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Soil compaction as a stressor, and its effect on cadmium toxicity to *Paronychiurus kimi* (Collembola)

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ABSTRACT

The effects of soil compaction alone or in combination with cadmium treatment on *Paronychiurus kimi* were investigated in OECD artificial soil in order to quantify how changes in compaction affect the behavior and reproduction of *P. kimi*. Four series of compaction levels including an uncompacted control were examined (0, 15.7, 28.0, and 73.7 g/cm^2). The cadmium concentration used in these experiments was 28.3 mg/kg (28-d EC_{50}-reproduction). Movement of *P. kimi* adults between soil surface and subsoil, which was expressed as the difference in the number found on the surface to those in the subsurface of the soil divided by the total (STS), was used as the behavioral endpoint. In the compaction only experiment, STS increased and reproduction decreased significantly in a compaction level dependent manner, indicating that compaction is an important stressor affecting the performance of *P. kimi*. In cadmium treated soils, the STS patterns was similar to those in the compaction only experiment, but the decrease in reproduction in the cadmium treated compacted soil was different. The most notable finding was that the change in the cadmium bioaccumulation factor was closely related to the relative reduction in reproduction ($r^2 = 0.81$), implying soil compaction affected the uptake and toxicity of cadmium in *P. kimi* by influencing the magnitude of exposure to cadmium through dermal contact. The present study clearly demonstrated that soil compaction is an important factor affecting the performance (behavior and reproduction) of *P. kimi* as well as the toxicity and bioavailability of cadmium to *P. kimi*. Therefore, soil compaction should be included in soil ecotoxicity tests and ecological risk assessments.

1. Introduction

Soil as a habitat for a variety of soil organisms is substantially heterogeneous with respect to the soil physico-chemical properties, which creates various types of habitats in the soil ecosystem. As a consequence of this heterogeneity in soil properties, the distribution and abundance of soil organisms may be restricted to habitats that they are best adapted because soil organisms vary in their tolerance to these soil properties (Whitford, 1996; Bardgett, 2005). Among the many soil physico-chemical properties, the impacts of pH, organic matter (OM) content, texture, water content, aeration, available nutrients and soil compaction on distribution and abundance of soil organisms have been most widely studied (Kevan, 1962; Kuhnelt, 1976; Heisler, 1991; Heisler and Kaiser, 1995; Schrader and Lingnau, 1997; Salmon and Ponge, 1999; Eaton et al., 2004; Son et al., 2009a,c).

Soil compaction causes a rearrangement of soil particles and as a result many properties of the soil are altered including the pore size distribution, total porosity, and the movement and content of heat, air, water and nutrients in the soil. Thus, soil compaction may be considered as a stressor that restricts the distribution and abundance of soil organisms. Several studies have reported that soil arthropods and microbial biomass are sensitive to compaction-induced changes in soil structure (Joschko, 1990; Heisler, 1991). Furthermore, Staempfli et al. (2007) reported that soil Collembola are more sensitive to contaminants in compacted soil than in uncompacted soil. Although soil compaction has been shown to negatively impact the environment and agriculture, little attention has been paid to understand the effect of soil compaction on soil organisms in ecotoxicological issues.

Among the soil arthropods, Collembola comprise the most widespread and abundant species found in many terrestrial ecosystems (Hopkin, 1997). They play important roles in decomposition processes and in forming soil microstructures (Whitford, 1996; Rusek, 1998) and they also serve as food for other soil organisms (Hopkin, 1997; Rusek, 1998). The abundance of Collembola in soils is closely linked to soil structure and function (Usher, 1976), and

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thus their abundance and distribution in a particular habitat can serve as an indicator of soil quality (ISO, 1999). Since Collembola are not able to make their own burrows and are entirely dependent on earthworm burrows and air-filled pores that have dimensions close to their body width, soils that are too fine or compact may not be suitable for burrowing and inhabiting. Thus, it is imperative to understand how soil structural changes influence the activity of Collembola, and how these factors may modify the toxicity effects of contaminants. Understanding the answers to these questions is fundamental to both ecological and toxicological research. If these factors significantly affect activity and toxicity, they should be considered when assessing the toxicity of contaminants to Collembola.

Recently, *Paronychiurus kimi* (Lee) (Collembola: Onychiuridae), a Collembola species native to Korean soils, was determined to be a suitable ecotoxicological test animal in accordance with the ISO guideline (1999) (Son et al., 2007, 2009a,b,c), and is listed as an alternative to *Folsomia candida* for toxicity tests (OECD, 2008). However, there is still a need to increase the number of toxicity tests by using soil invertebrates that represent a wider range of life histories in order to increase the ecological relevance of data and reduce uncertainty in ecological risk assessment (Van Straalen and Løkke, 1997; Løkke and van Gestel, 1998).

In this study, the effect of soil compaction on movement behavior from the soil surface to the subsoil and reproduction of *P. kimi* was investigated in standard OECD soils. The soil structure was altered by compaction in order to quantify how changes in habitable pore space affected the behavior and reproduction of *P. kimi*. To assess the effect of soil compaction on cadmium toxicity, a single concentration of cadmium (EC_{50}-reprodcution) was applied to the compacted soils. The accumulation of cadmium by *P. kimi* was measured to determine the effect of soil compaction on the bioavailability of cadmium to *P. kimi*.

2. Materials and methods

2.1. Test animal

Cultures of *P. kimi* were started from a population extracted by floatation in water from paddy soils in Korea (Choi et al., 2002). *P. kimi* were maintained in the laboratory using a method similar to the one developed for *F. candida* (Snider et al., 1969; Son et al., 2007). *P. kimi* were cultured on a moist substrate that was comprised of a 4:1:4 ratio (by volume) of plaster of Paris, activated charcoal and distilled water in plastic Petri dishes (9.5 cm in diameter, 1.5 cm in height), which were filled with approximately 0.5 cm of the media and incubated at 20 ± 1 °C under continuous darkness. The cultures were fed a few drops of Brewers yeast diluted with distilled water (100 mg granulated Brewers yeast in 30 ml distilled water) weekly. To obtain a large number of synchronized adult *P. kimi* (42–44 d old), hundreds of adults were separately introduced into several breeding substrates, and allowed to lay eggs. The adults were then removed after 3 d. As soon as the eggs hatched (after ≈ 14 d), the juveniles were cultured under the same conditions described above. Adult cohorts cultured from this method were used throughout the experiments.

2.2. Test soil and soil compaction

The artificial soil used in the test was composed of the same ingredients as those prescribed by OECD (1984), which consists (by dry weight) of 70% quartz sand, 20% kaolin clay, and 10% finely ground peat. The soil pH was adjusted to 6.0 ± 0.5 through the addition of $CaCO_3$. Distilled water was added in sufficient quantities to bring the water holding capacity up to 50%. Thirty grams of the soil (wet weight) was added to each polystyrene container (75 mm diameter × 63 mm height, 200 ml volume). Surface pressure was applied through a unidirectional force for 5 min to the top of soil with a cylindrical rod of approximately the same diameter of the container. Soil compaction level was quantified by dividing the weight of the soil by the volume of the soil.

Four different levels of soil compaction, including the uncompacted control (C_0) were prepared; 15.7, 28.0, and 73.7 g/cm^2, referred to as C_1, C_2, and C_3, respectively. All experiments were conducted in triplicate at each compaction level. After compaction, the soil moisture content was adjusted by replenishing the weight loss with the appropriate amount of deionized water, if necessary.

2.3. Soil bulk density and porosity

The post-compression height of the soil column was measured and the volume of the soil was calculated by multiplying the height by the cross sectional area of the container. The bulk density was then calculated as follows:

$$\text{Bulk density (g/cm}^3) = \frac{\text{dry weight of soil (g)}}{\text{volume of soil (cm}^3)} \quad (1)$$

The porosity of the compacted soil was calculated from the bulk density data by assuming that the particle density of the artificial soil was 2.65 g/cm^3 (Brady and Weil, 1996):

$$\text{Porosity (\%)} = \left(1 - \left(\frac{\text{bulk density}}{\text{particle density}}\right)\right) \times 100 \quad (2)$$

2.4. Effect of soil compaction on behavior and reproduction

The 28-d reproduction test with *P. kimi* was carried out according to the method of ISO 11267 (1999). Ten synchronized (42–44 d old) *P. kimi* adults were introduced on the soil surface of each container, which had been compacted as described in Section 2.2. The containers were kept in continuous darkness at 20 ± 1 °C during the test periods (28 d). The soil moisture content was adjusted weekly by replenishing the weight loss with the appropriate amount of deionized water. Granulated Brewers yeast was added to the soil surface as a food source two times during the test (at the beginning and 14 d after test). At the end of the test, the surviving adults and resulting juveniles were counted after floatation.

To investigate whether soil compaction affected the behavior of *P. kimi*, which should move from the surface to the subsurface of the soil, the number of adults found on the surface of the soil were counted at 4-d intervals throughout the duration of exposure. At the end of the test, the surviving adults and resulting juveniles were counted using the floatation method and recorded separately. In the controls (C_0, uncompacted soils), the number of juveniles always exceeded 100 juveniles per container and the mortality was less than 5%.

2.5. Effects of soil compaction on cadmium toxicity to P. kimi

The same compacted soils and methodologies described in sections 2.2 and 2.4 were used to assess the combined effects of soil compaction and cadmium toxicity on *P. kimi*.

2.5.1. Test chemical and soil treatment

The compacted soils were treated with a solution of cadmium chloride hemi pentahydrates ($CdCl_2 \cdot 2½H_2O$; CAS No. 7790-78-5; 98% purity; Sigma–Aldrich®) in deionized water, at a concentration of 28-d EC_{50}-reproduction = 28.3 (23.7–33.8; 95% confidence intervals) mg/kg (Son, 2009). The EC_{50}-reproduction corresponded to the 2% lethal concentration (LC_2). Equation used for this calculation was $y = 2.93\log(x) - 1.16$, where x = cadmium concentration and y = probit scale. This cadmium concentration ensures that adult

mortality was very low, but the expected reduction in the juvenile production was nearly 50%. The amount of water used was sufficient to moisten the soils to 50% of the water holding capacity.

Before the artificial soil was spiked with the cadmium solution, the cadmium concentration in the solution was measured using a Vista-PRO inductively coupled plasma-optic emission spectrometer (ICP-OES) at a wavelength of 228.9 nm (Varian, Australia) to confirm its nominal concentration. The nominal concentration was used as the total cadmium concentration in the soil, since the measured cadmium concentration in the stock solution did not differ from the nominal concentration by more than 5%. The cadmium solution was treated thoroughly with artificial soil 1 d before the start of the test. Four series of cadmium treated compacted soils ($C_0 - C_3$) were prepared, and are hereafter referred to as $C_0 + Cd$ (uncompacted soil + 28.3 mg/kg cadmium), $C_1 + Cd$, $C_2 + Cd$, and $C_3 + Cd$.

2.5.2. Bioaccumulation factor

All the surviving adults were collected separately according to the compaction level at the end of the experiments. The collected adults were oven dried at 70 °C for 24 h and then weighed to five decimal places using an A&D HR-202 semi-micro balance (A&D Company, Japan). The samples were digested in a 1 ml mixture of HNO_3 and $HClO_4$ (7:1, v/v) at 90 °C as described by Van Straalen and van Wensem (1986). The residues were dissolved in 5 ml 0.1% HNO_3 in 15 ml conical tube and cadmium concentrations were measured using a Varian 820-MS inductively coupled plasma mass spectrometer (ICP-MS) at a wavelength of 228.9 nm (Varian, Australia). The bioaccumulation factor (BAF) of cadmium in *P. kimi* at each compaction level was calculated from the measured cadmium concentrations in *P. kimi* using the following equation:

$$\text{BAF} = \frac{\text{total cadmium concentration in the } P.\ kimi}{\text{total cadmium concentration in the soil}}$$

2.6. Data analysis

The movement of *P. kimi* adults between the soil surface and subsoil, which was expressed as the difference in the number found on the surface to those in the subsurface of the soil divided by the total (STS), in each compacted soil was calculated as follows:

$$\text{STS}\ (\%) = \frac{(S - (N - S))}{N} \times 100, \quad (3)$$

where S is the number of adults found on the surface of soil at 4 d intervals; N is the total number of surviving adults at the end of the experiment (28 d). Since not all adults could survive in the cadmium treated soils and only adults observed on the surface of the soil could be counted at each time interval, N for the cadmium treated soil was assumed to be equal to the final number of surviving adults at the end of the experiment. The STS ranged from −100% to 100%. A value of STS = 100% indicates all the adults remained on the surface of the soil, while a value of STS = −100% indicates all adults were below the surface, and stayed below the soil surface. At STS = 0, 50% of individuals stayed on the surface soil, and the others were under the soil surface. Since the STS value does not satisfy the normality assumption, comparisons between compaction levels or between treatments were performed using nonparametric tests (SAS Institute, 1999). The Kruskal–Wallis test followed by the Tukey–Kramer multiple comparisons test was conducted to determine whether the STS of *P. kimi* was affected by the soil compaction level in the soils treated with or without cadmium, respectively. If significant, Mann–Whitney rank sum test was conducted to determine whether the STS of *P. kimi* was different between the cadmium treated and untreated soils at the same compaction level.

To determine the relationship between juvenile production and soil porosity, which depended on the soil compaction level, an exponential equation for cadmium untreated soils and second order polynomial equation for cadmium treated soils was used. The parameters of each equation were estimated using the least-squares method (SAS Institute, 1999), and the adequacy of the model was verified by calculating the coefficient of determination (r^2).

To determine whether the soil compaction level affected the cadmium toxicity for *P. kimi* reproduction, a chi-square goodness-of-fit (GOF) test was conducted (SAS Institute, 1999), and compared between the same compacted soils. Since the cadmium concentration used was EC_{50} for reproduction, the expected percentage of reduction in the reproduction in the cadmium treated soils in comparison to their corresponding untreated soils would be 50%, if soil compaction did not affect cadmium toxicity. If the reduction significantly deviated from the expected value (50%), then soil compaction did alter the response of *P. kimi* to cadmium toxicity.

3. Results

3.1. Effect of soil compaction and cadmium toxicity on the behavior of P. kimi

No adults mortalities were observed in the cadmium untreated compacted soils, but low mortalities were observed, as expected, in the cadmium treated soils, which ranged from 3 to 10%. Overall, the mortality in the cadmium treated soils was 6.7%, but the adult mortality did not significantly deviate from the expected mortality of 2% ($P > 0.05$). These results demonstrated that the STS values for the cadmium treated soils were valid, and comparable with the results obtained for the cadmium untreated compacted soils.

The STS values increased significantly as the compaction levels increased in cadmium untreated compacted soils throughout the experiment periods ($F = 77.84$, df = 3, $P < 0.0001$) (Fig. 1A), indicating that adult *P. kimi* tended to stay on the surface of the soil as soil compaction increased. Nearly all adults introduced to the uncompacted soil (C_0) immediately penetrated into the surface of the soil, and remained below the surface of the soil throughout the duration of the experiment. All STS values remained constant as increasing exposure time, with the exception of C_2 compaction. The reason why adults under the C_2 compaction condition moved from the subsurface region of the soil to the surface during a short time period (between observation days of 12–16) was not clear. The mean STS ± S.E. for C_0, C_1, C_2 and C_3 was -96.2 ± 1.8, -75.2 ± 6.9, -20.0 ± 9.2 and 37.1 ± 8.0, respectively.

Similar trends were observed for the cadmium treated soils, where compaction significantly affected the behavior of *P. kimi* ($F = 134.67$, df = 3, $P < 0.0001$) (Fig. 1B). The mean STS ± S.E. in the cadmium treated soil for $C_0 + Cd$, $C_1 + Cd$, $C_2 + Cd$ and $C_3 + Cd$ was -98.1 ± 1.3, -66.9 ± 4.8, -29.9 ± 6.0 and 34.8 ± 8, respectively.

When the STS values were compared between treatments at the same compaction level, no significant difference was observed between cadmium untreated and treated soil ($P > 0.05$), and only a marginal significant difference was observed between C_1 and $C_1 + Cd$ ($\chi^2 = 3.6317$, df = 1, $P = 0.0567$).

3.2. Effect of soil compaction and cadmium toxicity on the reproduction of P. kimi

In the assay without cadmium, soil compaction significantly affected the reproduction of *P. kimi*, and the reproduction decreased as soil compaction increased ($F = 41.22$, df = 3, $P < 0.0001$). The mean number of juveniles ± S.E. at C_0, C_1, C_2 and C_3 was 149.3 ± 2.0, 118.7 ± 6.5, 99.0 ± 2.5 and 84.3 ± 4.9, respectively (Fig. 2).

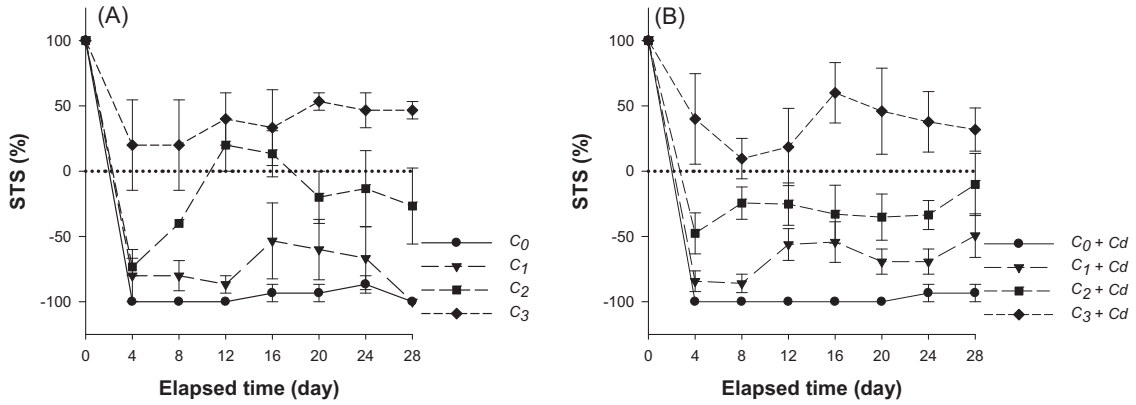

Fig. 1. The difference in the number of adults found between the surface and subsurface, expressed in percentage of the total number of *Paronychiurus kimi* adult (STS (%), mean ± S.E.) in the compacted soils treated without (A) and with (B) cadmium during a 28-d experiment. A positive value of STS indicates the number of adults found on the surface of the soil was larger than the number found below the surface, whereas a negative value of STS indicates the opposite trend.

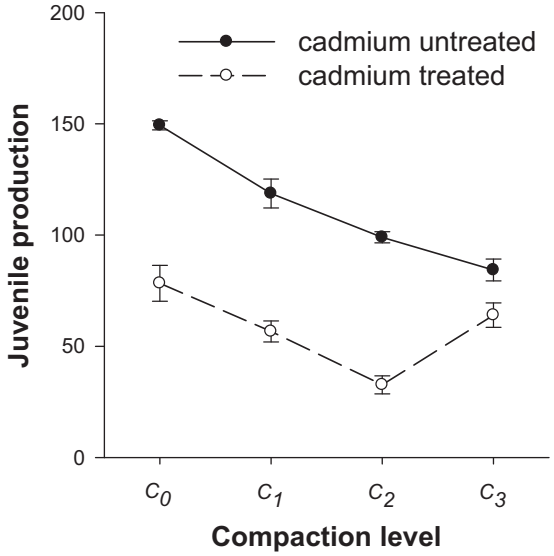

Fig. 2. Effect of soil compaction on the reproduction (mean ± S.E.) of *Paronychiurus kimi* in soil treated with or without cadmium at different compaction levels.

In the reproduction test with cadmium treatment, soil compaction was also shown to significantly affect the reproduction of *P. kimi* ($F = 10.85$, df = 3, $P < 0.0034$)), but the reduction in reproduction followed a different pattern relative to cadmium untreated soils. The reproduction decreased up to the $C_2 + Cd$ compaction level, and increased again at higher compaction pressures. The mean reproduction at $C_0 + Cd$, $C_1 + Cd$, $C_2 + Cd$ and $C_3 + Cd$ was 78.3 ± 8.1, 56.7 ± 4.7, 32.7 ± 4.1 and 64.0 ± 5.5, respectively (Fig. 2).

According to the χ^2-test, the observed reproduction at $C_0 + Cd$ was not significantly different from the expected reproduction (reduction by 50% compared to C_0) (Table 1). Based on these results, we performed further comparisons to find out whether the impacts of cadmium toxicity were related to soil compaction. At each compaction level, cadmium treatment reduced the reproduction of *P. kimi* by 47.5, 52.3, 67.0, and 24.1%, relative to the corresponding cadmium untreated soils at C_0, C_1, C_2, and C_3, respectively (Fig. 3). Interestingly, the reduction at $C_2 + Cd$ treatment was significantly higher than that of the expected reduction, whereas the reduction at $C_3 + Cd$ was significantly lower (Table 1).

The highest BAF was observed at $C_2 + Cd$, followed by $C_1 + Cd$, $C_0 + Cd$ and $C_3 + Cd$ (Fig. 3). A change of the BAF in *P. kimi* adults was closely related to the decrease in reproduction (Fig. 3). A good positive linear relationship with a high coefficient of determination ($r^2 = 0.81$) was observed when the BAFs were regressed with the percentage of reduction in reproduction, indicating that the increased toxicity to reproduction resulted from a greater accumulation of cadmium.

The bulk density of the soils was 0.50 ± 0.01, 0.58 ± 0.01, 0.70 ± 0.01 and 0.82 ± 0.01 g/cm^3 for C_0, C_1, C_2, and C_3, respectively. The porosity of compacted soils decreased gradually as the bulk density increased, which ranged from 69.03 ± 0.37% (C_3) to 81.31 ± 0.32% (C_0) (Fig. 4). The porosity was negatively related to the STS (Fig. 1A and B), where a smaller porosity resulted in a positive or close to positive STS and larger poros-

Table 1
Chi-square tests of the comparison of the reproduction of *Paronychiurus kimi* between cadmium treated and untreated compacted soils at different compaction levels.

Comparison[a]	df	χ^2-Value	Pr > χ^2
C_0 vs. $C_0 + Cd$	1	0.35	0.55
C_1 vs. $C_1 + Cd$	1	0.24	0.62
C_2 vs. $C_2 + Cd$	1	12.91	<0.001
C_3 vs. $C_3 + Cd$	1	19.28	<0.001

[a] Expected percentage of reduction in the reproduction in cadmium treated soils in comparison to their corresponding untreated soils would be 50%, if the soil compaction did not affect the cadmium toxicity.

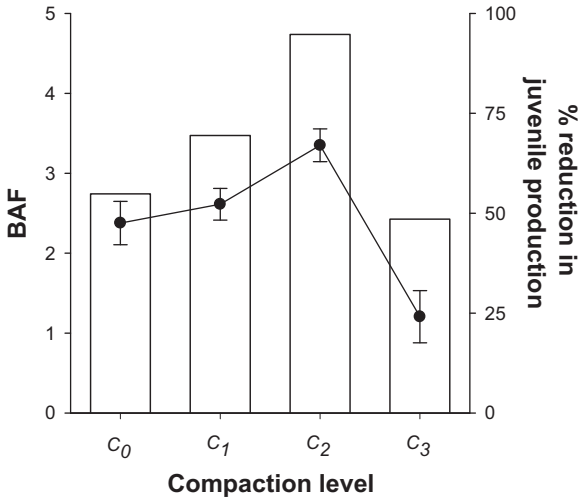

Fig. 3. Comparison of the relative reduction in the reproduction number (%) (line with closed circles) and the bioaccumulation factor (BAF) of cadmium in *Paronychiurus kimi* in the compacted soils treated with cadmium (bar graph). The relative reduction was calculated by comparing the numbers in cadmium untreated soils to those in cadmium treated soils.

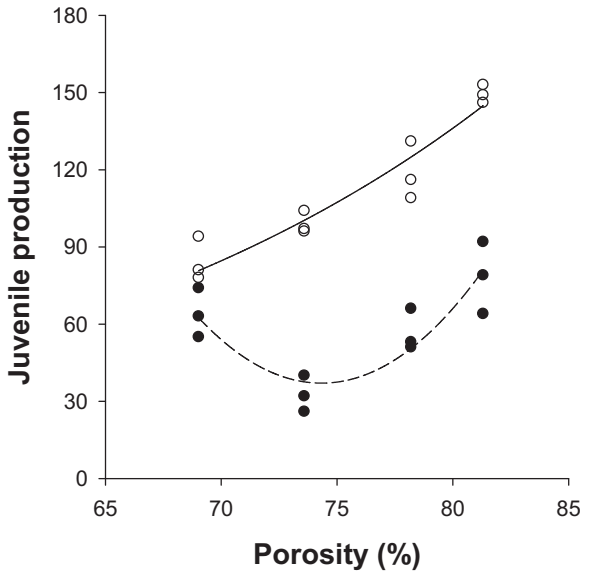

Fig. 4. The exponential and polynomial equations relating the juvenile production to soil porosity (%) in the soil treated without (solid line with open circles) and with cadmium (dotted line with closed circles) at different compaction levels.

ity resulted in a negative or close to negative STS. Exponential and polynomial models relating the reproduction of *P. kimi* to the porosity of compacted soils treated without or with cadmium were Juvenile production = $3.03 \times e^{(0.05 \times \text{porosity})}$ ($r^2 = 0.91$) and Juvenile production = $0.90 \times \text{porosity}^2 - 134 \times \text{porosity} + 5017$ ($r^2 = 0.75$), respectively (Fig. 4).

4. Discussion

Although soil compaction has been recognized as a major form of soil degradation that affects various processes in soil, its significance and implication for ecotoxicological studies has not been well reported in the literature. This study clearly demonstrates that the behavior and reproduction of *P. kimi* as well as cadmium toxicity are greatly affected by soil compaction. These results suggest that soil compaction is an important stressor for Collembola and must be included in soil ecological and ecotoxicological studies. Although a recent approach to incorporate various stressor factors into the ecological risk assessment procedure has been suggested by several researchers, most concerns have focused on the effect of soil physical and chemical parameters, such as pH, cation exchange capacity, clay, and organic matter content, but relatively little attention has been paid to the effect of soil structure on the toxicity of contaminants to date.

As shown in Fig. 1, the compaction-dependent increase in the STS (Eq. (3)) may be attributable to a decrease in the accessible and habitable pore space due to a decrease in the soil porosity, which may in turn result in a reduction in reproduction. Unlike other soil macrofauna, such as earthworms, most Collembola are sensitive to compaction-induced changes in soil structure (Heisler, 1991) because they live in the air-filled pore space of the soil and are not able to make their own burrows (Rusek, 1998). Furthermore, they avoid narrow pores so as to protect their wax coat against damage (Choudhuri, 1961). Thus, Collembola movement in the soil is largely dependent on the size, distribution and connectivity of the soil pore system (Van de Bund, 1970; Loranger et al., 1998). Several studies have also reported that the abundance and distribution of Collembola in soils were closely related to pore-size distribution and soil structure (Usher, 1976; Haarlov, 1955; Schrader et al., 1997; Larsen et al., 2004). In addition, Heisler and Kaiser (1995) reported that soil dwelling Collembola are sensitive to soil compaction, and any reduction in soil pore size produces a decrease in Collembola density. The reduction in reproduction at higher compaction levels was not related to adult mortality because no adult mortalities (cadmium untreated soils) or very few mortalities (cadmium treated soils) were observed during this study.

The most notable difference within cadmium treated soils was that the highest BAF was observed at the compaction level of $C_2 + Cd$, while the lowest BAF was observed at $C_3 + Cd$ (Fig. 3). The change in BAF strongly correlated with the relative reduction in reproduction. These findings are very interesting because the same concentration of cadmium (28.3 mg/kg) was present in all the compacted soils. It is generally believed that the toxicity and accumulation of metal is related to the bioaccessible fraction of a metal in a given soil and, more specifically, only to the fraction that is biologically available (Van Gestel et al., 1995; Alexander et al., 2003; Harmsen, 2007). The reason why the highest BAF and reproduction reduction were observed at $C_2 + Cd$ can be explained by a change in behavior of *P. kimi* depending on soil compaction (Fig. 1B). Structural changes induced by compaction might affect the pore-size distribution in the soil, and consequently *P. kimi* might experience different exposure conditions. In addition, these changes might affect the magnitude of exposure to cadmium through dermal contact. This is because Collembola are largely dependent on the presence of pore spaces that have dimensions similar to their body width. As the soil became more compacted from the $C_1 + Cd$ to the $C_2 + Cd$, the pore space decreased in a compaction dependent manner, while the cadmium uptake increased by increasing the magnitude of dermal contacts in the $C_2 + Cd$, which resulted in a higher BAF. The lowest BAF at $C_3 + Cd$ was expected because almost all of the adults were found on the surface of the soil during the experiment. Several researchers have reported that soil compaction can influence the sensitivity of Collembola to contaminants, where contaminants will most likely be more toxic to Collembola in compacted soil than in uncompacted soil (Rundgren and van Gestel, 1998; Campiche et al., 2006; Staempfli et al., 2007).

Even though the soil pore size was not measured at the different compaction levels in this study, the change in reproduction under both treatments (cadmium untreated and treated) in relation to porosity demonstrates that pore size is an important factor for determining reproduction and toxicity (Fig. 4). Son et al. (2009b) reported that the adult body width (headcapsule width) of *P. kimi* ranged between 0.25 and 0.35 mm, indicating that the pore size of the $C_2 + Cd$ soil might be close to 0.25–0.35 mm, but the pore size of the $C_3 + Cd$ might be less than these values. Larsen et al. (2004) also showed that the abundance and reproduction of various Collembola species could be affected by the relationship between the body widths and the soil pore size.

The present study suggests that soil compaction affects the uptake and toxicity of cadmium in *P. kimi* by influencing the magnitude of exposure to contaminants through dermal contact. Several researchers have reported that soil compaction can influence the sensitivity of Collembola to contaminants, where contaminants will most likely be more toxic to Collembola in compacted soil than in uncompacted soil (Rundgren and van Gestel, 1998; Campiche et al., 2006; Staempfli et al., 2007).

Standard toxicity tests conducted under specified and controlled conditions have been widely used to derive soil screening values or regulatory values for particular contaminants in ecological risk assessment. In most soil ecotoxicological studies that use Collembola, OECD artificial soil is used as a standardized substrate. Although the amount of ingredients used for artificial soil preparation is strictly defined, there is still significant variability in the soil properties, such as the organic matter content, structure and particle distribution, which produces a high variation in the toxicity results across different laboratories (Hofman et al., 2009). Furthermore, much of the research to date has examined the effect of these

factors on the toxicity of contaminants of concern as factors affecting the behavior of contaminants and resulting bioavailability to soil organisms. However, as shown in this study, the change in soil structure induced by soil compaction significantly affected not only the performance (i.e., behavior and reproduction) of *P. kimi* but also the uptake and toxicity of cadmium to *P. kimi*, highlighting its importance as a factor affecting the performance of *P. kimi* and in assessing the toxicity of contaminants. Therefore, these results suggest that soil compaction is one of the most important factors affecting soil structure and should be included in soil ecotoxicity test.

Based on the results presented in this study, it can be concluded that soil compaction can influence the behavior of *P. kimi*, which in turn negatively impacts the reproduction of *P. kimi* by altering accessible and habitable pore spaces in soil. These results suggest that soil compaction alone can act as a stressor itself, thereby affecting the fitness of soil organisms. This study also highlights the importance of soil compaction in assessing the toxicity of contaminants. Since soil compaction can affect the magnitude of exposure of contaminants and sensitivity of organisms to contaminants, it is suggested that soil compaction be taken into consideration to better understand the effect of contaminants on soil organisms and to facilitate ecological risk assessments. Since the distribution of contaminants in contaminated sites is not restricted to the soil surface, but heterogeneously distributed in terms of vertical and spatial content, further detailed studies on various soil samples from contaminated sites should include soil compaction as an important factor in the ecological risk assessment procedure.

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