

## Original Article



# Biological Effects of Cloth Containing Specific Ore Powder in Patients with Pollen Allergy\*

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## Abstract

**Objective** The custom-homebuilding company, Cosmic Garden Co. Ltd., located in Okayama City, Japan was established in 1997 and uses specific natural ore powder (SNOP) in wall materials and surveys customers in order to improve allergic symptoms.

**Methods** To investigate the biological effects of SNOP, patients with a pollen allergy were recruited to stay in a room surrounded by cloth containing SNOP (CCSNOP), and their symptoms and various biological parameters were compared with those of individuals staying in a room surrounded by control non-woven cloth (NWC). Each stay lasted 60 min. Before and immediately after the stay, a questionnaire regarding allergic symptoms, as well as POMS (Profile of Mood Status) and blood sampling, was performed. Post-stay minus pre-stay values were calculated and compared between CCSNOP and NWC groups.

**Results** Results indicated that some symptoms, such as nasal obstruction and lacrimation, improved, and POMS evaluation showed that patients were calmer following a stay in CCSNOP. Relative eosinophils, non-specific Ig E, epidermal growth factor, monocyte chemotactic protein-1, and tumor necrosis factor- $\alpha$  increased following a stay in CCSNOP.

**Conclusion** This ore powder improved allergic symptoms, and long-term monitoring involving 1 to 2 months may be necessary to fully explore the biological and physical effects of SNOP on allergic patients.

**Key words:** Specific Mineral; Cloth, Indoor air; Immunological effects; Pollen allergy

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## INTRODUCTION

It is a recognized social problem that indoor air conditions in the home, office, and other buildings impair human health and cause

ailments such as sick building syndrome<sup>[1-3]</sup>. In addition, it is known that certain allergic diseases, such as hypersensitivity pneumonitis, are caused by indoor antigens derived from fungus and trichosporon<sup>[4-6]</sup>, and that bronchial asthma is caused

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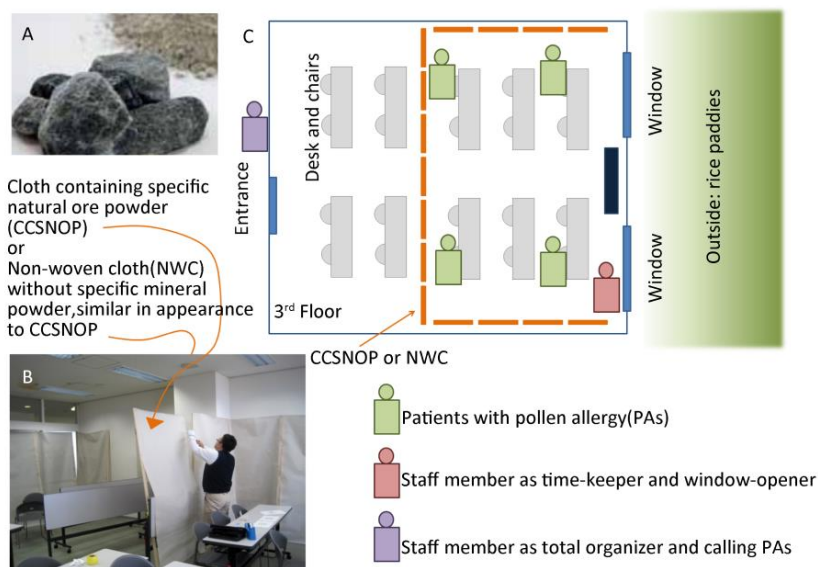
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by house dust and other agents<sup>[7-9]</sup>. On the basis of these facts, many custom-home builders have been trying to establish health-promoting indoor conditions. For example, we reported that negatively charged particle-dominant indoor air conditions enhance natural killer cell activity and result in a slight but significant elevation of serum epidermal growth factor (EGF)<sup>[10-13]</sup>. This indoor air condition probably promotes immune stimulation, particularly against cancer cells and virus-infected cells<sup>[10-13]</sup>.

The Cosmic Garden Co. Ltd. is a custom-home building company located in Okayama City, Japan and was established in 1997. The characteristics of products and homes they manufacture include a modified '2x4' construction method for aseismic capacity, the avoidance of chemical substances and smells when possible, as well as well-sealed, super-insulated, durable housing. In addition to these capabilities, this company uses specific natural ore powder (SNOP) within wall material when constructing housing. This natural ore is shown in Figure 1A and is obtained near Aso-mountain, Kumamoto prefecture, Japan, and is known to release far-infrared rays. This home-construction

company uses SNOP on all of the products they sell. The impressions of customers include an improvement of allergic symptoms, such as pollen allergy (PA), bronchial asthma, and atopic dermatitis, as well as improved sleep. In particular, the office room of this company is also built with SNOP and many customers have stated that symptoms of PA, such as nasal discharge and lacrimation, are improved immediately after entering the office. However, this company has not explored the biological effects of SNOP.

To support future product development using cloth containing SNOP (CCSNOP), this company obtained the Kirameki Creation Fund from the Okayama Prefecture Industrial Promotion Foundation for the period of January to September, 2014. Hence, we tried to explore the biological effects of CCSNOP on symptoms in patients with PA during the period of this disease. In addition to this trial, the far-infrared spectral radiation characteristics of the ore were examined by a Fourier transform infrared spectrometer at 100 °C using a Spectrum Gx FT-IR device (PerkinElmer, Inc., Waltham, MA, USA).



**Figure 1.** The experimental conditions. A. The appearance of natural ore obtained near Aso-mountain located on Kyushu Island, Japan. B. Arrangement of the cloth containing specific natural ore powder (CCSNOP) and non-woven cloth (NWC) in the experimental room before a stay involving patients with pollen allergies (PAs). C. Schematic constitution of the experimental room. PAs sat on a chair surrounded by CCSNOP or NWC. A staff member who acted as time-keeper and window-opener was sitting in the corner of the room. Another staff member was the overall organizer and controlled the waiting PAs who were sitting outside the rooms.

## METHODS

### *Chemical Analysis of Natural Ore Used in This Study*

The chemical analysis of natural ore used in this study was performed in the Hiruzen Institutes for Geology and Chronology<sup>[14]</sup>.

### *Subjects*

All 20 participants enrolled in this study were Japanese (average age  $\pm$  standard deviation:  $36.9 \pm 11.2$  years old). The eleven males (age:  $39.4 \pm 9.8$ ) and nine females (age:  $33.8 \pm 12.6$ ) participating in this study were patients with pollen allergies (PAs). They were diagnosed in certain clinics with symptoms of PA. It should be noted that all of the PAs were enrolled after being solicited through telephone by Mr. Okamoto (one of the authors, belonging to the Cosmic Garden Co. Ltd.) and included customers, suppliers, people from building and materials companies, and members of a real estate agency, as well as family members and employees of the Cosmic Garden Co. Ltd. All PAs had symptoms caused by PA during this study. At least one day prior to the commencement of the study, all PAs stopped taking their medication for PA, which included anti-histamine drugs. They are therefore aware of the possible impact of SNOP on their symptoms. Actually, all subjects complained about some of their symptoms, such as sneezing, nasal obstruction, nasal discharge, eye redness and lacrimation, which caused them difficulty in daily life. The project was accepted by the ethical committee of the Kawasaki Medical School (No. 1723) and all subjects provided written informed consent before enrolling in the study.

### *Study Protocol*

The experiments were performed on April 3 and 4, and then on April 10 and 11, 2014, in rooms on the 3rd floor of the Okayama South Fureai Center, located at 690-1 Fukuda, Minami-ku, Okayama City. This location is approximately 5 km south of Okayama Train Station (West Japan Railway Company) and in downtown central Okayama City. There are rice paddies and fields in the surrounding area. The dates of the experiments coincided with the timing of PA, and all subjects exhibited certain symptoms, such as nasal discharge and lacrimation, when they entered the experimental rooms. The weather on the four days of experiments was cloudy to sunny, and windy only on April 4. Although

detailed official pollen information was not collected, we were certain that pollen particles were present in this area because subjects and other people with PA showed symptoms of PA.

Ten of the subjects entered the experimental room on April 3 and 10, and the remaining 10 on April 4 and 11, with an interval of 7 days. We divided the subjects into two groups due to limitations regarding area/rooms where experiments were performed. All subjects were admitted to the room with control cloth, namely, non-woven cloth (NWC) without SNOP, on the first day (April 3 and 4 for the former and latter groups, respectively). They then entered the room with CCSNOP in the second week (April 10 and 11 for former and latter groups, respectively). Although the subjects did not know which date was set for the CCSNOP experiment, they were surrounded by CCSNOP during the first week and then entered a room in the second week with the control cloth, non-woven cloth (NWC) without SNOP, but which had a similar appearance to the CCSNOP room. As shown in Figure 1B, the CCSNOP and NWC rooms had a height of 2 m and a width of 1 m. The schematic positioning of the experimental room is shown in Figure 1C. The staying space was surrounded by CCSNOP or NWC. The subjects entered this room individually at 15-min intervals, since the pre-stay and post-stay biological measurements needed approximately 10 to 15 min for completion and we planned to set the staying time to an exact period of 60 min. A staff member at the entrance of the experimental room organized the progress of the experiment and controlled passage of PAs into the room. Another staff member stayed in the room to control the period of stay of each PA and to open the window for 1 min. This window opening was used to avoid air-tightening in the room and improve the symptoms of PA despite the presence of airborne pollen.

Every PA entering the experimental room was subjected to pre-stay biological measurements before entering the room. After staying in the room whilst watching a DVD video of calming landscapes from a railroad window and foreign historical scenes, and being allowed to drink mineral water (350 mL), they were subjected to post-stay biological measurements. These procedures are outlined in Figure 2.

The environmental situation on each experimental day (April 3, 4, 10, and 11) did not differ much with regard to temperature, humidity, or concentration of PM<sub>2.5</sub> according to details found

on the website of the Japanese Ministry of the Environment.

### Biological Measurement

For the pre- and post-stay biological measurement, all participants were subjected to the following protocol:

#### 1. Questionnaire for symptoms of PA

The questionnaire inquired about the (1) frequency of sneezing (a: quite often, b: often, c: low, d: none), (2) volume of nasal discharge(a: quite large, b: large, c: small, d: none), (3) degree of nasal obstruction (a: complete, b: severe, c: mild, d: none), (4) degree of eye redness (a: quite severe, b: severe, c: mild, d: none), (5) degree of lacrimation (a: complete, b: severe, c: mild, d: none), and the (6) difficulty of daily life, such as routine activity, work, study, housework (a: impossible, b: very difficult, c: slightly difficult, d: no problem).

#### 2. Present mental status

The present anxiety score was measured using a POMS (Profile Of Mood Status) questionnaire<sup>[15-16]</sup>.

#### 3. Stress marker

Mental stress was measured by salivary amylase<sup>[17-18]</sup> using CM-21 (Nipro, Co. Ltd., Osaka, Japan). Subjects held a CM-21 stick for 1 min under the

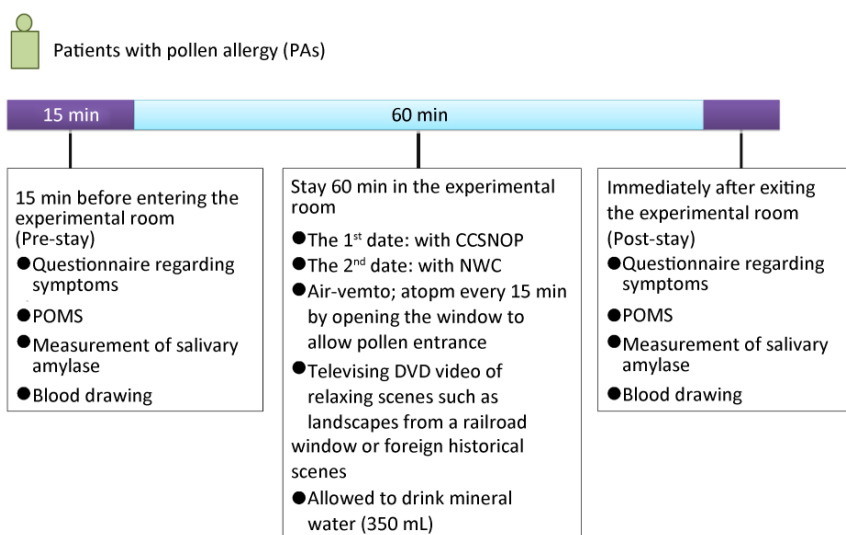
tongue, and then a measurement was performed in the machine for 60 s.

#### 4. Blood sampling

A 15-mL sample of peripheral venous blood was drawn from subjects and the following parameters were measured. (1) General condition: blood chemistry including liver (AST, ALT, and  $\gamma$ GTP) and kidney (BUN and creatinine) functions, blood sugar, HbA1C, lipids (including LDL and HDLcholesterols and triglycerides), and peripheral blood counts including differential white blood cell count, (2) immunoglobulins (Igs: IgG, A, M, and non-specific IgE), (3) multi-allergen specific Ig E (33 kinds), and (4) cytokines [29 kinds measured by a Luminex 29 Cytokine Plex Kit Human Cytokine/Chemokine Panel (HCYTMAg-60K-PX29, Merck Millipore, Billerica, MA)].

### Statistical Analysis

To compare the biological effects of CCSNOP and NWC conditions, all individual parameters were used to calculate a post-stay minus pre-stay value. CCSNOP data from all subjects for all parameters were then compared with data from NWC conditions. For each analysis,  $P < 0.05$  was considered significant and a value between 0.05 and 0.1 was considered to



**Figure 2.** The experimental schedule. The time schedule of PAs enrolled in this study. Participants were subjected to pre-stay and post-stay biological measurements for CCSNOP and NWC experiments. The biological measurements included a questionnaire regarding symptoms, POMS, measurement of salivary amylase, and blood sampling. During a stay in an experimental room with CCSNOP or NWC, airventilation was performed every 15 min by opening a window. PAs were sitting on a chair while watching a DVD video of calming landscapes from a railroad window and foreign historical scenes, and allowed to drink mineral water.

indicate a tendency towards significance. All statistical analyses were performed using SPSS ver. 22 (IBM Japan, Tokyo, Japan). The chi-square test was used to analyze changes in symptoms examined by the questionnaire and moods evaluated by POMS. Comparison between the two groups for white blood cell fractions, serum immunoglobulins (Ig) and cytokines was conducted using the Student's *t*-test.

RESULTS

Chemical Analyses of Natural Ore

The natural ore used in this study was divided into four dominant ore types, as represented by sample A-1, consisting of sandstone with quartz, potassium feldspar, plagioclase, and biotite; sample A-2, comprising mudstone with the same components as sample A-1, sample B-1 consisting of quartz, and illite; and sample B-2 comprising quartz, potassium feldspar, and plagioclase. The results of chemical analyses using an X-ray fluorescent method are shown in Table 1, and reveal no specific characteristics of the natural ore found in the surrounding area of Aso-mountain, one of the biggest volcanoes in Japan.

Far-infrared Spectral Radiation Characteristics

Results concerning far-infrared spectral radiation characteristics revealed approximately 80% release from the far-infrared spectrum at 100 °C (data not shown). This finding indicates that this natural ore is not specific regarding the far-infrared spectrum. The detailed features of this specific natural ore should be analyzed in the future.

However, the Cosmic Garden Co. Ltd. has been using the powder of this ore for all house products, and there have been no complaints concerning health matters, at least regarding the opinions and comments from customers. We therefore believe that SNOP does not induce adverse effects on human health.

Questionnaire

Analysis of questionnaire data was performed using the chi-square test. The answer [d] for each question was omitted from the data analyzed. The category 'improved' is defined as an answer [a] to [b or c] and [b] to [c], and 'getting worse' is an answer

Table 1. Chemical Analyses of Four Main Parts of the Natural ore Analyzed by the X-ray-fluorescence (XRF) Method Showing no Remarkable Characteristics

| Sample<br>(wt %)               | A-1       | A-2      | B-1      | B-2       |
|--------------------------------|-----------|----------|----------|-----------|
|                                | Sandstone | Mudstone | Mudstone | Sandstone |
| SiO <sub>2</sub>               | 73.27     | 71.42    | 63.94    | 75.06     |
| TiO <sub>2</sub>               | 0.43      | 0.49     | 0.76     | 0.24      |
| Al <sub>2</sub> O <sub>3</sub> | 13.64     | 1.77     | 18.01    | 10.47     |
| Fe <sub>2</sub> O <sub>3</sub> | 4.12      | 6.94     | 4.20     | 10.47     |
| MnO                            | 0.04      | 0.19     | 0.04     | 0.07      |
| MgO                            | 1.05      | 1.72     | 1.55     | 0.93      |
| CaO                            | 0.85      | 1.19     | 1.58     | 3.89      |
| Na <sub>2</sub> O              | 3.73      | 1.76     | 1.56     | 3.09      |
| K <sub>2</sub> O               | 2.33      | 3.10     | 5.41     | 1.50      |
| P <sub>2</sub> O <sub>5</sub>  | 0.07      | 0.11     | 0.13     | 0.05      |
| H <sub>2</sub> O <sup>-</sup>  | 0.05      | 0.04     | 0.09     | 0.02      |
| H <sub>2</sub> O <sup>+</sup>  | 0.42      | 0.26     | 2.72     | 1.47      |
| CO <sub>2</sub>                | -         | -        | -        | -         |
| Total                          | 100.00    | 100.00   | 100.00   | 100.00    |

Table 2. Results of a Questionnaire Regarding Pollen Allergy Symptoms Indicating Improvement of Nasal Obstruction, Lacrimation and Difficulty of Daily Life, but a Worsening of Eye Redness

| Condition |                                                                            | CCSNOP   |                               | NWC      |                                     | P Value<br>(χ <sup>2</sup> test) |
|-----------|----------------------------------------------------------------------------|----------|-------------------------------|----------|-------------------------------------|----------------------------------|
| Item      | Answer                                                                     | Improved | No Change or<br>Getting Worse | Improved | No Change<br>or<br>Getting<br>Worse |                                  |
| A         | Frequency of sneezing                                                      | 14       | 2                             | 13       | 5                                   | 0.271                            |
| B         | Volume of nasal juice                                                      | 12       | 6                             | 10       | 6                                   | 0.464                            |
| C*        | Degree of nasal obstruction                                                | 12       | 6                             | 9        | 8                                   | 0.003                            |
| D*        | Degree of eye redness                                                      | 4        | 7                             | 7        | 7                                   | 0.010                            |
| E*        | Degree of lacrimation                                                      | 7        | 4                             | 4        | 11                                  | <0.001                           |
| F*        | Difficulty of daily life, such as routine activity, work, study, housework | 11       | 5                             | 5        | 7                                   | 0.021                            |

**Note.** \*Statistically significant differences were found for items 'C', 'E' and 'F'. These items indicated that associated symptoms improved in CCSNOP compared to NWC. However, item 'D' showed an improvement that was significantly higher in NWC than in CCSNOP.

[c] to [a or b] and [b] to [a] when comparing pre-stay and post-stay data. As shown in Table 2, “improved” versus ‘no change + getting worse’ was compared for CCSNOP and NWC conditions. Results indicated that although the frequency of sneezing and volume of nasal discharge did not change significantly, the other three items, except the degree of eye redness, were significantly higher or ‘improved’ for CCSNOP compared with NWC. Eye redness was the only symptom that improved significantly when PAs stayed under NWC rather than CCSNOP conditions. However, the overall results indicate that many of the symptoms in PAs improved following a stay in CCSNOP, as NWC conditions did not involve any alteration of indoor air conditions.

POMS

As shown in Table 3, the results of POMS were analyzed using the chi-square test and revealed that the moods for ‘Tension and Anxiety’, ‘Depression’, and ‘Fatigue’ decreased significantly when PAs stayed under CCSNOP conditions compared to NWC.

In addition, the total mood disturbance (TMD) calculated by instruction provided by POMS showed a significant decrease in CCSNOP compared with NWC conditions. Because symptoms mostly improved under CCSNOP conditions, the mental moods of subjects might become more stable, particularly when all PAs stopped their medication at least 1 day before entering the rooms. The results of POMS may suggest that the improving symptoms induced consolation of moods in PAs.

Stress Marker (salivary amylase)

We used a simple measurement kit to measure salivary amylase as a stress marker as we previously used salivary amylase as well as IgA, cortisol and chromogranin A as stress markers to examine changes in stress during stays under negatively charged air particle-dominant indoor conditions. At that time, saliva was collected using a cotton swab. However, to provide an easier sampling and collection method for subjects, a simple kit was employed that required the subject to hold a thin stick under the tongue for 1 min. Results showed no difference in the values of salivary amylase calculated as [pre-stay minus post-stay] concentrations when comparing CCSNOP and NWC. In addition, there were no differences in pre-data entering SSNOP and NWC.

Serum Igs and Multi-allergen Specific IgE (33 kinds)

There were no differences in pre-data entering SSNOP and NWC. Among IgG, IgA, IgM, and non-specific IgE, only the IgE (IU/mL) value calculated as [post-stay minus pre-stay] showed a significant difference between CCSNOP and NWC. Although the difference was very small (average: 55.6 IU/mL), stays in CCSNOP showed a significantly higher change when compared with stays in NWC. In particular, Figure 3A shows that stays in CCSNOP did not alter serum IgE levels, whereas stays in NWC yielded a slight decrease and the difference was significant.

Among specific IgE against various antigens including *Cryptomeria japonica* and *Chamaecyparis obtusa*, the typical antigens for PA in Japan, all PAs were negative for most of the antigens measured. Few were positive for certain allergens and it was difficult

Table 3. Results of POMS Indicating Improvement of Items Such as Tension & Anxiety, Depression, Fatigue, as Well as Total Mood Disturbance

| Condition Score (change of score<br>[Post- Pre] x number of HVs) | CCSNOP   |          | NWC      |          | P value<br>(χ <sup>2</sup> test) |
|------------------------------------------------------------------|----------|----------|----------|----------|----------------------------------|
|                                                                  | Decrease | Increase | Decrease | Increase |                                  |
| T-A*: Tension & Anxiety                                          | -53      | 0        | -19      | 4        | <0.001                           |
| D*: Depression                                                   | -23      | 2        | -8       | 4        | 0.002                            |
| A-H: Anger & Hostility                                           | -11      | 3        | -8       | 1        | 0.440                            |
| V: Vigor                                                         | -29      | 6        | -30      | 10       | 0.274                            |
| F*: Fatigue                                                      | -40      | 9        | -19      | 10       | 0.010                            |
| C: Confusion                                                     | -21      | 8        | -23      | 3        | 0.050                            |
| Total Mood disturbance (TMD)                                     | -109     | 21       | -75      | 39       | <0.001                           |

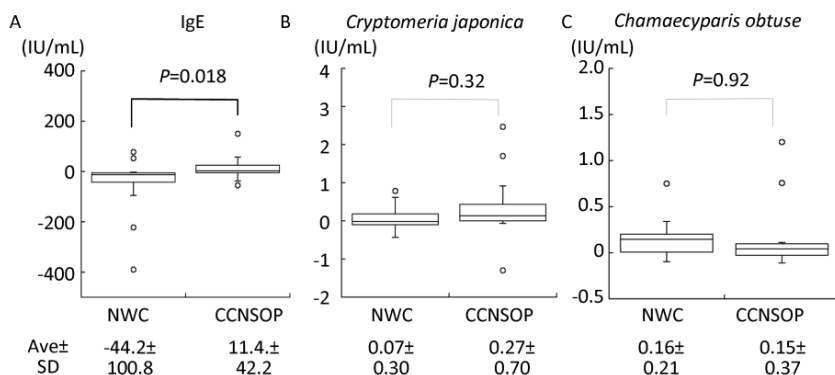
**Note.** Moods for ‘T-A’, ‘D’, and ‘F’ decreased significantly when PAs stayed in CCSNOP compared to NWC. In addition, TMD decreased significantly when PAs stayed in CCSNOP compared to NWC.

to compare changes between stays in CCSNOP and NWC. Results specific for *C. japonica* and *Chamaecyparis obtusa* are shown in Figure 3B and C, respectively. Specific antigen titers calculated as [post-stay minus pre-stay] values for CCSNOP and NWC for *Chamaecyparis obtusa* did not differ between stays in both rooms. However, those for *C. japonica* showed a slight but significant increase in CCSNOP compared with NWC.

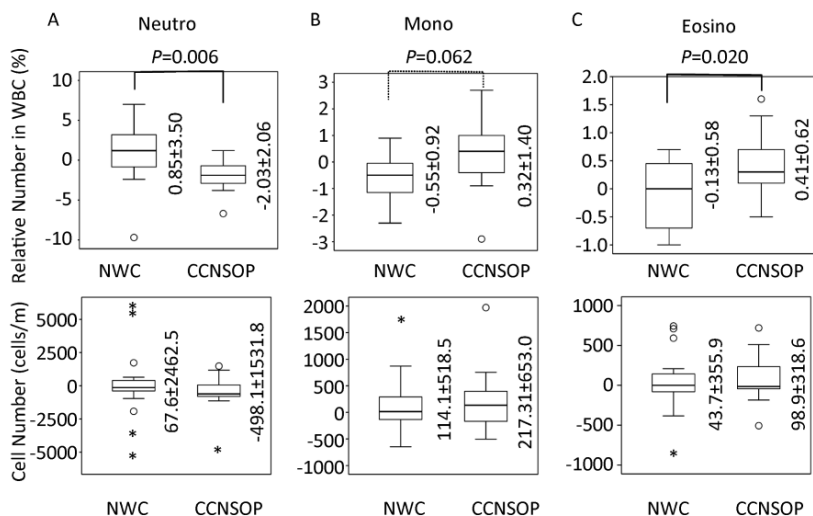
These results indicated that a stay in CCSNOP caused a slight activation of allergic reactions, although symptoms were improved as mentioned above.

### Differential White Blood Cell Count

There were no differences in pre-data entering SSNOP and NWC. Among the neutrophils, monocytes, basophils, eosinophils, and lymphocytes, the percentages of neutrophils decreased and monocytes and eosinophils increased following a stay in CCSNOP compared with NWC, as shown in Figure 4A, B, and C, respectively.



**Figure 3.** The distribution of non-specific IgE (A) and specific IgE for antigens of *Cryptomeria japonica* (B) and *Chamaecyparis obtusa* (C). Data comprise (post-stay minus pre-stay) values for CCSNOP and NWC, and statistically significant differences were found for non-specific IgE and specific IgE against antigens of *C. japonica*.



**Figure 4.** The distribution of percentages of neutrophils (A), monocytes (B), and eosinophils (C) in the peripheral blood of PAs. The data are shown as (post-stay minus pre-stay) values for CCSNOP and NWC,

and reveal that neutrophils decreased, while monocytes and eosinophils increased significantly when PAs stayed in a CCSNOP room compared with an NWC room.

These results also indicated that a stay in CCSNOP caused a slight activation of allergic reactions, although symptoms were improved as mentioned above.

These results also indicated that a stay in CCSNOP caused a slight activation of immune reactions, although symptoms were improved as mentioned above.

Changes in Cytokine Concentrations

Among the 29 kinds of cytokines investigated, significant differences in the changes in cytokine concentrations as calculated by [post-stay minus pre-stay] values for CCSNOP and NWC were found for EGF, monocyte chemotactic protein-1 (MCP-1), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), as shown in Figure 5A, B, and C, respectively.

Comparison of pre-data entering CCSNOP and NWC showed significant differences for eotaxin and TNF- $\alpha$ . Pre-data values for eotaxin before entering CCSNOP and NWC were 87.7 $\pm$ 46.7 and 57.4 $\pm$ 31.9 ng/mL, respectively. This difference is statistically significant (Student's *t*-test, *P*=0.022). Similarly, pre-data TNF- $\alpha$  values before entering CCSNOP and NWC were 8.94 $\pm$ 3.94 and 5.92 $\pm$ 3.45 ng/mL, respectively, and this difference was statistically significant (Student's *t*-test, *P*=0.014). Although these differences may affect the interpretation of results, all of the actual data concerning cytokines were in low ranges and the levels are understandably not pathological. Thus, even though these two cytokines showed differences in pre-data values for the two different conditions, the differences recorded for [post-stay minus pre-stay] values may be considered a reflection of the effects of CCSNOP.

All three cytokines were elevated when PAs stayed under CCSNOP conditions in comparison with NWC.

DISCUSSION

Customer comments and opinions regarding the wall building material that included the powder of a specific natural ore handled by Cosmic Garden Co. Ltd. suggested that this material improved allergic symptoms and reduced sleep disturbance in a portion of customers. This specific natural ore was obtained near Aso-mountain, located on Kyushu-island, and is an active volcano. In fact, Aso-mountain erupted in September of 2015.

Although customers mentioned beneficial effects in relation to various health problems, details of these health effects had not been explored. We therefore attempted to investigate some of the biological effects of this natural ore in patients with PA. This is the first trial to observe the biological effects of this ore, although the study protocol was limited by time, weather and the small number of patients. Even though customers have been living in homes built with material that incorporated this natural ore powder, there seems to be no adverse effects on the health of these individuals, and future studies should use short-stay trials involving indoor air conditions that include natural ore powder. In addition, it is difficult to enlist the participation of many patients with PA, and only 20 PAs agreed to be enrolled in this study. All of these PAs stayed in

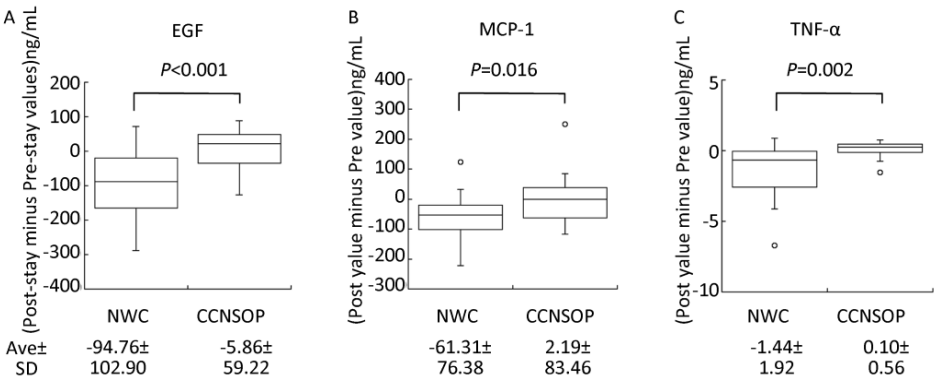


Figure 5. Distribution of changes in concentrations of EGF (A), MCP-1 (B), and TNF- $\alpha$  (C) in the

peripheral blood of PAs. Data are shown as [post-stay minus pre-stay] values for CCSNOP and NWC, and reveal that all three cytokines increased significantly when PAs stayed in a CCSNOP room compared with an NWC room.

CCSNOP and NWC rooms every other week, and we assumed that one week would be sufficient to observe any biological effects and the basic status of PA in participants. In addition, the time of this experiment was chosen to coincide with the season of the year that mostly affects PAs.

All subjects knew that the natural ore powder was used when Cosmic Garden Co. Ltd. built their homes. However, they did not know whether they were staying in a CCSNOP or NWC room during the first and second week of experiments. Additionally, the general consensus among individuals was that the overall status of the weather and symptoms of PA did not change during the experimental period in Okayama, Japan relative to those of previous periods.

Two interesting results arose from our experiments. The first was that CCSNOP induced an improvement of the symptoms of PA, as well as calmness of mental moods, probably as a consequence of the improved symptoms. The second is an enhancement of immunological and/or allergic biological reactions, reflected in an increase in non-specific IgE, specific IgE for an antigen of *C.japonica*, an elevation of the percentage of eosinophils, and an increased level of certain cytokines such as EGF, MCP-1, and TNF- $\alpha$ .

Unfortunately, we were not able to measure the number of pollen particles in the experimental CCSNOP and NWC rooms. Although we measured particulate matter in the size range 1 to 1000  $\mu\text{m}$ , we could not distinguish pollen particles from other indoor particulate matter. However, we did open the windows of the experimental rooms every 15 min to maintain the number of pollen particles in the room during experiments.

PA symptoms were improved under CCSNOP conditions when compared with NWC, presumably because CCSNOP may have decreased the number of pollen particles floating in the rooms. Another interpretation of the improved symptoms is the possibility that the biological mechanisms that yield nasal obstruction and lacrimation were immediately reduced by CCSNOP indoor air conditions. However, these mechanisms are natural biological reactions and may depend on the number of exposed pollen allergens at local lesion sites such as the nasal and eye mucosa. It is difficult to imagine that even if the number of allergens did not change, only the

symptoms were improved in certain environmental circumstances. Furthermore, all PAs were separated from the CCSNOP material by a distance of at least 1 m. In addition to these speculations, symptoms may be improved by the physical reduction of pollen allergens in the CCSNOP room.

All of the biological findings, such as the increase of non-specific IgE, specific IgE for an antigen of *C.japonica*, elevation of the percentage of eosinophils, and increased levels of certain cytokines, such as EGF, MCP-1, and TNF- $\alpha$ , indicated that these immunological and allergic reactions were enhanced during a stay in CCSNOP when compared with NWC, and symptoms were improved.

The most challenging interpretation of our results was the discrepancy between symptoms and biological data. Most of the symptoms were improved in the CCSNOP room, whereas the biological data showed a slight but significant worsening, such as the increase in IgE and eosinophils. We currently offer the following explanation. The findings concerning nonspecific and specific IgE, as well as eosinophils and monocytes, are assumed to have occurred as a reaction to allergens that suddenly disappeared from lesion sites where symptoms manifested, such as the nasal and eye mucosa. Because the duration of an experimental stay was 60 min, the biological reactions may have to maintain allergic reactions in order to combat the allergens, and symptoms therefore resulted. However, because there was an improvement in symptoms, it is possible that the allergens suddenly decreased in number.

The observed changes in cytokines, such as MCP-1 and TNF- $\alpha$ , may reflect the continuous maintenance of allergic reactions mentioned above due to the increasing level of monocytes<sup>[19-21]</sup>. However, an increase in EGF could not be explained by certain allergic reactions<sup>[22-24]</sup>. Although we have to consider the role of EGF in allergic reactions, one clue regarding the elevation of EGF during immunological activation caused by environmental circumstances is that indoor air conditions dominated by negatively charged particles (approximately 20 nm in diameter) have been shown to cause an elevation of EGF and natural killer cell activity, as we reported previously<sup>[13]</sup>. Thus, EGF may be one of the biomarkers reflecting a slight activation of immune and allergic reactions.

Due to the limited conditions used to explore the biological and physical effects of CCSNOP on PA, future studies should be performed using long-term experiments involving weekly or bi-weekly biological monitoring during a 1-to 2-month PA period in patients living in homes utilizing CCSNOP or NWC in sleeping or living rooms in order to fully evaluate the effect of this natural ore powder on indoor air conditions. If specific biological effects and physiological changes are found that improve allergic reactions in environments induced by this natural ore powder, patients suffering from various allergic diseases may benefit from the use of this material.

## CONCLUSIONS

All of the practical results indicated that some symptoms, such as nasal obstruction and lacrimation, improved and POMS evaluation showed patients to be calmer following a stay in CCSNOP. Relative eosinophils, non-specific IgE, epidermal growth factor, monocyte chemotactic protein-1, and tumor necrosis factor- $\alpha$  increased following a stay in CCSNOP. This ore powder improved allergic symptoms, and long-term monitoring involving 1-to 2-month stays may be necessary to fully explore the biological and physical effects of SNOP on allergic patients.

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## CONFLICT OF INTEREST

The authors FO and YF are employee and president of the Cosmic Garden Co. Ltd., Okayama city, Japan, respectively. The Department of Hygiene, Kawasaki Medical School, Kurashiki City, Japan was supplied the funds to measure the biological effects using the Kirameki Creation Fund from Okayama Prefecture Industrial Promotion Foundation from January to September, 2014, obtained by the Cosmic Garden Co. Ltd. The authors TS and YK contributed to the coordination of this project and declare no conflict of interest.

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