Bean sprouts (*Pisum sativum L.*) accumulate cadmium (Cd) in their foliage

Yumiko Nitta*

(Received on May 14, 2020)

ABSTRACT

To investigate the transfer of Cd from fertilizer into farm plants, a hydroponic experiment was performed with bean sprout. The concentrations of Cd in the foliage were measured by the Atomic Absorption Spectrometer. The bean sprout increased their weight for 1 week and then gradually decreased the rates of weight gain to the end of the experiment. When added the oyster shell powder (*Crassostrea gigas*) in the cultivation liquid as a source of the heavy metals, they accumulated Cd and zinc (Zn) in their foliage time dependently.

key words: bean sprout, cadmium (Cd), hydroponics, oyster shell, zinc (Zn)

INTRODUCTION

Cd and Zn are widespread ubiquitously with the Cd/Zn ratio of one hundredth in rocks, soils and seagrass beds[1–4]. Both Cd and Zn are present in the sediment and shells of oyster at the mouth of Otagawa river in Hiroshima bay, where the oysters were cultivated[5, 6]. There has been no regulation of the Cd contamination in oyster meat by the Food Safery Commission of Japan. No attention has been paid to the Cd contaminated in shells[7]. The cycle of Cd from benthic organisms to terrestiral organisms has received little attention. Meanwhile, several plants for recycling oyster shells as fertilizers began to operate in the Hiroshima Regional Urban Area.

The bean sprout are a nutritious vegetable that have been widely consumed in China, Korea and Japan[8]. The hydroponic cultivation enables the bean sprouts to enrich their nutritional value and allowed us to consume the vegetable throughtout year[9].

In order to confirm the transfer of Cd from oyster shells into vegetables, a hydroponic cultivation method was prefered for the bean sprout. This is a part of our project to make

* Department of the Health and Nutrition, Faculty of Health Sciences, Hiroshima Shudo University.

the circulation of Cd in the target districts visible, which could be informative for the health of resident people and the perspective of biodiversity.

MATERIALS AND METHODS

Oyster shell: Commercially available cultured oysters were used (Hiroshima Prefectural Union of Fishermen, Hiroshima, Japan). After removing meat, the shells were washed with running water, air-dried and ground by the stainless ball mill. The pulverized shells were sieved through a 2 mm screen to remove impurities. For the comparison of the appearance of the shell powder, three products from the three different companies were purchased.

Their concentrations of the metals were measured by the sediment determining methods of II-5.1 and II-5.4 for Cd and Zn, respectively. The mean value of the triplicated measurements was sued as representative for each sample.

Hydroponic cultivation of bean sprout: Commercially available bricks of bean sprout (Murakami Field Co, ltd., Hiroshima, Japan) were used. Each brick was placed in a 6.5L plastic tank of polysulfone (CL-0123-3, CLEA Japan Inc., Tokyo, Japan) with or without 100 g of oyster shell powder (Fig. 1). Deionized water of 0.5 L was added to cultivate bean sprouts hydroponically for 0, 1, 2 or 4 weeks in a laboratory room with natural light and temperatures from 11 to 20°C. The 0.5 L of deionized water was supplied every morning until the end of the observation duration. This every day's supply of water helped the irrigation of the hydroponic water in the tank. The hydroponic cultivation experiments were performed from December in 2018 to March in 2019. The oyster shell powder was prepared from the shells purchased in 2018.

Parts of the foliage were dried in a drying device (KM-300V, AS ONE, Osaka, Japan) overnight and then ground by a stainless ball mill and stored at −20°C until use. Drying ratios (mean ± SD) were 5.4 ± 0.8 times. For microwave digestion, 70% HNO_3 solution (reagent grade) was added to each sample. For drying, the sample was set on a hot plate at 180°C. After filled up to 10 ml with deionized water, the concentrations of their Cd and Zn were determined by the atomic absorption spectrometry (AA-6200, SHIMADZU, Kyoto, Japan). Values of the metals are presented per wet weight of the samples.

RESULTS

Cd concentration in oyster shell: The oyster shells cultured in Hiroshima bay contained 0.160 ± 0.071 mg/kg (mean ± SD) and 15.2 ± 9.1 mg/kg (mean ± SD) of Cd and Zn, respectively (Table 1). The 2018 powder contained 0.18 and 19.00 mg/kg of Cd and Zn, respectively.

Table 1. Concentrations of Cd and Zn in oyster shells.

	Cultured oyster (mean ± SD)	
	Hiroshima a)	Except Hiroshima b)
n	4	5
Cd (mg/kg)	0.16 ± 0.07	0.13 ± 0.03
Zn (mg/kg)	15.23 ± 9.10 c)	4.44 ± 1.80
Cd/Zn	0.013 ± 0.008	0.036 ± 0.024

a): Oysters were cultivated within the Hiroshima Regional Urban Area.
b): Oysters were cultivated in Hyogo, Miyagi (Ishinomaki and Onagawa), Miyazaki and Niigata prefectures.
c): $p < 0.02$ by *t*-test when compared to the Zn value of the prefectures except Hiroshima.

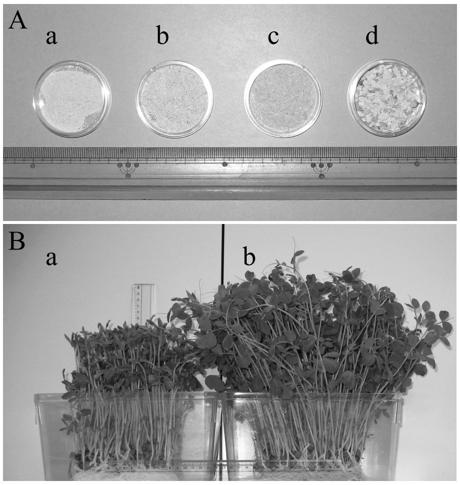

Fig. 1. Materials.
A: Oyster shell powders in ϕ30 mm dish. The experimentally used 2018 powder (**a**) and the other three commercially available oyster shell powders (**b**, **c**, **d**). **B**: Representative bricks of bean sprout hydroponically cultivated for 0 (**a**) and 1 (**b**) week. Plastic scales indicate 30 cm in maximum.

The oyster shell powder used in this experiment was composed with the finest particles compared to the others purchased commercially (Fig. 1-A). The color was whitest among the four kinds of powder products.

Cd concentration in bean sprout: The weight of the 52 bricks of bean sprout was 360.0 ± 35.4 (mean ± SD) g. Growth of the hydroponically cultured bean sprout was shown by the relative weight to the weight at 0 week (Fig. 2-A). The rates of weight

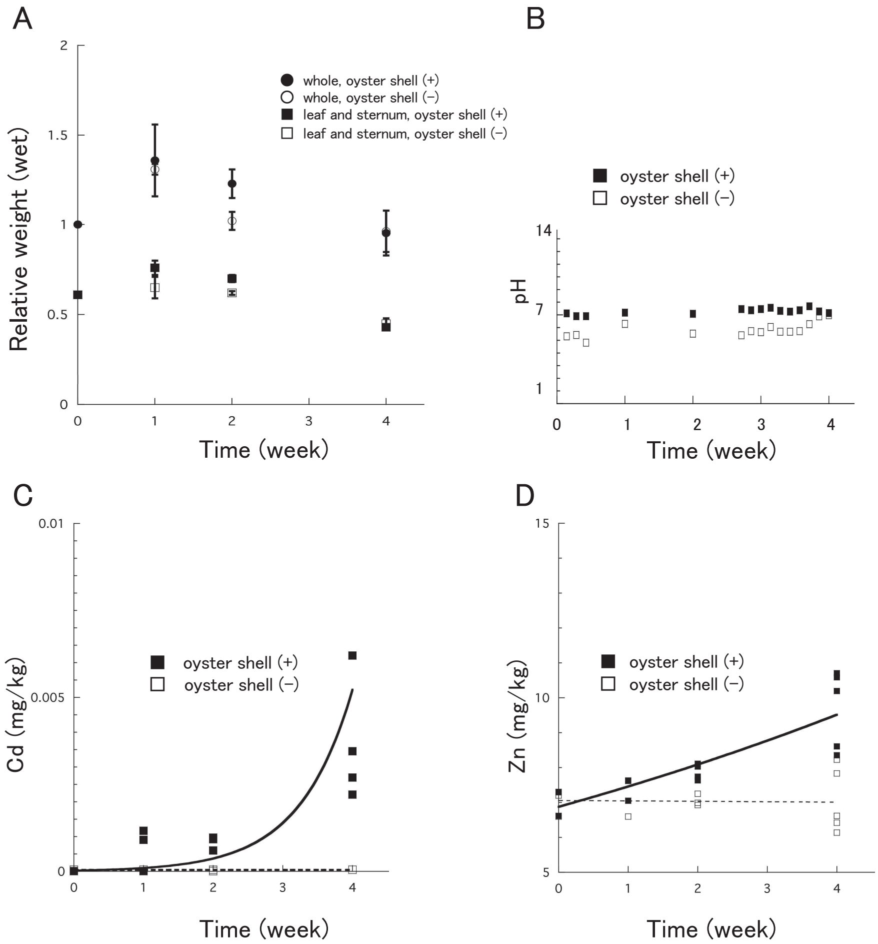

Fig. 2. Hypotonic cultivation of the bean sprout.
A: Weight changes of the bean sprout treated with or without oyster shell powder. Circle symbols (●, ○) indicate the weight of roots and foliage. Square symbols (■, □) indicate the weight of foliage. Vertical bars indicate standard deviation. **B**: pH transition of the cultivation solutions with or without oyster shell powder. **C**: Concentrations of Cd in the bean sprout. Solid line is expressed by the formula: $y=2.67 \cdot e^{-1.32 \cdot X}$ (R = 0.84). **D**: Concentrations of Zn in the bean sprout. Solid line is expressed by the formula: $y=6.80 \cdot e^{-0.69 \cdot X}$ (R = 0.85).

increase reached the highest at 1 week and then gradually decreased to the end of the observation period. There was no statistically significant difference in the relative weights between the two groups of with or without oyster shell except the culturing duration at 2 weeks.

The hydroponic condition monitored by the pH values was constant in the oyster shell treated group during the experimental periods (Fig. 2-B). While, the pH values increased after 3 weeks of cultivation in the oyster-non-treated group.

The bean sprouts accumulated Cd and Zn exponentially (Fig. 2-C, D). There was a time-dependency in accumulation.

DISCUSSION

The Cd in the oyster shells was able to be absorbed into the foliage of bean sprout experimentally in this study. This is one example of the flow of Cd from benthic organisms in sea to terrestiral organisms on land.

The measurement of Cd in oyster shells was informative to speculate the additional burden of Cd on the fields. Oysters biologically consentrates a certain amount of Cd from sea to the shell[8, 10]. The oyster shells cultured in Hiroshima bay showed the 0.013 ± 0.008 mg/kg of Cd, while, the fertilizers available in Japan contained 67 mg/kg of 45% Cd[11–14].

When the risks of heavy metal contamination for humans were assessed, foodstuffs are the main source of Cd[15–19]. Vegetables absorb Cd from soil[20–22]. Reported concentration of Cd in foods varied[23, 24]. Therefore, the exposure to Cd could be different personally[25]. The human activity to reuse oyster shells as one of the fertilizer components adds burden of Cd on our sanitary environment, since the oyster shell usage in the fields could be a contributor to the Cd circulation in land.

REFERENCES

1. Bouchon, C., Lemoine, S., Dromard, C. and Bouchon-Navaro, Y. (2016) Level of Contamination by Metallic Trace Elements and Organic Molecules in the Seagrass Beds of Guadeloupe Island. *Environ. Sci. Pollut. Res. Int.*, No. 23: 61–72.
2. Fishbein, L. (1981) Sources, Transport and Alterations of Metal Compounds: an Overview. 1. Arsenic, Beryllium, Cadmium, Chromium and Nickel. *Environ. Health Perspec.*, No. 40: 43–61.
3. Kubicka, K., Samecka-Cymerman, A., Kolon, K., Kosiba, P. and Kempers, A. J. (2015) Chromium

and nickel in Pteridium aquilinum from environments with various levels of these metals. *Environ. Sci. Pollut. Res. Int.*, No. 22: 527–534.

4. Zhu, X. M., Kuang, Y. W., Xi, D., Li, J. and Wang, F. G. (2013) Absorption of Hazardous Pollutants by a Medicinal Fern. *Blechnum Orientale L. Biomed. Res. Int.*, doi: 10.1155/2013/192986.
5. Nitta, Y. (2019) Oysters (C*rassostrea gigas*) in the Hiroshima Wide Area Urban Districts Concentrate Cadmium in Their Shells. *Studies Health Sci.,* No. 3: 1–8.
6. Nitta, Y., Miki, Y., Suenaga, M., Tanaka, H. and Katoh, K. (2019) The Measurement of Wildlife Exposure to Cadmium Contributes to Assess its Contamination in the Hiroshima Wide-area Urban Districts and the Human Health. *Hiroshima J. Vet. Med.*, No. 34: 77–84.
7. Lee, Y. H., Islam, M. S. A., Hong, S. J., Cho, K. M., Math, R. K., Heo, J. Y., Kim, H., and Yun, H. D. (2010) Composted Oyster Shell as Lime Fertilizer Is More Effective Than Fresh Oyster Shell. *Biosci. Biotechnol. Biochem.*, No. 74: 1517–1521.
8. Lee, E. J., Khan, M. S. I., Shim, J. and Kim, Y. J. (2018) Roles of oxides of nitrogen on quality enhancement of soybean sprout during hydroponicproduction using plasma discharged water recycling technology. *Sci Rep.*, doi: 10.1038/s41598-018-35385-5.
9. Xu, X., Yang, B., Qin, G., Wang, H., Zhu, Y., Zhang, K. and Yang, H. (2019) Growth, accumulation, and Antioxidative Responses of Two Salix Genotypes Exposed to Cadmium and Lead in Hydroponic Culture. *Environ. Sci. Pollut. Res.*, No. 26: 19770–19784.
10. Rodney, E., Herrera, P., Luxama, J., Boykin, M., Crawford, A., Carroll, M. A., and Catapane, E. J. (2007) Bioaccumulation and Tissue Distribution of Arsenic, Cadmium, Copper and Zinc in *Crassostrea virginica* Grown at Two Different Depths in Jamaica Bay, New York. *In Vivo.*, No. 29: 16–27.
11. Dharma-Wardana, M. W. C. (2018) Fertilizer Usage and Cadmium in Soils, Crops and Food. *Environ. Geochem.*, *Health*, doi.org/10.1007/s10653-018-0140-x
12. Lin, W., Lin, M., Zhou, H., Wu, H., Li, Z. and Lin, W. (2018) The Effects of Chemical and Organic Fertilizer Usage on Rhizosphere Soil in Tea Orchards. *PLos One,* doi: 10.1371/journal.pone.0217018.
13. Roberts, T. L. (2014) 2nd International Symposium on Innovation and Technology in the Phosphate Industry Cadmium and Phosphorous Fertilizers. *Issues Sci. Proc. Engineer.*, No. 83: 52–59.
14. Zhu, X., Beiyuan, J., Lau, A. Y. T., Chen, S. S., Tsang, D. C. W., Graham, N. J. D., Lin, D., Sun, J., Pan, Y., Yang, X. and Li, X. D. (2018) Sorption, Mobility, and Bioavailability of PBDEs in the Agricultural Soils, Roles of Co-existing Metals, Dissolved Organic Matter, and Fertilizers. *Sci. Total Environ.*, No. 619–620: 1153–1162.
15. Filippini, T., Cilloni, S., Malavolti, M., Violi, F., Malagoli, C., Tesauro, M., Bottecchi, I., Ferrari, A., Vescovi, L. and Vinceti, M. (2018) Dietary Intake of Cadmium, Chromium, Copper, Manganese, Selenium and Zinc in a Northern Italy Community. *J. Trace Elem. Med. Biol.*, No. 50: 508–517.
16. García, F., Ortega, A., Domingo, J. L. and Corbella, J. (2001) Accumulation of Metals In Autopsy Tissues of Subjects Living in Tarragona County, Spain. *J. Environ. Sci. Health*, No. 36: 1767–1786.
17. Hamid, A., Riaz, H., Akhtar, S. and Ahmad, S. R. (2016) Heavy metal contamination in vegetables, soil and water and potential health risk assessment. *American-Eurasian J. Agric. Environ. Sci.,* No. 16: 786–794.
18. Ikeda, M., Shimbo, S., Watanabe, T. and Yamagami, T. (2006) Correlation Among Cadmium Levels in River Sediment, in Rice, in Daily Foods and in Urine of Residents in 11 Prefectures in

Japan. *Int. Arch. Occup. Environ. Health*, No. 79: 365–370.
19. Pastorelli, A. A., Baldini, M., Stacchini, P., Baldini, G., Morelli, S., Sagratella, E., Zaza, S. and Ciardullo, S. (2012) Human exposure to lead, cadmium and mercury through fish and seafood product consumption in Italy: a pilot evaluation. *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess.*, No. 29: 1913–1921.
20. Casado, M., Anawar, H. M., Garcia-Sanchez, A. and Regina, I. S. (2008) Cadmium and Zinc in Polluted Mining Soils and Uptake by Plants. *Int. J. Environ. Pollut.*, No. 33: 146–159.
21. Guo, G., Lei, M., Wang, Y., Song, B., and Yang, J. (2018) Accumulation of As, Cd, and Pb in Sixteen Wheat Cultivars Grown in Contaminated Soils and Associated Health Risk Assessment. *Int. J. Environ. Res. Public Health,* doi: 10.3390/ijerph15112601.
22. Retamal-Salgado, J., Hirzel, J., Walter, I. and Matus, I. (2017) Bioabsorption and Bioaccumulation of Cadmium in the Straw and Grain of Maize (*Zea mays* L.) in Growing Soils Contaminated with Cadmium in Different Environment. *Int. J. Environ. Res. Public Health*, doi:10.3390/ijerph14111399
23. Kikuchi, Y., Nomiyama, T., Kumagai, N., Uemura, T. and Omae, K. (2002) Cadmium Concentration in Current Japanese Foods and Beverages. *J. Occup. Health,* No. 44: 240–247.
24. Tanaka, Y., Ikebe, K., Tanaka, R. and Kunida, N. (1974) Contents of Heavy Metals in Food (III). *Food Hyg. Saf. Sci.*, No. 15: 390–393. (in Japanese)
25. Marín, S., Pardo, O., Sánchez, A., Sanchis, Y., Vélez, D., Devesa, V., Font, G. and Yusà, V. (2018) Assessment of Metal Levels in Foodstuffs from the Region of Valencia (Spain). *Toxicol. Rep.*, No. 5: 654–670.

豆苗（*Pisum sativum L.*）はカキ殻肥料から カドミウムを吸収して茎・葉に蓄積する

新　田　由美子*

（受付：2020 年 5 月 14 日）

要　　　約

カドミウム（Cd）は環境汚染物質で，慢性曝露はヒトへ健康影響を及ぼす。農水畜産物の Cd 含有の実態を調査して情報提供することは，消費者の Cd 摂取量低減に寄与する。著者は，広島広域都市圏のマガキ（*Crassostrea gigas*）と野性動物とを定点観察し，Cd と亜鉛（Zn）の含有量を測定している。本稿では，カキ殻に含まれる Cd と Zn の野菜への移行を豆苗の水耕栽培で確認した。**材料及び方法**：2014～2018年に採集したカキ殻を粉末にして用いた。豆苗の茎と葉の Cd と Zn の濃度を原子吸光法で測定した。**成績**：①カキ殻に含まれる Cd と Zn は，0.16 mg/kg と15.23 mg/kg であった。②豆苗は Cd と Zn を栽培時間依存性に蓄積した。**考察**：広島広域都市圏でカキ殻を肥料として再利用する場合，カキ殻の含有する重金属量を把握しておくことは，ヒトの環境として公衆衛生上の安全性を担保し，ヒト以外の生物の環境として生物多様性への影響を考察するための基礎資料となる。

* 広島修道大学健康科学部