Distribution of Heavy Metal Content in Plants and Soil from a Korean Shooting Site

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ABSTRACT : In this research we determined the levels of heavy metals in soil and metal-accumulating plants from a D military shooting site in the Kyungkido district of Korea. The data obtained may be useful in the development of methods for the efficient phytoremediation of contaminated soil. The total Cd, Cu, Pb, and Zn concentrations in the soil were found to be 1.67~5.04 mg/kg, 52.51~106.26 mg/kg, 37.24~90.32 mg/kg, and 111.45~188.19 mg/kg, respectively. These results show that the soil is contaminated with Cd and Cu, and this contamination is particularly severe in the case of Cd because of its high bioavailability (25~57% of the total metal in the soil is exchangeable). The high concentrations of heavy metals in the shoots of *Persicaria thunbergii* and *Artemisia princeps* var. *orientalis* indicate that these plants (all perennial herbs) accumulate heavy metal efficiently. Further, these plants were found to contain more Cd in its shoots (>60% of the total metal found in the plant) than any other plant; these results indicate that these native species are particularly suited to use in Cd phytoextraction.

Key words : *Artemisia princeps* var. *orientalis*, Heavy metals, *Persicaria thunbergii*, Phytoremediation, Plant, Shooting site

INTRODUCTION

Phytoremediation is a site, soil, or water remediation process that employs plants to remove contamination. This strategy has the following advantages over physico-chemical remediation approaches: lower costs (Glass 2000), generation of a recyclable metal-rich plant residue, minimal environmental disturbance, and no production of secondary air or water-borne wastes (Nanda 1995). The two main categories of phytoremediation strategies are phytoextraction and phytostabilization. Phytoextraction removes the contaminants by harvesting the plants that accumulate them (Nanda *et al*. 1995), and phytostabilization restricts the bioavailability and movement within soil of heavy metals through their accumulation in plants tolerant to them (Salt *et al*. 1995). The degree of accumulation of heavy metals by plants and the rate of the movement of heavy metals to the easily harvestable shoots depend on both the nature of the contaminant and on the plant species (Laperche *et al*. 1997). Heavy metals are absorbed into the plants" roots from the soil and then transported through the xylem. Plants that take up heavy metals from the soil can be classified into three categories (Baker 1981). An accumulator is a plant in which high concentrations of heavy metals collect in its shoots, an excluder is a plant with low concentrations of heavy metals in its shoots due to the restricted transport of heavy metals within the plant, and an indicator is a plant in which the concentration of a heavy metal in its shoots is directly proportional to the concentration in the soil. Many experiments have been performed on plants from these different categories, particularly on accumulator plants. These experiments are also often accompanied by a search for native plants that accumulate large amounts of heavy metals at sites that have extensive soil contamination such as mines (Dahmani-Muller *et al*. 2000; Pichtel *et al*. 2000). Plants that have adapted and become tolerant to the conditions at contaminated sites are expected to maximize the efficiency of remediation, because these plants are expected to minimize the heavy metal bioavailability by their adaptation to specific soil characteristics such as pH, organic matter content, and soil texture (Saxena *et al*. 1999).

Until now, the effort to find such native plants has been limited to investigations at mines (Jung and Iain 1996; Merrington and Alloway 1994) and at landfill sites (Seo and Cho 2002). However, the division of Korea into North and South led to a large increase in the number of military bases in South Korea. These bases occupy about 1,400 km^2, which is 1.4% of the total national land area. This percentage increases to about 5.9% when military installations and

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their protective zones are taken into account. At military firing ranges, the buildup of metallic waste from sources such as shells, cartridges, and bullets causes severe metal contamination of the soils. Kim *et al*. (2002) showed that the concentration of cadmium in the soil of shooting ranges in Korea was on average 38 times higher than in nearby control areas. In previous studies, our research group has investigated various aspects of the soil ecologies of military bases, such as the concentrations of heavy metals and TNT, the activity of microbial enzymes, and heavy metal bioavailability (Park *et al*. 2003; Lee *et al*. 2002).

The present study was carried out to determine the uptake of metals by plants growing at the D shooting site located in the Kyunggi-do district in Korea and to find native herbaceous plants with potential as accumulators.

MATERIALS AND METHODS

Sampling site and sample collection

The study site is located in the D general shooting area in Korea. This area has a continental climate, making the weather very warm and rainy in the summer, and cold and dry in the winter. The mean temperature, precipitation, and evaporation are 10.2℃, 1,353 mm, and 1,127 mm respectively (Korea Meteorological Administration 2000). The site has been used as a firing range for 45 years, and is contaminated by heavy metals and TNT from cannonballs. The shooting range is situated on the west slope of a mountain that is 440 m high, and covers an area of about 1.2×10^6 m^2. There is a steep gradient down from the mountaintop to an altitude of 300 m, and flat grassland is connected to a stream at an altitude of 200 m. Craters generated by the shots are scattered throughout the area and remain flooded during rainy periods. The upper area, bald spot(BS) of the study site exhibits ground depression and supports no plants due to the heavy shooting. The nonvegetated spot(NS) is rarely vegetated. The lower area, vegetated spot(VS) is richly vegetated and borders a brook (Fig. 1).

Samples (soil and plants) were obtained in spring (March) and autumn (October) of 2002. Each survey grid measured 10×10 m^2. Soil cores to a depth of 10 cm were collected from three randomly selected quadrants (1×1 m^2) in each survey grid (n=3 per grid) using a 5 cm diameter soil corer. Soil cores were placed in a cooler and stored at 2~4℃ until shipped for analysis. The vegetation biomass was determined by collecting plants from 1 m^2 quadrants at the same sampling locations. All plants present in the survey grids were recorded and the dominant species were determined through measurements of their relative coverage.

Soil chemistry

Soil texture was determined by the pipette method. Soil pH was determined by adding soil to water at a ratio of 1:2.5 w/v and measuring the pH after 1 hour. Organic matter content was determined by measuring the loss on ignition in a furnace (MAS 7000, CEM). Dehydrogenase activity was measured by the INT assay (Tabatabai 1982). The substrate used for these assays was 2-[4-iodophenyl]-3-[4-nitrophenyl]-5-phenyltetrazolium chloride (INT). The mixtures (fresh soil 3 g) were incubated for 24 hours at 37℃. Reaction products were detected using a spectrophotometer (DR/3000 Spectrophotometer, HACH) at 485 nm.

The total metal (Cd, Cu, Pb, and Zn) content of the soils was determined by an acid digestion method. Each 10 g sample of air-dried soil (< 0.5 mm) was digested in a mixture of concentrated HNO_3 and HCl in automatic microwave digester (MDS-2000;CEM) (Nieuwenhuize and Poley-Vos 1991). The heavy metal content of each extract was then determined using a flame atomic absorption spectrometer (AAS analysis 100, Perkin Elmer Analyst 100). The system was calibrated using certified reference materials (MESS-2: Marine Sediment) obtained from the National Research Council of Canada (NRS-CNRC). To determine the levels of exchangeable metals (Cd, Cu, Pb, and Zn), 1 g samples of dried soil were extracted with 1 N ammonium acetate for 1 hr. To determine the levels of soluble metals (Cd, Cu, Pb, and Zn), 2.5 g soil samples were extracted with 0.01 M KNO_3 for 2 hr. The total concentrations of exchangeable and dissolved metals were determined using graphite-furnace AAS and flame AAS.

Fig. 1. Description of the sampling site.
BS=bald spot, NS=nonvegetated spot, VS=vegetated spot.

Determination of metal levels in plants

Plants were washed with distilled water for 2 min and then dried at 70℃ in a drying oven. The herbaceous plants were divided into two parts: shoots and roots. Plant samples were digested in HNO_3. Heavy metal content was then determined using a graphite-furnace Atomic Absorption Spectroscopy (Perkin Elmer HGA 800) and flame atomic absorption spectroscopy (Perkin Elmer Analyst 100). The system was calibrated using certified reference materials (NO 10-C: Rice Flour) obtained from the National Institute for Environmental Studies of Japan (NIES).

Data analysis

Statistical comparisons between the three sites were made by applying two-way analysis of variance (ANOVA) using the SAS Package. Correlation analyses were performed to determine relationships among edaphic factors and total metal concentrations.

RESULTS AND DISCUSSION

Soil chemical properties

Values of other physico-chemical factors also varied among the different sites (Table 1). Because heavy metal bioavailability depends on soil properties such as pH, organic matter, and soil texture (Seo and Cho 2002; Lim *et al*. 1991), it is very important to investigate the chemical properties of each soil sample. The soil pH was found to be in the weakly acidic range (6.16~6.50). The moisture content decreased from 50.50% in the vegetated spot to 7.07 % in the BS in spring, but was lowest in the BS in both spring and autumn. The organic matter content ranged from 3.40 to 5.57%, and the DHA, which is a measure of the microbial activity, was found to be lower during autumn.

Table 1. Physico-chemical characteristics of soils at the D general shooting range

Factor	Bald spot (BS)	Nonvegetated Spot (NS)	Vegetated spot (VS)	P-value
pH	6.16±0.12 (6.50±0.38)	6.19±0.05 (6.20±0.07)	6.20±0.11 (6.36±0.54)	<0.001 (<0.05)
Moisture content (%)	7.07±1.07 (12.56±0.46)	16.33±9.21 (13.31±3.69)	50.50±8.94 (47.52±23.28)	<0.001 (<0.001)
Organic matter content (%)	5.57±0.38 (3.40±0.40)	5.23±0.64 (4.10±0.10)	5.30±1.92 (3.57±1.46)	<0.05 (<0.05)
Clay content (%)	20.37±2.65 (39.83±2.97)	28.10±4.35 (34.27±7.26)	41.80±2.28 (47.23±20.41)	<0.001 (<0.01)
Dehydrogenase activity[a] (㎍/g)	10.08±3.80 (9.08±4.84)	16.86±2.11 (8.19±1.90)	20.24±9.02 (11.04±3.35)	<0.001 (<0.05)

[a] Dehydrogenase activity is expressed as ㎍/g fresh wt soil.
Values represent mean ± standard deviation of triplicate. Values shown are for both spring and autumn (in parentheses).

A correlation between total heavy metal and organic matter could not be established conclusively. Total heavy metal concentrations were significantly negatively correlated with moisture content (r= -0.86, p=0.01) and dehydrogenase activity (r=-0.79, p=0.05) (Fig. 2). These results are similar to those reported in a study investigating heavy metals and edaphic factors in the soils of Mt. Nam located in the center of Seoul city and a natural reserve forest site (Lee *et al*., 1998), and also with those of a study investigating the heavy metal accumulation by plants at iron mines and a military shooting range (Kim *et al*. 2002).

Soil heavy metal concentrations

To investigate the levels of metal contamination at the site, the concentrations in the soil of four heavy metals (Cd, Cu, Pb, and Zn)

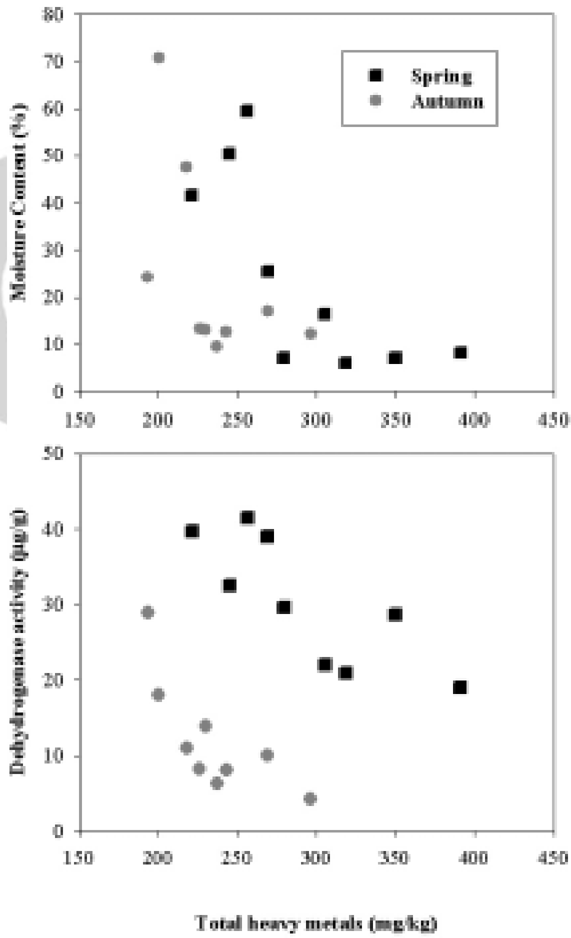

Fig. 2. Correlation between (a) moisture content and (b) dehydrogenase activity and the concentration of total heavy metals in soil at D general shooting range.

were determined (Table 2). The concentrations of the heavy metals varied significantly with sampling site, *i.e.* the heavy metal levels at each sampling site are dependent on the amount of shooting carried out there; the BS had particularly high concentrations of heavy metals due to the direct influence of concentrated shooting. The concentrations of Cd in the soil were found to be 1.67~3.84 mg/kg in spring and 2.37~5.14 mg/kg in autumn. Similarly, the concentrations of Pb in the soil were found to be 43.09~48.99 mg/kg and 46.59~50.92 mg/kg respectively, the concentrations of Cu were found to be 59.17~106.26 mg/kg and 52.40~74.38 mg/kg respectively, and in the case of Zn, 132.77~188.19 mg/kg and 111.45~124.42 mg/kg respectively. The levels of Cd, Cu and Zn in the soil of the shooting range are higher than the upper limits for non-polluted soil, as reported by Temmerman *et al.* (1984), *i.e.*, Cd 1 μg/g, Cu 25 μg/g and Zn 150 μg/g, and of most concern are the levels of Cd and Cu contamination, which exceed the Korean Anxiety Standard Levels of 1.5 mg/kg for Cd and 50 mg/kg for Cu by factors of about upto 3.4 and 2.1 respectively. As shown in Table 2, we found that the concentrations of the heavy metals (Cd, Cu and Zn) increased as we moved from the VS spot to the BS spot, *i.e.* towards the shooting target site.

Table 2. Total, exchangeable and soluble metal concentrations (mg kg^{-1} dry soil) at sampling sites

		Bald spot (BS)	Nonvegetated Spot (NS)	Vegetated spot (VS)	P-value
Cd	Total	3.84±1.00	2.51±0.74	1.67±.0.16	<0.001
		(5.14±1.45)	(2.91±0.73)	(2.37±1.03)	(<0.001)
	Exchangeable	2.06±0.65	0.98±0.28	0.95±0.59	<0.001
		(1.42±0.18)	(1.13±0.58)	(0.60±0.24)	(<0.001)
	Soluble	ND[a]	ND[a]	ND[a]	-
		(ND[a])	(ND[a])	(ND[a])	-
Cu	Total	106.26±26.29	62.38±23.97	59.17±12.51	<0.001
		(74.38±21.00)	(52.40±9.05)	(52.99±5.59)	(<0.01)
	Exchangeable	4.47±0.67	2.40±0.15	2.61±0.47	<0.001
		(1.60±0.19)	(3.39±1.08)	(1.18±0.18)	(<0.05)
	Soluble	0.71±0.0	0.57±0.05	0.54±0.00	<0.001
		(0.40±0.25)	(0.37±0.07)	(0.15±0.02)	(<0.001)
Pb	Total	47.14±5.71	43.09±5.07	48.99±5.97	<0.05
		(51.92±10.05)	(46.59±7.26)	(49.25±5.95)	(<0.05)
	Exchangeable	0.40±0.09	0.37±0.10	0.37±0.18	<0.05
		(0.52±0.44)	(1.24±0.97)	(0.06±0.07)	(<0.05)
	Soluble	ND[a]	ND[a]	ND[a]	-
		(ND[a])	(ND[a])	(ND[a])	-
Zn	Total	188.19±9.56	158.93±40.44	132.77±7.57	<0.001
		(111.45±0.61)	(124.42±12.86)	(113.29±4.34)	(<0.05)
	Exchangeable	3.15±0.85	2.02±0.77	0.83±0.38	<0.001
		(3.29±0.48)	(3.27±0.68)	(1.69±0.21)	(<0.001)
	Soluble	ND[a]	0.04±0.06	0.22±0.32	<0.05
		(0.68±0.32)	(0.51±0.01)	(0.06±0.01)	(<0.001)
Total heavy metals		349.90	269.31	245.21	-

Values represent mean ± standard deviation of triplicate. Values shown are for both spring and autumn (in parentheses).

[a] ND: not detected at detection limit of 0.01mg L^{-1}.

In order to evaluate the influence of these heavy metal levels in the soil on organisms, we determined the levels of exchangeable and soluble metals in the soil samples by treating them with an ammonium acetate solution and a KNO_3 solution respectively (Table 2). Ure (1995) have suggested that the most direct indication of the noxious effects of heavy metals on the environment can be obtained from estimations for soil samples of the soluble and exchangeable fractions of heavy metals. For Cu, Pb, and Zn, the levels of exchangeable metal in the soil samples considered here (2~6%, 0~4%, and 1~3% respectively of the total metal content) and the levels of soluble metal (< 1% of the total metal content) were found to be very low. This shows that only small amounts of the metals in the soil samples are soluble, so although the soils are highly contaminated by metals, only a small fraction of the total metal in the soil is in a bioavailable form. However, the concentrations of exchangeable Cd in the soil samples were in the range 25% to 57%, and thus there are concerns about the environmental hazard posed to this ecosystem by its contamination with Cd.

Uptake of heavy metals by the plant

To evaluate the levels of contamination by Cd in native plant species, we investigated the above-ground vegetation on the site by collecting plants from 1 m^2 quadrants (Table 3). The dominant grasses in VS spot were found to be *Ambrosia trifida*, *Ambrosia artemisiifolia* var. *elatior*, *Artemisia princeps* var. *orientalis*, *Glycine soja*, *Persicaria thunbergii* and *Typha orientalis*.

To find species suitable for use in phytoextraction, we determined the levels of accumulated heavy metals in the dominant plants, the uptake of metals by each tissue, and the metal partitioning between shoots and roots (Table 4). Phytoextraction is the harvesting of plants that take up heavy metals from the soil and transport them into the shoots. This remediation method is very efficient comparing to existing physico-chemical processes because combustibles of plant mass is less than 10 % of soil mass with equal heavy metal content (Glass 2000). Markert (1994) have reported that the concentrations of Cd, Cu, Pb, and Zn normally found in plants are 0.05 mg/kg, 10 mg/kg, 1 mg/kg, and 50 mg/kg

Table 3. Plants sampled from the shooting site

Scientific Name	Ecotype
Aeschynomene indica	annual
Ambrosia trifida [a]	perennial
Ambrosia artemisiifolia var. *elatiora*	perennial
Artemisia capillaries	perennial
Artemisia selengensis	perennial
Artemisia princeps var. *orientalis* [a]	perennial
Aster subulatus	annual
Bidens tripartite	annual
Bromus yezoensis	perennial
Calamagostis epigeios	perennial
Cardamine flexuosa var. *fallax*	biennial
Carsella bursa-pastoris	biennial
Chenopodium album var. *centrorubrum*	annual
Commelina communis	annual
Cyperus microiria	annual
Digitaria sangurialis	annual
Echinochloa crusgalli var. *crusgalli*	annual
Erigeron canadensis	biennial
Glycine sojaa	annual
Hemistepata lyrata	biennial
Humulus japonicus	annual
Jancus effuses var. *decipiens*	perennial
Kummerowia striata	annual
Miscanthus sacchariflorus	perennial
Oenanthe javanica	perennial
Oenothera odorata	biennial
Persicaria hydropiper	annual
Persicaria sieboldi	annual
Persicaria thunbergii [a]	annual
Plantago asiatica	perennial
Polygonum aviculare	annual
Potentilla kleiniana	perennial
Portulace oleracea	annual
Rorippa indica	perennial
Ranunculus japonicus	perennial
Setaria viridis	annual
Typha orientalis [a]	perennial
Thlaspi arvense	perennial

[a] represent dominant grasses.

respectively. The concentrations of Cd, Cu, Pb and Zn in the plants on the site were found to be higher than these normal concentrations. The ratios of S/R heavy metal concentrations were variable. In general, the concentrations of all metals were found to be 1.2~4 times higher in root tissues than in shoot tissues. However, *P. thunbergii* and *A. princeps* var. *orientalis* contained more Cd in its shoots (> 60% of the total Cd in the plant) in both seasons; the S/R ratios in *P. thunbergii* were 1.57 in spring and 1.52 in autumn and those in *A. princeps* var. *orientalis* were 3.12 in spring and 1.50 in autumn. The ratios of shoot/root heavy metal concentrations (S/R) in plants depend on the genetic characteristics of the species and on environmental conditions. Generally, a ratio of >1.0 for a given plant indicates that it accumulates heavy metals (Kim *et al*. 1999; Baker 1981) and that this species is suitable for use in phytoextraction. The levels of Cd in both plants were also high; *P. thunbergii* were 16.05 ppm in spring and 23.03 ppm in autumn and those in *A. princeps* var. *orientalis* were 7.09 ppm in spring and 12.50 ppm in autumn. The most important factor for success of phytoremediation is the capacity of the plants to accumulate metals in their shoots (Ernst 1996). *A. princep* var. *orientalis* is very well known as an indigenous metal accumulator that is suitable for use in phytoremediation (Nanda *et al*. 1995; Temmerman *et al*. 1984). Kim *et al*. (1999) concluded that *A. princep* var. *orientalis* has potential as a soil remediation plant from a survey in Korea of inactive and abandoned mining sites. *A. princep* var. *orientalis* is also a dominant heavy metal accumulator in landfill areas (Seo and Cho 2002) and perennial so has roots that persist through winter and have large biomass, which is important for phytoextraction because the quantities of heavy metals absorbed by plants with small biomasses are small (Ebbs *et al*. 1997). Taken together, these results indicate suggest that *P. thunbergii* and *A. princeps* var. *orientalis* are as suitable species for use in phytoextraction.

This study of a D military shooting range in the Kyungkido district of Korea has shown it to be contaminated with Cd and Cu; the contamination with Cd is especially serious because of the presence of high levels of exchangeable Cd. Of particular concern is the potential for the Cd to spread into the aquatic ecosystem through the brook bordering the lower area of the site. In cases where continuous contamination is unavoidable, such as in this shooting range, the efficiency of contaminant removal can be improved by the use of phytoextraction and the revegetation of the site with the appropriate herbaceous plants. Our analysis of the seasonal variation of the heavy metal concentrations in the plants enabled us to determine which plants contain high concentrations of heavy metals in each season. As a result of our survey at the D shooting site, *P. thunbergii* and *A. princeps* var. *orientalis* have been identified as suitable species for use in phytoextraction.

Table 4. Metal concentrations(mg/kg) in dominant plant species

Species	Organ	Cd	Cu	Pb	Zn	Total heavy metals
Glycine soja	shoot	0.49±0.28	39.51±20.51	3.54±0.26	67.21±17.11	110.75
	root	5.35±0.33	99.82±33.05	2.17±1.43	305.32±71.21	412.66
	S/R ratio	0.09(0.08)	0.40(0.20)	1.63(0.59)	0.22(0.34)	0.27(0.31)
Persicaria thunbergii	shoot	16.05±4.36	43.55± 6.39	4.47±2.51	54.89± 9.56	118.96
	root	10.22±4.35	69.12± 3.23	3.48±1.75	77.58±19.11	160.4
	S/R ratio	1.57(1.52)	0.63(0.54)	1.28(0.74)	0.71(0.93)	0.74(0.85)
Typha orientalis	shoot	0.29±0.16	21.93± 6.23	1.23±0.17	62.18±16.79	85.63
	root	0.33±0.14	115.74±53.05	8.01±4.58	119.16±32.98	243.24
	S/R ratio	0.88(0.79)	0.19 (0.21)	0.15(0.09)	0.52(0.21)	0.35(0.26)
Artemisia princeps var. *orientalis*	shoot	7.09±3.25	45.40±16.13	1.68±0.97	55.22± 8.16	109.39
	root	4.73±0.57	57.81±19.28	1.05±0.52	85.68±10.03	149.27
	S/R ratio	1.50(3.12)	0.79(1.02)	1.60(0.85)	0.64(1.40)	0.73(1.59)
Ambrosia trifida	shoot	7.61±4.34	56.39±24.38	3.16±1.24	88.83±36.23	155.99
	root	11.09±3.12	56.99±15.83	3.46±1.14	77.43±15.26	148.97
	S/R ratio	0.69(0.58)	0.99(0.10)	0.91(0.63)	1.15(0.27)	1.05(0.59)
Ambrosia artemisiifolia var. *elatior*	shoot	0.79±0.35	31.89±12.36	1.07±0.18	54.25±14.16	88.00
	root	3.74±1.67	77.27±13.80	2.16±1.02	115.63±14.41	198.80
	S/R ratio	0.21(0.11)	0.41(0.34)	0.50(0.36)	0.47(0.37)	0.44(0.40)

Values represent mean ± standard deviation of triplicate. Values shown are for spring.

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