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Perchlorate in Soybean Sprouts (*Glycine max* L. Merr.), Water Dropwort (*Oenanthe stolonifera* DC.), and Lotus (*Nelumbo nucifera* Gaertn.) Root in South Korea

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ABSTRACT: The occurrence of perchlorate in soybean sprouts (*Glycine max* L. Merr), water dropwort (*Oenanthe stolonifera* DC.), and lotus (*Nelumbo nucifera* Gaertn.) root, which are commonly consumed by people in South Korea, was determined by using an ion chromatograph coupled with a tandem mass spectrometer. For soybean sprouts (11 samples), perchlorate was detected in most (91%) of the samples at various concentrations of up to 78.4 μg/kg dry weight (DW); the mean concentration was 35.2 μg/kg DW. For water dropwort, of the 13 samples examined, four showed concentrations that were above the limit of quantification (LOQ). The mean perchlorate concentration was 20.7 μg/kg DW, and the highest perchlorate value was 39.9 μg/kg DW. Of the six lotus root samples examined, only one exhibited a detectable perchlorate concentration (17.3 μg/kg DW). For the accumulation experiments with artificially contaminated solutions, the concentrations of perchlorate in soybean sprouts gradually increased with the increase of perchlorate concentration in the solution. However, there was a decrease in the bioconcentration factor as the perchlorate concentration in the solution increased.

KEYWORDS: Perchlorate, soybean sprouts, water dropwort, lotus root, occurrence, South Korea

INTRODUCTION

Perchlorate, which has been detected in surface water, groundwater, and seawater, has become a serious concern in recent years with regard to its effect on public health, especially in areas where accidental spills and emissions from the use of military oxidizers and explosives have occurred.[1−5] Humans can be exposed to low concentrations of naturally occurring perchlorate, which is produced by natural atmospheric processes and distributed by precipitation.[6−9] However, it has been reported that in most cases, the perchlorate salts to which humans are exposed to originate from anthropogenic sources such as the solid salts of ammonium, potassium, and sodium perchlorate, which are commonly used in the manufacture of rockets, missiles, fireworks, and matchsticks.[10]

The chemical toxicity of perchlorate, which acts as a competitive inhibitor of the sodium iodide symporter (NIS) in the human body, has been found to negatively affect thyroid functions, leading to abnormal endocrine functions.[11,12] Perchlorate's main pathway into the human body is through the ingestion of contaminated food and water.[11−13] A decrease in thyroid hormones is known to have potentially negative effects on humans, especially fetuses and breast-fed infants, hampering normal growth and cognitive development in the brain.[13−15] Because perchlorate is a highly soluble, nonreactive, and poorly sorbed compound under aerobic ambient conditions, it can persist for decades in aqueous matrices.[8−10]

The U.S. Environmental Protection Agency (U.S. EPA) and a number of researchers investigated the status of perchlorate accumulation in vegetation after determining the amount of perchlorate in water.[16−20] Numerous studies have demonstrated that edible vegetables and fruits have been exposed to perchlorate at contaminated sites. Sanchez et al. reported that perchlorate was detected in different lettuce cultivars (*Lactuca sativa* L.) cultivated in the region of the Colorado River, which is contaminated with low levels of perchlorate (2−8 μg/L).[21] In a follow-up study, the perchlorate concentrations in spinach (*Spinacia oleracea* L.) and arugula (*Eurca sativa* Miller) in this region were 628 μg/kg fresh weight (FW) and 195 μg/kg FW, respectively.[22] In another study, perchlorate concentrations in *Citrus* sp. including the fruit (peel and pulp) and leaves were greater than that in irrigation water.[23] These results confirm that exposure to perchlorate occurs through the consumption of fresh vegetables and fruit as well as through drinking water because perchlorate concentrations in vegetables are closely correlated with exposure to contaminated water. Furthermore, vegetables and fruit containing high concentrations of perchlorate may constitute the primary source of perchlorate exposure, rather than drinking water.

In South Korea, perchlorate was recently detected in the Nakdong River (<1.0−60.0 μg/L), the Yeongsan watersheds (<1.0−2.5 μg/L), several sources of tap water (<1−6.1 μg/L), bottled water (0.04−0.29 μg/L), seawater (0.11−6.11 μg/L), dairy milk (1.99−6.41 μg/L), and powdered infant formula (1.49−33.30 μg/kg). Several studies have been conducted to examine the perchlorate contamination and to remediate contaminated water.[3,24,25] Nevertheless, little information exists regarding the perchlorate contamination of food. There is also limited data regarding the uptake of perchlorate by agricultural crops cultivated by contaminated water in South Korea.

Because of the potential harmful effects of perchlorate accumulation in plants and animals on human health, this study

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focused on demonstrating the presence of perchlorate in soybean sprouts (*Glycine max* L. Merr), water dropwort (*Oenanthe stolonifera* DC.), and lotus (*Nelumbo nucifera* Gaertn.) root sourced from many different provinces of South Korea. In addition, accumulation tests using soybean sprouts were conducted to verify the ability to accumulate perchlorate. Because soybean sprouts, water dropwort, and lotus root, which are common edible vegetables in South Korea, are cultivated hydroponically, they accumulate many elements and compounds from the water source, including contaminants. Therefore, we have tried to determine the perchlorate concentrations in these vegetables. A total of 11 soybean sprouts, 13 water dropwort, and 6 lotus root samples were obtained from local markets in different locations. These samples were analyzed for perchlorate using ion chromatography−tandem mass spectrometry (IC-MS/MS). To the best of our knowledge, this study is the first to report on the perchlorate levels in vegetables in South Korea.

MATERIALS AND METHODS

Sample Collection. Three vegetable species—soybean sprouts (*G. max* L. Merr), water dropwort (*O. stolonifera* DC.), and lotus (*N. nucifera* Gaertn.) root—which are local landraces in Korea and were not identified with specific cultivar names, were used for perchlorate determination. From December 2009 to August 2010, a total of 11 soybean sprout samples of 11 different brands were purchased from local markets in seven provinces. A total of 13 water dropwort samples were purchased from local merchants in 10 cities. For lotus root, a total of six samples from six different brands were collected from five different locations. Figure 1 shows the sampling locations of the soybean sprouts, water dropwort, and lotus root.

Chemicals. A perchlorate standard solution of 1000 mg/L was purchased from Accustandard, Inc. (New Haven, CT) and ^{18}O-enriched $NaClO_4$ (Icon Services Inc., NJ) was used as an internal standard (IS) for perchlorate analysis to provide accurate quantification by compensating for matrix effects. Glacial acetic acid and high-performance liquid chromatography (HPLC) grade acetonitrile (ACN) were purchased from JT Baker (Phillipsburg, NJ). The glacial acetic acid was diluted with distilled water to form a 1% (v/v) solution for use in the extraction. All solutions and dilutions were prepared using ultrapure water purified with an MQ water purification system (Milli-Q, Millipore, Molsheim, France).

Sample Preparation. In this study, an extraction procedure for the determination of perchlorate in vegetable samples by IC-MS/MS was carried out with modifications of the extraction methods developed previously.[26−28] All of the vegetable samples were freeze-dried in a freeze-drier (FDT 8606, Operon, Kimpo, Korea). They normally required 48 h for complete freeze-drying. All freeze-dried materials were then ground and stored in airtight containers at 2 °C until undergoing analysis. Three grams of the ground sample was weighed into a 50 mL polypropylene conical tube, and 1.6 mL of the 300 μg/L IS solution was pipetted. After adding 20 mL of 1% acetic acid, the tightly capped tube was shaken using a mechanical shaker (SHO-2D, WiseShake, Daihan Scientific Co., Ltd., Seoul, Korea) at a speed of 250 rpm for 10 min at room temperature. Next, 20 mL of ACN was added to the tube and vortexed for 1 min. The mixture was then centrifuged at 4000 rpm for 20 min, and the supernatant was passed through a preconditioned Supelclean Envi-Carb SPE cartridge and filtered through a 0.20 μm pore size polytetrafluoroethylene (PTFE) syringe filter for IC-MS/MS analysis. For the soybean sprouts, samples were treated with serially placed OnGuard II cartridges (Ba and H).

Instrumental Analysis. The perchlorate concentrations were analyzed by IC-MS/MS (Dionex ICS 2100 and Agilent 6410 triple quadrupole mass spectrometers). The separation was performed using

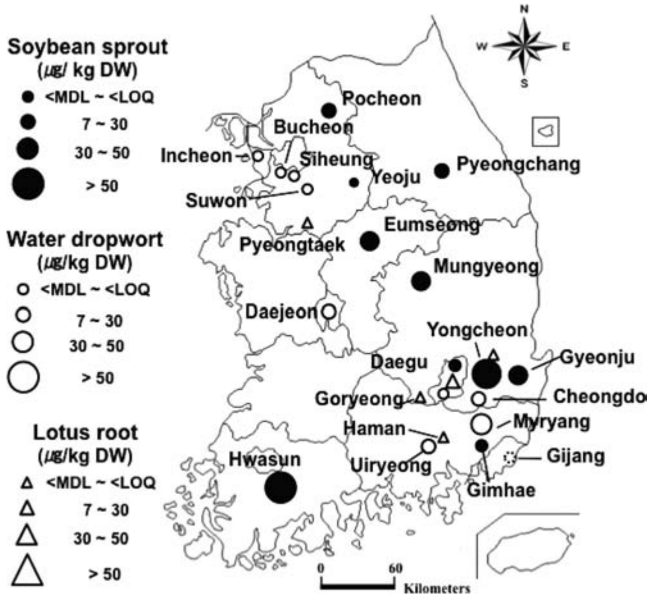

Figure 1. Sampling locations and distribution of the average perchlorate concentrations for soybean sprouts, water dropwort, and lotus root in South Korea.

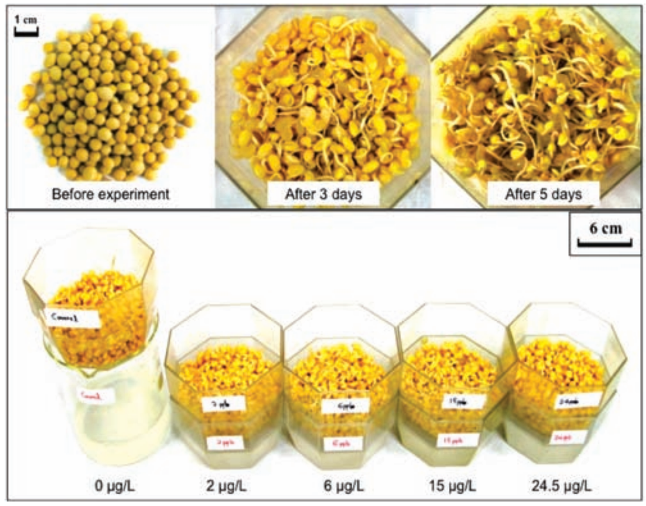

Figure 2. Photographs of the accumulation experiment using soybean sprouts with five different perchlorate concentrations in artificial water.

a Dionex ICS 2100 with a GS50 gradient pump, an ASRS 300 2 mm suppressor, an IonPac AG21 guard column (2 mm × 50 mm), and an IonPac AS21 separation column (2 mm × 250 mm). A 15 mM potassium hydroxide (KOH) solution flowing at a rate of 0.35 mL/min was used as an eluent. The detection was performed using an Agilent 6410 triple quadrupole mass spectrometer operated in the electrospray ionization (ESI) mode. Conditions for negative ESI MS/MS were as follows: polarity, negative ion mode; capillary voltage, 3.5 kV; chamber current, 0.7 μA; gas temperature, 350 °C; gas flow, 11 L/min; nebulizer pressure, 310 Pa; MS1 heater temperature, 100 °C; and MS2 heater temperature, 100 °C. The transition from ^{35}Cl-containing species was monitored under multiple reaction monitoring modes: m/z 99 → 83 for $[^{35}Cl^{16}O_4]^-$ to $[^{35}Cl^{16}O_4-^{16}O]^-$ for quantification; m/z 107 → 89 for $[^{35}Cl^{18}O_4]^-$ to $[^{35}Cl^{18}O_4-^{18}O]^-$. Perchlorate was quantified using the area ratio of perchlorate to the internal isotopic perchlorate standard.

The method detection limit (MDL) was determined by consecutively injecting 1.3 μg/L into the column seven times for the soybean sprouts extracts. The MDL was calculated according to the following equation: MDL = standard deviation of measured concentration × 3.143, and the LOQ was then calculated by multiplying MDL by 7.[29]

Perchlorate Accumulation Tests with Artificially Contaminated Water. In accumulation experiments, the soybean sprout (*G. max* L. Merr) was selected to verify the accumulation capability of perchlorate in the plant body. The seeds were purchased from a local seed market. The whole process of the accumulation test simulated (on a laboratory scale) the condition of an actual process in soybean sprout cultivating plants. The accumulation tests were performed using glass jars (12 cm × 12 cm × 8 cm) containing 300 mL of water artificially contaminated with perchlorate. The soybean seeds were soaked in the contaminated water for 12 h. After the soaking phase, the seeds were placed in a glass jar that contained a hole in the base. The jar containing the hole was then stacked on top of a second jar, in order for the contaminated water to drain and collect at the bottom (Figure 2). The geminated seeds were rinsed eight times a day to keep them from fermenting, until they developed a root length of about 4 in. The water artificially contaminated with perchlorate was replaced with new contaminated water every 24 h. The entire cultivation process was conducted for 1 week in a growth chamber at 25 °C (60% relative humidity) in the darkness. For the accumulation experiments, a standard solution of perchlorate of 1000 mg/L purchased from Accustandard (United States) was diluted with deionized−distilled water. Five solutions (0, 2, 6, 15, and 25 μg/L) of perchlorate were used, and no artificial nutrients were added to the solution during the tests. For each experiment, 6 mL of solution was sampled from the jar before and after the replacement of new contaminated solution every day for 6 days to investigate how the perchlorate concentration levels changed before and after the soybean sprouts were rinsed. Then, the perchlorate concentrations were analyzed by the IC-MS/MS. After the accumulation tests, the sample processing and analysis procedures were the same as in the previous experiments.

RESULTS AND DISCUSSION

IC-MS/MS Analysis. The calculated MDL for the soybean sprouts extracts was 0.16 μg/L. The LOQ for the soybean sprouts extracts was set to 1.2 μg/L. On the basis of these results, the LOQ for the soybean sprouts samples was estimated to be 7.2 μg/kg dry weight (DW) by IC-MS/MS using our extraction ratio.

A fortifying test was also conducted. The results of the perchlorate levels for the soybean sprouts, water dropwort, and lotus root samples fortified with 5 and 10 μg/kg are presented in Table 1. The accuracy and precision were estimated as the mean percent recovery and the relative standard deviation (RSD), respectively. The results reveal that 98% of the perchlorate in soybean sprouts, 104% in the water dropwort, and 96% in the lotus root are recovered with an RSD of 2.4−10.6%.

Perchlorate Concentration in Vegetables. For three vegetable cultivars, the results of perchlorate concentrations with standard deviations for individual samples are presented in Table 2. The perchlorate values are averages of triplicate extractions. In addition, the comparative results for the soybean sprouts, water dropwort, and lotus root are shown in Figure 1.

The perchlorate levels were detected at different concentrations in most soybean sprout samples (rate of detection, 91%), ranging from ND to 78.4 μg/kg DW, with an average concentration of 35.2 μg/kg DW. The highest perchlorate value of 78.4 μg/kg DW was detected in the sample from Yongcheon. Only one of the 11 soybean sprout samples was below the LOQ.

Table 1. Recoveries of Perchlorate Anion Fortified in Soybean Sprouts, Water Dropwort, and Lotus Root

commodity	fortification level (μg/kg)	average % recovery ($n = 3$)	RSD (%)
soybean sprouts (brand F)	5.0	98.7	6.0
	10.0	96.8	3.0
water dropwort (brand II)	5.0	102.4	10.6
	10.0	106.4	2.4
lotus root (brand I)	5.0	98.4	6.1
	10.0	94.3	5.7

The perchlorate levels of water dropwort and lotus root were below the LOQ in most (74%) of the samples collected. For water dropwort, of the 13 sites sampled, only four exceeded the LOQ (rate of detection, 31%). The concentrations of perchlorate in these samples were generally lower than those found in the soybean sprouts samples, with a mean concentration of 20.7 μg/kg DW. It is assumed that this is due to the water dropwort being cultivated mostly in mountainous regions, which are not influenced by perchlorate contaminated irrigation water. However, the single water dropwort sample obtained from Myryang contained 39.9 μg/kg DW perchlorate. Analysis of the six lotus root samples revealed that only one sample (rate of detection, 17%), cultivated in Goryeong, contained 17.31 μg/kg DW perchlorate. It is speculated that the reason why perchlorate was rarely detected in the lotus root samples is that most of the perchlorate tends to accumulate in leaves; transpiration might be the major mechanism for transporting perchlorate from the root to the top of the plants.[17,20] Perchlorate accumulation has been shown to be greatest in actively transpiring tissue, and previous studies have reported significantly lower concentrations in roots as compared to leaves.[16,17,20,30]

The results indicate that perchlorate exposure in vegetables exists in South Korea. The gathered data on perchlorate concentrations provide an indication of the perchlorate pollution status in South Korea. It is speculated that some vegetables that have considerable perchlorate concentrations might be irrigated directly with perchlorate-contaminated surface water, groundwater, or tap water. Although the data regarding the regional dispersion of perchlorate in South Korea are fairly limited, previous studies have demonstrated that perchlorate was found in various concentrations ranging from 0.05−60 μg/L in surface water (i.e., river mainstream and tributary), 0.56−22 μg/L in wastewater treatment plant (WWTP) effluent, 1.0−6.1 μg/L in tap water, 0.04−0.29 μg/L in bottled water, and 0.11−6.11 μg/L in seawater.[3,24,25] Because a huge amount of water is necessary for soybean sprouts, water dropwort, and lotus root cultivation, all of which are grown hydroponically, many manufacturers utilize surface water and groundwater near big rivers instead of tap water. Consequently, it can be assumed that since irrigation water for the vegetables is always local origin, they can be affected directly when perchlorate pollution occurs in surface water. Although the detection of perchlorate in specific vegetable samples has been confirmed in this study, we believe that more extensive investigations are necessary to determine the sources of perchlorate contamination and to examine the status of perchlorate accumulation in vegetation.

Perchlorate Accumulation Experiment. The results of the change in perchlorate concentrations using soybean sprouts with

Table 2. Perchlorate Concentrations in Soybean Sprouts, Water Dropwort, and Lotus Root from South Korea

soybean sprouts		water dropwort		lotus root[d]	
description	perchlorate (μg/kg DW ± SD)	description	perchlorate (μg/kg DW ± SD)	description	perchlorate (μg/kg DW ± SD)
brand A	78.4 ± 7.8	brand I	39.9 ± 7.5	brand 1	17.3 ± 2.3
brand B	40.1 ± 2.2	brand II	19.7 ± 3.0	brand 2	ND[a]
brand C	20.2 ± 3.8	brand III	ND	brand 3	ND
brand D	32.7 ± 5.1	brand IV	ND	brand 4	ND
brand E	26.5 ± 3.6	brand V	12.8 ± 2.5	brand 5	ND
brand F	21.5 ± 6.7	brand VI	ND	brand 6	ND
brand G	38.2 ± 6.0	brand VII	ND	detection rate	17%
brand H	18.5 ± 4.9	brand VIII	ND		
brand I	16.4 ± 11.5	brand IX	ND		
brand J	ND	brand X	ND		
brand K	60.0 ± 9.4	brand XI	10.6 ± 1.2		
mean[b]	35.2 ± 20.2	brand XII	ND		
min.[c]	16.4 ± 11.5	brand XIII	ND		
max.	78.4 ± 7.8	mean[b]	24.1 ± 12.9		
detection rate	91%	min.[c]	10.6 ± 1.2		
		max.	39.9 ± 7.5		
		detection rate	31%		

[a] ND = not detected. [b] Mean values were calculated from all data except for ND. [c] Minimum values were based on all data except for ND. [d] Mean, minimum, and maximum values for lotus root were not calculated because only one result was available.

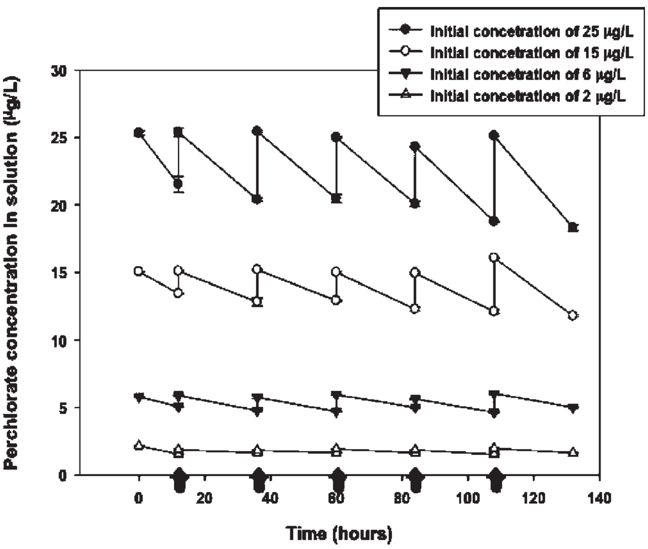

Figure 3. Results of the accumulation experiment using soybean sprouts with five different concentrations in artificial water. Arrows indicate replacement of fresh artificial water.

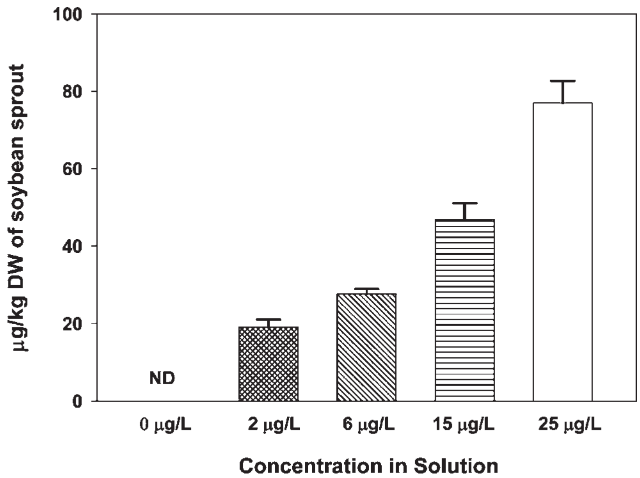

Figure 4. Results of perchlorate accumulation in soybean sprouts at different initial perchlorate concentrations of artificial solution.

five different perchlorate contaminated solutions are shown in Figure 3. At each of the five different perchlorate solutions, soybean sprouts removed about 18% of the perchlorate from the solution every 24 h (8 times rinsed). As the perchlorate concentration in the solution was increased from 2 to 25 μg/L, the concentration of perchlorate remaining in the solution decreased from 84 to 79% of the initial concentration for each day. In addition, the perchlorate removal efficiency increased with the increase in growth of the soybean sprouts in all of the perchlorate contaminated solutions. The results from the accumulation experiment using soybean sprouts with five different perchlorate solutions are shown in Figure 4. Perchlorate was not detected in soybean sprouts exposed to the control solution. The concentrations of perchlorate in the soybean sprouts increased gradually as the perchlorate concentration in the solution was increased. When the initial perchlorate concentration in the solution was 25 μg/L, the concentration in the soybean sprouts was 76.9 μg/kg DW. Despite the low concentration (2 μg/L) of perchlorate in the solution, the perchlorate concentration in the soybean sprouts was approximately 10 times more than the exposure concentration. Previous studies have shown that various plants including soybean are capable of perchlorate

Table 3. Perchlorate Concentration in Artificially Contaminated Solution and Perchlorate Bioaccumulation Factors of Soybean Sprout after Growth for 6 Days

ClO_4^- in solution (μg/L)	ClO_4^- in soybean sprout (μg/kg DW ± SD)	BCF
control[a]	ND[b]	NC[c]
2	19.1 ± 1.9[d]	9.6 ± 1.0[d]
6	27.6 ± 1.4	4.6 ± 0.2
15	46.9 ± 4.2	3.1 ± 0.3
25	76.9 ± 5.7	3.1 ± 0.2

[a] Control = without perchlorate. [b] ND = not detected. [c] NC = not calculated. [d] All values are means of triplicates ± SDs.

uptake if exposed to perchlorate.[19,20,31] A separate study demonstrated that perchlorate was readily taken up in soybean leaves and roots in the 4 week soybean uptake experiment. Although perchlorate concentrations in soybean leaves were significantly higher than those in soybean roots at the end of experiment, the large amount of perchlorate accumulated in the roots before transported to the leaves.[30] In our study, the results of these experiments indicate that soybean sprouts have a strong capability to accumulate perchlorate from the water resources, even if the solution has low levels of perchlorate concentration, supporting the previous studies.

The bioconcentration factor (BCF), defined as the ratio of the perchlorate concentration in the plants to that in the solution, provides an index of the ability of the plant to absorb and accumulate perchlorate with respect to its concentration in the contaminated water. Table 3 shows that the BCF was found to be more than 3.1 for all perchlorate solutions. A difference in BCF values was observed depending on the exposure concentration. There was a general decrease in the BCF with increasing perchlorate concentration in the solution after 6 days of growth. When the initial perchlorate concentration of the solution was 2 μg/L, the BCF was high (9.6) and then decreased with the increase in perchlorate concentration (3.1 at 25 μg/L). As a result, the accumulation of perchlorate did not follow a linear relationship with the perchlorate concentration in the solution.

When the results from the accumulation tests are compared with the survey data of vegetables from our study, an assumption can be made that soybean sprouts, which were obtained from local markets, might be exposed to perchlorate contaminated irrigation water with various concentrations ranging from 2 to 25 μg/L. However, for the market survey, it is plausible that the soybean seeds were contaminated with perchlorate before they were sprouted. Therefore, although the detection of perchlorate in soybean sprout samples and their ability to accumulate perchlorate from water sources have been established in this study, more extensive research is necessary to determine the extent of perchlorate contamination of vegetables.

In conclusion, the results of the market survey and the accumulation tests suggest that in South Korea, exposure to perchlorate presumably occurs not only through drinking water but also through consuming vegetables. Perchlorate intake from vegetables is of great importance for humans because high levels of perchlorate in vegetables are attributed to trace levels of perchlorate in water sources, and these vegetables are ultimately consumed by humans. In addition, the water sources for vegetables are always of local origin, and the perchlorate levels in vegetables are closely related to the perchlorate that is dispersed in the local environment; hence, more extensive research is needed in the future to determine the source and fate of perchlorate in surface water and groundwater.

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