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Uptake, translocation and distribution of three veterinary antibiotics in *Zea mays* L.☆

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ABSTRACT

Frequently detected residuals of antibiotics in crops has drawn increasing attention from research community and the general public. This study was conducted under the controlled environmental conditions to investigate the uptake, translocation and distribution of three different veterinary antibiotics (VAs) in plants of *Zea mays* L. (maize, the third largest crop in the world, especially in China) and the associated mechanisms. The distribution color-maps of mixed-VAs showed that the highest RCF (root concentration factors) values of chlortetracycline (CTC) and sulfamethoxazole (SMZ) were found in the 0.5–2.0 mm zone (cell division zone), while the highest RCF value of sulfathiazole (ST) was in the 6.0–8.0 mm zone (elongation zone) of root tips (0.5–10.0 mm) after 120 h of exposure to VAs. The translocation factor (TF) of CTC was greater than 1.0, but the TFs of SMZ and ST were less than 1.0 under addition of single antibiotic. However, the TFs of three VAs were all greater than 1.0 at the end of exposure under addition of mixed-VAs. The dissipation of antibiotics by maize was also demonstrated by harvesting all plant parts in an enclosed system. The possible mechanisms for uptake and translocation of VAs in maize were investigated by adding multiple respiration inhibitors into the culture solution. The RCFs of VAs were suppressed heavily by salicylhydroxamic acid (SHAM) and sodium azide (NaN_3), which indicates that the uptake of VAs was an active process. The results of TFs and stem concentration factors (SCFs) of CTC and SMZ in $HgCl_2$ treatments revealed that the translocation of VAs was associated with the aquaporin activity in maize. The findings from this study will have significant implications for the management of crop food contamination by VAs and for the development of phytoremediation technology for antibiotics in the environment.

1. Introduction

Veterinary antibiotics (VAs) are widely used to treat disease, protect the health and promote growth of animals. According to the Ninth BRICS Summit, many countries had shifted toward highly cost-efficient and vertically integrated intensive livestock production systems (Li, 2017). Thus, using veterinary antibiotics to maintain productivity and rising incomes has become the most important way to accelerate the speed of animal husbandry (Boeckel et al., 2015). It has been reported that China is the largest producer and user of antibiotics in the world based on the market sales data (Hvistendahl, 2012). The annual usage amount of VAs was about 84.2 million kg for China (Zhang et al., 2015), apparently higher than 14.0 million kg for USA (US-FDA, 2017). The antibiotics in this paper is limited to tetracycline and sulfonamide groups due to their globally widespread uses (Mathews and Reinhold, 2013), and their frequently detected in environment (Li et al., 2013). VAs can enter the environment in many ways, such as land application of animal manures, large-scale discharge of wastewater from city and pharmaceutical factory, improper disposal of VAs and so on (Pan and Chu, 2017a). In general, most VAs are degradable organic pollutant (Gulkowska et al., 2008). However, when accumulated

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constantly or combined with other pollutants in the environment, VAs are degraded tardily and have been considered to be pseudo-POPs (persistent organic pollutants) in the environment (Boeckel et al., 2015; Inc, 2017). Now, VAs are widely detected in different environmental mediums. For examples, the concentrations of VAs were between 23 and 1420 mg/kg in livestock manure, 0–0.003 mg/L in river water, 0.28–1.051 mg/kg in soil, and 0–0.495 mg/kg in plants with details in Table S1 (Awad et al., 2014; Boxall et al., 2006; Dolliver et al., 2007; Goldstein et al., 2014; Hurtado et al., 2016; Li et al., 2015; Ling et al., 2010; Pan and Chu, 2017a; Wu et al., 2013; Yao et al., 2015). In addition, the residual antibiotics in the environment from livestock applications have contributed to the development and spread of antibiotic resistance, which poses unimaginable risks to human and animal health globally (Chaudhary, 2016). Nowadays, great concerns have been raised about residual VAs and antibiotic resistance due to their potential harmful impacts on terrestrial and aquatic ecosystems.

Without doubt, VAs can be absorbed by plants and enriched in plant tissues and organs (Hu et al., 2010; Michelini et al., 2012). There are many reports of VAs detected in spinach, lettuce, maize, tomato, onion, etc. (Azanu et al., 2016; Bassil et al., 2013; Boxall et al., 2006; Grote et al., 2007; Kang et al., 2013; Qiu et al., 2019). However, the accumulation of VAs in plants is complicated, and varies with different VAs, plant species and tissues (An et al., 2009; Hillis et al., 2011; Migliore et al., 1996; Zhang et al., 2017). Most VAs are low volatile compounds, which could only be absorbed by plants through the roots, so their effects on plants are also initiated through the root tips (Kumar et al., 2005; Li et al., 2013; Xie et al., 2011; Zhang et al., 2017). Root tips, the most active part of the roots, can be divided into three zones: a zone of cell division (0.5–4.0 mm), a zone of elongation (4.0–8.0 mm), and a zone of maturation (above 8.0 mm) (Chen and Guo, 1988; Ye et al., 2007). All three zones are located in approximately the first centimeter of the root tips (Ichikawa et al., 2014; Wang et al., 2016). Only a few studies focused on the accumulation of VAs in root tips other than the toxicity of VAs to root tips. Liao (2012) found the concentration of sulfonamides in tomato xylem fluids decreased with height from root up to top. Zhan et al. (2013) found that there was a significant correlation between the uptake of polycyclic aromatic hydroxy (PAH) and the number of root tips. To gain good knowledge of the distribution of VAs in plants, it's necessary to study the regularity of VAs accumulation in root tips. Maize was chosen because it is the third largest production and consumption crop in the world, and the first largest one in China. (Li et al., 2017). In this study, we hypothesized that the accumulation of VAs in root tips would be associated with the distance from root tips.

Furthermore, there are few studies focused on the mechanism of uptake and translocation of VAs by higher plants. It was supposed that the uptake of oxytetracycline (OTC) by alfalfa was an energy-dependent process, which was suppressed by some respiration inhibitors (Chopra and Roberts, 2001; Kong et al., 2007). However, Goldstein et al. (2014) inferred it as a non-energy dependent process due to the detection of tetracyclines in xylem fluids. We suspect these conflicting results might result from different types of antibiotics and experimental conditions. Future studies are warranted to investigate the mechanisms of uptake and translocation of different types of VAs by higher plants grown under controlled conditions. In this study, we hypothesized that the uptake of VAs by maize roots would be an energy-dependent active process. Therefore, the objectives of the present study were (1) to characterize the accumulation of three VAs along the root tips of maize, (2) to quantify the degradation of three VAs by maize and (3) to investigate the possible mechanisms of uptake and translocation of three VAs in maize.

2. Materials and methods

2.1. Reagents

Three typical antibiotics were selected, including chlorotetracycline (CTC), sulfamethoxazole (SMZ) and sulfathiazole (ST). These antibiotics were obtained from Dr. Ehrenstorfer (purity > 98.0%, Augsburg, Germany), and their selected properties are shown in Table S2 (Feng et al., 2016). The isotope internal standards of sulfamethoxazole-^{13}C and tetracycline-D^6 were purchased from WITEGA Laboratories (Berlin, Germany) and TRC (Toronto, Canada), respectively. Methanol (MeOH), acetonitrile (ACN) and acetic acid (CH_3COOH) were all HPLC grade from Fisher (Geel, Belgium). Formic acid (HCOOH, purity > 98.0%, LC-MS grade) were purchased from Tokyo Chemical Industry (TCI). The other chemicals used in the study were of analytical grade. Distilled water was used in all the experiments. Milli-Q water from a Milli-Q Advantage A10 system (Millipore, USA) was used when ultrapure water was required.

The filters used in extraction process were 0.45-μm and 0.22-μm PVDF syringe filters (Tianjin Jingteng, China). The rotary evaporator (Heidolph Digital, Germany) was used to remove a solvent. The Oasis HLB cartridges (6 cm^3, 500 mg) from Waters (MILLFORD, MA) were used to purify and concentrate the extracted VAs.

Each of three antibiotics was accurately weighed (0.001 g) into individual 100-mL amber volumetric flasks, dissolved and filled up to the mark with MeOH, to give individual stock standard solutions with the concentration of 100 mg/L. The stock standard solutions were diluted with MeOH to get the concentrations of 0.01, 0.05, 0.1, 0.5, 1, 5, 10 and 100 mg/L as working standard solutions, which were prepared weekly and stored at 4 °C.

2.2. Plant growth

Maize (*Zea mays* L.) seeds were obtained from the Chinese Academy of Agricultural Sciences (CAAS), Beijing, China. Dry maize seeds of similar size were selected and surface-sterilized with 70% (v/v) ethyl alcohol for 30 min, followed by thoroughly washing with plenty of distilled water, and subsequently soaked in distilled water for 12 h. The seeds were germinated at 30 °C on a humid filter paper in a manual climatic box. After 3 days, uniformly (about 5 cm in height) germinated seedlings were transferred to white enamel square tray with silica sand (the diameter of silica sand <0.25 mm, the thickness of sand layer < 3 cm). The seedlings were nurtured with half-strength Hoagland nutrient solution to trefoil stage in the silica sand for 7 days before they were used in following experiments. The Hoagland nutrient solution had the following composition of macronutrients (g/L): 0.493 $MgSO_4 \cdot 7H_2O$, 0.506 KNO_3, 0.136 KH_2PO_4, 1.181 $Ca(NO_3)_2 \cdot 7H_2O$; and of micronutrients (mg/L): 2.78/3.73 $FeSO_4$/EDTA-Na_2, 1.180 $MnCl_2 \cdot 4H_2O$, 0.063 $H_2MoO_4 \cdot 4H_2O$, 0.220 $ZnSO_4 \cdot 7H_2O$, 0.079 $CuSO_4 \cdot 7H_2O$, 2.860 H_3BO_3.

2.3. Accumulation of three VAs in roots

The hydroponic simulation experiment was conducted in the greenhouse of CAAS in May 2016, with photoperiod of 15 h light/9 h dark; temperature of 25–28 °C/18–20 °C at day/night; relative humidity of 50–60%. The plastic containers, with a dimension of 390 × 284 × 142 mm (length × width × height), were used to grow maize hydroponically. According to the toxicity tests results on maize (Jing et al., 2015; Wen et al., 2012) and the high concentration of VAs contamination emergencies (Yu et al., 2012), the lower toxic concentration (1 mg/L) and higher toxic concentration (10 mg/L) to maize roots were selected. In addition, the differences among the effects of different concentration proportions of VAs on maize were

also studied. There were 5 treatments (P1-P5) with different combinations of CTC, SMZ and ST concentrations (Table S3). There were 3 replications for each treatment and there were 3 containers in each replication for sampling at 24, 72 and 120 h after treatment. Each container was planted with 24 maize seedlings. All containers were arranged in a randomized complete block design.

Each treatment solution was prepared immediately prior to exposure and mixed thoroughly. During the experimental period, the treatment solution was changed every day to keep the relatively stable supply of CTC, SMZ and ST in the medium. After harvest at 24, 72 and 120 h, the whole maize seedling was carefully divided into 3 parts, stems, main roots and root tips (0.5–10.0 mm). The root caps at the forefront (0.0–0.5 mm) of root tips, not accumulating any VAs (Haerizadeh et al., 2011; Zhan et al., 2013), were discarded. After that root tips (0.5–10.0 mm) were cut into 5 parts by using coordinate papers: 0.5–2.0 mm, 2.0–4.0 mm, 4.0–6.0 mm, 6.0–8.0 mm and 8.0–10.0 mm. The remnant roots without root tips were collected together as the main roots to represent the maize roots in this experiment.

The concentrations of VAs in the main roots were expressed as root concentration factors (RCF), the concentrations of VAs in maize stems were expressed as stem concentration factors (SCF), and the transport of VAs in maize body from root to stems were expressed as translocation factors (TF). The factors were calculated as follows:

$$RCF = \frac{C_R}{C_L} \tag{1}$$

$$SCF = \frac{C_S}{C_L} \tag{2}$$

$$TF(\%) = \frac{SCF}{RCF} \times 100 = \frac{C_S}{C_R} \times 100 \tag{3}$$

Where C_R (mg/kg) was the concentration of each VAs in maize roots, C_L (mg/L) was the concentration of each VAs in the nutrient solution, C_S (mg/kg) was the concentration of each VAs in maize stems.

2.4. The dynamic changes of three VAs in plants and solutions

This experiment was conducted in a climate chamber under the controlled conditions (photoperiod of 15 h light/9 h dark; temperature of 25/20 °C at day/night; relative humidity of 60%; light intensity of 400 Lux). In this study, we put the solution in a closed flask and did not change the solution until harvest. The closed containers used for this experiment were angled neck cell culture flasks (75 cm^2 in cross sectional area, 300 mL in volume). Those glass flasks were covered with tinfoil, and one maize seedling was planted and fixed in each flask with degreasing cotton. There were 6 treatments with different combinations of 3 VAs (CTC, SMZ and ST) and maize seedlings (Table S4). There were 3 replications for each treatment and there were 10 flasks in each replication for sampling at 24, 48 and 72 h after treatment. All flasks were arranged in one-factor completely random design in the plant growth chambers. After harvest at 24, 72 and 120 h, the solution samples were filtrated with 0.22-μm filter and stored into 1.5-ml amber screw autosampler vial. The whole maize seedling was divided into 2 parts, the stems and the roots. All samples were stored in dark plastic bags and kept in a freezer (−20 °C) for a maximum of 1 month before analysis.

The degradation of each VAs in maize-solution were calculated as follows:

$$D_{Maize}(\%) = \frac{V_{LCK} \times C_{LCK} - (M_R \times C_R + M_S \times C_S + V_L \times C_L)}{V_0 \times C_0} \times 100 \tag{4}$$

$$D_{Nature}(\%) = \frac{V_0 \times C_0 - V_{LCK} \times C_{LCK}}{V_0 \times C_0} \times 100 \tag{5}$$

$$P_{Stable}(\%) = (1 - D_{Nature} - D_{Maize}) \times 100 \tag{6}$$

Where D_{Maize} (%) was the degradation percentage of each VAs in maize, D_{Nature} (%) was the nature degradation percentage of each VAs in blank control solution, P_{Stable} (%) was the percentage of stable VAs in maize-solution system; V_0 (L) was the initial volume of liquid in maize-solution systems, C_0 (mg/L) was the initial concentration of VAs in maize-solution system; V_{LCK} (L) was the average volume of liquid in blank control solution, C_{LCK} (mg/L) was the average concentration of each VAs in blank control solution; M_R (kg) was the average mass of maize roots, C_R (mg/kg) was the average concentration of each VAs in maize roots; M_S (kg) was the average mass of maize stems, and C_S (mg/kg) was the average concentration of each VAs in maize stems; V_L (L) was the average volume of liquid in maize flasks, C_L (mg/L) was the average concentration of each VAs in the solution with maize.

2.5. The influence of metabolic inhibitors on uptake and translocation of two VAs in plants

This experiment was performed in the intelligent greenhouse of CAAS, with controlled conditions (photoperiod 15 h light/9 h dark; temperature 25/20 °C at day/night; relative humidity 60%; light intensity 400 Lux). NaN_3, malonic acid and SHAM were selected as specific inhibitors to investigate the possible mechanisms of uptake and translocation of two VAs (CTC and SMZ) in plants. The glass containers were angled neck cell culture flasks (75 cm^2 in cross sectional area, 300 mL in volume). Those glass flasks were covered by tinfoil, and the maize seedlings were fixed by degreasing cotton. CTC, SMZ and other inhibitors were all diluted in distilled water, and then added into the Hoagland nutrient solution. Each solution was prepared immediately prior to exposure and mixed thoroughly. As shown in Table S5, there were 12 treatment combinations. Each treatment had three replicates, and each replicate had 10 flasks. Maize seedlings were harvested after 72 h of exposure, and the solution sample from each replicate were collected at the same time. To prevent photolysis of antibiotics, all samples were stored in dark plastic bags and kept in a freezer (−20 °C).

2.6. Sample extraction and analysis

A HPLC-MS/MS system was used to detect and quantify the antibiotics. Extraction of CTC, SMZ and ST in plant samples was based on the methods of our preliminary research (Feng et al., 2018). As shown in Fig. S1, the plants samples were lyophilized by a vacuum freeze drier and sieved through a 2-mm sieve. Multiple extractants were all prepared in a water bath with ultrasound. Clean-up steps were performed using solid-phase extraction. Finally, 1.0 mL of the eluent was filtered through a 0.22-μm hydrophobic PVDF membrane into a 1.5-ml amber vial and stored (less than 1 week) at −20 °C until HPLC-MS/MS analysis. An Agilent 1200 series HPLC and an Agilent 6410 Triple Quadrupole mass spectrometer (MS/MS) equipped with an electrospray ionization source (Agilent Technologies, USA) were used for the analysis of CTC, SMZ and ST. The detailed description of the analytical procedures can be found in our previous research (Feng et al., 2018). Some of the mass spectrometer parameters for analysis of three

VAs are illustrated in Table S6.

2.7. Data analysis

All the data were analyzed by using Excel (Microsoft Office 2016), Predictive Analytics Software Statistics (PASW Statistics 18.0, attached to SPSS) and Statistical Analysis System (SAS 9.4) for Windows. One-way analyses of variance (ANOVA) with Duncan's multiple-comparison test or least-significant difference (LSD) were performed to test the significant differences among the treatments. P values < 0.05 were considered as significant when compared with different samples. The fitting parameters in equations and the figures were managed by OriginPro 8.5 software (OriginLab Corporation).

3. Results

3.1. Accumulation of three VAs along the root tips (0.5–10 mm)

The accumulation results of the three VAs (CTC, SMZ and ST) along the root tips (0.5–10.0 mm) over time (120 h) are presented in Fig. 1, and the peak values of RCFs over time are summarized in Fig. S2. There was a similar variation tendency for the RCF peak

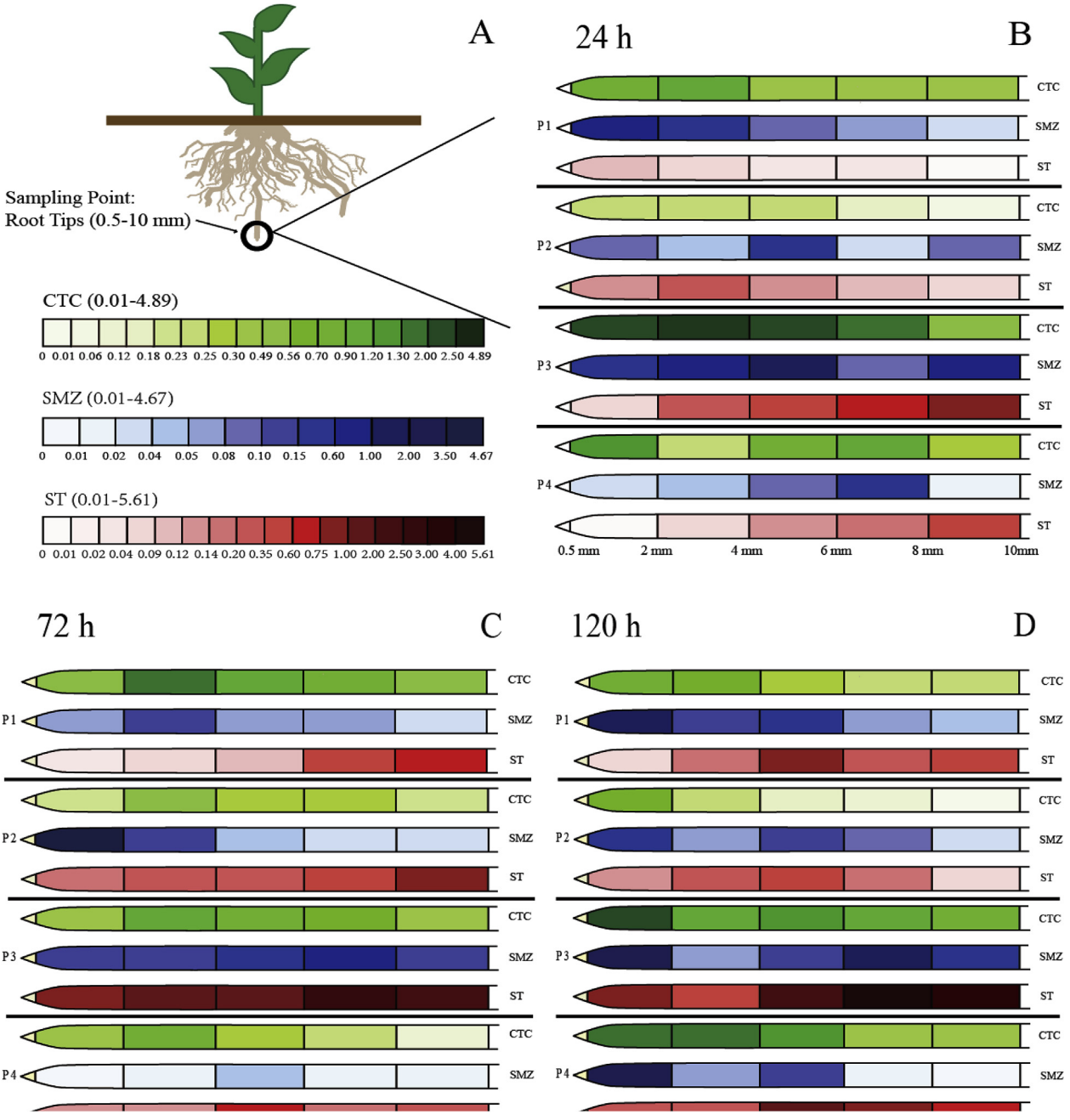

Fig. 1. The accumulation map of veterinary antibiotics (VAs) in maize root tips (0.5–10 mm) (A) the sampling point in maize and the CMKY color model matched with root concentration factor (RCF) of each VAs. The green color scheme is for chlortetracycline (CTC), blue for sulfamethoxazole (SMZ) and red for sulfathiazole (ST). (B) the RCF of VAs in the root tips after exposure for 24 h. (C) the RCF of VAs in the root tips after exposure for 72 h. (D) the RCF of VAs in the root tips after exposure for 120 h. P1 treatment: 1 mg/L CTC +1 mg/L SMZ +1 mg/L ST in Hoagland nutrient solution; P2 treatment: 1 mg/L CTC +10 mg/L SMZ +10 mg/L ST in Hoagland nutrient solution; P3 treatment: 10 mg/L CTC +1 mg/L SMZ +1 mg/L ST in Hoagland nutrient solution; P4 treatment: 10 mg/L CTC +10 mg/L SMZ +10 mg/L ST in Hoagland nutrient solution.

zone of CTC, SMZ and ST in maize root tips, transferred disorderly initially until the exposure times of 120 h, and then stably distributed, where there might be distribution equilibriums of VAs between uptake and transport in root tips. The distribution equilibriums of CTC, SMZ and ST were reached at 72 h, 120 h and probably longer than 120 h, respectively.

The changes in total RCF of each VA with different treatments are shown in Fig. 2. The sums of RCF for each treatment of SMZ were all below 1.6, while the sums of RCF for CTC and ST were all lower than 16; with a broader range of the sums of RCF observed for CTC and ST than for SMZ. As shown in Fig. 2, the maximum values of the sums of RCF for each VA were all found in the P3 treatment (10 mg/L of CTC, 1 mg/L of SMZ and ST).

The sum of RCF for CTC declined with time in the P1 and P2 treatments, but decreased first and then increased with time in the P3 treatment (Fig. 2A). The sum of RCF for CTC in P1 (1 mg/L of the three VAs) and P3 treatments were higher than those in P2 (1 mg/L of CTC, 10 mg/L of SMZ and ST) and P4 (10 mg/L of the three VAs) treatments within 72 h, while the sum of RCF for CTC in P3 and P4 treatments were higher than those in P1 and P2 treatments at 120 h. The sum of RCF for SMZ in each treatment are shown in Fig. 2B, the accumulation of SMZ in root tips for each treatment at both 24 h and 120 h decreased in the following order: P3 > P1 > P2 and P4, and the sums of RCF for SMZ in root tips were highest at 120 h. The sums of RCF for ST increased with time, but decreased in the following treatment order: P3 > P4 > P1 and P2 at 120 h (Fig. 2C).

3.2. Accumulation of three VAs in main roots without the root tips (0.5–10 mm)

The RCF and TF of the three VAs in main roots (without the root tips) are shown in Fig. 3 and Fig. 4, respectively. Across all treatments, the RCF of the three VAs declined over time, while the TF of the three VAs increased over time.

For all the treatments at all times (Fig. 3), the mean values of RCF for the three VAs in main roots followed the order: CTC (0.21) > SMZ (0.08) > ST (0.04). Across all the time, the RCF of CTC and SMZ in P1 and P2 treatments were higher than those in P3 and P4 treatments, and the RCF of ST deceased in the order of P4 > P2 > P1 ≥ P3. For all the treatments at all times (Fig. 4), the mean values of TF for three VAs followed the order: SMZ (0.79) > CTC (0.73) > ST (0.69). In addition, the maximum values of the TF for each VA were all found in the P3 treatment. The TF of CTC decreased in the order: P3 > P1 ≥ P2 > P4. As for SMZ, the TFs in P4 and P3 treatments were higher than those in P1 and P2 treatments. Across all the time, the TF of ST in P1 and P3 treatments were higher than those in P2 and P4 treatments.

3.3. The dynamic changes of three VAs contents in plants and solutions over time

There were no significant differences in the dry weights of stems and roots among the treatments applied with different concentrations of three VAs over time (Table S7). As shown in Table S8, there were no significant differences among the solution concentrations of the three VAs in the no-maize control within 72 h, while the solution concentrations of three VAs in the treatments with maize plants decreased significantly over time. The concentrations of CTC and ST in maize roots and stems decreased markedly over time, while the changes of the SMZ concentration in maize roots and stems were complicated.

The dynamic changes and the degradation percentages in total amount of each VA in plant parts and solution are shown in Fig. 5 and Fig. 6, respectively. The total amount of CTC in the maize - nutrient solution system was about 2.4 mg on average initially, which decreased significantly with time (Fig. 5A). About 0.3 mg of CTC was taken up by maize over 24 h, and the contents of CTC peaked at 24 h in both maize roots and stems and then gradually decreased with time. As presented in Fig. 5 (B), the initial total

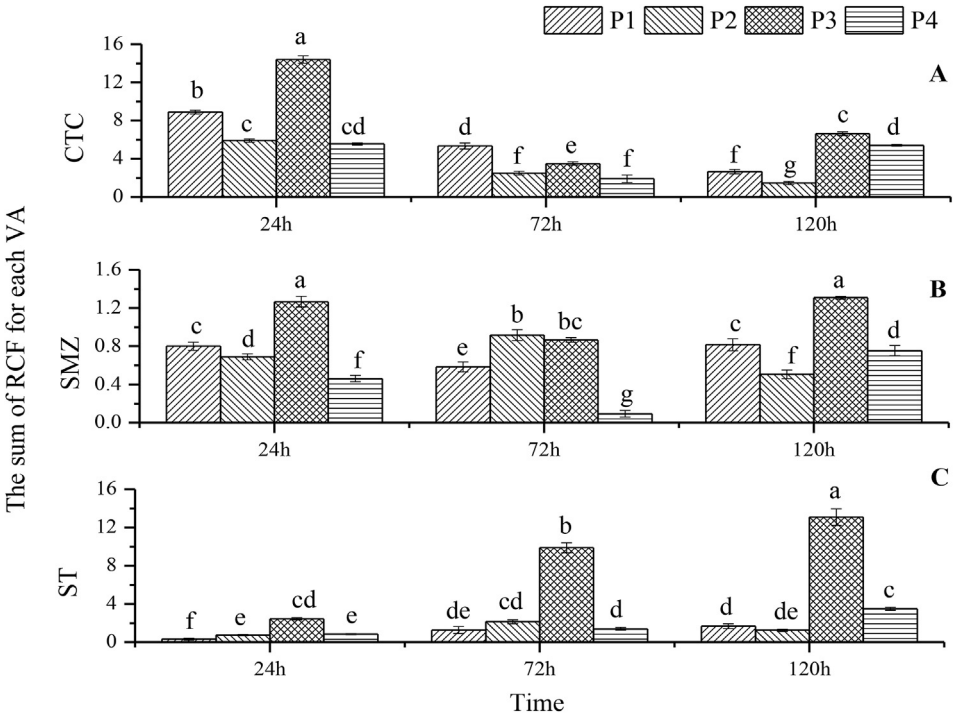

Fig. 2. The sum of RCF of each VA with different treatments in root tips (0.5–10.0 mm) over time. (A) The sum of RCF of CTC. (B) The sum of RCF of SMZ. (C) The sum of RCF of ST. The error bars represent ± standard error of the mean (n = 3). The different lowercase letters indicate significant difference ($P < 0.05$) among all the sums of RCF of each VA.

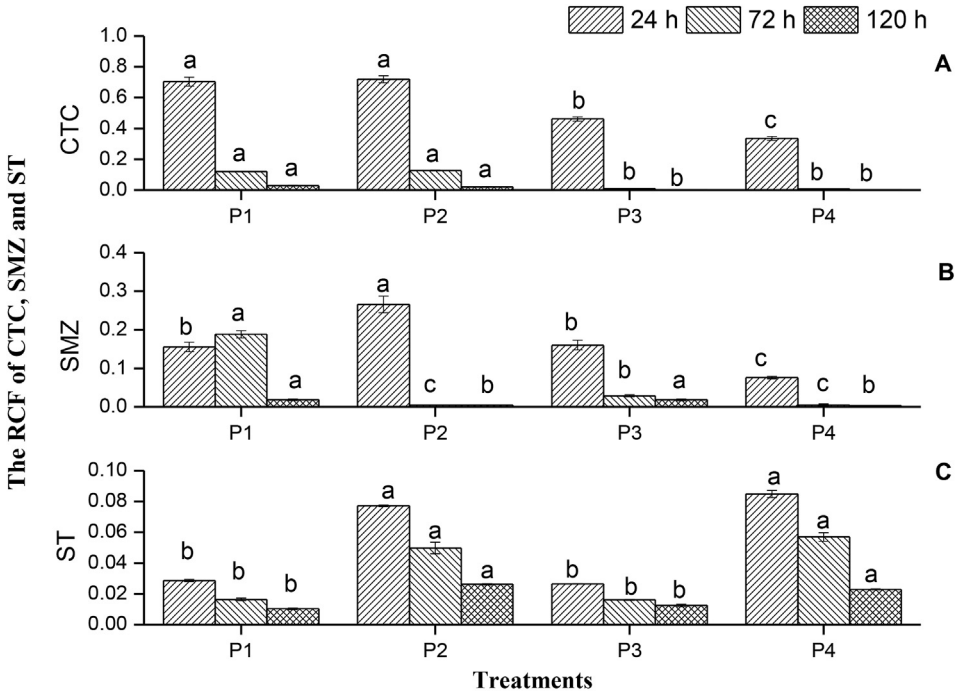

Fig. 3. The RCF values of three VAs in main roots without root tips (0.5–10.0 mm). (A) The RCF of CTC in maize roots. (B) The RCF of SMZ in maize roots. (C) The RCF of ST in maize roots. The error bars represent ± standard error of the mean (n = 3). The different lowercase letters indicate significant difference ($P < 0.05$) between treatments for the RCF values at a given time.

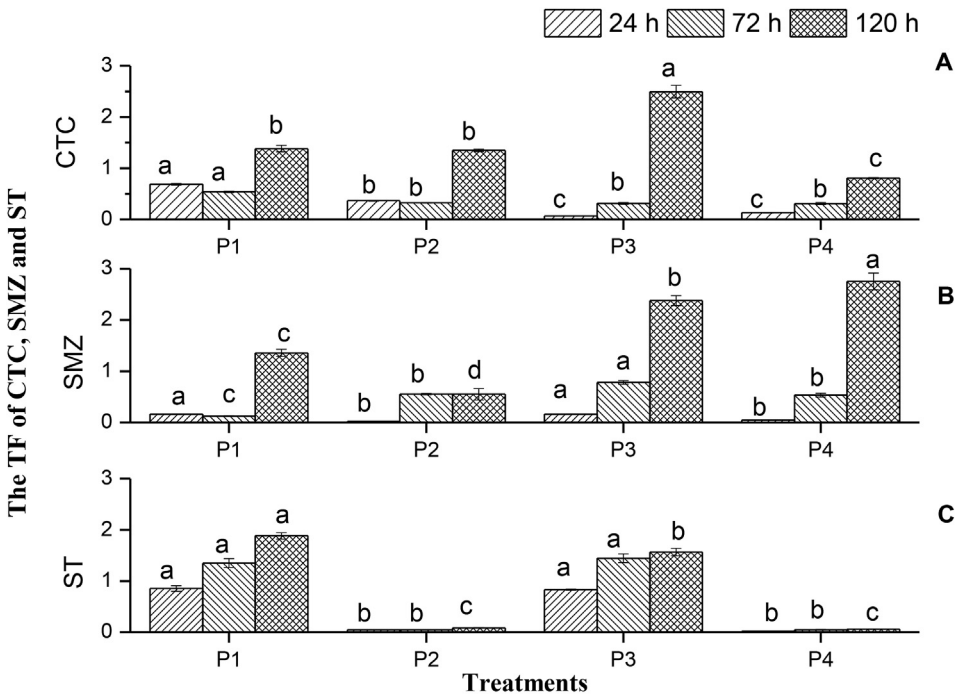

Fig. 4. The translocation factor (TF) values of three VAs in main roots without root tips (0.5–10.0 mm). (A) The TF of CTC in maize roots. (B) The TF of SMZ in maize roots. (C) The TF of ST in maize roots. The error bars represent ± standard error of the mean (n = 3). The different lowercase letters indicate significant difference ($P < 0.05$) between treatments for the RCF values at a given time.

amount of SMZ was also about 2.4 mg on average in the maize - nutrient solution system, and the total amount and the detected amount in solution were decreased with time. There were 0.32 mg of SMZ entered into maize body over 24 h. The amount of SMZ peaked at 48 h in maize root, while peaked at 24 h in maize stem. Similarly, there was about 2.3 mg of ST in the solution initially, and about 0.06 mg of ST was absorbed by maize over 24 h (Fig. 5C). The total amount (in the maize - nutrient solution system) and detected amount of ST in the culture solution all decreased with time, while the amount of ST in both maize roots and stems increased until 24 h

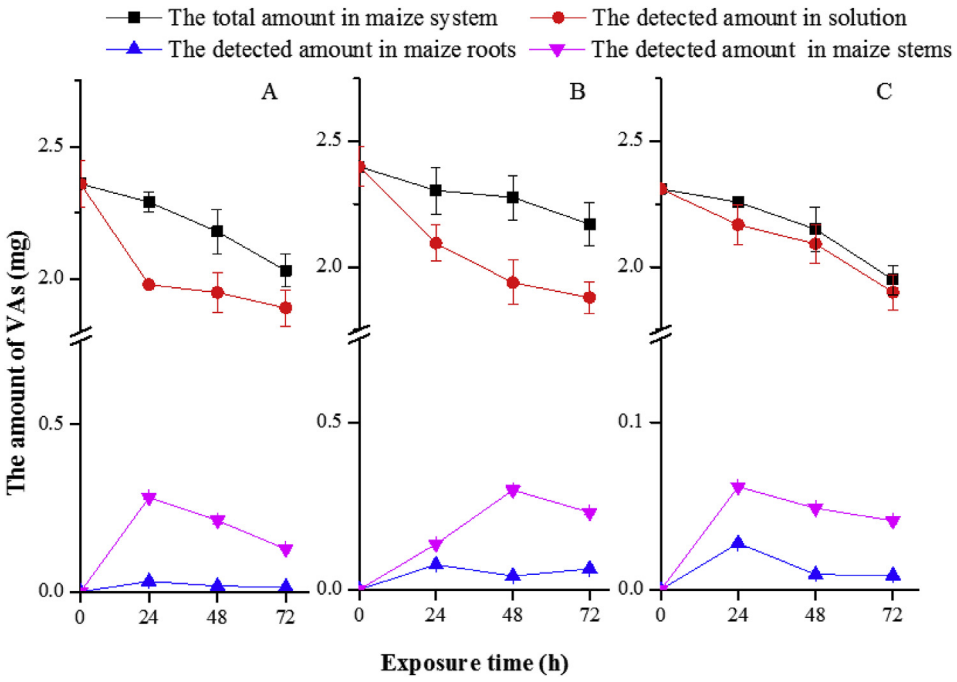

Fig. 5. The changes in total amount and detected amount (mg) of VAs in the maize-nutrient solution system. (A) CTC; (B) SMZ; (C) ST. The error bars represent ± standard error of the mean (n = 3).

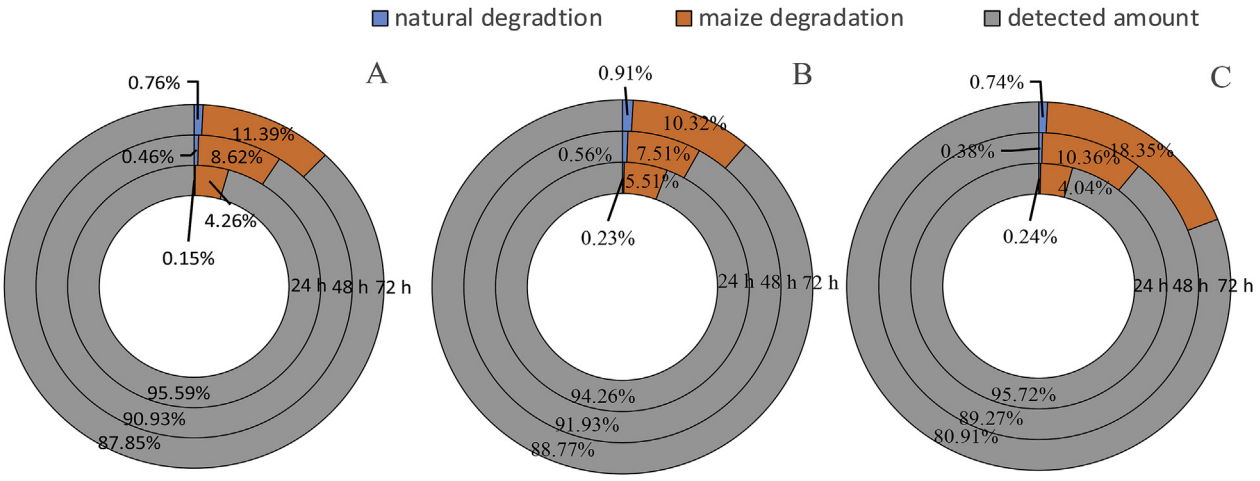

Fig. 6. The degradation percentages of the three VAs in the maize-nutrient solution system at different time. (A) CTC; (B) SMZ; (C) ST.

and then dropped. According to Fig. 5, the absorption capacity of the three VAs by maize were different, being CTC ≥ SMZ > ST. The amount of VAs in stems were higher than in roots, although the concentration of VAs in stems were lower than that in roots.

As shown in Fig. 6, the natural degradations of the three VAs in the nutrient solution were very slow within 72 h, but the degradations of these VAs by maize were relatively fast. Within 72 h, the degradation percentages of CTC, SMZ and ST by maize were 11.39%, 10.32% and 18.35%, respectively. The degradation percentages of the three VAs were all increased with time, which were ST > CTC > SMZ at 72 h.

3.4. The influence of metabolic inhibitors on uptake and translocation of two VAs in plants

Of all the treatments, the highest RCF of CTC was found in the NaN_3 treatment (8.22%), and the lowest one in the SHAM + NaN_3 treatment (0.06%). All other treatments had the RCF values of 0.65–2.2% (Fig. 7A). The RCF value of CTC in the CK treatment was significantly lower than that in the NaN_3 treatment, but significantly higher than that in the SHAM + NaN_3 treatment. In contrast, the SCF values of CTC decreased in the following order: CK ≥ NaN_3 ≥ malonic acid > $HgCl_2$ ≥ SHAM > SHAM + NaN_3. Interestingly, the TF values of CTC in the NaN_3 and $HgCl_2$ treatments were much lower than those in the other treatments (Fig. 8). There were no significant differences in the RCF, SCF and TF values of CTC between the Malonic acid and CK treatments.

The RCF values of SMZ in maize seedlings decreased in the following order: $HgCl_2$ > CK > NaN_3 > Malonic acid > SHAM ≥ SHAM + NaN_3, with the maximum and minimum RCF being 48.71% and 0.05% (Fig. 7B). The SCF values of SMZ in maize seedlings decreased in the following order: Malonic acid

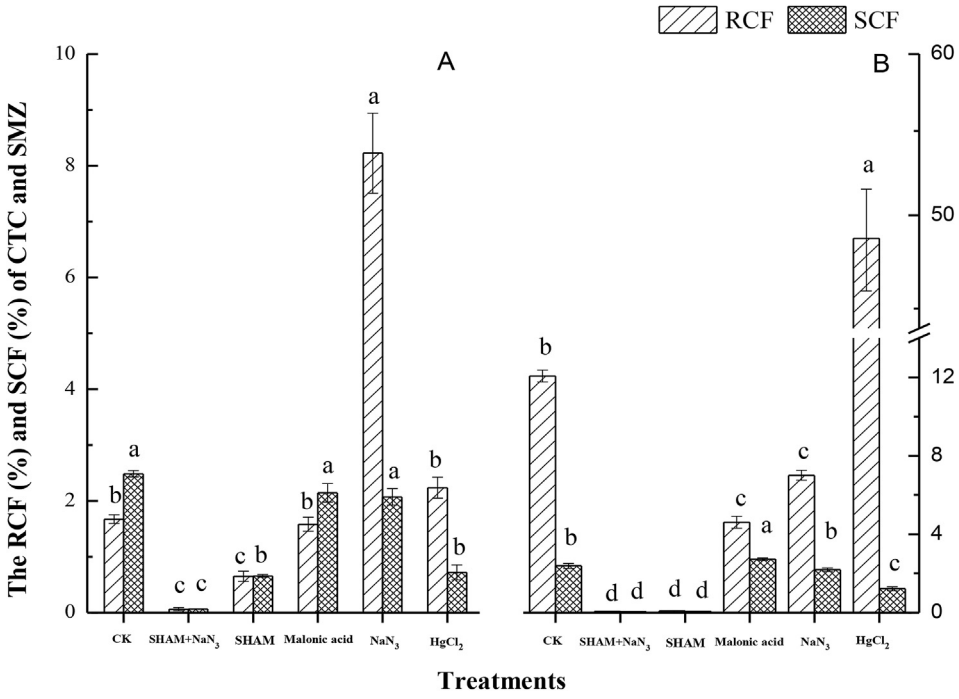

Fig. 7. The effects of different metabolic inhibitors on RCF (%) and stem concentration factor (SCF) (%) values of CTC (Fig. 7A) and SMZ (Fig. 7B) in maize seedlings. Note: The error bars represent ± standard error of the mean (n = 3). The different lowercase letters indicate significant difference ($P < 0.05$) between different treatments for an index.

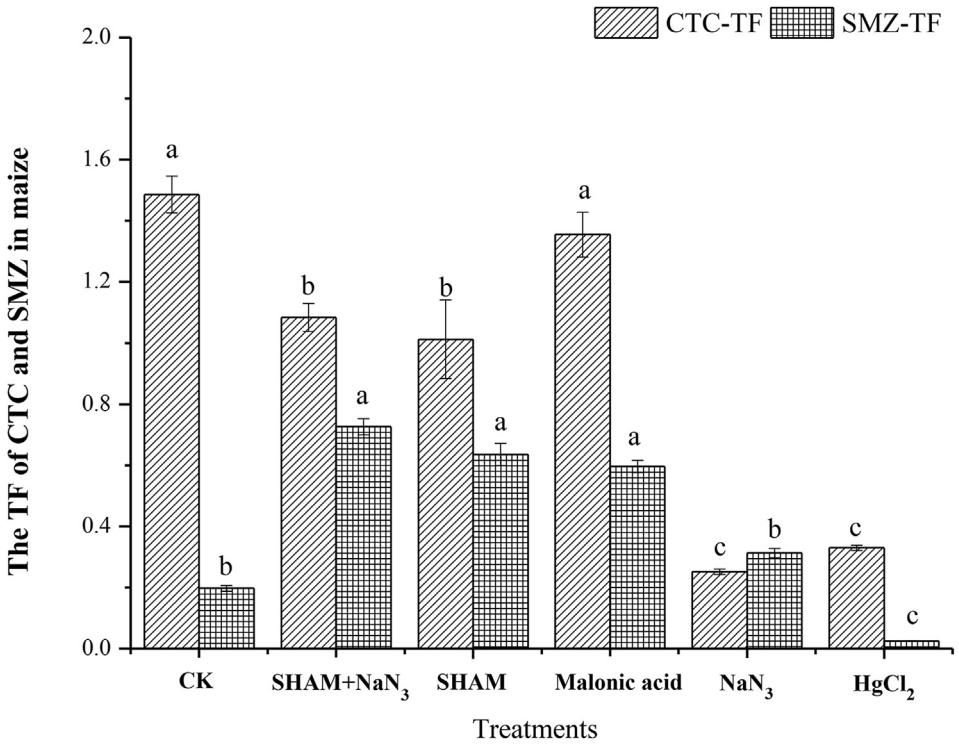

Fig. 8. The effects of different metabolic inhibitors on the TF values of CTC and SMZ in maize seedlings. Note: The error bars represent ± standard error of the mean (n = 3). The different lowercase letters indicate significant difference ($P < 0.05$) between different treatments for an index.

(2.72%) > CK ≥ NaN_3 > $HgCl_2$ > SHAM ≥ SHAM + NaN_3 (0.04%). In addition, the TF values of SMZ in maize seedlings decreased in the following order: SHAM + NaN_3 (0.73) ≥SHAM ≥ Malonic acid > NaN_3 ≥ CK > $HgCl_2$ (0.03).

4. Discussion

A large number of studies have reported on the accumulation of VAs in plant roots (Kong et al., 2007; Migliore et al., 2003). Liao (2012) had tested SMZ in the whole xylem of tomato and made

some predictions on the absorption and accumulation of VAs in plant roots. The results from the present study showed that, either by single or multiple antibiotic treatment, SMZ and ST were more likely to be enriched in roots than stems, while CTC were more easily to be transferred to stems (Figs. 4 and 7). This might be associated with the lower Log K_{ow} (octanol/water partition coefficient) of CTC than SMZ and ST (Table S2), which is consistent with the root uptake of antibiotics observed by Mathews and Reinhold (2013) and Wen et al. (2012).

We further demonstrated that the three VAs were mainly accumulated in the cell division zone, elongation zone and maturation zone of root tips (0.5–10.0 mm), which were varied with different types of antibiotics. Both CTC and SMZ were accumulated steadily on the 0.5–2.0 mm zone (cell division zone), while ST on the 4.0–6.0 mm zone (a part of elongation zone). This suggests that there might be specific locations for accumulation of different antibiotics in maize root tips. These may be associated with the Log K_{ow} of each VA and the extractable lipid content in different parts of root tips, which were consistent with the positive correlation between organic pollutant content and lipid content in plant roots reported by Gao et al. (2005).

Compared the sums of RCF for the P3 (10 mg/L of CTC, 1 mg/L of SMZ and ST) and P4 (10 mg/L of three VAs) treatments at a given time (Figs. 1 and 2), it revealed that the more the SMZ and ST were in nutrient solution, the less the SMZ and ST were in root tips. This might imply that there were some competitive relationships between SMZ and ST for accumulation in maize root tips, which may result from the competition for the same sites caused by the similar structure and physicochemical property (Ezra et al., 1982; Li et al., 2013; Mathews and Reinhold, 2013). Compared the sums of RCF with P1 (1 mg/L of three VAs) and P3 treatments at a given time, the more the CTC was in nutrient solution, the more the CTC was in root tips. In addition, the sums of RCF of VAs in root tips would show as decrease for CTC and increase for ST varied with time as the maize grows (Fig. 2). Decrease of RCF for CTC with time may be caused by higher translocation and degradation in maize (Pan and Chu, 2017a). This study confirmed that the RCF of VAs in root tips could be associated with the concentrations of VAs, the types of VAs, the number of VAs and the exposure time of treatment, which are similar to the reports of Li et al. (2005).

It was reported that the TF values were greater than 1.0 for most tetracyclines, and less than 1.0 for most sulfonamides in soil (Miller et al., 2016; Pan and Chu, 2017a), which is consistent with the TF values in this study for single CTC or SMZ in hydroponic solution (Fig. 8). Although hydroponic experiments with nutrient solution yielded higher RCF of VAs than those in realistic soil condition, similar TF of VAs were observed. This implies the hydroponic experiments in this study might be used as a simplified verification test. In addition, the hydroponic experiment was used in lots of previous studies as a very useful method to investigate the uptake of organic pollutants or nutrients by plants (Lamshoeft et al., 2018; Silberbush, 2002; Xu et al., 2016). However, the greater peak values of TF were observed in this study for CTC, SMZ and ST (2.5, 2.75 and 1.90, respectively, Fig. 4), indicating greater translocation of these antibiotics from roots to stems when multiple VAs coexisted in nutrient solution. There might be a synergistic effect of the three VAs on their translocation, but few paper had reported this phenomena. In this study, the decreasing of RCF values for each antibiotic coincided with an increase in the SCF values over time. This indicates these antibiotics could be easily taken up and accumulated in maize roots initially and then gradually translocated to the stems (Figs. 3 and 4) under the stable supply of VAs in nutrient solution. The increased TF values over time (Fig. 4) further supported our observation.

This study revealed the slow natural attenuation of three VAs in the medium and the accelerated degradation of these VAs by maize system. The higher concetrations of VAs ($\geq$10 mg/L) in solution and maize body might trigger the defense system and accelerate the metabolic process of VAs (Table S8 and Fig. 6), so the dissipation percentage of VAs in maize stems increased over time. This is in agreement with recent resports (Pan and Chu, 2017b; Xie et al., 2010; Xu et al., 2016). The increased degradation of VAs over time also happened in roots, even at a stable supply of VAs in solution. The total amount in the maize - nutrient solution system and the amount in culture solution of three VAs in the enclosed system were all decreased (Fig. 5). The reduced VAs in roots might be due to the translocation of these antibiotics to stems or their degradation by plants. This study convincingly demonstrated that the decreased contents of VAs in plants were mainly caused by phytodegradation and to a lesser extent by natural degradation.

Kong et al. (2007) reported that the uptake of OTC by alfalfa was an energy-dependent and aquaporins-independent process. Miller et al. (2016) reported that the root uptake kinetics of tetracycline antibiotics in rice were consistent with non-facilitated passive uptake (Miller et al., 2016). However, different plant species might have different uptake pathways. Our study investigated the uptake and translocation of VAs in maize by using 3 respiration inhibitors (NaN_3, malonic acid, SHAM) and 1 aquaporin protein inhibitor ($HgCl_2$) to understand the underlying mechanisms. As shown in Fig. 7 (A), it showed significant inhibitory effects on RCF for CTC in the SHAM and SHAM + NaN_3 treatments compared with the CK treatment. In other words, the main respiration pathway in maize was cyanide resistant respiration pathway, accompanied with a bit cytochrome pathway. Malonic acid had no effect on the uptake of CTC by maize roots, which indicates that suppressing the electron transferring from succinic acid to flavin adenine dinucleotide had no influence on the energy production in maize. However, the RCF in the NaN_3 treatment was much higher than that in the CK treatment, suggesting that the cyanide resistant respiration of maize would be much more active when suffering with NaN_3 and 10 mg/L-CTC together, which might be caused by cyanide resistant respiration in maize (Anderson and Beardall, 1991; Taiz and Zeiger, 2002). In a word, the uptake of CTC by maize root was an initiative-uptake-process, an energy-dependent process, and it was influenced more by cyanide resistant respiration. But the low SCF with normal RCF (low value of TF) in $HgCl_2$ treatment might indicate that, CTC could not enter into the root through water channels but the translocation of CTC in maize was influenced by aquaporin. Our findings agree with the results of Kong et al. (2007).

For the RCF and SCF of SMZ in maize, all the respiration inhibitors made a difference (Fig. 7 B). However, different inhibitors had different magnitudes of impact. The cyanide resistant respiration dominated the absorption of SMZ by maize roots, with the RCFs being the lowest in the SHAM and SHAM + NaN_3 treatments. The RCF in NaN_3 treatment was higher than that in Malonic acid treatment, suggesting that restraining in the tricarboxylic acid cycle could have stronger suppression of the uptake for SMZ by maize than restraining in respiratory electron-transport chain. The cyanide resistant respiration of maize became much more active with both SMZ and $HgCl_2$ in solution ($HgCl_2$ treatment). Therefore, the aquaporin activity may affect the translocation of SMZ from root to stem in maize. In brief, when suffering from higher concentrations (10 mg/L) of SMZ, cyanide resistant and cytochrome pathway respiration could be active at the same time, but cyanide resistant respiration still dominated. The translocation of SMZ might be a process connected with the transport of water, which was supported by a previous study that all the sulfonamides were detected in xylem of tomato, but part of sulfonamides were detected in phloem (Liao, 2012). Our results are in agreement with the findings that compounds were transported via the Casparian strip in roots

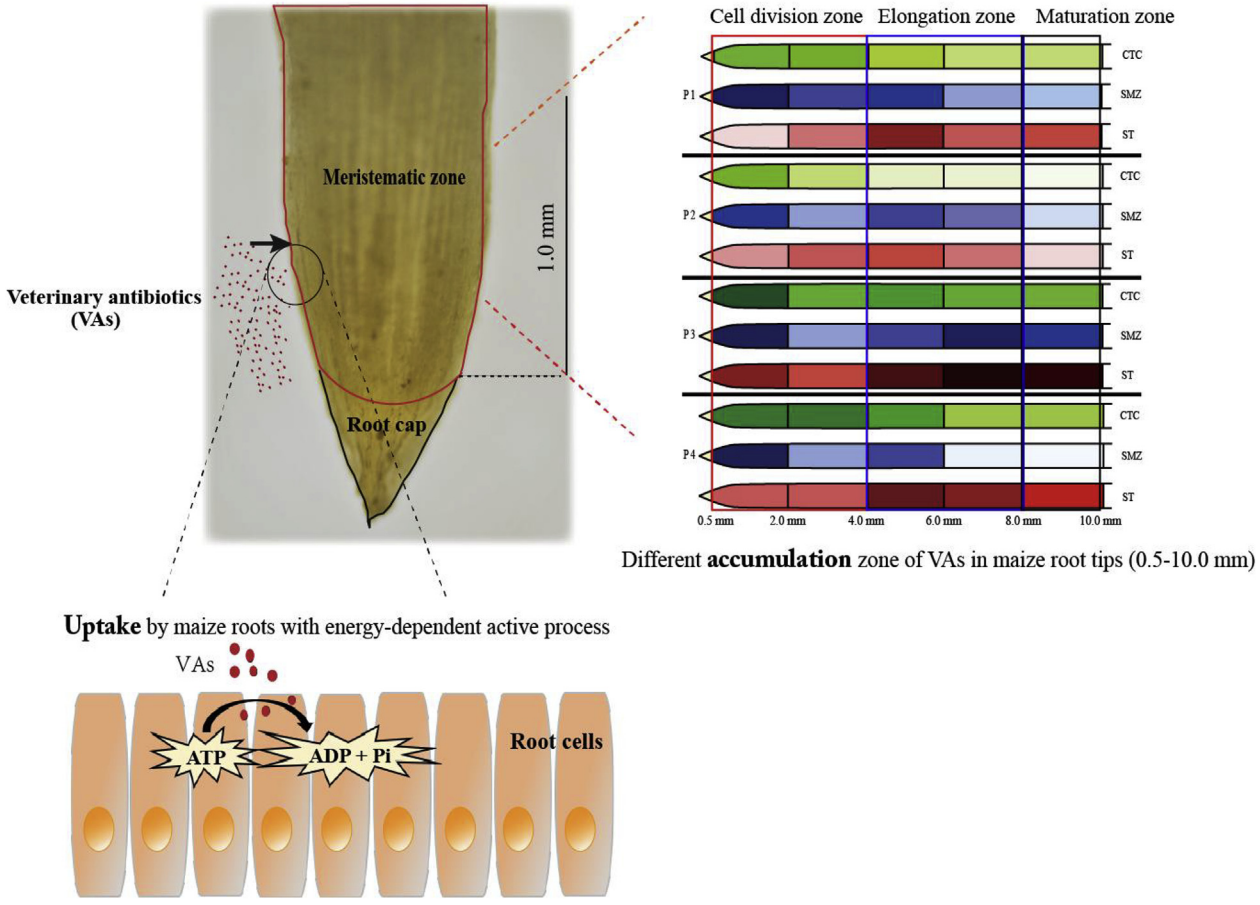

Fig. 9. The uptake and accumulation of VAs in maize roots.

and then to leaves through the xylem or to fruit through the phloem (Pan and Chu, 2017a).

Given all the above, tetracyclines and sulfonamides could enter into the root tips (0.5–10.0 mm) and root cells by active process (Fig. 9), then might be transferred to the upper root and vascular tissue via symplastic or transmembrane pathway. They might be transported to stems with water through the vessel of xylem, which might be controlled by aquaporin activities. Further research is warranted to investigate the more specific molecular mechanism and the protein-mediated uptake of veterinary antibiotics by plant roots.

5. Conclusion

Three VAs tested in this study showed different accumulation in root tips (0.5–10.0 mm) dependent on their properties: the accumulation of CTC and SMZ tended to be in the 0.5–2.0 mm zone (cell division zone), while ST tended to be in the 6.0–8.0 mm zone (elongation zone). There were competitive and synergistic relationships among the three VAs absorbed by maize grown in the solution with mixed VAs. The biodegradations of the three antibiotics by maize were also found in this study. Three VAs could be easily taken up by maize and be translocated from roots to stems. The uptake of VAs by maize was an active process, and the translocation of VAs within plants was associated with the root aquaporin activity in maize. The findings from our study could have significant implications for the management of crop food contamination by VAs and for the development of phytoremediation technology for antibiotics in the environment.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2019.03.110.

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