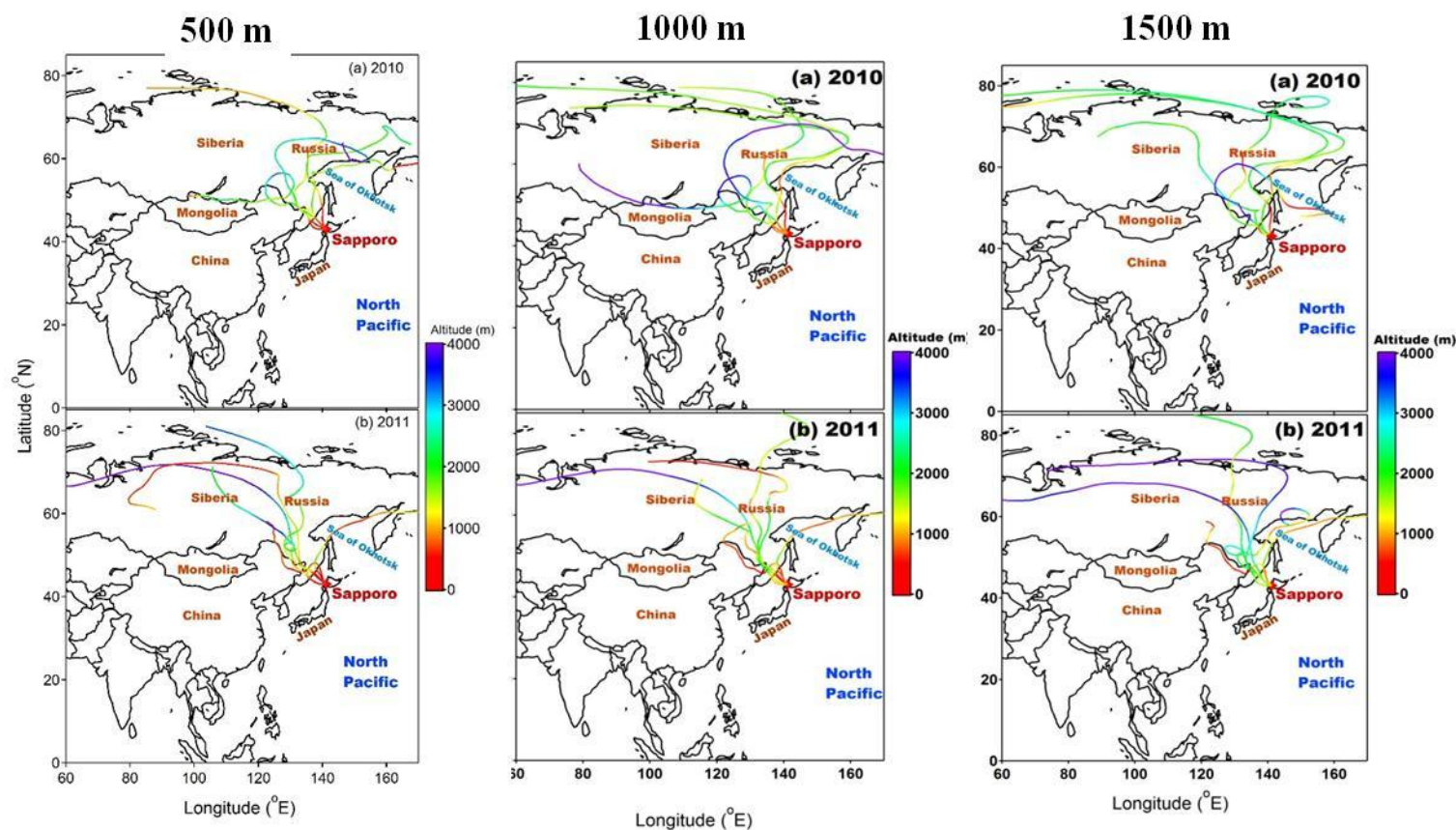


Fig.7.1. Air mass back-trajectory cluster at arrival heights of 500, 1000 and 1500 m AGL (above ground level) for the sampling days in winters of (a) 2010 and (b) 2011.

7.3.2. Mass concentrations

Homologues series of α -, β - and ω -hydroxy FAs were detected in fresh snow samples collected from Sapporo. Their mass concentrations are summarized in Table 7.1 and Table 7.2 for winter 2010 and 2011, respectively. Based on two-year seasonal data on hydroxy FAs, concentrations of α -hydroxy FAs were found to be significantly higher than β - and ω -hydroxy FAs. The predominance of α -hydroxy FAs can be explained by the α -oxidation pathway of FAs, which generally occurs in plants, animals and bacteria [Cranwell, 1981 and references therein] whereas β - and ω -oxidation is specific to bacteria [Lehninger, 1975]. α -Hydroxy FAs, in particular high molecular weight ones, come from the epicuticular waxes of higher plants as well from algae. However, higher abundance of α -hydroxy FAs were also found in the biomass burning aerosols collected over Mt. Tai, China, possibly due to photochemical oxidation of higher molecular weight fatty acids. Such a possibility of in-situ formation of α -hydroxy FAs has also been reported in the hydrolysis products of leaf

waxes and wood, and in microalgae and sea grasses [Feng *et al.*, 2015]. Further, microbial oxidation could also be a possible source of α -hydroxy FAs [Eglinton *et al.*, 1968] in the snow samples studied. Hence, it is suggested that α -hydroxy FAs cannot be employed as the tracers of plant waxes only, as they can come from microbial/photochemical oxidation of higher molecular weight fatty acids during long-range atmospheric transport.

A characteristic feature of our data is the predominance of C₁₆ hydroxy FAs in all the types of hydroxy FAs measured. However, significant shifts were observed in the carbon numbers of the second most abundant β -hydroxy FAs (mostly C number >16) and ω -hydroxy FAs (*i.e.*, C number <16; see Tables 7.1 and 7.2). A likely explanation for this observation is that β -hydroxy FAs above C₁₆ were formed by β -oxidation of long chain FAs, which is a more common in microorganisms as discussed previously. In contrast, ω -hydroxy FAs <C₁₆ are present in plants and microbes [Cardoso and Eglinton, 1983], in which ω -oxidation of fatty acids is secondary choice for microbial oxidation.

Table 7.1. Mass concentrations (in ng kg⁻¹) of α -, β - and ω -Hydroxy fatty acids (FAs) measured in snow samples (N=5) collected from Sapporo during winter 2010.

C-number	2010								
	α -Hydroxy FAs			β -Hydroxy FAs			ω -Hydroxy FAs		
	range	mean $\pm$ S.E.	median	range	mean $\pm$ S.E.	median	range	mean $\pm$ S.E.	median
C ₉	b.d.-7.1	2.4 $\pm$ 1.3	1.7	0.5-2.7	1.8 $\pm$ 0.47	2	b.d.-1.7	0.97 $\pm$ 0.4	1.4
C ₁₀	b.d.-37.3	14.6 $\pm$ 7.6	10.9	1.7-6.5	4.6 $\pm$ 1.2	5.1	b.d.-5.1	1.7 $\pm$ 1.1	0
C ₁₁	b.d.-35.1	21 $\pm$ 6.5	21.1	3.4-7.9	6.1 $\pm$ 0.8	6.2	b.d.-6.4	2.2 $\pm$ 1.4	0
C ₁₂	b.d.-46.7	25.3 $\pm$ 7.8	22.6	8-10.1	9.2 $\pm$ 0.4	9.8	b.d.-95.6	47.2 $\pm$ 17.8	32.7
C ₁₃	b.d.-45.2	20 $\pm$ 7.3	18	3.5-11.9	7.1 $\pm$ 1.8	6	b.d.-5.1	3.7 $\pm$ 0.9	4.4
C ₁₄	b.d.-53.4	27.1 $\pm$ 8.5	27.6	16.6-40.9	23.5 $\pm$ 4.4	19.6	b.d.-196.7	101 $\pm$ 34.7	79.8
C ₁₅	b.d.-44	18.6 $\pm$ 7.2	16.4	2.9-10.8	6.8 $\pm$ 1.4	6.7	b.d.-17	9.6 $\pm$ 3.1	12.8
C ₁₆	b.d.-139	89.2 $\pm$ 23.6	97.8	21.7-79.4	45.1 $\pm$ 9.4	4.4	2.3-754.1	296 $\pm$ 129	256.3
C ₁₇	b.d.-26.5	12.4 $\pm$ 4.4	10	3.1-10.7	7.5 $\pm$ 1.3	8.4	b.d.-12.6	7.1 $\pm$ 2	8.1
C ₁₈	b.d.-44.7	26.2 $\pm$ 8.1	26.3	23.4-52.3	33.5 $\pm$ 6.6	29.1	b.d.-43.9	21.2 $\pm$ 6.9	21
C ₁₉	b.d.-20.1	11.5 $\pm$ 3.4	11.5	5.3-21.7	10.4 $\pm$ 3.8	7.3	b.d.-12.2	5.5 $\pm$ 2	5.7
C ₂₀	b.d.-46.6	25 $\pm$ 7.8	21.5	14.4-120	48.3 $\pm$ 25	29.2	0.2-45.6	17.2 $\pm$ 7.6	13.5
C ₂₁	b.d.-21.1	12.1 $\pm$ 3.7	11.2	5.6-28.8	14.8 $\pm$ 5.4	13	b.d.-8.7	3.6 $\pm$ 1.4	3
C ₂₂	b.d.-73.7	40.8 $\pm$ 13.1	37.7	11.2-30.4	19.5 $\pm$ 4.1	18.2	b.d.-318	96.4 $\pm$ 56.5	50.7
C ₂₃	b.d.-32.8	18.5 $\pm$ 5.8	18.3	2.8-33.9	13.2 $\pm$ 7.1	8.1	b.d.-9.2	3.8 $\pm$ 1.6	3.6
C ₂₄	b.d.-145	64 $\pm$ 25	56.8	6.2-29	15 $\pm$ 5.1	12.3	b.d.-72.4	24.1 $\pm$ 12.7	13
C ₂₅	b.d.-39.1	18.4 $\pm$ 6.7	15.4	1.4-17.4	7.7 $\pm$ 3.4	5.9	b.d.-2.6	1.02 $\pm$ 0.5	1.2
C ₂₆	b.d.-49.3	18.6 $\pm$ 9	15.8	b.d.-18	7.5 $\pm$ 3.8	6	b.d.-3.2	0.6 $\pm$ 0.6	0
C ₂₇	b.d.-14.4	4.4 $\pm$ 2.8	1.1	b.d.-2.7	0.7 $\pm$ 0.7	0	b.d.-0.2	0.03 $\pm$ 0.03	0
C ₂₈	b.d.-10.9	4 $\pm$ 2.5	0	b.d.-1.6	0.3 $\pm$ 0.3	0			
C ₂₉	b.d.-0.54	0.1 $\pm$ 0.1	0						
C ₃₀	b.d.-0.32	0.06 $\pm$ 0.06	0						
Total	432-774	593 $\pm$ 88	582	70-379	247 $\pm$ 52	252	2-1411	643 $\pm$ 228	530

Note: b.d.= below detection limit ≤ 0.02 ng kg⁻¹; S.E. (Standard Error) = $\sigma/N^{1/2}$, where σ refers to standard deviation of total samples (N).

Table 7.2. Mass concentrations (in ng kg⁻¹) of α -, β - and ω -Hydroxy fatty acids (FAs) measured in snow samples (N=6) collected from Sapporo during winter 2011.

C-number	2011								
	α -Hydroxy FAs			β -Hydroxy FAs			ω -Hydroxy FAs		
	range	mean $\pm$ S.E.	median	range	mean $\pm$ S.E.	median	range	mean $\pm$ S.E.	median
C ₉	b.d.-27.2	14.2 $\pm$ 5.7	13.8	1-8.5	5.1 $\pm$ 1.3	6	b.d.-16.4	11.0 $\pm$ 2.6	12.9
C ₁₀	b.d.-65.4	30.9 $\pm$ 11.2	33.3	1.7-12.7	8.1 $\pm$ 1.8	8.8	b.d.-4.7	0.8 $\pm$ 0.8	0
C ₁₁	19.8-66.6	34.2 $\pm$ 8.5	28.5	1.7-13.3	9.2 $\pm$ 1.9	10.1	b.d.-4.7	0.8 $\pm$ 0.8	0
C ₁₂	20.7-60.4	36.5 $\pm$ 6.6	32.9	1.3-15.3	8.7 $\pm$ 2.2	8.8	b.d.-13.4	4.3 $\pm$ 2.7	0
C ₁₃	b.d.-49.2	21.5 $\pm$ 8.2	21.8	4.5-15.8	9.1 $\pm$ 2.1	8.6	b.d.-7.3	2.1 $\pm$ 1.2	1
C ₁₄	7.5-55.3	28.6 $\pm$ 7.7	28.4	4.5-25.5	13.7 $\pm$ 4	16.6	b.d.-61.5	17.7 $\pm$ 9.3	9.1
C ₁₅	b.d.-77.6	29.2 $\pm$ 13.1	23.3	1.9-11.1	6.3 $\pm$ 1.8	7.7	b.d.-12.1	4.0 $\pm$ 2.2	3.9
C ₁₆	14.3-186	94.0 $\pm$ 29.3	92.5	2.8-55.8	30.5 $\pm$ 10.2	32.8	b.d.-159	42.9 $\pm$ 24.7	19.4
C ₁₇	2.8-29.3	15.3 $\pm$ 4.3	14.5	1.6-12.2	7.7 $\pm$ 2.2	9	b.d.-8.2	1.9 $\pm$ 1.3	0.3
C ₁₈	8.0-55.8	31.3 $\pm$ 8.2	29.9	0.6-31.4	14.4 $\pm$ 5.3	13.6	b.d.-18.2	5.8 $\pm$ 2.8	3.9
C ₁₉	b.d.-22.4	6.2 $\pm$ 4.4	0	1.9-10.9	6.5 $\pm$ 1.5	7.1	b.d.-6.5	1.5 $\pm$ 1.0	0.5
C ₂₀	11.5-97.9	53.5 $\pm$ 18.6	47.3	1.2-43.4	23 $\pm$ 8.6	27.4	b.d.-10.5	3.3 $\pm$ 1.5	2.3
C ₂₁	b.d.-95.2	29.1 $\pm$ 17.2	13	1.0-16.6	8.8 $\pm$ 3.2	8.8	b.d.-3.4	1.0 $\pm$ 0.5	0.6
C ₂₂	13.4-109	60.8 $\pm$ 19.9	56.1	1.6-27.2	19.8 $\pm$ 6.1	25.2	b.d.-48.1	13.7 $\pm$ 7.4	8.2
C ₂₃	8.1-58.1	32.2 $\pm$ 10.1	26.3	5.7-11.6	9.1 $\pm$ 1.7	10	b.d.-6.8	1.2 $\pm$ 1.1	0
C ₂₄	12.3-92.2	74.9 $\pm$ 34	34	19.1-24.3	22.2 $\pm$ 1.6	23.1	b.d.-38	9.1 $\pm$ 6.0	3.2
C ₂₅	2.6-51.3	18.4 $\pm$ 8.9	9.8	3.3-11.1	8.5 $\pm$ 2.6	11.1	b.d.-3.7	1.0 $\pm$ 0.6	0
C ₂₆	2.6-52.0	24.2 $\pm$ 9	23.5	b.d.-15.9	6.4 $\pm$ 3.1	4	b.d.-10	2.2 $\pm$ 1.6	0.1
C ₂₇	b.d.-5.6	2 $\pm$ 1.3	0	b.d.-9.2	3.3 $\pm$ 1.6	2.1			
C ₂₈	b.d.-4.8	1.4 $\pm$ 0.9	0	b.d.-10.6	4.3 $\pm$ 2.1	2.3	b.d.-1.4	0.2 $\pm$ 0.2	0
C ₂₉	b.d.-3.35	0.7 $\pm$ 0.67	0						
C ₃₀	bd.-0.60	0.12 $\pm$ 0.12	0						
Total	169-1279	639 $\pm$ 187	651	6-354	179 $\pm$ 64	170	27-422	149 $\pm$ 73	102

Note: b.d.= below detection limit ≤ 0.06 ng kg⁻¹. S.E. (Standard Error) = $\sigma/N^{1/2}$, where σ refers to standard deviation of total samples (N).

7.3.3. Molecular distributions

Fig. 7.2 presents molecular distributions of α -hydroxy (C_9 to C_{30}), β - and ω -hydroxy FAs (C_9 to C_{28}) in snow samples from Sapporo during winter 2010 and 2011. Even carbon number predominance is noteworthy for α -, β - and ω -hydroxy FAs. α -Hydroxy FAs show molecular distributions with the order $C_{16} > C_{24} > C_{22}$ in both years (Fig. 7.2a). Likewise, β -hydroxy FAs show the predominance of C_{16} followed by C_{18} or C_{20} and then by C_{14} in both winters. However, we found the predominance of C_{20} β -hydroxy FA over C_{16} in one snow sample during 2010. Similarly, ω -hydroxy FAs showed dominance of C_{16} followed by the others as $C_{14} > C_{12} \sim C_{22} \sim C_{24}$ during snowfall in both the years.

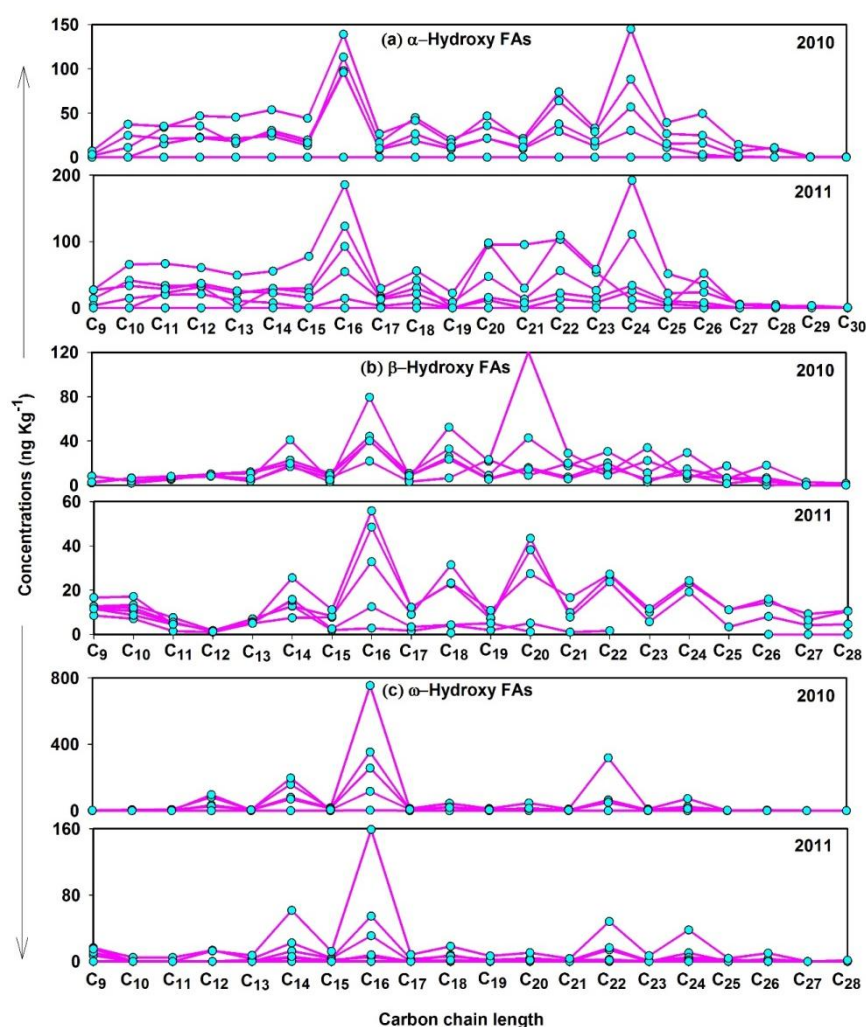


Fig.7.2. Molecular distributions of (a) α -Hydroxy fatty acids (FAs) (C_9 - C_{30}), (b) β -Hydroxy FAs (C_9 - C_{28}) and, (c) ω -Hydroxy FAs (C_9 - C_{28}) in the snow samples collected from Sapporo during winter 2010 and 2011.

Table 7.3 describes the statistically significant differences in the ratios of even-to-odd carbon numbers for α -, β -, and ω -hydroxy FAs in snow samples based on two-tailed unpaired t test. No significant differences were observed between 2010 and 2011 for the ratios of even-to-odd carbon number α -hydroxy FAs. In contrast, the difference is statistically significant between 2010 and 2011 for β - and ω -hydroxy FAs. In fact, the difference is extremely larger for ω -hydroxy FAs than that for β -isomers. In 2010 winter, AMBTs show atmospheric transport from the continents at 500, 1000 and 1500 m above ground, however, at the same heights in 2011 winter, the air masses came from the oceans during one sample collection. Higher plants in the continents contribute to higher abundances of hydroxy FAs than the oceans, and thus explain higher abundances of β - and ω -hydroxy FAs in 2010 than 2011. On average, even carbon numbered α -, β - and ω -hydroxy FAs in their total mass concentrations account for ~69, 68 and 84%, respectively. The even carbon number predominance is also found in recent marine and lacustrine sediments [Cardoso and Eglinton, 1983; Goossens *et al.*, 1986; Kawamura, 1995; Zhang *et al.*, 2014].

Table 7.3. Two-tailed unpaired t test to ascertain the statistical significance of ratio of relative abundances of even to odd carbon numbered hydroxy FAs in snow samples collected from the Sapporo between winter-2010 and 2011.

even/odd	2010	2011	t-score, df, P-value
α -hydroxy FAs	2.4 ± 0.3	2.2 ± 0.3	1.4, 7, > 0.05
β -hydroxy FAs	2.9 ± 0.8	1.8 ± 0.6	2.5, 9, < 0.05
ω -hydroxy FAs	15.8 ± 4.5	3.2 ± 1.8	5.8, 7, < 0.05

Similar to present study, Volkman *et al.* [1980] documented the bimodal distribution of α -hydroxy FAs with peaks at C_{16} and C_{24} in the intertidal sediments from Victoria, Australia and attributed their contribution from sea grass (*i.e.*, *Zostera muelleri*) detritus owing to similar distribution pattern. However, it is noteworthy that our AMBTs show a continental origin rather than the oceanic origin. Therefore, it is possible that waxes emitted from continental grasses *via* wind abrasion can be transported to Sapporo through the atmosphere. α -Hydroxy FAs (C_{16} - C_{28}) in Sapporo snow can be speculate to be used as a tracer of plant waxes. Likewise, higher plant derived cutin and suberin have been suggested as a significant source of C_{16} to C_{22} α -, β - and ω -hydroxy FAs [Cardoso and Eglinton, 1983]. In a similar way, it has been proposed that hydroxy FAs (C_{20} - C_{30}) are principally derived

from terrestrial higher plants [Kawamura and Ishiwatari, 1984]. Therefore, α -, β - and ω -hydroxy FAs (C_{16} - C_{22}) in snow samples can be related to their sources from terrestrial higher plants through long-range atmospheric transport.

Previous studies documented ubiquitous occurrence of these hydroxy FAs in soil microbes such as yeast and fungi [Van Dyk *et al.*, 1994 and references therein] and in the LPS of GNB [Lee *et al.*, 2007]. Moreover, prior studies focusing on β -hydroxy FAs with the predominance of C_{16} and C_{18} , suggested the contributions from yeast and fungi [Stodola, 1967; Van Dyk *et al.*, 1994 and references therein]. Molecular distributions of β -hydroxy FAs show a predominance of C_{16} followed by C_{18} or C_{20} (see Fig. 7.2b), suggesting that they have been derived from soil microbes. Likewise, FAs $<C_{20}$ are derived from marine phytoplankton [Kawamura, 1995 and references therein]. β -Hydroxy FAs (C_{10} - C_{18}) have been proposed as a biomarker for soil microbes as they are the constituents of LPS of GNB [Szponar *et al.*, 2002; Lee *et al.*, 2004]. Hence, it is likely that β -hydroxy FAs in snow samples may have been significantly influenced by GNB and terrestrial higher plant metabolites.

Fig. 7.3 depicts bar graphs, showing the relative abundances of α -, β - and ω -hydroxy FAs in the snow samples from Sapporo during winter. The proportions of two classified groups (LMW C_9 - C_{19} and HMW C_{20} - C_{30} or C_{20} - C_{28}) of α -, β - and ω -hydroxy FAs were found to be very similar between 2010 and 2011 (Fig. 7.3). This observation is perhaps related to their common sources/transport pathways of α -, β - and ω -hydroxy FAs over Sapporo. This inference is further supported by the AMBTs computed at arrival heights of 500, 1000 and 1500 m (Fig. 7.1), indicating similar air mass transport pathway from Russia and Siberia.

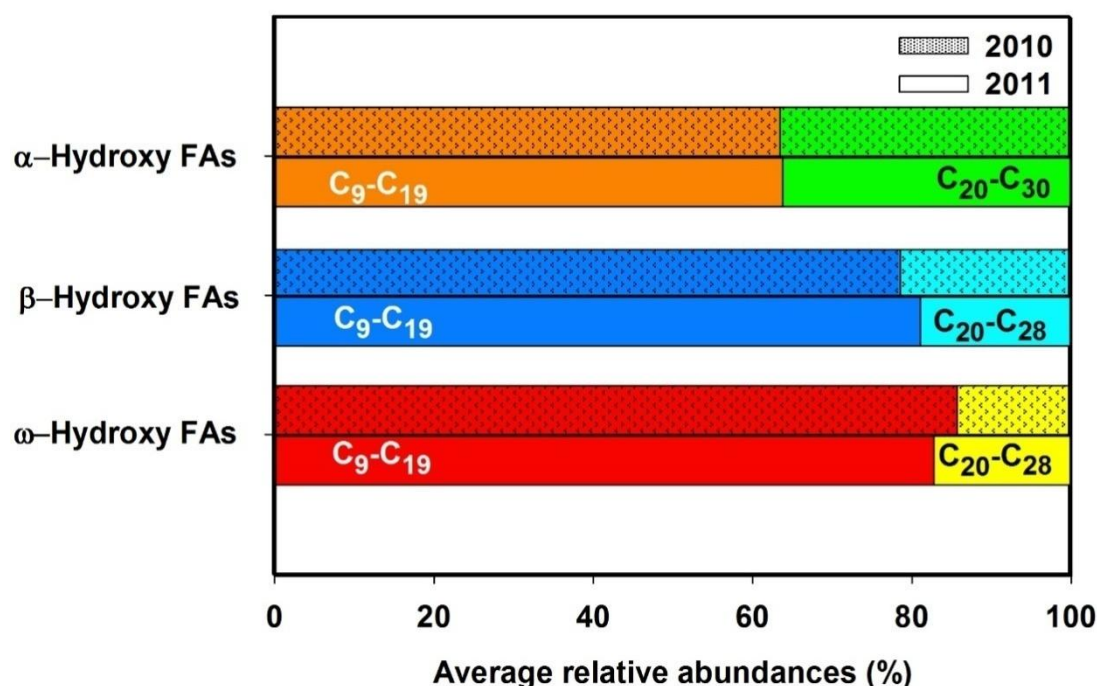


Fig.7.3. Bar graph, showing the relative abundances of low molecular weight (C₉-C₁₉), and high molecular weight hydroxy fatty acids (C₂₀-C₃₀ for α -isomers; C₂₀-C₂₈ for β - and ω -isomers) in their total mass for the snow samples collected during winter 2010 and 2011. The upper and lower horizontal bars for each type of hydroxy fatty acids indicate the data for 2010 and 2011, respectively.

7.3.4. Endotoxin potency of GNB-impact via aeolian transport

Endotoxin in GNB determines their viability and potentially causes pathological effects on mammals [Westphal, 1975; Lüderitz *et al.*, 1981]. In particular, GNB contain LPS in their outer membrane. When bacteria multiply, die and lyse, LPS are released from the surface as a potential bacterial toxin, and therefore called as endotoxin [Westphal, 1975]. In addition to intact bacterial cells, this endotoxin can trigger to cause allergies, respiratory problems and infections. The β -hydroxy FAs, marker for endotoxin/LPS, were assayed in various environmental samples such as dust [Andersson *et al.*, 1999; Hines *et al.*, 2000], aerosols [Walters *et al.*, 1994; Lee *et al.*, 2004; Lee *et al.*, 2007], soils [Keinänen *et al.*, 2003], sewage [Spaan *et al.*, 2008] and marine dissolved organic matter [Wakeham, 1999].

As detailed in chapter 2, the abundances of endotoxin and mass loading of GNB were estimated in fresh snow samples. This quantification is indeed crucial for assessing a likely allergic impact of endotoxin globally *via* long-range atmospheric transport. Here, the estimated endotoxin concentrations in snow varied from 424 to 1080 ng kg⁻¹ (av. 789±237 ng kg⁻¹) in 2010 and 36 to 1100 ng kg⁻¹ (av. 579±435 ng kg⁻¹) in 2011 samples. The estimated lower limits of endotoxin in Table 7.1 and Table 7.2 are calculated based on the minimum concentration of β -hydroxy FAs (C₁₀-C₁₈), which are specific to Gram-negative bacteria (GNB). Thus, the endotoxin concentrations in snow samples were estimated based on the abundances of β -hydroxy FAs having carbon chain length from 10 to 18. Being consistent with this study, *Lee et al.* [2004] also reported endotoxin concentration based on β -hydroxy FAs (C₁₀-C₁₈). Although relative abundances of endotoxin during winter 2010 (*N*=5) are higher than those of 2011 samples (*N*=6), the two-tailed *t* test revealed no significant differences (*t*=0.96; *df* = 9; *P* > 0.05) with regard to mean concentrations of the two years.

In this study, dry mass concentrations of GNB were estimated in snow samples to be 26.3±7.9 μ g kg⁻¹ in 2010 vs. 19.3±1.4 μ g kg⁻¹ in 2011. *Lee et al.* [2007] reported that airborne endotoxin is of crustal origin and thus can be transported long distances to the outflow region. Since AMBTs reveal the impact of long-range transport from Russia and Siberia during the study period, we infer that estimated endotoxin concentrations and dry cell weight of GNB over Sapporo are derived from those source regions. Recently, *Golokhvast* [2014] documented the airborne biogenic particles in snow from Russian Far East that cause allergy for the pedestrians. The airborne biogenic particles can be scavenged efficiently by both wet precipitation and snow fall. Therefore, literature describing the occurrence of GNB in rainwater was searched for comparison with the study on Sapporo snow. Towards this, *Gould* [1999] and *Lye* [2002] have documented the presence of various GNB (*e.g.* *Salmonella*, *Shigella*, *Vibrio*, *Legionella* and *Campylobacter spp.*) species in rainwater. Likewise, *Kawamura and Kaplan* [1983] also reported the presence of β -hydroxy FAs in rain water samples collected from Los Angeles (USA) and attributed their sources as bacterial membrane. So far, no literature is available on endotoxin and GNB concentrations in snow samples from East Asia in order to make a comprehensive comparison with the present study.

Overall, the presence of endotoxin and GNB in snow affirms that biogenic particles of soil microbes and their potential health impact should not be overlooked. Routine and long-term measurements of airborne chemical markers (hydroxy FAs in this study) could aid

the monitoring of the microbial content in long-range transported air masses. Further studies are required to examine their distributions in the atmospheric environment and health effects on human beings in the regional and global perspectives during long-range atmospheric transport.

7.4 Summary and Conclusions

Hydroxy fatty acids (FAs) in fresh snow from Sapporo, one of the heaviest snowfall regions in the world, have been studied to ascertain the airborne bacterial endotoxin concentrations and their biomass. The presence of β -hydroxy FAs (C_9 - C_{28}), constituents of Gram-negative bacteria (GNB), suggests long-range atmospheric transport of soil microbes. Likewise, the occurrence of α - and ω -hydroxy FAs (C_9 - C_{30} and C_9 - C_{28} , respectively) in snow reveals their contribution from epicuticular waxes and soil microorganisms. Estimated endotoxin and GNB mass can aid in assessing their possible impacts on the diversity and functioning of aquatic and terrestrial ecosystems, as well as lethal effects on pedestrians through dispersal of microbes. Air mass back trajectories together with hydroxy FAs unveil their sources from Siberia, Russian Far East and North China by the Asian monsoon. This study highlights the role of fresh snow that reduces the human health risk of GNB and endotoxin by the scavenging from air.

Although low temperature is considered to be a limiting factor for bacterial activity in air/snow, some studies have shown that bacteria can be metabolically active even at subzero temperatures [Polymenakou, 2012 and references therein]. Fig. 7.4 summarized the whole idea, which was addressed in this study. It can be concluded that fresh snow in Japan acts as a filter, which aids in reducing the burden of pathogenic microbes from the atmosphere *via* wet scavenging of these particles. Owing to prolonged winters and thus, snow fall in Sapporo, it is likely that ambient bacterial endotoxin (LPS) is largely scavenged from the atmosphere by snow, which can decrease their effect on human health via inhalation [Jacobs, 1989; Milton, 1996]. However, without snow scavenging, ambient bacterial endotoxin levels may stay high; having an influence on human health as well can be transported to further long distances (North Pacific). Overall, bacteria and their debris (biomass) can be evaluated in aerosols that are scavenged by snow in free troposphere without prior culture by the determination of hydroxy FAs for both LPS and GNB.

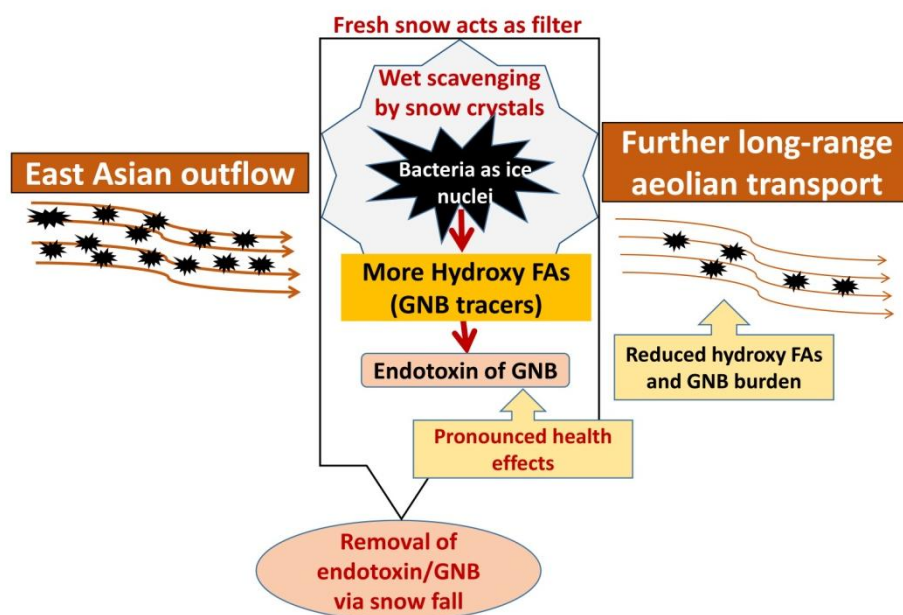


Fig.7.4. A conceptual model to explain the scavenging of hydroxy fatty acids (FAs) by fresh snow in the free troposphere. Snow fall in north Japan acts as a filter in reducing the hydroxy FAs (tracers of Gram-negative bacteria; GNB), which in turn results in the removal of endotoxin from the atmosphere and reduction in their health effects during long-range aeolian dust transport.

Chapter 8

Hydroxy Fatty Acids in Snow Pit Samples from Mt. Tateyama in Central Japan: Implication for Atmospheric Transport of Microorganisms and Plant Waxes Associated with Asian Dust

Chapter 8 presents the results of hydroxy FAs in snowpack samples collected from Mt. Tateyama (central Japan). The molecular distributions in snowpack samples were compared with those observed in fresh snow and marine aerosols. These results are interpreted in terms of below-cloud scavenging and in-cloud processes of hydroxy FAs over East Asia.

8.1 Introduction

Several billion tons of Asian dust are annually transported to Japan from the Taklamakan and Gobi Deserts and Loess Plateau [Yamaguchi *et al.*, 2014]. These dust particles transport microorganisms long distances, possibly affecting human health and ecosystems [Griffin, 2007]. Molecular and culture-based techniques have been employed to identify the bacterial communities in soil surfaces [Hua *et al.*, 2007] and snow layers in Japan [Yamaguchi *et al.*, 2014]. However, among culture independent techniques, the chemical marker-based approach is considered as a suitable tool for the rapid and comparative analysis of bacterial dry biomass and their endotoxins in a variety of environmental samples. Furthermore, our previous studies showed that atmospheric hydroxy fatty acids (FAs) can be used as chemical markers to trace soil microbes and higher plant metabolites in aerosol and snow samples. These results indicate that higher atmospheric abundances of hydroxy FAs are associated with Asian dust episodes.

α -, β - and ω -isomers of hydroxy FAs ($<C_{18}$) have been studied in environmental samples such as sediments [Kawamura and Ishiwatari, 1981; 1982; Ratledge and Wilkinson, 1988; Wakeham *et al.*, 2003], marine aerosols [Kawamura, 1995] and snow as tracers of microorganisms such as algae, fungi, bacteria, protozoan and epicuticular waxes of higher plants. β -Hydroxy FAs from C_{10} to C_{18} have been used as chemical markers for Gram-negative bacteria (GNB) and their lipopolysaccharides (LPS) in previous studies of indoor aerosols [Lee *et al.*, 2004; Sebastian *et al.*, 2006], dust [Reynolds *et al.*, 2005; Saraf *et al.*, 1997] and snow [Tyagi *et al.*, 2015b]. Positional isomers of short-chain α - and ω -homologues of hydroxy FAs have a potential as tracers of soil microorganisms (*e.g.* bacteria, fungi, yeasts, protozoa) [Ratledge and Wilkinson, 1988], whereas long-chain (from C_{16} to C_{32}) homologues are used for the microalgae and cyanobacteria [Matsumoto and Nagashima, 1984; Matsumoto *et al.*, 1984], sea grasses [Volkman *et al.*, 1999], and plant waxes [Rogge *et al.*, 1993; Simoneit, 1989]. More specifically, bound α -hydroxy FAs from C_{16} to C_{18} are present in microorganisms as well as in higher plants but free α -isomers from C_{22} to C_{26} are present in higher plants only and can be decomposed [Cranwell, 1981]. Bound β -hydroxy FAs from C_{22} to C_{26} were suggested to be incorporated into higher plant bound lipids after the β -oxidation of long-chain FAs [Cranwell, 1981]. Moreover, these α -, β - and ω -hydroxy FAs can also act as intermediate products of photochemical as well as microbial oxidation of long-chain monocarboxylic acids to dicarboxylic acids in the sediments [Kawamura and Ishiwatari,

1981; Volkman *et al.*, 1998; Wakeham, 1999]. Furthermore, specificity of hydroxylation of fatty acids depends on the type of bacterium involved [Wakeham, 1999]. As intermediate oxidation products, these hydroxy FAs can either incorporate into higher plant fatty acids in bound lipids or decompose or remain free before preserving into sediments [Cranwell, 1981].

High mountain sites provide a means to assess the sources and transport pathways of atmospheric aerosols. Mt. Tateyama is located in central Japan and is a unique observation point for aerosol research because the mountain faces the Sea of Japan and northeast China. The Murodo-Daira site located near the summit of Mt. Tateyama is usually covered by thick snow (5–8 m) from November to July reaching ≥ 6 m in April. Since tourism-related activities are scarce from winter to early spring, this site is ideal for the study of long-range atmospheric transport of microorganisms without local emissions. The snowpack over Mt. Tateyama is a receptor surface and storage compartment for the deposition of Asian dust [Kawamura *et al.*, 2012; Mochizuki *et al.*, 2015]. The snow cover over Mt. Tateyama consists of deposited as well as scavenged Asian dust particles [Osada *et al.*, 2004]. Although previous studies have been conducted to assess the atmospheric chemistry over Mt. Tateyama [Maki *et al.*, 2011; Mochizuki *et al.*, 2015; Osada *et al.*, 2004; Watanabe *et al.*, 2010], detailed analysis of bacterial transport and associated endotoxins is still lacking.

Hydroxy FAs in the snowpack are important to evaluate the contribution of soil- and plant-associated microbes to long-range transported aerosols as they can act as cloud condensation nuclei (CCN) and ice nuclei, affecting the climate [Christner *et al.*, 2008]. Furthermore, Asian dust events can facilitate the dispersal of airborne bacteria and their metabolites over the Pacific Ocean, spreading the potential pathogens that are associated with human allergies and infections. As snow can scavenge the airborne particles in the free troposphere, hydroxy FAs in snowpack over Mt. Tateyama can be employed as chemical markers to assess the presence, sources, and transport of microorganisms and plant metabolites. In this study, we measured hydroxy FAs in the Asian dust deposited in snow layers of Mt. Tateyama. The molecular distribution of hydroxy FAs was analyzed and estimated the airborne endotoxin and bacterial dry mass to understand the long-range atmospheric transport and scavenging of Asian dust-associated microorganisms and plant waxes.

8.2 Experimental methods

8.2.1 Site description and snow sample collection

Snow samples were collected from two snow pits, which were dug at the Murodo-Daira site (36.58 °N, 137.36 °E, 2450 m above mean sea level) on the western plateau of Mt. Tateyama (3015 m above sea level), Toyama prefecture, central Japan (Fig. 8.1). This site faces the Sea of Japan and is located on the outflow pathway of the Asian dust transport. On April 18, 2009 and April 17, 2011, two snow pit holes were dug to the depth of 6.0 and 6.5 m, respectively, and snowpack samples were collected using a pre-cleaned shovel to avoid possible contamination. Dust-containing snow layers were found in the snow pits, which showed a light yellow-brown color and were later characterized by high Ca^{2+} concentrations, suggesting long-range transport of Asian dust to the sampling site [Mochizuki *et al.*, 2015]. Previous studies [Kawamura *et al.*, 2012; Maki *et al.*, 2011; Osada *et al.*, 2004; Tanaka *et al.*, 2011] also suggested that the snow samples collected from the same site were influenced by Asian dust.

Six and eight snow samples including dust layers were collected from the snow pits in 2009 and 2011, respectively, and placed in a pre-cleaned glass jar (8 L), to which HgCl_2 was added prior to sample collection to prevent any microbial activity. The thickness of snow layer collected was approximately 10 cm in 2009 and 5–20 cm in 2011. The detailed description of snow sample collection and the results of non-sea-salt Ca^{2+} (nss- Ca^{2+}) concentrations are given in our previous publication [Mochizuki *et al.*, 2015] and summarized in Table 8.1. Since large aliquots of samples (*i.e.*, almost all of the snowpack melt water) were used for the determination of hydroxy FAs (α -, β -, ω -) and, therefore, unfortunately not much attempts were made to characterize other organic molecular markers in the snowpack samples from Mt. Tateyama. The analytical protocol used for extracting total hydroxy FAs (LPS-bound and free) from the snowmelt water is described in chapter 2.

Table 8.1. Description of the snow layers and measured nss-Ca^{2+} concentrations in the snowpack samples collected from Murodo-Daira of Mt. Tateyama, central Japan in 2009 and 2011.

Sampling date	Sample ID	Depth from the surface in cm	Snow layer description	nss-Ca^{2+} in $\mu\text{g kg}^{-1}$
18-Apr-09	1	Surface snow layer	Granular snow	-
	2	325-335	Asian-dust containing snow	3.0
	3	410-420	Clean snow	0.43
	4	520-530	Asian-dust containing snow	1.54
	4'	520-530	Asian-dust containing snow, 4' collected from different snow pit parallel to sample 4	1.82
	5	530-540	Granular snow	0.46
17-Apr-11	6	115-125	Granular snow with ice plate	1.45
	7	169-178	Dusty and granular snow	0.17
	8	290-300	Compacted snow layer	0.57
	9	390-400	Compacted snow layer	0.12
	10	400-410	Dusty and compacted snow	0.12
	11	507-527	Compacted snow with ice plate	0.97
	12	542-548	Dusty and compacted snow	-
	13	630-635	Granular snow	0.53

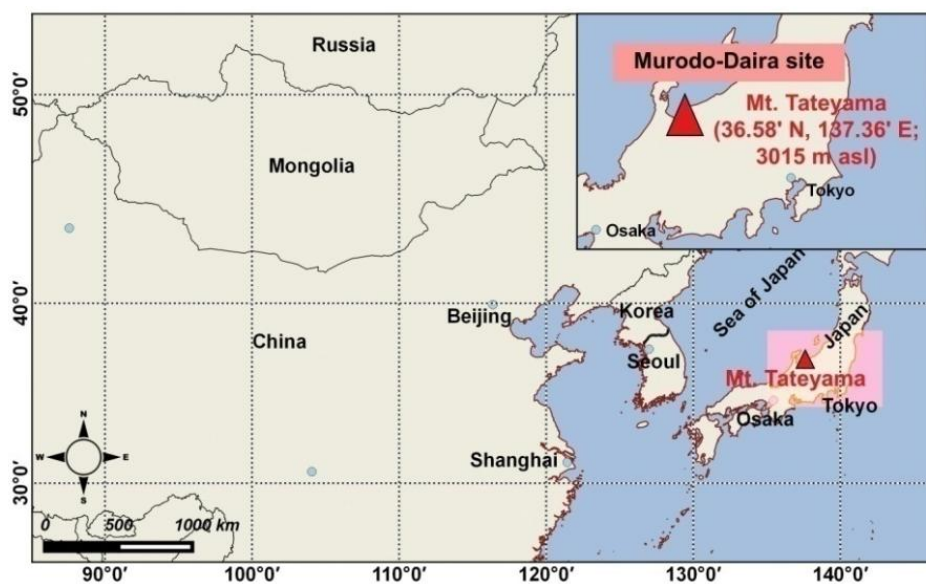


Fig.8.1. Map showing the geographical location of Mt. Tateyama in central Japan. The inset shows the Murodo-Daira site on Mt. Tateyama, where snowpack samples were collected.

8.2.3. Backward-trajectory analysis

To assess the probable source regions of Asian dust and its transport pathway to the Murodo-Daira sampling site on Mt. Tateyama, we computed air mass back-trajectories at an arrival heights of using the computer based Hybrid Single Particle Lagrangian Integrated Trajectory (HYSPLIT, version 4) [Draxler and Rolph, 2013]. This HYSPLIT model used archived meteorological datasets (NCEP/NCAR Reanalysis) from the National Oceanic and Atmospheric Administration (NOAA) Air Resources Laboratory as an input file. Backward-trajectories were computed for air masses arriving at an altitude of 100 m above ground level for the previous 5-day period (Fig. 8.2).

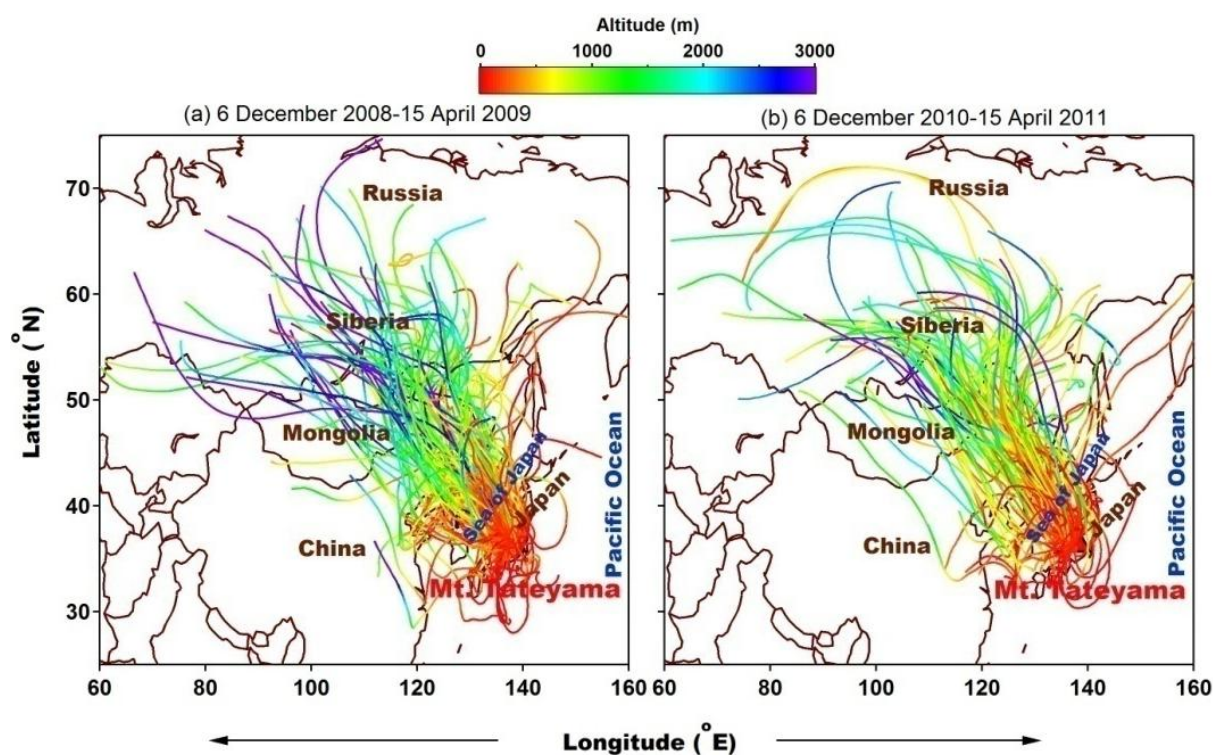


Fig.8.2. 5-day backward-trajectories of air masses are arriving at the height of 100 m above ground level over the Murodo-Daira site near Mt. Tateyama, central Japan. The color scale indicates the heights of the air mass parcels arriving from the source regions in the Asian continent.

8.3 Results and Discussion

8.3.1. Hydroxy fatty acids

A statistical summary of hydroxy FAs in the snowpack samples from spring 2009 and 2011 is presented in Tables 8.2 and 8.3, respectively. A series of saturated α -hydroxy FAs was found, for which C-chain length of these organic compounds varied from 16 to 30 in 2009 and from 16 to 31 in 2011. In contrast, short-chain homologues of β -hydroxy FAs were found, ranging from C-number 9 to 20, in both the years. Interestingly, a wide carbon chain length of ω -hydroxy FAs was found with C-numbers from 9 to 30 in both 2009 and 2011. All these hydroxy FAs showed strong even to odd carbon predominance for the snowpack samples collected in April 2009 and April 2011; indicating their source as terrestrial lipids from vascular plants/soil microbes [Gagosian and Peltzer, 1986].

The even carbon predominance of fatty acids is a characteristic feature of biological processes involved in the synthesis of these lipid compounds in higher plants and soil microorganisms [Simoneit, 1985; Xie *et al.*, 2003]. The wind abrasion of epicuticular waxes of higher plants contributes to even C-predominance of fatty acids ranging from C_{22} to C_{32} with characteristic abundant peaks of either C_{24} or C_{26} . Alternately, lipids from soil microbes mostly contribute to lower homologues of fatty acids, typically ranging from C_{10} or C_{12} to C_{22} [Xie *et al.*, 2003]. Therefore, the even to odd carbon abundance ratio of hydroxy FAs was estimated in snow pit samples that ranged from 2.6 to 5.1 (3.9 ± 1.0) for α -hydroxy FAs, from 1.3 to 14.1 (4.7 ± 4.8) for β -hydroxy FAs, from 2.6 to 59.3 (16 ± 22) for ω -hydroxy FAs in 2009, while the abundance ratio varied from 1.4 to 6.2 (2.6 ± 1.6) for α -hydroxy FAs, from 0.6 to 6.8 (2.2 ± 2.0) for β -hydroxy FAs, from 4.1 to 17.1 for ω -hydroxy FAs in 2011. These entire ratios indicate their significant contribution of lipids from plant waxes/soil microbes.

8.3.1.1. Concentrations of hydroxy FAs in different snowpack

In 2009, median concentration of total hydroxy FAs was of comparable magnitude ($1.13 \mu\text{g kg}^{-1}$) with that observed in 2011 ($0.96 \mu\text{g kg}^{-1}$). Similar feature was reflected in the median concentrations of total α -, total β - and total ω -hydroxy FAs, which were consistent between 2009 ($0.22 \mu\text{g kg}^{-1}$, $0.53 \mu\text{g kg}^{-1}$ and $0.47 \mu\text{g kg}^{-1}$, respectively) and 2011 ($0.14 \mu\text{g kg}^{-1}$, $0.57 \mu\text{g kg}^{-1}$ and $0.32 \mu\text{g kg}^{-1}$, respectively). The overall median distributions of

hydroxy FAs in snowpack overlapped between 2009 and 2011, suggesting their similar provenance (or source region, as also supported by the AMBTs). However, the median concentration of water-soluble non-sea-salt Ca^{2+} (a proxy for mineral dust) in 2009 ($1.5 \mu\text{g kg}^{-1}$) was 7 fold higher than that found in 2011 ($0.19 \mu\text{g kg}^{-1}$) snowpack samples (see for ranges and mean, Table 8.1). Significant linear relationship was found between nss-Ca^{2+} and total β -hydroxy FAs for the snowpack samples collected in 2011 ($R^2 = 0.73$; regression: $0.050x + 32.1$). However, such correlation was not observed as the nss-Ca^{2+} data only available for four samples. As reported in previous studies [Mori *et al.*, 2002; Nishikawa *et al.*, 2000; Osada *et al.*, 2004], water-soluble Ca^{2+} is a major ionic species detected in Asian and Chinese loess dust samples, and thus it can be inferred that hydroxy FAs in snowpack samples were strongly influenced by the atmospheric input of Asian dust.

It has been suggested that Asian dust is a significant source of atmospheric water-soluble organic nitrogen (WSON) [Shi *et al.*, 2010; Mochizuki *et al.*, 2015]. A moderately significant linear correlation was also observed between WSON and nss-Ca^{2+} in the snowpack samples from Mt. Tateyama collected during spring 2009 and 2011 [Mochizuki *et al.*, 2015], suggesting their probable source as Asian dust. However, their study suggested that WSON subjected to intense oxidation during transport before reaching to Mt. Tateyama. This is inferred to explain the occurrence of relatively low abundance of WSON observed over Mt. Tateyama than those found for Asian dust [Mochizuki *et al.*, 2015]. Likewise, water-soluble nss-Ca^{2+} concentration in the aerosols is highly influenced by the aging processes (or chemical reactions), unlike the lipid biomarker compounds, *via* the reactive uptake of acidic species such as anthropogenic HNO_3 and H_2SO_4 on atmospheric mineral dust during long-range transport [Sullivan *et al.*, 2007; Kim and Kim, 2008].

To investigate the effect of aging processes during transport, we have examined the linear relationship between water-soluble nss-Ca^{2+} and NO_3^- in snowpack samples from spring 2009 and 2011. A good correlation was found between nss-Ca^{2+} and NO_3^- with differing slopes (Fig. 8.3), indicating different sources and/or processing effects (*i.e.*, extent of aging). Also the mass contribution of individual water-soluble ions to ΣWSIC in snowpack samples collected during Spring 2009 and 2011 differ significantly (Fig. 8.4) with former samples which have more contribution from the Asian dust (as supported by the higher abundances of Ca^{2+} and Na^{2+}) while the latter samples are more influenced by the anthropogenic emissions. Since the β -hydroxy FAs measured in this study come either from

bound/free lipids or both (due to the analytical protocol adapted), it is, however, not logical to expect a statistical correlation of these organic compounds with water-soluble nss-Ca^{2+} despite being originated from similar dust source regions in East Asia. Also, the sample size of snowpack ($N=6$ and 8 for 2009 and 2011, respectively) analyzed here is rather small to infer about the statistical relations between these two chemical entities (*i.e.*, β -hydroxy FAs and nss-Ca^{2+}). However, the occurrence of nss-Ca^{2+} has been used effectively in the past studies from East Asia to ascertain the influence of Asian dust events.

Interestingly, it is evident from Fig. 8.4 that the reference materials from the source regions of mineral dust in East Asia (Certified reference material for Chinese Loess: CJ-1 and Simulated Asian mineral dust: CJ-2) contain abundant quantities of water-soluble nss-Ca^{2+} . Therefore, high concentrations of water-soluble nss-Ca^{2+} along with air mass back-trajectories indicate the significant contribution of Asian dust to snowpack samples collected from Mt. Tateyama. However, significant mismatch in the relative abundances of individual water-soluble inorganic ions to their total mass concentration (ΣWSIC : $\text{Na}^+ + \text{NH}_4^+ + \text{K}^+ + \text{Ca}^{2+} + \text{Mg}^{2+} + \text{F}^- + \text{NO}_3^- + \text{SO}_4^{2-}$) between the snowpack samples and certified reference materials (CRMs) analyzed could be due to the grain size fractionation as well as the aging effects (or oxidation processes) during transport.

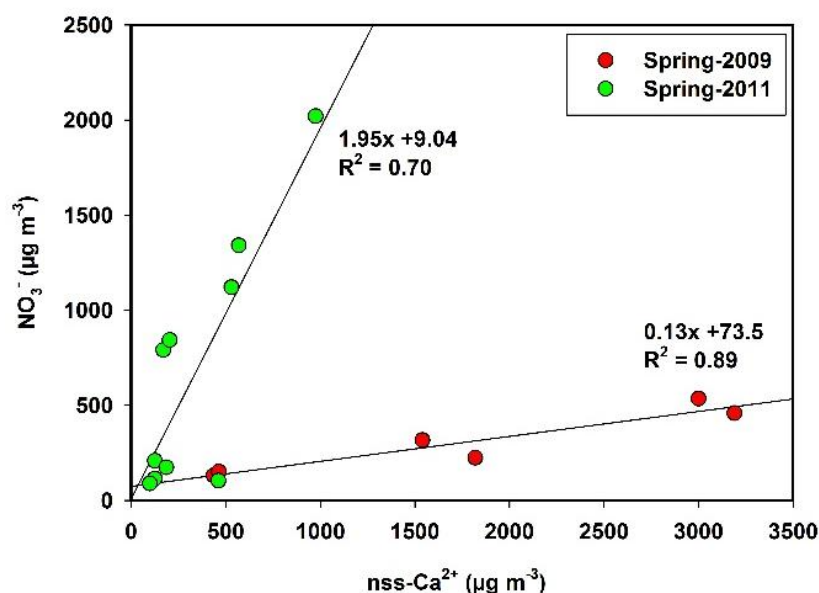


Fig.8.3: Linear regression analysis between concentrations of water-soluble non-sea-salt Ca^{2+} and nitrate in the snowpack samples collected during spring 2009 and 2011 over Mt. Tateyama.

Table 8.2. Statistical concentrations of α - β - and ω -hydroxy FAs in snowpack samples collected in 2009 from the Murodo-Daira, Mt. Tateyama, Japan.

18 April, 2009 (in $\mu\text{g kg}^{-1}$; N=6)								
Carbon chain length	α -Hydroxy FAs		β -Hydroxy FAs		ω -Hydroxy FAs		Σ (α , β , ω) hydroxy FAs	
	Av. $\pm$ SE	Min.-Max. (Median)	Av. $\pm$ SE	Min.-Max. (Median)	Av. $\pm$ SE	Min.-Max. (Median)	Av. $\pm$ SE	Min.-Max. (Median)
C ₉			0.16 $\pm$ 0.04	0.07-0.32 (0.12)	0.11 $\pm$ 0.09	0.00-0.29 (0.04)	0.59 $\pm$ 0.25	0.34-0.84 (0.59)
C ₁₀			0.36 $\pm$ 0.25	0.06-1.12 (0.12)	0.14 $\pm$ 0.1	0.00-0.28 (0.14)	0.86 $\pm$ 0.57	0.29-1.43 (0.86)
C ₁₁			0.04 $\pm$ 0.03	0.00-0.1 (0.02)	0.12 $\pm$ 0.1	0.00-0.26 (0.13)	0.2 $\pm$ 0.07	0.13-0.27 (0.2)
C ₁₂			0.15 $\pm$ 0.08	0.02-0.54 (0.07)	0.78 $\pm$ 0.66	0.02-4.12 (0.08)	2.82 $\pm$ 1.9	0.92-4.73 (2.82)
C ₁₃			0.02 $\pm$ 0.00	0.00-0.04 (0.01)	0.04 $\pm$ 0.03	0.00-0.16 (0.01)	0.16 $\pm$ 0.06	0.1-0.22 (0.16)
C ₁₄			0.13 $\pm$ 0.05	0.03-0.38 (0.06)	0.53 $\pm$ 0.43	0.02-2.67 (0.1)	1.98 $\pm$ 1.2	0.78-3.18 (1.98)
C ₁₅			0.02 $\pm$ 0.01	0.00-0.08 (0.02)	0.59 $\pm$ 0.04	0.00-0.27 (0.01)	0.26 $\pm$ 0.09	0.17-0.35 (0.26)
C ₁₆	0.09 $\pm$ 0.02	0.07-0.12 (0.01)	0.15 $\pm$ 0.09	0.02-0.61 (0.06)	1.34 $\pm$ 1.15	0.04-7.1 (0.11)	3.06 $\pm$ 2.52	0.2-8.08 (0.9)
C ₁₇			0.02 $\pm$ 0.01	0.00-0.08 (0.01)	0.03 $\pm$ 0.02	0.00-0.14 (0.01)	0.11 $\pm$ 0.06	0.00-0.2 (0.12)
C ₁₈	0.04 $\pm$ 0.01	0.02-0.06 (0.02)	0.08 $\pm$ 0.05	0.00-0.36 (0.03)	0.1 $\pm$ 0.08	0.00-0.4 (0.1)	0.39 $\pm$ 0.14	0.11-0.53 (0.52)
C ₁₉	0.01 $\pm$ 0.00	0.00-0.01 (0.01)	0.02 $\pm$ 0.02	0.00-0.08 (0.01)	0.07 $\pm$ 0.06	0.00-0.19 (0.01)	0.1 $\pm$ 0.05	0.02-0.2 (0.09)
C ₂₀	0.03 $\pm$ 0.01	0.00-0.07 (0.02)	0.14 $\pm$ 0.1	0.01-0.45 (0.05)	0.18 $\pm$ 0.14	0.01-0.76 (0.04)	0.54 $\pm$ 0.22	0.15-0.91 (0.57)
C ₂₁	0.01 $\pm$ 0.00	0.00-0.02 (0.01)			0.18 $\pm$ 0.17	0.00-0.8 (0.01)	0.49 $\pm$ 0.43	0.06-0.92 (0.49)
C ₂₂	0.05 $\pm$ 0.01	0.01-0.1 (0.05)			0.38 $\pm$ 0.34	0.01-2.08 (0.02)	1.29 $\pm$ 0.98	0.31-2.26 (1.29)
C ₂₃	0.03 $\pm$ 0.01	0.01-0.1 (0.01)			0.42 $\pm$ 0.41	0.00-2.1 (0.01)	1.15 $\pm$ 0.97	0.18-2.11 (1.15)
C ₂₄	0.13 $\pm$ 0.07	0.02-0.4 (0.01)			0.23 $\pm$ 0.21	0.01-1.05 (0.02)	0.95 $\pm$ 0.17	0.78-1.12 (0.95)
C ₂₅	0.03 $\pm$ 0.01	0.00-0.01 (0.02)			0.56 $\pm$ 0.55	0.00-2.2 (0.01)	1.22 $\pm$ 1.04	0.18-2.26 (1.22)
C ₂₆	0.03 $\pm$ 0.01	0.01-0.07 (0.02)			0.2 $\pm$ 0.2	0.00-0.6 (0.00)	0.41 $\pm$ 0.2	0.2-0.61 (0.41)
C ₂₇	0.01 $\pm$ 0.00	0.00-0.04 (0.01)			0.38 $\pm$ 0.37	0.00-0.76 (0.38)	0.41 $\pm$ 0.35	0.07-0.76 (0.41)
C ₂₈	0.03 $\pm$ 0.01	0.00-0.07 (0.02)			0.08 $\pm$ 0.07	0.00-0.24 (0.01)	0.18 $\pm$ 0.07	0.11-0.25 (0.18)
C ₂₉	0.02 $\pm$ 0.00	0.00-0.02 (0.01)			0.06 $\pm$ 0.05	0.00-0.11 (0.06)	0.08 $\pm$ 0.04	0.05-0.12 (0.08)
C ₃₀	0.02 $\pm$ 0.00	0.00-0.02 (0.01)			0.04 $\pm$ 0.03	0.01-0.08 (0.43)	0.07 $\pm$ 0.02	0.05-0.09 (0.07)
Total	0.4 $\pm$ 0.2	0.1-1.2 (0.22)	1.04 $\pm$ 0.6	0.1-3.8 (0.51)	4.96 $\pm$ 4.3	0.16-26.8 (0.47)	1.77 $\pm$ 0.46	0.13-9.18 (1.07)

Av. (Average), SE (Standard error) = $\sigma/\text{SQRT}(N)$ where N is total number of samples.

Table 8.3. Statistical concentrations of α - β - and ω -hydroxy FAs in snowpack samples collected in 2011 at the Murodo-Daira, Mt. Tateyama, Japan.

17 April, 2011 (in $\mu\text{g kg}^{-1}$; N=8)								
Carbon chain length	α -Hydroxy FAs		β -Hydroxy FAs		ω -Hydroxy FAs		Σ (α , β , ω) hydroxy FAs	
	Av. $\pm$ SE	Min.-Max. (Median)	Av. $\pm$ SE	Min.-Max. (Median)	Av. $\pm$ SE	Min.-Max. (Median)	Av. $\pm$ SE	Min.-Max. (Median)
C ₉			0.15 $\pm$ 0.02	0.08-0.3 (0.13)	0.01 $\pm$ 0.00	0.00-0.01 (0.01)	0.61 $\pm$ 0.59	0.02-1.21 (0.61)
C ₁₀			0.16 $\pm$ 0.04	0.07-0.39 (0.12)	0.04 $\pm$ 0.02	0.00-0.12 (0.01)	0.72 $\pm$ 0.54	0.19-1.26 (0.72)
C ₁₁			0.03 $\pm$ 0.01	0.01-0.06 (0.03)	0.00 $\pm$ 0.00	0.00-0.00 (0.00)	0.14 $\pm$ 0.13	0.00-0.27 (0.14)
C ₁₂			0.08 $\pm$ 0.02	0.01-0.19 (0.04)	0.06 $\pm$ 0.02	0.02-0.19 (0.04)	0.57 $\pm$ 0.05	0.51-0.62 (0.57)
C ₁₃			0.01 $\pm$ 0.00	0.00-0.03 (0.01)	0.01 $\pm$ 0.00	0.00-0.01 (0.00)	0.08 $\pm$ 0.03	0.05-0.1 (0.08)
C ₁₄			0.04 $\pm$ 0.01	0.00-0.08 (0.03)	0.06 $\pm$ 0.02	0.01-0.2 (0.04)	0.42 $\pm$ 0.12	0.3-0.54 (0.42)
C ₁₅			0.01 $\pm$ 0.00	0.00-0.03 (0.01)	0.00 $\pm$ 0.00	0.00-0.01 (0.00)	0.05 $\pm$ 0.02	0.04-0.07 (0.05)
C ₁₆	0.08 $\pm$ 0.02	0.05-0.1 (0.08)	0.07 $\pm$ 0.01	0.02-0.14 (0.06)	0.09 $\pm$ 0.02	0.000.23 (0.04)	0.5 $\pm$ 0.17	0.16-0.75 (0.5)
C ₁₇	0.04 $\pm$ 0.00	0.00-0.04 (0.04)	0.01 $\pm$ 0.00	0.00-0.02 (0.01)	0.00 $\pm$ 0.00	0.00-0.01 (0.00)	0.05 $\pm$ 0.01	0.04-0.07 (0.05)
C ₁₈	0.03 $\pm$ 0.01	0.02-0.04 (0.03)	0.03 $\pm$ 0.00	0.01-0.06 (0.02)	0.01 $\pm$ 0.00	0.00-0.03 (0.01)	0.15 $\pm$ 0.05	0.07-0.25 (0.15)
C ₁₉			0.01 $\pm$ 0.00	0.00-0.01 (0.01)	0.01 $\pm$ 0.00	0.00-0.02 (0.00)	0.03 $\pm$ 0.01	0.00-0.04 (0.03)
C ₂₀	0.02 $\pm$ 0.01	0.01-0.03 (0.02)	0.02 $\pm$ 0.00	0.01-0.04 (0.01)	0.02 $\pm$ 0.00	0.00-0.05 (0.01)	0.13 $\pm$ 0.04	0.05-0.19 (0.13)
C ₂₁	0.02 $\pm$ 0.00	0.01-0.02 (0.02)			0.01 $\pm$ 0.00	0.00-0.05 (0.00)	0.08 $\pm$ 0.03	0.04-0.11 (0.08)
C ₂₂	0.02 $\pm$ 0.00	0.00-0.05(0.01)			0.03 $\pm$ 0.01	0.01-0.1(0.02)	0.24 $\pm$ 0.06	0.18-0.3 (0.24)
C ₂₃	0.02 $\pm$ 0.00	0.00-0.05 (0.02)			0.01 $\pm$ 0.00	0.00-0.03 (0.01)	0.11 $\pm$ 0.05	0.07-0.16 (0.11)
C ₂₄	0.06 $\pm$ 0.01	0.02-0.14 (0.04)			0.02 $\pm$ 0.00	0.00-0.06 (0.01)	0.32 $\pm$ 0.16	0.17-0.48 (0.32)
C ₂₅	0.03 $\pm$ 0.00	0.00-0.06 (0.02)			0.01 $\pm$ 0.00	0.00-0.01 (0.01)	0.13 $\pm$ 0.11	0.02-0.23 (0.13)
C ₂₆	0.02 $\pm$ 0.00	0.01-0.06 (0.02)			0.02 $\pm$ 0.1	0.00-0.06 (0.02)	0.16 $\pm$ 0.05	0.11-0.21 (0.16)
C ₂₇	0.02 $\pm$ 0.00	0.01-0.03 (0.02)			0.01 $\pm$ 0.00	0.00-0.01 (0.01)	0.05 $\pm$ 0.04	0.02-0.09 (0.05)
C ₂₈	0.02 $\pm$ 0.00	0.01-0.03 (0.02)			0.02 $\pm$ 0.01	0.00-0.03 (0.01)	0.06 $\pm$ 0.00	0.05-0.06 (0.06)
C ₂₉	0.01 $\pm$ 0.00	0.00-0.01 (0.01)			0.01 $\pm$ 0.00	0.00-0.01 (0.01)	0.02 $\pm$ 0.00	0.00-0.02 (0.02)
C ₃₀	0.01 $\pm$ 0.00	0.00-0.02 (0.01)			0.00 $\pm$ 0.00	0.00-0.01 (0.00)	0.03 $\pm$ 0.00	0.00-0.03 (0.03)
C ₃₁	0.01 $\pm$ 0.00	0.00-0.02 (0.01)					0.02 $\pm$ 0.00	0.00-0.02 (0.02)
Total	0.23 $\pm$ 0.07	0.06-0.67 (0.14)	0.62 $\pm$ 0.08	0.3-1.01 (0.57)	0.42 $\pm$ 0.13	0.11-1.17 (0.32)	0.44 $\pm$ 0.09	0.02-1.49 (0.257)

Av. (Average), SE (Standard error) = $\sigma/\text{SQRT}(N)$ where N is total number of samples.

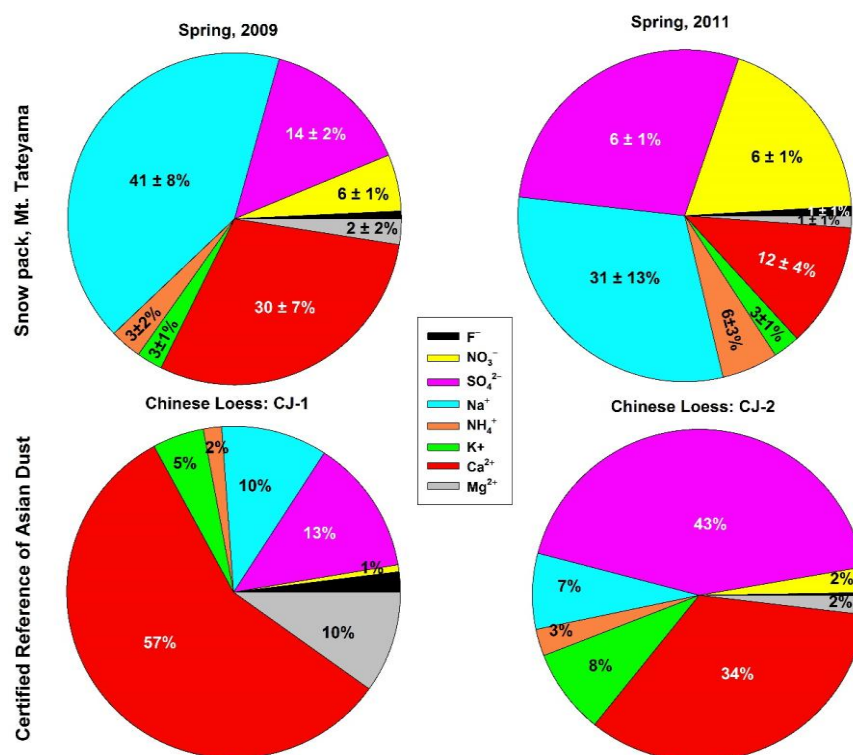


Fig.8.4: A comparison of fractional contribution of individual inorganic ions in their total mass concentrations for the snowpack samples collected during spring 2009 and 2011 with Certified Reference Materials from Chinese loess (CJ-1 and CJ-2).

A comparison of mass concentrations of total β - and total ω -hydroxy FAs in the dusty and non-dusty layers (classified based on the visual appearance and nss-Ca^{2+} conc., see Table 8.1) of snowpack samples collected in 2009 (β -isomers: $1.04 \mu\text{g kg}^{-1}$; ω -isomers: $4.96 \mu\text{g kg}^{-1}$) and 2011 (β -isomers: $0.62 \mu\text{g kg}^{-1}$; ω -isomers: $0.42 \mu\text{g kg}^{-1}$) were significantly higher than those reported in the fresh snow samples collected from Sapporo (northern Japan) in the Winter seasons of 2010 (β -isomers: $0.24 \mu\text{g kg}^{-1}$; ω -isomers: $0.64 \mu\text{g kg}^{-1}$) and 2011 (β -isomers: $0.18 \mu\text{g kg}^{-1}$; ω -isomers: $0.15 \mu\text{g kg}^{-1}$). This is due to a strong influence by the atmospheric input of Asian dust in the snowpack samples at the Murodo-Daira site. These hydroxy FAs (especially β -isomers) from soil microbes such as bacteria can adhere to dust particles during strong wind conditions and can be carried out long distances. Therefore, snow samples collected during 2009 and 2011 from Mt. Tateyama were strongly influenced by Asian dust, showing higher inputs of soil microorganisms transported from the Asian continent.

α -Hydroxy FAs measured in 2009 ($0.4 \mu\text{g kg}^{-1}$) and 2011 ($0.23 \mu\text{g kg}^{-1}$) were found to be less abundant than those detected in fresh snow from Sapporo (2010: $0.59 \mu\text{g kg}^{-1}$ and 2011: $0.64 \mu\text{g kg}^{-1}$). This could be due to photochemical/microbial degradation of long-chain fatty acids present in the cloud water by the α -oxidation pathway to short-chain α -hydroxy FAs detected in fresh snow samples. *Cranwell* [1981] suggested that the lesser abundances of long chain α -hydroxy FAs from C_{22} to C_{26} (*i.e.*, which are contributed from higher plants; allochthonous) as well as short-chain α -hydroxy FAs from C_{14} to C_{18} (*i.e.*, derived from marine biota; autochthonous) due to their oxidation/degradation processes before preserving into pelagic sediments. Thus, α -isomers of hydroxy FAs cannot be employed as specific tracers of higher plant metabolites in atmospheric samples as they are more susceptible to degradation during transport and have wide variety of formation pathways such as photochemical/microbial oxidation of high molecular weight fatty acids [*Eglinton et al.*, 1968].

8.3.1.2. Inter-annual variability of hydroxy fatty acids

Fig. 8.5 shows the mean concentrations of hydroxy FAs (α -, β - and ω -) measured in snow layers from the Murodo-Daira site on Mt. Tateyama. In 2009, maximum mean concentrations of α -, β - and ω -hydroxy FAs ($1.09 \mu\text{g kg}^{-1}$, $4.2 \mu\text{g kg}^{-1}$ and $26.8 \mu\text{g kg}^{-1}$, respectively) were detected in the snow layer collected at the depth of 520–530 cm (sample No. 4). This layer was identified as a dusty layer with high concentration of nss-Ca^{2+} and influenced with air masses derived from the arid regions in western China, Mongolia and Siberia (Fig. 8.2a). Another dusty snow sample was collected (sample No. 4') parallel to the sample No 4 at the same depth. This sample also showed higher mean concentrations of hydroxy FAs and nss-Ca^{2+} , receiving air masses from the same regions in the Asian continent. Significant concentrations of α - and β -hydroxy FAs was also observed in sample No. 2 collected at the depth of 325–335 cm. However, this snow layer was clean as reported by *Mochizuki et al.* [2015] and also characterized by lower abundance of nss-Ca^{2+} . As the Asian dust is enriched with calcium minerals (see Fig. 8.4 for nss-Ca^{2+} content in CJ-1 and CJ-2), the lower concentration of nss-Ca^{2+} and higher abundances of hydroxy FAs indicate different source contribution of these lipid biomarkers than those originated from Chinese loess.

Although no clear trend was observed in the abundances of hydroxy FAs with depth, however, we found the high abundances of hydroxy FAs in the sample Nos. 4, 4' and 5,

which were also characterized by the dusty color and high concentrations of nss-Ca^{2+} (a dust tracer). This could be explained by the occurrence of Asian dust events, which were suggested by the previous studies [Kawamura *et al.*, 2012; Mochizuki *et al.*, 2015; Tanaka *et al.*, 2011] over Mt. Tateyama. At the same sampling site, Tanaka *et al.* [2011] and Maki *et al.* [2011] reported higher values of bacterial cell counts in the snow layers containing Asian dust than in other layers collected during the same period and at the same sampling site. They also assigned the sources of bacteria in the snowpack layers as the Asian dust observed in early spring of 2008 and 2009. These results are consistent with our findings that hydroxy FAs are the constituents of soil microbes and their abundances in the snow layers can be strongly influenced by long-range transport of Asian dust over Mt. Tateyama.

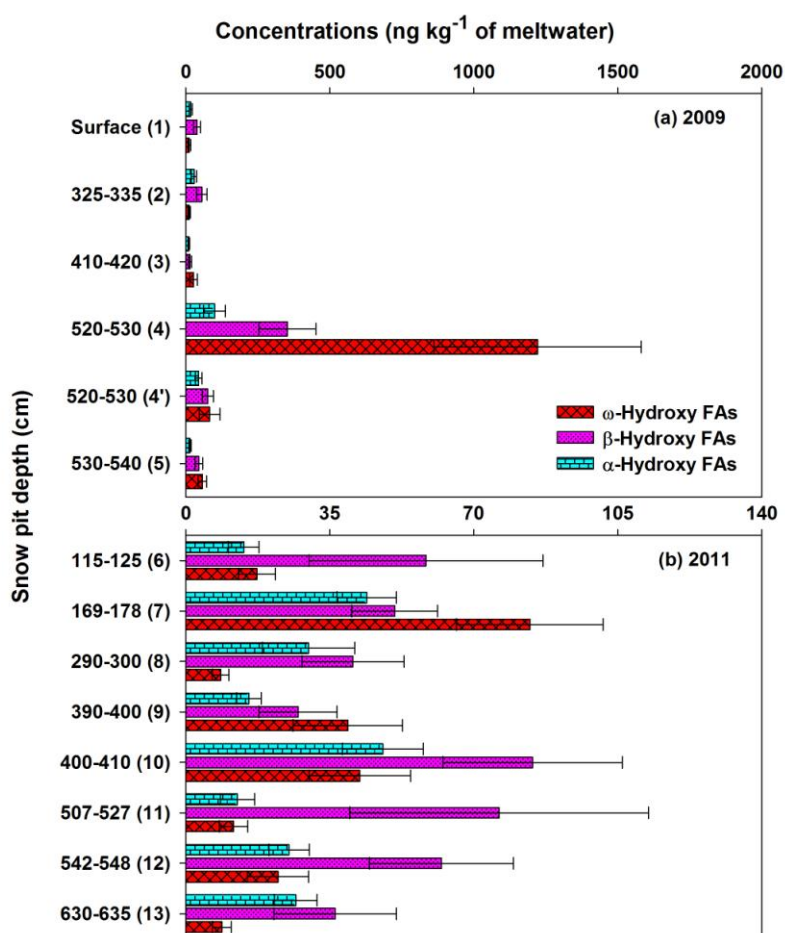


Fig.8.5. Bar graphs showing the mean concentrations of α -, β - and ω -hydroxy fatty acids in the Asian dust containing and underlying snow layers collected in (a) 2009, and (b) 2011 from Mt. Tateyama in Japan. Numbers in the brackets on the y-axis are the sample IDs of the snow layers as given in Table 8.1.

In the snowpack samples collected in April 2011, high abundances of hydroxy FAs were detected in the sample Nos. 7, 10 and 12 (Fig. 8.5), which were also characterized by yellow/dusty color and high abundances of nss-Ca^{2+} . In our previous reports [Mochizuki *et al.*, 2015], high abundances of water soluble organic nitrogen (WSO_N) was also found in the dust containing-snow layers at the same sampling site, which clearly shows the inputs of biological sources such as bacteria, algae and plant debris from the terrestrial sources. There is also a possibility of the photochemical production of these hydroxy FAs during the melting process of surface snow at the Murodo-Daira site when it was exposed to high solar radiation and warmer temperatures. However, hydroxy FAs found in underlying dusty snow layers should better reflect the atmospheric inputs of Asian dust containing soil microorganisms and higher plant metabolites during the outflow of East Asian aerosols. Similar to 2009, dust events were also observed during 2010–2011 (Fig. 8.2b), which explain high abundances of inorganic ions and hydroxy FAs in the snowpack layers.

Interestingly, maximum concentrations of hydroxy FAs were detected in the snowpack samples collected at the middle depths (such as sample Nos. 4 and 10). The sunlit surface snow layers are usually exposed to fluctuations in the ambient insolation, temperature and relative humidity and, thereby, having a possibility of intense photochemical oxidation of organic matter (here, hydroxy FAs) [Grannas *et al.*, 2004]. Unlike surface snow, occurrence of high concentrations of C_{10} – C_{20} β -hydroxy FAs in the middle layers (or the intermediate depths) of the snowpack because of the fact that these are the bound cell membrane lipids of soil bacteria, which could survive even at low temperatures through the consumption of other organic matter within the snow. Presumably bacteria and other soil microorganisms can digest organic acids and can survive for longer time in the middle snow layers by avoiding environmental stress factors [Kawamura *et al.*, 2012]. This idea is further supported by Maki *et al.* [2011], who also reported the presence of viable halotrophic and oligotrophic bacteria associated with kosa particles in the snowpack samples collected at middle depths from Mt. Tateyama. Thus, hydroxy FAs in the dusty snow layers serve as the best proxies for tracing the dust- and plant-associated bacteria (such as *Pseudomonas syringae*, *Pseudomonas viridiflava*, *Pseudomonas fluorescens*, *Pantoea agglomerans*, and *Xanthomonas campestris*) [Christner *et al.*, 2008].

8.3.2. Molecular distributions

Homologues series of saturated α -(C₁₆–C₃₁), β -(C₉–C₂₀) and ω -(C₉–C₃₀)-hydroxy FAs was detected in the snowpack samples collected from Mt. Tateyama in 2009 and 2011 (Figs. 8.6, 8.7, 8.8 and 8.9). The subsequent section describes about the molecular distributions of measured three classes of hydroxy FAs with an emphasis on their even carbon predominance, peak maximum and their possible sources by relating them to those documented for plant waxes/soil microbes. These results were also compared with those obtained in fresh snow collected from Sapporo, Japan.

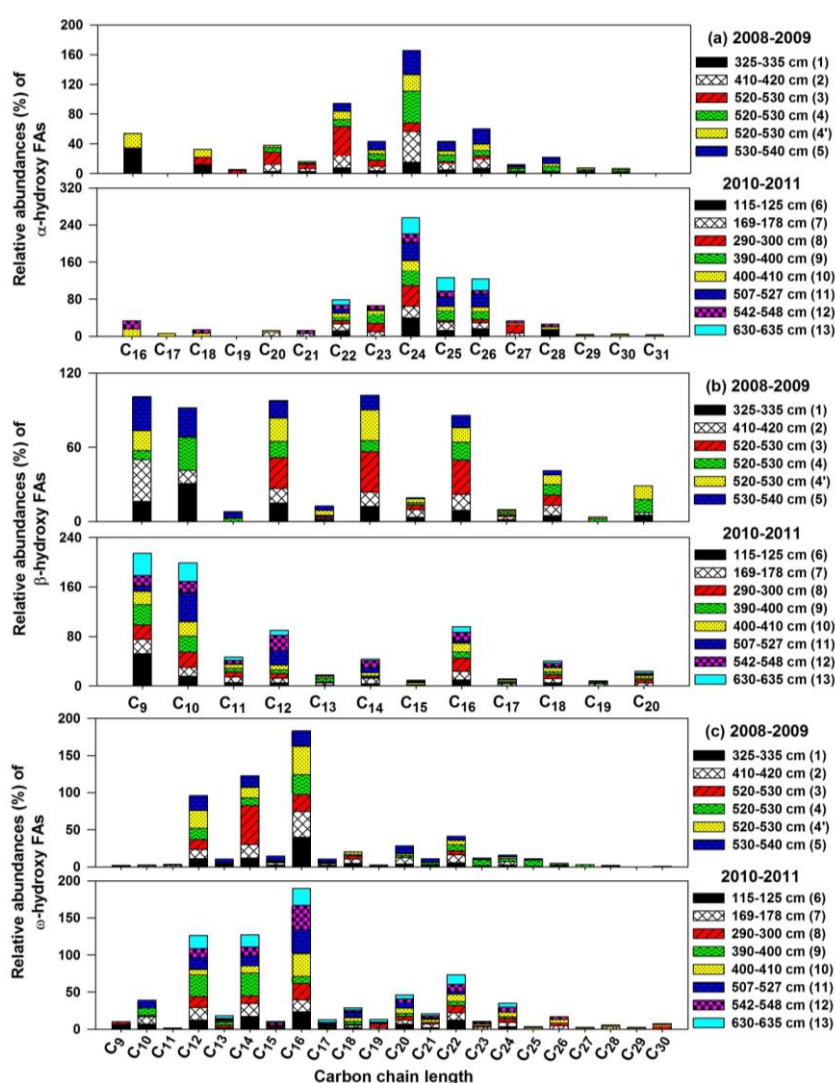


Fig. 8.6. Molecular distribution of (a) α -hydroxy FAs (C₁₆–C₃₁), (b) β -hydroxy FAs (C₉–C₂₀), and (c) ω -hydroxy FAs (C₉–C₃₀) in the snowpack samples collected at different depths from the Murodo-Daira site on the western slope of Mt. Tateyama in central Japan. The numbers in the parenthesis indicate the sample ID as given in Table 8.1.

8.3.2.1. α -Hydroxy fatty acids

A homologues series of saturated α -hydroxy FAs from C₁₆ to C₃₀ (Fig. 8.7a) and from C₁₆ to C₃₁ (Fig. 8.7b) were observed for the snowpack samples collected in 2009 and 2011, respectively. Although the molecular distributions of α -hydroxy FAs were characterized by the even C-predominance for the snowpack samples collected during 2009 and 2011, however, occurrence of odd carbon compounds is also possible (in less abundance); perhaps related to their contribution from differences in the source emissions (*e.g.* types of plant/microorganisms) or oxidation/degradation processes. In snowpack samples collected at different depths from the Murodo-Daira site on Mt. Tateyama (Fig. 8.6a), the molecular distributions of α -hydroxy FAs were dominated by C₂₄, which is found as a major component in epicuticular waxes of higher plants along with C₂₂, C₁₆, and C₁₈ homologues. These results are consistent with our previous study in fresh snow samples collected from Sapporo, northern Japan, where even C-number predominance of α -hydroxy FAs was observed with maxima at C₁₆, C₂₄ and C₂₂.

Interestingly, predominance of odd C-numbered C₂₅, C₂₇ and C₂₃ α -hydroxy FAs (Figs. 8.6a, 8.7a and 8.7b) was also found in the Asian dust and ice plate snow containing compacted snow layers (sample Nos. 4, 7, 8, 9 and 13) from Mt. Tateyama. These odd C-numbered species could be derived from photochemical/microbial oxidation of mono- and dicarboxylic acids during the transport of polluted air masses from North China due to an increased anthropogenic emission [Cranwell, 1981; Osada *et al.*, 2009]. It has been reported that α -oxidation is one of the pathways in the degradation of fatty acids in biological, geological and atmospheric circumstances [Stumpf and Barber, 1960]. This oxidation pathway is utilized in yeasts to produce α -hydroxy FAs as intermediates of fatty acid biosynthesis [Fulco, 1967]. This situation is further complicated because derivation of α -hydroxy FAs (C₁₆ to C₂₈) from higher plant waxes (cutin and suberin) [Cardoso and Eglinton, 1983] and seagrass (*Zostera muelleri*) [Volkman *et al.*, 1980]. Thus, it can be inferred that α -hydroxy FAs with a variation in wide chain length (C-numbers from 16 to 31) found in dusty snowpack samples may have mixed sources such as contribution from direct emissions of fatty acids originated from soil microbes and plant waxes, which are associated with the atmospheric mineral dust, as well as their photochemical oxidation products.

This inference on mixed dust sources can be further corroborated by the decrease in relative abundance of mineral dust derived Ca^{2+} over NO_3^- for the snowpack samples in 2011 than those found for spring 2009. Also, the percentage relative abundances of water-soluble inorganic ions dominated by sulphate (in which non sea salt-fraction account for more than 90%) and sodium in spring 2011 samples than those in Spring 2009 (*i.e.*, Na^+ and Ca^{2+} are the dominant water-soluble inorganic species). Therefore, observed molecular distribution of C_{16} to C_{31} α -hydroxy FAs in spring 2011 snowpack samples are likely result of increased processing of mineral dust containing soil microbes/plant waxes with anthropogenic pollutants during long-range atmospheric transport.

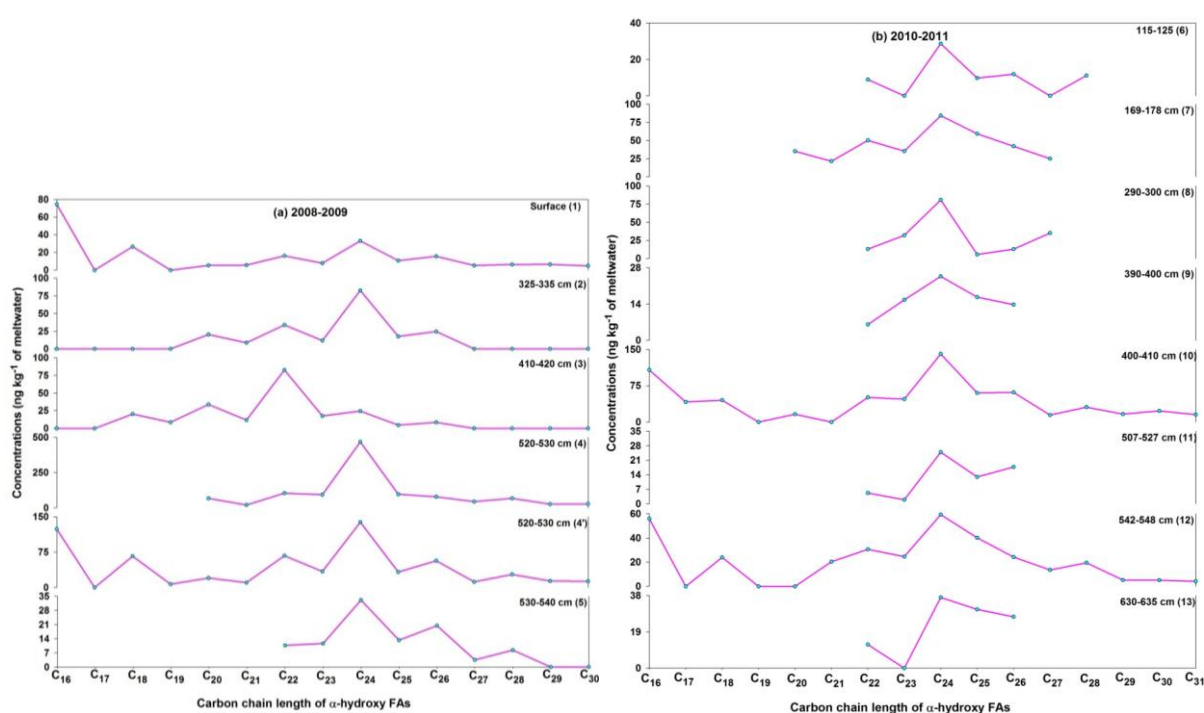


Fig.8.7. Molecular distributions of α -hydroxy FAs (C_{16} – C_{31}) in the snowpack samples collected at various depths at the Murodo-Daira site of Mt. Tateyama (a) 2009 and (b) 2011 in central Japan. The numbers in the parenthesis indicate the sample IDs as given in Table 8.1.

8.3.2.2. β -Hydroxy fatty acids

Molecular distributions of β -hydroxy FAs are relatively simple; with even C-numbered short-chain species (C_{14} , C_{12} , C_{10} and C_{16}) being predominant (Figs. 8.6b, 8.8a and 8.8b). These results are consistent with our previous studies conducted in fresh snow and marine aerosols where similar C-number predominance was observed. Odd C-numbered β -hydroxy FAs were present in very small amounts, with C_9 species being the most abundant in

two snowpack samples collected in 2009 (sample Nos. 1 and 4') and all the samples collected in 2011. It was found that the relative abundance of C₉ β-hydroxy FAs can be explained by the formation either *via* photochemical oxidation of long-chain unsaturated fatty acids such as oleic acid or bacterial β-hydroxylation of C₉ fatty acid during long-range atmospheric transport [Kawamura, 1995]. Some inter-annual variability was also observed in the molecular distributions of β-hydroxy FAs. The snow layers collected in 2009 showed a predominance of even C-numbered C₁₄, C₁₂, C₁₀, C₉ and C₁₆ β-hydroxy FAs. However, in the snow layers collected during 2011, we observed the predominance of C₉ or C₁₀ β-hydroxy FAs, except for sample No. 12 where C₁₂ was the dominant β-isomer. This difference in the molecular distributions could be due to a strong and frequent influence of atmospheric inputs from Asian dust in 2009 as compared to those in 2011. Moreover, snow layers collected during 2011 were mostly clean and having an ice plate (Table 8.1). This may suggest a possibility of photochemical degradation and bacterial modification of hydroxy FAs during re-freezing of melt water in the snowpack samples collected in April 2011 [Kawamura *et al.*, 2012].

Short-chain β-hydroxy FAs (typically <C₂₀) with a predominance of C₁₄ or C₁₂ are found in the LPS present in the cell wall of Gram-negative bacteria (GNB). Goossens *et al.* [1986] reported C₁₄, C₁₂, C₁₆ and C₁₈ β-hydroxy FAs in the GNB cultures. Pomorska *et al.* [2007] demonstrated that β-hydroxy FAs from C₁₄ to C₁₈ detected in aerosols collected from animal houses are most prevalent in the *Enterobacteriaceae* family of the GNB. Furthermore, β-hydroxy FAs can be produced as intermediates by β-oxidation of fatty acids during the synthesis or degradation processes in microorganisms. β-Oxidation is more important than α- and ω-oxidation pathways [Lehninger, 1975; Volkman *et al.*, 1998], which might have accounted for the greater abundances of β-isomers over α- and ω-isomers observed in the snowpack samples collected from Mt. Tateyama. Further, Tanaka *et al.* [2011] and Maki *et al.* [2011] evaluated bacterial diversity and abundance in the Asian dust containing snow layers over Mt. Tateyama and attributed their source regions as the Gobi and Taklamakan Deserts and Loess Plateau *via* atmospheric transport over the Korean Peninsula. Our results clearly show that β-hydroxy FAs of GNB origin are long-range transported in the atmosphere from the arid regions of the Asian continent and scavenged by snowflakes over Mt. Tateyama.

In a previous study, *Zelles and Bai* [1994] documented the predominant occurrence of α - and β -hydroxy FAs in grassland and aggregated soils, respectively, while ω -hydroxy FAs dominate (70–80%) the forest soils. Furthermore, β -hydroxy FAs account for 70%, 27% and 1% in aggregated-, grassland-, and forest-soil samples, respectively, with maximum abundance peak centered on C₁₀ or C₁₂ or C₁₄ [Zelles and Bai, 1994]. Their study emphasized that LPS derived β -hydroxy FAs in soil samples mostly represent GNB species. Since the samples collected from Mt. Tateyama were significantly influenced by the Asian dust outbreaks (as inferred from AMBTs and concurrent occurrence of higher abundances of water-soluble nss-Ca²⁺), we inferred that measured β -hydroxy FAs are indeed representative of soil GNB associated with mineral dust. Furthermore, given the close agreement of molecular distributions of β -hydroxy FAs with even C-predominance and higher abundances of C₁₀ or C₁₂ in the snowpack samples with certified reference material of Chinese loess (CJ-1) and simulated Asian mineral dust (CJ-2) altogether indicate their representative source signatures (Fig. 8.9).

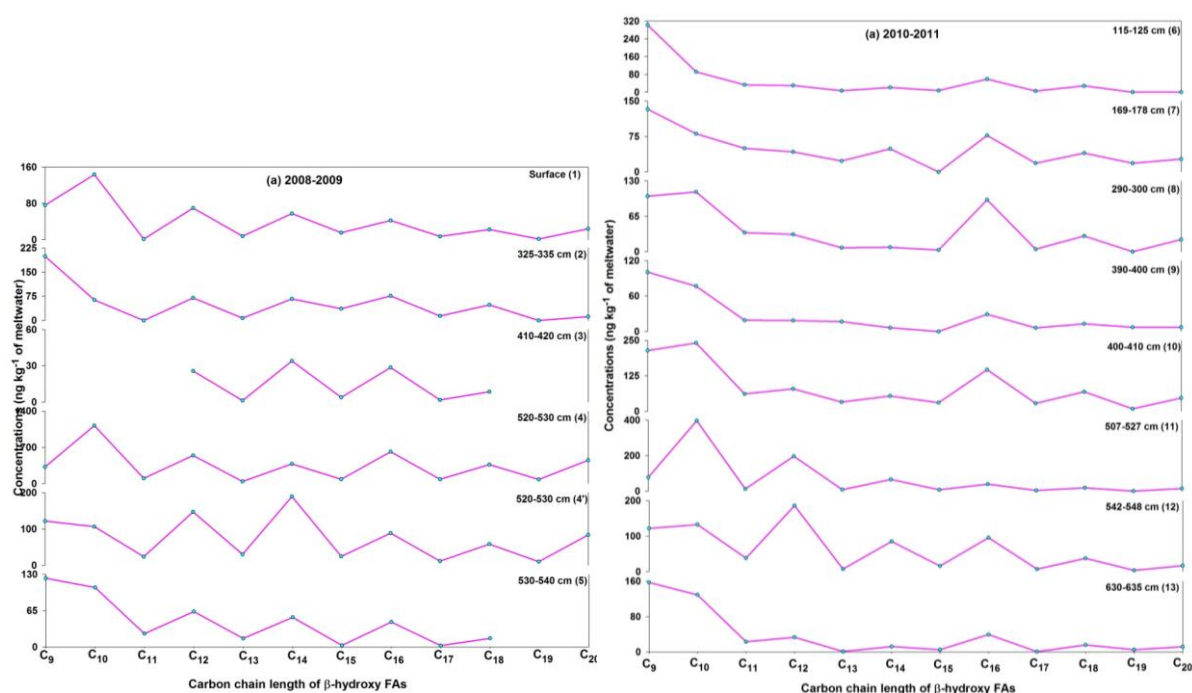


Fig.8.7. Molecular distributions of β -hydroxy FAs (C₉–C₂₀) in snowpack samples collected at various depths at the Murodo-Daira site of Mt. Tateyama in (a) 2009 and (b) 2011. The numbers in the parenthesis indicate the sample IDs as given in Table 8.1.

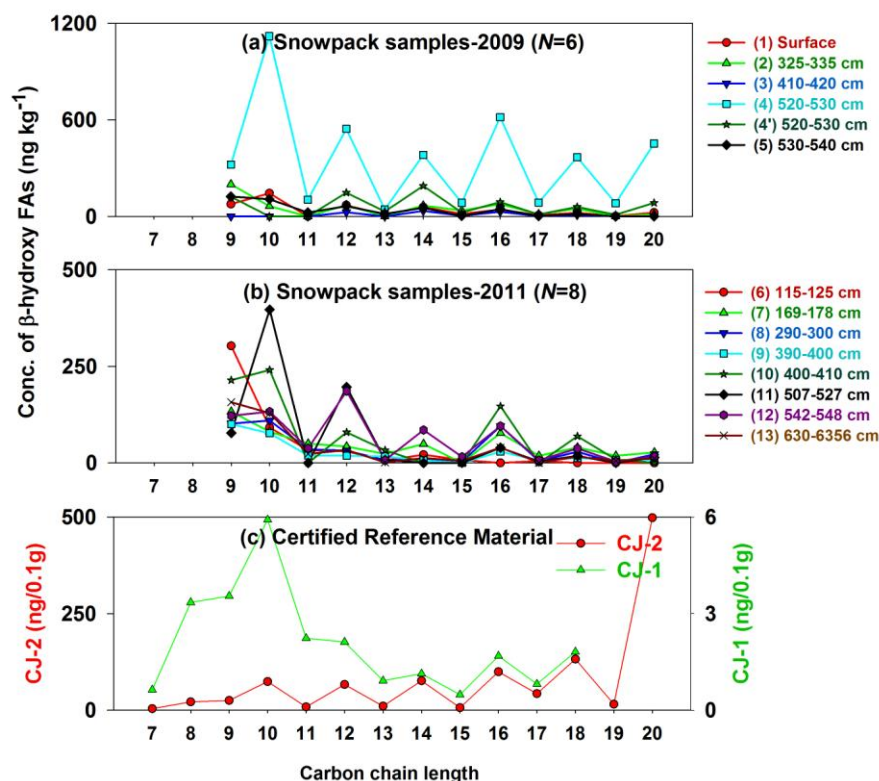


Fig.8.9. Comparison of molecular distributions of C_9 – C_{20} β -hydroxy FAs in the snowpack samples collected in (a) 2009 (b) 2011 with (c) those documented for Certified Reference Materials of Chinese loess and Asian dust mineral standards (CJ-1 and CJ-2).

8.3.2.3. ω -Hydroxy fatty acids

ω -Hydroxy FAs were detected in a wide range of C_9 to C_{30} in snowpack samples (Fig. 8.6c). Their relative abundances and molecular distributions are shown in Figs. 8.10a and 8.10b, respectively. Short-chain ω -hydroxy FAs from C_9 to C_{19} were characterized by the predominance of even C-numbers with maxima at C_{16} followed by C_{14} , C_{12} and C_{10} . In contrast, long-chain ω -hydroxy FAs (C_{20} – C_{30}) showed a characteristic pattern of $C_{22} > C_{20} \sim C_{24} > C_{26}$. These results are consistent with the similar molecular distributions which were observed in our previous studies. Long-chain ω -hydroxy FAs have been used as markers of higher plant waxes in sediments and aerosols [Eglinton *et al.*, 1968; Kawamura *et al.*, 2003].

ω -Hydroxy FAs (C_{20} – C_{26}) have been detected as important constituents of suberin, which occurs in the cork layer of the woody parts of plants. Furthermore, ω -hydroxy FAs are characterized by the dominance of C_{16} and C_{18} species in cutin, which makes part of the cuticle of leaves and fruits [Eglinton *et al.*, 1968]. It is noteworthy that both suberin and cutin

can contribute to short-chain ω -hydroxy FAs whereas the occurrence of long-chain isomers ($>C_{20}$) is supposed to be the characteristic of a suberin contribution [Cardoso and Eglinton, 1983]. Previous studies have reported that terminal oxidation of fatty acids by microorganisms can also produce ω -hydroxy FAs as the intermediates [Wakeham, 1999 and references therein]. These hydroxy FAs can survive geological conditions due to their ubiquitous occurrence in woody parts; thus, we employ them as geochemical tracers of epicuticular waxes of higher plants and soil bacteria in the snowpack samples collected from Mt. Tateyama.

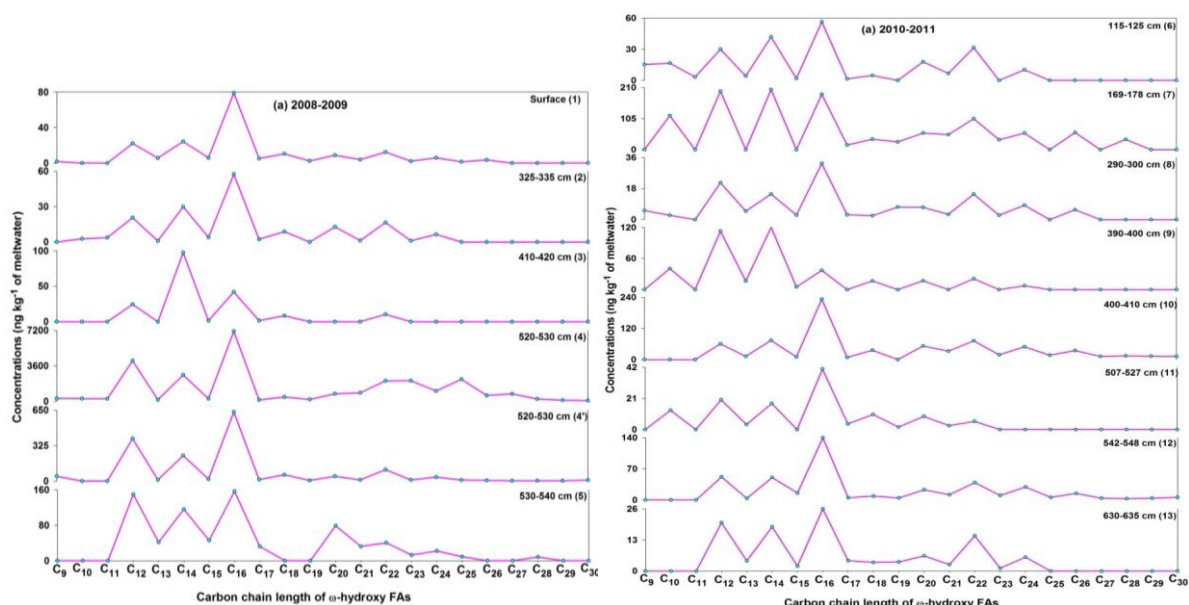


Fig.8.10. Molecular distributions of β -hydroxy FAs (C_9 – C_{20}) in the different snowpack samples collected at various depths in (a) 2009 and (b) 2011 at the Murodo-Daira site of Mt. Tateyama in central Japan. The numbers in the parenthesis indicate the sample IDs as given in the Table 8.1.

8.3.3. Relative abundances

In snowpack samples collected during spring 2009, high relative abundances of ω -isomers (45 ± 25 %) was found followed by β - (35 ± 19 %) and α -isomers (20 ± 14 %). In contrast, snowpack samples collected in 2011 showed a predominance of β -isomers (54 ± 8 %) over ω - and α -isomers (30 ± 16 % and 16 ± 8 %, respectively). However, the differences between 2009 and 2011 snowpack samples were not statistically significant (Table 8.4). This observation indicates their similar sources of soil microbes/plant waxes or their common long-range atmospheric transport to Mt. Tateyama. The Asian dust can transport not only soil microorganisms but also the epicuticular waxes of higher plants, fruits and leaves from East

Asia, explaining the high atmospheric abundances of ω -hydroxy FAs in the Asian dust-containing snow layers of snowpack samples collected in this study.

Table 8.4. A statistical comparison of mean relative abundances of individual type of hydroxy FA in their total mass concentration, and also the short-chain ω - or long-chain α -hydroxy FAs in their total ω - and total α -hydroxy FA mass in snowpack samples collected in April 2009 and 2011.

Compounds	2009	2011	t-score, df, p-value
α -hydroxy FAs	20 $\pm$ 14%	16 $\pm$ 8%	0.65, 12, >0.05
β -hydroxy FAs	35 $\pm$ 19%	54 $\pm$ 18%	1.89, 12, >0.05
ω -hydroxy FAs	45 $\pm$ 25%	30 $\pm$ 16%	1.38, 12, >0.05
α -C ₂₀ –C ₃₁ OHFAs/ α -C ₁₆ –C ₃₁ hydroxy FAs	85 $\pm$ 20%	93 $\pm$ 12%	1.0, 12, >0.05
ω -C ₉ –C ₁₉ OHFAs/ ω -C ₉ –C ₃₀ hydroxy FAs	78 $\pm$ 12%	72 $\pm$ 11%	0.98, 12, >0.05

The relative abundances of measured hydroxy FAs in snowpack samples collected from Mt. Tateyama were also compared with those obtained in fresh snow from Sapporo, Japan (Fig. 8.11). For comparison each type of hydroxy FAs, were classified into short-chain (C₁₆–C₁₉ for α -hydroxy FAs and C₉–C₂₀ or C₉–C₁₉ for β - or ω -hydroxy FAs, respectively) and long-chain compounds (C₂₀–C₃₁ and C₂₀–C₃₀ for α - and ω -hydroxy FAs, respectively) due to their differences in the sources/formation pathways. It has been suggested that the long-chain saturated homologues of hydroxy FAs originate mainly from the higher plant waxes [Cranwell, 1981; Rogge *et al.*, 1993], while their photo-degradation/oxidation during transport and/or as well as contribution from soil microbes account for the occurrence of corresponding short-chain compounds [Cranwell, 1981; Rogge *et al.*, 1993; Řezanka and Sigler, 2009]. Therefore, the relative dominance of long-chain α -hydroxy FAs (>C₂₀; Fig. 8.11) in snowpack samples could be due to their contribution from epicuticular plant waxes, which are associated with the atmospheric mineral dust and are scavenged by the snowflakes during transit as well as their dry-deposition to the snow layers over Mt. Tateyama. In contrast, the predominance of short-chain α -hydroxy FAs (<C₂₀) in fresh snow could be either due to in-cloud α -oxidation of long-chain fatty acids by airborne bacteria or contribution from soil microbes in cloud water (see for more detailed discussion in section 8.3.5).

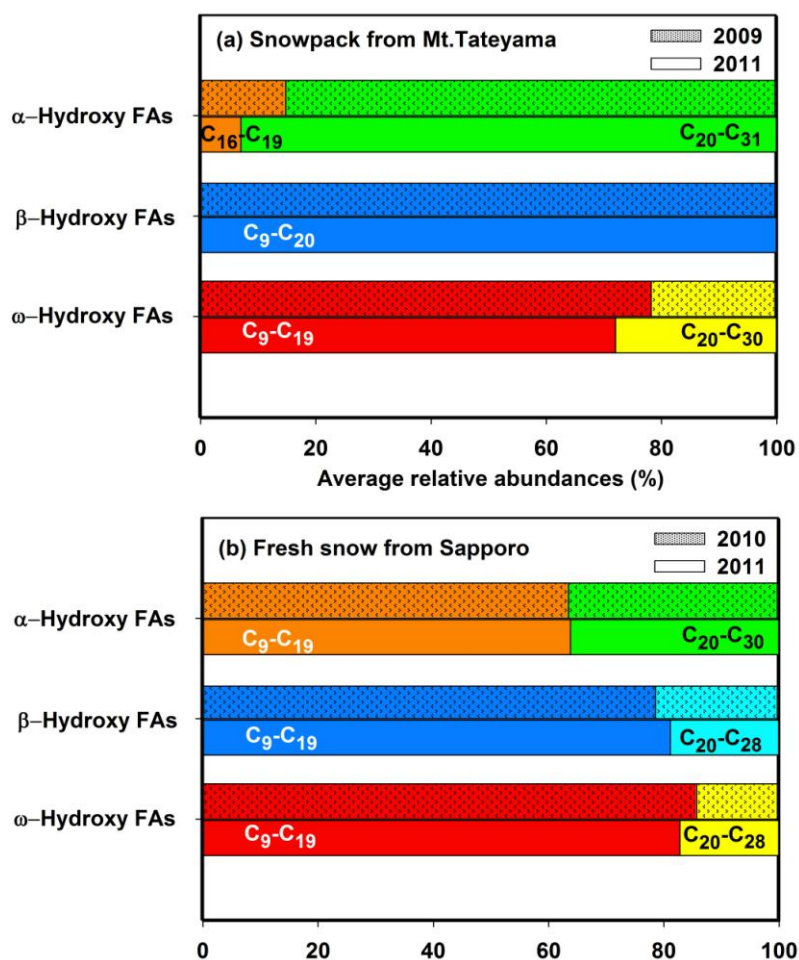


Fig.8.11. The relative abundances of short-chain (<C₂₀) and long-chain (>C₂₀) α-, β- and ω-hydroxy FAs in their respective total mass concentration for (a) snowpack samples from Mt. Tateyama (this study), and (b) fresh snow from Sapporo (chapter 7).

Atmospheric mineral dust is a carrier of both plant waxes and soil microorganisms, which are the important CCN [Möhler *et al.*, 2007]. It has been suggested that fatty acids and other dicarboxylic acids in highly acidic cloud waters serve as nutrients for airborne microorganisms and, thus, subjected to their intense biological degradation [Deguillaume *et al.*, 2008; Väitilingom *et al.*, 2010; Väitilingom *et al.*, 2013]. Since the fresh snow samples collected from Sapporo during non-dust season (December–February), it is likely that cloud water has dominant contribution from airborne plant waxes as CCN than those of dust origin. This inference is based on the predominance of ω-hydroxy FAs (derived mostly from the epicuticular plant waxes; [Kolattukudy, 1980]) over β-hydroxy FAs (originate from soil microbes; [Zelles, 1997]) in fresh snow from Sapporo and vice-versa is observed in snowpack samples from Mt. Tateyama. In a previous study, Hamberg *et al.* [1999] documented the preferential α-oxidation of fatty acids in higher plants by plant pathogens. Also, it has been

suggested that microbe-mediated oxidation of organic matter in clouds dominates (90–99%) the free-radical based reactions especially during nighttime, however, also contributes significantly during daytime (2–37 %) [Vaithilingom *et al.*, 2010]. All these observations highlight the potential role of microbial oxidation processes in clouds and consequent shifts in the overall relative abundances of α -hydroxy FAs from long-chain to short-chain homologues in fresh snow.

The snowpack samples collected from Mt. Tateyama are significantly influenced by the Asian dust outbreaks for which we observed only short-chain homologues of β -hydroxy FAs (100%). This observation clearly indicates the strong input of soil microorganisms in the Asian dust to snowpack collected from Mt. Tateyama. In contrast, the fresh snow samples collected from Sapporo are influenced by both plant waxes and soil microbes and, thus, explain the occurrence of both short-chain as well as long-chain β -hydroxy FAs (Fig. 8.11). The relative abundances of ω -hydroxy FAs were dominated by short-chain isomers in both fresh snow and snowpack samples collected from Sapporo and Mt. Tateyama, respectively. This is perhaps due to short-chain ω -hydroxy FAs also originated from higher plants, for instance some studies documented the predominant occurrence of C₁₆ followed by C₁₈ and/or C₁₄ in higher plants [Kawamura *et al.*, 2003; Simoneit *et al.*, 2004]. Similarly, the photochemical oxidation of long-chain ω -hydroxy FAs during transport from Siberia and Russian Far East could be a potential source of their short-chain homologues to both fresh snow (Sapporo) and snowpack samples (Mt. Tateyama).

8.3.4. LPS mass and GNB dry mass in snow layers

Previous studies suggested that β -hydroxy FAs, as determined by GC/MS, can be used as molecular tracers for the presence/absence of airborne LPS, which are also known as pyrogen (fever causing toxin) [Maitra *et al.*, 1986; Paba *et al.*, 2013; Pomorska *et al.*, 2007; Saraf *et al.*, 1997]. The most common route of exposure to airborne LPS is inhalation, which has been associated with a variety of clinical symptoms, including respiratory disorders [Mielniczuk *et al.*, 1993 and references therein]. Airborne LPS mass from GNB is usually measured by extracting the filter samples with water/buffer and analyzing with the Limulus Amebocyte Lysate (LAL) test. Although LAL based assaying of endotoxin/LPS from GNB [Saraf *et al.*, 1999] have been employed in occupational environments, however, they are poorly reproducible and only limited to culturable soil GNB. In the literature, the LAL assay

is a widely adopted analytical technique that essentially relies on clotting the enzyme activity through an artificial chromogenic substrate and, thereby, leads to the formation of a gel clot [Levin and Bang, 1968; Iwanaga *et al.*, 1978]. However, there has been substantial variability in the endotoxin levels with different GNB species and, thus, the LAL assay may not reflect all these differences. Moreover, this method also subjected to strong interference from β -1-3 glucans from airborne moulds and plant fragments present in organic dust. Thus, measurement technique based on a detection of specific LPS molecules, *i.e.*, homologues of β -hydroxy FAs with carbon chain length from C₁₀ to C₁₈ may present a precise alternative to the LAL test.

Unlike LAL assay, the estimation of LPS-mass from GNB using β -hydroxy FAs is free of interferences from β -1-3-glucans [Saraf *et al.*, 1999]. Moreover, Saraf *et al.* [1999] observed significant linear correlations between LAL assay and the measured mass concentrations of C₁₀ to C₁₈ β -hydroxy FAs in 30 house dust samples. Therefore, chemical marker based assessment of “LPS mass of GNB” serves as a pseudo first order approach and can be employed to both culturable and non-culturable soil GNB. Furthermore, this approach provides some information about the source of LPS due to the fact that the molecular distributions of β -hydroxy FAs from different GNB species vary considerably [Wilkinson, 1988]. Although this approach is semi-quantitative, in light of paucity of datasets related to quantification of LPS from GNB in the literature, the GC-MS based quantification serves as fast and reliable technique (in terms of reproducibility) owing to its large throughput of samples.

In a previous study by Segawa *et al.* [2005] documented the occurrence of two dominant GNB species (*Janthinobacterium lividum* and *Variovorax paradoxus*) in the dust impacted layers of snow pit samples from Mt. Tateyama, based on the analyses of 16S rRNA gene sequencing and real-time PCR. Moreover, their study also observed that the bacterial biomass component of both Gram-positive (*e.g.* *C. psychrophilum*) and Gram-negative (*J. lividum*) increased significantly from March to April, as a result of enhanced supply of mineral dust transport from Asian deserts. Likewise, Tanaka *et al.* [2011] also observed the presence of both Gram-negative species (*e.g.* *Methylobacterium sp.*) and Gram-positive species (*e.g.* *Bacillus sp.*) in the snow pit samples collected during spring 2009 from Mt. Tateyama. However, the total bacterial cell counts reported by Tanaka *et al.* [2011] for the snow pit samples collected from Mt. Tateyama during April 2009 were consistent with those

collected during March 1998 by *Segawa et al.* [2005]. Therefore, we presumed that the snow pit samples analyzed for hydroxy FAs in this study are significantly influenced by the soil microorganisms associated with Asian dust storms.

In this study, we estimated the amount of “LPS from GNB”, on the basis of β -hydroxy FAs detected in Asian dust-containing and underlying snow layers collected at the Murodo-Daira site of Mt. Tateyama in 2009 and 2011 (Fig. 8.12). Maximum mean concentrations of LPS mass from GNB was found in sample Nos. 4 ($29.7 \mu\text{g kg}^{-1}$ in 2009), 10 ($6.60 \mu\text{g kg}^{-1}$ in 2011), 11 ($7.3 \mu\text{g kg}^{-1}$ in 2011) and 12 ($5.4 \mu\text{g kg}^{-1}$ in 2011), which are heavily influenced with dust and characterized by yellow to brown color and high abundances of nss-Ca^{2+} . Although the mean concentration of LPS-mass from GNB in the snowpack samples collected in 2009 ($7.4 \mu\text{g kg}^{-1}$) is almost twice as high as those reported in snow layers in 2011 ($4.0 \mu\text{g kg}^{-1}$), however, their median are consistent between 2009 ($3.3 \mu\text{g kg}^{-1}$) and 2011 ($3.1 \mu\text{g kg}^{-1}$). These concentrations are also much higher than those reported in our previous study in the fresh snow samples ($\sim 0.79 \mu\text{g kg}^{-1}$ in 2010 and $\sim 0.59 \mu\text{g kg}^{-1}$ in 2011) from Sapporo. Several studies have been conducted on the determination of bacterial LPS-mass using β -hydroxy FAs as biomarkers in the indoor environments [*Hines et al.*, 2003; *Pomorska et al.*, 2007; *Reynolds et al.*, 2005], however, very few studies are present in relation to their atmospheric transport. Although there are not many studies performed on the measurement of LPS-mass in snow and marine aerosols, this quantification is indeed crucial for assessing a likely global allergic impact of LPS from GNB *via* long-range atmospheric transport.

The mass loading of airborne GNB was estimated using the marker-to-microbial mass conversion factors as described in the section 2.3 (Fig. 8.12). Dry cell mass of GNB in snow layers were found to vary from $28 \mu\text{g kg}^{-1}$ to $990 \mu\text{g kg}^{-1}$ (av. $248 \mu\text{g kg}^{-1}$) in 2009 and from $57 \mu\text{g kg}^{-1}$ to $241 \mu\text{g kg}^{-1}$ (av. $134 \mu\text{g kg}^{-1}$) in 2011. Similar to LPS-mass, estimated concentrations of GNB dry cell mass are higher than those obtained in our earlier study conducted for fresh snow samples from Sapporo (av. $26.3 \mu\text{g kg}^{-1}$ in 2010 and $19.3 \mu\text{g kg}^{-1}$ in 2011). *Golokhvast* [2014] reported that airborne biogenic particles scavenged by snow can cause allergies to the pedestrians in the Russian Far East. Additionally, *Lee et al.* [2007] showed a crustal origin for airborne endotoxin, bacterial and fungal markers. In our study, air masses associated with Asian dust might have transported from the Gobi and Taklamakan Deserts and Loess Plateau in 2009 and 2011. Thus, source regions of these enhanced LPS and

bacterial dry mass could be the arid regions of Mongolia, Siberia, northern China and the Korean Peninsula during the study period.

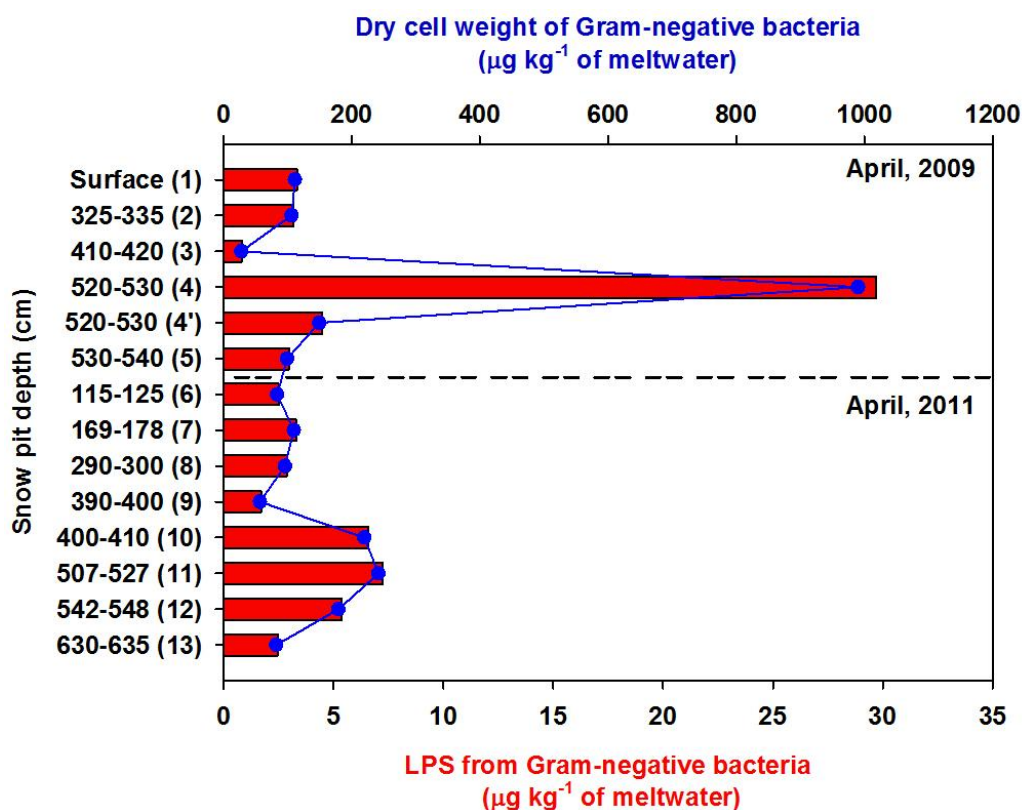


Fig. 8.12. Bar graph represents airborne LPS mass (bottom x-axis, red bars) and blue line shows dry cell weight of Gram-negative bacteria (GNB) (top x-axis, blue circles) estimated in the snowpack samples deposited from winter to early spring of 2008-2009 and 2010-2011 at the Murodo-Daira site on Mt. Tateyama in central Japan. Numbers in the parenthesis on the left y-axis represent the sample IDs as given in Table 8.1.

Studies have shown that bacteria can be metabolically active even at subzero temperatures [Polymenakou, 2012 and references therein]. Snow can efficiently scavenge these bacterial particles from the atmosphere and, hence, reduce their associated health burden. β -Hydroxy FAs, cell membrane constituents of GNB, can be used to quantify the dry cell weight in these snowpack samples. High abundances of LPS content and GNB dry cell mass were estimated in the Asian dust-containing snow layers, which clearly affirm their atmospheric transport along with the Asian dust particles. High abundances of LPS and dry cell mass of GNB were found in the Asian dust contained snowpack samples confirm that bacterial particles and their associated health impacts should be studied for long-range transported air masses on a global scale. Our study has used chemical markers (hydroxy FAs)

for the assessment of biogenic particles in the snowpack, which could assist monitoring the microbial content during their aeolian transport. The results presented here along with previous studies prove the robustness, sensitivity, and wide applicability of hydroxy FAs as molecular tracers, which allow their application to other airborne particulates during a long-range transport.

8.3.5 Inference on “below cloud” and “in-cloud oxidation” processes

A comparison of relative abundances of hydroxy FAs in snowpack samples collected from Mt. Tateyama with fresh snow samples from Sapporo reveal that the former samples are characterized by the predominance of β - or ω -hydroxy FAs whereas α -hydroxy FAs dominate in the latter samples (Fig. 8.13). As mentioned earlier, the dominant occurrence of short-chain (C_9 – C_{19}) homologues of α -hydroxy FAs in fresh snow from Sapporo, whereas long-chain (C_{20} – C_{30}) homologues mostly contribute to snowpack samples from Mt. Tateyama (present study). Furthermore, the overall predominance of β - and/or ω -hydroxy FAs in snowpack samples from Mt. Tateyama (collected in spring of 2009 and 2011) also showed a good agreement with dust-laden air masses sampled over the remote marine island (Chichijima) in the western North Pacific (chapter 3). Similarly, molecular distributions of these hydroxy FAs regarding the predominance of β - and/or ω -hydroxy FAs together with occurrence of long-chain α -hydroxy FAs (C_{16} – C_{30}) in snowpack samples from Mt. Tateyama were also consistent with the dust events (kosa) collected from Jeju Island (Gosan, South Korea; chapter 4). In addition, similar even C-predominance of C_{10} followed by C_{12} or C_{16} β -hydroxy FAs in snowpack samples was observed in certified reference materials from the source regions of East Asian Deserts, Chinese loess (CJ-1) and Asian dust (CJ-2). All these observations hint the below cloud oxidation process of α -hydroxy FAs.

As snow essentially comes from cloud water and, therefore, occurrence of both short-chain and long-chain α -hydroxy FAs in fresh snow could be a result of direct emissions/in-situ oxidation processes of microbes in cloud water. A close observation of results from chapter 7 with respect to predominance of α -hydroxy FAs followed by ω -isomers together with their similar even C-predominance of C_{16} followed by C_{22} or C_{24} in fresh snow, suggesting their in-cloud oxidation. This inference can be further supported by the higher relative abundances of short-chain homologues (C_9 – C_{20}) accounting for ~65% of total (C_9 – C_{30}) α -hydroxy FAs in fresh snow (chapter 7), which indicates their production through

α -oxidation. The microbial oxidation of fatty acids in cloud water could be likely explanation for the observed predominance of α -hydroxy FAs (as discussed in section 8.3.2.1.) in fresh snow than those observed for dust-laden snowpack samples (this study) and aerosols (chapter 3). All these observations highlight potential role of microbial oxidation processes in clouds.

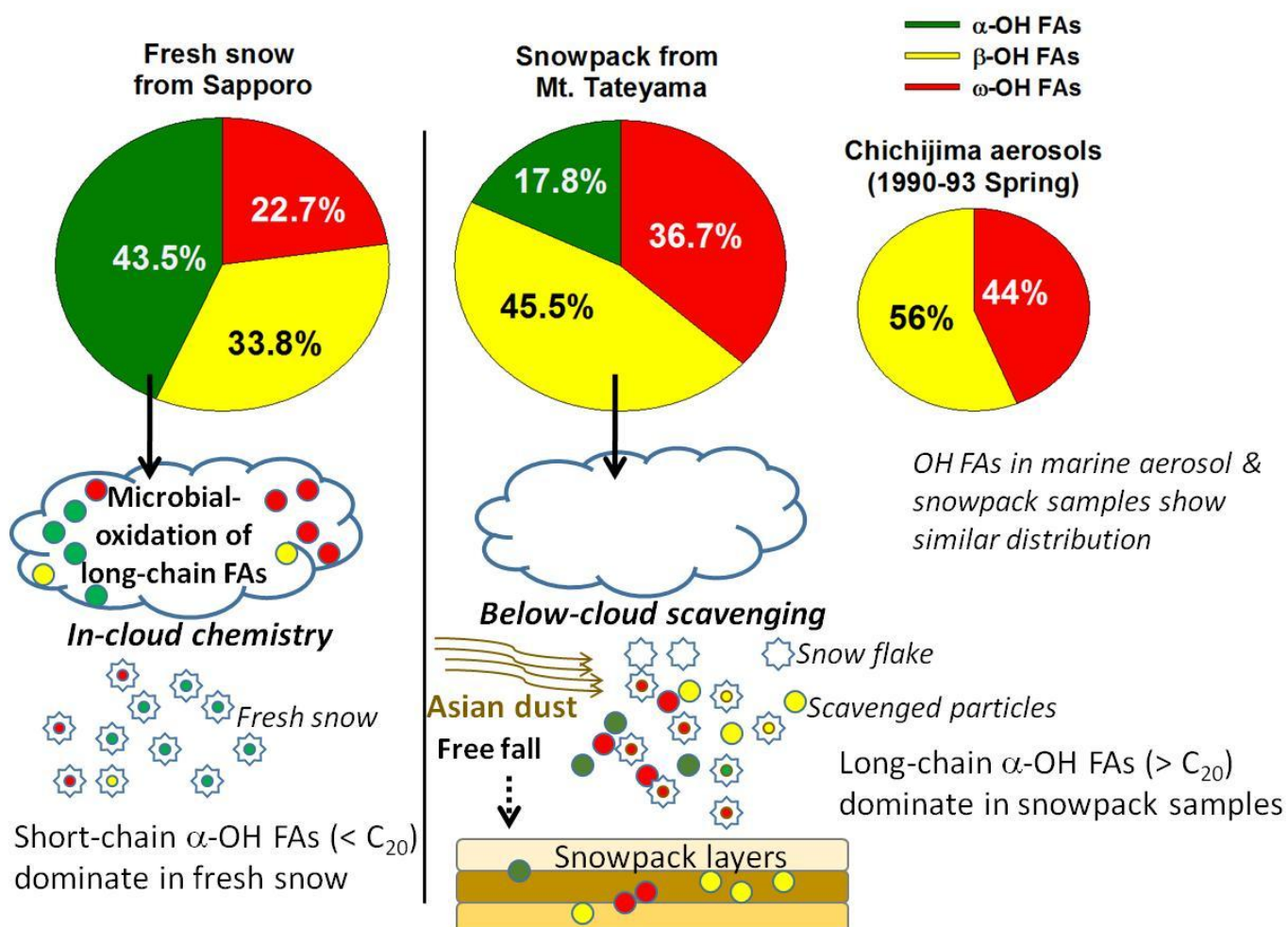


Fig.8.13. A conceptual diagram showing a comparison of relative abundances of hydroxy fatty acids (OH FAs) in snowpack samples collected from Mt. Tateyama (central Japan) with fresh snow from Sapporo (northern Japan) and aerosols from Chichijima (remote Marine Island in the western North Pacific).

8.4. Summary and Conclusions

A homologues series of three positional isomers of hydroxy FAs, i.e., α -(C_{16} – C_{31}), β -(C_9 – C_{20}) and ω -(C_9 – C_{30}), were determined in snowpack samples collected from snow pit sequences at the Murodo-Daira site in Mt. Tateyama, central Japan. The molecular distributions of measured hydroxy FAs were characterized by even C-number predominance,

which is a characteristic of lipid biomarkers from soil microorganisms and higher plant waxes. The occurrence of only short-chain homologues (*i.e.*, specific to soil GNB) of β -hydroxy FAs and their significant linear relationship with water-soluble nss-Ca^{2+} indicate that snowpack samples are significantly influenced by the contribution of soil microbes from Asian dust outbreaks. Moreover, the air mass back-trajectories and the co-variability of hydroxy FAs and nss-Ca^{2+} in snowpack samples collected from Mt. Tateyama revealed a long-range atmospheric transport of soil microbes and plant waxes from the Asian continent including Siberia, Mongolia and Russian Far East.

Several studies have documented the occurrence of GNB and soil microbes in cloud water (as CCN) and ice nuclei. Cloud waters are generally more acidic ($\text{pH} \sim 2\text{--}4$) and often contains high amount of oxygenated water-soluble species and free radicals. A close resemblance in the overall predominance of ω - and β -hydroxy FAs between snowpack samples (present study) and marine aerosols indicates, conceivably, that Mt. Tateyama represents “*below cloud scavenging*” of transit dust particles and associated soil microbes by snow in the East Asian outflow during Spring/Winter. Moreover, these dust particles can be engulfed as ice nuclei and CCN to form the snowflakes and, also can be scavenged during snowfall. We attribute the, predominance of α -hydroxy FAs in fresh snow to enhanced microbial oxidation (degradation) of long-chain hydroxy FAs “*within the cloud water*” to form short-chain homologues. Therefore, simultaneous comparison of distributions of hydroxy FAs in snowpack samples from Mt. Tateyama with fresh snow from Sapporo and marine aerosols from Chichijima provided additional insights regarding the aeolian transport of soil microbes in the East-Asian regions to the western North Pacific. Overall, our study is one of the first to employ hydroxy FAs as proxies to trace and quantify bacteria and their debris (biomass) as well as plant waxes in the Asian dust-containing and underlying snow layers in Mt. Tateyama. Our results along with other culture-dependent and culture-independent approaches can aid to provide a better understanding of long-range transport of Asian dust-associated soil-borne microorganisms.

Chapter 9

Synthesis and Future Directions

Chapter 9 presents the overall summary and conclusions of this thesis regarding atmospheric abundances of hydroxy FAs, their molecular distributions, long-range transport, sources and their emission strength in the East Asian outflow to the North Pacific.

9.1 Synthesis

The impact of terrestrial lipids in the East Asian outflow to the North Pacific is essential to evaluate their role in carbon cycling in surface waters and their export to deep sea sediments. Apart from the chemical composition of aerosol (inorganic and organic constituents), rather few studies exist in the literature focusing on the characterization of these terrestrial lipid compounds in atmospheric outflow from East Asia. However, none of these studies have focused on the quantification and seasonality of hydroxy FAs (lipids specific to soil microbes and epicuticular plant waxes) in the East Asian outflow to the North Pacific. The paleoclimatic studies have documented the occurrence of these hydroxy FAs in sediments and attributed their sources as soil microbes and plant waxes. However, no depth study is available in the literature with regard to their sources, atmospheric transport (whether occurring on a seasonal/annual basis) and transformations (formation/degradation) during transport before reaching the sediment floor through air-sea deposition. It is, therefore, essential to understand their atmospheric abundances, transport pathways, heterogeneity in the sources (or variability; loess sediment vs. desert dust), and temporal/seasonal variability (if any). This study also compares the observed molecular distributions in aerosols from source region to remote oceanic islands to observe the possible changes due to ambient photochemical processes. Furthermore, the observed molecular distribution in marine aerosols is compared with those reported for sediments, thereby, establishing the source specific signatures of these lipid compounds associated with soil microbes and higher plant waxes.

The aerosol samples investigated from Gosan and Chichijima showed pronounced temporal as well as seasonal variability. The year round observations from Gosan indicate higher concentrations of both β - and ω -hydroxy FAs (tracers of soil microbes and plant waxes, respectively) in spring season followed by winter and autumn, whereas lowest concentrations are observed for summer. Since Gosan is influenced by the Asian dust storms (from China and Mongolia) in spring, the observed higher abundances of β -hydroxy FAs indicate enhanced contribution of soil microbes during this period. Likewise, enhanced contribution of organic aerosols from forest fires in Siberia during spring could explain the occurrence of ω -hydroxy FAs. In winter season, the transport of loess sediments from China (inferred from AMBTs) and biomass burning/biogenic emissions (ascertained from MODIS fire count data) over East Asia contribute to atmospheric abundances of β - and ω -hydroxy

FAs, respectively. The contribution from loess sediment in winter samples is further strengthened by comparing the molecular distributions of β -hydroxy FAs in ambient aerosols with those observed in CRM samples (CJ-1 and CJ-2; Chinese loess soil standard).

The seasonal variability of hydroxy FAs over Gosan is further supported by other dust and biogenic emission tracers (*e.g.*, water soluble nss-Ca^{2+} , trehalose, and methanesulfonic acid, respectively). The water soluble nss-Ca^{2+} (a proxy for mineral dust) and trehalose (a tracer for fungal spores associated with soil dust) both show similar seasonal variability that are consistent with β -hydroxy FAs. The enhanced contribution of biogenic emissions in spring, compared to other seasons, is supported by the higher atmospheric concentrations of methanesulfonic acid (a proxy for biogenic emissions). In contrast to β - and ω -hydroxy FAs, α -isomers show high concentrations in winter followed spring and autumn, while low concentrations in summer. The observed lowest of measured hydroxy FAs in summer over Gosan is due to the predominant contribution from oceanic sources in the former sampling period, while mixed origin of air masses (*i.e.*, from ocean and continent) account for their lower abundances in autumn.

The long term observations of β -hydroxy FAs from Chichijima during 1990–1991 and 2001–2003 showed similar seasonal variability as that observed over Gosan; also reflected through higher nss-Ca^{2+} concentrations in spring/winter than summer/autumn over Chichijima. These observations clearly emphasize the significance of atmospheric transport of soil microbes associated with mineral dust from East Asia to the remote oceanic islands in the North Pacific during spring. However, the ω -hydroxy FAs over Chichijima showed high concentrations in winter than spring; suggest a different source in the East Asian outflow than those contributed to Gosan. Higher abundances of ω -hydroxy FAs in winter than spring season are also consistent with levoglucosan concentrations (a proxy for biomass combustion source). Combining this information with AMBTs and fire count data reveal that biomass burning emissions in northern China contribute to higher abundances of ω -hydroxy FAs and levoglucosan over Chichijima.

Another interesting feature related to minimum concentrations of hydroxy FAs over Chichijima occurs in autumn than those observed in summer over Gosan. The oceanic air masses mostly dominate over both the sampling sites in summer and autumn. However, continental sources in the East Asian outflow account for slightly higher concentrations in autumn (transition of winds from land to ocean) over Gosan (neritic island) than summer. On

the other hand, the influences of continental sources over Chichijima (pelagic island) during autumn are rather weak. Therefore, microbial sources in surface waters (*e.g.* algal excreta, phytoplankton debris) of the North Pacific and island based vegetation could be a major source of atmospheric hydroxy FAs in summer and autumn over Chichijima.

A comparison of molecular distributions of β -hydroxy FAs among various seasons between Gosan and Chichijima reveals their common source and transport pattern in spring, characterized by the unique feature of C_{12} predominance. A closer look at the measurements of β -hydroxy FAs indicate that the TSP collected in April month at Gosan showed rather high concentrations of C_{12} - β -hydroxy FA. These samples are, indeed, influenced by the dust episodes (also referred as kosa events), as inferred from the higher abundances of Ca^{2+} (a proxy for mineral dust) in TSP samples. Likewise, the molecular distribution of β -hydroxy FAs showed similar temporal trends in summer and autumn at both the receptor sites, characterized by the predominance of C_{16} followed by C_{10} or C_{18} β -hydroxy FAs. The observed shifts in the molecular distribution of β -hydroxy FAs between spring and summer/autumn are mostly due to differences in the contributing source type (*i.e.* soil bacteria and/or marine dissolved organic matter, respectively). Since easterly wind regimes dominate over Chichijima and Gosan during summer and fall, therefore, observed molecular distributions with C_{16} predominance in marine aerosols could be attributed to their contribution from marine derived organic matter. These molecular distributions observed here are consistent with those documented for marine sediments in the literature.

The TSP samples collected from Mt. Tai provided the significance of hydroxy FAs from the biomass burning emissions (BBEs) in the North China Plain (NCP). Higher concentrations of hydroxy FAs were observed during high-BBEs than during low-BBEs. Estimated endotoxin concentrations during H-BBEs are also higher than the health-based occupational guidance limit ($\sim 90 \text{ EU m}^{-3}$) suggested by Dutch expert committee on Occupational Standards (DECOS) 2010 in Netherlands. Furthermore, these results are consistent with other organic tracers from biomass burning (*e.g.*, levoglucosan), and soil microbes (*e.g.*, trehalose and mannitol). This observation highlights the significance of re-suspended dust from charred agricultural soils during fire emissions, which can act as a principal source of airborne soil microbes. Overall, temporal shifts in the molecular distribution of hydroxy FAs and other tracer compounds are likely due to high temperatures occurred during agricultural residue combustion. However, the molecular distributions of

hydroxy FAs from Mt. Tai are different with respect to carbon number predominance than those documented from the remote island (Chichijima) in the North Pacific, which also receives soil-borne microbes from the Asian dust. Therefore, it has been inferred that any type of biomass burning (outdoor/indoor) can severely influence the emission of soil- and air-borne microbes, and increase the risk of exposure to their potent pathogenic particles (endotoxin) regionally as well as globally.

A comparison of relative abundances of hydroxy FAs in snowpack samples collected from Mt. Tateyama with fresh snow from Sapporo reveals that the former samples are characterized by the predominance of β - and ω -hydroxy FAs whereas the α -hydroxy FAs dominate in the latter samples. Another interesting feature of our data is the occurrence of short-chain (C_9 - C_{20}) homologous of α -hydroxy FAs in fresh snow from Sapporo, whereas long-chain (C_{16} - C_{30}) homologous of α -hydroxy FAs dominates in snowpack samples from Mt. Tateyama. Furthermore, the overall predominance of β - and ω -hydroxy FAs in snowpack samples from Mt. Tateyama (collected in spring of 2009 and 2011) also showed a good agreement with dust-laden air masses sampled over Gosan and Chichijima.

A close resemblance in the overall predominance of β - and ω -hydroxy FAs between snowpack samples and marine aerosols indicates that Mt. Tateyama represents “*below cloud scavenging*” of transit dust particles and associated soil microbes by snow in the East Asian outflow during spring/winter. Moreover, these dust particles can be engulfed as ice nuclei and CCN to form the snowflakes and, also can be scavenged during snowfall. On the contrary, predominance of α -hydroxy FAs in fresh snow could be due to enhanced microbial oxidation (degradation) of long-chain hydroxy FAs “*within the cloud water*” to form short-chain homologues. Therefore, simultaneous comparison of distributions of hydroxy FAs in snowpack samples from Mt. Tateyama with fresh snow from Sapporo and marine aerosols from Chichijima provided additional insights regarding the atmospheric transport of soil microbes in the East Asian regions to the western North Pacific.

9.2 Future directions

Atmosphere is a complex and heterogeneous ecosystem and its composition imposes significant challenges to the researchers. The presence and composition of microbial communities in the atmosphere makes the situation worse as these are still not well-defined, and also taxonomic studies of microbial diversity in the open-air have just started to appear.

More importantly, our knowledge about the potential techniques to collect and identify (qualitatively and quantitatively) the air microbiota is limited. This knowledge gap is due to the poor combination of micro- and molecular biology with atmospheric sciences.

It is not possible to culture/grow all the bacteria (<1.0% is culturable) in the laboratory which are present in the atmosphere. Similarly, analysis based on the quantification of biomarkers (organic compounds present as the structural and functional moieties in the bacterial cell) have their own limitations. The specific biomarkers of bacteria (hydroxy FAs for Gram-negative bacteria used in this study) can degrade and form during their long-range atmospheric transport. Still there is no report on degradation of hydroxy fatty acids (FAs), but formation is possible as observed in our study on snow. The intervention of nucleic acid amplification protocols such as PCR-based assays is efficient in tracing and quantifying the bacterial communities present in very low numbers. However, these assays are costly and require expertise in sequence interpretations. Thus, there is always a need to establish more specific, reliable and reproducible techniques with less error to estimate and measure the role of soil- and air-borne bacteria as well as to trace their atmospheric movement.

Gram-negative bacteria (GNB) are widely studied as they can act as ice nuclei and CCN. Several strains of plant pathogenic GNB (*Erwinia sp.*, *Pseudomonas sp.*, and *Xanthomonas sp.*) have been identified as ice nucleation active bacteria. In my thesis, hydroxy FAs have been employed as the potential biomarkers for the quantification of GNB in during the long-range transport of Asian dust to the North Pacific. Biomarker to microbial mass conversion factors was used to estimate the GNB and their associated toxic compounds. However, this kind of approach includes chances of under/over estimation of GNB dry mass. Thus, to validate hydroxy FAs as biomarkers for GNB in the environmental samples, I would like to use existing nucleic acid sequence based amplification and compare the results with biomarker based approach. Integrated culture-independent (biomarker and molecular techniques) technique will be used in my future studies to evaluate the applicability of hydroxy FAs as biomarkers to trace the atmospheric transport of bacteria as carriers of potentially harmful genetic elements.

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