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Effect of Soil Organic Matter on Adsorption and Insecticidal Activity of Toxins from *Bacillus thuringiensis*

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ABSTRACT

With the large-scale cultivation of transgenic crops expressing *Bacillus thuringiensis* (*Bt*) insecticidal toxin in the world, the problem of environmental safety caused by these *Bt* crops has received extensive attention. The effects of soil organic matter (SOM) on the adsorption and insecticidal activity of *Bt* toxin in variable- and constant-charge soils (red and brown soils, respectively) were studied. Organic carbon in the soils was removed using hydrogen peroxide (H_2O_2). After H_2O_2 treatment, the SOM in the red and brown soils decreased by 71.26% and 82.82%, respectively. Mineral composition of the H_2O_2-treated soils showed no significant changes, but soil texture showed a slight change. After SOM removal, the cation exchange capacity (CEC) and pH decreased, while the specific surface area (SSA), point of zero charge (PZC), and zeta potential increased. The adsorption isotherm experiment showed that the *Bt* toxin adsorption on the natural and H_2O_2-treated soils fitted both the Langmuir model ($R^2 \geq 0.985\,7$) and the Freundlich model ($R^2 \geq 0.984\,1$), and the amount of toxin adsorbed on the H_2O_2-treated soils was higher than that on the natural soils. There was a high correlation between the maximum adsorption of *Bt* toxin and the PZC of soils ($R^2 = 0.935\,7$); thus, *Bt* toxin adsorption was not only influenced by SOM content, but also by soil texture, as well as the SSA, CEC, PZC, and zeta potential. The LC_{50} (lethal concentration required to kill 50% of the larvae) values for *Bt* toxin in the H_2O_2-treated soils were slightly lower than those in the natural soils, suggesting that the environmental risk from *Bt* toxin may increase if SOM decreases. As the measurement of insecticidal activity using insects is expensive and time consuming, a rapid and convenient *in vitro* method of enzyme-linked immunosorbent assays is recommended for evaluating *Bt* toxin degradation in soils in future studies.

Key Words: *Bt* toxin, point of zero charge, soil texture, specific surface area, zeta potential

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INTRODUCTION

Genetically modified (GM) crops were first approved for commercialization in 1994 and for large scale commercialization in USA in 1996 (Hung *et al.*, 2016). The plantation area of GM crops has reached 179.7 million hectares (ISAAA, 2016). There is considerable public concern about the environmental and health impacts of their use. Approximately 25% of GM crops contain the insecticidal trait, derived from the spore-forming bacterium *Bacillus thuringiensis* (*Bt*) (Helassa *et al.*, 2011). There is a wide range of issues being discussed in the context of risk assessment of these *Bt* plants. Transgenic plants encoding *Bt* toxins release these proteins into soil through root exudates during the vegetative period and through the incorporation of root and aerial plant biomass into soil after harvest (Stotzky, 2005), and some input comes from pollen (Kim *et al.*, 2008). The toxin accumulates in soil when its concentration exceeds the consumption by insect larvae and the degradation by soil microbes (Stotzky, 2005). In a large-scale agricultural field, the Cry1Ab protein expressed by *Bt* maize was transported to waterways adjacent to agricultural fields by runoff and the Cry1Ab protein persisted for up to 2 months (Strain and Lydy, 2015). The persistence of the *Bt* toxin in soil is further enhanced through association with soil particles. Previous studies showed that the toxin could rapidly bind to clay minerals, humic acids, and organo-mineral complexes (Stotzky, 2000; Zhou *et al.*, 2007, 2011; Fu *et al.*, 2012), and the content of clay minerals in soil had a significant influence on the adsorption and desorption of *Bt* protein; the vertical movement of *Bt* protein in the soil generally decreased as the clay content increased (Saxena *et al.*, 2002). The toxins bound to soil components had decreased bioa-

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vailability and were less susceptible to degradation. Therefore, the accumulated toxins were exposed to non-target organisms in the soil environment (Muchaonyerwa *et al.*, 2006).

Soil organic matter (SOM) shows high sorption characteristics because of its high specific surface area (SSA) and cation exchange capacity (CEC). Therefore, SOM could play a major role in the interactions of *Bt* toxins with soil particles (Crecchio and Stotzky, 1998). Because soil adsorbing surfaces are highly heterogeneous, the influence of soil components on the adsorption of toxins can be examined in two different ways. One involves studying adsorption in simple semisynthetic systems associated with various soil colloid materials such as clays (Tapp *et al.*, 1994), organic matter (Crecchio and Stotzky, 1998), oxides (Sander *et al.*, 2010; Madliger *et al.*, 2011), and organomineral associations (Crecchio and Stotzky, 2001). The other involves removing a specific soil component in order to establish its effect on adsorption (Pagel-Wieder *et al.*, 2007; Pérez-Novo *et al.*, 2008; Fu *et al.*, 2012). Soil structure can be defined as an arrangement of mineral soil particles and organic compounds that form aggregates of different sizes and stability. Pagel-Wieder *et al.* (2007) reported that destruction of organic matter with hydrogen peroxide (H_2O_2) was related to an increase in the adsorption of Cry1Ab protein. However, Muchaonyerwa *et al.* (2006) observed that the clay fraction of H_2O_2-treated Vertisol adsorbed less toxin than the natural clay fraction. The varying results from these two studies indicate that more detailed studies are needed to confirm the influence of organic matter on toxin adsorption, as well as on the physicochemical properties of soils.

In this study, we examined the adsorption and insecticidal activity of *Bt* toxin in the natural and H_2O_2-treated soils. In order to compare different soil types, variable- (Ultisol) and constant-charge (Alfisol) soils were chosen. The objective of this study was to identify changes in the adsorption patterns and insecticidal activity of *Bt* toxin due to a reduction in the organic matter content of soils.

MATERIALS AND METHODS

Bt toxin and soil samples

The transgenic insecticidal toxin (Cry1Ab) was provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. The Cry1Ab protein has a molecular weight of 66 kDa and consists of 618 amino acid residues.

Red soil (Ultisol) was collected from the 0–40 cm layer of cultivated land in Xianning, Hubei Province, China. Brown soil (Alfisol) was sampled from the 0–40 cm layer of forest land in Tianwai Village, Taishan, Shandong Province, China. Neither soil was planted with *Bt* crops. After air-drying, the soils were ground to pass a 0.2-mm mesh. Soil pH was measured in a soil:water ratio of 1:2.5 (weight:volume). The point of zero charge (PZC) was determined using the salt titration method (Gillman and Uehara, 1980). Mineralogy of the soils was determined by X-ray diffraction (XRD) using a diffractometer with Cu K_α radiation (Rigaku D/max-2500, Japan). The apparent zeta potential of the soil particles was estimated from electrophoretic mobility measurements with a Zetasizer Nano-ZS90 (Malvern Instruments, UK). The CEC was determined by extraction with $NH_4C_2H_3O_2$. The SSA was measured using the N_2 adsorption method. The texture of soil samples was analyzed with a wet sieving and sedimentation technique.

For SOM removal, 20 g soil was transferred into a beaker (500 mL), and mixed with 200 mL deionized water. Five milliliters of H_2O_2 solution (30%, weight:weight) were added. The suspension was stirred periodically at room temperature until bubbling ceased, and then 5 mL H_2O_2 solution (30%, weight:weight) was added again. This procedure was repeated until no visible frothing was observed after H_2O_2 addition, indicating a completed reaction (Mikutta *et al.*, 2005). The soil slurry was heated to 70–90 °C to remove excess H_2O_2. The samples were washed out with deionized water and centrifuged at 5 000 × *g* for 10 min. This washing procedure was repeated 5 times. Finally, the samples were air-dried and stored at room temperature for further use. Soil organic carbon was analyzed by using the $K_2Cr_2O_7$ digestion method. The surface morphology of soils before and after H_2O_2 treatment was examined in a Hitachi S-4800 scanning electron microscope (Hitachi, Japan) equipped with an X-ray dispersive analyzer. The basic characteristics of the natural and H_2O_2-treated soils are summarized in Table I.

Adsorption kinetics and isotherm experiments

The *Bt* toxin was dissolved in *Tris*-HCl buffer (pH 7.0). The adsorption kinetics was determined at a toxin concentration of 0.2 g L^{-1} and a soil concentration of 0.5 g L^{-1}. The mixtures of soil and *Bt* toxin were shaken at 300 r min^{-1} and 25 ± 1 °C, pipelined out at designated time intervals (from 0.5 to 180 min), and then centrifuged at 20 000 × *g* for 20 min; the absorbance of supernatants was measured at 280 nm (Zhou *et al.*, 2007). The amount of *Bt* toxin adsorbed was calcula-

TABLE I

Basic characteristics of the natural and H_2O_2-treated red and brown soils

Soil	Clay	Silt	Sand			pH
			Total	Coarse	Fine	
			%			
Natural red soil	54.2	29.4	16.4	3.9	12.5	4.61
Treated red soil	54.7	33.2	12.1	1.6	10.5	4.05
Natural brown soil	17.7	36.7	45.6	10.5	35.1	5.99
Treated brown soil	20.4	37.6	42.0	7.1	34.9	4.87

ted from the concentration difference.

Adsorption isotherm experiment was carried out with an initial concentration of *Bt* toxin from 0 to 1.0 g L^{-1} (pH 8.0) and of soil at 1.0 g L^{-1}. The mixtures of soil and *Bt* toxin were shaken at 200 r min^{-1} and 25 ± 1 °C, and a 3-h adsorption period was sufficient for equilibrium conditions to be reached. The mixtures were centrifuged at 20 000 × *g* for 20 min and the amount of *Bt* toxin adsorbed was calculated according to the adsorption kinetics experiment.

Determination of insecticidal activities

Heliothis armigera (first instar) with a length of 2 to 3 mm was used to determine the insecticidal activity of *Bt* toxin. Free and adsorbed toxins were diluted to 200, 100, 50, 25, and 12.5 mg L^{-1}. Each concentration of toxin needed one incubation plate with 24 wells (1.7 cm high, 1.6 cm in diameter); therefore, five concentrations of each sample needed five incubation plates (120 wells in total). The inflection feed was prepared as follows: agar powder (4.5 g) was dispersed in 220 mL sterilized water, and maintained at boiling temperature for 10 min; yeast powder (12 g), soybean powder (24 g), sodium benzoate (0.42 g), and vitamin C (1.5 g) were mixed and soaked with 80 mL sterilized water, and then transmitted into the agar solution; during stirring, the temperature of the suspension solution dropped to 65 °C, and 3.9 mL acetic acid (36%, weight:weight) was added; the inflection feed was obtained by mixing 2 mL *Bt* toxin samples with 18 mL suspension feed. Twenty milliliters of inflection feed were dispersed into the 24 wells of incubation plate. After air-drying for 12 h, water on the walls of the wells disappeared (feed was still wet), and one larva was placed into each well and incubated at 30 ± 2 °C for 72 h. The incubation plate was covered tightly with a lid.

The mortality was then determined using a sterilized toothpick. If the insect body became black or could not move, it was confirmed as dead. The mortality of the testing sample needed rectification using the following equation:

$$M_R = M_D/(1 - M_C) \quad (1)$$

where M_R is the rectification mortality of larvae in the testing sample, and M_D is the direct mortality of larvae in the testing sample, M_C is the mortality of larvae in the control sample. The soils were used as the control, and the mortality in it was almost zero.

To calculate the lethal concentration required to kill 50% of the larvae (LC_{50}), the mortality values were converted into probability values. A plot of probability values *versus* the logarithmic concentrations of *Bt* toxin yielded a straight line. The LC_{50} values were obtained using the least square method. The bioassay was repeated three times; each soil sample required 15 incubation plates and 270 mL liquid feed in total.

Data analysis

Two isotherm equations were tested in the present study. The Langmuir model assumes that adsorption occurs at specific homogeneous sites on the adsorbent surface and has been successfully applied to many monolayer adsorption processes. The Freundlich model is an empirical relationship describing the adsorption of solutes from a liquid to a solid surface, and assumes that different sites with various adsorption energies are involved. The equations for the two models are given below:

$$q_{e,L} = \frac{q_{max} \cdot K_L \cdot C_{eq}}{1 + K_L \cdot C_{eq}} \quad (2)$$

$$q_{e,F} = K_F (C_{eq})^{\frac{1}{n}} \quad (3)$$

where $q_{e,L}$ and $q_{e,F}$ are the equilibrium adsorption capacity of the adsorbent in the Langmuir and Freundlich models, respectively (g g^{-1}), q_{max} is the maximum adsorption capacity of the adsorbent (g g^{-1}), K_L is the Langmuir adsorption constant related to free energy of adsorption (L g^{-1}), C_{eq} is the equilibrium concentration of *Bt* toxin in solution (g L^{-1}), and K_F and n are the Freundlich constants. The experimental data for *Bt* toxin adsorption were analyzed using the Langmuir and Freundlich models with SigmaPlot software (Systat Software Inc., USA). The parameters of the Langmuir and Freundlich models can be easily obtained by choosing the modified hyperbola I and power equations, respectively.

All the experiments were conducted in three replications. Data are expressed as means ± standard errors.

RESULTS AND DISCUSSION

Selected properties of natural and H_2O_2-treated soils

Total carbon of the natural brown soil was much higher than that of the natural red soil (Table II). After treatment with H_2O_2, the amount of carbon in the red and brown soils decreased by 71.26% and 82.82%, respectively. With respect to soil texture, the sand content (including coarse and fine sand) slightly decreased, while the clay and silt contents slightly increased. There was no significant difference in the mineral composition of the natural soils and H_2O_2-treated soils according to the XRD identification (Fig. 1). After removing SOM, the CEC and pH decreased, and the SSA, PZC, and zeta potential increased (Table II).

Adsorption kinetics

It was found that the adsorption rate of *Bt* toxin on the H_2O_2-treated soils was higher than that on the natural soils (Fig. 2). Furthermore, the equilibrium time

TABLE II

Selected properties[a)] of the natural and H_2O_2-treated red and brown soils

Soil	SSA	CEC	PZC	Zeta potential	TOC	Clay mineral composition
	$m^2\ g^{-1}$	cmol kg^{-1}		mV	g kg^{-1}	
Natural red soil	48.5	21.2	3.3	−18.4	14.73	Hydromica (20%), 1.4-nm mineral (35%), kaolinite (45%)
Treated red soil	52.7	17.5	3.6	−16.3	4.23	Hydromica (20%), 1.4-nm mineral (35%), kaolinite (45%)
Natural brown soil	31.7	46.2	4.1	−17.6	26.33	Hydromica (45%), vermiculite (35%), kaolinite (20%)
Treated brown soil	37.5	41.2	4.3	−15.8	4.52	Hydromica (45%), vermiculite (35%), kaolinite (20%)

[a)]SSA = specific surface area; CEC = cation exchange capacity; PZC = point of zero charge; TOC = total organic carbon.

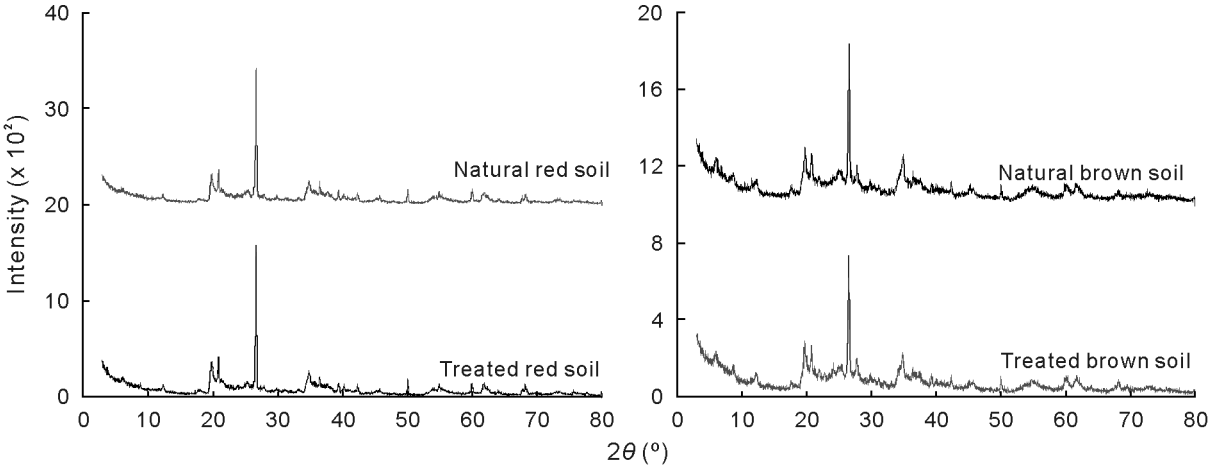

Fig. 1 X-ray diffraction analysis of the natural and H_2O_2-treated red and brown soils.

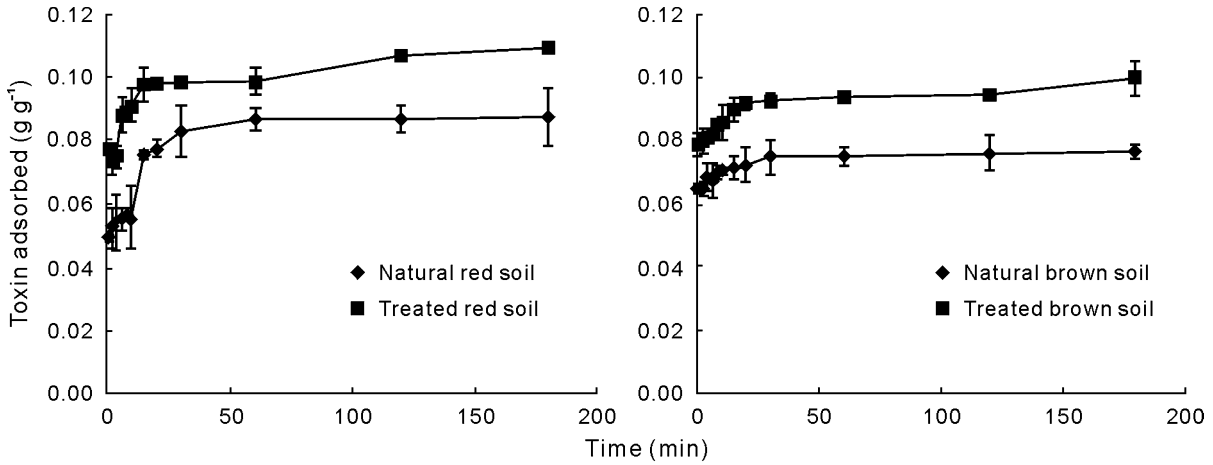

Fig. 2 Adsorption kinectics of toxin from *Bacillus thuringiensis* on the natural and H_2O_2-treated red and brown soils. Vertical bars indicate standard errors of the means ($n = 4$).

for adsorption of *Bt* toxin became shorter. Adsorption of *Bt* toxin on the H_2O_2-treated soils was rapid up to 15 min and then reached equilibrium. For the natural soils, the adsorption of *Bt* toxin reached equilibrium at around 30 min.

Adsorption isotherms

The adsorption isotherm is the equilibrium relationship between the concentrations in the fluid phase and in the adsorbent surface at a given temperature. Within the range of *Bt* toxin concentrations studied (0–1.0 g L^{-1}), the adsorption amount increased rapidly at the initial stage and then gradually reached equilibrium (Fig. 3).

The results in Table III indicate that the adsorption isotherm can be described by both the Langmuir ($R^2 \geq 0.987\,5$) and Freundlich models ($R^2 \geq 0.984\,1$). The maximum adsorption amount of *Bt* toxin on the H_2O_2-treated soil was higher than that on the natural soils. For example, the maximum adsorptions of *Bt* toxin on the natural and treated red soils were 0.426 7 and 0.498 7 g g^{-1}, respectively, and on the natural and treated brown soils were 0.535 3 and 0.599 4 g g^{-1}, respectively. The results of the present study are in contrast to the study of Muchaonyerwa *et al.* (2006), who reported that the adsorption of toxin from *Btt* (*Bacillus thurigiensis* subsp. *tenebrionis*) on the carbon fraction was much higher than that on clay minerals. In view of the contrasting results, we speculate that the effects of SOM on adsorption of *Bt* toxin may not be limited to the carbon content, and are also attributed to soil texture and other factors, such as the SSA, CEC, PZC, and zeta potential.

The Freundlich constants K_F and n indicate the adsorption capacity and adsorption intensity, respectively (Özcan *et al.*, 2005; Bulut and Tez, 2007). The K_F and $1/n$ values of the H_2O_2-treated soils were higher than those of the natural soils, which is consistent with the variation in q_{max} (Table III). The K_L value from the Langmuir model is also used for expressing the adsorption affinity (Cai *et al.*, 2006). However, in the present study, the K_L value for the H_2O_2-treated brown soil was lower than that for the natural brown soil, which is in contrast to the variation in q_{max}. Therefore, for adsorption affinity, the Freundlich model had a better fit than the Langmuir model. It is also reported that the coefficient of determination (R^2) of adsorption data can be used to determine which model

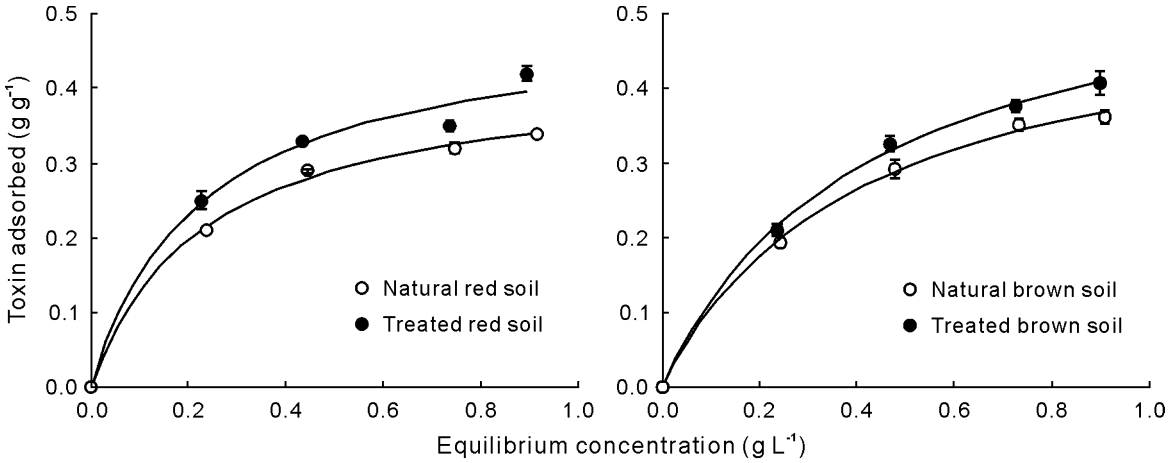

Fig. 3 Adsorption isotherms of toxin from *Bacillus thuringiensis* on the natural and H_2O_2-treated red and brown soils. Vertical bars indicate standard errors of the means ($n = 4$).

TABLE III

Parameters[a)] of the Langmuir and Freundlich models used for the natural and H_2O_2-treated red and brown soils

Soil	Langmuir model			Freundlich model		
	q_{max}	K_L	R^2	K_F	$1/n$	R^2
	g g^{-1}	L g^{-1}				
Natural red soil	0.426 7	4.290	0.997 3	0.355 0	0.328 8	0.991 7
Treated red soil	0.498 7	4.331	0.987 5	0.418 5	0.338 5	0.984 1
Natural brown soil	0.535 3	2.424	0.997 5	0.390 4	0.456 0	0.990 8
Treated brown soil	0.599 4	2.364	0.998 3	0.435 2	0.466 2	0.992 9

[a)] q_{max} is the maximum adsorption capacity of the adsorbent; K_L is the Langmuir adsorption constant related to free energy of adsorption; R^2 is the coefficient of determination; K_F and n are the Freundlich constants.

has a better fit (Chen *et al.*, 2007). As shown in Table III, the R^2 values of the Langmuir model were higher than those of the Freundlich model. According to our experimental results, both models had different advantages. As the adsorbent had a limited adsorption capacity, the amount of maximum adsorption could be better described by the Langmuir model than by the Freundlich model. Nevertheless, the Freundlich model was better for the expression of adsorption affinity.

Insecticidal activity of adsorbed Bt toxin

Both free and adsorbed toxins were toxic to the larvae of *H. armigera* (Table IV). The LC_{50} values for the adsorbed *Bt* toxin were lower than that of free *Bt* toxin; *i.e.*, the insecticidal activity of adsorbed *Bt* toxin increased, which is in agreement with a previous study (Crecchio and Stotzky, 2001). The LC_{50} values for *Bt* toxin in the H_2O_2-treated soils were slightly lower than those in the natural soils, suggesting that the environmental risk from toxins could increase if the organic matter of soils decreased. The measurement of insecticidal activity using insects used in this study is expensive and time consuming. A rapid and convenient *in vitro* method of enzyme-linked immunosorbent assays (ELISA) (Mueting *et al.*, 2014) has currently been used for evaluating toxin degradation in soils.

TABLE IV

Insecticidal activity of toxin from *Bacillus thuringiensis* before and after adsorption by natural and H_2O_2-treated red and brown soils

Toxin	LC_{50}[a)]
	μg mL^{-1}
Before adsorption	9.12 ± 1.39
After adsorption	
Natural red soil	3.08 ± 1.13
Treated red soil	2.76 ± 0.94
Natural brown soil	4.16 ± 1.50
Treated brown soil	3.24 ± 1.22

[a)] Lethal concentration required to kill 50% of the larvae of *Heliothis armigera*.

Effects of SOM removal on mineral structure

There are three reagents used for SOM removal: H_2O_2, sodium hypochloride (NaOCl), and disodium peroxodisulfate ($Na_2S_2O_8$) (Mikutta *et al.*, 2005). Hydrogen peroxide was introduced by Robinson (1922) for soil texture analysis and has become the most widely used chemical reagent for organic matter destruction. Generally, NaOCl and $Na_2S_2O_8$ are more effective in organic carbon removal than H_2O_2. The NaOCl procedure is carried out at pH 9.5, with three consecutive cycles including boiling each for 15 min (Anderson, 1963). Despite the higher electronic potential of NaOCl with decreasing pH, carbon removal efficiency is maximal at pH 9.5. However, the NaOCl procedure (pH 9.5) may dissolve Al-hydroxides, although alkaline conditions favor the precipitation of metals released upon destruction of organic matter (Mikutta *et al.*, 2005). Furthermore, at higher temperatures, the NaOCl treatment may induce changes in extractable pedogenic Al and Fe due to transformation into more crystalline forms. The efficiency of $Na_2S_2O_8$ in removing organic matter has been proposed as superior to H_2O_2 and NaOCl (Meier and Menegatti, 1997). With the measurements using XRD, infrared spectroscopy, and N_2 adsorption, no structural alteration of $Na_2S_2O_8$-treated illite, kaolinite, and montmorillonite reference minerals was observed (Menegatti *et al.*, 1999). The red soil used in the present study contains iron-aluminum oxides; therefore, the $Na_2S_2O_8$ procedure was not suitable for our experiment.

Both red and brown soils are composed of nonuniform particles. After treatment with H_2O_2, the size of soil particles decreased (Fig. 4), supporting the results that the SSA increased after SOM removal (Table II), but the basal spacing of soil minerals was not observed (Fig. 1).

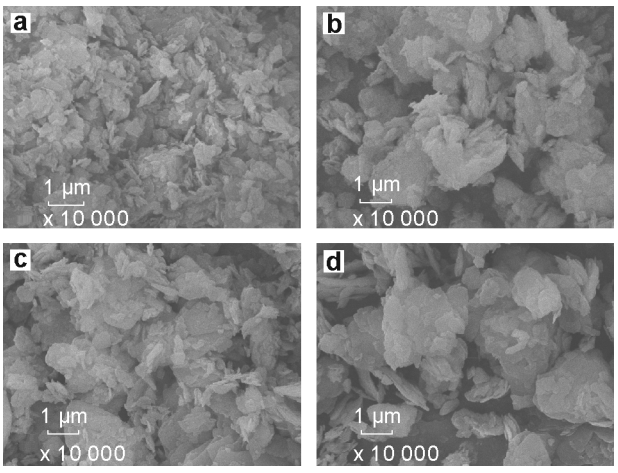

Fig. 4 Scanning electron microscope images of the H_2O_2-treated red soil (a), natural red soil (b), H_2O_2-treated brown soil (c), and natural brown soil (d).

Effects of SOM removal on Bt toxin adsorption

There are several potential mechanisms for interactions between *Bt* proteins and the surfaces of soil particles. It can be assumed that electrostatic forces (Sander *et al.*, 2010), ligand exchange (Fu *et al.*, 2007), hydrogen bridges (Crecchio and Stotzky, 2001), ionic bonds and complex formation (Tapp *et al.*, 1994), hydrophobic interactions (Helassa *et al.*, 2011), and van der Waals forces are responsible for the binding

of *Bt* toxin. The adsorptive forces operating between the bound toxin and the clay-sized fractions are not clearly understood. Sander *et al.* (2010) reported that electrostatic interactions governed the adsorption of monomeric Cry1Ab toxin to charged polar surfaces, whereas Helassa *et al.* (2011) reported that hydrophobic interactions may play an important role in Cry1Ab toxin adsorption.

The isoelectric point (IEP) of Cry1Ab toxin was between 6.0 and 6.4 (Sander *et al.*, 2010), and the PZC of the studied soils ranged from 3.3 to 4.3 (Table II). At the experimental pH of 7.0, the soils and Cry1Ab toxin are negatively charged, and there are repulsive electrostatic interactions between the negatively charged surfaces of soil particles and the anionic functional groups of the toxin. When the negative charges on the surface of soil particles decrease, the electrostatic repulsion between toxin and soil particles weakens, resulting in an increasing affinity of toxin for soil particles. The zeta potential of the H_2O_2-treated soils was higher than that of the natural soils (Table II), which is consistent with the results of toxin adsorption (Table III). On the other hand, when the pH value is above the IEP of soils, the lower the PZC values are, the higher the net negative charge on their surface is (McLaren *et al.*, 1958). We found a high correlation between the $q_{\max}$ and PZC values ($R^2 = 0.935\,7$); therefore, our results support the electrostatic driving mechanism for toxin adsorption.

Cry1Ab toxin has a higher affinity for hydrophobized surfaces compared to hydrophibic ones (Janot *et al.*, 2010). Soil organic matter may considerably enhance hydrophobicity, leading to macroscopic changes in soil properties (Doerr *et al.*, 2007). However, the adsorption capacity of *Bt* toxin in the H_2O_2-treated soils (lower hydrophobility) was higher than that in the natural soils (higher hydrophobility) (Table III), which does not appear to support the hydrophobic interaction mechanism for toxin adsorption (Helassa *et al.*, 2011). There are two possible reasons for the contradictory results. First, the SSA of the H_2O_2-treated soils was higher than that of the natural soils (Table II). The adsorption amount for a biomacromolecule depends on its size in the medium and the SSA of the adsorbent. The relationship between the adsorption amount and the SSA can be described as in Eq. 4 when the section area of the adsorbate is constant (Physical Chemistry Teaching and Research Section of Tianjing University, 1983).

$$\Gamma_\infty = \frac{A_\mathrm{w}}{L \cdot A_\mathrm{m}} \tag{4}$$

where Γ_∞ is the saturation adsorption amount (mol kg^{-1}), A_w is the specific surface area of the adsorbent ($\mathrm{m}^2\ \mathrm{kg}^{-1}$), L is the Avogadro's number, and A_m is the section area of the adsorbate molecule (m^2). It can be seen that the greater the SSA is, the higher the adsorption amount is. Although the hydrophobicity of the H_2O_2-treated soils may decrease, the SSA increases due to the changes in soil texture. If a decrease in the hydrophobicity and an increase in the SSA work against each other, the adsorption may show an increase or a decrease, depending on which factor is predominant. Second, the PZC and zeta potential of the soils increased after SOM removal, resulting in a decrease of electrostatic repulsion between toxin and soil particles.

Effects of SOM removal on insecticidal activity

Previous studies showed that the insecticidal activity of bound toxin was higher than that of free toxin (Tapp and Stotzky, 1995; Crecchio and Stotzky, 2001). The increase in apparent toxicity may have been a result of the toxin being localized by binding on the complexes; *i.e.*, more toxin was ingested by larvae as clay-toxin complexes than the free toxin uniformly spread over the surface of food medium (Crecchio and Stotzky, 1998, 2001). Zhou *et al.* (2005) assumed that the adsorbed toxin showed higher insecticidal activity due to its higher stability. However, Hung *et al.* (2016) suggested that toxicity may not have been enhanced by adsorption, but the larvae consumed less feed when it contained soluble Cry than when Cry was adsorbed on soils. Their results confirmed that the consumption amount of feed containing adsorbed toxin was 7% higher than that of feed containing soluble toxin. Another reason for the enhanced toxicity of adsorbed toxin was due to the less marked mandible paralysis of larvae and hence less cessation of feeding for adsorbed Cry with respect to soluble Cry in feed (Hung *et al.*, 2016). The adsorbed Cry would not be solubilized in the insect mouth, but could be desorbed in the alkaline solution of the midgut. In the present study, the toxin adsorbed on the H_2O_2-treated soils had a higher toxicity than that on the natural soils. The possible reason is that the H_2O_2-treated soils had a higher adsorption capacity of toxin, leading to a lower desorption of toxin in feed, and thus larvae consumed more feed, resulting in higher mortality.

CONCLUSIONS

After the removal of SOM by H_2O_2 treatment, the mineral composition of the soils showed no significant changes, but there was a slight change in soil texture;

the CEC and pH decreased, while the SSA, PZC, and zeta potential increased. The adsorption isotherm experiment showed that the toxin adsorption on the natural and H_2O_2-treated soils fitted both the Langmuir model ($R^2 \geq 0.985\,7$) and the Freundlich model ($R^2 \geq 0.984\,1$). The adsorption amount of toxin on the H_2O_2-treated soils was higher than that on the natural soils, and there was a high correlation between the maximum adsorption of toxin and the PZC of soils ($R^2 = 0.935\,7$). Therefore, the adsorption of *Bt* toxin was not only influenced by the SOM content, but also by soil texture as well as the SSA, CEC, PZC, and zeta potential. The current study supports the electrostatic driving and cation exchange mechanisms for toxin adsorption. The LC_{50} values for the toxin in the H_2O_2-treated soils were slightly lower than those in the natural soils, suggesting that the environmental risk from the toxin could increase if the organic matter of soils decreased. Because the measurement of insecticidal activity using insects is expensive and time consuming, a rapid and convenient *in vitro* method of ELISA is recommended for evaluating the toxin degradation in soils in future studies.

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