J. Korean Soc. Appl. Biol. Chem. **52**(4), 389-396 (2009)

Effect of Inorganic and Organic Germanium Treatments on the Growth of Lettuce (*Lactuca sativa*)

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Received January 13, 2009; Accepted May 26, 2009

Germanium (Ge) is a rare heavy metal and is known to toxic to plants at high level. However, there is little evidence about the Ge effect on plant growth. Here, we investigated the effect of inorganic (GeO_2) and organic (Ge-132) germanium on lettuce growth by treatment with various concentrations of GeO_2 and Ge-132. Under GeO_2 treatment, lettuce growth was not much inhibited at 2.5 mg/L concentration and then significantly inhibited at 5 mg/L concentration. However, under Ge-132 treatment, lettuce growth was not much inhibited by concentrations up to 10 mg/L. Relative fresh weight of lettuce at 2.5, 5, 10 and 25 mg/L concentrations was 99, 76, 65 and 35% in GeO_2 treatments and was 105, 99, 97 and 75% in Ge-132 treatments, respectively. In GeO_2 treatments, Ge was highly accumulated in the roots at concentration below 10 mg/L and in the shoots at concentration above 25 mg/L. However, Ge was primarily accumulated in the roots at all Ge-132 concentrations. Accumulated Ge amounts of plants under GeO_2 treatment were 0.72 mg/ g DW in roots and 0.27 mg/ g DW in shoots at a 10 mg/L concentration. At a 50 mg/L concentration of GeO_2, the Ge content was 0.77 mg/g DW in roots and 1.58 mg/g DW in shoots, respectively. Based on our results, inorganic germanium is more toxic for lettuce growth than organic germanium. Upper critical toxic levels for lettuce growth were 2.5 to 5 mg/L concentrations in GeO_2 treatments and 10 to 25 mg/L concentration in Ge-132 treatments, respectively.

Key words: *distribution, hydroponically grown, inorganic and organic germanium, lettuce growth, toxicity, uptake*

The concentration of heavy metals in soils and in water sources has increased continuously through time due to diverse human activities [Antosiewicz, 1992]. In high concentrations, many of those metals can be harmful to plants [Kabata-Pendias and Pendias, 2001]. Germanium (Ge) is a rare element that belongs to the fourth group of the periodic table along with carbon (C), silicon (Si), and lead (Pb). It exists in the Earth's crust (about 1.6 ppm Ge crustal average). It has been reported that Ge exhibits similar biogeochemical behavior to Si [Rosenberg, 2009]. Germanium can be divided into general inorganic compounds including elemental germanium (Ge) and germanium dioxide (GeO_2), and organic compounds including Ge-132, spiro-germanium and proxi-germanium etc. [Adams and Thomas, 1994; Kaplan *et al.,* 2004]. Furthermore, Ge exists in all biomaterials including plants and animals [Rosenberg, 2009]. Interestingly, generally recognized therapeutic plants such as herbs

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Abbreviations: DMSO, dimethyl sulphoxide; ICP, inductively coupled plasma; ICP-MS, inductively coupled plasma-mass spectrometry; ppb, parts per billion; ppm, parts per million

doi:10.3839/jksabc.2009.069

occasionally accumulate high amounts of Ge in their tissues dependent upon the geological distribution.

In terms of the biological effects to human health, several studies have reported that long-term intake of GeO_2 causes several clinical symptoms, such as renal damage, cranial neuropathy and cancer formation [Obera *et al.,* 1991]. However, an organic Ge compound, Ge-132 (2-carboxyethylgermanium-sesquioxide) has been used as a dietary supplement and its therapeutic attributes include fighting cancer and improving the immune system etc [Goodman, 1988: Li *et al.,* 1993; Tang *et al.,* 1997].

Regarding plants, a few studies have reported the effects of Ge treatment on plant growth and its uptake by barley [Halperin *et al.,* 1995], rice [Lim *et al.,* 2008], lettuce [Lee *et al.,* 2005], Chinese cabbage [Han *et al.,* 2007] and ginseng seedlings [Yu *et al.,* 2005]. Barley seedlings accumulated Ge in the roots and shoots, and the shoots accumulated Ge linearly as medium Ge concentration increased. This demonstrates that barley plants can take up Ge and it is not toxic at the levels that might occur in areas where Ge is normally mined [Halperin *et al.,* 1995]. In hydroponically-grown lettuce treated with a low concentration of GeO_2, Ge was taken up by the roost and barely moved to the shoots. Furthermore, in all ranges of the Ge treatment, the plants showed no phenotypic changes in leaf shape and color, including necrosis or chlorosis [Lee *et al.,* 2005]. Cakmak *et al.* [1995] showed a substitution effect of Ge in boron (B)-deficient sunflowers. In sunflower plants, Ge was found to temporarily alleviate boron-deficiency symptoms and to significantly increase the growth of sunflower seedlings in the absence of B. However, Ge is not capable of completely substituting B, particularly if plants have a high boron requirement under growing conditions [McIllrath and Skok, 1966]. Similar results were reported by other research groups for tomatoes [Brown and Jones, 1972] and pumpkins [Ishii *et al.,* 2002].

Although the uptake and transport of Ge in plants is limited compared to silicon, it has been obviously demonstrated that Ge uptake and transport are very similar to Si metabolism [Blecker *et al.,* 2007; Nikolic *et al.,* 2007]. In particular, Ge mimics the metabolic pathway of silicon in cases of low concentration, while it affects silicon transport and uptake by acting as a classical competitive inhibitor at high concentrations in plants. Although several papers have reported on the toxicity and uptake of Ge in plants, there is still little evidence regarding the effect of Ge on plant growth and the distribution and accumulation of Ge in inorganic or organic Ge treatments.

In this study, we investigated the toxicity of Ge on lettuce growth, the uptake properties under GeO_2 and Ge-132 treatments and its differential accumulation and distribution in hydroponically-grown lettuce plants.

Materials and Methods

Plant material and growth conditions. For early seedling analysis, sterilized lettuce seeds were placed in Petri dishes (90×15 mm) containing GeO_2 or Ge-132 solutions at various concentrations (0, 5, 10, 25, 50, and 100 mg/L). The seeds were then incubated in growth chamber conditions (14 h/10 h of light/dark cycle and at 23°C/22°C) for 7 days. For an analysis of two week old lettuce, the sterilized lettuce seeds of lettuce were sown into plug plates filled with peatmoss and perlite (2:1 v/v). Ten seedlings of two week-old uniform plants were transferred into glass jars (2 cm in diameter×20 cm in height) wrapped with aluminum foil to be hydroponically grown in a continuously aerated 1/5 Hoagland nutrient solution [2 mM KNO_3, 1 mM $Ca(NO_3)_2$, 0.4 mM $MgSO_4$, 0.4 mM $(NH_4)H_2PO_4$, 20 μM Fe-EDTA, 3 μM H_3BO_3, 0.5 μM $MnCl_2$, 0.2 μM $CuSO_4$, 10 μM $ZnSO_4$, and 1 μM $(NH_4)_6Mo_7O_{24}$], with a pH 6.0 [Benzari *et al.,* 2008]. Plastic tripods (5 cm in diameter and 5 cm in height) with openings at the top were placed inside the jars to hold the seedlings. The hydroponic units were aerated using an air pump connected to flexible plastic tubes immersed in the solution. The growth chamber was maintained under long day conditions (14 h light/10 h dark cycle) at 22°C. The volume of nutrient solution was kept at a constant throughout the experiments by adding sterile distilled water when necessary. For the Ge treatments, GeO_2 or Ge-132 was added to the nutrient solution at various concentrations at 1 week after transplanting and then harvested at 2 weeks after Ge treatment for analyzing both the growth parameters and Ge accumulation in the lettuce. Ge-132 and GeO_2 were purchased from Sigma Chemical Co. (St. Louis, MO). All experiments were triplicated.

Determination of Ge contents. Samples were always handled using plastic gloves and clean plastic or ceramic tools. The plant samples were ultrasonically cleaned with distilled water, oven-dried at 70°C for 72 h and ground to a fine powder with an agate mortar. The powders with particle size under 100 mm were collected for the following experiments. A 0.5 g portion of the powder material was transferred into 25 mL Falcon tube and then digested by 10 mL of Suprapur-grade HNO_3 (Merck, Darmstadt, Germany) at 90°C for 6 h. When the digestion was complete, the residues were filtered with a filter paper (Whatman No. 2) and the solutions were diluted to a final volume of 50 mL. Ge content was determined with some

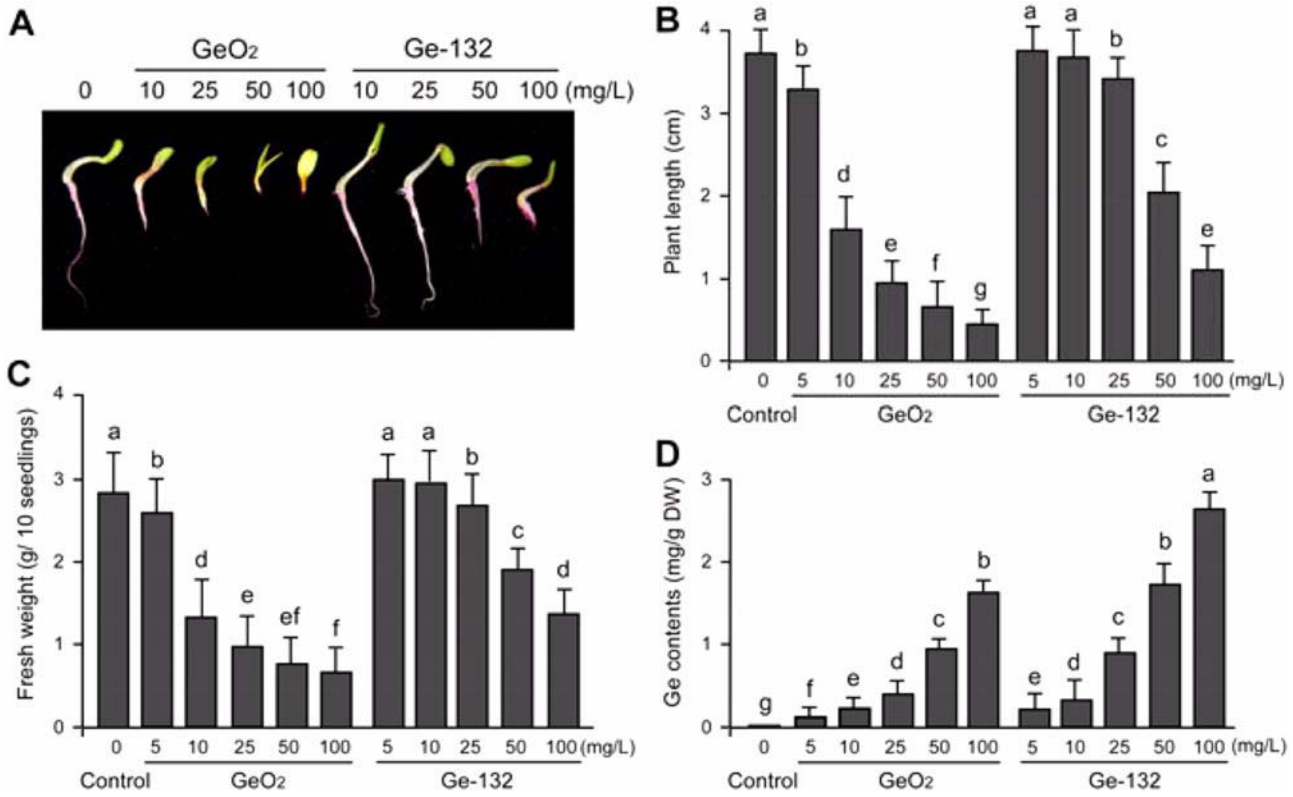

Fig. 1. Effect of different concentrations of GeO_2 and Ge-132 on lettuce seedling growth and its uptake properties. (A), photographs of lettuce seedlings; (B), plant length of lettuce seedlings; (C), fresh weight of lettuce seedlings, and (D), Ge contents in lettuce seedlings. Each bar is the mean of three independent replicates with 10 plants per each replicate (n=30). Error bars represent±SE. For each parameter, the data presented in each bar followed by dissimilar letters differs significantly at ($p<0.05$).

modification, as described in Jin *et al.* [1991] and Li *et al.* [1998]. ICP (ICPE-9000, Shimadzu, Japan) and ICP-MS (Agilent 7500, Santa Clara, CA) were used for analysis at the ppm or ppb level, respectively. An National Institute of Standard and technology (NIST) traceable ICP inorganic germanium standard solution (ICP-GE; 1,000 mg/L: germanium dioxide dissolved in water) was purchased from AnApex Co., Ltd., Korea and then diluted standard solutions were standardized using ICP or ICP-MS. Ge contents were assayed in triplicate and measurements were repeated three times. All concentrations were expressed on a dry weight basis. Data quality was checked by concurrent digestion and analysis of Standard Reference Material (SRM) 1547 peach leaves from the NIST in the USA. Before determining Ge amounts, we measured amounts of elements using SRM 1547 to verify the experiment procedure and the determination system. The values of most measured elements did not differ more than 10% from the certified values.

Statistical analysis. All statistical analyses were performed using the Statistical Analysis Software (SAS) version 6.08 (SAS Institute, 1990). Treatment means are the average of three replicates. The least significant difference (LSD) among mean values was calculated at a $p<0.05$ confidence level.

Results and Discussion

It has been reported that Ge exists in the Earth's crust as one of race elements. Several studies have reported the effects of Ge treatment on plant growth and its uptake in barley, rice, lettuce, and ginseng seedlings [Halperin *et al.,* 1995; Lee *et al.,* 2005; Yu *et al.,* 2005; Lim *et al.,* 2008]. However, there is little evidence about the different effects of inorganic and organic Ge on plant growth. Here, we investigated growth characteristics and Ge accumulation properties with GeO_2 and Ge-132 treatments to determine the effect of Ge on plant seedlings growth and its the upper critical toxic level.

Effects of Ge treatment on lettuce seedling growth. To investigate the effect of GeO_2 and Ge-132 treatment on early lettuce seedlings, the growth characteristics and Ge contents of plants were examined at various concentrations of GeO_2 and Ge-132 (0, 5, 10, 25, 50, and 100 mg/L) for 7 days, respectively. As shown in Fig. 1A, lettuce seedling growth was not much inhibited until 10

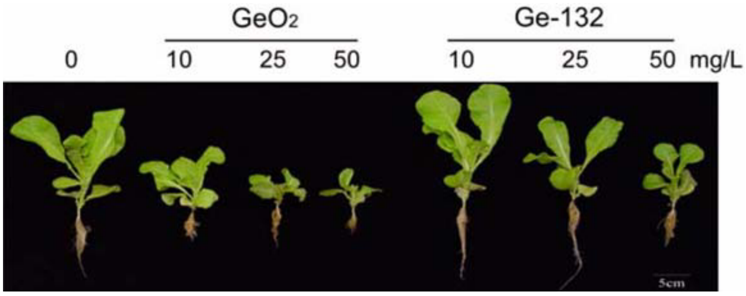

Fig. 2. Photographs of mature lettuce with GeO_2 and Ge-132 treatments. Two week-old lettuce plants were treated by adding several different concentrations of GeO_2 and Ge-132 in hydroponics solutions for an additional 2 weeks, and then a photograph was taken. Bar=5 cm.

and 25 mg/L concentrations under Ge-132 treatment compared to controls. However, lettuce seedlings under GeO_2 treatment were significantly inhibited at 10 and 25 mg/L concentrations, compared to controls. Lettuce seedlings were severely inhibited by 50 and 100 mg/L concentrations of both GeO_2 and Ge-132 treatments.

To determine the effect of Ge on lettuce growth in detail, plant length and fresh weight were measured under both GeO_2 and Ge-132 treatments, respectively (Fig. 1B and 1C). Under treatment of GeO_2, plant length was significantly inhibited at all concentrations. At a 10 mg/L concentration of GeO_2 treatments, more than 50% of plants were inhibited compared to controls (Fig. 1B). The fresh weight of lettuce seedlings also showed significant inhibition by treatment of GeO_2 (Fig.1C). Compared to controls, more than 50% and 70% of fresh weight was inhibited at 10 and 50 mg/L concentrations of GeO_2 treatment, respectively. However, unlike GeO_2 treatment, plant length and fresh weight were not much inhibited until a 25 mg/L concentration of Ge-132 treatment (Fig. 1B and 1C). These results suggest that Ge-132 treatment is not much toxic on lettuce seedling growth at a low concentration (10 mg/L) although Ge-132 would be toxic to plant growth at a high concentration, and that GeO_2 is very toxic for plant growth. Total Ge contents in early lettuce seedlings were much highly accumulated in plants treated with Ge-132 compared to those treated with GeO_2 (Fig. 1D). Ge content in plants treated with GeO_2 and Ge-132 treatment was also linearly accumulated in lettuce seedlings from 5 to 100 mg/L concentrations, respectively.

Recently, Han *et al.* [2007] reported that vegetables, such as leaf mustard, Chinese cabbage and pak-choi, could be affected by Ge treatment during seed germination and seedling growth. Ge-132 treatment did not inhibit seed germination or seedling growth, whereas GeO_2 might have been toxic to plant growth. These results are very similar to our results, as shown in Fig. 1.

Mature lettuce growth and different accumulations of Ge. In order to further determine the effect of Ge on mature lettuce growth and Ge accumulation, 2-week-old lettuce plants were treated with various GeO_2 or Ge-132 solutions (0, 2.5, 5, 10, 25, and 50 mg/L) for 14 days in hydroponics cultures. Growth characteristics, including fresh weight and plant length, were then measured (Fig. 2-4). As shown in Fig. 2, the growth of mature lettuce was significantly inhibited by GeO_2 treatment, but was not much inhibited by a 10 mg/L concentration of Ge-132 treatment. However, lettuce growth would be significantly inhibited by a high concentration (25 and 50 mg/L) of Ge-132 treatment.

To compare the different effects of GeO_2 and Ge-132 on lettuce growth and to establish the upper critical toxic levels of GeO_2 and Ge-132 treatment, we measured the shoot length, root length and fresh weight at various concentrations (0, 2.5, 5, 10, 25, and 50 mg/L) of Ge. At a low level (2.5 mg/L) of GeO_2 treatment, growth characteristics were not much inhibited compared to controls. However, at a 5 mg/L of GeO_2 treatment, growth characteristics were shown to be significantly inhibited compared to controls (Fig. 3 and 4). At a 25 mg/L concentration of GeO_2 treatment, more than 50%, 40% and 70% inhibition was observed in shoot length, root length and total fresh weight, respectively. However, under Ge-132 treatment, growth characteristics were not much different compared to controls until a 10 mg/L concentration of Ge-132 treatment (Fig. 3 and 4). A 25 mg/L concentration of Ge-132 treatment slightly inhibited lettuce growth. These results showed GeO_2 treatment inhibited lettuce growth even at a 5 mg/L concentration, whereas Ge-132 treatment didn't much inhibit lettuce growth until a 10 mg/L concentration, suggesting that inorganic germanium is more toxic than organic germanium for lettuce growth. Upper critical toxic levels would be between 2.5 and 5 mg/L concentrations of GeO_2 treatments and between 10 and 25 mg/L concentrations of Ge-132 treatments for lettuce growth.

To determine the properties of Ge accumulation and distribution for GeO_2 and Ge-132 treatments, we

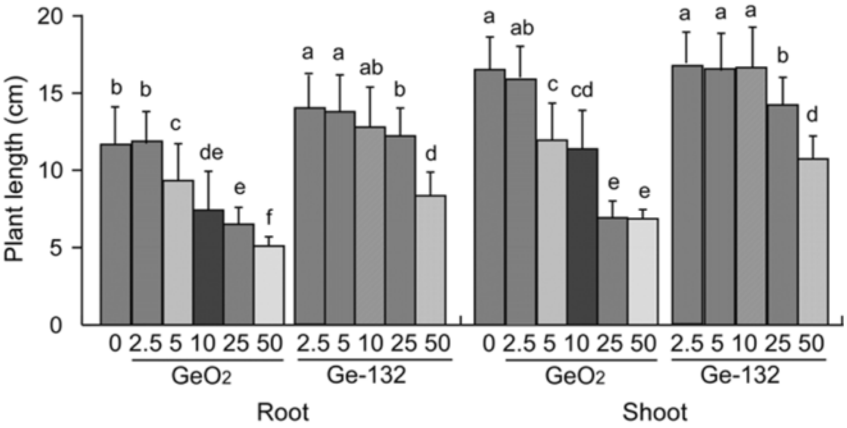

Fig. 3. Effect of GeO_2 and Ge-132 treatment on the length of mature lettuce. Each bar is the mean of three independent replicates with five plants per each replicate (n=15). Error bars represent±SE. For each parameter, the data presented in each bar followed by dissimilar letters differs significantly at ($p<0.05$).

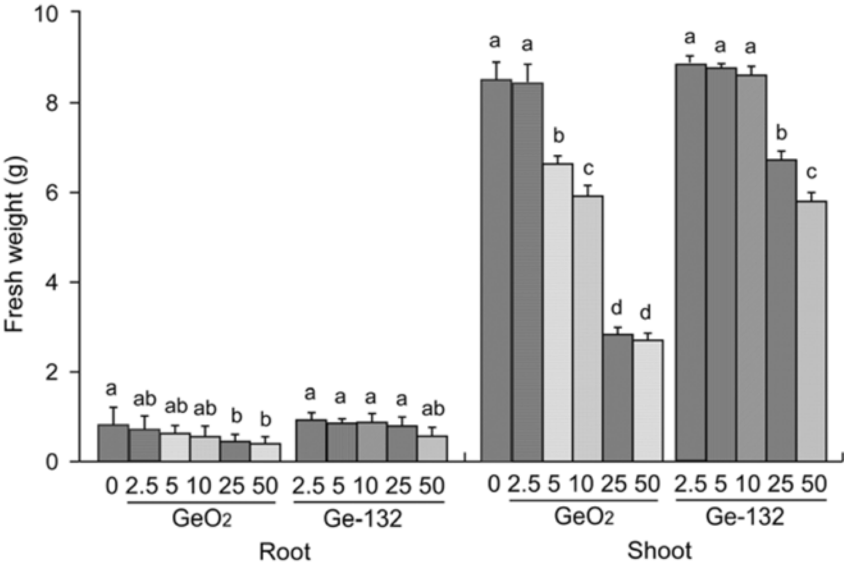

Fig. 4. Effect of GeO_2 and Ge-132 treatment on the fresh weight of mature lettuce. Each bar is the mean of three independent replicates with five plants per each replicate (n=15). Error bars represent±SE. For each parameter, the data presented in each bar followed by dissimilar letters differs significantly at ($p<0.05$).

fractionated lettuce samples into shoots and roots and then measured the Ge contents in both parts (Fig. 5). Ge was highly accumulated in roots at low level (below 10 mg/L) and highly accumulated in shoots at high level (above 25 mg/L) under GeO_2 treatments. However, Ge primarily accumulated in roots at all concentrations of Ge-132 treatments, suggesting that GeO_2 may be easily transported from roots to shoots by xylem loading after uptake of the solution by the root system. However, Ge-132 may be hard to transport from roots to shoots by xylem loading because Ge-132 may act as an organic compounds in plants. Accumulated Ge amounts in plants treated with GeO_2 were 0.72 mg/g DW in roots and 0.27 mg/g DW in shoots at 10 mg/L concentrations. At a 50 mg/L concentration of GeO_2 treatment, the Ge contents were 0.77 mg/g DW in roots and 1.58 mg/g DW in shoots, respectively. Total Ge amounts in plant at a 50 mg/L concentrations were highly observed (~2 fold) in plants treated with Ge-132 compared to those treated with GeO_2.

To further analyze the different distributions in detail, we treated the lettuce with 10 mg/L concentration of GeO_2 and Ge-132 for 2 weeks, fractionated the lettuce seedlings into young leaves, old leaves, stems, primary roots and lateral roots, and then measured their Ge contents. As shown in Fig. 6, Ge primarily accumulated in lateral and primary roots under both GeO_2 and Ge-132 treatments.

Recently, Lee *et al.* [2005] reported that Ge linearly accumulated in the plants, primarily in the roots, as GeO_2

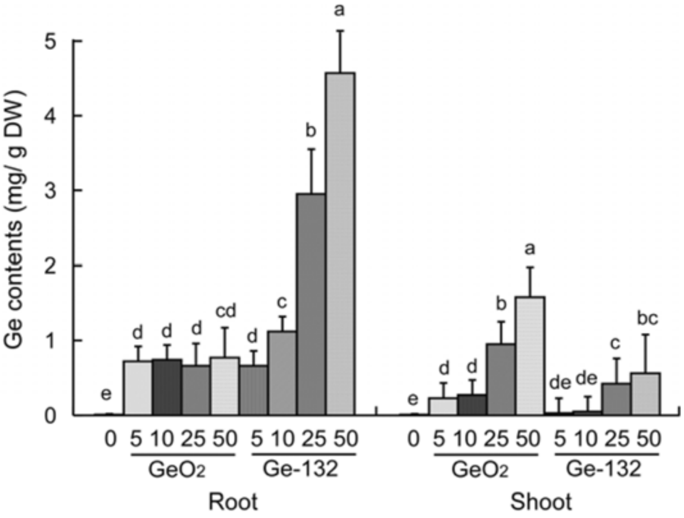

Fig. 5. Ge contents in mature lettuce plant under GeO_2 and Ge-132 treatment. Each bar is the mean of three independent replicates with five plants per each replicate (n=15). Error bars represent±SE. For each parameter, the data presented in each bar followed by dissimilar letters differs significantly at ($p<0.05$).

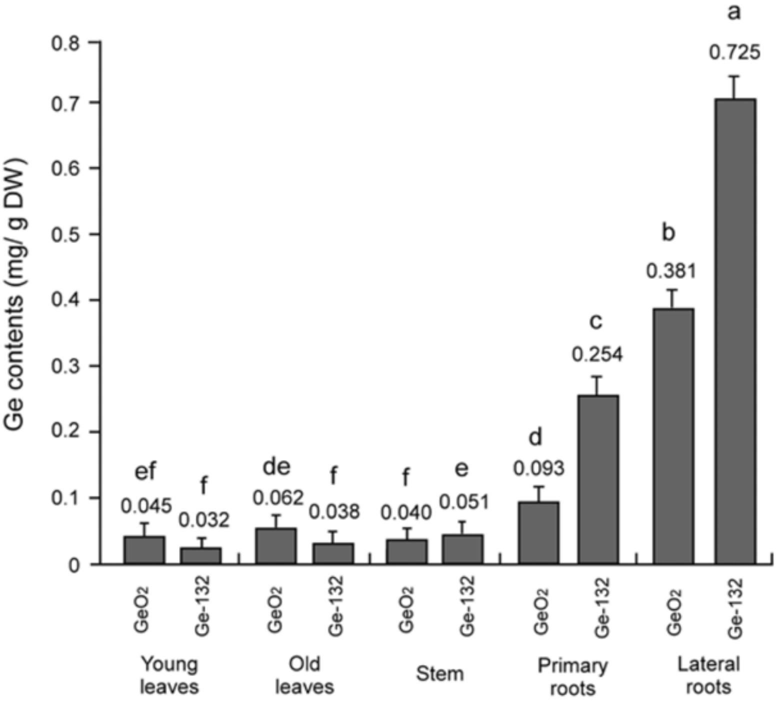

Fig. 6. Ge contents in young leaves, old leaves, stem, primary roots, and lateral roots of lettuce plants. Each bar is the mean of three independent replicates with five plants per each replicate (n=15). Error bars represent±SE. Numbers above bar represent the average value of Ge contents accumulated in plants. For each parameter, the data presented in each bar followed by dissimilar letters differs significantly at ($p<0.05$).

concentration in the nutrient solution increased from 1.0 to 8.0 mg/L, suggesting that Ge once taken up by the roots hardly moved to the shoots. In all ranges of the Ge treatment, the plants showed no phenotypic changes, but the roots were stunted and thickened. Lee *et al.* [2005] also showed that roots treated with GeO_2 at a 6 or 8 mg/L concentration showed a thickened cortex and the Ge-treated leaf looked harder and more greenish than the control. Our results also showed that Ge was primarily accumulated in the roots at a GeO_2 concentration of below 10 mg/L. Taken together, we could suggest that Ge once taken up by the roots of plants treated with GeO_2 may be hard to transport until roots become saturated with Ge. At that point, Ge might be easily transported from roots to shoots by xylem loading, when enough Ge is taken up from the solution by the root system. However, Ge-132 may be hard to transport from roots to shoots by xylem loading because Ge-132 may act as an organic compound in plants.

Several other earlier studies also reported that Ge is toxic to plants at high levels [Halperin *et al.,* 1995] and has physiological functions at trace levels [Loomis and Durst, 1992; Cakmak *et al.,* 1995]. It has also been reported that when Ge was supplied to the cultivating medium (e.g., in the form of GeO_2 or germanium sesquioxide), it was readily taken up, most likely due to the presence of polyacids, sugars and polyphenols that have strong complexing properties for germanium [Babula *et al.* , 2008]. Recently, Yu *et al.* [2005] reported that organic germanium stimulated the growth of ginseng roots and ginsenoside production at high concentrations, suggesting organic germanium could act as an elicitor for biomass enhancement and ginsenoside accumulation in plant cell cultures.

Germanium exhibits similar to Si in chemical properties. Although roles of Si in plants, including increasing mechanical strength, resistance to diseases and pests and salt, cold, heavy metals resistance etc, are well known [Epstein, 1999; 2001], the role of Ge and its metabolism is still unknown. Silicon could be taken up in plants by passive (diffusion) as well as active transports, and then transported from roots to aerial parts [Ma and Yamaji, 2006; 2008]. After transport, Si could be precipitated in cell walls, in intercellular spaces and in cells in the form of SiO_2 [Hodson *et al.,* 2005]. Although studies about Ge uptake and transport are very limited when compared to silicon, it is obvious that Ge is similar to Si metabolism [Blecker *et al.,* 2007; Nikolic *et al.,* 2007]. It has also been reported that organic selenium (Se) compound (SeMet) and selenate could be taken up by and transported in plants by an active process mechanism, whereas selenite might be accumulated through passive diffusion [Sors *et al.,* 2005]. Thus, we can suggest that Ge-132 might be translocated into plants via an active process.

Regarding physiological effects and toxicity for human health, long-term intake of GeO_2 causes severe clinical symptoms [Obera *et al.,* 1991]. However, an organic Ge compound, Ge-132, has been used to be potent drugs to fight cancer and improve the immune system [Goodman, 1988: Li *et al.*, 1993; Tang *et al.,* 1997]. Ge-132 has proven not to be toxic to animals at a dose of 5g/kg for 3 months [Sugiya *et al.,* 1986]. Some organogermanium compounds, such as benzyl and furyl germanium derivatives, also showed significant biological effects such as neurotropic activity, anti-cancer activity and a protective effect against radiation [Ignatovich *et al.,* 2002]. It has been reported that most Ge compounds have acute LD_{50} (lethal dose, 50%) values in the range of 100-1,000 mg/kg for parenteral and 500-5,000 mg/kg for oral application [Lukevics and Ignatovich, 2002]. Furthermore, we tested the single dose toxicity of germanium-fortified lettuces in mouse. A single oral dose of germanium-fortified lettuces containing 60 mg/kg of Ge (treated with GeO2 or Ge-132 at a 10 mg/L concentration) given to mice did not cause any apparent toxicological change at a dose of 2,000 mg/kg body weight [Kim *et al*., 2009]. Based on the risk assessment, accumulated Ge concentration in lettuces could thus be relevant for crop safety.

In conclusion, lettuce growth was highly inhibited at most concentrations under GeO_2 treatments, but was not inhibited at Ge-132 concentrations of less than 10 mg/L. Properties of Ge accumulation in plants showed the difference between GeO_2 and Ge-132 concentrations. Ge content was highly accumulated in the roots at a low level (below 10 mg/L) and in the shoots at a high level (above 25 mg/L) for plants treated with GeO_2. However, Ge primarily accumulated in the roots at all Ge-132 concentrations. Our results showed that inorganic germanium is much more toxic than organic germanium for lettuce growth. Upper critical toxic levels for lettuce growth were between 2.5 and 5 mg/L concentrations for plants treated with GeO_2 and between 10 and 25 mg/L concentrations for those treated with Ge-132. Thus, we can suggest that solutions containing Ge can be used for plant growth using a 2.5 mg/L concentration of GeO_2 and a 10 mg/L concentration of Ge-132.

Acknowledgments. This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea. This work was also supported in part by a grant (20080401034018) from BioGreen 21 program, Rural Development Administration, Republic of Korea and from Sunchon National University to YHC.

References

Adams JH and Thomas D (1994) Germanium and germanium compounds. In *Encyclopedia of Chemical Technology*, Kirk-Othmer (ed.), pp. 540-555. Wiley, New York, NY, U.S.A.

Antosiewicz DM (1992) Adaptation of plants to environment polluted with heavy metals. *Acta Soc Bot Pol* **61**, 281-299.

Babula P, Adam V, Opatrilova R, Zehnalek J, Havel L, and Kizek R (2008) Uncommon heavy metals, metalloids and their plant toxicity: a review. *Environ Chem Lett* **6**, 189-213.

Benzarti S, Mohri S, and Ono Y (2008) Plant response to heavy metal toxicity: comparative study between the hyperaccumulator *Thlaspi caerulescen*s (ecotype Ganges) and nonaccumulator plants: lettuce, radish, and alfalfa. *Environ Toxicol* **23**, 607-616.

Blecker SW, King SL, Derry LA, Chadwick OA, Ippolito JA, and Kelly EF (2007) The ratio of germanium to silicon in plant phytoliths: quantification of biological discrimination under controlled experimental conditions. *Biogeochemistry* **86**, 189-199.

Brown JC and Jones WE (1972) Effect of germanium on utilization of boron in tomato (*Lycopersicon esculentum* Mill.). *Plant Physiol* **49**, 651-653.

Cakmak I, Kurz H, and Marschner H (1995) Short-term effects of boron, germanium and high light-intensity on membrane permeability in boron deficient leaves of sunflower. *Physiol Plant* **95**, 11-18.

Epstein E (1999). Silicon. *Ann Rev Plant Physiol Plant Mol Biol* **50**, 641-664.

Epstein E (2001) Silicon in plants: facts vs. concepts. In *Silicon in agriculture*, Datnoff LE, Snyder GH, and Korndorfer GH (eds.), pp. 1-16. Elsevier, New York, NY, U.S.A.

Goodman S (1988) Therapeutic effects of organic germanium. *Med Hypoth* **26**, 207-215.

Han MJ, Kim SU, Seo DC, Cheong YH, Lee DJ, Park MS, Rim YS, Sohn BK, Heo JS, and Cho JS (2007) Uptake properties of germanium to vegetable plants and its effect on seed germination and on early stage growth. *Kor J Environ Agric* **26**, 217-222.

Halperin SJ, Barzilay A, Carson M, Roberts C, and Lynch J (1995) Germanium accumulation and toxicity in barley. *J Plant Nutr* **18**, 1417-1426.

Hodson MJ, White PJ, Mead A, and Broadley MR (2005) Phylogenetic variation in the silicon composition of plants. *Ann Bot* **96**, 1027-1046.

Ignatovich L, Zarina D, Shestakova I, Germane S, and Lukevics E (2002) Synthesis and biological activity of silyl- and germylsubstituted trifluoroacetylfurans. *Met Based Drugs* **8**, 211-214.

Ishii T, Matsunaga T, Iwai H, Satoh S, and Taoshita J (2002) Germanium does not substitute for boron in cross-linking of Rhamnogalacturonan II in pumpkin cell walls. *Plant Physiol* **130**, 1967-1973.

Jin K, Shibata Y, and Morita M (1991) Determimation of germanium species by hydride gerneration-inductively coupled argon plasma mass spectrometry. *Anal Chem* **63**, 986-989.

Kabata-Pendias A and Pendias H (2001) In *Trace Elements in Soils and Plants,* (3rd ed.). CRC Press LLC, Boca Ratón, FL, U.S.A.

Kaplan BJ, Parish WW, Andrus GM, Simpson JSA, and Field CJ (2004) Germane facts. About germanium sesquioxide: I. Chemistry and anticancer properties. *J Altern Complement Med NY* **10**, 337-344.

Kim JJ, Choi J, Cho JS, Heo JS, and Yee ST (2009) Oral single dose toxicity studies on germanium-fortified lettuce in moyse. *Korean J Environ Agric* **28**, 59-68.

Lee PG, Park HS, Won JH, and Park KW (2005) Effect of GeO_2 concentration on hydroponically-grown lettuce (*Lactuca sativa*). *J Korean Soc Hort Sci* **46**, 113-118.

Li JF, Kirchgessner M, and Steinruck U (1993) Growth promoting effect and toxicity of germanium in chickens. *Arch Geflugelkunde* **57**, 205210.

Li YC, Jiang SJ, and Chen SF (1998) Determination of Ge, As, Se, Cd and Pb in plant materials by slurry sampling-electrothermal vaporization0inductively coupled plasma-mass spectrometry. *Anal Chim Acta* **372**, 365-372.

Lim JS, Seo DC, Park WY, Cheon YS, Lee ST, Cho JS, and Heo JS (2008) Effects of soil texture on germanium uptake and growth in rice plant by soil application with germanium. *Korean J Environ Agric* **27**, 245-252.

Loomis WD and Durst RW (1992) Chemistry and biology of boron. *Biofactors* **3**, 229-239.

Lukevics E and Ignatovich L (2002) Biological activity of organogermanium compounds. In *The chemistry of organic germanium, tin and lead compounds*, Rappoport Z (ed.), pp. 1653-1683. Wiley, Chichester, UK.

Ma JF and Yamaji N (2006) Silicon uptake and accumulation in higher plants. *Trends in Plant Sci* **11**, 392-397.

Ma JF and Yamaji N (2008) Functions and transport of silicon in plants. *Cell Mol Life Sci* **65**, 3049-3057.

McIllrath WJ and Skok J (1966) Substitution of germanium for boron in plant growth. *Plant Physiol* **41**, 1209-1212.

Nikolic M, Nikolic N, Liang YC, Kirkby EA, and Romheld V (2007) Germanium-68 as an adequate tracer for silicon transport in characterization of silicon uptake in different crop species. *Plant Physiol* **143**, 495-503.

Obera K, Saito T, Sato H, Yamakage K, Watanabe T, Kakizawa M, Tsukamoto T, Kobayashi K, Hongo M, and Yoshinaga K (1991) Germanium poisoning: clinical symptoms and renal damage caused by long-term intake of germanium. *Japanese J Med* **30**, 67-72.

Rosenberg E (2009) Germanium: environmental occurrence, importance and speciation. *Rev Environ Sci Biotechnol* **8**, 29-57.

Schroeder HA and Balassa JJ (1967) Arsenic, germanium, tin, and vanadium in mice: Effects on growth, survival, and tissue levels. *J Nutr* **92**, 245252.

Sors TG, Ellis DR and Salt DE (2005) Selenium uptake, translocation, assimilation and metabolite fate in plants. *Photosynth Res* **86**, 373-389.

Sugiya Y, Sakamaki S, and Satoh H. (1986) Reversibility study in rats after two month's oral administration of carboxyethylgermanium sesquioxide (Ge-132). *Pharmacometrics* **31**, 1191-1200.

Tang Z, Zhang GH, Wang ZL, Shi Y, Tang ZX, Zhang GH, Wang ZL, and Shi, Y (1997) Effects of an organogermanium compound on antioxidant function of MDV-infected chickens. *Chinese J Vet Sci* **17**, 173176.

Yu KW, Murthy HN, Jeong CS, Hahn EJ, and Paek KY (2005) Organic germanium stimulated the growth of ginseng adventitious roots and ginsenoside production. *Proc Biochem* **40**, 2959-2961.