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Graphical Abstract

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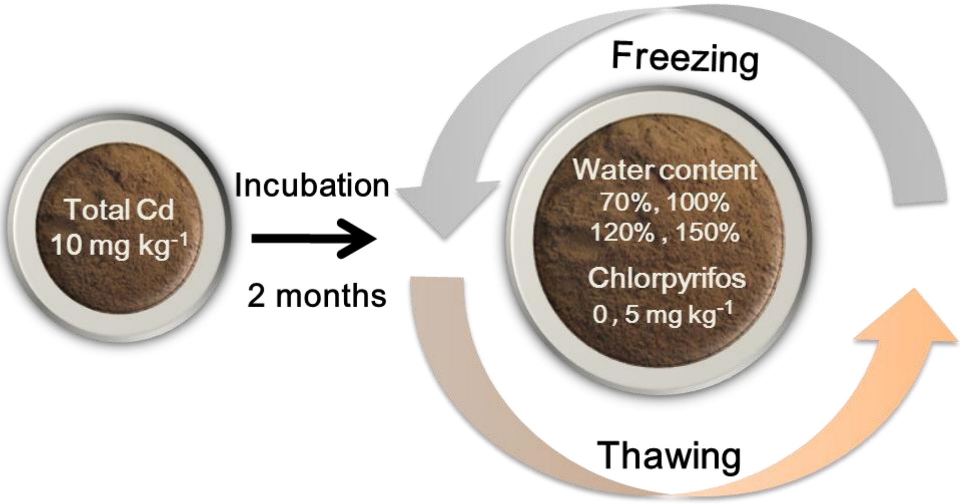

The effect of one natural factor was weakened by the binary interactions, which were further weakened by the ternary interactions. The significant effect was computed via Design of Experiments.

Multivariate interactions of natural and anthropogenic factors on Cd behavior in arable soil

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ABSTRACT

Multivariate interactions are far more complex between natural factors and pollutants resulting from anthropogenic practices than between pollutants themselves. But little attention is drawn to address the complex interpretation of multivariate interactions. To bridge this research gap, this study aimed to identify the interactive effect of multiple affecting factors including freeze-thaw cycles (FT), soil water (SW) content, and chlorpyrifos (CP) on soil Cd behavior in arable soil, based on the analysis of changes in Cd fractionations and Cd availability. Moreover, the significant effect was computed via Design of Experiments. The content of Cd fractions and the evaluated index of Cd availability were obtained employing the modified six-step sequential extraction method. The results showed that the main effect of FT, SW and CP on Cd fractionation and availability was significant. The binary interactions weakened the main effect of FT or SW, but enhanced the main effect of CP on Cd fractionation. The ternary interactions further weakened the binary interactions, whereas CP enhanced the interaction between SW and FT. The interaction between SW and CP had a negative effect on residual Cd, but positively affected water-soluble and organic matter-bound Cd. The binary interaction between CP and FT had a positive effect on residual Cd (21.0%), but negatively affected water-soluble and Fe-Mn-oxide-associated Cd (25.9% and 21.1%). These results covered more innovative information on the multivariate interactions between natural and anthropogenic factors on Cd behavior in arable soil. A possibly new way to quantify the significant impact of multivariate factors also was provided.

Keywords: Freeze-thaw cycles; Soil water content; Chlorpyrifos; Cd frationation; Cd availability; Multivariate interactions

1. INTRODUCTION

Anthropogenic practices have generally caused the release of heavy metals (HMs) and pesticides in the environment. The interactions between HMs and pesticides in arable soil have caused world-wide concern, as they have been linked to many ecological and human health risks[1]. Previously, such interactions (mainly including synergism and antagonism) have been reported in many literatures[2]. For instance, synergism was observed in the binary mixture of Cd and dimethoate[1], while antagonism was discovered that chlorpyrifos could reduce the toxicity of nickel by competing for the same binding sites[3]. The effect of copper-carbendazim on reproduction of *Caenorhabditis elegans* was synergistic at low dose levels but antagonistic at high dose levels[4]. The aforementioned studies focusing on the interactions between HMs and pesticides are mostly investigated in laboratory experiments under well-controlled conditions[2]. However, the actual field condition is much more complicated than that in laboratory due to the large fluctuations in natural environmental factors. Moreover, the impact of natural environmental factors on the interactions between HMs and pesticides are more serious than that of pollutants themselves[5]. Nevertheless, studies addressing this topic are really scarce.

Seasonal freeze-thaw (FT) is fairly common in mid-high latitude regions among these natural factors. FT and soil water content (SW) play an import role in controlling the mass transport and energy exchange in the soil-plant-atmosphere-climate system[6]. Also, both FT and SW can alter soil physio-chemical and biological properties[5,7], such as soil pH, the dissolved organic matter (DOM) and the activity of soil organisms, which are easily affected by pesticides[8]. At least two of these properties are usually observed to control HMs behavior[9]. For instance, FT can destroy soil aggregates, and then increase the content of DOM, which will subsequently alter the capacities of soil to bind HMs[10-11]. The increased SW can enhance the extent to which soil aggregates are damaged by FT, which will decrease when SW exceeds the saturation value[12]. A significant increase in the total amount of free amino acids and sugars caused by freezing is combined with an increment in soil respiration and dehydrogenase activities[13], which can easily be affected by pesticides[14]. The above phenomenon indicates that interactions between the two natural factors (FT and SW) and pesticides are far more complex in the mechanism, which may directly/indirectly further affect the behavior of pollutants themselves. Therefore, a scientific basis should also be provided to consider the impact of the natural environmental factors, especially on that how natural stressors interact with chemical stressors. Further researches are desired to conduct focusing on the elaborate experimental design and the complex interpretation of multivariate interactions[2].

Design of experiments (DOE) has been reported to provide maximized information of experimental statistics and to get unambiguous results taking minimized efforts[15]. 2^n full factorial design in DOE is a statistical technique for designing experiments where n affecting factors are controlled[16]. The effects of various affecting factors are investigated at each of two levels, containing the minimal and the maximal input–output data pairs[17]. On this basis, the effect of main effects and interactions was assessed by the outputs viz the values of estimated effect (E). A higher E revealed a stronger effect. The main effect reveals the impact of one changed controlling factor, while the interaction reveals the combined impact of the multivariate affecting factors[16]. This method has been widely used in behavior sciences to analyse a random response of output variables to a set of various affecting factors.

Therefore, to bridge the research gap in multivariate interactions between natural and anthropogenic factors on HMs behavior, based on the analysis of changes in HMs fractionations and HMs availability, this study aims to: (1) evaluate the potential binary and ternary interactions between natural factors (FT and SW) and pesticides on HMs behavior in arable soil; (2) provide a possible way to quantify the significant effect of these affecting factors on the basis of DOE analysis. Cd and chlorpyrifos (CP) were selected as the representatives due to their intrinsic properties, such as the tendency of accumulation[18], the most toxic and mobile in arable soil[19] and the special chemical structure to inhibit the activity of acetylcholinesterase[8,14]. Cd fractionation and Cd availability were selected to investigate because they have been reported to provide valuable information on represent Cd behavior[9]. Soil samples were collected from an arable soil in a typical seasonal frozen region of northeast China. A 2^3 full factorial design was carried out based on the hypothesis that Cd behavior was influenced by the interactive effects of FT, SW and CP.

2. MATERIALS AND METHODS

Soil sampling

The location of sampling site was illustrated in Figure 1. Soil samples were collected from an arable soil located in northeastern China (47 °24′N, 134 °05′E), where soils were regularly exposed to sub-zero temperatures for approximately six months[20]. The minimum and maximum air temperatures of the studied area were -20.2 ℃ in January and 21.7 ℃ in July. The average depth of the frozen soil was 141 cm[6]. Rain-fed agriculture was the dominant production system of the study maize-field region[20], and CP was widely applied to corn and soybean in this seasonal frozen region. The total Cd in the soil has been reported to present in a trend of accumulation due to the long-term fertilization of rock phosphate and application of pesticides[18].Surface soils (< 20 cm) were collected with five sampling replicates in a “W” shape. All five soil replicates were blended, air-dried and ground.

Place Figure 1 here

Experimental preparation

Parts of soil samples were sieved with a 0.147-mm nylon sieve. The soil pH (1:2.5, soil:water) was determined using a combination electrode. The maximal water holding capacity was determined following the method described by Muhammad et al[21]. After the soil saturation and later drainage in a water-saturated atmosphere, the water content was determined. The soil was a silt loam and classified as burozem[22], with a maximal water holding capacity of 41.2% and a pH of 5.68.

Prior to Cd analysis, a HF-HNO_3-$HClO_4$ acid mixture was used for the digestion of the ground soil and the constant-volume acid mixture was determined by inductively coupled plasma optical emission spectrometry (ICP-OES, IRIS Intrepid II XSP, Thermo Electron, USA). The concentration of total Cd in the soil was 0.009 mg kg^{-1}.

Parts of soil samples were sieved with a 2-mm nylon sieve. 1 kg of soil was weighed into a plastic box. 10 mg L^{-1} of Cd^{2+} solution was added into the soil with a volume of 1 L. To homogenize Cd in soil, the mixture was incubated under a room temperature for approximately 2 months. The total content of Cd in soil thereby was adjusted to 10 mg kg^{-1}. Then, the soil was air-dried and sieved by a 2-mm mesh sieve again and subsample specimens (1 g each) were weighed in a plastic bag.

Experimental design

As shown in Figure 2, the study was conducted in a completely randomised design with four levels of soil water content (SW1-70%, SW2-100%, SW3-120% and SW4-150% of the maximal water holding capacity) and two levels of CP (CP0 and CP1, 0 and 5 mg kg^{-1}). The subsamples were subsequently subjected to the FT process with a frequency of 1 (F1), 3 (F3), 6 (F6) or 9 (F9). Meantime, the subsamples were kept under the thawing temperature was set as the control group (F0). Each treatment was replicated four times. The total number of the experimental groups and samples was 40 and 160, respectively. Freezing and thawing were carried out over a period of 24 hours each (the FT frequency was set at 2 days) and the soil temperature was set in accordance with the air temperature. In order to simulate field conditions, this study accordingly used a freezing temperature of -10 ℃ and a thawing temperature of 20 ℃. This was based on the fact that the soil temperature exceeded the air temperature by approximately 5-10 ℃ when the surface layer of the soil began to freeze[23].

Place Figure 2 here

Modified six-step sequential extractions and Cd availability calculation

Cd fractionations were determined using modified Tessier six-step sequential extractions method, which was reported in 2007[24]. Soil samples were sequentially extracted by (1) deionised water for 1 h (20 ℃); (2) 1 M NH_4OAc (pH 7.0) for 2 h; (3) 1 M NH_4OAc (pH 5.0) for 2 h; (4) 0.04 M NH_2OH-HCl in 25% HOAc for 6 h (water bath, 60 ℃); (5) 30% H_2O_2 (pH 2, adjusted with HNO_3) for 5.5 h (water bath, 80 ℃) + 3.2 M NH_4OAc in 20% HNO_3 for 30 min, and (6) HF-HNO_3-$HClO_4$ acid mixture (1:2:2); in order to obtain six fractions: water-soluble Cd (*Wat-Cd*), exchangeable Cd (*Exc-Cd*), carbonate-bound Cd (*Car-Cd*), Fe-Mn-oxide-associated Cd (*Oxi-Cd*), organic matter-bound Cd (*Org-Cd*) and residual Cd (*Res-Cd*), respectively[24]. The Cd concentrations in all extracts were determined by ICP-OES, with an average recovery of 98.4%±5%. Triplicate samples were analysed, and the data were expressed as the means. The results showed that the standard error was within 10%. The content of each fraction of Cd in soil was calculated as the ratio of each fraction to the sum of the extracted Cd in all six-sequential fractions.

The Cd availability in soils was usually determined by the potential bioavailability and mobility of Cd. The potential Cd bioavailability reflected the portion of the total Cd that could be taken up directly or indirectly by organisms in the soil. On this basis, the index of potential Cd bioavailability was obtained using the following equation: $K\ (\%) = (Wat\text{-}Cd + Exc\text{-}Cd + Car\text{-}Cd) / (Wat\text{-}Cd + Exc\text{-}Cd + Car\text{-}Cd + Oxi\text{-}Cd + Org\text{-}Cd + Res\text{-}Cd)$, where K was the relative content of the potential bioavailable fractions including the sum of *Wat-Cd*, *Exc-Cd* and *Car-Cd*[25]. The potential Cd mobility in soil usually indicated the absolute and the relative content of fractions weakly bound to soil components[21]. Therefore, the relative index of potential Cd mobility was calculated using the following equation: $M\ (\%) = (Wat\text{-}Cd + Exc\text{-}Cd) / (Wat\text{-}Cd + Exc\text{-}Cd + Car\text{-}Cd + Oxi\text{-}Cd + Org\text{-}Cd + Res\text{-}Cd)$, where M was the relative content of the potential mobile fractions including the sum of *Wat-Cd* and *Exc-Cd*.

Quality assurance and quality control were assessed using duplicates, method blanks and standard reference materials (GBW07401) from the Chinese Academy of Measurement Sciences for each batch of samples. All chemicals were of analytical grade or better, and all glassware and centrifuge tubes were previously soaked in acid (10% HNO_3) and rinsed with deionised water.

Statistical analysis

Descriptive data and statistical analyses were carried out using Minitab v.16.0 and Origin 8.0 software, providing the means ± S.D. (standard deviation) of four replicates. A variance analysis of all the data was carried out by the general linear model (GLM) using SPSS v.16.0 with a significant level of 0.05. This model defined the relationship between a dependent continuous outcome variable (six Cd fractions and two availability indexes) and multiple independent variables (5-level FT, 4-level SW and 2-level CP)[26]. It performed ANOVA by using the least squares regression to fit general linear models[27]. In the procedure, the relationships between dependent and independent variables were also given as the main effects and the interactions, similar to the case in the DOE analysis. But the magnitude of the impact was evaluated by the partial eta squared values (*eta*) of between-subjects in the GLM process[28]. In detail, a higher value of the *eta* referred to a stronger effect. When the value of the *eta* was greater than or equal to 0.14 or 0.06, the impact was considered to be strong or moderate, respectively.

Furthermore, a 2^3 full factorial design was carried out to identify the impact of affecting factors (FT, SW and CP) on Cd fractionation and Cd availability. Based on the output results, the contribution rate of various factors as well as their higher-order interactions was calculated using the following equation: R_i (%) = E_i / (E_1 + E_2 + ⋯), where R_i was the relative percentage of the *i* th estimated effect (*E*) of the significantly positive/negative factor in the sum of the estimated effects of all the significantly positive/negative factors.

3. RESULTS

Cd fractionation and Cd availability in soil response to FT, SW and CP manipulations

As indicated in Figure 3, *Exc-Cd* (22.0%-33.1%) and *Res-Cd* (21.9%-41.3%) were the predominant fractions in the soil, while small amounts of *Wat-Cd* (0.25%-1.48%) and *Org-Cd* (2.53%-7.02%) were also detected. *Wat-Cd* reached the maximum almost two FT cycles earlier in soils with CP than in soils without CP, while the minimal *Car-Cd* and the maximal *Res-Cd* emerged two or three FT cycles later. Interestingly, the irregular distribution changes in the soil were observed due to the variation in SW content as a function of FT and CP. In particular, the content of *Wat-Cd*, *Exc-Cd* and *Org-Cd* was significantly ($P < 0.05$) increased by circa 16.0%, 4.67% and 8.93% due to the addition of CP, respectively, whereas the content of *Oxi-Cd* was significantly ($P < 0.05$) decreased by circa 10.8%.

Place Figure 3 here

The *K* values slightly decreased to a minimum at F1, then gradually increased as a result of the increased FT frequency. A notable decrease in the *K* and *M* values (approximately 10.3% and 14.7%, respectively) was observed as SW content increased. However, there was no significant variation once the saturation value was exceeded. It was five FT cycles earlier for the minimal *K* value emerging due to the addition of CP, which also caused the *K* and *M* values significantly ($P <$ 0.05) to increase by circa 3.49% and 4.96%, respectively (Figure 4, Table 1).

Place Figure 4 here

Intra-group differences in Cd fractionation and Cd availability resulting from FT, SW and CP

As shown in Table 1, there was a significant difference between each two treatments regarding the *Wat-Cd* content with FT frequency increasing, and its maximum emerged at F1. The maximal *Exc-Cd* content was at F9, much higher than at F0 and F1. Although both the *Car-Cd* and the *Oxi-Cd* contents showed a descending trend, no significant changes in the *Car-Cd* content were observed after F1. The maximal *Org-Cd* content was at F6, significantly higher than at other frequencies. The *Res-Cd* content related to F1 and F3 was markedly higher than that at F0 and F9 in spite that an uptrend was observed in the *Res-Cd* content. The *M* value was significantly higher at F9 than at F0.

Place Table 1 here

Variations in Cd fractions in soil were observed due to the different SW contents. Interestingly, an increase in the SW content caused a notable decrease in the *Wat-Cd* content. The maximal *Exc-Cd* content at SW1 was significantly higher than that under other SW content conditions. The *Car-Cd* content was markedly higher at SW1 than at SW4. The *Oxi-Cd* content at SW2 was the highest among the four-level of SW contents. A significant difference was observed in the *Org-Cd* content under various SW treatments. In detail, the minimum and the maximum was at SW1 and SW2, respectively. The *Res-Cd* content increased with the increase of the SW content. The *K* and *M* values were higher at SW1 than at other SW contents. The addition of CP caused a significant increase in the content of *Wat-Cd*, *Exc-Cd*, and *Org-Cd*, but a notable decrease in the *Oxi-Cd* content. In particular, the *K* and *M* values strongly increased due to the addition of CP, while no visible changes in the content of *Car-Cd* or *Res-Cd* was detected.

Inter-group differences in Cd fractionation and Cd availability among FT, SW and CP

The data presented in Table 2 demonstrated that each affecting factor (i.e., FT, SW, and CP) had significant effect on Cd fractionation and Cd availability. The relatively low *eta* value (< 0.14) indicated a moderate effect of FT on the *Car-Cd* content, which also was observed for the effect of CP on the *Exc-Cd* content and Cd availability. No significant effects of SW on the *Car-Cd* content and of CP on the content of *Car-Cd* and *Res-Cd* were observed.

Place Table 2 here

The binary interactions weakened the effects of FT and SW on the soil Cd fractionation and Cd availability. However, they enhanced the effects of CP on Cd fractionation except for *Exc-Cd*. The ternary interactions further weakened the binary interactions, while CP enhanced the binary interaction between SW and FT (SW*FT). The distribution showed a significant variation in the content of *Wat-Cd*, *Exc-Cd* and *Org-Cd* in soil as a result of SW*FT. The fractions of Cd except for *Exc-Cd* and *Car-Cd* in soil were remarkably affected by the binary interaction between CP and SW/FT (SW*CP and CP*FT). The ternary interaction of SW*CP*FT weakened the binary interactions on the *Wat-Cd* content. The *M* values were significantly affected only by SW*FT (Figure 5).

Place Figure 5 here

Contribution rate of FT, SW and CP on Cd fractionation and Cd availability in soil

According to the results of the full factorial design, there was a significant effect ($P < 0.05$) on Cd fractionation and Cd availability resulting from the main and interactive effect of FT, SW and CP. In detail, the binary interactions had a significant effect ($P < 0.05$) on the content of *Wat-Cd*, *Oxi-Cd*, *Org-Cd* and *Res-Cd*. No significant ternary interactions ($P > 0.05$) were observed.

Table 3 showed the estimated effects and percent distributions of each term. The percent contribution of the significant effects of SW, CP and FT was calculated based on the data presented in Table 3, which was displayed in Figure 6. The main effect of SW significantly and positively ($P < 0.05$) influenced the Res-Cd content and its contribution was 79.0%, while it had a significant negative effect ($P < 0.05$) on the content of *Wat-Cd*, *Exc-Cd*, *Car-Cd*, *Oxi-Cd*, the *K* and the *M* values, with a contribution of 51.3%, 100%, 100%, 44.9%, 100% and 100%, respectively. The main effect of CP negatively influenced the *Oxi-Cd* content ($P < 0.05$), accounting for 34.0% of the total negative effect on the *Oxi-Cd* content. Nevertheless, it positively influenced ($P < 0.05$) the content of *Exc-Cd*, *Org-Cd*, the *K* and the *M* values, which accounted for 36.8%, 39.7%, 54.2% and 39.7% of the total positive effect on the corresponding variables, respectively.

Furthermore, the contribution generated from the binary interaction between SW and CP to the negative effect ($P < 0.05$) on the *Res-Cd* content was 100%, while its contribution to the positive effect ($P < 0.05$) on the content of *Wat-Cd* and *Org-Cd* was 100% and 60.3%, respectively. The binary interaction between CP and FT accounted for 21.0% of the positive effect ($P < 0.05$) on the *Res-Cd* content, and it caused a negative ($P < 0.05$) effect on the contents of *Wat-Cd* and *Oxi-Cd*, with a contribution of 25.9% and 21.1%, respectively. No other significant effects ($P > 0.05$) of the higher-order interaction on Cd fractionation and Cd availability were observed.

Place Table 3 and Figure 6 here

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4. DISCUSSION

Main and interactive effect of FT, SW and CP on Cd fractionation and Cd availability in soil

The FT process can occur with high levels of soil moisture and over a wide range of temperatures[23]. Conversely, the FT process can alter the soil temperature, thermal and hydraulic conditions as well as water and energy exchanges at the land-atmosphere surface[29]. These changing environmental factors can further influence Cd fractionation and Cd availability in soil[9]. Thereby in this study, a reduction of the main effects on Cd fractionation in soil was observed when SW and FT interacted with each other. The main effect of CP on the *K* and *M* values indicated that the potential Cd availability moderately responded to the addition of CP. One possible reason is due to the fact that CP competes with Cd for sorption sites via electrostatic interactions and hydrogen bonding[30]. On the other hand, CP can also affect Cd fractionation and availability via initial biotic transformation into chlorpyrifos-oxon[14]. Chlorpyrifos-oxon can significantly alter the activity of soil organisms and enzymes[8]. This can cause great changes in soil SOM and pH, which are key factors controlling Cd fractionation and availability[9]. When CP interacted with FT or SW, the pesticide weakened the main effects of FT or SW, while the main effects of FT on the *Wat-Cd* content were enhanced. Simultaneously, the main effect of CP on the Cd fractionation in soil was altered when CP interacted with FT or SW. This phenomenon again suggested that natural environmental factors can alter the effects of chemicals due to changes in controlled conditions[5].

The results indicated that the binary interactions were further weakened by the ternary interactions. For example, SW*CP*FT further affected the binary interactions on the *Wat-Cd* content. It also suggested that natural environmental factors not only modify effects of chemicals directly but also influence them through interactions among them indirectly. This conclusion was also demonstrated in the previous study[2]. One possible mechanism is the fact that CP inhibits the activity of soil enzymes or reduces the buffering capacity of soil[14], and thereby reduces the sensitivity of the potential Cd bioavailability in soil to FT and SW.

Acting mechanism of FT, SW and CP on Cd fractionation and Cd availability in soil

FT can alter soil surface area by destroying soil aggregates or by gathering fine particles around medium particles[31]. In response, the number of soil sorption sites of H^+ is increased or reduced, leading to changes in soil pH, which is a key factor dominating Cd behaviors in soil. Moreover, the soil aggregates destruction causes the release of Fe-Mn oxides and an increase in the DOM content. This potentially results in an increase of Cd availability/solubility in soil via the formation of soluble complexes[11,32]. The decrease of soil pH caused by FT can also increase soil Cd availability/solubility. On this background, FT had a positive effect on the increase of labile Cd content, including *Wat-Cd*, *Car-Cd*, *Oxi-Cd*, and part of *Org-Cd*, as well as Cd availability. However, such increase may be inhibited by the elevated soil pH as function of FT. The above mechanism can also interpret the conclusion of this work that the contents of the different Cd fractions (especially the labile ones) varied with different FT frequencies.

Moreover, the increased SW can enhance the extent to which soil aggregates are damaged by FT and thereby can promote the effects of FT on Cd fractionation and availability, which will decrease once SW exceeds the saturation value[12]. Labile Cd fractions and Cd availability will decrease because the cationic Cd is more immobilized in the saturated soils with higher pH, lower Eh and potentially contain sulphides, compared to unsaturated soils[33]. Cationic Cd dissolved in the soil solution can also migrate with soil water. As a function of FT, the unfrozen water with the dissolved Cd will migrate to the freeze front, beside the in-situ water that is frozen during the freezing process[29]. Moreover, an increase in temperature during the thawing process can be beneficial for ion exchange sorption but detrimental to specific sorption, which results in a process that is faster than the ion exchange sorption[34]. Based on this information, FT can substantially affect the contents of Cd fractions. This is mainly due to the formation of chelates and precipitates on soil surface by combining with aluminosilicate and Fe-Mn oxides or by bonding with the oxygen or hydroxyl molecules within soil particles[35].

Furthermore, FT can also affect Cd behavior via the alteration of the microbial structure and functions of soil, which readily respond to the soil humidity level and temperature[3,9]. Simultaneously, the freezing process can cause a significant increase in the total amount of free amino acids and sugars, in combination with an increase in soil respiration and dehydrogenase activity[13]. Nevertheless, either the microbial functions or the enzymatic activity can be easily affected by CP via ecological effects, organic effects and the coordination reaction[14]. The complexation ability of the organic ligands that originate from CP can control the hysteresis quality of the Cd desorption process in soil, together with the soil colloid content[36-37]. This is the reason that CP can alter the emerging time of visible changes in Cd fractions resulting from FT. The ability of CP that increased the potential Cd mobility coincided with the findings of other previous studies[32,38], where the presence of chloride and SOM promoted this process by forming soluble complexes.

Changes in Cd fractionation and Cd availability in soil caused by various treatments

In this study, the predominant *Res-Cd* content showed that most of the added Cd^{2+} could be adsorbed by the soil, whereas the relatively high *Exc-Cd* content suggested that Cd in the soil had a relatively strong potential bioavailability and mobility. These findings were consistent with those reported in other studies on the distribution of Cd in soil[39]. Once added to soil, *Wat-Cd* is converted to relatively less soluble compounds within a very short period by inner-sphere surface complexation[40]. Cd retention in the more persistent fractions over time results from reductions in the loosely bound fractions[41]. Nevertheless, the transformation from *Exc-Cd* can be barricaded because *Exc-Cd* is likely hydrated by Cd^{2+} and adsorbed via outer-sphere surface complexation[42]. The high *K* and *M* values observed in this study support this inference. Furthermore, the *M* values showed a variation similar to that of *Exc-Cd*. This phenomena could be explained by the higher content of *Exc-Cd* compared to the *Wat-Cd* content.

5. CONCLUSIONS

The main effect of FT, SW and CP on Cd fractionation and availability was significant. The binary interactions weakened the main effect of FT or SW on Cd fractionation and availability, but enhanced the main effect of CP on Cd fractionation except for *Exc-Cd*. The ternary interactions further weakened the binary interactions, whereas CP enhanced the binary interaction between SW and FT. The binary interaction between SW and CP had a negative effect on *Res-Cd* (100%), but positively affected *Wat-Cd* and *Org-Cd* (100% and 60.3%). The bianry interaction between CP and FT had a positive effect on the *Res-Cd* content, but negatively affected *Wat-Cd* and *Oxi-Cd* (25.9% and 21.1%). No other significant effects of the higher-order interaction on Cd fractionation and Cd availability were observed. These results not only covered more innovative information to facilitate the identification of the multivariate interactions between natural and anthropogenic factors on Cd behavior in arable soil, but also showed a possibly new way to quantify the significant influence of multivariate factors was also given.

ACKNOWLEDGEMENTS

This research benefited from financial support from the National Natural Science Foundation of China (Grant No. 41371018, 41271463), the “Twelfth Five-year Plan” for Sci & Tech Research of China (Grant No. 2012BAD15B05), and the GLOCOM project (Grant No.

PIRSES-GA-2010-269233). Besides, the authors are very grateful for the assistance with data requirements provided by the Bawujiu Farm in Heilongjiang Province, China.

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Figure captions

Figure 1 Sampling site.

Figure 2 Experimental design.

Figure 3 Percentage distribution of Cd in each fraction of the six-step sequential extraction for variously treated soils.

Figure 4 Impact of FT and SW on Cd availability in soil in the presence of CP.

Figure 5 Interactions of FT, SW and CP on Cd fractionation and availability in soil.

Figure 6 Contribution of main effects and interactions of FT, SW and CP on Cd fractionation and Cd availability in soil.

Table 1. Intra-group differences in Cd fractionations and Cd availability resulting from various treatments. Average data and standard deviation are shown (%).

Treatments	*N*	*Wat-Cd*	*Exc-Cd*	*Car-Cd*	*Oxi-Cd*	*Org-Cd*	*Res-Cd*	*K*	*M*
F0	32	0.47±0.14c	25.2±2.40c	20.8±1.98a	19.2±2.48a	4.63±1.08b	29.6±4.45c	46.5±3.60a	25.7±2.48c
F1	32	0.76±0.42a	25.7±2.07bc	19.6±1.87b	16.9±2.84c	4.23±1.12c	32.8±4.46a	46.1±3.38a	26.5±2.15bc
F3	32	0.52±0.22b	26.6±3.25ab	19.5±1.54b	16.9±3.09c	3.37±0.67d	33.0±5.30a	46.6±3.86a	27.1±3.38ab
F6	32	0.44±0.12d	26.7±2.76ab	19.4±1.39b	16.5±3.36c	4.82±1.00a	32.0±4.76ab	46.6±3.35a	27.2±2.83ab
F9	32	0.51±0.09b	27.2±2.81a	20.0±1.48ab	18.0±3.30b	3.42±0.74d	30.8±4.15bc	47.7±3.33a	27.7±2.85a
SW1	40	0.67±0.17A	29.2±2.77A	20.5±1.76A	17.7±1.73B	3.54±0.64D	28.4±3.04B	50.4±3.19A	29.9±2.75A
SW2	40	0.58±0.31B	25.6±2.41B	19.7±1.73AB	20.5±3.98A	5.05±1.30A	28.7±4.18B	45.8±3.05B	26.1±2.46B
SW3	40	0.49±0.28C	25.1±1.79B	19.9±1.57AB	16.3±1.96C	4.07±0.92B	34.2±3.70A	45.4±2.48B	25.5±1.88B
SW4	40	0.41±0.15D	25.4±1.61B	19.5±1.73B	15.6±2.00C	3.73±0.82C	35.4±3.40A	45.2±2.48B	25.8±1.61B
CP0	80	0.50±0.21	25.7±2.83	19.7±1.73	18.5±3.71	3.92±1.32	31.7±5.41	45.9±3.37	26.2±2.94
CP1	80	0.58±0.29*	26.9±2.56*	20.1±1.71	16.5±2.05*	4.27±0.82*	31.7±4.04	47.5±3.47*	27.5±2.56*

Different small and capital letters in the same column indicate significant differences between the freeze-thaw and water content treatments, respectively ($P < 0.05$). Asterisk indicates that there is a significant difference relative to the data before adding CP ($P < 0.05$).

Table 2. Inter-group differences in Cd fractionations and Cd availability among various treatments.

Source		*Wat-Cd*	*Exc-Cd*	*Car-Cd*	*Oxi-Cd*	*Org-Cd*	*Res-Cd*	*K*	*M*
SW	F	314	42.0	2.65	77.5	130	64.0	31.4	45.9
	P	0	0	0.05	0	0	0	0	0
	eta[a]	0.89	0.51	0.06	0.66	0.77	0.62	0.44	0.54
CP	F	181	16.3	1.74	71.2	34.5	0	14.4	18.6
	P	0	0	0.19	0	0	0.97	0	0
	eta	0.60	0.12	0.01	0.37	0.22	0	0.11	0.13
FT	F	314	5.95	3.44	16.9	105	8.03	1.56	5.47
	P	0	0	0.01	0	0	0	0.19	0
	eta	0.91	0.17	0.10	0.36	0.78	0.21	0.05	0.15
SW*CP[e]	F	304	0.68	0.18	43.7	85.6	19.8	0.19	0.77
	P	0	0.57	0.91	0	0	0	0.90	0.51
	eta	0.88	0.02	0	0.52	0.68	0.33	0.01	0.02
SW*FT	F	38.7	2.12	0.33	1.45	12.9	1.02	1.02	2.36
	P	0	0.02	0.98	0.15	0	0.44	0.44	0.01
	eta	0.80	0.18	0.03	0.13	0.56	0.09	0.09	0.19
CP*FT	F	432	1.52	0.33	6.43	5.60	4.29	1.09	2.13
	P	0	0.20	0.86	0	0	0	0.37	0.08
	eta	0.94	0.05	0.01	0.18	0.16	0.13	0.04	0.07
SW*CP*FT	F	60.8	1.32	0.55	1.12	0.96	0.51	0.35	1.13
	P	0	0.22	0.88	0.35	0.49	0.90	0.98	0.34
	eta	0.86	0.12	0.05	0.10	0.09	0.05	0.03	0.10

[a] the partial eta squared values were computed using alpha = 0.05, when $P < 0.05$, there was a significant difference, $P = 0$ implied P was below 0.001.

Table 3. *E* values of the main and interactive effect of SW, CP and FT on Cd fractionation and Cd availability.

Source		*Wat-Cd*	*Exc-Cd*	*Car-Cd*	*Oxi-Cd*	*Org-Cd*	*Res-Cd*	*K*	*M*
SW	*E*	-0.26	-3.69	-0.80	-2.98	-0.15	7.87	-4.75	-3.94
	T	-5.4	-7.65	-2.18	-5.06	-0.66	9.87	-7.52	-8.08
	P	0	0	0.03	0	0.51	0	0	0
CP	*E*	0.07	1.08	0.35	-2.26	0.40	0.34	1.49	1.15
	T	1.86	3.01	1.26	-5.13	2.36	0.58	3.18	3.15
	P	0.06	0	0.21	0	0.02	0.56	0	0
FT	*E*	-0.11	1.86	-0.48	-0.59	-0.61	-0.06	1.26	1.74
	T	-2.43	3.89	-1.31	-1	-2.69	-0.07	2.02	3.6
	P	0.02	0	0.19	0.32	0.01	0.94	0.04	0
SW*CP	*E*	0.14	-0.33	0.20	1.06	0.61	-1.70	0.01	-0.19
	T	2.97	-0.69	0.55	1.81	2.68	-2.13	0.02	-0.39
	P	0	0.49	0.58	0.07	0.01	0.03	0.98	0.70
SW*FT	*E*	0.03	-0.64	0.49	0.39	-0.16	-0.12	-0.11	-0.60
	T	0.54	-0.99	1	0.49	-0.52	-0.11	-0.13	-0.93
	P	0.59	0.32	0.32	0.62	0.60	0.91	0.90	0.35
CP*FT	*E*	-0.13	-0.82	-0.11	-1.40	0.38	2.09	-1.06	-0.95
	T	-2.77	-1.72	-0.30	-2.4	1.65	2.64	-1.69	-1.97
	P	0.01	0.09	0.76	0.02	0.10	0.01	0.09	0.05
SW*CP*FT	*E*	-0.08	0.98	-0.36	-0.70	0.04	0.11	0.54	0.90
	T	-1.26	1.53	-0.73	-0.89	0.14	0.11	0.65	1.39
	P	0.21	0.13	0.47	0.37	0.89	0.91	0.52	0.17

[a] *E*, computed using alpha = 0.05 via DOE, when $P < 0.05$, there was a significant difference; $P = 0$ implied *P* was below 0.001. T was the statistical value for evaluating the probability distribution, according to which *P* was obtained.

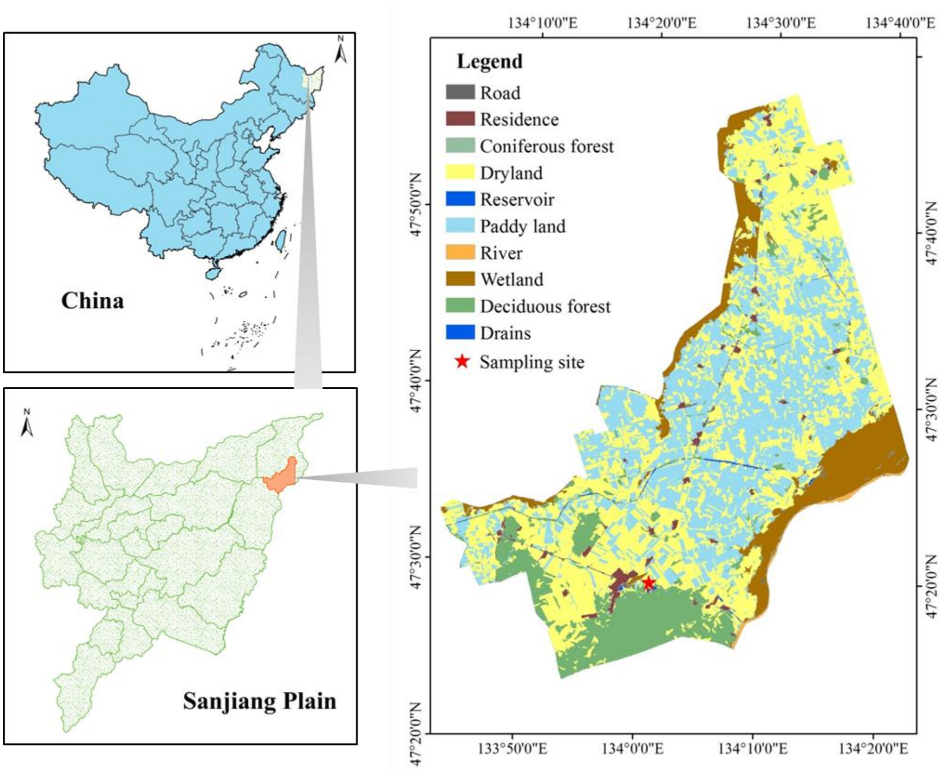

Figure 1 Sampling site.

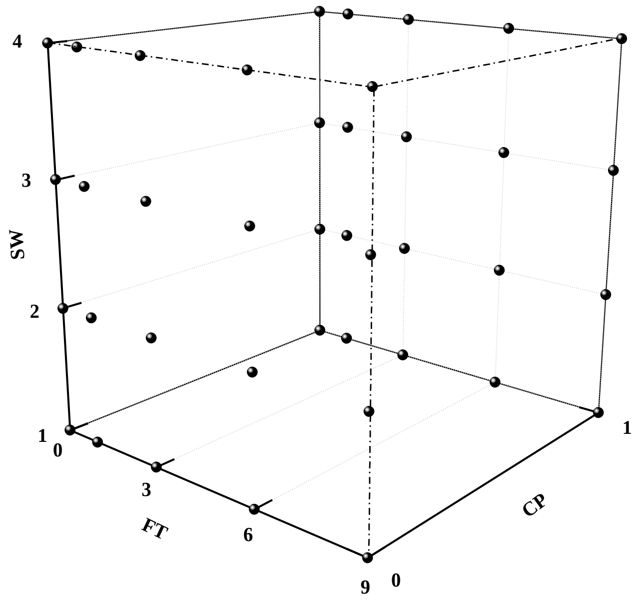

Figure 2 Experimental design.

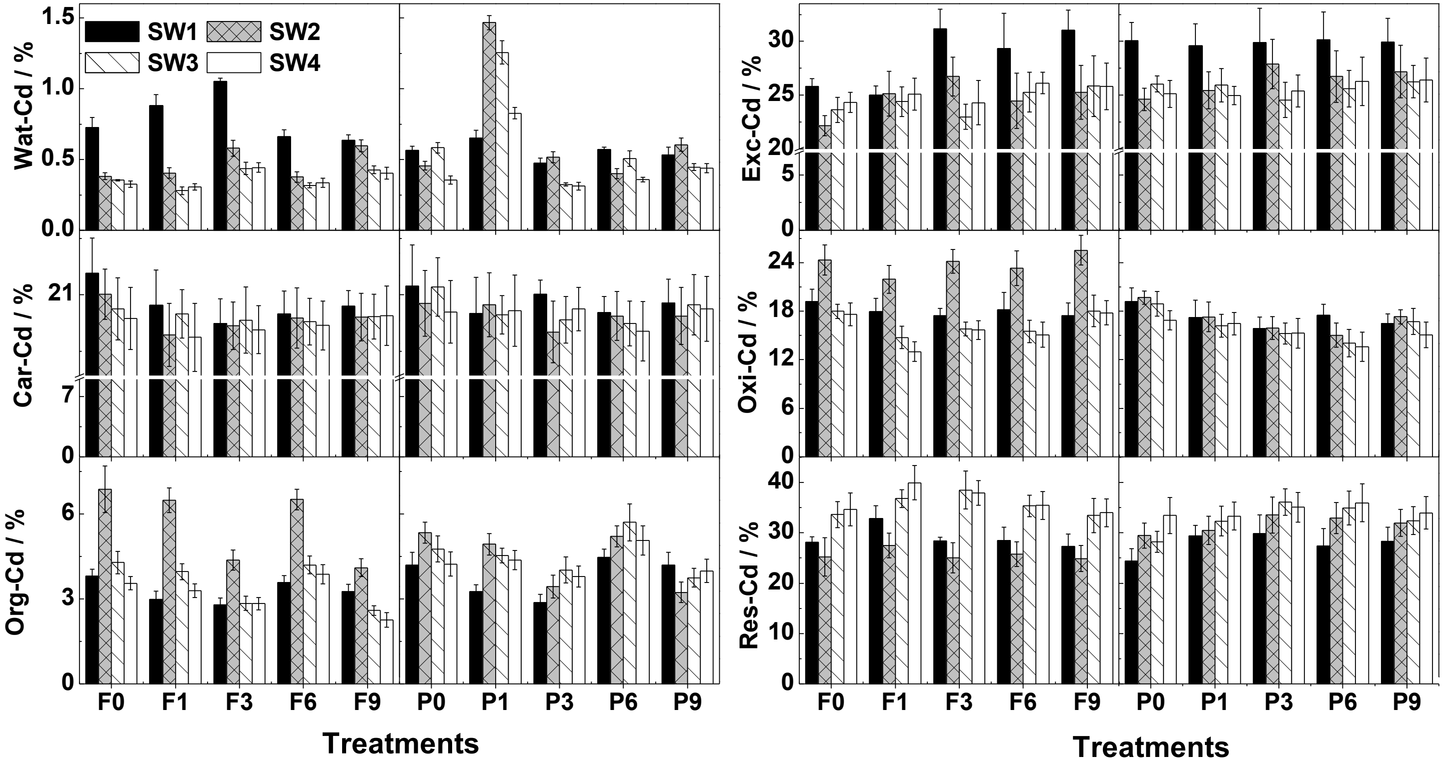

Figure 3 Percentage distribution of Cd in each fraction of the six-step sequential extraction for variously treated soils. Error bars represented ± standard error of the mean of four replicates. F0-9 and P0-9 represented the frequencies of the freeze-thaw cycles, i.e., 0, 1, 3, 6 and 9 in soils without and with chlorpyrifos pesticide.

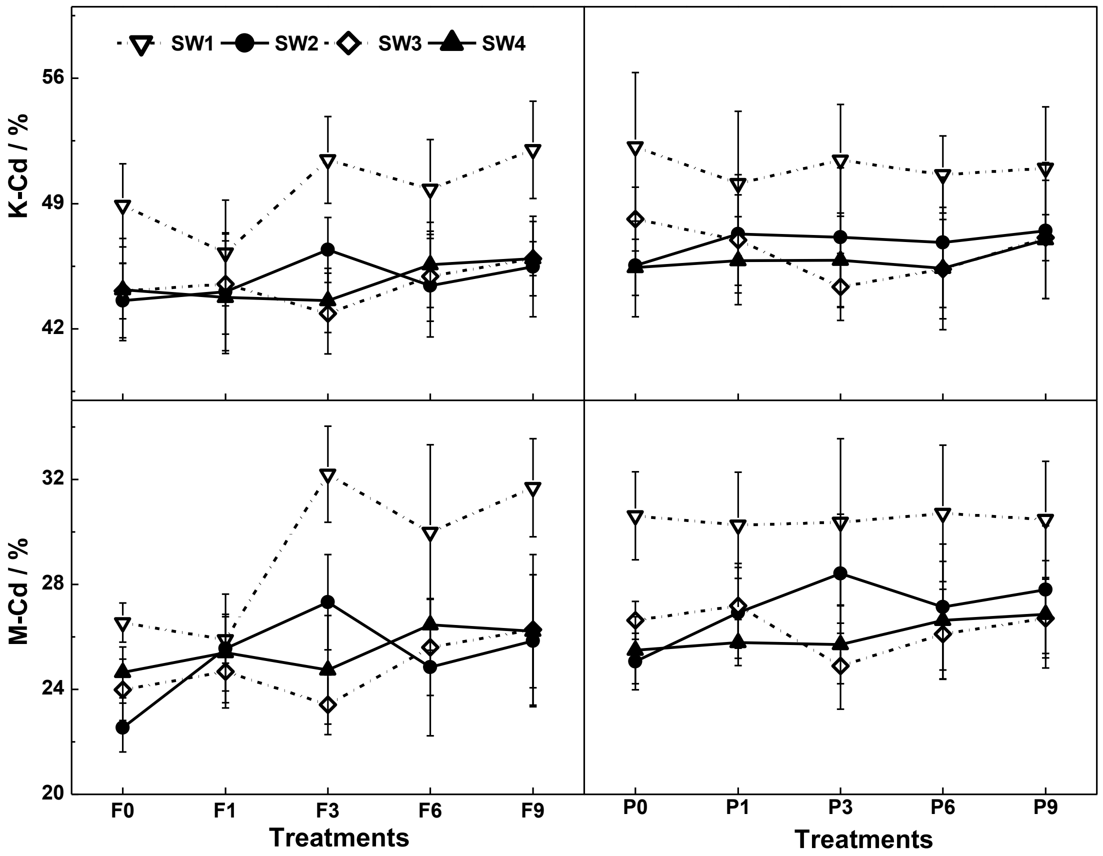

Figure 4 Impact of FT and SW on Cd availability in soil in the presence of CP. Error bars represented ± standard error of the mean of four replicates. F0-9 and P0-9 represented the frequencies of the freeze-thaw cycles, i.e., 0, 1, 3, 6 and 9 in soils without and with CP.

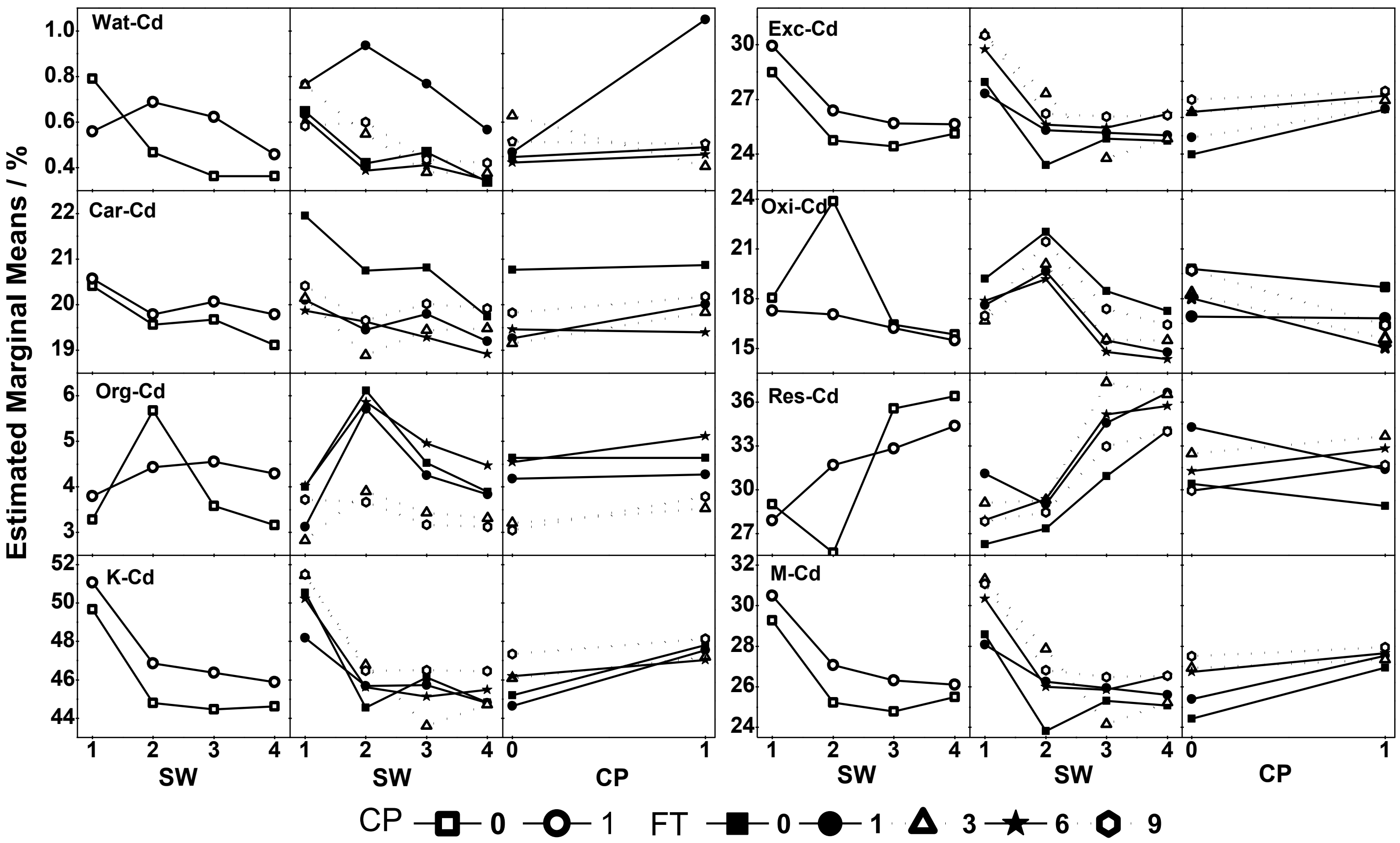

Figure 5 Interactions of FT, SW and CP on Cd fractionation and Cd availability in soil. If the lines of two levels crossed each other, there was thought to be an interactive effect.

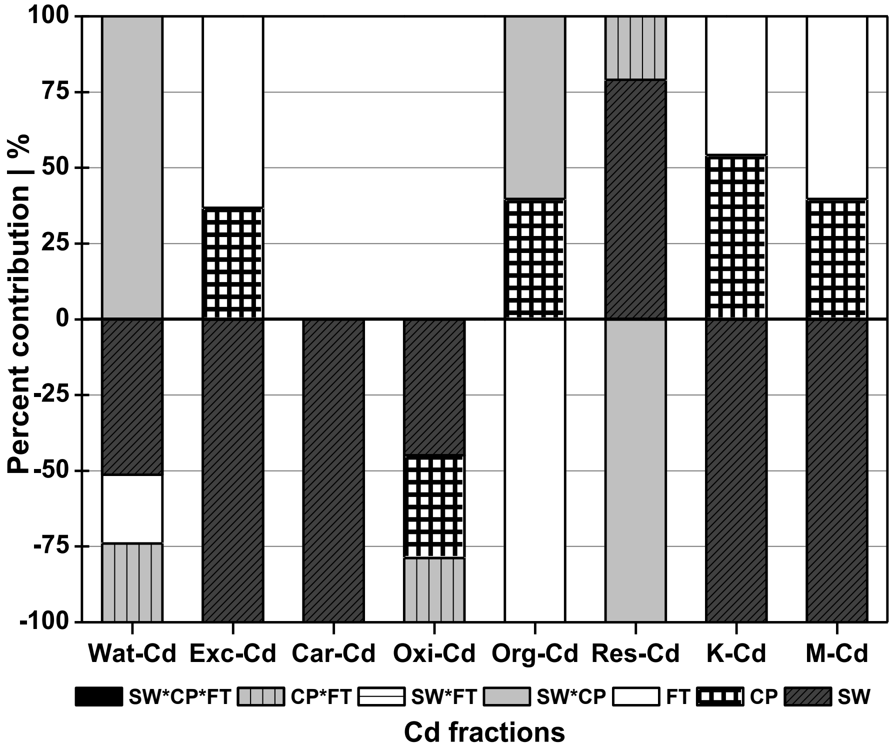

Figure 6 Contribution of main effects and interactions of FT, SW and CP on Cd fractionation and Cd availability in soil.