Journal of
Bioremediation & Biodegradation
ISSN: 2155-6199

Zhao et al., J Bioremediat Biodegrad 2016, 7:3
DOI: 10.4172/2155-6199.1000353

Rapid Commentary Open Access

Estimation of the Seedling Vigor Index of Sunflowers Treated with Various Heavy Metals

Xin Zhao[1], Jin Chul Joo[2], Dongwook Kim[3], Jung-Kul Lee[4] and Jae Young Kim[1]*

[1]Department of Civil and Environmental Engineering, College of Engineering, Seoul National University, 1 Gwanak-ro, Gwanakgu, Seoul 08826, Republic of Korea
[2]Department of Civil and Environmental Engineering, Hanbat National University, Dongseo-daero 125, Yuseong-gu, Daejeon 34158, Republic of Korea
[3]DOWOO Environment and Agriculture Technology, 408 Moving Techno Gwan, Wonkwang University, 460 Iksandae-ro, Iksan, Jeonbuk 54538, Republic of Korea
[4]Department of Chemical Engineering, Konkuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea

Abstract

In order to quantitatively describe the relative effect of the concentrations of heavy metals (i.e., Cd, Pb, Zn, and Ni) on the germination and seedling growth of plants, the seed germination tests were conducted using sunflower seeds treated with various heavy metals, and a mathematical model to estimate the seedling vigor index (SVI) is proposed. From the results of germination tests, a decrease in the seed germination percentage with an increase in the heavy metal concentration was observed, and the inhibitions of seedling growth were clearly detectable above certain critical concentrations (e.g., 50 mg-Cd/l, 50 mg-Ni/l, 100 mg-Zn/l, and 1,000 mg-Pb/l). According to both the IC_{50} and a_1 values, the resulting order of phytotoxicity for the heavy metals on sunflower seed germination was Cd>Ni>Zn>Pb. The SVI estimation model developed in this study rigorously explained the relationship between the heavy metal concentration and the SVI values. Thus, SVI values for a certain plants can be, *a priori*, obtained, with the heavy metal concentrations in the aqueous phase without performing the germination tests. Although the coefficients of SVI estimation model changed from different species of plants, the empirical SVI estimation model derived from theoretical background can be utilized for other species of plants.

Keywords: Germination; Heavy metals; Mathematical model; Phytotoxicity; Seedling vigor index; Sunflower

Introduction

Phytoremediation is an emerging cost-effective and environmentally sound alternative to conventional remediation technologies through the efficient use of plants to remove or immobilize environmental contaminates in complex matrices (soil, water or sediments) [1-3]. Since phytoremediation involves growing plants in a contaminated matrix, various types of plants have been evaluated. For example, many fast-growing plants for their ability to tolerate and accumulate metals have been investigated, including rapeseed (*Brassica napus* L.), Indian mustard (*B. juncea*), tumbleweed (*Salsola kali* L.) and so on [4,5]. Among the various types of plants, sunflowers (*Helianthus annuus* L.) have been commonly used in numerous phytoremediation studies due to their high tolerance to heavy metals and their ability to use the seeds as raw materials for biodiesel production [6-8].

The phytoremediation of heavy metals and metalloids such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn) in various environment has been reported [9], and the phytotoxicity of various heavy metals on different plant species has been investigated [10,11]. Based on previous studies, the variation in phytotoxicity of various heavy metals is mainly attributed to the differences in both the inherent toxicity of the various trace metals and the tolerances among plant species [12].

In order to identify the appropriate plant seeds that can remove or immobilize heavy metals in different matrices, the phytotoxicity of heavy metals on plant seeds has been investigated by monitoring both the inhibition of seed germination and the retardation of plant growth [13,14]. As yet, the most widely used acute phytotoxicity tests are both seed germination test (a direct exposure method) and the root elongation test [15].

According to previous studies [16-19], the germination of seeds was found to be greatly affected by both the type and concentration of heavy metals [20], and the lengths of both root and shoot were reduced with an increase in the target heavy metal concentrations [21]. Since the effective concentrations of heavy metals for a certain degree of inhibition are different, variously-reduced seed germination rates and differently-inhibited growth of root and shoot with increasing concentrations have been observed.

Recently, the seedling vigor index (SVI) has been used as a phytotoxicity index to evaluate the effect of heavy metal on seedling growth [22,23]. Seedling vigor is a measure of the extent of damage that accumulates as viability declines, and the damage accumulates in seeds until the seeds are unable to germinate and eventually die [24]. Numerous studies have been done to investigate the effects of various species and concentrations of heavy metals on the early growth of seedlings. Nonetheless, no mathematical model to quantitatively describe the relative effect of the concentrations of heavy metals on germination and seedling growth of plants has been developed.

In this study, a mathematical model to estimate SVI values is proposed to quantitatively describe the relative effect of the concentrations of heavy metals on the germination and seedling growth of plants. The specific objectives of this study were to compare the effects of heavy metals (i.e., Cd, Pb, Zn, and Ni) with different concentrations on seed germination of sunflower, and to develop a mathematical model to estimate the SVI values using the heavy metal concentrations.

***Corresponding author:** Jae Young Kim, Department of Civil and Environmental Engineering, College of Engineering, Seoul National University, 1 Gwanak-ro, Gwanakgu, Seoul 08826, Republic of Korea, Tel: +8228808364; Fax: +8228732684; E-mail: jaeykim@snu.ac.kr

Received April 25, 2016; **Accepted** May 05, 2016; **Published** May 10, 2016

Citation: Zhao X, Joo JC, Kim D, Lee JK, Kim JY (2016) Estimation of the Seedling Vigor Index of Sunflowers Treated with Various Heavy Metals. J Bioremediat Biodegrad 7: 353. doi: 10.4172/2155-6199.1000353

Materials and Methods

Germination tests in heavy metal solutions

Sunflower was selected as a hyperaccumulator plant in this study because sunflower can accumulate some heavy metals from the soil [25,26]. Sunflower seeds were obtained from a commercial market because the species and cultivar are frequently and widely cultivated in the Republic of Korea. Heavy metals [i.e., Cd (II), Ni (II), Pb (II), and Zn (II)] evaluated in this study were obtained from the salts of $CdCl_2{\cdot}2H_2O$; $NiCl_2{\cdot}6H_2O$; $PbCl_2$; and $ZnCl_2$, respectively, and purchased from Sigma-Aldrich Co., USA and DaeJung Chemicals & Metals Co. Ltd., Republic of Korea.

Since this study focused on the effects of heavy metals on the early growth of sunflower seeds, no other elements were added to eliminate others effects when using the heavy metal solutions. The concentrations of the heavy metals used in this study were determined based on a previous study [27], reporting that the concentration ranges of Cd (II), Ni (II), Pb (II), and Zn (II) in which seeds can germinate are significantly different. Deionized water was selected as the control (pH=7.14) test. The heavy metal concentrations of the solutions was decided from previous studies and ranged up to 500 mg/l for Cd and Ni, 3,000 mg/l for Pb, and 900 mg/l for Zn, respectively. The average pH values of Cd, Ni, Pb, and Zn solution were 5.31 ± 0.19, 5.69 ± 0.09, 4.76 ± 0.23, and 6.58 ± 0.12, respectively.

The phytotoxicity of the target heavy metals were evaluated using the seed germination test described by the Environment Protection Agency (EPA, OPPTS 850.4200, 1996a). For each seed germination test, 15 sunflower seeds were exposed to heavy metal solutions with different concentrations and placed in a multi spin shaker (VS-8480MX4, Vision Scientific Co., Ltd., Republic of Korea) under dark condition at 25 ± 1°C. The detailed experimental methods are presented in Table 1. After seven days, the seed germination percentage and the lengths of both root and shoot were measured. All the values are presented as the average values ± standard deviation (SD) obtained from at least three replicates. The heavy metal concentrations in the sunflower tissues were not analyzed because the growth amounts of sunflower tissues were too small for analyses.

The IBM SPSS statistics (Version 21.0) analysis program was used for the statistical analyses. The data were analyzed with one-way analysis of variance (ANOVA) to determine the effect of the treatments, and Duncan's multiple range tests was applied to test the statistical significance of the differences in the averages of the treatments. Linear regression analysis was also performed among the heavy metal concentrations, seed germination percentages, and elongations of the roots and shoots.

	Germination test
Test species	Sunflower (*Helianthus annuus* L.)
Pretreatment	10% sodium hypochlorite solution for 10 min.
Temperature	25 ± 1°C
Light quality	Dark
Test vessel	Whatman No. 42 filter paper over 185 × 30 mm (D × H) Culture dish
Test solution/soil	20 ml/vessel
Specimens	15 seeds/vessel
Replicates	3
Water control and dilution water	Deionized water
Test duration	7 days
Germination decision criteria	Growth of 5 mm or more from primary root

Table 1: Experimental design of the seed germination tests.

Model development

A germination test determines the maximum germination potential, or viability, of a seed and can be used to estimate the seed vigor, which is the extent of damage to a seed. Both mean root and shoot length were calculated after the seedling length was measured, and seed germination percentage is the percentage ratio of the number of germinated seeds to initial fifteen test seeds. The model presented here is based on the seedling vigor index [28] and calculated as follows:

Seedling vigor index (SVI)=[Mean root length (L_r)+Mean shoot length (L_s)] × Percentage of seed germination (GP) (1)

Where; GP is the seed germination percentage (%); L_r is the root length (L); L_s is the shoot length (L).

When the heavy metal concentration increased to certain levels (i.e., 1.008-9.968 μg-Cd/l, 4.968-9.936 μg-Pb/l and 19.89-159.05 μg-Zn/l), the growth of *A. paniculata* was enhanced, and no toxicity symptoms were observed [29]. However, above the critical levels of heavy metals, the linear correlation between the inhibitory rate of the root and shoot elongation versus tested heavy metal concentrations has been well described [30]. Based on these linear correlation between the inhibitory rate of the root and shoot elongation versus heavy metal concentrations above the certain critical levels, the germination percentage, root length, and shoot length can be expressed as follows:

$$GP=GP_0-a_1C \quad (2)$$

$$L_r=L_{r,0}-a_2C \quad (3)$$

$$L_s=L_{s,0}-a_3C \quad (4)$$

Where, GP_0 is the initial seed germination percentage (%), $L_{r,0}$ is the initial root length (L), $L_{s,o}$ is the initial shoot length (L), C is the heavy metal concentration (M/L^3), and a_1, a_2 and a_3 are the fitting coefficients.

Equations (2), (3) and (4) can be combined in equation (1) giving the following:

$$SV1=(a_2+a_3)a_1C^2-[(L_{r,o}+L_{s,o})a_1+(a_2+a_3)GP_0]C+(L_{r,o}+L_{s,o})GP_0 \quad (5)$$

Equation (5) is the quadratic function theoretically derived in this study. Based on the nature of the quadratic function, for the range of heavy metal concentrations in this study, the necessary conditions for the model was established as follows:

$$(L_{r,0}+L_{s,0})GP_0 \le \frac{\left[\left(L_{r,0}+L_{s,0}\right)a_1+(a_2+a_3)GP_0\right]^2}{4\left(a_2+a_3\right)a_1} \quad (6)$$

According to the nature of the quadratic function, the coefficient '$(a_2+a_3)a_1$' should be positive, which means that the SVI value decreases as the concentration of the heavy metals increases. When the absolute value of '$(a_2+a_3)a_1$' is higher, the SVI value decreases faster, and the phytotoxicity of the heavy metal is greater.

Results and Discussion

Effects of heavy metal concentrations on seed germination percentage

Germination started three days after the germination tests, and germination percentages of the sunflower seeds in both the control and heavy metal treatments were monitored seven days after the germination tests. The effects of Cd, Ni, Pb, and Zn in various aqueous

Cd		Ni		Pb		Zn	
Dose (mg/l)	**Germination percentage (%)**	**Dose (mg/l)**	**Germination percentage (%)**	**Dose (mg/l)**	**Germination percentage (%)**	**Dose (mg/l)**	**Germination percentage (%)**
0	75.56 ± 10.18*a**	**0**	75.56 ± 10.18a	**0**	75.56 ± 10.18a	**0**	75.56 ± 10.18a
50	57.78 ± 7.70ab	**50**	57.78 ± 16.78a	**1,000**	51.11 ± 20.37b	**50**	62.22 ± 7.70ab
100	48.89 ± 16.78bc	**100**	66.67 ± 13.33a	**1,500**	44.44 ± 7.70b	**100**	75.56 ± 10.18a
150	33.33 ± 11.55c	**200**	37.78 ± 3.85b	**2,500**	2.22 ± 3.85c	**300**	64.44 ± 7.70ab
300	11.11 ± 13.88d	**300**	13.33 ± 6.67c	**3,000**	0.00 ± 0.00c	**500**	53.33 ± 13.33b
500	0.00 ± 0.00d	**500**	0.00 ± 0.00c			**900**	0.00 ± 0.00c

*: Results are presented as the means ± SD.
**: Means with different letters are significantly different from each other ($P < 0.05$) according to the Duncan test (n = 3).

Table 2: Effects of heavy metal concentrations on the seed germination of sunflower seeds.

concentrations on the germination percentage of sunflower seeds are presented in Table 2, and a decrease in the seed germination percentage with an increase in the heavy metal concentration was observed. Similar results have been reported in previous studies on *Albizia lebbeck* and *Oryza sativa* L. with Cd, Pb, and Hg in aqueous solutions [31,32].

The low concentrations of Cd (i.e., 50 mg/l), Ni (i.e., 50 and 100 mg/l), and Zn (i.e., 50, 100 and 300 mg/l) evaluated in this study had no impact on the germination percentage compared to the control group. However, the sunflower seeds did not germinate at concentrations above 500 mg-Cd/l, 500 mg-Ni/l, 3,000 mg-Pb/l, and 900 mg-Zn/l. Similar to this study, the inhibition of germination of sunflower seeds at higher concentrations of Cd and Ni has been reported [33].

Heavy metal	Linear equation	R^2	IC_{50}* (mg/l)
Cd	GP = -0.1458C + 64.6902	0.9091	102.21
	Lr = -0.0051C + 2.1161	0.4351	
	Ls = -0.0094C + 4.8945	0.9514	
Ni	GP = -0.1570C + 71.9451	0.9249	139.78
	Lr = -0.0057C + 2.4436	0.5807	
	Ls = -0.0090C + 4.4347	0.9819	
Pb	GP = -0.0270C + 77.8947	0.9693	1033.14
	Lr = -0.0013C + 3.5482	0.9318	
	Ls = -0.0016C + 5.5148	0.7199	
Zn	GP = -0.0767C + 78.8412	0.8683	376.03
	Lr = -0.0046C + 3.5911	0.8737	
	Ls = -0.0051C + 4.6603	0.9083	

*: Half of the maximal inhibitory concentration, which was estimated with the GP linear function.

Table 3: Correlations between heavy metal concentrations and germination percentage, root length, and shoot length (C: heavy metal concentrations in solutions).

As summarized in Table 3, a linear regression between the heavy metal concentrations and germination percentages was obtained with R^2 values greater than 0.86. Based on this linear regression, the IC_{50} (half of the maximal inhibitory concentration) values of Cd, Ni, Pb, and Zn on sunflower seeds were estimated as 100.07 mg-Cd/l, 139.78 mg-Ni/l, 1033.14 mg-Pb/l, and 376.03 mg-Zn/l, respectively. The coefficient a_1 of GP for Cd, Ni, Pb, and Zn were also estimated as 0.146, 0.157, 0.027, and 0.767, respectively. According to both the IC_{50} and a_1 values, the resulting order of toxicity for the heavy metals on sunflower seed germination was Cd>Ni>Zn>Pb.

Effects of heavy metal concentrations on seedling growth

Heavy metal treatments inhibited both seed germination and the elongation of the root and shoot. Figure 1 shows the effects of the Zn concentrations on the length of the roots and shoots of the sunflowers. Regression analysis between the root length and Zn concentrations, as well as the shoot length and Zn concentrations showed a robust linear correlation with R^2>0.87. Similar results were also observed in the germination tests with the Cd, Pb, and Ni solutions (see Table

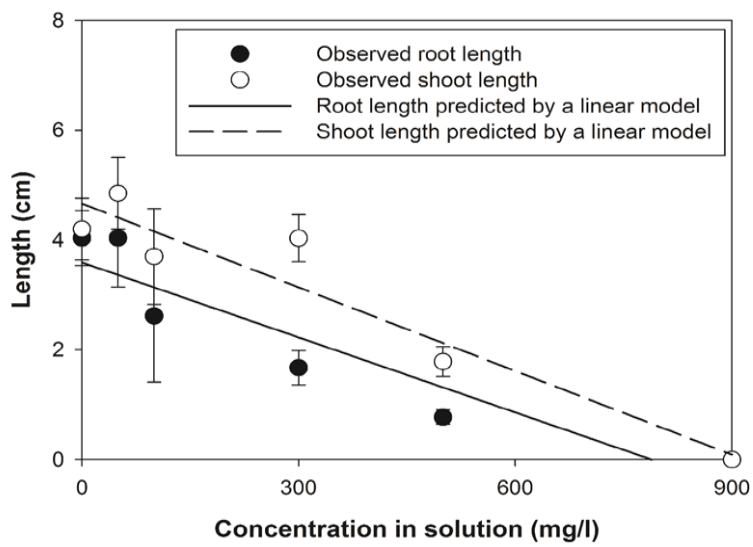

Figure 1: Effects of the Zn concentrations on the sunflower seedling growth.

3). As summarized in Tables 4 and 5, the lengths of both the root and shoot significantly decreased with the treatments of Cd, Ni, Pb, and Zn compared to the control group. Especially, the inhibitions were clearly detectable above certain critical concentrations (e.g., 50 mg-Cd/l, 50 mg-Ni/l, 100 mg-Zn/l, and 1,000 mg-Pb/l).

Because Cd is a highly toxic contaminant that affects many plant metabolic processes [34], treatments at 50 mg-Cd/l produced significant ($P<0.05$) effects on root growth compared to those at 50 mg/l for Ni, Pb, and Zn. All root elongations in the Cd treatments at various concentrations were shorter than those in the Ni, Pb, and Zn treatments. Considering that sunflower roots can accumulate significant amounts of these heavy metals, shorter root elongations in the Cd treatments indicate that the sunflower roots accumulated Cd to a higher degree than the other heavy metals. These results are consistent with a previous study using sunflower seeds treated with Cd and Cu solutions [35].

Interestingly, the shoot lengths exposed to the 50 mg-Cd/l, 1,000 mg-Pb/l, and 50 mg-Zn/l solutions exhibited a growth increase compared to those of the control by 16.32%, 15.57%, and 15.55%, respectively. This may be attributed to the metal hormesis, in which small doses of metal can increase the shoot growth [36]. Similar results have also been reported in previous studies using *Dorycnium pentaphyllum*, *Medicago sativa* L., and *Salosla kali* L. with Cd, Zn, Ni, and Pb [5,37,38].

Generally, the effects of heavy metals on seedling growth depend on the amount of toxic substance extracted from a given environment. As the concentrations of Cd, Pb, Zn, and Ni increased the seedling growth of sunflowers decreased. Similar tendencies have also been reported in other studies with *A. lebbeck* in Cd and Pb contaminated conditions [32]. For the phytotoxicity of heavy metals, root elongation was more sensitive than shoot elongation because the heavy metals show further accumulation in root than in shoot. For instance, Zn was mainly restricted to the radicle while Cd had an even distribution between the radicle and shoots [37].

Mathematical model

Using the inhibition of the germination percentage, root elongation, and shoot elongation as an index to describe the relationship between the heavy metal concentration and early seed growth has its limitations because the process of seed germination can be affected by various interactions of factors including temperature, water availability, oxygen, light, substrate, maturity of seed, and physiological age of seed [39]. Thus, the seedling vigor index (SVI) is required to compare the relative phytotoxicity in plants.

The relationship of the heavy metal concentrations and the seedling vigor index of the sunflower seeds are shown in Figure 2. Compared to those of the control, the sunflower seedlings showed a low tolerance of Cd, Ni, Pb, and Zn in their corresponding treatments. The relationship among the coefficients of the linear equations for GP, L_r, and L_s presented in Table 3 satisfies equation 6, which denotes the necessary conditions for the mathematical model.

The SVI estimation model developed in this study rigorously explained the relationship between the heavy metal concentration and the SVI values, as shown in Figure 2. Moreover, all R^2 values in the SVI estimation model using the heavy metal concentrations are greater than 0.90, indicating that the SVI estimation model to describe heavy metal phytotoxicity on seedling growth is reasonable. Thus, the SVI estimation model developed in this study was proved to have a high level of precision in describing the phytotoxicity of Cd, Ni, Pb, and Zn on sunflower seedling growth.

The coefficient $(a_2+a_3)a_1$ of the SVI estimation model for Cd, Ni, Pb, and Zn were 0.0041, 0.0035, 2.8341e-005, and 0.0005, respectively. According to the nature of the quadratic function, the heavy metal inhibition on early sunflower seedling growth was on the order of Cd>Ni>Zn>Pb. Similarly, heavy metal phytotoxicity follows the following trend: Pb ≈ Hg>Cu>Cd ≈ As>≈ Ni ≈ Zn>Mn [12]. Although similar heavy metal phytotoxicity order was obtained in this study, the

Cd		Ni		Pb		Zn	
Dose (mg/l)	**Root length (cm)**	**Dose (mg/l)**	**Root length (cm)**	**Dose (mg/l)**	**Root length (cm)**	**Dose (mg/l)**	**Root length (cm)**
0	4.03 ± 0.50*a**	**0**	4.03 ± 0.50a	**0**	4.03 ± 0.50a	**0**	4.03 ± 0.50a
50	0.75 ± 0.11b	**50**	1.28 ± 0.19b	**1,000**	1.71 ± 0.42b	**50**	4.03 ± 0.89a
100	0.92 ± 0.08b	**100**	1.18 ± 0.12b	**1,500**	1.37 ± 0.13b	**100**	2.61 ± 1.20b
150	0.86 ± 0.19b	**200**	0.94 ± 0.12bc	**2,500**	0.23 ± 0.40c	**300**	1.67 ± 0.31bc
300	0.54 ± 0.47b	**300**	0.63 ± 0.06c	**3,000**	0.00 ± 0.00c	**500**	0.77 ± 0.13cd
500	0.00 ± 0.00c	**500**	0.00 ± 0.00d			**900**	0.00 ± 0.00d

*: Results are presented as the means ± SD.
**: Means with different letters are significantly different from each other (P < 0.05) according to the Duncan test (n = 3).

Table 4: Effects of heavy metal concentrations on the root length of sunflower seedlings.

Cd		Ni		Pb		Zn	
Dose (mg/l)	**Shoot length (cm)**	**Dose (mg/l)**	**Shoot length (cm)**	**Dose (mg/l)**	**Shoot length (cm)**	**Dose (mg/l)**	**Shoot length (cm)**
0	4.20 ± 0.56*a**	**0**	4.20 ± 0.56a	**0**	4.20 ± 0.56a	**0**	4.20 ± 0.56a
50	4.88 ± 0.38a	**50**	4.07 ± 0.59a	**1,000**	4.85 ± 0.95a	**50**	4.85 ± 0.65ab
100	4.12 ± 0.24a	**100**	3.85 ± 0.64a	**1,500**	4.65 ± 1.06a	**100**	3.70 ± 0.87b
150	3.64 ± 0.57ab	**200**	2.66 ± 0.28b	**2,500**	1.10 ± 1.91b	**300**	4.03 ± 0.43ab
300	2.13 ± 1.91b	**300**	1.43 ± 0.08c	**3,000**	0.00 ± 0.00b	**500**	1.78 ± 0.27c
500	0.00 ± 0.00c	**500**	0.00 ± 0.00d			**900**	0.00 ± 0.00d

*: Results are presented as the means ± SD.
**: Means with different letters are significantly different from each other (P < 0.05) according to the Duncan test (n = 3).

Table 5: Effects of heavy metal concentrations on the shoot length of sunflower seedlings.

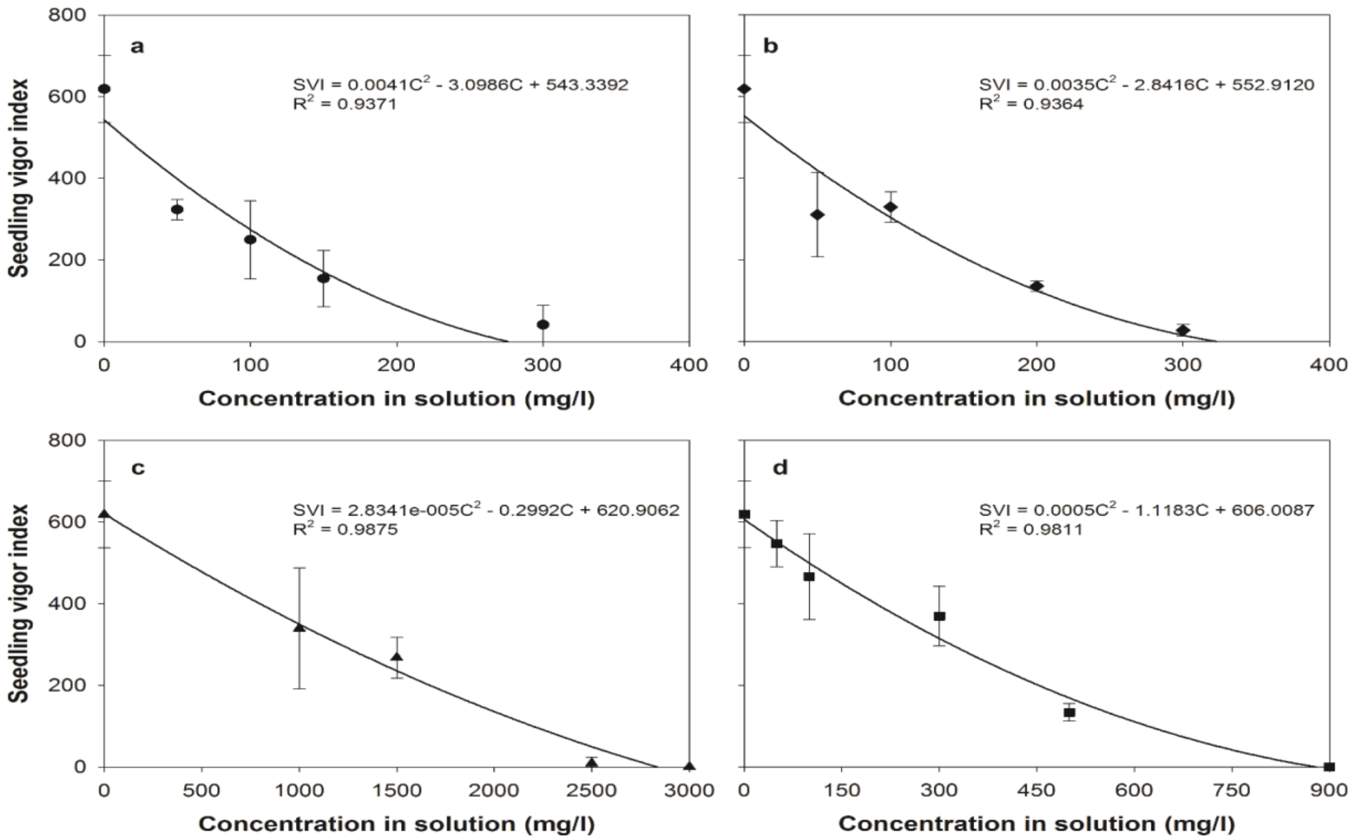

Figure 2: Effects of the Cd (a), Ni (b), Pb (c), and Zn (d) concentrations on the seedling vigor index of sunflower seeds.

slightly different results from this study were mainly attributed to the difference in the target plant species and the range of the heavy metal concentrations.

The SVI estimation model developed in this study is readily available in predicting SVI values when the value of the heavy metal concentration is within concentration ranges that sunflowers can germinate (In this study, the Cd, Ni, Pb, and Zn concentration range is 0-500 mg/l, 0-500 mg/l, 0-3,000 mg/L, and 0-900 mg/l, respectively). Thus, SVI values for a certain plants can be, *a priori*, obtained, with the heavy metal concentrations in the aqueous phase without performing the germination tests. Although the coefficients of SVI estimation model changed from different species of plants, the empirical SVI estimation model derived from theoretical background can be used for other species of plants.

Conclusions

The phytotoxicity of heavy metals on seed germination and seedling growth of the model species *Helianthus annuus* L. was evaluated. According to the results, Cd, Ni, Pb, and Zn treatments had significantly negative impacts on the germination percentage, seedling growth and seedling vigor index of sunflower seeds, compared to the control tests. Liner relationships can be drawn between the heavy metal concentrations and germination percentage and length of root and shoot within the range, and a quadratic function model between the heavy metal concentrations and the seedling vigor index was successfully derived. The SVI estimation model developed in this study rigorously explained the relationship between the heavy metal concentration and the SVI values. Thus, SVI values for a certain plants can be, *a priori*, obtained, with the heavy metal concentrations in the aqueous phase without performing the germination tests. The resulting order of phytotoxicity of heavy metals on sunflower seedling growth was Cd>Ni>Zn>Pb. Although the coefficients of SVI estimation model changed from different species of plants, the SVI estimation model can be used to explain the impact of heavy metals in soils on the germination and growth of hyperaccumulator plants. Future research is warranted to elucidate the universal usage of the SVI estimation model with various hyperaccumulator plants treated with heavy metals.

Acknowledgements

This work was supported by the Korea Ministry of the Environment as "The GAIA (Geo-Advanced Innovative Action) Project" and the Korea Ministry of Education through the Brain Korea 21 Plus research program. We also appreciate the technical support of the Institute of Construction and Environmental Engineering and Engineering Research Institute, Seoul National University.

References

1. Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2001) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30: 1919-1926.
2. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees--a review. Environ Int 29: 529-540.
3. Ali H, Khan E, Sajad MA (2013) Phytoremediation of heavy metals--concepts and applications. Chemosphere 91: 869-881.
4. Kumar PN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environmental science & technology 29: 1232-1238.
5. De La Rosa G, Peralta-Videa JR, Cruz-Jimenez G, Duarte-Gardea M, Martinez-Martinez, et al. (2007) Role of ethylenediaminetetraacetic acid on lead uptake and translocation by tumbleweed (Salsola kali L.). Environmental Toxicology and Chemistry 26: 1033-1039.

6. Schmidt U (2003) Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. J Environ Qual 32: 1939-1954.

7. Tang S, Xi L, Zheng J, Li H (2003) Response to elevated CO2 of Indian mustard and sunflower growing on copper contaminated soil. Bull Environ Contam Toxicol 71: 988-997.

8. Bastianoni S, Coppola F, Tiezzi E, Colacevich A, Borghini F, et al. (2008) Biofuel potential production from the Orbetello lagoon macroalgae: a comparison with sunflower feedstock. Biomass and Bioenergy 32: 619-628.

9. Lasat MM (2002) Phytoextraction of toxic metals: a review of biological mechanisms. J Environ Qual 31: 109-120.

10. Munzuroglu O, Geckil H (2002) Effects of metals on seed germination, root elongation, and coleoptile and hypocotyl growth in Triticum aestivum and Cucumis sativus. Arch Environ Contam Toxicol 43: 203-213.

11. Clabeaux BL, Navarro DA, Aga DS, Bisson MA (2011) Cd tolerance and accumulation in the aquatic macrophyte, Chara australis: potential use for charophytes in phytoremediation. Environmental science & technology 45: 5332-5338.

12. Kopittke PM, Blamey FP, Asher CJ, Menzies NW (2010) Trace metal phytotoxicity in solution culture: a review. J Exp Bot 61: 945-954.

13. Wong M, Bradshaw A (1982) A comparison of the toxicity of heavy metals, using root elongation of rye grass, Lolium perenne. New Phytologist 91: 255-261.

14. Wang W, Keturi PH (1990) Comparative seed germination tests using ten plant species for toxicity assessment of a metal engraving effluent sample. Water, Air, and Soil Pollution 52: 369-376.

15. Kapustka L, Reporter M (1993) Terrestrial primary producers. Handbook of ecotoxicology, pp: 278-298.

16. Lin J, Jiang W, Liu DC (2003) Accumulation of copper by roots, hypocotyls, cotyledons and leaves of sunflower (Helianthus annuus L.). Bioresour Technol 86: 151-155.

17. Groppa MD, Zawoznik MS, Tomaro ML, Benavides MP (2008) Inhibition of root growth and polyamine metabolism in sunflower (Helianthus annuus) seedlings under cadmium and copper stress. Biol Trace Elem Res 126: 246-256.

18. Lee KK, Cho HS, Moon YC, Ban SJ, Kim JY (2013) Cadmium and lead uptake capacity of energy crops and distribution of metals within the plant structures. KSCE Journal of Civil Engineering 17: 44-50.

19. Kötschau A, Büchel G, Einax J, Mirgorodsky D, Meißner R, et al. (2014) Element contents in shoots of sunflower (Helianthus annuus): Prediction versus measuring. Chemie der Erde-Geochemistry 74: 385-391.

20. Chakravarty B, Srivastava S (1992) Toxicity of some heavy metals in vivo and in vitro in Helianthus annuus. Mutat Res 283: 287-294.

21. Jadia CD, Fulekar MH (2008) Phytoremediation: the application of vermicompost to remove zinc, cadmium, copper, nickel and lead by sunflower plant. Environmental Engineering and Management Journal 7: 547-558.

22. Srivastava S, Thakur IS (2006) Evaluation of bioremediation and detoxification potentiality of Aspergillus niger for removal of hexavalent chromium in soil microcosm. Soil Biology and Biochemistry 38: 1904-1911.

23. Kabir M, Iqbal MZ, Shafiq M, Farooqi Z (2008) Reduction in germination and seedling growth of Thespesia populnea L., caused by lead and cadmium treatments. Pak J Bot 40: 2419-2426.

24. Copeland LO, McDonald M (2012) Principles of seed science and technology. Springer Science & Business Media.

25. Boonyapookana B, Parkpian P, Techapinyawat S, DeLaune RD, Jugsujinda A (2005) Phytoaccumulation of lead by sunflower (Helianthus annuus), tobacco (Nicotiana tabacum), and vetiver (Vetiveria zizanioides). J Environ Sci Health A Tox Hazard Subst Environ Eng 40: 117-137.

26. January MC, Cutright TJ, Van Keulen H, Wei R (2008) Hydroponic phytoremediation of Cd, Cr, Ni, As, and Fe: can Helianthus annuus hyperaccumulate multiple heavy metals? Chemosphere 70: 531-537.

27. Zhao X, Lee J, Han J, Kim D, Kim JY (2013) Comparison of Cd, Pb, Ni, Zn and As phytotoxicities under aqueous and soil conditions. 10th International Phytotechnologies Conference, International Phytotechnology Society, Syracuse, USA.

28. Abdul-Baki AA, Anderson JD (1973) Vigor determination in soybean seed by multiple criteria. Crop science 13: 630-633.

29. Tang YT, Qiu RL, Zeng XW, Ying RR, Yu FM, et al. (2009) Lead, zinc, cadmium hyperaccumulation and growth stimulation in Arabis paniculata Franch. Environmental and Experimental Botany 66: 126-134.

30. Wang M, Zhou Q (2005) Single and joint toxicity of chlorimuron-ethyl, cadmium, and copper acting on wheat Triticum aestivum. Ecotoxicol Environ Saf 60: 169-175.

31. Mishra A, Choudhuri M (1999) Monitoring of phytotoxicity of lead and mercury from germination and early seedling growth indices in two rice cultivars. Water, Air & Soil Pollution 114: 339-346.

32. Farooqi Z, Iqbal MZ, Kabir M, Shafiq M (2009) Toxic effects of lead and cadmium on germination and seedling growth of Albizia lebbeck (L.) Benth. Pak J Bot 41: 27-33.

33. Khan S, Moheman A (2006) Effect of heavy metals (cadmium & nickel) on the seed germination, growth and metals uptake by Chilli (Capsicum frutescens) and sunflower plants (Helianthus annuus). Pollution Research 25: 99.

34. Li M, Zhang LJ, Tao L, Li W (2008) Ecophysiological responses of Jussiaea rapens to cadmium exposure. Aquatic Botany 88: 347-352.

35. Groppa MD, Ianuzzo MP, Tomaro ML, Benavides MP (2007) Polyamine metabolism in sunflower plants under long-term cadmium or copper stress. Amino Acids 32: 265-275.

36. Calabrese EJ, Baldwin LA (1999) Reevaluation of the Fundamental Dose–Response Relationship A new database suggests that the U-shaped, rather than the sigmoidal, curve predominates. BioScience 49: 725-732.

37. Lefèvre I, Marchal G, Corréal E, Zanuzzi A, Lutts S (2009) Variation in response to heavy metals during vegetative growth in Dorycnium pentaphyllum Scop. Plant Growth Regulation 59: 1-11.

38. Peralta JR, Gardea-Torresdey JL, Tiemann KJ, Gomez E, Arteaga S, et al. (2001) Uptake and effects of five heavy metals on seed germination and plant growth in alfalfa (Medicago sativa L.). Bull Environ Contam Toxicol 66: 727-734.

39. Smýkal P, Vernoud V, Blair MW, Soukup A, Thompson RD (2014) The role of the testa during development and in establishment of dormancy of the legume seed. Front Plant Sci 5: 351.