International Journal of GEOMATE, Oct., 2019 Vol.17, Issue 62, pp. 188 - 194
ISSN: 2186-2982 (P), 2186-2990 (O), Japan, DOI: https://doi.org/10.21660/2019.62.50812
Special Issue on Science, Engineering & Environment

DIAZINON ABSORPTION AND BIOACCUMULATION IN THE GARDEN RADISH (RAPHANUS RAPHANISTRUM SSP. SATIVUS)

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*Corresponding Author, Received: 28 Feb. 2019, Revised: 19 April. 2019, Accepted: 05 May. 2019

ABSTRACT: In order to evaluate the absorption and bioconcentration profiles for diazinon (DZN) in the garden radish, radishes were grown in DZN-containing soil under two sets of conditions: early exposure and late exposure. In early exposure, seeds were planted in contaminated soil that DZN levels immediately after seeding ranged from 843-6650 ng/g-ww. DZN levels during growing period ranged from 2.11-2050 ng/g-ww in soil and from undetectable to 360 ng/g-ww in radish. In late exposure, seeds were planted in normal soil, to which DZN was added 28 days later. DZN levels in soil and radish ranged from 1480-9080 ng/g-ww and 110-3280 ng/g-ww. The levels of DZN in the roots and leaves were higher than those in the stems. The relationship between DZN levels in soil and radishes observed in these experiments were strongly positive, with radishes taking up greater quantities of DZN the higher its concentration in soil. The concentration factor (radish-to-soil; Cr/Cs) decreased at an exponential rate with days elapsed since DZN addition. This suggests that the degree of DZN taken up by radishes is influenced by exposure time. Based on the findings for the time course of tissue-specific DZN concentration ratios and DZN contents, it seems that DZN in the soil were absorbed through the roots, and then transported through the stem into the leaves: after this, DZN primarily bioaccumulates in the garden radish in its roots and leaves circulating within the plant.

Keywords: Diazinon, Garden radish, Absorption, Bioaccumulation

1. INTRODUCTION

Outbreaks of Minamata disease and Itai-itai disease in Japan in the 1950s and 1960s were caused by the improper handling of industrial waste, contaminating food and water resources with toxic chemicals, and seriously damaging the health of those who consumed them [1-4]. Bioconcentration of hazardous compounds—in these cases, mercury and cadmium—plays a major role in the epidemiology of such pollution-related diseases. Eliminating such hazards from society will require authorities to collect large volumes of data on the toxicity and environmental exposure of such pollutants, and use them to estimate health risks in human populations [5,6]. While plenty of empirical data has been accrued by a number of laboratories and researchers [7-15], this task is hardly complete. In particular, much more information still needs to be collected on how toxic chemicals concentrate in water, air, foodstuffs, and other environmental media in order to construct accurate exposure and risk profiles.

Here, we report bioaccumulation data for the toxic compound diazinon, using the common garden radish (Raphanus raphanistrum ssp. sativus) as a model vegetable. Originally domesticated in Europe, it belongs to the same family (Brassicaceae) as the daikon radish, the fourth most popular vegetable in Japan by annual domestic freight volume. When grown at ~20 ℃, the plant can be harvested about one month after sowing. The edible root is red and globular: for large individuals, it measures about 3 cm in diameter, and the leaves are ~30 cm in length. We selected this species because of its relatively short maturation time and ease to cultivate. Originally developed in 1952 by the former Swiss pharmaceutical company Ciba-Geigy AG, diazinon (DZN) is a pesticide used in large quantities in the 1970s to kill agricultural pests such as leafhopper and cabbageworm, and ward off domestic pests such as cockroaches and flies [16,17]. This compound was selected because even today it remains a common ingredient present in organophosphate insecticide formulations [18]. In a series of experiments, we assessed this compound's absorption and bioconcentration profiles in the garden radish when grown in contaminated soil: namely, its absorption over time, and its migration and localization in the plant's tissues.

2. EXPERIMENTAL METHODS

2.1 Study Overview

Radishes were grown under two sets of conditions: *early exposure* and *late exposure*. In the former, seeds were planted in DZN-containing soil; in the latter, seeds were planted in normal soil, to which DZN was added 28 days later. Radishes were grown in the 10 planters (P1-P10) detailed in Table

1. Individuals exposed as seeds were grown in three batches: from 20 Oct to 3 Nov 2016 (P1-P4), from 7 May to 11 Jun 2017 (P5-P6), and from 29 Jun to 27 Jul 2017 (P7-P9). Individuals exposed when mature were grown in one batch from 1 Sep to 11 Oct 2018 (P10). The average air temperatures of the growing room during each cultivation period were as follows: P1-P4, not measured; P5-P6, 26.8°C; P7-P9, 33.7°C; P10, 26.6°C. (Daily mean outdoor temperatures reported by the Japan Meteorological Agency [JMA] for the region over these periods were respectively 16.7 °C, 21.1 °C, 28.5 °C, and 23.7°C.)

Table 1 Radish growing conditions

Planter	Growing period	Air	Soil
P1-P4	20 Oct – 3 Nov 2016	16.7 (N.A.)	N.A.
P5-P6	7 May – 11 Jun 2017	21.1 (26.8)	25.9
P7-P9	29 Jun – 27 Jul 2017	28.5 (33.7)	29.7
P10	01 Sep – 11 Oct 2018	23.7 (26.6)	25.6

Note: Air temperature (˚C): Average of daily mean outdoor air temperatures during the growing period for the region reported by the JMA; parenthetical values are average temperatures actually recorded inside the growing room during the same period (during watering, between 0800-1000 each day). Soil temperature (˚C): mean of five measurements (planter center and four corners; also measured during watering). N/A: not measured.

2.2 Radish Cultivation

Radishes were cultivated in planters in a sunny room with windows on all four sides. Each planter was 45 cm wide, 30 cm long, and 18 cm deep. Figure 1 depicts the planter set-up. Gravel (~400 g) was first laid evenly at the bottom, and then potting soil (~400 g) added to a depth of ~8 cm. Next, a mixture of soil (~1600 g), fertilizer (~15 g), and DZN (P1-P4, P6-P7: 1.0 g, P5: 0.4 g, P8-P9: 1.2 g, P10: none) was laid on top, another ~8 cm deep. Once these preparations were complete, the planter was sprinkled with water (~2 L), and about 60 seeds were sown in two rows and then covered with soil. Each planter was watered nearly every day (~300-500 mL) during the growing period. Windows were left open when the weather was particularly hot to moderate the indoor temperature, except in the event of strong wind and rain. For P10—the only late-exposed batch—a DZN solution was sprinkled around the plants' roots 28 days after sowing (technically a suspension, since the DZN granules did not dissolve completely in water).

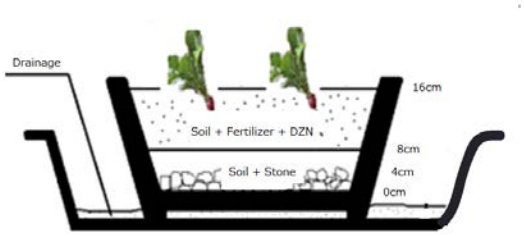

Fig.1 Planter set-up

2.3 Sampling

Radish and soil samples for the early exposure condition were collected according to the following schedule. P1-P4, 2 times: immediately after sowing (Day 0) and 14 days later (Day 14); P5-P6, 6 times: Day 0, 7, 14, 21, 28, and 35; P7-P9, 3 times: Day 0, 6, and 14. On Days 6 and 7, 15-30 small sprouts were collected; subsequently, 9-15 individuals were harvested on Day 14, 4-5 on Day 21, 2 on Day 28, and 1 on Day 35. On each occasion, five soil specimens were taken from five locations in each planter: the center, and near each of the four corners. Roughly cylindrical (~3×3 cm) sections were collected and mixed well before analysis.

For the late-exposure condition (P10), radish and soil specimens were collected just before adding DZN (Day 0*), and 1*, 2*, 3*, 4*, 6*, and 13* days later. (Here, day numbers with an asterisk denote days after exposure; thus, Day 1* = Day 29 after seeding, Day 2* = Day 30, etc.) One plant was harvested each time. The soil surrounding the root, roughly a ~5×5 cm cylinder in shape, was also collected and mixed well before analysis.

2.4 Analysis

Radish specimens were rinsed well with water, wiped off, and weighed using an electronic balance. They were cut into sections of a few mm in length, and re-weighed after placing them in cellulose thimbles (Whatman, UK) to ensure accurate readings following ultrasound-assisted hexane extraction. Plants harvested from P5 and P6 at Day 35, and from P10 at each time point, had grown quite large: they were divided into root, stem, and leaf specimens, with each tissue cut into few-mm sections. Similarly, some of each of these tissue samples were placed in cellulose thimbles and weighed. Soil specimens were also weighed in cellulose thimbles.

Specimen-containing thimbles were placed in 260-mL bottles, and filtered with 150 mL of hexane for pesticide residue and polychlorinated biphenyl analysis (Wako Pure Chemical Industries, Ltd.) under ultrasonication for 15 min to extract organic compounds. The extract was dehydrated with anhydrous Na_2SO_4 for 30 min, and then

concentrated to a volume of ~2 mL using a rotary evaporator. The concentrate was removed, and the evaporator rinsed with hexane: the two liquids were combined, and again treated with anhydrous Na_2SO_4 to remove moisture. Remaining liquid was passed through a cylindrical filter (Whatman Puradisc 25 TF; GE Healthcare Bio-Sciences, Piscataway NJ, USA), and then concentrated to a volume of 1.5 mL under N_2 flow. Gas chromatography/mass spectrometry (GC/MS) specimens were prepared by the addition of 0.2 mL of an internal standard solution (100× dilution of 3 Internal Standards Mixture Solution: Wako Pure Chemical Industries, Ltd.), followed by hexane for a total volume of exactly 2.0 mL.

GC/MS parameters were as follows. Device: 5975B inert XL E/CI MSD (Agilent Technologies), capillary column: HP-5MS (30 m × 0.25 mm × 0.25 μm), inlet temperature: 250 °C, injection method: splitless, injection volume: 2 μL, column temperature program: 70 °C (1.5 min) → 20 °C/min → 180 °C (0 min) → 5 °C/min → 290 °C (10 min), carrier gas: He, interface temperature: 230 °C, MS: electron impact ionization mode, ionization voltage: 70 eV. For qualitative analysis, signal intensities were measured at two m/z values each for DZN (137.1, 152.1) and an internal standard (9-bromoanthracene: 256.1, 176.1). For quantitative analysis, signal intensities at the most sensitive values in the mass spectra (respectively, 137.1 and 256.1) were used to construct internal standard calibration curves. Standard DZN solutions were prepared by dilution of diazinon reference material (Wako Pure Chemical Industries, Ltd.).

3. RESULTS AND DISCUSSION

3.1 Early DZN Exposure

Table 2 displays the DZN levels measured in radishes seeded in DZN-contaminated soil (P1-P9), as well as in the soil itself. Planters P1-P4 contained 1.0 g DZN distributed in the top layer of soil; the mean outdoor temperature was 16.7 °C during the growing period. DZN's soil concentration immediately after seeding and watering ranged from 3620-5960 ng/g-ww. On Day 14, it ranged from 219-387 ng/g-ww, and in radishes, from undetectable to 16.3 ng/g-ww.

Planters P5 and P6 respectively contained 0.4 and 1.0 g DZN; the mean outdoor temperature was 21.1 °C during the growing period. DZN levels in the soil and plants at six time points over 35 days are plotted in Figure 2. DZN concentrations in soil immediately after seeding and watering were 843 (P5) and 3570 ng/g-ww (P6), but fell at an exponential rate thereafter. In radishes, DZN levels were 63.5 (P5) and 360ng/g-ww (P6) on Day 7, and 2.13 (P5) and 14.6ng/g-ww (P6) on Day 14.

Planters P7-P9 respectively contained 1.0, 1.2, and 1.2 g DZN; the mean outdoor temperature was 28.5 °C during the growing period. DZN's soil concentrations immediately after seeding and watering were respectively 5680, 6650, and 6090 ng/g-ww. On Day 6, they ranged from 403-2050

Planter	DZN	Sample type	Day (after seeding)						
			0	6	7	14	21	28	35
P1	1.0g	Soil	4420			219			
		Radish				16.3			
P2	1.0g	Soil	4510			387			
		Radish				16.2			
P3	1.0g	Soil	5960			277			
		Radish				N.D.			
P4	1.0g	Soil	3620			350			
		Radish				14.0			
P5	0.4g	Soil	843		389	65.9	19.2	3.58	2.11
		Radish			63.5	2.13	N.D.	N.D.	N.D.
P6	1.0g	Soil	3570		1840	235	40.0	6.59	5.28
		Radish			360	14.6	N.D.	N.D.	N.D.
P7	1.0g	Soil	5680	403		29.2			
		Radish		44.0		N.D.			
P8	1.2g	Soil	6650	921		82.4			
		Radish		178		N.D.			
P9	1.2g	Soil	6090	2050		41.5			
		Radish		171		N.D.			

Table 2. DZN levels in soil and radishes under early exposure conditions (Units: ng/g-ww)

Note: N.D. denotes 'not detected'. Blank cells indicate no measurement taken at that time point.

ng/g-ww, and in radishes, from 44.0-178 ng/g-ww. On Day 14, they ranged from 29.2-82.4 ng/g-ww, but were undetectable in all radish specimens.

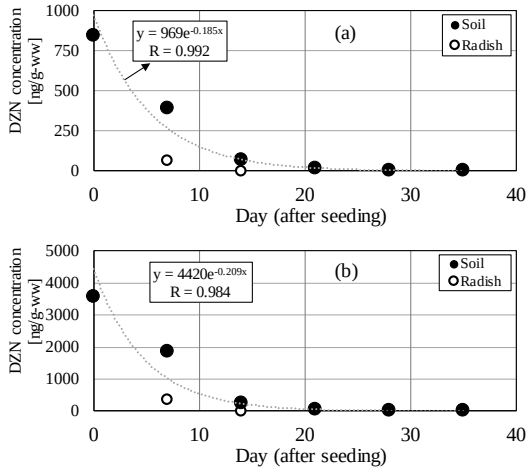

Fig.2 DZN concentrations in soil and radish for (a) P5 and (b) P6

3.2 Late DZN Exposure

Table 3 displays the DZN levels measured in radishes exposed to DZN after four weeks of normal growth (P10). Separate values are reported for entire plants, by tissue, and for the soil itself. Plants were initially seeded in uncontaminated soil, and a DZN solution was sprinkled around the root on Day 28 (Day 0*); the mean outdoor temperature during the growing period was 23.7 °C.In soil, DZN was most concentrated the day after it was added (Day 1*(29): 9080 ng/g-ww), and lowest 13 days after (Day 13*(41): 1480 ng/g-ww). DZN levels in soil fell gradually over time, although an unusually low value was recorded on Day 2*(30) (3210 ng/g-ww).

Similarly, DZN levels in radishes started to decline a few days after exposure: they were greatest on Day 1*(29) (3280 ng/g-ww) and lowest on Day 13*(41) (110 ng/g-ww). Similar trends were evident in tissue-specific observations: DZN levels were highest the day after first exposure (root: 11300, stem: 1350, leaf: 2350 ng/g-ww), and lowest 13 days later (respectively, 90.0, 61.9, and 172 ng/g-ww).

3.3 Relationship between DZN Concentrations in Soil versus Radish Plants

Figure 3 depicts the relationship between DZN concentrations in soil and radishes observed in these experiments. Data were plotted as the common logarithm of DZN concentrations in soil (x-axis) versus in radish plants (y-axis) for P1-P10 (N=16). Linear regression analysis showed a strong positive correlation (y = 1.37x - 2.08; R=0.966), with radishes taking up greater quantities of DZN the higher its concentration in soil.

Table 3. DZN levels in soil and radishes under late exposure conditions

Sample type	Day						
	0* (28)	1* (29)	2* (30)	3* (31)	4* (32)	9* (37)	13* (41)
Soil	N.D.	9080	3210	8360	9020	3630	1480
Radish	N.D.	3280 (11.5g)	1270 (13.7g)	2310 (9.50g)	1300 (19.8g)	263 (34.2g)	110 (34.9g)
Root	N.D.	11300 (1.65g)	1630 (3.28g)	2100 (3.73g)	1290 (8.68g)	312 (15.7g)	90.9 (20.6g)
Stem	N.D.	1350 (4.10g)	579 (4.08g)	1360 (2.08g)	499 (4.12g)	82.7 (7.14g)	61.9 (4.38g)
Leaf	N.D.	2350 (5.71g)	1540 (6.33g)	3070 (3.69g)	1790 (7.01g)	308 (11.4g)	172 (9.88g)

Note: Units are ng/g-ww, unless otherwise noted. N.D. denotes 'not detected'. Asterisks (*) denote the number of days after DZN was added to soil; parenthetical values indicate the number of days after seeding. DZN concentrations and weights were measured separately for root, stem, and leaf samples. "Radish" denotes DZN measurements for the whole plant: concentration was multiplied by weight for each tissue, the products added together, and the sum divided by total weight. Upper values in each cell are concentrations (ng/g-ww); lower parenthetical values are masses (g).

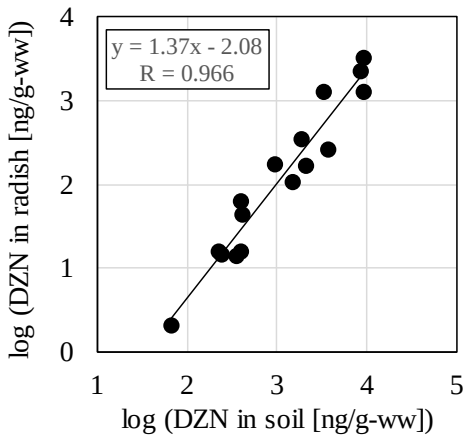

Fig.3 DZN concentration in soil v. radish plants

Figure 4 depicts the relationship between days elapsed since DZN addition and concentration factor (radish-to-soil; Cr/Cs). Black points indicate data from P10 (late exposure). The concentration factor decreased at an exponential rate, as described by the regression function $y = 0.395 \times e^{-0.151x}$, where y = Cr/Cs and x = days after DZN addition (R=0.916). White points indicate data from P1-P9 (early exposure). Some data points (i.e. Day 6) deviate from the equation slightly, but most lie close to the regression curve. These data suggest that the degree of DZN taken up by radishes is influenced by exposure time.

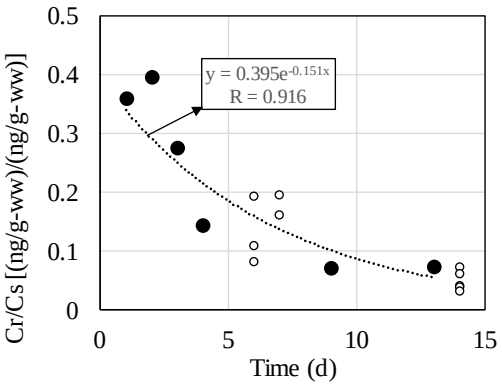

Fig.4 Days since DZN exposure versus concentration ratio (radish/soil)

3.4 Tissue-specific DZN Concentration Ratios and Relative Content

Figure 5 shows the time course of tissue-specific DZN concentration ratios under late exposure conditions (P10). The root/leaf ratio was highest on Day 1* (8.37), but had sharply declined by Day 3 (1.54). By Day 9*, it had risen slightly (3.77), but then again fell by Day 13* (1.47). The leaf/stem ratio, on the other hand, was quite low on Day 1* (1.74): after rising through Day 9* (3.72) with some fluctuation, it had dropped lower by Day 13* (2.78).

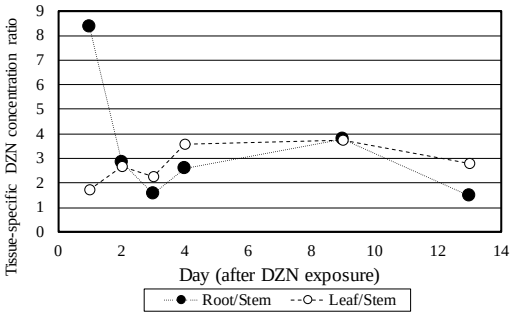

Fig.5 Time course of tissue-specific DZN concentration ratios (P10)

Figure 6 shows how DZN content (% mass) was distributed between the three radish tissues over time. Roots contained 49.5% of absorbed DZN on Day 1*, but this dropped rapidly to 30.7% by Day 2*: thereafter, content steadily rose to 54.4% by Day 9*, then dropped slightly to 48.8% by Day 13*. Leaves contained 35.8% of absorbed DZN on Day 1*, rising rapidly to 55.7% by Day 2*: content steadily fell to 39.0% by Day 9*, then rose slightly to 44.2% by Day 13*. Stems contained 14.7% of absorbed DZN on Day 1*, but this content continued to gradually decrease thereafter: to 6.6% by Day 9*, and 7.1% by Day 13*. Based on these findings, it seems that DZN molecules in the soil were absorbed through the roots, and then transported through the stem into the leaves: after this, the compound appears to be carried back to the roots. In essence, DZN primarily bioaccumulates in the garden radish in its roots and leaves, but still circulates within the plant over time.

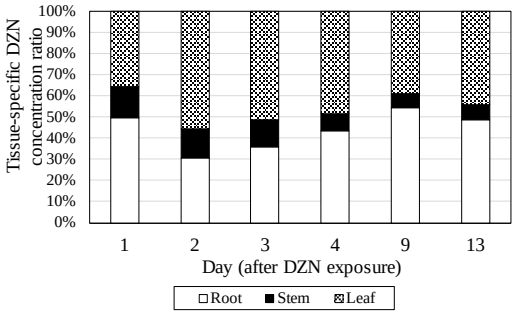

Fig.6 Time course of relative DZN content by tissue (P10)

4. CONCLUSIONS

The steady accumulation of data on how a variety of environmental pollutants and chemicals are absorbed and accumulate in various vegetables is an important task to assess the health risks they pose to humans. Here, we analyzed empirical data for diazinon absorption and bioaccumulation in the common garden radish (Raphanus raphanistrum ssp.

sativus), and considered their implications. We can summarize the study findings as follows.

(1) In early exposure experiments, seeds were planted in contaminated soil that DZN concentrations immediately after seeding ranged from 843-6650 ng/g-ww. DZN levels during growing period ranged from 2.11-2050 ng/g-ww in soil and from undetectable to 360 ng/g-ww in radish.

(2) In late exposure experiments, seeds were planted in normal soil, to which DZN was added 28 days later. DZN levels in soil and radish ranged from 1480-9080 ng/g-ww and 110-3280 ng/g-ww. The levels of DZN in the roots and leaves were greater than those in the stems.

(3) The relationship between DZN levels in soil and radishes observed in these experiments were strongly positive. Radishes took up greater DZN quantities the higher its concentration in soil.

(4) The concentration factor (Cr/Cs) decreased at an exponential rate with days elapsed since DZN addition. This suggests that the degree of DZN taken up by radishes is influenced by exposure time.

(5) Based on the findings for the time course of tissue-specific DZN concentration ratios and DZN contents, it seems that DZN in the soil were absorbed through the roots, and then transported through the stem into the leaves: after this, DZN primarily bioaccumulates in the garden radish in its roots and leaves circulating within the plant.

Constructing a system to assess the relative health risks of various pollutants and chemicals in human populations would be an effective approach to limiting their potential damage. Accordingly, the process by which they enter the human body from the environment needs to be elucidated in greater detail. Hereafter, the absorption and bioaccumulation of many more toxic compounds still need to be studied, in a variety of different vegetables. For now, we hope that our work will serve as a valuable first step towards a paradigm for assessing the health hazards caused by their exposure.

5. ACKNOWLEDGMENTS

The author would like to thank Kindai University for providing the grant to complete this research. The same appreciate to Mr. Shohei Otsu, Ms. Ayaka Nitta, and Mr. Takurou Kashino for their help in experiments.

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