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Inflammatory and degranulation effects of yellow sand on RBL-2H3 cells in relation to chemical and biological constituents

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ABSTRACT

Recent studie pointed out that allergic diseases have increased during the Asian dust storm event (ADSE) in Japan. Daily observations and the atmospheric concentrations of yellow sand (YS) aerosol have been increasing. In this study, YS samples collected from three sites of Japan during ADSE in 2009-2010 were used. The particles were analyzed by X-ray photoelectron spectroscopy (XPS) and X-ray fluorescence - energy dispersive spectrometer (XRF-EDS). We investigate ability of YS extract on enhancing the chemical mediator release and cytokine production from rat basophilic leukemia (RBL-2H3) cells. The dust particles at Fukuoka and Tsukuba were abundant in aluminum (Al), iron (Fe), potassium (K) and titan (Ti) than those at Naha. Concentration of the trace endotoxin and *Cryptomeria japonica* pollen allergen (Cry j 1) were measured in YS extract. After exposure of RBL-2H3 cells to YS extract, the β-hexosaminidase (β-hex) release, tumor necrosis factor-alpha (TNF-α) production were enhanced in RBL-2H3 cells. This process depends on endotoxin, Cry j 1 and other allergen present in the YS extract. YS water extract also show a strong cytotoxic effect on the cells. This data suggest that low levels of endotoxin and Cry j 1 in YS may cause allergy during the ADSE.

Keywords

Allergen, Asian dust storm, β-hexosaminidase, RBL-2H3, Yellow sand

Abbreviations

ADSE, Asian dust storm event; β-hex, β-hexosaminidase; Cry j 1, *Cryptomeria japonica* pollen

allergen; Fuku., Fukuoka; LPS, Lipopolysaccharide; MTT, 3-(4, 5-dimethylthiazolyl)-2, 5-diphenyltetrazolium bromide; PM, Particulate matter; RBL-2H3, Rat basophilic leukemia cells; TNF-α, Tumor necroses factor-α; Tsu., Tsukuba; YS, Yellow sand.

57 **1. Introduction**

58

59 The prevalence and morbidity of asthma and other allergic diseases have increased
60 dramatically during the last 30 years, particularly in industrial countries (Narita et al., 2007). A
61 lot of studies have demonstrated that allergens commonly associated with dust storms include
62 fungal spores, plant and grass pollens, anthropogenic emissions, and organic detritus (Griffin
63 2007). Recently, it was clarified that dust event enables atmospheric long distance transport of
64 bacteria, fungi, viruses, pollen etc. (Kellogg and Griffin 2006; Griffin 2010). Risk of
65 hospitalization was increased during the Sahara dust event in European cities (Middleton et al.,
66 2008). There was an association between the increase in paediatric asthma admissions and the
67 increased Saharan dust event in Caribbean island (Gyan et al., 2005). Recently, daily
68 observations and the atmospheric concentrations of YS aerosol have been increasing steadily in
69 the eastern Asia region, including Japan (Goto et al., 2010). YS increase the risk of mortality
70 and hospitalization in cardiovascular and respiratory patients during the ADSE in China, Korea,
71 and Japan (Kwon et al., 2002; Meng and Lu 2007). Total fungi and particulate matter (PM)
72 had significantly higher concentrations during the period affected by ADSE in Taiwan (Ho et al.,
73 2005). In Japan, it was pointed out that fine YS particles make people's allergy more severe
74 from February to May. Hua et al. (2007) have detected some bacteria, such as *Bacillus subtilis,*
75 *B. licheniformis* in YS collected in Hiroshima, Japan during ADSE, and provided evidence that
76 ADSE can transport these microorganisms in Northeast Asia.

77 Endotoxin, the lipopolysaccharide (LPS) components of the outer membrane of
78 Gram-negative bacteria, has been associated with an increase in asthma symptoms, asthma

79 medications, and reductions in lung function in patients with atopic or asthma. Airborne
80 endotoxin appears to be a risk factor for clinically symptomatic respiratory illnesses (Dales et al.,
81 2006) and allergic inflammation (Imrich et al., 2000; Gon et al., 2005), and induces or
82 aggravates a variety of respiratory diseases (Tulic et al., 2000). On the other hand, in recent
83 decades in Japan, the number of Japanese cedar (Cryptomeria Japonica, JC) hay fever patients
84 has increased and an increase in JC pollen is a probable principal cause (Sakurai et al., 2002).
85 The pollen grains of JC pollen (Cry j 1) usually exist as coarse particles about 30 μm.
86 However, the conglomeration of dust-storm particles with sea salt forms larger hygroscopic
87 particles during the long distance transport (Ma et al., 2004). Suspended PM (SPM) is present
88 in the aqueous phase and induces the release of allergen particles (Wang et al., 2012). It was
89 supposed that the major allergen Cry j 1 could be release to the atmosphere as respirable-sized
90 particles and modified by some air pollutants during airborne transportation (Wang et al.,
91 2009a).

92 An increase in ambient antigen is one of the most probable reasons for the increase in
93 allergic disorders such as allergic rhinitis, bronchial asthma, and atopic dermatitis. Allergic
94 diseases are immunologic disorders, traditionally referred to as immediate or type I
95 hypersensitivity reactions with IgE playing an important role. Crosslinking of the FcεRI
96 (high-affinity IgE receptor) induced by complex formation of IgE with an antigenic protein is an
97 essential event in the IgE-mediated allergic reaction (Beaven and Metzger 1993), The
98 interaction of IgE with allergen on mast cells or basophils leads to allergic reactions causing the
99 release of an array of inflammatory mediators resulting in the inflammation of airway mucus
100 membrane leading to clinical symptoms in the target organ (Novak et al. 2001). Mast cells and

101 basophils play essential roles in the pathogenesis of allergic reactions and in protection against
102 Gram-negative bacteria. RBL-2H3 cells, a tumor analog of mast cells, display characteristics
103 of mucosal-type mast cells and express several hundred thousand FcεRI on the membrane
104 surface. After sensitization with IgE, the cells respond to the antigen and release histamine.
105 β-Hex, which is stored in the secretiory granules of mast cells is released concomitantly with
106 histamine when mast cells are immunologically activated (Ortega et al. 1988; Schroeder et al.
107 1995). Thus, β-hex activity in the medium is used as a marker of mast cell degranulation
108 (Yamada et al., 2010). Therefore, RBL-2H3 cells are considered as a good tool for studying
109 the effect of environmental pollutant on chemical mediator release activity.
110 Recent studies regarding the impact of microbiological factors contained in YS on the air
111 passage by Yanagisawa et al. (2007) and Ichinose et al. (2008) have indicated that YS has the
112 potential of exacerbating symptoms in the human respiratory apparatus, eyes, and nose. Even
113 though there are few reports concerning the biological activity of particular dust on macrophage
114 and rat lung (Becker et al., 1996; Kim et al., 2003; Meng and Zhang 2007), and pulmonary
115 toxicity and inflammatory allergy induced by administered of YS in mice and guinea pigs
116 (Ichinose et al., 2005; 2009), there is no experimental evidence available regarding YS
117 extract-mediated chemical mediator release by immediate type allergic model cells collected
118 during ADSE in Japan. In this study, YS was collected from Naha, Fukuoka, and Tsukuba
119 during ADSE in 2009 and 2010 of Japan, the chemical composition of YS was analyzed, and
120 the endotoxin and Cry j 1 concentrations in the YS extracts were investigated. Furthermore,
121 degranulation, cytokine production activity, and the cytotoxicity of YS extract on RBL-2H3
122 cells were examined.

2. Materials and methods

2.1. Chemicals and cells

LPS was obtained from Alexis Biochemical (Enzo Life Sciences, Inc., San Diego, CA). Fetal bovine serum (FBS) was obtained from Gibco BRL, Paisley, Scotland. The enzyme-linked immunosorbent assays (ELISA) for analysis of rat TNF-α and IL-13 were obtained from Invitrogen Co., CA. USA. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) was obtained from Dojindo, Japan. RBL-2H3 cells were purchased from JCRB Cell Bank, Japan. The cells were maintained in MEM supplemented with 10%FBS and 2 mM L-glutamine, and incubated at 37^{o}C in a 5%CO_2 .

2.2. Sampling and sample location

YS samples were obtained using a high volume sampler (HV-1000F, Shibata Science, Tokyo, Japan) set on the open top floor of building (15-20 m) during March to June, 2009-2010 based on the information on the YS storm movement reported by the Japan Meteorological Agency. Wind with aerosols including sands was sucked into sampler at a rate of 1000 L /min and the aerosols that adhered onto the filter (Quartz Fiber Filters, Adventec QR-100, Toyo Roshi Kaisha, Ltd.), which was set in the sampler were obtained. Three samples collected in 2009 and three samples collected in 2010, from Naha city (127°40'44"E, 26°12'44"N), Okinawa

145 prefecture (named Naha09 and Naha10, collected during April 27th to June 10th in 2009 and
146 March 10th to April 14th in 2010), Fukuoka city (130°24'06"E, 33°35'24"N), Fukuoka prefecture
147 (samples Fuku.09 and Fuku.10, collected during March 16th to 19th in 2009 and March 20th to
148 24th in 2010) and Tsukuba city (140°10'37"E, 36°14'00"N), Ibaraki prefecture (samples Tsu.09
149 and Tsu.10, collected during March 16th to 20th in 2009 and March 19th to 23th in 2010), Japan
150 (Fig. 1), during the ADSE (MEGJ, 2010a) were used in this study. The sampling period,
151 particle concentration, and particle density are shown in Table 1. The first sampling site in the
152 city of Tsukuba (approximately 0.2 million inhabitants a population density of 284.07
153 inhabitants/km^2) is located in an industrialized area near Tokyo, and the samples from Fukuoka
154 City, Fukuoka prefecture (approximately 1.45 million inhabitants, 341.32 inhabitants/km^2)
155 situated in an industrialized area near the Japanese sea, whereas the samples from Naha City,
156 Okinawa prefecture (approximately 0.3 million inhabitants, 39.24 inhabitants/km^2) represented a
157 rural site. The geographical distances between Naha and Fukuoka sampling sites is about 860
158 km, while Fukuoka and Tsukuba sampling site about 940 km.
159
160 *2.3. Analysis of sand components*
161
162 The collected samples were separated carefully from the filters and subjected to component
163 analysis. First, the chemical components on the upper most 5 nm surface of the sands were
164 analyzed by XPS with ESCA-300 (VG Scienta AB, Uppsala, Sweden). Then this surface
165 layer was separated from bulk sands. The components of bulk sands were analyzed by
166 XRF-EDS with JSX-3220 (JEOL Ltd., Tokyo, Japan).

167

168 *2.4. Extraction of LPS from YS sample*

169

170 The filters used to collect the dust samples were cut out and transferred into 15-ml Costar
171 plastic tubes with 2 mL distilled MilliQ water. The tubes were then placed in an ultrasonic bath
172 for 30 min. Then centrifugation was carried out at 1000 × g for 20 min at room temperature,
173 and the supernatant (called water extract) were used for the determination of the endotoxin
174 concentration, β-hex release, cytokine assay, and MTT assay.

175

176 *2.5. Extraction of Cry j 1 from YS sample*

177

178 The filters used to collect dust samples were cut out and transferred into 15-mL Costar
179 plastic tubes with 2 mL Cry j1 extract buffer of TAC® Cry j 1 ELISA Kit (AKCJ1-010,
180 Shibayagi, Gunma, Japan). After standing the solution in 4 °C for 2 h, shaking was carried out
181 at room temperature for 30 min. Centrifugation was then carried out at 1200 × g for 30 min at
182 room temperature, and the supernatant (called Cry j 1 extract) was used as the samples for the
183 pollen allergen Cry j 1 determination and the β-hex release assay after filter-sterilization (0.45
184 μm Millipore filter).

185

186 *2.6. LPS determination*

187

188 Endotoxin in the water extract was measured by a quantitative kinetic chromogenic

189 Limulus Amoebocyte Lysate (LAL) method using Endospecy ES-50M (Seikagaku Co., Tokyo,
190 Japan) at 37°C. Analyses were performed with an automated microtitre plate reader (Power
191 Scan HT; Dainippon Pharmaceutical Co., Ltd.). Standard Endotoxin CSE-Kit (Seikagaku Co.,
192 Tokyo, Japan) was used to determine the amount of endotoxin present in the sample. The
193 detection limit was 0.002 EU/ml. The endotoxin concentration in each sample was calculated
194 using a standard curve, and expressed as units per milliliters of sample (EU/mL). Endotoxin
195 concentrations were reported as endotoxin units (EU) per mL of extract where 10 EU is
196 equivalent to 1.25 ng of the reference standard endotoxin.
197
198 *2.7. Cry j 1 determination*
199
200 Cry j 1 concentration in the sample was quantified by an ELISA method using TAC® Cry j
201 1 ELISA Kit according to the manufacturer's instructions. Finally, the absorbance of each
202 solution obtained by ELISA was converted into Cry j 1 concentration (pg/mL) of the Cry j 1
203 extract.
204
205 *2.8. Degranulation assay*
206
207 The β-hex release assay using RBL-2H3 cells was performed as previously described
208 (Yamada et al., 2010). RBL-2H3 cells were seeded onto 96-well plates at 5.0×10^4 cells/well in
209 100 μL of medium. Cells were incubated for 24 h at 37°C, then washed twice with PBS (-) to
210 eliminate medium, and then the cells were exposed to various concentrations of water extract (0,

211 1/125, 1/100, 1/75, 1/50 dilution in medium) for 16 h at 37°C, 5%CO_2 incubator. As positive
212 and negative controls, 5.0 ng/mL LPS and PBS (-) were used respectively. For Cry j 1
213 extract-treated cells, cells were sensitized with 0.3 μg/mL anti-DNP-IgE antibody before
214 incubation for 24 h at 37°C, then washed twice with PBS (-) to eliminate the free IgE, and then
215 the cells were exposed to various concentration of Cry j 1 extract (0, 1/100, 1/50, 1/25, 1/10, 1.0
216 dilution in medium) for 1 h. As positive and negative controls, 0.3 μg/mL DNP-BSA and PBS
217 (-) were used respectively. The β-hex in the supernatant was measured as described in the
218 previous study (Yamada et al., 2010).
219
220 *2.9. Cytokine assay*
221
222 To test the amount of TNF-α and IL-13, RBL-2H3 cells were seeded onto 24-well plates at
223 2.5×10^5 cells/well in 500 μL of medium. Cells were incubated for 16 h in the presence of a
224 Naha 09 and Tsu.09 water extract at a final dilution of 1/100 in growth medium. For positive
225 and negative controls, 5.0 ng/mL LPS and PBS (-) were used, respectively. Levels of TNF-α
226 and IL-13 were measured in RBL-2H3 cells' supernatant using ELISA kits (Inivitrogen Co.,
227 UAS, CA) according to the manufacturer's instructions. The study was performed in triplicate
228 using cells from three different passages.
229
230 *2.10. MTT assay*
231
232 The MTT reduction assay is the most frequently used method for quantitative cell viability

233 (Yamada et al., 2007). RBL-2H3 cells were seeded on to 96-well plates at 5.0 x 10^4 cells/well
234 in 100 μL of medium. After an overnight incubation, the growth medium was removed
235 followed by addition 100 μL of the water extract (dilution 0, 1/1000, 1/100, 1/10, 1/2, 1.0 with
236 medium). For the highest concentrations, additional controls (1, 5 and 10% PBS (-), data not
237 shown) were considered as references in order to consider the sample effect exclusively on the
238 survival and proliferation of RBL-2H3 cells. The cells were incubated for 24 h, and cell
239 viability was measured as described in the previous study (Yamada et al., 2010).
240
241 *2.11. Statistical analysis*
242
243 Results are expressed as the mean ± standard deviation of at least three independent
244 experiments. Experiments were realized in triplicates. Comparisons with the control were
245 performed by analysis of variance (ANOVA), followed by the Newman-Keuls post hos test. *p*
246 values of less than 0.05-0.01 were considered significant.
247
248 **3. Results**
249
250 *3.1. Chemical compositions of YS*
251
252 To confirm chemical composition of collected sample during ADSE, the results of
253 component analysis of YS by XPS and XRF-EDS collected in 2009 were shown in Table 2.
254 The upper most surface mainly consists of carbon (C) and oxygen (O). In all 3 samples, there

255 are much calcium (Ca) and sulfur (S) contents. The dust particles frequently contained Al and
256 Fe besides silicon (Si) as indicators of dust particles (Nishikawa et al., 2000), and the percentage
257 were higher in Fuku.09 and Tsu.09 compared with Naha09. Our observation was correlated
258 with ASDE information of Japan Meteorological Agency (MEGJ, 2010a). Other minerals
259 components such as K, and magnesium (Mg) were also detected. A few amount nickel (Ni)
260 and zinc (Zn) were detected in Naha and Fuku.09. The percentage of Ti was higher in Tsu.09
261 compared with Naha09 and Fuku.09.
262
263 *3.2. Concentration of LPS in water extract of YS*
264
265 To examine the endotoxin concentration in the collected sample during ADSE, we
266 measured the endotoxin concentrations of each water extract (Table 3). The results showed
267 that all water extracts contained LPS, Tsu.10 (9.49 ± 1.82 EU/mL), Fuku.10 (16.25 ± 2.34
268 EU/mL), and Naha10 (22.62 ± 1.96 EU/mL) contained the highest level of LPS, followed by
269 Tsu.09 (8.39 ± 1.81 EU/mL), Fuku.09 (7.20 ± 0.36 EU/mL), and Naha09 (3.4 ± 0.26 EU/mL).
270 The LPS level of the sample collected in 2010 was higher than the sample collected in 2009
271 (Table 3).
272
273 *3.3. Concentrations of Cry j 1 in Cry j 1 extract of YS*
274
275 To examine the Cry j 1 concentration in the collected sample during ADSE, the Cry j 1
276 concentration of each Cry j 1 extract was measured (Table 3). The results showed that all Cry j

277 1 extract contained Cry j 1. Fuku.10 (19.7 ± 2.1 pg/m^3) contained the highest level of Cry j 1,
278 followed by Tsu.10 (13.4 ± 2.3 pg/m^3) and Naha10 (1.7 ± 0.3 pg/m^3). Tsu.09 also contained
279 the a high level of Cry j 1 (17.2 ± 2.3 pg/m^3) (Table 3).
280
281 *3.4. YS extract induces β-hex release from RBL-2H3 cells*
282
283 We performed a series of experiments to screen for the effects of water extract on
284 RBL-2H3 cells degranulation over a range of noncytotoxic concentrations (dilution rate
285 1/125-1/50). The β-hex release from RBL-2H3 cells as caused by treatment with the water
286 extract is shown in Fig. 2. The water extract collected in 2009 and 2010 from each sampling
287 site induced the β-hex release from RBL-2H3 cells after 16 h of exposure, the effect being
288 significant at 1/100 treatment (except Fuku.10 sample, Fig. 2B) (p<0.05 - p<0.01 vs. the
289 negative control of PBS (-)), and only Fuku.10 sample showed significant effect at 1/125
290 treatment (Fig. 2B). For comparison, a LPS (Escherichia coli 0111:B4) induced approximately
291 150% release of β-hex at 5 ng/mL concentration (Fig. 2D).
292 To confirm the effect of Cry j 1 extract on β-hex release, we performed a series of
293 experiments to screen for the effects of various concentrations (dilution rate 1/100-1) of Cry j 1
294 extract on RBL-2H3 cells degranulation, and the result is shown in Fig. 3. For comparison,
295 BSA induced approximately 193% β-hex release at 0.3 μg/mL treatment. The Cry j 1 extract
296 collected in 2009 and 2010 from each sampling site induced the β-hex release from RBL-2H3
297 cells, the effect being significant at all treatment ranges with Fuku.10 sample (Fig. 3B), 1/10-1

298 treatment with Naha10 sample (Fig. 3A), and treated with Tsu.09 sample (Fig. 3C) (p<0.05 -
299 p<0.01 vs. the negative control of PBS (-)).
300
301 *3.5. Water extract induces cytokine production in RBL-2H3 cells*
302
303 The production of TNF-α and IL-13 following 16 h incubation with water extracts by both
304 Naha09 and Tsu.09 analyzed using ELISA assay are shown in Fig. 4. TNF-α production was
305 increased RBL-2H3 cells treated with noncytotoxic concentrations of water extract (1/100
306 dilution rate). The TNF-α production was 7.76 ± 1.24 pg/mL after stimulation with Tsu.09 for
307 16 h, and Naha09 was 7.66 ± 0.62 pg/mL, the effect being significantly (p<0.05-p<0.01)
308 different from the control (3.06 ± 0.53 pg/mL). Furthermore, the TNF-α production was 9.28
309 ± 0.15 pg/mL after stimulation with 5 ng/mL LPS, and the effect being significant (p<0.01),
310 while there was no significant different in the IL-13 production after stimulation with water
311 extract (data not shown).
312
313 *3.6. Water extract exhibits cytotoxic effects on basophil cells*
314
315 To characterize YS-induced cytotoxicity, cell viability was determined in water extract- and
316 Cry j 1 extract-stimulated RBL-2H3 cells using the MTT assay. The results showed that
317 exposure to water extract at different concentration rate for 24 h reduced the cell viability in a
318 concentration-dependent manner. The dilution rate of 1/10-1 can decrease the cell viability to
319 Naha09 was 69.2% ± 0.9% (Fig. 5A), Fuku.09 was 82.0% ± 1.7% (Fig. 5B), Tsu.09 was 79.8%

320 ± 1.1% (Fig. 5C), Naha10 was 64.2% ± 1.0% (Fig. 5A), Fuku.10 was 73.6% ± 1.2% (Fig. 5B),
321 and Tsu.10 was 67.6% ± 1.4% (Fig. 5C), the effect being significant compared with the control
322 ($p<0.05$ – $p<0.01$ vs. the negative control of PBS (-)). Among the samples, those collected in
323 2010 showed the strongest toxic effect on RBL-2H3 cells compared with the 2009 samples (Fig.
324 5). However, Cry j 1 extract did not cause significant reduction in cell viability (data not
325 shown).
326
327 **4. Discussion**
328
329 *4.1. Allergenic*
330
331 The presence of water-soluble material around dust-storm particles over Japan has been
332 reported by previous research reports (Iwasaka et al., 2004; Iwasaka et al., 2008). Most of the
333 dust particles are coated with water-soluble materials after their long-range transport from China
334 to Japan, and that the dust storm particles were modified by sea-salt and/or anthropogenic
335 pollutants and cloud process (Zhou et al., 1996). Our results showed that, YS extracts caused
336 degranulation of RBL-2H3 cells. Biological species, such as pollen allergens and endotoxin,
337 as well as transition metals in acidic environments are water soluble (Monn and Becker 1999).
338 In concordance with several other studies (Becker et al., 1996; Dong et al., 1996; Long et al.
339 2001), our data suggest the possible involvement of endotoxin in promoting allergy after long
340 exposure to low level of LPS (Fig. 2D). The endotoxin levels of water extract of samples
341 collected from 2010 was higher than those of the 2009 samples from all sampling pointe (Table

342 3), and the differences in LPS content were weakly associated with the increase in filter density
343 (R^2 = 0.5888, Fig. 6A), and only the Fukuoka and the Tsukuba samples were strongly correlated
344 (R^2 = 0.9592, Fig. 6B). However, LPS content were weakly associated with the β-hex release
345 rate (R^2 = 0.461, Fig. 6C). As shown in Fig. 2D, the effect of low level LPS on β-hex release
346 was not dose-dependent, there is an optimal concentration. In addition, long-range transported
347 pollutants such as nitrogen oxides (NOx) and sulfur oxides (SOx) were likely to interact with
348 aeroallergens (Ichinose et al. 2005) that induce degranulation. Furthermore, metal elements
349 have inhibitory effect (Tanaka et al., 1991) or induce and enhance (Walczak-Drzewiecka et al.,
350 2003) action on chemical mediator release by mast cells. Therefore, YS water extract may
351 have several different ways of inducing chemical mediator release from RBL-2H3 cells,
352 suggesting the complexity of its action, although most of the mechanisms remain to be studied.
353 It is well known that JC pollinosis patients who start their medication several weeks before
354 the first day of the pollen season can spend the pollen season without severe symptoms.
355 Takahashi et al. (2007) found that the total pollen counts and the total Cry j 1 amounts during the
356 pollen season (Jan. – May. in 2003 – 2006) collected from Yamagata prefecture of Japan did not
357 correlate with pollen precisely, and the Cry j 1 levels reached 1 pg/m^3 2-3 weeks before the first
358 day of the pollen season (5-25 ng/m^3). We hypothesize that the higher concentration of Cry j 1
359 collected before pollen season was probably transported from other sites to the sampling sites by
360 ADSE. This study has shown that there was no correlation between the Cry j 1 contents (Table
361 3) and the increase in filter particle density (Table 1) (R^2=0.0236, Fig. 6D). Comparing
362 samples from Naha and Fukuoka, the Cry j 1 concentration were increased when the filter
363 particle density increased, but this result do not correlate with the pollen count information

364 (MEGJ, 2010b). On the other hand, the results of this study have shown that Cry j 1 extract
365 exhibited an effect on the β-hex release in both 2009 and 2010 (Fig. 3), and the differences in
366 β-hex release rate were weakly associated with the increase in the Cry j 1 content ($R^2 = 0.5168$,
367 Fig. 6E), but higher correlation for the Naha and Fukuoka samples were observed ($R^2 = 0.9455$,
368 Fig. 6F). This result was consistent with Wang et al. (2009a) observation. This suggests that
369 ADSE can transport, absorb or concentrate Cry j 1 allergen, and these have the possibility of
370 contributing to the cause of the allergic reaction (Mori et al., 2003; Takahashi et al., 2007).
371 Sandstorm dust is a prolific source of potential triggers of allergic and non-allergic
372 respiratory ailments (Kwaasi et al., 1998). Many of the cellular signals activated by
373 Gram-negative bacteria are attributed to TLR4-mediated recognition of LPS (Gon et al., 2005).
374 LPS is an agonist of TLR4, although TLR4 receptors are expressed on the RBL-2H3 cell
375 surface (Passante et al., 2009). In this study, the role of endotoxin as a stimulant of cytokine
376 production was confirmed (Becker et al., 1996; Dong et al., 1996; Long et al., 2001). The
377 results have shown the possibility that low level LPS can induce TNF-α production in
378 RBL-2H3 cells. Cytokine release showed a similar pattern only for TNF-α after stimulation
379 with Tsu.09 and Naha09 samples (Fig. 4), while there was no effect on IL-13 expression.
380 Mitogen activated protein kinase (MAPK) JNK was involved in LPS-induced IL-13, but not in
381 TNF-α synthesis (Gon et al., 2005). This difference may be contributed by the
382 proinflammatory activity of water extract. However, the endotoxin levels of water extract
383 from Tsu.09 was 2-fold higher than Naha09 (Table 3). These results indicate that the
384 cytokine-inducing capacity of Naha09 and Tsu.09 are not only due to the low amount of LPS
385 present in these water extract. It was hypothesized that there are other proinflammatory

386 components, ambient particles (Imrich et al., 2000) or other mechanism (Shoenfelt et al., 2009),
387 and there may be greater synergism between endotoxin and components of ambient particles
388 (Long et al., 2001). Dong et al. (1996) demonstrated that there was no cytokine induction by
389 diesel particles. This suggests that there are other proinflammatory components in the water
390 extract that may be responsible for the effects observed in cells treated with YS.
391
392 *4.2. Cytotoxicity*
393
394 Our results suggest that YS water extract exhibited cytotoxicity, with the cell treated with
395 2010 samples exhibiting higher cell viability than the 2009 samples (Fig. 5A, B, C). The
396 differences in the cell viability were weakly associated with the increase in filter particle density
397 (R^2 = 0.6711, Fig. 5D), but higher correlation for the Naha and Fukuoka samples was observed
398 (R^2 = 0.9253, Fig. 5E). These results agree with the results of the study of Meng and Zhang
399 (2007) which showed that the effect of PM on health is greater during dust storms because
400 airborne PM's mass is high. Ichinose et al. (2005) found that inflammatory lung injury was
401 induced by microbiological materials, such as β-glucan, and by chemical materials such as SO_4^{2-}.
402 Therefore, these materials adsorbed onto dust particles, are implicated in the pathogenesis of
403 human respiratory disorders during a dust event. YS exhibits a cytotoxic effect on
404 pneumocytes *in vitro*, and reactive oxygen species (ROS), fenton activity, reactive nitrogen
405 species (RNS), and titania (TiO_2) are involved in this toxicity (Kim et al., 2003). Becker et al.
406 (1996) also found that TiO_2 is toxic to both human and rat alveolar macrophage (AM). Monn
407 and Becker (1999) reported that LPS is cytotoxic on human monocytes and rat AM. Toxicity

in macrophages has also been associated with metals, and higher metal concentrations are usually found in the PM (Monn and Becker 1999; Schins et al., 2004). Some studies also suggest that metals, either water-soluble of PM can catalyze reactions involved in oxidative stress and DNA damage (Meng and Zhang 2007). Nicel (Ni) has slight cytotoxic effect on CHO cells (Fredj et al., 2010), and significant positive dose response for LDH, a marker of cell death (Cho et al., 2010). In this study, composition analysis results showed that YS surface contained Al, C, Fe, nitrogen (N), Ni, S, Ti, and Zn (Table 2). Previous studies showed that transition metals such as Fe and Zn, components that might be bound to the fine particles, endotoxins, other organic compounds, and polycyclic aromatic hydrocarbon content of particles participated in the production of ROS and was correlated with oxidative stress in macrophages (Ortiz et al., 2006). Effect of diesel particulate matters on ROS production in human alveolar epithelial cells was observed (Patel et al., 2011). YS contain ultrafine particles from combustion sources, such as diesel and gasoline engines, and coal fired power plants, and concentrated during the long distance transport (Zhou et al., 1996; Mori et al., 2003; Ichinose et al., 2005). This was evident in the cytotoxic effect of the water extract which could have been induced by their constituents and enhanced by ADSE.

5. Conclusion

In conclusion, YS can rapidly enhance preformed chemical mediator release in basophilic cells. This process may depend on endotoxin, Cry j 1, and other airborne allergen content of YS. These findings may help explain the increase in the incidence of allergic diseases during

ADSE in East Asia. The detailed mechanism behind the enhancement of allergic affect of YS will be the subject of our future study. It is recommended that patients suffering from pollinosis, and physicians be made aware of the pollen dispersal that may be cause by YS during the ADSE.

Acknowledgements

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601

Figure captions

Fig. 1. A meteorological map showing areas in Japan, Korea and China impacted by YS. The sampling sites, Tsukuba, Fukuoka and Naha are indicated.

Fig. 2. Effect of water extract on β-hex release from RBL-2H3 cells. The cells (5.0 x 10^4 cells/well in 100 μg/mL) were preincubated with each extract at 37°C for 16 h. Results represent one trial (n = 6). Tree additional trials show similar results. *Significantly different from the negative control (PBS (-)) (*: $p<0.05$, **: $p<0.01$).

Fig. 3. Effect of Cry j 1 extract on β-hex release from RBL-2H3 cells. The cells (5.0 x 10^4 cell/well in 100 μg/mL) were incubated with each extract at 37°C for 1 h after IgE sensitization. Results represent one trial (n = 6). Tree additional trials show similar results. *Significantly different from the negative control (PBS (-)) (*: $p<0.05$, **: $p<0.01$).

Fig. 4. Effect of water extract on TNF-α production in RBL-2H3 cells. After exposed with extract (1/100 dilution) and LPS (5 ng/mL) for 16 h, conditioned media were collected for TNF-α ELISA Kit. Results represent one trial (n = 3). Tree additional trials show similar results. Tsu.09: collected from Tsukuba city during ADSE in 2009; Naha09: collected from Naha city during ADSE in 2009. *Significantly different from the negative control (PBS (-)) (*: $p<0.05$, **: $p<0.01$).

Fig. 5. Effect of water extract at different concentration on the cell viability of RBL-2H3 cells. After 24 h incubation, cell viability was determined using the MTT assay. The percent cell viability was calculated relative to the untreated control. The cells (5.0 x 10^4 cells/well) were incubated with yellow sand extract at 37°C for 24 h in 5%CO_2. Results represent one trial (n = 6). Tree additional trials show similar results. *Significantly different from the negative control (PBS (-)) (*: $p<0.05$). A, B, and C: Cell viability of water extract; D and E: Correlation between cell viability and filter particle density.

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632 **Fig. 6.** A and B: Correlation between LPS concentration of water extract and filter particle
633 density; C: Correlation between β-hex release at 1/100 dilution and LPS concentration of water
634 extract; D: Correlation between Cry j1 concentration of Cry j1 extract and filter particle density;
635 E and F: Correlation between β-hex release at 1/100 dilution and Cry j 1 concentration of Cry j 1
636 extract.

637

Fig. 1.

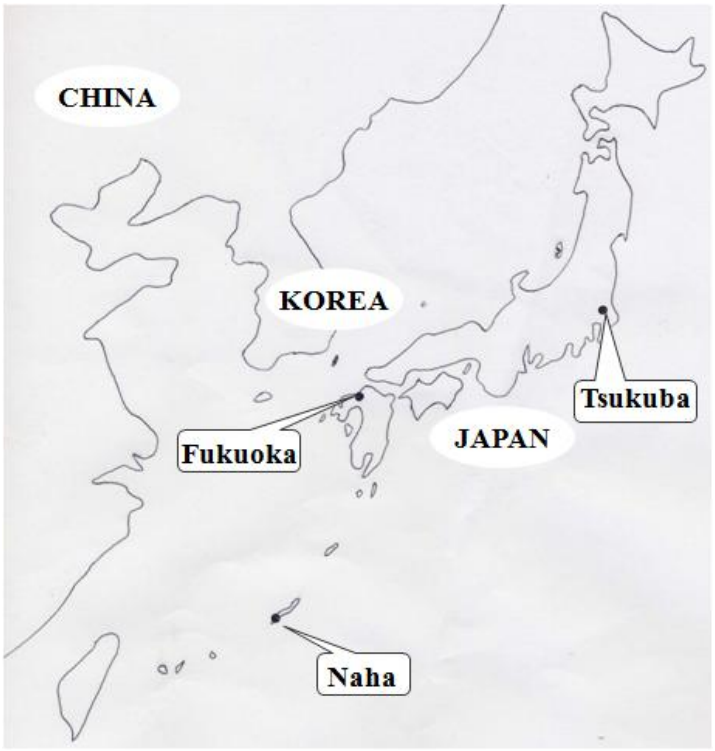


Fig. 2.

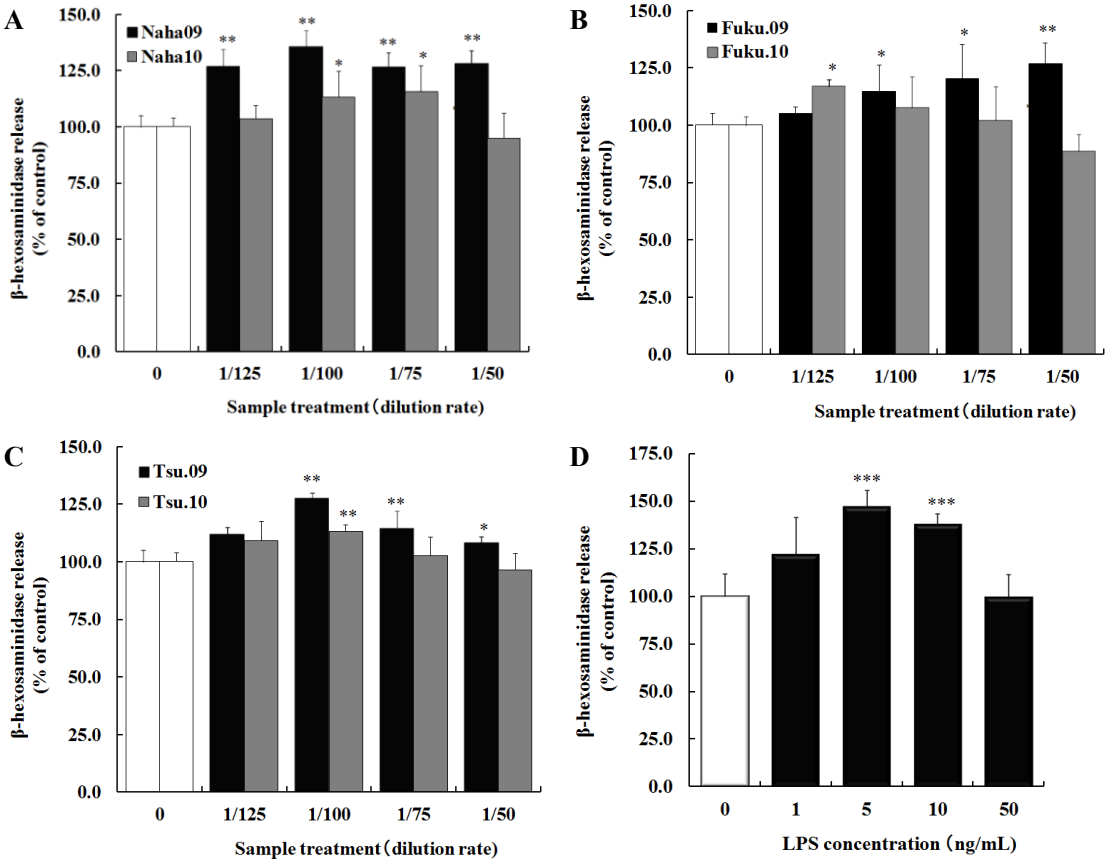

A
Naha09
Naha10
β-hexosaminidase release (% of control)
150.0
125.0
100.0
75.0
50.0
25.0
0.0
0
1/125
1/100
1/75
1/50
Sample treatment (dilution rate)
B
Fuku.09
Fuku.10
β-hexosaminidase release (% of control)
Sample treatment (dilution rate)
C
Tsu.09
Tsu.10
β-hexosaminidase release (% of control)
Sample treatment (dilution rate)
D
175.0
β-hexosaminidase release (% of control)
0
1
5
10
50
LPS concentration (ng/mL)

Fig. 3.

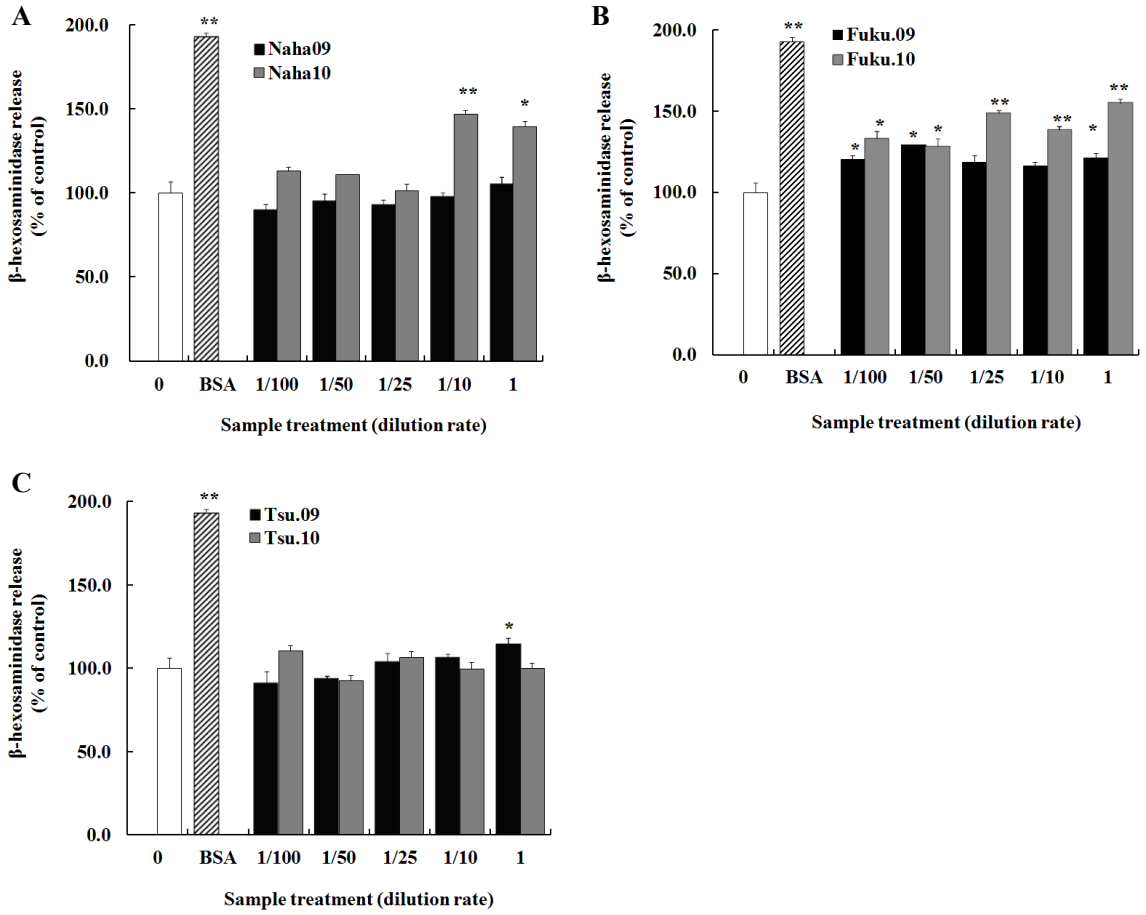

A
β-hexosaminidase release (% of control)
200.0
150.0
100.0
50.0
0.0
Naha09
Naha10
0
BSA
1/100
1/50
1/25
1/10
1
Sample treatment (dilution rate)
B
Fuku.09
Fuku.10
C
Tsu.09
Tsu.10

Fig. 4.

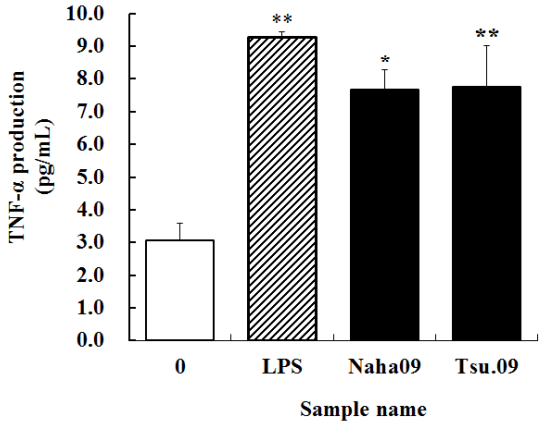
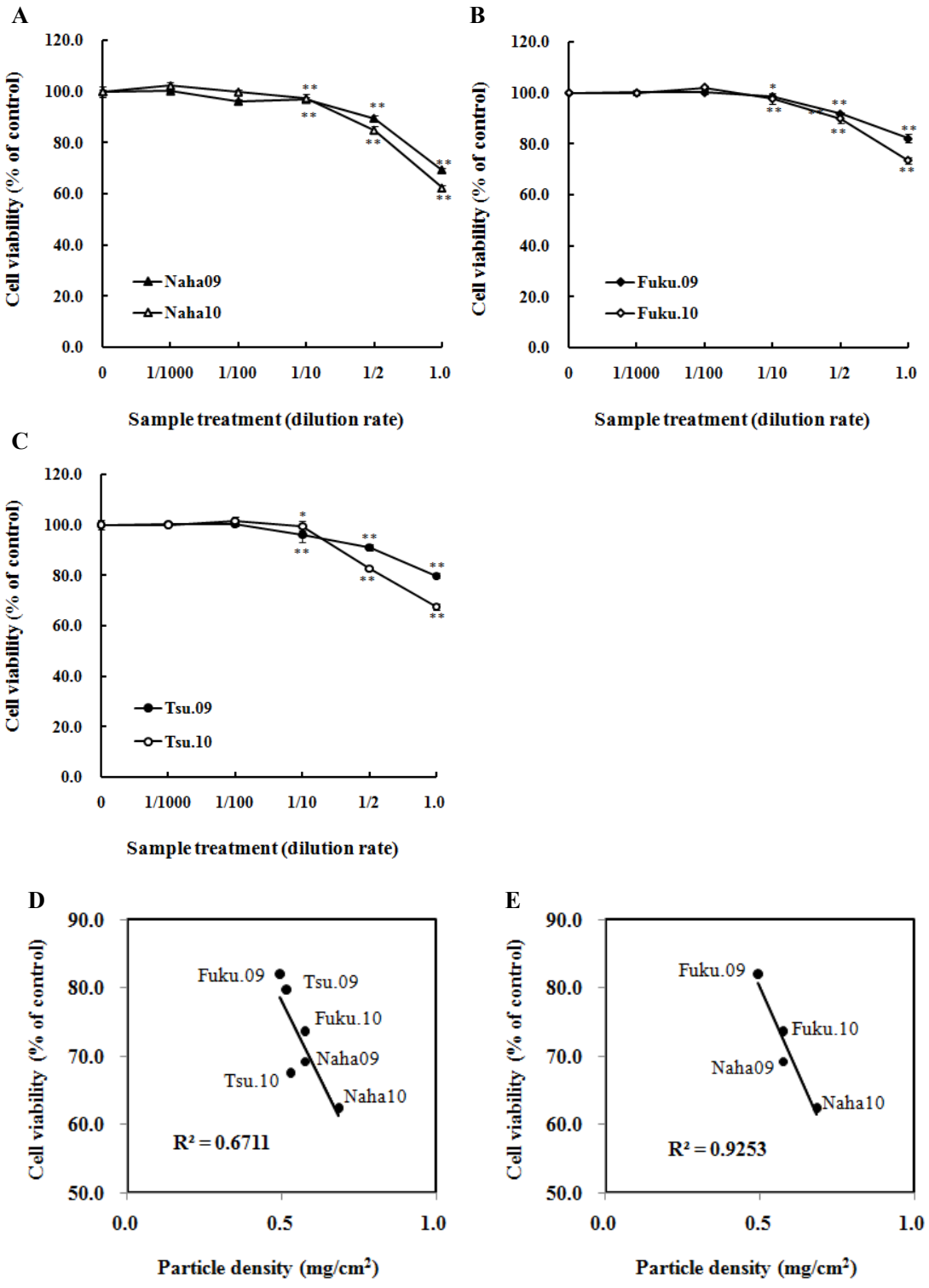

TNF-α production (pg/mL)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
**
*
**
0
LPS
Naha09
Tsu.09
Sample name

Fig. 5.


A
Cell viability (% of control)
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Naha09
Naha10
0
1/1000
1/100
1/10
1/2
1.0
Sample treatment (dilution rate)
B
Cell viability (% of control)
Fuku.09
Fuku.10
Sample treatment (dilution rate)
C
Cell viability (% of control)
Tsu.09
Tsu.10
Sample treatment (dilution rate)
D
Cell viability (% of control)
90.0
80.0
70.0
60.0
50.0
Fuku.09
Tsu.09
Fuku.10
Naha09
Tsu.10
Naha10
R² = 0.6711
0.0
0.5
1.0
Particle density (mg/cm²)
E
Cell viability (% of control)
Fuku.09
Fuku.10
Naha09
Naha10
R² = 0.9253
Particle density (mg/cm²)

741 **Fig. 6.**

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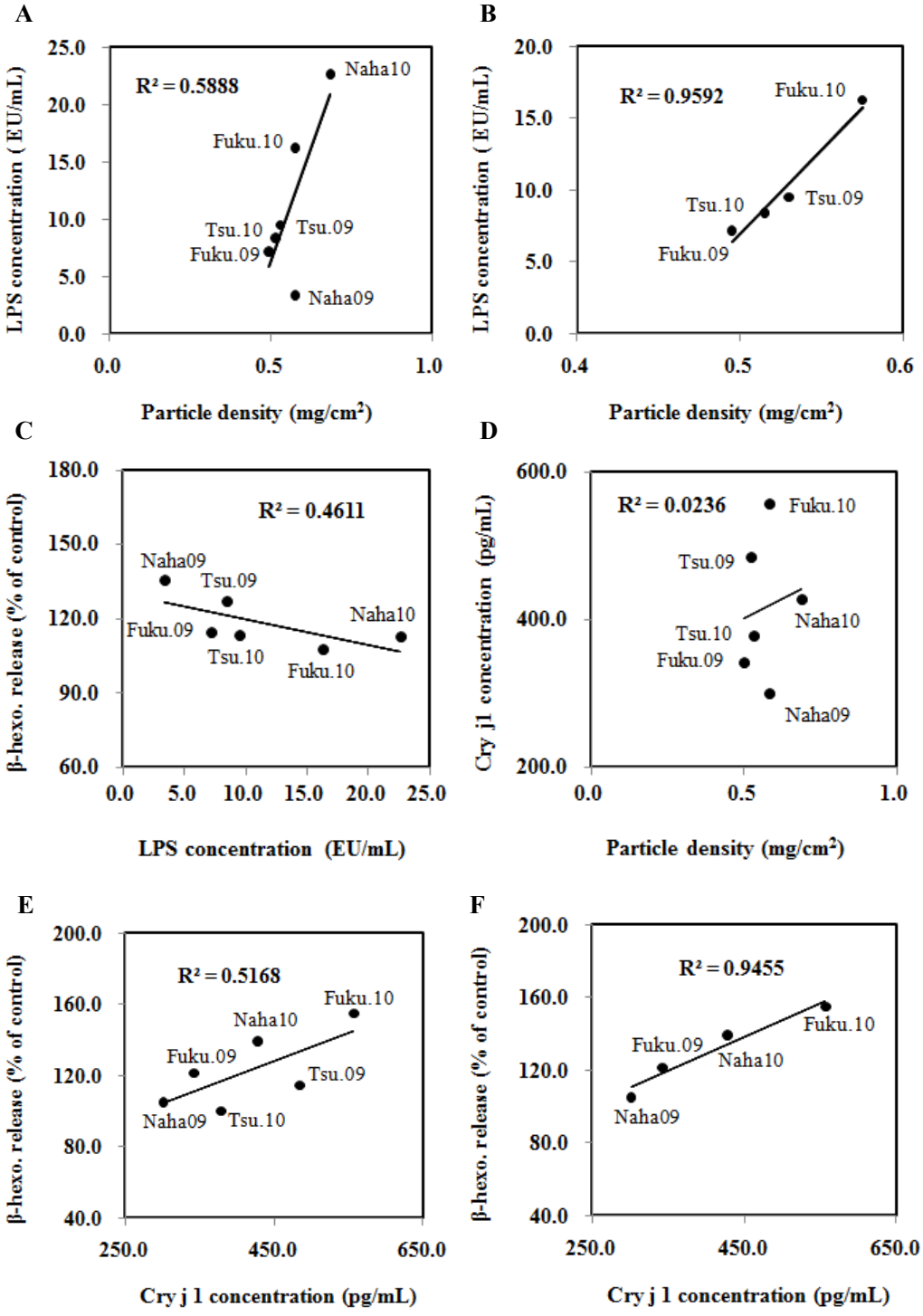

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Table 1

Table 1

Sampling site and collection days during the ADSE.

Year	Sample Name	Site	Days	Periods (days)	Particle conc. ($\mu g/m^3$)	Particle density (mg/cm^2)
2009	Naha09	Naha	4/27 - 6/10	45	4.6 ± 0.1	0.58 ± 0.01
	Fuku.09	Fukuoka	3/16 - 3/19	3	59.7 ± 3.6	0.50 ± 0.03
	Tsu.09	Tsukuba	3/16 - 3/20	4	46.6 ± 1.8	0.52 ± 0.02
2010	Naha10	Naha	3/10 - 4/14	35	7.1 ± 0.4	0.69 ± 0.04
	Fuku.10	Fukuoka	3/20 - 3/24	4	52.0 ± 1.8	0.58 ± 0.02
	Tsu.10	Tsukuba	3/19 - 3/23	4	47.5 ± 0.9	0.53 ± 0.01

779 **Table 2**

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Table 2

Components analysis of YS by XPS and XRF-EDS collected in 2009.

XRF-EDS analysis				XPS analysis				
	Naha	Fuku.	Tsu.			Naha	Fuku.	Tsu.
Na	13.91	7.58	7.75	C	1s1/2	40.57	38.31	45.75
Mg	2.83	5.66	5.45	N	1s1/2	3.65	3.29	2.34
Al	9.45	13.42	17.87	O	1s1/2	38.84	34.81	33.58
P	1.12	1.96	2.05	Na	1s1/2	4.32	1.42	1.38
S	35.78	24.90	30.80	Mg	2s1/2	0.76	3.68	3.06
Cl	11.60	13.89	– *	Al	2s1/2	2.62	2.20	2.92
K	6.76	7.89	9.23	Si	2s1/2	4.36	7.77	6.55
Ca	13.90	19.24	18.73	S	2s1/2	2.76	2.41	1.58
Ti	0.65	0.81	1.14	Cl	2s1/2	0.39	2.85	0.57
Cr	0.06	0.08	0.09	K	2p3/2	0.08	0.77	0.62
Mn	0.09	0.14	0.18	Ca	2p3/2	1.05	1.84	0.88
Fe	3.59	4.37	6.58	Fe	3s1/2	0.59	0.65	0.77
Ni	0.02	0.01	–					
Zn	0.25	0.07	0.13					
Total	100.00	100.00	100.00	Total		100.00	100.00	100.00
			(atm%)					*(atm%)*

781 * – : No detectable.

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794 **Table 3**

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Table 3

LPS and Cry j 1 content of YS extract.

Sample Name	LPS (EU/mL)	Cry j 1 (pg/m^3)	Cry j 1 (pg/mL)
Naha09	3.40 ± 0.26	1.0 ± 0.2	301.1 ± 68.1
Fuku.09	7.20 ± 0.36	16.2 ± 2.8	342.6 ± 58.9
Tsu.09	8.39 ± 1.81	17.2 ± 2.3	484.4 ± 65.3
Naha10	22.62 ± 1.96	1.7 ± 0.3	428.7 ± 74.5
Fuku.10	16.25 ± 2.34	19.7 ± 2.1	556.8 ± 60.0
Tsu.10	9.49 ± 1.82	13.4 ± 2.3	378.8 ± 64.9

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