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Antibiotics in the agricultural soils from the Yangtze River Delta

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ABSTRACT

This study focused on the occurrence and spatial distribution of 13 common antibiotics in the agricultural soils of the Yangtze River Delta (YRD), China. Antibiotics were detected in all the 241 soil samples (i.e., 100% detection rate) with the total concentrations ranging from 4.55 to 2,010 ng/g dry weight. The concentrations of three antibiotic classes decreased in the order: quinolones (mean 48.8 ng/ g) > tetracyclines (mean 34.9 ng/g) > sulfonamides (mean 2.35 ng/g). Ciprofloxacin was the prevalent compound with a mean concentration of 27.7 ng/g, followed by oxytetracycline (mean of 18.9 ng/g). A distinct spatial distribution was observed, where high concentrations of antibiotics were detected in the sites adjacent to the livestock and poultry farms. The potential sources of antibiotics in the agricultural soils were the application of manure and wastewater irrigation in this region. Risk assessment for single antibiotic compound indicated that tetracyclines and quinolones could pose a potential risk, in which doxycycline and ciprofloxacin had the most severe ecological effect in the agricultural soils. Antibiotic resistance genes (ARGs), such as tetA, sulI, and qnrS, were detected in 15 analyzed soil samples, and sulI showed significant correlations with quinolones, tetracyclines, copper, and zinc. Further studies on the distribution of other ARGs in agricultural soil at a region-scale are needed for the risk management of extensively used antibiotics and major ARGs.

INTRODUCTION

Antibiotics have become the most popular antimicrobial drug for human disease control and livestock growth promotion for a long time. China is one of the largest producers and consumers of anti- biotics in the world. The total usage of antibiotics was 92,700 tons in 2013, and an estimated 54,000 tons of the antibiotics was discharged after human and animals use (Zhang et al., 2015). A large number of these antibiotics cannot be metabolized in vivo, 30 - 90% of which will be excreted into the environment (Hu et al., 2010; Zhao et al., 2010). Due to the use of organic manure/sludge and irrigation of wastewater or reclaimed water in the agricultural land, a significant amount of antibiotics, such as tetracyclines and quinolones, are found in soils (Wang et al., 2014a, 2014b; Hou et al., 2015).

Many antibiotics are biologically active in the environment (Zhou et al., 2006, 2013; Xu et al., 2013), which may pose a pressure on antibiotic resistance genes (ARGs) and bacterial communities (Ding and He, 2010; Tello et al., 2012; Xiong et al., 2015). The occurrence of ARGs in sediments, soil, and water has been observed in different countries (Pruden et al., 2006; Knapp et al., 2010; Chen et al., 2016a), which may be enriched by antibiotic residues (Cheng et al., 2016; Yang et al., 2017) and transferred into different environmental media (Chen et al., 2016a,b; Xie et al., 2016). Therefore, antibiotic may cause significant impacts on the environment and human (Tello et al., 2012).

It has been reported that soil is an important reservoir for antibiotics. For example, the levels of several antibiotics in soils in farmland around feedlots in Shanghai were 3.27e33.4 mg/g (Hu et al., 2010). The detected concentrations of antibiotics in organic farms ranged from 0.1 to 2,683 ng/g in soils (Xiang et al., 2016). Antibiotics with high concentration were detected in vegetable farmlands adjacent to livestock farms, and the detected concentration of quinolones can reach up to 1,540 ng/g (Li et al., 2011). This indicated that livestock manure is an important source of antibiotic residues in soil. After the application of manure/sludge contaminated with antibiotics to agricultural soil, the residues of antibiotics in soil can be accumulated by vegetables that are consumed by human (Hu et al., 2010).

The Yangtze River Delta (YRD) is a developed and populated region in China. The YRD region include Shanghai Municipality, Zhejiang province and Jiangsu province. As a result, the increasing need for agricultural produce from urban population led to booming agriculture in the region (Sun et al., 2016b). Our previous studies showed that organochlorine pesticides, phthalate esters, polybrominated diphenyl ethers (Sun et al., 2016b), and poly- chlorinated biphenyls (Sun et al., 2016a) were widely detected, which may pose ecological risks in the YRD region. Yet, little information is known on the distribution of commonly-used antibiotics in agricultural soils of the YRD region. It is important to evaluate the regional-scale distribution and the potential ecological risks of antibiotics to facilitate sustainable economic development for this rapidly developing region.

This study characterizes the concentrations and distribution patterns of tetracycline, sulfonamide and quinolone residues in agricultural soils from the YRD region of China and their potential ecological risks, as well as typical ARGs in a number of soil samples. The regional-scale evaluation of multiple antibiotics pollution in agricultural soils will provide useful information for the management of antibiotics contamination in China.

MATERIALS AND METHODS

Chemicals and reagents

The targeted antibiotics in this study were chosen according to their application in livestock industry in China. There are three classes of antibiotics, i.e., tetracyclines (TCs), quinolones (QNs) and sulfonamides (SAs). Three TCs included tetracycline (TC), doxycy- cline (DC), and oxytetracycline (OTC). Four QNs included ofloxacin (OFL), ciprofloxacin (CIP), norfloxacin

(NOR), and enrofloxacin (ENR). Six SAs included sulfadimethoxine (SDM), sulfamethazine (SMZ), sulfamethizole (SMTZ), sulfamerazine (SMR), sulfamethoxazole (SMX), and sulfadiazine (SDZ). Simeton was used as the internal standard. The standards were obtained from J & K Chemical Ltd., USA. Acetonitrile and methanol (HPLC grade) were obtained from Sigma-Aldrich. Oasis HLB Cartridge used for purification were purchased from Waters. Deionized water (18.2 MΩ) from a Milli-Q system was used.

Sample collection

The YRD region is located in east China. In June 2014, 241 agricultural soils were collected in this region. The studied region covered approximately 45,800 km^2. The detailed information about sample collection were presented in our previous report (Sun et al., 2016b). The types of land-use of the sampling sites included paddy fields, vegetable fields, forests, uncultivated lands, and other agricultural uses.

Sample extraction and clean-up

The extraction and cleanup procedures for antibiotics were conducted according to the methods reported previously (Uslu. et al., 2007), with small modifications. Briefly, all the soil samples were purified using solid phase extraction (SPE) method after freeze drying, homogenizing and sieving through a 75 mesh sieve.

Two grams of soil were extracted by using acetonitrile (3 mL) and potassium phosphate buffer (3 mL) (pH = 4) containing 0.2 g of EDTA in a 15-mL polypropylene centrifuge tube. After being vortexed for 1 min, the sample was treated ultrasonically for 20 min, followed by centrifugation at 2600 rpm for 10 min. Afterwards, the supernatants were moved carefully to a new tube. This extraction procedure was repeated three more times with additions of 5, 5 and 4 mL of reagents. The extracts were combined and transferred to new glass bottles. Then, these extracts were concentrated with a rotary evaporator, and diluted to 500 mL to lower the concentration of organic solvents to below 5%.

The Oasis HLB Cartridge was used for the purification of each soil sample. The cartridges were preconditioned sequentially with 6 mL of methanol and 6 mL of deionized water. Then, the dilute supernatants were loaded on the cartridges at a rate of about 5 mL/min. The cartridges were then vacuum-dried after being rinsed with 12 mL of deionized water. The elution on the cartridges were performed with 0.1% acidified methanol (9 mL) at a rate of 1.0 mL/min. The analytes were then concentrated to near dryness under a gentle flow of nitrogen gas. Simeton was added and methanol was used to dissolve the final extract to 1 mL. The final extract was filtered through syringe filters (0.22 μm) before further instrumental analysis.

Instrumental analysis

The determination of antibiotics in this study was performed by using a liquid chromatographye-tandem mass spectrometry system (Agilent 1260 - 6460), according to a previous study (Wu et al., 2014) with some modifications. A C18 column (5 μm, 2.1 x 150 mm) was used for the chromatographic separation. The mobile phase was methanoleacetonitrile (1:1, v/v) and 0.3% formic acid/water (containing 0.1% ammonium formate, v/v). The column temperature was 40℃, and the injection volume was 20 μL. The detection was conducted with ESI source in the mode of multiple- reaction monitoring (MRM). The detailed information on instrumental parameters is listed in Supporting Information (Table S1).

DNA extraction and real-time qPCR

The current study analyzed three common ARGs (tetA, sulI, and qnrS) in the selected 15 sampling sites from the northeast of the YRD region, where high levels of antibiotics were found. The methods of DNA extraction and real-time qPCR were adopted from the previous literature (Chen and Zhang, 2013). Briefly, DNA extraction from soil samples was conducted with the PowerSoil DNA Isolation Kit following the instructions. Three ARGs (tetA, sulI, and qnrS) were analyzed by PCR and agarose electrophoresis, quantified by real-time qPCR. The reaction system of real-time qPCR comprised 7.5 μL of SYBR Premix Ex Taq™ (TaKaRa), 0.3 μL of ROX reference dye for correction, 0.3 μL of each 10 μM primer, 2 μL of template DNA and 4.6 μL of ddH2O. The temperature programming of qPCR contained a hold for 30 s at 95℃, followed by 40 cycles of 5 s at 95℃ and 30s at 55℃ for annealing, then 30 s at 72℃ for extension and a fluorescence acquisition step at 72℃, a final melt curve stage conducted from 60 to 95℃. The information of the qPCR primers and standard curves are shown in Table S2.

Quality control and quality assurance

A spiked blank, a procedural blank, and a duplicate of soil were processed in parallel in each batch of ten soils. The relative standard deviation (RSD) in the measured concentrations of duplicate samples was less than 15%. The average recoveries of 13 target com- pounds in soil samples ranged from 73 to 108%. The coefficients of determination of the working calibration curve

(0.1e100 ng/g) were all >0.999. The limit of detection (LOD) for antibiotics were calculated and fell in the range of 0.10e0.80 ng/g (Table S3). Statistical analysis with Microsoft European Commission, 2003 and SPSS (IBM SPSS Statistics 20) was conducted. Spatial distributions of the antibiotics were simulated by using universal Kriging in ArcGIS 10.2 (ESRI, Redlands, CA, USA). The measured concentra- tions of antibiotics were interpolated to the whole study area using ordinary kriging with normal score transformation (Deutsch and Journel, 1998), which assumed the normal distributions. Back- transformation was applied to the interpolated results for obtaining the spatial maps of the antibiotics concentrations.

RESULTS AND DISCUSSION

Concentrations and profiles of antibiotics

The concentrations of the total antibiotics in agricultural soils were in the range from 4.55 to 2,010 ng/g, with a mean value of 86.1 ng/g (n = 241), indicating widely-spread antibiotic contamination in the agricultural soils of the region. The concentrations of 13 antibiotics in 241 agricultural soil samples of the YRD region are summarized in Table 1. Three antibiotic classes, including quinolones (OFL, ENR, CIP and NOR), tetracyclines (DC, OTC and TC), and sulfonamides (SDZ, SMZ, SMTZ, SDM, SMX and SMR) were detected, of which the detection rates ranged from 0.8% to 95% across the study area. The concentrations of total quinolones (∑ QNs), total tetracyclines (∑ TCs) and total sulfonamides (∑ SAs) ranged from <LOD to 1410 ng/g, from <LOD to 809 ng/g, and from <LOD to 111 ng/g, respectively. The mean values of three antibiotic classes decreased in the following order: QNs (48.8 ng/g) > TCs (34.9 ng/ g) > SAs (2.35 ng/g) in the soils.

Significant differences in the concentrations of three classes of antibiotics were observed in the YRD agricultural soils. The TCs and QNs were prevalent antibiotics with higher frequencies (99.0% and 99.6%, respectively) than SAs (66.8%). This may be caused by the chemical properties of diverse antibiotic compounds. Compared with most SAs, TCs and QNs can be more strongly absorbed by soil particles in the absence of biodegradation (Moreno-Bondi et al., 2009), and as a result they are more persistent in the environment (Hu et al., 2010).

The detection frequencies ranged from 72% to 83% for the four kinds of QNs. The CIP was the dominant compound in the class of QNs, followed by NOR, OFL and ENR. The mean concentration of CIP, NOR, OFL and ENR was 27.7 ng/g 11.2 ng/g, 5.86 ng/g and 3.98 ng/g, respectively. The maximum concentration levels of four QNs were 1030 ng/g for CIP, 505 ng/g for NOR, 498 ng/g for OFL, and 237 ng/g for ENR. Several previous studies reported the occurrence of QNs in agricultural soils. The level of QNs in this study was higher than the concentration detected in soils from organic vegetable farms in Guangdong, in which the average concentration of QNs was 14.0 ng/g (Wu et al., 2014). The mean concentration of QNs in the YRD region was lower than that from some other regions of China. For example, In the Pearl River Delta region in south China, the levels of four QNs in soils collected from vegetable farmlands fell in the range of 27.8 - 1530 ng/g (Li et al., 2011). The detected levels of QNs ranged from <LOD to 290 ng/g and from <LOD to 649 ng/g in Nanjing and Beijing greenhouse soils, respectively (Fang et al.,2015; Li et al., 2015).

The detection frequencies ranged from 37% to 95% for TCs. The mean values of individual TCs decreased in the following order: OTC (18.9 ng/g) > DC (13.9 ng/g) > TC (2.07 ng/g). The maximum concentrations were 530 ng/g for OTC, 105 ng/g for DC, and 197 ng/g for TC. It was reported that the mean values of TCs, including DC, TC and OTC, ranged from 2.38 to 21.9 ng/g in agricultural soils of organic farms in south China (Xiang et al., 2016). Besides, in Fuyang, Zhejiang province, the mean concentration of TCs was 23 ng/g, with OTC as the dominant compound in different soil samples (Wu et al., 2013). In this research, the mean value of TCs was higher than these reported values in agricultural soils. As for SAs, the detection rate ranged from 0.8% to 44%, and six SAs were detected at very low levels (<LOD to 21.5 ng/g), which was consistent with the survey on the agricultural soils of Beijing with the mean concentration of 1.1 ng/g for SAs (Li et al., 2015). However, the detected levels of SDZ, SMX and SMZ in the current investigation were much lower than the concentrations in agricultural soils nearby feedlots (5.85 - 33.37 μg/g) (Ji et al., 2012). In our previous studies, various organic pollutants in these soils were detected (Sun et al., 2016a, b). There were significant differences between the levels of total antibiotics and phthalate esters (PAEs) ($p < 0.001$), polybrominated diphenyl ethers (PBDEs) ($p < 0.001$), and polychlorinated biphenyls (PCBs) ($p < 0.001$). There was no significant difference between the concentrations of antibiotics and organochlorine pesticides (OCPs) ($p = 0.211$). These results reflect the extensive contamination of antibiotics and historical agricultural use of OCPs in this area. Significantly positive correlations were found between the levels of antibiotic and PCBs ($r = 0.207$, $p < 0.05$). The combined organic pollution in soil was widely detected, however, antibiotics used in China might originate from different sources.

The Veterinary International Committee on Harmonization sets the trigger value (100 μg/kg) that can cause ecotoxicity effects of soil antibiotics on a range of organisms (Huang et al., 2013). In this study, it should be noted that the concentrations of 15.4% $\sum$ antibiotics and 3.7% of individual antibiotics in soil samples exceeded the trigger value. The proportion of sites in which the concentrations of antibiotics exceeded the trigger value was 0.4%, 0.4%, 1.2%, 0.4%, 0.4%, 1.2%, 1.7%, and 0.8% for SMTZ, DC, OTC, TC, OFL, ENR, CIP, and NOR, respectively.

Spatial distribution and potential sources

The spatial distribution pattern of three classes of antibiotics and total antibiotics are shown in Fig. 1. Antibiotics are used for disease control and growth promotion for livestock and fishing farms (Wu et al., 2014; Liu et al., 2017), and they can be continuously excreted into the environment. Distinct spatial distribution was observed that the total concentrations of antibiotics were much higher in Jiangsu and Shanghai than in Zhejiang (Fig. 1A). This indicated the different contamination status of antibiotics in agricultural soil of different areas. The soil antibiotic concentrations were strongly influenced by proximity to the sources. The levels of QNs in agricultural soils were higher in Shanghai Municipality and Jiangsu province than in Zhejiang province (Fig. 1B). As for TCs, the levels in soil were higher in the border of Shanghai and Jiangsu (Fig. 1C). High levels of both TCs and QNs were detected in north- east of the YRD region, including Taicang, Jiading, Qingpu and Baoshan, where the livestock and fishing farming industry were well-developed (Hu et al., 2010; Li et al., 2015). Thus, the high levels of antibiotics might be originated from the extensive use of organic fertilizer and wastewater containing animal excreta in agricultural soils. There was a negative correlation between antibiotics and population density in the YRD region ($r = 0.187$, $p < 0.01$). This indicated the consumption of antibiotics by human beings may not be the main source of antibiotic contamination in agricultural soils. On the contrary, the excessive use of antibiotics in the livestock and fishing farming industry in rural areas may contribute to the widespread contamination of antibiotics in these soils.

Moreover, the exacerbation of agricultural land use had intensified the application of fertilizers as well as the wastewater and reclaimed water irrigation, which could result in severe antibiotic contamination. It has been reported that there were high concentrations of antibiotics in wastewater and reclaimed water (Wang et al., 2014a; Wu et al., 2015; Ostman et al., 2017). In the coastal water of the South Yellow Sea in China, 25 antibiotics were widely present in the region with the total concentration up to 1350 ng/L (Du et al., 2017). In the YRD region, the SAs concentrations in soils were higher in Jiangsu province than in Shanghai and Zhejiang, which might result from farm irrigation with water containing SAs (Luo et al., 2011). In aquatic environments of Indochina, the concentrations of SMX and SMZ ranged from <LOD to 6.06 μg/g (Suzuki and Hoa, 2012). However, the levels of SAs in all the soils of the studied region were relatively low (Fig. 1D). The distribution pattern of a total of 13 antibiotics was consistent with that of QNs (Fig. 1A). The soil physicochemical properties, including total organic carbon (TOC), total phosphorous (TP), total nitrogen (TN), soil moisture content, and pH (Table S4) were reported in our previous studies (Sun et al., 2016a, b). However, no significant correlations were found between the total antibiotics and soil physicochemical properties ($0.09 < p < 0.85$). These results indi- cated that other factors, such as human activity, might have greater influence on the residues of antibiotics. The microbial communities of the studied soils were previously reported (Sun et al., 2016a). In this study, the impacts of antibiotics on soil microbial communities were assessed, and no clear correlation was found between anti- biotic concentrations and the abundance of bacteria, fungi, and actinomycin in soil.

In this study, the cluster analysis was conducted to determine the combined characteristics of all the target antibiotics in the region (Fig. 2). The 13 antibiotics were divided to two groups. The first major group contained seven antibiotics including OTC, ENR, CIP, NOR, SDM, SMTZ, and SMX. The second groups contained six antibiotics including DC, OFL, SMZ, SMR, SDZ, and TC. In general, the chemicals in the same branch of the same group have a closer correlation, such as CIP and NOR, indicating their positive correlation in soils. It may be deduced for coexisting compounds based on the cluster analysis that the compounds share common source or have analogical sorption affinity even though they are in different classes of pollutants. On the contrary, a class of chemicals may not be divided into the same group. For instance, ENR, CIP and NOR were in one group while OFL was in another group. This indicated that ENR, CIP and NOR showed similar environmental source and fate in soils, whereas there might be distinct input sources of OFL. The results of cluster analysis can provide reference for combined antibiotic pollution in the regional-scale assessment.

Potential ecological risk and occurrence of ARGs

The potential ecological risk of contaminants such as antibiotics in the environment could be assessed by using the mean values of risk quotient (RQs). In the present study, the RQ value was equal to the quotient between the measured environmental concentration (MEC) and the predicted no effect concentration (PNEC) (European Commission, 2003). High levels of antibiotics, such as the TCs and QNs, were frequently detected in the agricultural soils, and their ecological risk should be determined. The PNEC values were obtained from toxicity assessment (Li et al., 2013; Zhang et al., 2013). The values of PNEC of OTC and TC were obtained directly from the published literature (Vaclavik et al., 2004; Thiele-Bruhn and Beck, 2005). As for QNs and DC, only a small percentage of the literature reported the ecological risks to soils (Gao et al., 2008). Thus, it is impossible to obtain the values of PNEC in soils to calculate the RQs. In our study, PNEC values in soils were calculated from the PNEC values in water ($PNEC_{water}$) as shown in Table S5.

For the assessment of ecological risk of antibiotics in agricultural soils, RQ values were divided into three risk levels: high (RQ 1), medium (0.1 RQ < 1), and low (0.01 RQ < 0.1) (Martin et al., 2012; Zhang et al., 2013). In this study, DC, OTC, TC, OFL, ENR, CIP, and NOR may cause high ecological risk, in 64.3%, 5.4%, 1.2%, 4.6%, 2.1%, 30.7%, and 7.5% of the studied agricultural soils, respectively (Fig. 3). The potential ecological risk of TC is the lowest, with 93.4% of soil samples pose a low or no risk. In the YRD region, DC and CIP showed the most potential ecological effects. In greenhouse soil samples from Beijing, OTC, NOR, CIP and ENR might pose high ecological risk with the proportions of 39.3%, 10.7%, 17.9%, and 41.1%, respectively (Li et al., 2015). It was reported that DC may cause high risk in 44.3% of the soil samples collected in organic farms of south China (Xiang et al., 2016), which was similar to the condition in this study.

According to these calculations, it was considered that the measured levels and distribution of antibiotics can result in different degrees of ecological risks the region, and the risks should receive more attention especially for antibiotic resistance genes in the environment. Nevertheless, the risk was calculated through the concentration of individual analytes without the consideration of coexisting pollutants (Zhu et al., 2013), such as PCBs and OCPs in studied area (Sun et al., 2016a, 2016b; Cai et al., 2017). The actual risk may be higher than expected (Chen and Zhou, 2014).

The analysis of ARGs in a portion of these soils was conducted in this study to provide a preliminary result of the occurrence of ARGs in the region. The three ARGs (i.e., tetA, sulI, and qnrS) were all detected in 15 soil samples with a concentration of

3.5 10^5 - 2.1 x 10^8 copies per dry soil for sulI, 3.6 x 10^6 - 8.1 x 10^7 copies per dry soil for tetA, and 2.5 105e4.2 107 copies per dry soil for qnrS, respectively. The relative abundance of tetA and qnrS were more than that of sulI gene in most soils, which might due to the comparatively higher concentration of TCs and QNs. The cor- relation analysis between antibiotics and ARGs indicated that total antibiotics, TCs, and QNs showed significant positive correlation with sulI in 15 soil samples ($p < 0.01$). However, SAs and QNs showed no significant correlation with sulI and qnrS, respectively. These results may be caused by cross-selection of the studied an- tibiotics to the related ARGs (McKinney et al., 2010; Shen et al., 2008). The environmental behavior of antibiotics and ARGs may be influenced by many factors. Here, we only assessed several important parameters which may cause impact on the target an- tibiotics and ARGs. Redundancy analysis of the relative abundance of ARGs and environmental variables was shown in Fig. 4. The data were obtained from the correlation analysis of different compo- nents included in this study (Table S6). The measurement of Cu and Zn in these soils were reported in a previous study (Shao et al., 2016). Both Cu and Zn were positively correlated with sulI, which reflect the potential impact of metals on the abundance of this ARG (Hu et al., 2017). The soil properties such as TOC and TN were positively correlated with sulI, whereas pH showed a negative correlation with sulI. Comparatively, there was no significant cor- relation between tetA and antibiotics. Soil properties, Cu, and Zn also showed no clear influence on tetA and qnrS (Fig. 4). ARGs could be affected by many factors and qnrS and tetA may be imported directly into soils from other media such as manure and waste- water. In the selected 15 sampling sites, NE6, NE8, and NE12 are all rice paddy fields, and other sites are vegetable field, fruit garden or uncultivated field. The abundance of three ARGs were predominant in paddy soils of sites NE6, NE8 and NE12, among 15 soil samples (Fig. S1). Moreover, the highest concentration of antibiotics was also found in NE6. These results indicated that water irrigation in paddy fields may intensify the contamination of antibiotics and ARGs (Chen et al., 2016a,b). The abundance of three ARGs covered from 2.5 x 10^5 to 2.1 x 10^8 copies per dry soil and were lower than that in sewage sludge (Xie et al., 2016) and swine wastewater (Sui et al., 2016). In agricultural soils, ARGs had many sources and could be influenced by antibiotics or heavy metals (Luo et al., 2017). In addition, with more application of manure, sewage sludge and wastewater in agricultural soils, there will be aggravation of ARGs dissemination from the environment to the agricultural system, such as soils and crops (Zhu et al., 2017). As a result, the antibiotic pollution in soils and their potential damage to ecosystem and human health require more systematic research in the future, and further data on the distribution of ARGs in the studied region will provide more information for the risk management of antibiotics in the environment.

CONCLUSION

This study revealed the occurrence and distribution of antibi- otics including QNs, TCs and SAs and their associated ecological risk assessment by analyzing soil samples from the agricultural fields in the YRD, China. Across the studied area, the levels of total antibi- otics fell in the range 4.55 - 2,010 ng/g dry weight with a detection rate of 100%. The concentrations of total antibiotics were higher in Shanghai and Jiangsu than in Zhejiang. The mean concentrations of three classes of antibiotics measured in all soil samples were in the following order: QNs (48.8 ng/g) > TCs (34.9 ng/g) > SAs (2.35 ng/g). Ecological risk assessment based on the risk quotient values revealed that tetracyclines and quinolones could pose potential ecological risks, and doxycycline showed the higher risk, followed by ciprofloxacin. These results suggest that regular monitoring of antibiotics in regional-scale agricultural soils is needed to prevent the risks from the excessive use of antibiotics in the livestock and fishing farming industry, and proper management of their dis- charges to the environment.

ACKNOWLEDGMENTS

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.09.040

LIST OF TABLES AND FIGURES

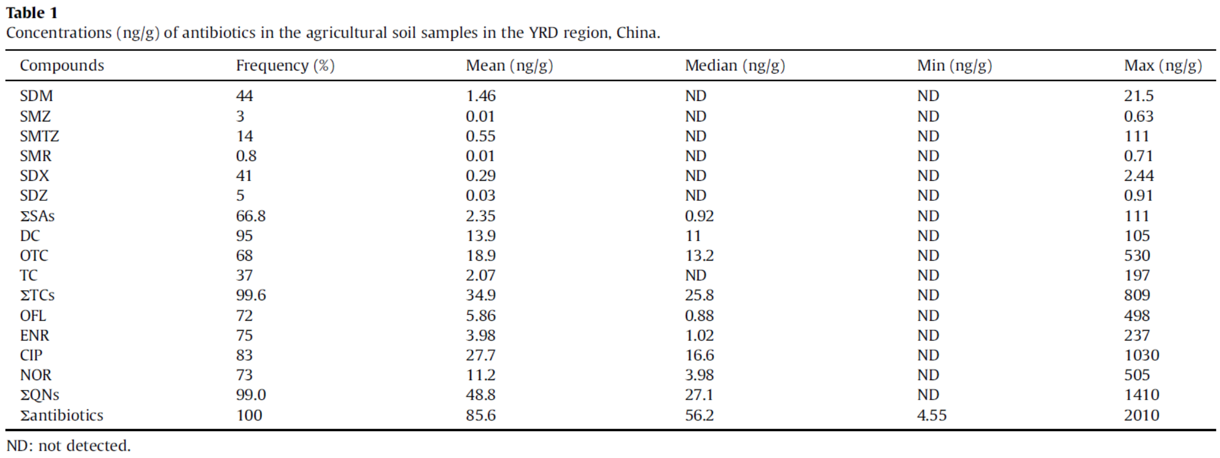

Table 1
Concentrations (ng/g) of antibiotics in the agricultural soil samples in the YRD region, China.

Compounds	Frequency (%)	Mean (ng/g)	Median (ng/g)	Min (ng/g)	Max (ng/g)
SDM	44	1.46	ND	ND	21.5
SMZ	3	0.01	ND	ND	0.63
SMTZ	14	0.55	ND	ND	111
SMR	0.8	0.01	ND	ND	0.71
SDX	41	0.29	ND	ND	2.44
SDZ	5	0.03	ND	ND	0.91
ΣSAs	66.8	2.35	0.92	ND	111
DC	95	13.9	11	ND	105
OTC	68	18.9	13.2	ND	530
TC	37	2.07	ND	ND	197
ΣTCs	99.6	34.9	25.8	ND	809
OFL	72	5.86	0.88	ND	498
ENR	75	3.98	1.02	ND	237
CIP	83	27.7	16.6	ND	1030
NOR	73	11.2	3.98	ND	505
ΣQNs	99.0	48.8	27.1	ND	1410
Σantibiotics	100	85.6	56.2	4.55	2010

ND: not detected.

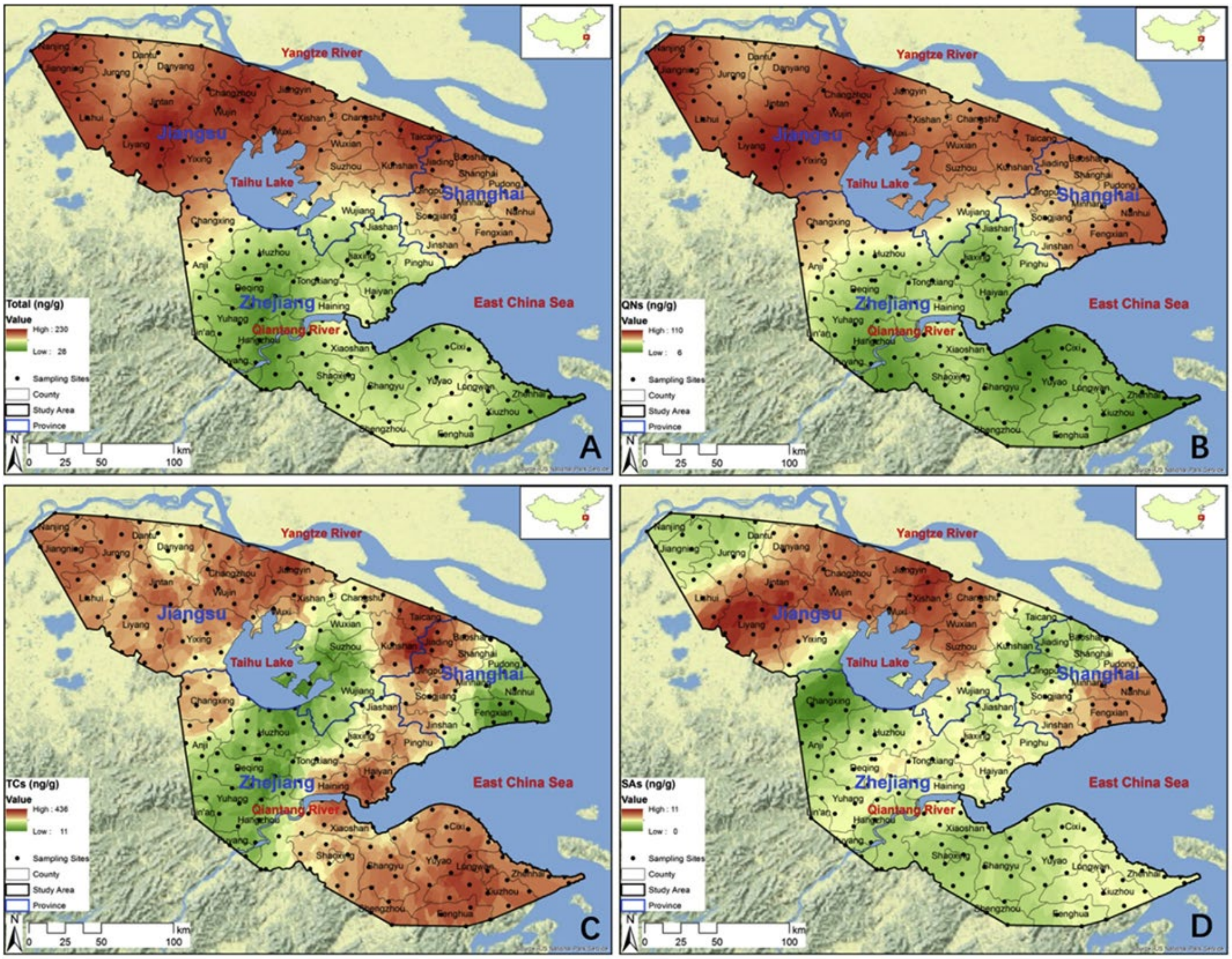

Fig. 1. Spatial distributions of (A) total antibiotics, (B) QNs, (C) TCs, and (D) SAs in the agricultural soils of the YRD region

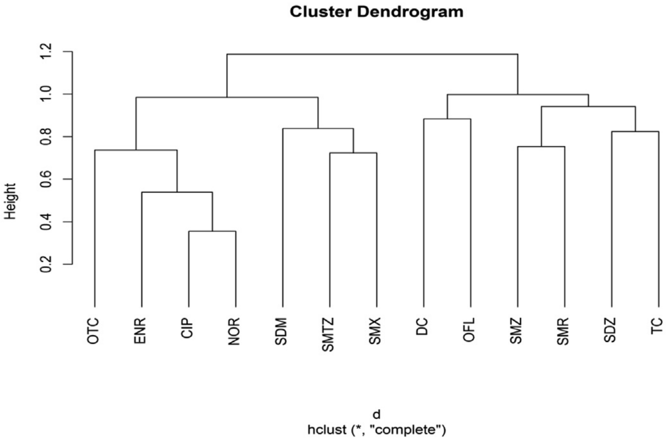

Fig. 2. Cluster dendrogram for 241 soil samples from the YRD region through cluster analysis.

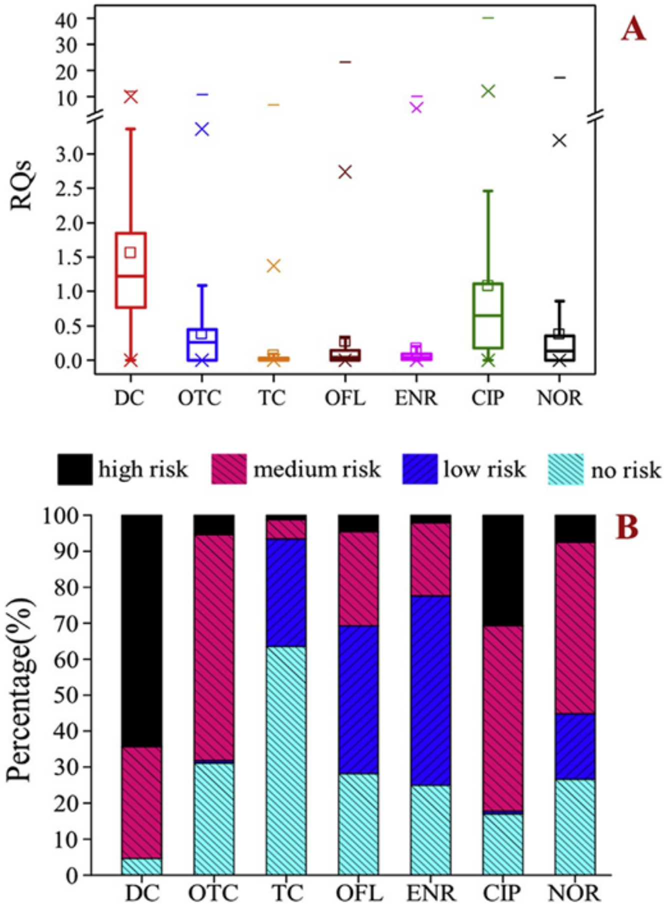

Fig. 3. Risk quotients (A) and percentages (B) for QNs and TCs detected in the agricultural soils of the YRD region

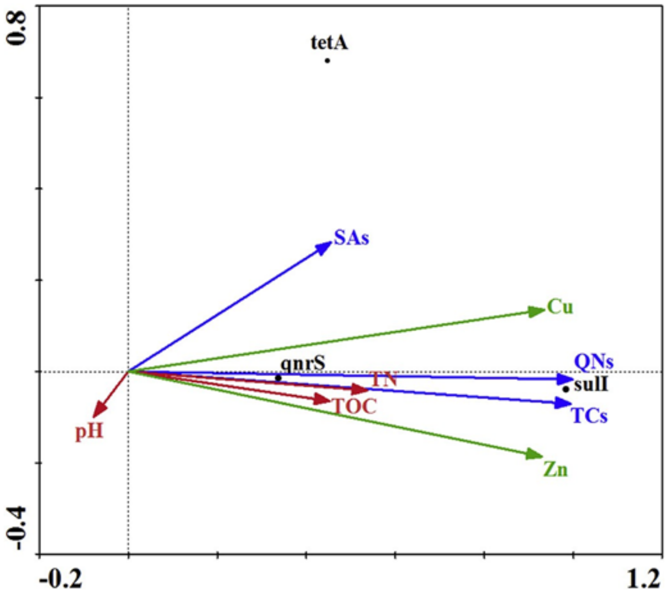

Fig. 4. Redundancy analysis of the relative abundance of ARGs and environmental variables (including various antibiotics, copper, zinc, pH, TOC, and total nitrogen) in 15 soil samples from northeast of the YRD region.

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