ARSENIC CONTENTS AND SPECIATION AT DIFFERENT GROWTH STAGES OF *SARGASSUM FUSIFORME* [HARV.] SETCHELL (HIJIKI), AN EDIBLE SEAWEED

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Abstract. *Sargassum fusiforme* (hijiki) is a popular edible seaweed in some Asian countries. However, it has been shown to have high concentrations of arsenic, mainly the more toxic inorganic arsenic. In this study, we determined the concentration, species, distribution, and absorption kinetics of arsenic (As), and the influence of different exogenous substances on its absorption. Arsenic content in the mature stage of *hijiki* reached up to 84.37 mg/kg dry weight. Inorganic arsenic (iAs) accounted for 68% of the total arsenic, while Arsenate (As (V)) accounted for more than 50% of total inorganic arsenic. The contents of arsenic in different *hijiki* organs decreased in the following order rhizoid > stem > leaf > airbag. Subcellular distribution of arsenic in untreated *hijiki* decreased in the order: cell walls > cell organelles > cytoplasm. In *hijiki,* arsenate had a higher absorption rate than arsenite (As (III)), with V_{max} and k_m about 2 times and 3 times those of As (III), respectively. The absorption of As (V) was inhibited by phosphorus, but was not affected by glycerol. The opposite was true in the case of As (III) adsorption. Sodium vanadate significantly increased the efflux of As (V), but had no effect on As (III). Carbonylcyanide-p-chlorophenyl hydrazone (CCCP) and glycerol inhibited the arsenite efflux, but had no influence on arsenate.

Keywords: *arsenic speciation, arsenic distribution, arsenic absorption, influx, efflux*

Introduction

Arsenic is a semi-metal or metalloid which is widely distributed in the environment and it is present in both terrestrial and aquatic systems (Wang et al., 2019). It is a known human carcinogen (Rosen, 2002; García Salgado et al., 2008; Carey et al., 2012). The Agency for Toxic Substances and Disease Registry (ATSDR) ranked arsenic at the top of the substance priority list (ATSDR, 2018). The toxicity of arsenic is especially associated with liver, bladder, lung, and skin cancer (Rose et al., 2007). This element occurs in various chemical forms with different toxicological characteristics in the environment and organisms (Kohlmeyer et al., 2003; Panuccio et al., 2012). Arsenic speciation in the environment is complex and includes both inorganic and organic forms. Inorganic arsenic comprises of arsenate and arsenite. Organic arsenicals include monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), trimethylarsine (TMA), tetramethylarsonium ion (TMA), arsenobetaine (AsBet), arsenocholine (AsCho), and arsenosugars (Kohlmeyer et al., 2003; Quaghebeur et al., 2005). Moreover, inter-conversions between species regulated by both biotic and abiotic processes have been reported (Carey et al., 2012).

Generally speaking, inorganic arsenicals are more toxic than their organic counterparts (Nogueira et al., 2018), and arsenite is much more toxic, soluble, and

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mobile than arsenate (Quaghebeur et al., 2005; Raab et al., 2007; Yang et al., 2016). Organic arsenicals such as DMA and MMA usually have lower toxicities. Therefore, determination of arsenic speciation is essential for understanding and evaluating the safety of edible organisms which accumulate arsenic.

Marine algae are usually thought of as "health food" and consumed directly as such. This is because of the nutritional and therapeutic benefits that they provide. *Sargassum fusiforme* (*hijiki*), a brown edible algae (*Phaeophyta*, *Sargassum*), is traditionally consumed by the Japanese as one of seaweed foodstuffs due to its richness in essential minerals, and dietary fibre content. The total fiber in *hijiki* was much higher compared with other seaweeds, such as *Laminaria japonica*, *Porphyra tenera*, which showed more benefits to human health especially to intestines. (Zheng et al., 2013) However, marine algae have a greater ability to accumulate arsenic and usually have higher arsenic contents than terrestrial organisms (Hanaoka et al., 2006). In terrestrial organisms, the arsenic content rarely exceeds 1 μg/g (dry weight), while it ranges from 1 to 100 μg/g in marine organisms (Ichikawa et al., 2006).

The concentration of arsenic varies in different organisms, as well as its species. In rice, the predominant inorganic arsenic is arsenite, whereas in marine algae, inorganic arsenic is present mostly as arsenate (Kohlmeyer et al., 2003). It has been reported that the arsenic species in marine organisms are usually organic arsenicals such as arsenobetaine and arsenosugars which are forms considered less toxic than the inorganic forms of arsenic (Ichikawa et al., 2010). However, there are exceptions. In *hijiki*, the concentration of iAs is up to 135 mg/kg of dry weight, while the content of inorganic arsenic may range from 50 to 80% of its total arsenic content (Almela et al., 2005; Yokoi et al., 2012). Thus, the high contents of inorganic arsenic in hijiki have raised serious toxicological concerns among consumers in the past decades (Wondimu et al., 2007). Indeed, the Food Inspection Agency of Canada (2001), the UK Food Standards Agency (2004), and similar institutions in other countries, have warned consumers not to eat hijiki (Wondimu et al., 2007; Yokoi et al., 2012).

In this study, we determined the concentration, species, distribution, and absorption kinetics of arsenic, and the influence of different exogenous substances on its absorption.

Materials and Methods

Sample preparation

The research was carried out in the year of 2016. The samples used in this research were cultivated, in Dongtou breeding base of Zhejiang Mariculture Research Institute (Dongtou County, Zhejiang Province, China), under natural conditions. In order to track the changes of arsenic content in hijiki, we took samples at monthly interval from January to May, that was cover the growth period of hijiki from seedling to maturity. There were two varieties of hijiki were sampled. Fresh weight of the native species (250 g of whole plant) was sampled monthly, whereas 250 g of each Korean sample (whole plant) was collected from January to March. Three separate samples were collected each time. The samples were washed in seawater, kept in plastic bags, and immediately transported to the laboratory. All samples were then washed with tap water, followed by washing three times in ultra-pure water. Thereafter, each sample was freeze-dried to a constant mass and ground to homogenous powder before use. For the

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study of arsenic distribution, different organs (stem, leaf, air sac and rhizoid) were separated before freeze-drying, and then ground to homogenous powder.

Determination of total arsenic in hijiki (S. fusiforme) and different hijiki organs

Milled sample of *hijiki* (0.2 g) was added to 6 ml concentrated nitric acid. The mixture was left for 12 h, and then digested in a MARS 5 microwave oven. Each sample was digested in triplicate. The following conditions were used in the microwave digestion which was modified by Han et al. (2009): the temperature was increased to 120°C in 5 min, held for 5 min, then increased to 160°C in 5 min, kept constant for 20 min, and finally increased to 180°C for 20 min. After dilution and filtering, the digest was brought to a final volume of 25 ml with ultra-pure water. Then, total arsenic was measured using hydride generation-flame atomic absorption spectrometry (HVG-FAAS).

Determination of inorganic arsenic

The inorganic arsenic was extracted in accordance with the procedure of National Standards of China (National Food Safety standard, 2003).

Hijiki powder (1.00 g) was weighed into a 25 ml test tube, and 20 ml of 50% (v/v) hydrochloric acid was added. The mixture was kept in a water bath at 60°C for 18 h, during which time it was shaken several times in order to complete the extraction. On cooling to room temperature, the solution was filtered, and 1 ml potassium iodide (10% m/v) - thiocarbamide (5% m/v) was added to 4 ml of the filtrate. The volume of the solution was made up to 10 ml with ultra-pure water. Inorganic arsenic was determined using HVG-FAAS.

Determination of arsenic speciation

Arsenic speciation was determined using the method referred to in the manufacturer's manual of the SA-10 Atomic Fluorescence Speciation Analyzer. Each *hijiki* sample was weighed (0.5 g) and put in a test tube, 4 ml of 10% (v/v) HCl was added. Then, the sample was spun at 100 rpm in a water bath at 70°C for 1 h, and 4 ml of ultra-pure water was added. The mixture was warmed in a water bath at 70°C for 1 h, and centrifuged at 3000 rpm for 15 min. The supernatant (2 ml) was mixed with 2 ml 20% (v/v) hydrogen peroxide and put into a water bath at 70°C for 20 min. Thereafter, the mixture was filtered through a 0.45 μm filter membrane before injection into a chromatographic column. The speciation was analysed using SA-10 Atomic Fluorescence Speciation Analyzer (SA-10 AFSA). Triplicate analyses were performed for each sample.

Subcellular distribution of arsenic in hijiki

Fresh sample (0.5 g) and samples treated with 5 μmol/L and 50 μmol/L A sodium arsenate (As) for 24 h were used for subcellular distribution experiments. The samples were homogenized with precooled homogenate, and all homogenization and separation processes were carried out on ice. The total volume of homogenate and tissue was controlled at about 20 ml. The homogenate was transferred into a 50-ml centrifuge tube, and ultrasonic cell breaker was used for ultrasonic crushing. Thereafter, it was centrifuged in a high-speed freezing centrifuge for 30 sec at 300 g, and the precipitate (cell wall component) was collected. The supernatant was centrifuged in a high-speed

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freezing centrifuge for 45 min at 20000 g. The bottom debris contained the cytosolic organelles, while the supernatant was the cytosolic portion containing macromolecular organic matter and inorganic ions in the cytoplasm and vacuole. Each of the portions from centrifugation was subjected to microwave digestion, and the content of arsenic was determined as described earlier.

Kinetics of arsenic uptake by hijiki

Fresh *hijiki* samples were cleaned with ultra-pure water and dried with absorbent paper. About 15-g samples of *hijiki* were weighed and put in 0.5 L artificial seawater with different concentrations of arsenate or arsenite (0, 10, 20, 40, 60 and 80 μmol/L), each of which was repeated 3 times. After 30 min of treatment, all samples were soaked in phosphate buffer solution (1 mmol/L K_2HPO_4, 5 mmol/L MES, 0.5 mmol/L $CaCl_2$, pH 6.0) for 10 min to wash off the As on the surface (Xu et al., 2007). Then, samples were washed with ultra-pure water, and the surface water was removed with absorbent paper, followed by drying. Finally, the arsenic content was determined as described earlier.

Arsenic influx and efflux in hijiki

This experiment was used to determine if arsenate and arsenite are taken up via the P and aquaporin transporters, respectively. The effect of P ($K_2HPO_4 \cdot 3H_2O$) and glycerol on arsenic influx were investigated. Shoots of the plant (about 15 g) were subjected to three treatments: control (50 μmol/L sodium arsenate As (Ⅴ) or sodium arsenite As (Ⅲ) solution +100 μmol/L P ($K_2HPO_4 \cdot 3H_2O$) or + 20 mmol/L glycerol. After treating for 1 h, the samples were immersed in 1 mmol/L K_2HPO_4 solution containing 5 mmol/L MES and 0.5 mmol/L $CaCl_2$, pH 6.0, in ice bath for 10 min to wash off the surface As (Xue et al., 2012). Then, the samples were washed and dried to constant weight, and ground into powder. The arsenic content was determined after microwave digestion.

The metabolic inhibitor carbonyl cyanide m-chlorophenylhydrazone (CCCP), P-type ATPase inhibitor sodium vanadate, and glycerol were used to investigate whether the efflux of As by *hijiki* followed the same transport pathway as its influx. The samples of *hijiki* treated with 50 μmol/L arsenate As (V) or arsenite As (III) for 24 h were soaked in 1 mmol/L K_2HPO_4 solution containing 5 mmol/L MES and 0.5 mmol/L $CaCl_2$, pH 6.0, in ice bath for 10 min (Xue et al., 2012). Then the samples were cleaned and divided into 4 groups, and exposed to fresh artificial seawater with different treatments: control (artificial seawater), 1 μmol/L CCCP, 200 μmol/L sodium vanadate, or 10 mmol/L glycerol. After 2 hours of treatment, 25 ml water samples were taken from each group, and the arsenic content was determined after filtration with 0.45 μmol/L microporous membrane.

Triplicate analyses were performed for each sample. The final results were expressed in mean±SD.

Results

Total and inorganic arsenic contents

The total and inorganic arsenic contents at different growth stages of the two *hijiki* varieties (Chinese and Korean) are shown in *Table 1*. The total arsenic concentration varied from 35.05 to 84.37 mg/kg (dry weight) at different growth stages from January

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to May. With growth of the seaweed, the total arsenic concentration increased. The variation in the levels of inorganic arsenic was in accord with that of total arsenic concentration, varying from 24.21 to 58.00 mg/kg dry weight. Thus, the arsenic concentration in the two hijiki varieties increased with growth.

Table 1. *Arsenic contents at different stages of growth of two Sargassum fusiforme varieties*

Stage of growth	Native variety			Korean variety		
	t-As	i-As	% i- As	t-As	i-As	% i- As
Jan	48.72±7.98a	40.29±4.27f	82.69%	35.05±2.56d	24.21±1.89h	69.07%
Feb	56.98±7.97a	49.18±11.21fg	86.31%	38.31±4.72d	37.99±2.80fgi	99.16%
Mar	68.54±6.48b	51.30±4.55g	74.85%	56.97±3.67ae	45.44±1.14gj	79.76%
Apr	72.29±4.17b	54.08±2.21g	74.81%	ND	ND	ND
May	84.37±8.27c	58.00±0.91g	68.74%	ND	ND	ND

t-As: total arsenic. i-As: inorganic arsenic. percentage of i- As: percentage of inorganic arsenic. ND: not determined. Data are expressed in mg/kg dry weight, and are mean of triplicate assays. The final results were expressed in mean±SD

The percentage of inorganic arsenic varied from 68.74 to 86.31% in the native variety. In general, in native *hijiki*, the percentage of inorganic arsenic decreased with growth. This trend was close to those reported previously i.e. 69 to 72% (Rose et al., 2007).

Arsenic contents in different hijiki organs

The arsenic contents of different *hijiki* organs showed a lot of variation. The arsenic levels in the rhizoid, leaf, air sac and stem were 31.92, 27.23, 20.66 and 14.66 mg/kg dry weight, respectively. These results are shown in *Figure 1*.

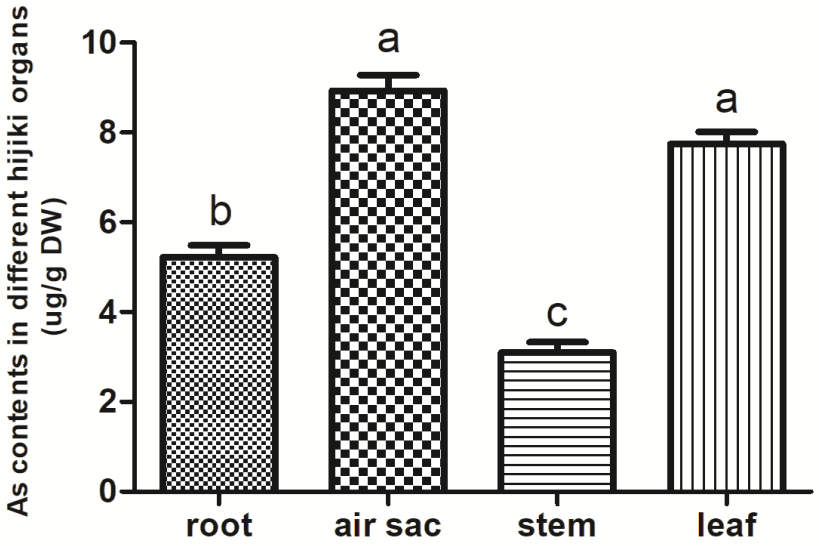

Figure 1. *Arsenic contents in different organs of Sargassum fusiforme*

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Subcellular distribution of arsenic in hijiki

The subcellular distribution of arsenic in *hijiki* is shown in *Figure 2*. The results showed that the subcellular distribution and compartmentalization of arsenic in *hijiki* differed greatly. In the untreated *hijiki*, the content of arsenic decreased in the order: cell wall > organelle > cell fluid, accounting for 48, 33 and 19% of total arsenic, respectively. However, the arsenic content in each component of *hijiki* increased after 5 and 50 μmol/L arsenic treatment. After 5 μmol/L sodium arsenate treatment, the order of arsenic content was: cell wall > cell fluid > organelle, accounting for 53, 24 and 23% of the total, respectively. After 50 μmol/L As treatment, the content of arsenic in each part was in the order of cell wall > cell fluid > organelle, accounting for 51, 11 and 38% of the total, respectively. The content of arsenic varied in the order of cell wall > cell fluid > organelle after treatment with 5 μmol/L As and 50 μmol/L As, with highest proportion of arsenic in cell wall. Moreover, the increment in arsenic level in cell wall was the largest after 5 μmol/L As treatment, but the increment of arsenic in cell fluid was the largest after 50 μmol/L As treatment. Thus, the increase in arsenic was mainly concentrated in the cell wall of *hijiki* under low concentration of arsenic, while the increase in arsenic was mainly concentrated in the cell fluid after treatment with high concentration of arsenic.

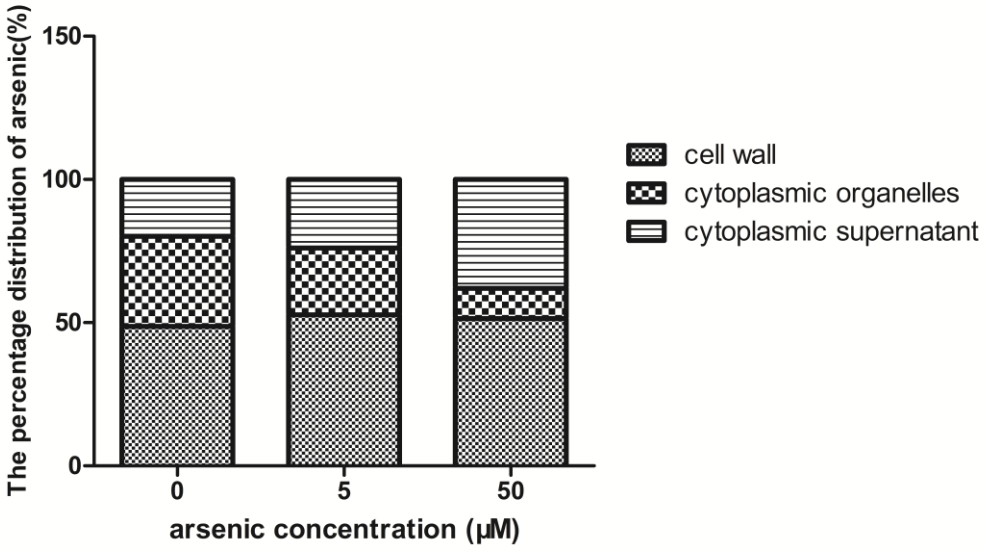

Figure 2. *Subcellular distribution of arsenic in Sargassum fusiforme*

Results of arsenic speciation analysis

The species of arsenic were determined with SA-10 Atomic Fluorescence Speciation Analyzer. The contents of arsenic species in each growth stage of the two *hijiki* are shown in *Table 2*. In this study, the extraction efficiency ranged from 75 to 90%. Irrespective of the growth stage, inorganic arsenic (especially arsenate) was always the main form of arsenic present, accounting for about 77 to 88% of the total arsenic.

The contents of four arsenic species decreased in the order: AsV > AsIII > DMA > MMA. The organic arsenic content was negligible: the DMA and MMA contents were 1 - 4% and 1 - 3% of the total arsenic, respectively.

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Table 2. *Contents of four arsenical species in different growth stages of the two Sargassum fusiforme varieties*

Growth stage	t-As	As (Ⅴ)	As (Ⅲ)	DMA	MMA
Native					
Jan	48.72±7.98	30.06±2.10	6.21±1.32	0.68±0.05	0.22±0.01
Feb	56.98±17.97	34.23±6.59	7.2±0.48	1.24±0.12	0.34±0.07
Mar	68.54±16.48	42.46±10.13	7.78±1.09	0.49±0.10	0.21±0.08
Apr	72.29±22.17	56.92±10.52	6.25±0.74	0.92±0.09	0.26±0.09
May	84.37±8.27	67.63±8.43	7.18±1.22	1.18±0.14	1.02±0.02
Korean					
Jan	35.05±2.56	24.75±2.40	6.75±1.56	1.04±0.06	0.19±0.16
Feb	38.31±4.72	28.24±0.25	7.47±0.45	0.73±0.05	0.32±0.07
Mar	56.97±3.67	34.24±9.35	7.67±2.72	1.66±0.08	0.39±0.04

Native: native *hijiki* variety. Korean: Korean *hijiki* variety. t-As: total arsenic. As (Ⅴ): arsenate. As (Ⅲ): arsenite. DMA: dimethylarsinic acid. MMA: monomethylarsonic acid. Results expressed in mg/kg dry weight and as mean of triplicate assays

Kinetics of arsenic uptake

The results showed that with increase in As concentration, arsenic absorption increased gradually, and the trivalent arsenic tended to be stable when the arsenic concentration reached 20 μmol/L. The absorption of the pentavalent arsenic tended to be stable after the arsenic concentration reached 60 μmol/L (*Figure 3*). The kinetics of arsenic and arsenate were in accord with the Michaelis–Menten equation, with R^2 values of 0.9744 and 0.9624 for arsenic and arsenate, respectively. The V_{max} for arsenate uptake (1202 ng/g DW min) was about twice that of arsenic uptake (619 ng/DW min). The K_m value of arsenate (12.91 μmol/L) was about three times that of arsenic (4.591 μmol/L).

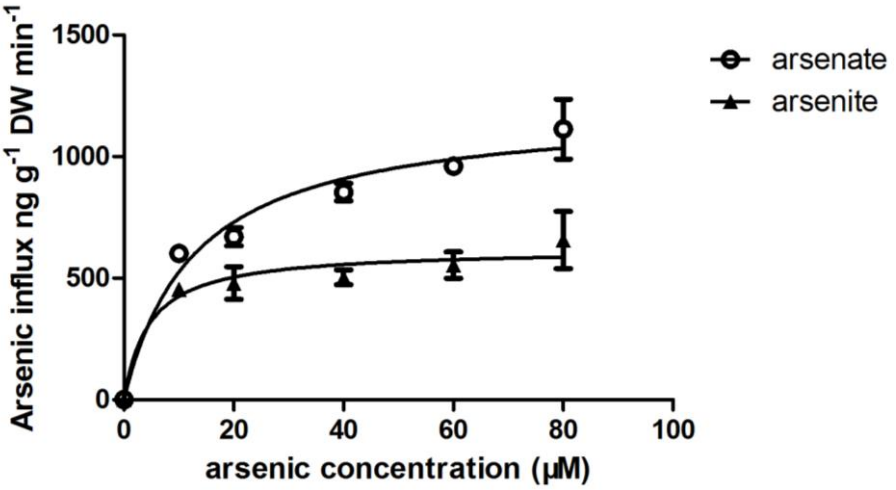

Figure 3. *Concentration-dependent kinetics for arsenate (open circles) and arsenite (closed triangles) uptake in Sargassum fusiforme*

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Arsenic influx and efflux in hijiki

After treatment with 50 μmol/L As for 1 h, the contents of arsenic and arsenate in *hijiki* were 61.8 and 39.9 μg/g dry weight, respectively. The absorption of arsenic was significantly higher than that of arsenate, about 1.5 times higher. The absorption of arsenate was obviously inhibited by phosphorus, while arsenic was not inhibited by phosphorus. Although glycerol inhibited the absorption of arsenic, it had little effect on the absorption of arsenate (*Figure 4*). In the control group, the amount of arsenic in *hijiki* was slightly higher than that of arsenate. After CCCP, sodium vanadate and glycerol were added to the solution. Sodium vanadate significantly increased the efflux of arsenate, but it had no effect on the efflux of arsenic. Treatment with CCCP and glycerol inhibited the efflux of arsenic, but there was no significant difference between them, and they had little effect on the efflux of arsenate (*Figure 5*).

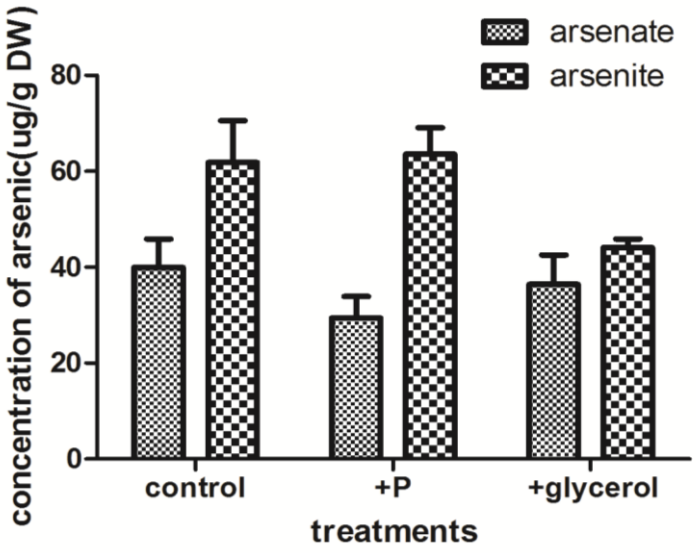

Figure 4. *Influence of P (100 μmol/L) and glycerol (20 mmol/L) on the uptake of arsenate and arsenite*

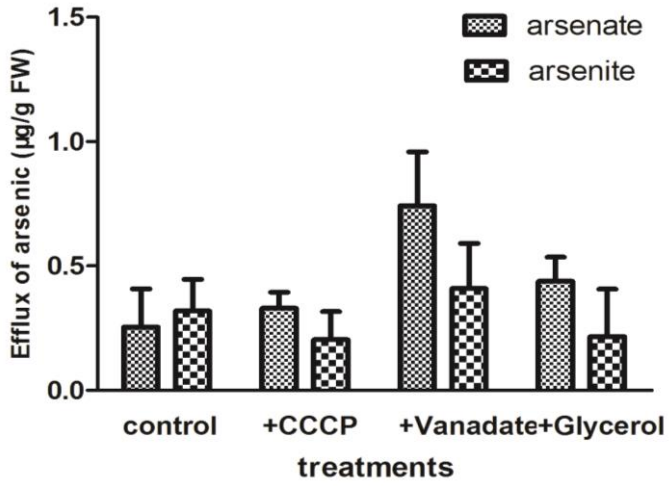

Figure 5. *Effects of CCCP (1μmol/L), sodium vanadate (200 μmol/L), and glycerol (10 mmol/L) on the efflux of arsenate and arsenite*

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Discussion

Several studies have reported total and inorganic arsenic contents in seaweeds. A study on a wide variety of seaweeds found that the concentrations of total arsenic varied from 2.2 to 149 mg/kg dry weight, and that the total arsenic content was related to the type of seaweed, decreasing in the order: brown seaweed > red seaweed > green seaweed (Almela et al., 2002). The arsenic contents of *hijiki* were especially high (68.3 to 149 mg/kg dry weight), as had been reported in the literature, which indicated that it could attain 179 mg/kg dry weight (Almela et al., 2002, 2006; Rose et al., 2007; Hamano-Nagaoka et al., 2008; Yokoi et al., 2012). Thus, the contents detected in the present study were high, but were still low, when compared to values reported in other studies. In the organs, the levels decreased in the order: rhizoid > leaf > air sac > stem. Since rhizoid is an inedible part of *hijiki*, this result suggests that *hijiki* with more stem and leaves than air sac should be selected.

In this research, the total arsenic content of the native *hijiki* was always higher than that of the Korean *hijiki*. These results differ from those of Ichikawa (2006), in which the ranges of total arsenic concentrations in *hijiki* gathered from the shores in Japan, South Korea, and China were 41.7- 46.7, 65.6 - 79.8, and 36.0 - 48.6 mg As/kg, respectively. Besides, arsenic concentrations in *hijiki* grown in gulfs were higher in than those in *hijiki* grown in coastline of open sea. This may have been influenced by lower pollutant concentrations and increased circulatory flow in the coastline of open sea. However, the arsenic concentrations in *hijiki* increased with growth. In this study, we also detected the arsenic content in the seawater from where the *hijiki* planted. The results showed that the content of arsenic in seawater is very low (1-7 ug/L). So we think that the arsenic contents in *hijiki* were not consistent with that in the seawater. The high content of arsenic in *hijiki* is not caused by the amount of arsenic in seawater. This may indicate that the accumulation of high arsenic content in *hijiki* was not caused by the arsenic content of seawater but by the high ability of arsenic absorption of hijiki.

Marine organisms have been examined extensively for arsenic species in recent decades. Marine animals contain mainly arsenobetaine, and nearly no MMA, DMA, TMA, AsCho, or TMAs (Kohlmeyer et al., 2003). Arsenosugars and arsenobetaine have been found in bivalves (Lai et al., 2001). In fish, there is hardly any inorganic arsenic or arsenosugars but trimethylarsoniopropionate has been detected in fish muscle (Francesconi et al., 2000). Many scholars have reported that the main arsenic species in *hijiki* is arsenate, and the level can reach approximately 65% of the total arsenic (Kohlmeyer et al., 2003; Raab et al., 2005; Hanaoka et al., 2006; Ichikawa et al., 2006; Hamano-Nagaoka et al., 2008). Park et al. (2019) also found that arsenate was the predominant arsenic species in *hijiki*, accounting for approximately 60% of the total arsenic content. These are similar to the results of this study, although the proportion of arsenate was greater (77 to 88%): the proportion of arsenite was between 10 to 20%. The concentration of organic arsenic was low, the DMA content being just 1 to 4% of the total arsenic present, and the MMA content was much smaller. These data are in agreement with the findings of Ichikawa et al. (2006).

In this study, after treatment with 50 μmol/L arsenic and arsenate for 1 h, the content of arsenate in *hijiki* was higher than that of arsenic. Compared with arsenic, arsenate had higher V*max* and smaller K_m value in *hijiki*, and the absorption of arsenate was higher than that of arsenic. Compared with other aquatic plants such as *Azolla caroliniana*, *Azolla filiculoides* and *Ceratophyllum demersum*, *hijiki* has higher degree of absorption and lower K_m value for arsenate (Xue et al., 2012). This also shows that *hijiki* has a

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transport system with high affinity for arsenate. In the study of Xue et al. (2012), the V_{max} values of arsenic and arsenate in *C. demersum* were 214 and 128 nmol/L/g DW min, respectively, which were lower than that in *hijiki.* The K_m values of arsenic and arsenate in *C. demersum* were 18 and 120 μmol/L, respectively, which were about 1.5 and 25 times, respectively higher than that of *hijiki.* Compared with *C. demersum, hijiki* had higher affinity for arsenic and arsenate.

Phosphorus and arsenic are homologous elements. The chemical properties of phosphate and arsenate are similar. Therefore, in higher plants, arsenate enters plants by sharing the same uptake and transport protein with phosphate (Asher and Reay, 1979). The absorption process is mainly completed by the coordinated transport of arsenate or phosphate ($H_2PO_4^-/H_2AsO_4^-$) and protons. The results of this study also showed that the absorption of arsenic in *hijiki* was inhibited by P in the environment, indicating that As and P share the same absorption channel in *hijiki*. Some plants can adjust the expression of phosphate transporters to reduce the absorption of arsenate, thereby adapting to the high concentration of arsenic pollution in soil (Meharg and Hartley Whitaker, 2002).

Unlike arsenate, the absorption of arsenic is not affected by phosphate. Arsenic has been shown to be able to enter the cell through water channel proteins in many plants (Meharg and Jardine, 2003; Zhao et al., 2009). Xue et al. (2012) found that the absorption of arsenate by *C. demersum* was significantly inhibited by P ($K_2HPO_4 \cdot 3H_2O$) in solution, but was not inhibited by glycerol and Sb ($C_4H_4KO_7Sb \cdot 1/2H_2O$) in solution, while the absorption of arsenic was the opposite. This also shows that the uptake of arsenate in *C. demersum* shares the same transport protein with phosphate, and arsenic can enter the cells of *C. demersum* through water channels. In this study, it was found that the absorption and efflux of arsenic in *hijiki* were inhibited by glycerol, which is consistent with the previous results for *Saccharomyces cerevisiae* and rice (Wysocki et al., 2001; Meharg and Jardine, 2003). It can also be concluded that, like other plants, arsenate is mainly transported in and out of cells by active transport, while arsenic is mainly transported in and out of cells by passive transport.

Conclusion

Overall, the results support the view that *hijiki* has a high concentration of inorganic arsenic. The dominant arsenic species in *hijiki* was found to be arsenate. To guarantee public food safety, besides warning people not to eat *hijiki,* future research into the selection of a *hijiki* variety which accumulates less arsenic, such as the Korean *hijiki* studied here, is recommended. Moreover, it is important to select a variety which has more leaves and stem instead of varieties with more air sacs. Harvesting *hijiki* a little earlier may also be a way of ensuring decreased accumulation of arsenic.

Research on the absorption and metabolism of arsenic can help in understanding of the mechanism of arsenic uptake and transport. This study lays the foundation for As control technology in *hijiki*, and is important for the safety of edible *hijiki*, and the development of *hijiki* industry. But what does the mechanism of arsenic absorption in *hijiki*? How to reduce the arsenic absorption of *hijiki*? These are all issues that need further study in the future.

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