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

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ABSTRACT

To investigate the transfer of Cd from fertilizer to plants, a hydroponic cultivation experiment was performed with bean sprout conditioned with oyster shell fertilizer. The concentrations of Cd in the foliage were measured. The bean sprout increased foliage weight for 4 week. The foliage weight increased by hydroponic culativation higly followed by farm and pot-cultivation. Concentration of Cd was the highest in the foliage cultured by farm followed by hydroponic and pot.

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

INTRODUCTION

Among several heavy metals, Cd and zinc (Zn) are the serious contaminants affecting plant and animal lives. Environmental contamination of these metals has resulted from improper disposal or specific chemicals to be accumulated in organisms by food chain. However, transfer of Cd from benthic organisms to terrestrial organisms has been paid little attention. And, the industrial plants for recycling oyster shells as fertilizers began to operate in the Hiroshima Regional Urban Area.

It was not deniable to suspect any anthropogenic condition influencing on an inconsistent matter of the Cd and Zn accumulations in the Area (Nitta *et al.*, 2019; Nitta and Katoh, 2020). The inconsistency is the no correlation of the concentrations of the two metals in the farm cultivated plants but a high correlation between the two metals in the wild pteridophytes just outside the farm. Phosphate fertilizers are the major source of Cd in agricultural soils (Dharma-Wardana *et al.*, 2018; Długaszek *et al.*, 2017; Ikeda *et al.*, 2006; Zhu *et al.*, 2018) depending on the conditions of each soil (Argullo *et al.*, 2019; He *et al.*, 2005; Lin *et al.*, 2018; Roberts, T. L., 2014; Xu *et al.*, 2019).

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The transfer of Cd from oyster shells (*Crassostrea gigas*) into a plant was examined by the farm-, pot- or hydroponic cultivation using the bean sprout (*Pisum sativum* L.), which was available with constant quality enough for this experiment in the Hiroshima Regional Urban Area (Nitta, Y., 2020; Nitta *et al*., 2021).

Bean sprout is a nutritious vegetable that have been widely consumed in China, Korea and Japan (Lee *et al.*, 2018). Hydroponic cultivation enables for the plant to enrich their nutritional value and allow us to examine the transfer of water-soluble contaminants from water to plant body (Xu *et al.*, 2019).

MATERIALS AND METHODS

Study area: The farm-, pot- and hydroponic cultivation experiments were performed in the Hiroshima Regional Urban Area (**Fig. 1**).

Measurements of Cd and Zn: Solid samples for the measurements of Cd and Zn were prepared (**Table 1**). The instrument of Atomic Absorption Spectrometry (AA-6200, SHIMADZU, Kyoto, Japan) was used with the sediment determining methods (https://www.env.go.jp/water/teishitsu-chousa/00_full.pdf). The concentrations of Cd and Zn in

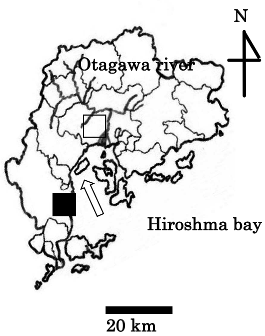

Fig. 1. A rough map of the Hiroshima Regional Urban Area.
Pot- and hydroponic cultivations were performed at Ozuka (□), N34°44' 04.28, E 132°41' 10.46. Farm cultivation was performed at Oogi (■), N34°19' 35.65, E 132°19' 43.04. Arrow indicates the Itsukushima island. The Hiroshima Regional Urban Area has increased the area since April the 1st in 2021 (https://www.city.hiroshima.lg.jp/site/kouiki/15648.html). Miyoshi city is included now. Present study was performed from December in 2020 to April in 2021, therefore, the Miyoshi city is not shown.

Table 1. Methods of Cd and Zn measurements.

		Soil and oyster shell	Bean sprout
Sample preparation	At the time of collection	Original samples were kept in polyethylene bag and stored at −70°C until use.	Original plant samples were brought to laboratory, washed with running water to remove soil particles and kept in vinyl bag and stored at −70°C until use. Others were directly kept in vinyl bag and stored at −70°C until use.
	Dried sample preparation	(1) The original soil samples were air-dried. The original oyster shell samples were dreid in a drying oven at 110°C for several hours and grounded into powder. (2) All the samples were sifted to a 2 mm sieve made of synthetic resin to be dried in a drying oven at 110°C for 2 hours. (3) The obtained powder was used as the dried sample.	(1) The samples were dried in a drying oven at 110°C overnight. (2) The dried samples were grounded into powder and used as the dried samples.
	Test sample preparation	Wet decomposition method was used. (1) Weight 0.1～5 g of the dried sample. (2) Add 10 ml of nitric acid and 20 ml of hydrochloric acid. (3) Heat at 200°C adding 10 ml of nitric acid. (4) Cooling. (5) Add 20 ml of nitric acid and 5 ml of perchloric acid. (6) Heat at 200°C. (7) Evaporative solidification. (8) Add 2 ml of nitric acidand 50 ml of deionized water. (9) Heat at 100°C. (10) Cooling. (11) Filtering using quaritative filter paper. (12) The obtained filtration was used as the test sample.	Pressure vessel method was used. (1) Weigh 0.1～0.5 g of the powderd sample. (2) Add 5 ml of nitric acid and 2 ml of hydrochloric acid. (3) Heat at 180°C. (4) Cooling. (5) Heat at 200°C. (6) Add 2 ml of nitric acidand 50 ml of deionized water. (7) Heat at 100°C. (8) Cooling. (9) Filtering using quaritative filter paper. (10) The obtained filtration was used as the test sample.
Determination of Cd and Zn	Instrument	Frame atomic absorption spectrometer (AA-6200, SHIMADZU, Kyoto, Japan)	Inductively coupled plasma emission spectrometer (ICP-OES730-ES, Agilent, Tokyo, Japan)
	Wave length	Wave lengths of the resonance line were set at 228.8 and 213.9 nm for Cd and Zn, respectively.	Wave lengths of the emission line were 214.4 and 213.9 nm for Cd and Zn, respectively.
	Thermal media	air-acetylene	argon
	Analytical quality control	(1) All the reagents used were of analytical grade. (2) Deionized water was used for all dilutions. (3) The procedure was repeated 3 times.	(1) All the reagents used were of analytical grade. (2) Deionized water was used for all dilutions. (3) The procedure was repeated 3 times.
	Obtaining quantitative value	Calibration curve method was used. (1) Reference standard solutions were purcased. The working standard solutions were prepared by apprpriate dilution with deionized water. (2) The blank sample was prepared by deionized water.	Calibration curve method was used. (1) Reference standard solutions were purcased. The working standard solutions were prepared by apprpriate dilution with deionized water. (2) The blank sample was prepared by deionized water.
	Concentration	Calculated on a dry weight basis.	Calculated on a wet weight basis.

organisms were determined by Inductively Coupled Plasma Atomic Spectrometry (ICP-OES 730-ES, Agilent, Tokyo, Japan) (https://www.mlit.go.jp/river/shishin_guideline/kasen/suishitsu/pdf/s06.pdf).

Farm-cultivation experiment: The commercially available brick of bean sprout (Murakami Field Co. Ltd., Hiroshima, Japan) was planted on the farm from January to March 2021. The handmade oyster shell powder had scattered on farm with a dose of 1 kg/m^2 in March, 2020. No other specific treatment was performed on the farm till the end of the experiment.

Pot-cultivation experiment: Commercially available bricks of bean sprout were used. Each brick was placed in pot filled with Kanuma soil per pot (φ: 21 cm; depth: 18 cm) from

Table 2. Cd and Zn concentrations in soils.

	Farm	Pot			Hydropnic		
		Shell	Kanuma	Shell + Kanuma[a)]	Shell	Kanuma	Water + Shell + Kanuma[b)]
Number	3	4	1	4	4	1	4
Cd (mg/kg)	0.11 ± 0.06	0.07 ± 0.02	< 0.05	0.04 ± 0.04	0.07 ± 0.02	< 0.05	0.04 ± 0.04
Zn (mg/kg)	127.5 ± 52.5	79.8 ± 4.19	16.0	28.7 ± 0.8	79.8 ± 4.19	16.0	28.7 ± 0.8

Metal concentrations in the agricultural soil and the cocktailed soil were measured before the experiment.
a): The weight ratio of shell and Kanuma in the cocktail per pot was 1:4;
b): The weight ratio of water, shell and Kanuma in the cocktail per tank was 5:1:4.

February to April in 2021. The brick cultivation was conditioned with or without the oyster shell powder. The calculated concentrations of Cd and Zn in each pot are shown in **Table 2**.

Hydroponic cultivation experiment: Commercially available bricks of bean sprout were used. Each brick was placed in a 6.5 L plastic tank of polysulfone (CL-0123-3, CLEA Japan Inc., Tokyo, Japan) conditioned with or without the 100 g of oyster shell powder (Nitta Y., 2020). Deionized water of 0.5 L was supplied every morning for 0, 1, 2 or 4 weeks in a laboratory room under the condition of natural light and temperature.

The experiment was performed from February to April in 2021 with room temperatures between 16 to 22°C. The conditioning groups of Kanuma soil alone and of Kanuma soil plus oyster shell were prepared. The concentrations of Cd and Zn of each tank are shown in **Table 2**.

Statistical analysis: For the comparison of the concentrations of Cd, Zn and Cd/Zn of perilla and bean sprout, the log-normal distribution was assumed. After calculating the logarithm of individual data, their statistical differences were compared by the Student's t-test. The KaleidaGraph software, version 3.6 (HULYNKS, Tokyo, Japan) was used.

RESULTS

Cd and Zn concentrations in the farm-, pot- and hydroponically cultivated bean sprouts: Weights of the 59 bricks of bean sprout were 320.8 ± 40.4 (mean ± SD) g. Bean sprouts increased their foliage weights by the 4 weeks of cultivation ($p < 0.01$) (**Table 3**). The ratio of weight increase was the highest in the hydroponic cultivation followed by the farm and pot- cultivations ($p < 0.05$ and $p < 0.01$, respectively).

Table 3. Growth of bean sprout by three types of cultivation.

Cultivation: Duration (week)	0	4		
Cultivation: Style	NC[a)]	Farm	Pot	Hydroponic
Number of bricks	8	6	10	6
Growth (g/brick)	89.6 ± 11.7	232.6 ± 48.2[b)]	230.6 ± 55.9[b)]	310.0 ± 31.8[b)]
Growth ratio	1.00 ± 0.08	2.59 ± 0.54[c)]	2.57 ± 0.65[d)]	3.46 ± 0.35

a): not cultivated;
b): $p < 0.01$ when compared to the 0-week-group;
c): $p < 0.05$ when compared to the hydroponic group;
d): $p < 0.01$ when compared to the hydroponic group.

Table 4. Accumulation of Cd and Zn from environment into foliage in bean sprout.

Cultivation: Duration (week)	0	4		
Cultivation: Style	NC[a)]	Farm	Pot	Hydroponic
Number of bricks	6	6	10	6
Cd (μg/brick)	0.111 ± 0.010	0.942 ± 0.652 **	0.279 ± 0.72	0.609 ± 0.312 *
Zn (mg/brick)	0.63 ± 0.08	1.93 ± 0.25 **	1.69 ± 0.48 **	1.40 ± 0.14 **
Cd/Zn (x100)	0.0179 ± 0.015	0.0479 ± 0.0292	0.0169 ± 0.0020	0.0447 ± 0.0023 *

a): not cultivated;
*: $p < 0.05$ when compared to the 0-week group by *t*-test;
**: $p < 0.01$ when compared to the 0-week group by *t*-test.

The amounts of Cd and Zn accumulated from environment into foliage are shown in **Table 4.** The farm cultivation accumulated Cd into the foliage more highly than the pot-cultivation. Whereas, the three ways of cultivations accumulated Zn into foliage with similar amount.

DISCUSSION

The first experiment of hydroponic cultivation with bean sprout had suggested that the Cd contained in oyster shells was transferred to the plants (Nitta, Y., 2020). Results from the three types of cultivation of bean sprout confirmed the results of the first experiment. The accumulation of Cd into foliage strengthened the hypothesis of the Cd flow from benthic organism in sea to terrestrial organism on land.

Commercially available bean sprout is a suitable organism for the experiments in order to examine the transfer of Cd contained in oyster shell fertilizer.

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Yumiko Nitta: Sequel: Bean sprouts (*Pisum sativum* L.) accumulate cadmium (Cd) in their foliage

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（その２）

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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 (<u>*Crassostrea gigas*</u>) 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 の豆苗への移行を水耕栽培，鉢栽培，圃場栽培で確認した。**材料及び方法**：豆苗とカキ殻粉末を用いた。水耕，鉢，圃場の方法で栽培した。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豆苗地上部の重量増加率は，水耕＞圃場＞鉢栽培の順に高かった。②豆苗地上部は Cd と Zn を蓄積した。③Cd は圃場＞水耕＞鉢栽培の順に高かった。**考察**：広島広域都市圏でカキ殻肥料を利用する場合，カキ殻の含有する重金属量を把握しておくことは，ヒトが生活する環境の公衆衛生上の安全性を担保し，ヒト以外の生物の生息環境として生態系への影響を考察するための基礎資料を提供することに寄与する。

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