Bull Environ Contam Toxicol (2012) 89:649–653
DOI 10.1007/s00128-012-0746-0

# Residues and Dynamics of Kasugamycin in Chilli and Soil

**Lei Lu · Shancang Zhao · Ligang Deng · Yebing Chen · Xue Liu · Dapeng Li**

Received: 30 March 2012 / Accepted: 10 July 2012 / Published online: 21 July 2012


**Abstract** A simple and efficient method for determination of kasugamycin in chilli and soil was developed, and the fate of kasugamycin in chilli field ecosystem was also studied. Kasugamycin residues were extracted from sample, cleaned up by solid phase extraction and chromatographic column and then determined by ultra performance liquid chromatography with tandem mass spectrometry detection. The method got recoveries ranged from 77.82 % to 83.35 % with relative standard deviations of 2.20 %–6.54 %. As far as the accuracy and precision was concerned, the method met certain standard. The LODs of kasugamycin calculated as a sample concentration(S/N ratio of 3) was 2.50 μg $kg^{-1}$. The degradation of kasugamycin in chilli and soil was determined. The results showed that kasugamycin degradation in chilli plant and soil followed the first-order kinetics. The half-lives of kasugamycin in chilli and soil was 2.76–3.77 and 3.07–3.91 days, respectively. The final kasugamycin residues in chilli and soil were undetectable at levels of recommended and 1.5 times recommended dosage with an interval of 21 days.

L. Lu · X. Liu · D. Li (✉)
College of Food Science and Engineering, Shandong Agricultural University, Taian 271018, Shandong, China
e-mail: dpli73@sdau.edu.cn

L. Lu
e-mail: luleibendan@126.com

S. Zhao · L. Deng · Y. Chen
Central Laboratory of Shandong Academy of Agricultural Sciences, Key Laboratory of Test Technology on Food Quality and Safety of Shandong Province, Jinan 250100, Shandong, China

**Keywords** Kasugamycin · Residue · Dynamics · Chilli · UPLC–MS/MS

Kasugamycin ($C_{14}H_{25}O_9N_3$) is an effective fungicide for prevention of chilli disease by anthrax bacteria (Yoo et al. 2009). Improper and extensive use of the pesticide not only pollutes the cultivated soil and groundwater, but also leads to accumulation in the plants, and the chillies for import and export must be free of chemical residues (Chanchaichaovivat et al. 2008). And nowadays public concern over pesticide residues has become an important consideration. Kasugamycin is a systemic fungicide with water solubility of 125 g $L^{-1}$ (25°C), it is not a toxic compound with a LD50 value larger than 5,000 mg $kg^{-1}$ in rats (Tomlin 1994), but as its strong inabsorbability, it may leave residues in chilli. Because of potential risk of kasugamycin residues to human and environment, a determination method for analyzing the residues of kasugamycin in chilli should be set up.

Developing and validating analytical methods of target compounds (may vary according to pesticide residue definition) is precondition for conducting supervised residual study (Wang et al. 2011). Even though official product analysis is conducted by a bioassay method, it is time-consuming and non-selective, Gas chromatography (GC) is not suitable to analyze the kasugamycin because of its high melting point (Lo et al. 1996). To the contrary, LC is proved to be a powerful technique for residue analysis (Turnipseed et al. 2005; Sagratini et al. 2007; Mezcua et al. 2009; Nanita et al. 2009). Several literatures reported the determination of kasugamycin, such as kasugamycin in agricultural products by HPCE (Lo Hsiao 1996), kasugamycin in fermentation broth, extract liquor and product by RP-HPLC and HPCE (Niu et al. 2001), kasugamycin in

water by HPLC (Sheu et al. 2010). Despite these studies, no development of the fate of kasugamycin during degradation in chilli fields has been observed. Thus, an efficient and selective UPLC–MS/MS method for analyzing the residues of kasugamycin in chilli was set up and research on the degradation dynamics of kasugamycin was carried out to evaluate the impact of using kasugamycin in chilli field ecosystem, so as to reveal the possible approaches of its degradation. This would help to provide basic information for developing regulations to guard a safe use of kasugamycin in chilli fields and to prevent any health problem from consumers.

## Materials and Methods

Kasugamycin hydrochloride hydrate (purity, 79.0 %) was purchased from Dr. Ehrenstorfer (Augsburg, Germany). 4 % Kasugamycin WP was obtained from yanbian biological Pharmaceutical Co., Ltd. (Jilin, People's Republic of China). LC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Methanol, analytical grade was purchased from Guang Cheng Chemical Reagent Co. (Tianjin, China). Formic acid (97 %) was purchased from Alfa Aesar (26 Parkridge Road Ward Hill, MA 01835 USA). Ammonia solution, analytical grade was purchased from Qiang Sheng Chemical Co., Ltd (Jiangsu, China). Water was purified using a Milli-Q system from Millipore (Bedford, MA, USA). Oasis® MCX extraction cartridges (3 cc, 60 mg) were purchased from Waters (Milford, USA).Stock solutions were prepared in methanol: water (1:1, v/v) at 1.0 mg $mL^{-1}$ and stored in the dark at 4°C. Working solutions were freshly obtained by appropriate dilutions with methanol: water (1:1, v/v).

Field experiment was carried out in Jinan, Hangzhou and Shijiazhuang during 2008–2009. The experiment was carried out in nine plots, each with an area of 30 $m^2$. A complete randomized block design (CRD) was applied with three replicates. Kasugamycin WP was applied at two doses 1,410 g a.i.$hm^2$(recommended)and 2,115 g a.i.$hm^2$ (1.5 times recommended). 1.5 times recommended dosage was applied as dynamics residue experiment, the representative samples were taken about 1 h, 1, 2, 3, 5, 7, 9, 12, 14 and 21 days after application of kasugamycin. The final experiment was carried out with recommended dosage and 1.5 times recommended dosage, each with spraying of two and three times, spraying interval of 7 days. The representative samples were taken about 7, 14 and 21 days after application of kasugamycin. The collected samples were placed in a freezer at −20°C until analysis.

5 g of homogenized chilli was weighed in a 50-mL centrifuge tube. After addition of 20 mL methanol, the extraction was shaken for 1 min in a vortex mixer, and then pH was adjusted to 5.0 with formic acid. The supernate was finally applied to Oasis® MCX extraction cartridges after centrifugation at 4,000 rpm for 5 min.

5 g of soil was weighed in a 50-mL centrifuge tube. After addition of 20 mL water, the extraction ultrasound 30 min, the supernate was collected in 50 mL volumetric flask after centrifugation at 4,000 rpm for 5 min, and then addition of 10 mL water, the extraction ultrasound 15 min, and repeated three times, all supernate was collected in the same volumetric flask. The supernate was constanted with water after pH was adjusted to 5.0 with formic acid. Then the supernate was finally applied to Oasis® MCX extraction cartridges.

The cartridge was firstly conditioned with 5 mL methanol and 5 mL water sequentially. After application of chilli supernate (5 mL) and soil supernate (20 mL), the cartridge was washed by 5 mL water, 5 mL methanol and dried with vacuum for 2 min. Finally, elution was carried out with 6 mL methanol containing 5 % ammonia. The eluate was evaporated with $N_2$ at 40°C and reconstituted to 2 mL with methanol: water (1:1, v/v) for analysis.

Chromatographic separation was performed on a Waters ultra performance liquid chromatogramphy system (Waters, Milford, MA, USA). Chromatography was performed by ACQUITY UPLC BEH AMIDE column (100 mm × 2.1 mm, 1.7 μm). Sample temperature and the column temperature were set at 10 and 30°C, respectively. The mobile phase used were 0.2 % (v/v) formic acid in water (A) and acetonitrile (B) with a flow rate of 0.25 mL $min^{-1}$. The linear gradient elution (A: B, v/v) was 30:70 at initial time, 50:50 at 2–4 min and 30:70 at 5 min. The injection volume was 5 μL.

Quattro Premier™ triple quadrupole mass spectrometer (Micromass, Milford, MA, USA) was used for MS/MS analyses in ESI (+). Data was acquired in multiple reactions monitoring mode (MRM). Source working conditions were as follows: capillary voltage, 3.5 kV; cone voltage, 35 V, source temperature, 110°C; desolvation temperature, 450°C; cone gas (argon, 99.9999 % purity) flow rate, 90 L $h^{-1}$; desolvation gas (nitrogen, 99.9 % purity) flow rate, 600 L $h^{-1}$. MRM transitions, retention time,

**Table 1** Retention time and optimized spectrometric parameters of kasugamycin

| Compound | Retention time (min) | Qual trace (m/z) | Quan trace (m/z) | Collisional energy (eV) |
|---|---|---|---|---|
| Kasugamycin | 3.2 | 380.33/200.21 | 380.33/200.21 | 7 |
| | | 380.33/112.30 | | 13 |

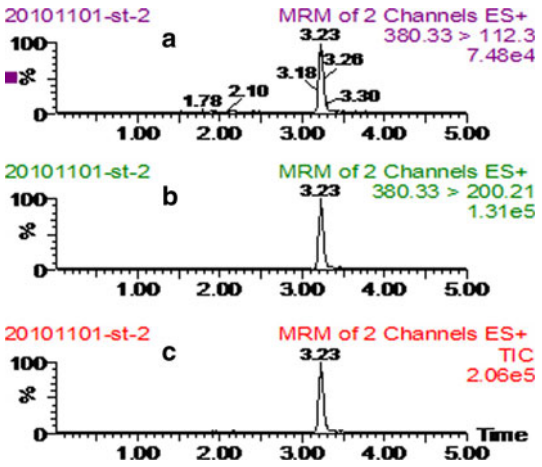


**Fig. 1** MRM chromatograms of kasugamycin standard. **a** Chromatogram of qualitative ion, **b** chromatogram of quantitative ion, **c** chromatogram of total ion

individual and collision energy applied for the analysis were listed in Table 1. The dwell time established for each transition was 0.1 s and the interscan delay time was set as 0.1 ms. The interchannel delay time was 0.02 s. Data acquisition was carried out by MassLynx V4.0 software. The UPLC–MS/MS chromatogram of standard solution of kaugamycin under these conditions is shown in Fig. 1.

## Results and Discussion

Trace analytes are often concentrated before detection (Shao et al. 2009). However, the matrix interference was also enriched while the analytes were concentrated. Ion suppression phenomenon could possibly influence assay sensitivity, linearity, accuracy and limit of quantification in UPLC–MS/MS (Maurer 2005; Taylor 2005; Buhrman et al. 1996). In this paper, standard solution curves and matrix-matched standard curves were used to study ion suppression in UPLC–MS/MS. The method of calculating ion suppression was obtained as follows: one subtracted the ratio between the slope of matrix-matched standard curves and the slope of standard solution curves (Shao et al. 2009). The linear ranges were obtained by plotting the calibration curves using six different concentration levels in the range of 2.0–100.0 μg $kg^{-1}$. Table 2 shows the results of ion suppression rates. In concentrated extracts of chilli and soil after SPE enrichment, matrix induced signal suppression is usually 40 % and 50 %. So matrix-matched standard curves were used to quantify the analyte in chilli.

The recovery levels, precision and limits of detection of the analytical method were investigated. The recoveries of kasugamycin were obtained by spiking a series (n = 5) of blank samples. As shown in Table 3, the average

**Table 2** Calibration curve, correlation coefficient ($r^2$) and ion suppression of kasugamycin

| | Calibration curve | ($r^2$) | Ion suppression (%) |
|---|---|---|---|
| Standard solution curve | Y = 77.26X ± 69.07 | 0.9993 | |
| Chilli-matched curve | Y = 46.83X ± 26.11 | 0.9997 | 39.40 |
| Soil-matched curves | Y = 37.49X ± 34.39 | 0.9987 | 51.48 |

**Table 3** Recovery and RSD (n = 5) of kasugamycin at the spiked level in blank sample

| Spiked level (μg $kg^{-1}$) | Chilli | | Soil | |
|---|---|---|---|---|
| | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| 8.0 | 80.00 | 6.54 | 81.42 | 3.57 |
| 40.0 | 77.82 | 2.20 | 82.36 | 4.45 |
| 120.0 | 82.33 | 4.30 | 83.35 | 2.96 |

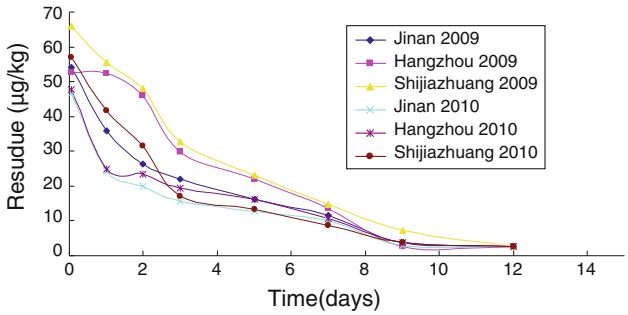


**Fig. 2** Dynamics of kasugamycin in soil in Jinan, Hangzhou and Shijiazhuaang

recoveries of kasugamycin were in the range of 77.82 %–83.35 %, the precision of the method in terms of relative standard deviations (RSD) was from 2.20 % to 6.54 %. The LODs, based on a signal- to-noise ratio (S/N) of 3, was 2.50 μg $kg^{-1}$. The recovery and precision results showed that it was a reliable method for determination of kasugamycin in chilli and soil samples.

Figure 2 shows the residue of kasugamycin in soil over the testing period. The original residues in soil were 46.67–66.00 μg $kg^{-1}$. The half-lives of kasugamycin in soil were 2.40–3.05 days. As expected, a gradual and continuous degradation of the fungicide was observed as a function of time after application. According to Fig. 2 it also can be seen that the major deterioration of kasugamycin took place within the first week after application.

**Table 4** Degradation dynamic of kasugamycin in chilli and soil

| Test sites | Test (years) | Chilli | | | Soil | | |
|---|---|---|---|---|---|---|---|
| | | Digestion equation | Correlation coefficient (r) | Half life (days) | Digestion equation | Correlation coefficient (r) | Half life (days) |
| Jinan | 2009 | $C = 73.786e^{-0.2028t}$ | −0.8728 | 3.42 | $C = 49.428e^{-0.2494t}$ | −0.9822 | 2.78 |
| | 2010 | $C = 108.83e^{-0.2686t}$ | −0.9609 | 2.58 | $C = 36.283e^{-0.232t}$ | −0.9675 | 2.99 |
| | 2009 | $C = 112.51e^{-0.237t}$ | −0.8310 | 2.92 | $C = 70.37e^{-0.2883t}$ | −0.9643 | 2.40 |
| Hangzhou | 2010 | $C = 92.676e^{-0.1971t}$ | −0.8954 | 3.52 | $C = 40.266e^{-0.2269t}$ | −0.9756 | 3.05 |
| | 2009 | $C = 141.69e^{-0.3218t}$ | −0.9664 | 2.15 | $C = 76.141e^{-0.2638t}$ | −0.9932 | 2.63 |
| Shijiazhang | 2010 | $C = 127.05e^{-0.3027t}$ | −0.9413 | 2.29 | $C = 50.198e^{-0.2619t}$ | −0.9864 | 2.65 |

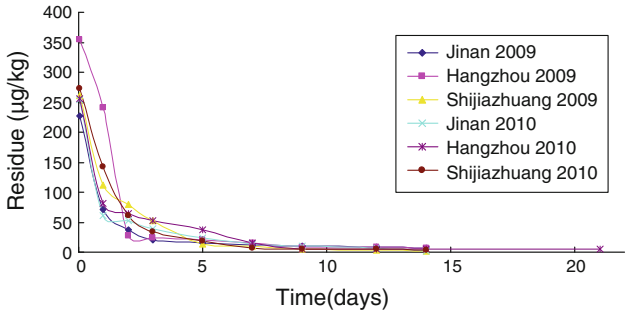


**Fig. 3** Dynamics of kasugamycin in chilli in Jinan, Hangzhou and Shijiazhuang

However, a further degradation at a slower rate occurred during the next several days. All applied kasugamycin became undetectable after 12 days application. The dynamics could be described by the following equation $C_t = C_0e^{-kt}$. Table 4 shows the first-order kinetics and half-life of Jinan, Hangzhou, and Shijiazhuang in 2 years.

Figure 3 provides the residue of kasugamycin in chilli over the testing period. The initial concentration of kasugamycin in chilli were 227.83–355.00 μg $kg^{-1}$ after 2 h of application. Then a sharp decrease of kasugamycin residues occurred within the first day. The kasugamycin residue in chilli was undetectable after 14 days application. In the degradation phase, the half-lives of kasugamycin in chilli were 2.15–3.52 days and Table 4 shows the first-order kinetics and half-life of Jinan, Hangzhou, and Shijiazhuang in 2 years.

The ultimate kasugamycin residue in chilli and soil was shown in Table 5, the residue of kasugamycin in soil was undetectable at levels of recommended (1,410 g a.i.$hm^{-2}$) and 1.5 times (2,115 g a.i.$hm^{-2}$) dosage after 7 days application. The maximum residues of kasugamycin in chilli were 35.17 μg $kg^{-1}$ after 7 days application and 27.17 μg $kg^{-1}$

**Table 5** The final residues of kasugamycin in chilli and soil

| Pesticide dose (g $mu^{-1}$) | Times | Interval time (days) | Residues (μg $kg^{-1}$) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Jinan | | | | Hangzhou | | | | Shijiazhuang | | | |
| | | | 2009 | | 2010 | | 2009 | | 2010 | | 2009 | | 2010 | |
| | | | Chilli | Soil | Chilli | Soil | Chilli | Soil | Chilli | Soil | Chilli | Soil | Chilli | Soil |
| 1,410 | 2 | 7 | 13.67 | <2.5 | 11.17 | <2.5 | 17.67 | <2.5 | 15.33 | <2.5 | 18.50 | <2.5 | 14.17 | <2.5 |
| 1,410 | 2 | 14 | 10.00 | <2.5 | 7.50 | <2.5 | 12.67 | <2.5 | 11.67 | <2.5 | 10.83 | <2.5 | 11.17 | <2.5 |
| 1,410 | 2 | 21 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 |
| 1,410 | 3 | 7 | 31.33 | <2.5 | 25.50 | <2.5 | 28.50 | <2.5 | 29.50 | <2.5 | 35.17 | <2.5 | 26.00 | <2.5 |
| 1,410 | 3 | 14 | 16.17 | <2.5 | 12.17 | <2.5 | 18.17 | <2.5 | 17.83 | <2.5 | 20.00 | <2.5 | 15.17 | <2.5 |
| 1,410 | 3 | 21 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 |
| 2,115 | 2 | 7 | 29.17 | <2.5 | 23.33 | <2.5 | 29.00 | <2.5 | 28.50 | <2.5 | 30.67 | <2.5 | 27.17 | <2.5 |
| 2,115 | 2 | 14 | 11.00 | <2.5 | 9.17 | <2.5 | 15.17 | <2.5 | 12.00 | <2.5 | 13.33 | <2.5 | 11.50 | <2.5 |
| 2,115 | 2 | 21 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 |
| 2,115 | 3 | 7 | 31.33 | <2.5 | 27.50 | <2.5 | 31.00 | <2.5 | 29.00 | <2.5 | 30.50 | <2.5 | 29.17 | <2.5 |
| 2,115 | 3 | 14 | 14.50 | <2.5 | 19.33 | <2.5 | 16.50 | <2.5 | 21.17 | <2.5 | 27.17 | <2.5 | 25.33 | <2.5 |
| 2,115 | 3 | 21 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 | <2.5 |

after 14 days application, and was undetectable after 21 days application.

In conclusion, the degradation rate of kasugamycin was fast and kasugamycin is a low residual and easily degradable pesticide. The kasugamycin final residue was not detected in chilli field ecosystem. In some countries MRLs of kasugamycin are reported, such as 50.0 μg $kg^{-1}$ in tomato (Spain and China), 30.0 μg $kg^{-1}$ in tomato (Japan), 40.0 μg $kg^{-1}$ in vegetables (USA), 25.0 μg $kg^{-1}$ in pome fruit (EPA), 100.0 μg $kg^{-1}$ in rice (China). Therefore, 14 days interval between application and harvest was thought to be safe due to its lower toxicity to biotic population, its short half-life and the final residue which below the MRL. This would contribute to establish adequate monitoring of the residue of this fungicide and its judicious incorporation in pest management strategies in vegetable fields and to prevent any health problem to consumers.

**Acknowledgments** The financial supportion and the Funding of High-tech Self-creation from Shandong Academy of Agricultural Sciences (2007YCX029) and National Key Technology R&D Program in the 12th 5 year Plan of china (2012BAK17B05) are gratefully acknowledged.

## References

Buhrman DL, Price PI, Rudewicz PJ (1996) Quantitation of SR 27417 in human plasma using electrospray liquid chromatography-tandem mass spectrometry: a study of ion suppression. J Am Soc Mass Spectrom 7:1099–1105

Chanchaichaovivat A, Panijpan B, Ruenwongsa P (2008) Putative modes of action of *Pichia guilliermondii* strain R13 in controlling chilli anthracnose after harvest. Biol Control 47:207–215

Lo CC, Hsiao YM (1996) High-performance capillary electrophoretic method for the determination of antibiotic fungicide kasugamycin in formulated products. J Agr Food Chem 44:2231–2234

Lo CC, Lee YJ, Chang CJ (1996) High-performance liquid chromatographic method for the determination of Blasticidin S in formulated products with photodiode array detection. J Agric Food Chem 44:153–158

Maurer HH (2005) Advances in analytical toxicology: the current role of liquid chromatography-mass spectrometry in drug quantification in blood and oral fluid. Anal Bioanal Chem 381:110–118

Mezcua M, Malato O, Garci'a-Reyes JF, Molina-Diaz A, Fernandez-Alba AR (2009) Accurate-mass databases for comprehensive screening of pesticide residues in food by fast liquid chromatography time-of-flight mass spectrometry. Anal Chem 81:913–929

Nanita SC, Pentz AM, Bramble FQ (2009) High-throughput pesticide residue quantitative analysis achieved by tandem mass spectrometry with automated flow injection. Anal Chem 81:3134–3142

Niu CQ, Zhu SQ, Zhang HM (2001) Determination of kasugamycin by ion-pair reverse phase high performance liquid chromatography and high performance capillary electrophoresis. Chin J Antibiot 26(348–350):400

Sagratini G, Manes J, Giardina D, Damiani P, Yolada P (2007) Analysis of carbamate and phenylurea pesticide residues in fruit juices by solid-phase microextraction and liquid chromatography-mass spectrometry. J Chromatogr A 1147:135–143

Shao B, Jia X, Zhang J, Meng J, Wu Y, Duan H, Tu X (2009) Multi-residual analysis of 16 b-agonists in pig liver, kidney and muscle by ultra performance liquid chromatography tandem mass spectrometry. Food Chem 114:1115–1121

Sheu C, Chen SC, Lo CC (2010) Analysis of antibiotic fungicide kasugamycin in irrigation water by high performance liquid chromatography. J Environ Sci Health B 45:478–484

Taylor PJ (2005) Matrix effects: the Achilles heel of quantitative high-performance liquid chromatography-electrospray-tandem mass spectrometry. Clin Biochem 38:328–334

Tomlin C (1994) The pesticide manual, 10th edn. The Bath Press, Cambridge

Turnipseed SB, Roybal JE, Andersen WC, Kuck LR (2005) Analysis of avermectin and moxidectin residues in milk by liquid chromatography-tandem mass spectrometry using an atmospheric pressure chemical ionization/atmospheric pressure photoionization source. Anal Chim Acta 529:159–165

Wang J, Zhao LL, Li XS, Jiang YP, Li N, Qin ZH, Pan CP (2011) Residue dynamic of pyrimorph on tomatoes, cucumbers and soil under greenhouse trails. Bull Environ Contam Toxicol 86:326–330

Yoo JH, Lee YJ, Seo JS, Ryu GH, Joo JH (2009) Biocontrol of anthracnose on peppers caused by Colletotrichum gloeosporioides by *Bacillus subtilis*. In: The 9th international conference of the east and Southeast Asia federation of soil science societies, pp 579–580