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A Study on Reduction and Transport of Cr(VI) in Two-dimensional Soils with Root Systems

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ABSTRACT

This paper presents reduction effect and evaluation of Cr(VI)-contaminated soil by two kinds of plants on various initial concentration on laboratory testing. In this experiment, we prepared semi-two-dimensional experimental apparatus which could evaluate movement of water and pollutant and growth of plants two-dimensionally. Simulated polluted soil mixed with $K_2Cr_2O_7$ powder as pollutant homogeneously and compacted. Selected plants were komatsuna (*Brassica rapa* var. *perviridis*) and sunflower (*Helianthus annuus*). Effect of reduction was confirmed by checking water content, pH, standard oxidation-reduction potential Eh, water soluble Cr(VI) of soil separated in 10 cm depth and 5-7.5 cm width respectively with changing time. Cr(VI) in plant body was also investigated at the end of test. 1) The higher initial concentration of Cr(VI), the lower root length density which describes root length in unit weight of soil for both of plant. 2) For komatsuna, reduction rate of Cr(VI) in the soil was ranged between 16-48 %. For sunflower, reduction rate of Cr(VI) in the soil is ranged between 27-45 %. It differed from initial concentration of Cr(VI).

Keywords: phytoremediation, hexavalent chromium (Cr(VI)), Standard oxidation reduction potential

1 INTRODUCTION

Soil contamination by toxic heavy metals are one of the worldwide serious geo-environmental problems. They are come out by human activity such as mining, production of fuel, smelting, making fertilizer, military activities and so on. They brought serious health problem to human body if it flows out of ground and groundwater. Hexavalent chromium (Cr(VI)) are one of the most dangerous heavy metal to human body which is the cause of ulcer and carcinogen formation on dermoid, respiratory and digestive system (WHO, 2000) (Cambridge plating company, 2007). In addition, it has high toxicity though its amounts are small, its leaching standard and content standard are under 0.05 mg/L and 250 mg/L respectively in Japan (Ministry of the Environment, 2015).

An usual way of removing Cr(VI) from ground are immobilization of them to solidified by cement, using insolubilization material, and removing contaminated soil by excavation from field. They might be effective within a short period, they also have problems such as redissolve due to changes of soil pH and standard oxidation-reduction potential (Eh), cost of detoxicating of contaminated soil, and tightening of disposal site (Cunningham et al., 1996).

A new technology of reduction and clean-up contaminants from soil which utilizes living plant is paid attention nowadays. Properties of absorption varies from kinds of plants, so that some screening investigation

were conducted from 1970's (Timperley, et al, 1970).

Some experimental studies have been conducted for confirming effect of absorbing water and Cr(VI) of plants and reduction of Cr(VI) in soil even today (Maria, et al, 2013) (Oh et al, 2013). However, the reduction effects of Cr(VI) evaluated partially, there were few investigated distributions and its time-space changings of Cr(VI) in experimental system considering absorption of root.

This paper presents to evaluate reduction effect of Cr(VI)-contaminated soil by two kinds of plants on various initial concentration through laboratory testing. For soil environment, Cr(VI) content of soil, soil pH and Eh affected to valence of chromium were measured periodically. For growth of plant, root length density and Cr(VI) content in stems and roots of plant were measured.

2 MATERIALS AND METHODS

2.1 Experimental Apparatus

Figure 1 indicates schematic diagram of experimental apparatus. Cr(VI)-mixed soil was put in rectangular parallelepiped box which was 500 mm height, 300 mm width, and 50 mm length. The length of experimental box was designed 6 - 10 times thinner than that of height and width which can restrict movement of materials in the length direction. A plant was set on the center of each box. 28 holes were made at the bottom of each box to allow drainage from the bottom. Diameter of the holes

were 5 mm. A non-woven fabric was put at the bottom of each box to prevent soil runoff. Run-off water could catch a vessel set under the box, which was 50 mm height, 300 mm width, and 50 mm length.

2.2 Soil Preparation

Simulated contaminated soil was used for the experiment. Base soil was decomposed granite soil sieved under 4.75 mm. 0.75 g/kg of cow dung compost and 0.15 g/kgdry of Bokashi (extended slowly-active fertilizer) were added and mixed with decomposed granite soil. Figure 2 shows particle size accumulation curve of used material. Simulated contaminated soil prepared 12.8 kN/m³ of unit dry mass and initial water content was set as 10 % (see Table 1). Table 2 shows initial content of 4 kinds of exchangeable cations (Ca²⁺, Mg²⁺, K⁺, Na⁺) and cation exchange capacity (CEC). CEC means ability of exchange cations absorbing soil particle to cations in pore water, and the value of CEC can be described as total amount of 4 exchangeable cations (Ca²⁺, Mg²⁺, K⁺, Na⁺). As shown in Table 2, CEC of used soil was 0.2 meq/gdry, which can be regarded as “low” capacity of absorbing nutrient and cations (Letica et al., 2017).

In regard to initial additive Cr(VI), K₂Cr₂O₇ powder was added to mixed soil mentioned above and mixed evenly. Initial contents of Cr(VI) differed from kinds of plants mentioned in 2-3, 0,10, 25, 50 mg/kgdry and 0,25,50,75 mg/kgdry respectively indicated in Table 4. For control, non-vegetation conditions at 0, 25, 50, 75 mg/kgdry of initial contents of Cr(VI) were also prepared.

2.3 Climatic conditions

Experimental boxes were set in thermostatic chamber. Table 3 shows climatic conditions. Light period was 16 hour in a day which illuminance was 10000 lx by metal halide lamp, and dark period was 8 hour in a day. Temperature and relative humidity were constant through the experimental period, 25 °C and 65 % respectively. Distilled water as irrigation was given 200 ml per 2 days from the top of each box.

2.4 Plant Conditions

Table 4 shows used plant and initial content of Cr(VI). *Brassica rapa* var. *perviridis* and *Helianthus annuus* were used for the experiment. The former is a standard species used germination experiment for checking qualities of compost and growth assay in Japan (Hase, et al, 2012), thus the specie was chosen. In regard to the latter, Oh et al. (2013) found that it was a hyperaccumulator plant especially Cd at hydroponic experiment. It also reported that hyperaccumulative against Cr through cultural experiment on fine-grained soil. When 0 - 10000 µg/L of K₂Cr₂O₇ solution had been given to it for 3 month, its roots, shoots, leaves and blossoms accumulated Cr(VI). Its roots accumulated 500 mg/gdry Cr(VI) in case that 5000 and 10000 µg/L of Cr(VI) solution was given (Vasiliki et al., 2017).

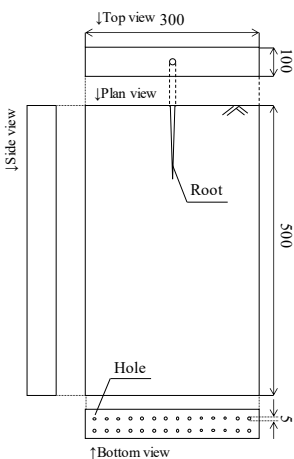


Fig. 1. Schematic design of soil box

Table 1. Initial soil conditions

Materials	Dry density	Initial volumetric water content
	kN/m ³	%
Decomposed granite soil (sieved under 4.75 mm) +Fermented excrements of cows (0.75 g/kgdry) +Fermented organic fertilizer (0.15 g/kgdry)	12.8	13.0

Table 2. Initial chemical contents of mixed decomposed granite soil

Ca ²⁺	mg/kgdry	338.26
Mg ²⁺		65.37
K ⁺		4.12
Na ⁺		18.61
CEC	meq/gdry	0.23

Table 3. Climatic conditions

Light period	hour	16
Dark period		8
Illuminance	lx	10000
Temperature	°C	25
Relative humidity	%	65
Illigation	ml/2days	200

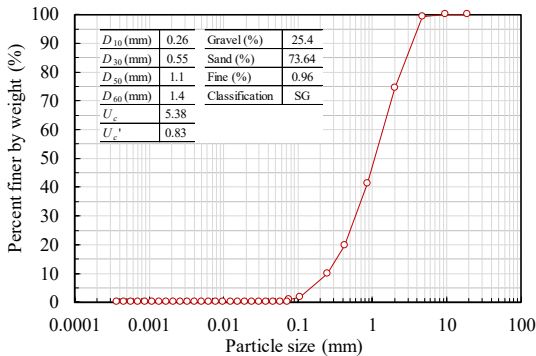


Fig. 2. Particle size accumulation curve of used soil material

Table 4. Used plant and initial content of Cr(VI)

Plant	Cr ⁶⁺ compound	Experimental Period
	mg/kgdry	day
<i>Brassica rapa</i> var. <i>perviridis</i> (Komatsuna)	0, 10, 25,50, 100	15, 30, 60
<i>Helianthus annuus</i> (Sunflower)	0, 25, 50, 75	
None		

Therefore, it was chosen for this experimental study.

Both kinds of seeds were put in plug pot filled with culture soil to obtain sample seedlings. Growth period was 2 or 3 weeks, until seed germinated and got true leaves.

A Seedling was put on the center of each experimental soil explained in 2.1. 3 samples were set in each

condition. Experimental periods were 0.5, 1, and 2 months after putting seedlings on the apparatus.

3 MEASUREMENTS

3.1 Soil water and chemical conditions

3 individuals were prepared for each experimental condition. Water content, volumetric water content, pH, oxidation reduction potential (ORP), water soluble and exchangeable Cr(VI) were measured following way of measurement. Soil were dividedly sampled with width and depth on the day after experimental period indicated in 2.4 was elapsed. In case of *Brassica rapa* var. *perviridis*, soil was divided 10 cm depth, 10 cm width and 5 cm length from surface. Totally, 15 samples were collected. For *Helianthus annuus* and non-vegetation condition, soil was divided 10 cm depth, 7.5 cm width and 5 cm length from surface. Half of soil from center of long side were sampled. Totally, 10 samples were collected. Measurement of soil pH conformed to JGS-0211 (JIS A 1226) (JGS, 2010) using pH electrode (9625-10D, HORIBA Scientific). For measuring soil ORP, soil and distilled water were mixed at the ratio of 1:5 on dry weight. After 30 minutes of mixing, ORP of suspension was measured by ORP electrode (9300-10D, HORIBA Scientific). Same suspensions were subjected to measure pH and ORP.

For measuring contents of water soluble Cr(VI) in soil, soil and distilled water were mixed at the ratio of 1:5 on weight. The mixtures were shaken for 1 hour at 200 times/min, and vacuum filtrated by 0.22 μm pore filter. Contents of water soluble Cr(VI) were determined with atomic absorption spectrophotometer (ANA-182, Tokyo Photoelectric, Co. Ltd.) by measuring filtered samples. Measuring wavelength was 357 nm. (SSAMMC, 2003.)

For measuring contents of exchangeable Cr(VI) in soil, soil and 1 N ammonium acetate were mixed at the ratio of 1:20 on weight. The mixtures were shaken for 1 hour at 200 times/min, and vacuum filtrated by 0.22 μm pore filter. Contents of exchangeable Cr(VI) were determined with atomic absorption spectrophotometer by measuring filtered samples. Measuring wavelength was 357 nm.

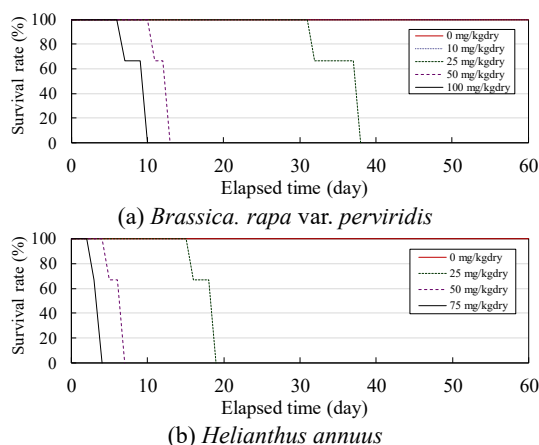


Figure 3. Elapsed time versus survival rate

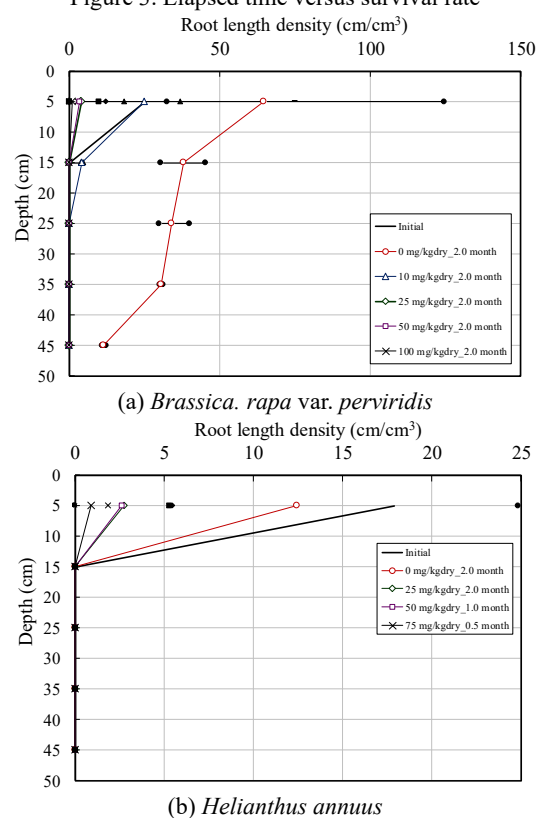


Fig. 4. Distributions of root length density

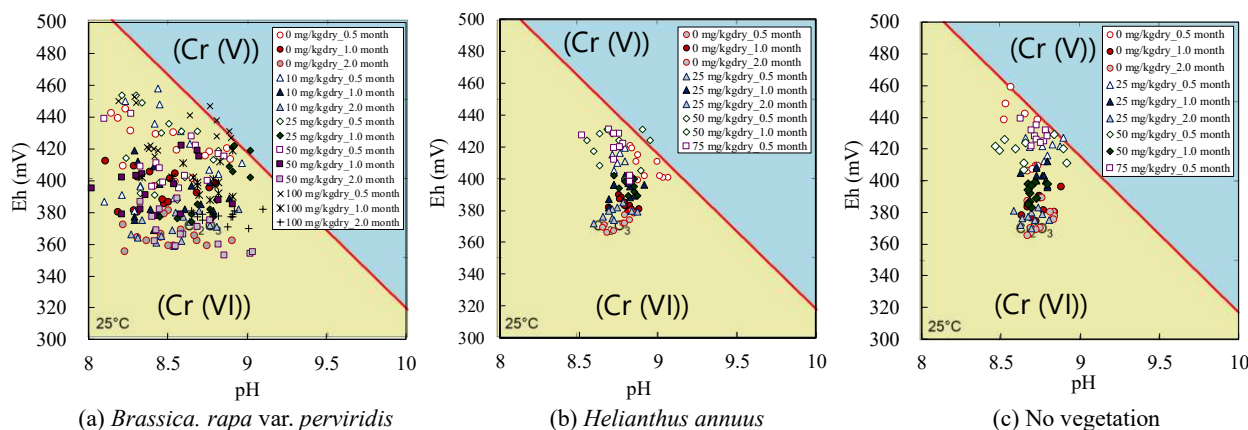
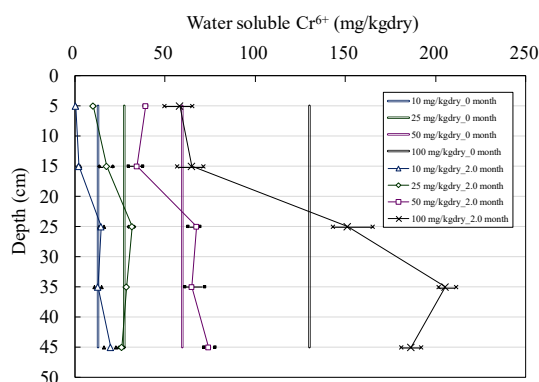
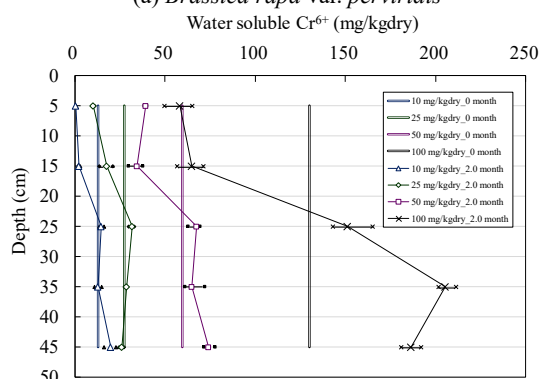


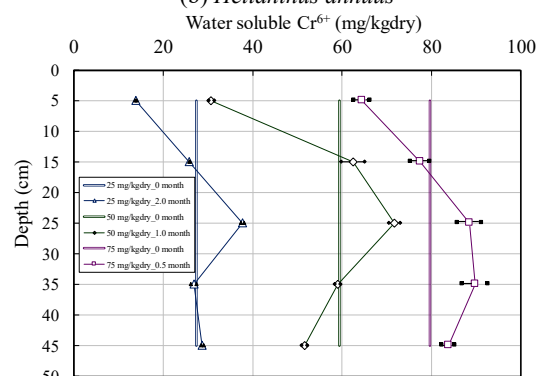
Fig. 5. pH-Eh diagram



(a) *Brassica rapa* var. *perviridis*



(b) *Helianthus annuus*

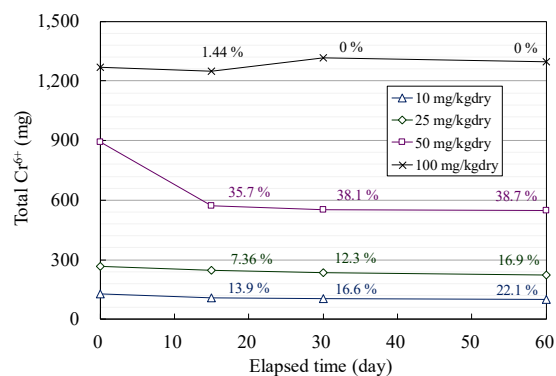


(c) No vegetation

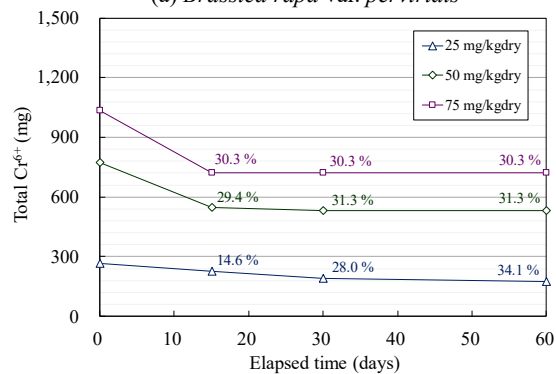
Fig. 6. Distributions of water soluble Cr(VI)

3.2 Plants growth and chemical contents

As growth parameters of plant, dry weight of root, root length and root length density were measured in each polluted and plant conditions. Root length density were derived by collecting roots and measuring their total length in each sampling soil indicated in 3.1. In regard to content of Cr(VI) in the plant, plant and 0.1 % (by weight) of hydrochloric acid were mixed at the ratio of 1:20 on dry weight (Nishiguchi, et al., 2007). The mixtures were shaken for 1 hour at 200 times/min, and vacuum filtrated by 0.22 μm pore filter. Contents of Cr(VI) content were determined with atomic absorption spectrophotometer by measuring filtered samples. Measured wavelength was 357 nm. Roots, stems and leaves were dividedly measured. Amount of Cr(VI) in roots and stems could be calculated from measured values.



(a) *Brassica rapa* var. *perviridis*



(b) *Helianthus annuus*

Fig. 7. Reduction rate of Cr(VI)

4 RESULTS AND DISCUSSIONS

4.1 Growth properties of plants on initial content of Cr(VI)

Figure 3 (a) and (b) show longevity of plants and initial content of Cr(VI). The figures also indicate minimum and maximum longevity on each condition. It can be found that *Brassica rapa* var. *perviridis* and *Helianthus annuus* had been lived when the experiment was finished (60 days) in case that there is no initial Cr(VI) and 10 mg/kgdry of Cr(VI) was added with *Brassica rapa* var. *perviridis*. The higher the initial content of Cr(VI) was, the shorter the longevity became. Longevity of *Brassica rapa* var. *perviridis* at 50 mg/kgdry of initial Cr(VI) was 0.34 times lower compared with that of 25 mg/kgdry. Compared with the condition of 50 mg/kgdry and 100 mg/kgdry, longevity of *Brassica rapa* var. *perviridis* at 100 mg/kgdry of initial Cr(VI) was 0.63-0.76 times lower than that of 50 mg/kgdry. For longevity of *Helianthus annuus*, compared with the condition of 25 mg/kgdry and 50 mg/kgdry, longevity of *Brassica rapa* var. *perviridis* at 50 mg/kgdry of initial Cr(VI) was 0.31-0.36 times lower than that of 50 mg/kgdry. Compared with the condition of 50 mg/kgdry and 100 mg/kgdry, longevity of *Brassica rapa* var. *perviridis* at 100 mg/kgdry of initial Cr(VI) was 0.57-0.60 times lower than that of 50 mg/kgdry.

Figure 4 (a) and (b) indicate average root length density of *Brassica rapa* var. *perviridis* and *Helianthus annuus* in each depth. For figure 4 (a), root which was brought on the conditions of 0 mg/kgdry Cr(VI) fulfilled

and reached bottom of the apparatus (50 cm). On the contrary to that, root reached shallower than 20 - 30 cm from soil surface in case that Cr(VI) was added more than 10 mg/kgdry. The smaller verage values of root length density in whole area of apparatus became, the higher initial content of Cr(VI) was. The values were 0.165, 0.023, 0.019 and 0.005 times compared with that of 0 mg/kgdry. For root length density of *Helianthus annuus* represented in figure 4 (b), root reached shallower than 20 cm from soil surface in case that Cr(VI) was added more than 25 mg/kgdry. It is shown in a same trend of *Brassica rapa* var. *perviridis*, the values were 0.21, 0.19, and 0.07 times compared with that of 0 mg/kgdry.

Volumetric water content has changed between 10 - 20 % on 5 cm depth between the experimental period because it was strongly affected by irrigation and evaporation. Regarding volumetric water content on 25 cm and 45 cm depth, there were a little changes of volumetric water content which were between 13-14 %. In addition, there was no runoff water at the experimental period, so that water consumption in the apparatus was due to evaporation from soil surface, evapotranspiration and absorption by plant.

4.2 Distributions of water soluble Cr(VI)

Figure 5 (a) - (c) are relationships between soil pH and Eh (Standard oxidation-reduction potential) converted from ORP values on each experimental conditions. Background colors of the figures represent valance of chromium. This figure means pH-Eh equilibrium diagram under the experimental condition. Plots in yellow background area might be existed as hexavalent, and plots in blue background area might be existed as pentavalent. According to figure 5, pH in the apparatus during the experimental period were between 8 - 10, and Eh in the apparatus during the experimental period were between 350-450 mV, thus chromium in the apparatus had been existed as hexavalent on the whole. Therefore, it can be assumed that reduction of Cr(VI) do not have to consider changings of valance of chromium, and the fluctuation was due to absorption of plants and advection by irrigation. Figure 6 (a)-(c) indicate distributions of water soluble Cr(VI) with depth. Maximum average, and minimum values are plotted with depth. Because of absorption of root and reaching effect by irrigation. Data of figure 6 (c) means changes of Cr(IV) in mixed decomposed granite soil with time without plant. For the reason why measured initial values were higher than decided addition, it could be assumed that measured three points were partially higher although it was tried to be uniformly mixed. The initial contents of Cr(IV) For *Brassica rapa* var. *perviridis*, water soluble Cr(VI) from surface to 15 cm depth where the roots existed reduced to 23.3 - 96.1 % compared with initial values. For *Helianthus annuus*, water soluble Cr(VI) from surface to 15 cm depth reduced to 21.8 - 64.5 % compared with initial values.

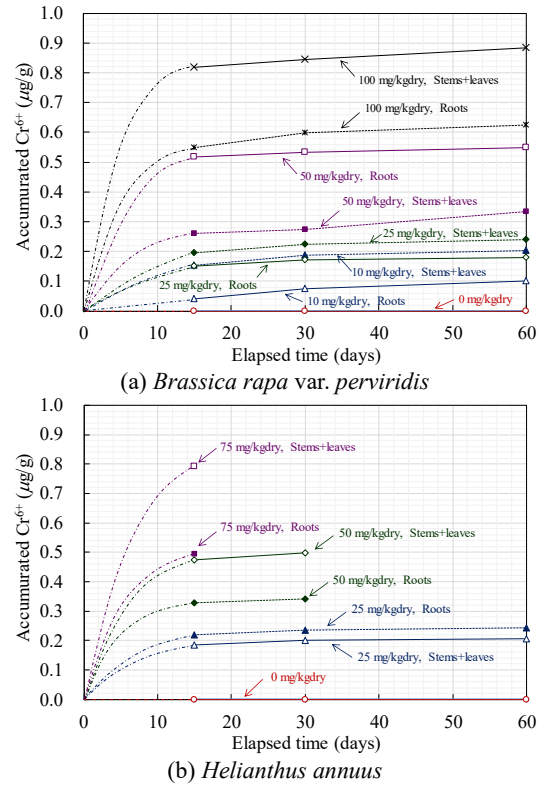


Fig. 8. Accumulation of Cr(VI) in plant body

As for the case of 25 mg/kg of initial Cr(VI) which both plant survived for 60 days, water soluble Cr(VI) on 60 days reduced to 23.3 - 64.2 %, 29.1 - 55.5 % from initial value respectively. In regard to deeper part than 15 cm depth where no root existed, content of water soluble Cr(VI) increased 1-28 % compared to initial values because absorption of roots might not work and Cr(VI) existed on upper part flew down to deeper part.

4.3 Absorption of Cr(VI) by plants

Figure 7 (a) represents total amount of Cr(VI) and reduction rate of Cr(VI) in soils versus experimental period with the case of planting *Brassica rapa* var. *perviridis*. Reduction rate written on each plot is defined as total amount of Cr(VI) at a targeted time divided by initial amount of Cr(VI). (see equation (1))

$$R = (1 - \text{Cr(VI)}(t) / \text{Cr(VI)}(0)) \times 100 \quad (1)$$

Where, R : Reduction rate of Cr(VI) (%), $\text{Cr(VI)}(t)$: Total amount of Cr(VI) in a soil box at arbitrary time t (g), $\text{Cr(VI)}(0)$: Initial total amount of Cr(VI) in a soil box (g).

Compared with initial Cr(VI) concentrations, total amount of Cr(VI) at 100 mg/kgdry of initial Cr(VI) was almost stable with the experimental period and reduction rate was almost 0 % through the whole experimental period. Therefore, In case of *Brassica rapa* var. *perviridis*, it might absorb Cr(VI) at less than 50 mg/kgdry of soil Cr(VI), and reduction rate ranged 16-38 %.

Figure 7 (b) represents total amount of Cr(VI) and reduction rate of Cr(VI) in soils versus experimental period with the case of planting *Helianthus annuus*.

Reduction rate is defined as total amount of Cr(VI) at a targeted time divided by initial amount of Cr(VI). In case of *Helianthus annuus*, it might absorb Cr(VI) at less than 25 mg/kgdry of soil Cr(VI), and reduction rate ranged 27-45 %.

Figure 8 (a) - (d) are accumulation of Cr(VI) in stems, leaves and roots. According to figure 7, it was found that amount of accumulation increased over time with the range of 10-100 mg/kgdry of Cr(VI) content. In regard to *Brassica rapa* var. *perviridis*, accumulation in stems and leaves was higher than that of root at the condition of initial content more than 50 mg/kgdry. Cr(VI) accumulation of stem after 2 months were 1.34-1.55 times higher than that of roots. Regarding total accumulation amount, when the values Paula (2013) obtained in 120 days are converted to values equivalent to 2 months (60 days), accumulation amount in roots were 24-34 $\mu\text{g/gdry}$ and accumulation amount in stem were 12-15 $\mu\text{g/gdry}$. That means the reduction rate was about 50 times higher than this experiment. When the values Unnikannan (2013) obtained in 120 days are converted to values equivalent to 2 months (60 days), accumulation amount in root were 25 $\mu\text{g/gdry}$ and accumulation amount in stem were 50 $\mu\text{g/gdry}$. That means the reduction rate was about 50-100 times higher than this experiment.

5 CONCLUSIONS

This paper presents to evaluate reduction effect of Cr(VI)-contaminated soil by two kinds of plants on various initial concentration through laboratory testing. Following conclusion were obtained from this experimental study.

- 1) The smaller average values of root length density in whole area of apparatus became, the higher initial content of Cr(VI) was. For *Brassica rapa* var. *perviridis*, on the condition of 10, 25, 50, 100 mg/kgdry, the values were 0.165, 0.023, 0.019 and 0.005 times respectively compared with that of 0 mg/kgdry. For root length density of *Helianthus annuus*, root reached shallower than 20 cm from soil surface in case that Cr(VI) was added more than 25 mg/kgdry. It is shown in a same trend of *Brassica rapa* var. *perviridis*, on the condition of 25, 50, 75 mg/kgdry, the values were 0.21, 0.19, and 0.07 times respectively compared with that of 0 mg/kgdry.
- 2) pH in the apparatus during the experimental period were between 8 - 10, and Eh in the apparatus during the experimental period were between 350-50 mV, thus chromium in the apparatus had been existed as hexavalent on the whole. In case of *Brassica rapa* var. *perviridis*, it might absorb Cr(VI) at less than 50 mg/kgdry of Cr(VI) in the soil, and reduction rate were 16-38 %. In case of *Helianthus annuus*, it might absorb Cr(VI) at less than 25 mg/kgdry of Cr(VI) in the soil, and reduction rate were 27 - 45%.
- 3) Both plants had a same trend that the higher root

length density was, the lower content of water soluble Cr(VI) was. There was a weak correlation root length density and content of water soluble Cr(VI).

- 4) It was found that amount of accumulation increased over time with the range of 10 - 100 mg/kgdry of Cr(VI) content. In regard to *Brassica rapa* var. *perviridis*, accumulation in stem was higher than that of root at the condition of initial content more than 50 mg/kgdry. Cr(VI) accumulation of stem after 2 months were 1.34-1.55 times higher than that of roots.

ACKNOWLEDGEMENTS

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REFERENCES

- 1) Cambridge Plating Company, Evaluation of Environmental Concerns and Cancer Incidence in Belmont and Surrounding Communities, Middlesex County, Massachusetts 1982-2003, ATSDR, 2007.
- 2) Cunningham S.D., and D. W. Ow, Promises and prospects phytoremediation, Plant Phys., Vol. 110, pp. 715-719, 1996.
- 3) Hase, T., and Kawamura, K., Germination test on *Komatsuna* (*B.rapa* var.*perviridis*) seed using water extract from compost for evaluating compost maturity: evaluating criteria for germination and effects of maturity, *J Mater Cycles Waste Management*, No. 14, pp. 334-340, 2012.
- 4) Japanese Geotechnical Society, JGS Standards, Vol. 3, JGS-0211, Maruzen Publishing, 2018.
- 5) Letica S. S., David, E. K., and Uttam, S., Cation Exchange Capacity and Base Saturation, University of Georgia extension, Circular 1010 pp. 1-4, 2017.
- 6) Maria Paula Escobar and Jenny Dussan, Phytoremediation Potential of Chromium and Lead by *Alnus acuminata* subsp. *acuminata*, Environmental Progress & Sustainable Energy (Vol.35, No.4) doi 10.1002/ep, pp. 942-948, 2013.
- 7) M. H. Timperley, R. R. Brooks and P. J. Peterson, The Significance of Essential and Non-Essential Trace Elements in Plants in Relation to Biogeochemical Prospecting, *Journal of Applied Ecology*, Vol. 7, No. 3, pp. 429-439, 1970.
- 8) Ministry of the Environment, National Effluent Standards, 2015.
- 9) Nishiguchi, Y., Ando S., Hayasaka, K., Ikeda, J., Hori, K., Suga, Y., and Fukunaga, A., Rapid Analysis of Magnesium, Potassium and Calcium Contents in Feed Stuffs, *Bulletin of The National Agricultural Research Center for Western Region*, No.6, pp. 113-139, 2007 (in Japanese).
- 10) Oh, K., Li, T., Cheng, H., He, X., and Yonemochi, S., Study on Tolerance and Accumulation Potential of Biofuel Crops for Phytoremediation of Heavy Metals, *International Journal of Environmental Science and Development*, Vol. 4, No. 2, 2013.
- 11) Soil Standard Analysis and Measurement Methods Committee, Soil Standard Analysis and Measurement Methods, pp.139-139, 155-160, 2003 (in Japanese).
- 12) Vasiliki, S., Vangelis, A., Sotiris, S., Marios, G.,K., Sofia, A., Niko laos S.T., and Ioannis Z., Metal Uptake by Sunflower (*Helianthus annuus*) Irrigated with Water Polluted Chromium and Nickel, *Foods*, Vol.6, No. 51, pp. 1-14, 2017.
- 13) WHO, Chromium in drinking-water. Background document for preparation of WHO Guidelines for drinking-water casualty. Geneva, WHO/SDE/WSH/03.04/4, 2000.