Science of the Total Environment 521–522 (2015) 101–107

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Occurrence of antibiotics in soils and manures from greenhouse vegetable production bases of Beijing, China and an associated risk assessment

Cheng Li [a,b], Jiayi Chen [a,b], Jihua Wang [a,b], Zhihong Ma [a,b], Ping Han [a,b], Yunxia Luan [a,b], Anxiang Lu [a,b,c,*]

[a] *Beijing Research Center for Agricultural Standards and Testing, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China*
[b] *Risk Assessment Lab for Agro-products (Beijing), Ministry of Agriculture, Beijing 100097, China*
[c] *Collaborative Innovation Center for Key Technology of Smart Irrigation District in Hubei, Yichang 443002, China*

HIGHLIGHTS

- High levels of antibiotics were found in soils from vegetable greenhouses.
- Concentrations of antibiotics were higher in greenhouse soils than in open fields.
- Manure application was the primary source of antibiotics in greenhouse soils.
- Several antibiotics in greenhouse soils pose a high ecological risk.

ARTICLE INFO

Article history:
Received 15 November 2014
Received in revised form 18 March 2015
Accepted 18 March 2015
Available online xxxx

Editor: Kevin V. Thomas

Keywords:
Antibiotics
Greenhouse vegetable production base
Manure
Risk assessment
Soil

ABSTRACT

The occurrence of 15 antibiotics in soil and manure samples from 11 large-scale greenhouse vegetable production (GVP) bases in Beijing, China was investigated. Results showed that the greenhouse soils were ubiquitously contaminated with antibiotics, and that antibiotic concentrations were significantly higher in greenhouses than in open field soils. The mean concentrations of four antibiotic classes decreased in the following order: tetracyclines (102 μg/kg) > quinolones (86 μg/kg) > sulfonamides (1.1 μg/kg) > macrolides (0.62 μg/kg). This investigation also indicated that fertilization with manure and especially animal feces might be the primary source of antibiotics. A risk assessment based on the calculated risk quotients (RQs) demonstrated that oxytetracycline, chlortetracycline, norfloxacin, ciprofloxacin and enrofloxacin could pose a high risk to soil organisms. These results suggested that the ecological effects of antibiotic contamination in GVP bases and their potential adverse risks on human health need to be given special attention.

1. Introduction

Antibiotics have been widely used to treat bacterial infections and promote the growth of livestock animals for several decades (Thiele-Bruhn, 2003). China is the world's largest producer and consumer of antibiotics. An estimated 210,000 t of antibiotics is produced every year, of which 48% are used in the agricultural and livestock industries. Most animals cannot completely metabolize the antibiotics they receive, and the animals excrete them into the environment as intact bioactive substances or metabolites. Researchers have reported that antibiotic residues in the environment can affect terrestrial organisms, alter microbial activity and community composition in soil, and promote the development of antibiotic resistant genes (ARGs), which represent a risk to human and animal health (Ashbolt et al., 2013).

Previous studies have frequently reported high concentrations of antibiotics in animal manure. Martínez-Carballo et al. (2007) reported that the concentrations of chlortetracycline, oxytetracycline and tetracycline in 30 pig manure of Austria were up to 46 mg/kg, 29 mg/kg and 23 mg/kg, respectively, whereas the concentrations as high as 225 and 1420 mg/kg of norfloxacin and enrofloxacin were found in chicken manures from China (Zhao et al., 2010). Current environmental legislation fails to cover these antibiotics; the antibiotics could lead to undesirable effects on ecosystems (Li et al., 2012a). Furthermore, the high concentrations of antibiotics in manure can be associated with an increased presence of resistant bacteria in environment, which is a major public health concern, due to the increased occurrence

* Corresponding author at: Beijing Research Center for Agricultural Standards and Testing, Beijing Academy of Agriculture and Forestry Sciences, Beijing 100097, China.
E-mail address: anxiang_lu@hotmail.com (A. Lu).

http://dx.doi.org/10.1016/j.scitotenv.2015.03.070

of associated clinical infections (Ashbolt et al., 2013; Luo et al., 2010). Most livestock manure is directly or indirectly applied on agricultural land as fertilizers, which constitute the main source of the dissemination of these chemicals in soil environments (Zhou et al., 2013). Hamscher et al. (2002) detected concentrations of tetracyclines up to 0.3 g/kg in an agricultural field fertilized with liquid manure. In addition, Li et al. (2011) found high antibiotic concentrations in vegetable farmlands affiliated with livestock farms and detected maximum concentrations of tetracyclines, sulfonamides, and quinolones of 242.6, 321.4, and 1537.4 μg/kg, respectively.

Market demands and economic incentives have resulted in intensified greenhouse vegetable production (GVP), particularly in developing countries; this intensive form of agriculture has expanded rapidly worldwide. China accounts for more than 90% of the global GVP operations, where GVP area had expanded to almost 4.67 million ha by 2010 (Chang et al., 2013). GVP can result in improved vegetable yields by extending the growing seasons and intensifying agriculture compared with conventional vegetable production. However, heavy fertilization strongly increases production and is always adopted under GVP conditions to achieve high vegetable production. In addition, growers of organic vegetables primarily use manure in GVP systems. Most GVP involves large and repeated amounts of animal manure application and probably increases the risk of antibiotic contamination, which could also have adverse effects to human health due to the consumption of these vegetables contaminated with antibiotics and antibiotic-resistant bacteria. Presently, no regulations address the presence of antibiotics in soil as well as in the manure applied to certified vegetable production systems. This has created an urgent problem related to the contamination of antibiotics in GVP systems.

Beijing, the capital of China, supports a resident population of about 20 million. In recent years, large-scale GVP bases have been widely developed on the outskirts of Beijing, to a total area of 1531.49 ha by 2011. With China's emphasis on food security and sustainability, an increasing demand exists for organic vegetables from GVP bases, where most fertilizer comes from manure. This cultivation technique may elevate the contamination of foods by antibiotics relative to conventional farming methods. To date, limited information is available on the occurrence of antibiotics in the GVP bases of Beijing. Therefore, the objective of this study was to improve our understanding of the general status of antibiotic pollution in the GVP bases. The data collected can also be used to evaluate the ecological risks created by antibiotics in GVP systems.

2. Materials and methods

2.1. Sampling sites and sample collection

Eleven large-scale GVP bases were selected in the suburbs of Beijing based on geographic location, cultivated area, history and environment. Bases are defined as large-scale areas with numerous greenhouses; sample sites are defined as the various sample points within those bases. The sampling bases were distributed across four districts, including Changping, Daxing, Shunyi and Yanqing, which covered a relatively large area. These vegetable bases were registered with the local Department of Agriculture, and the vegetable products from these bases were transported throughout of Beijing area. Therefore, the selected bases could reflect the whole situation of GVP bases in Beijing. The GVP bases were generally operated in the form of enterprises and only manure was applied in the process of production. And, groundwater was used for irrigation. Sampling work occurred in May–July 2013 (Fig. 1). Fifty-six vegetable greenhouses were selected from these GVP bases, with estimated areas ranging from 20 to 66.7 ha. Three to nine samples were evenly sampled in each GVP base, the exact number depending on the total area of each base. Fig. 1(B) shows Site YQ1 as an example showing the layout of plots. In addition, soils from the open fields near each GVP base were also collected as controls. These open fields were applied to plant seasonal vegetables and cultivated by local farmers. A combination of chemical fertilizers and organic fertilizers was generally used in the open fields. Topsoil was sampled at a depth of 0–20 cm using a stainless-steel auger. Five sampling sites were distributed in an S-shaped pattern in each greenhouse; these were mixed as a single sample. Furthermore, manure samples ($N = 17$) that were actively being applied in the studied GVP bases, were also gathered for potential source analysis. The categories of the manure include chicken, duck, pig, and cow manure as well as commercial organic fertilizers which were produced through microbial fermentation using the mixture of livestock dungs and some farm waste such as corn stalks or wheat shafts as raw materials. Soil and manure samples were air dried at ambient temperature, ground and homogenized by sieving through a stainless steel sieve (60-mesh) after removing stones and residual roots; samples were then sealed in brown glass bottles and stored at −20 °C prior to analysis.

2.2. Chemicals and standards

The antibiotics analyzed here were selected based mainly on the extent of their use in animal production in China. These drugs belonged to four different antibacterial families. Six sulfonamides (SAs) included sulfamerazine (SMR), sulfamethazine (SMZ), sulfadiazine (SDZ), sulfameter (SM), sulfadimethoxine (SDM), and sulfamethoxazole (SMX). Three tetracyclines (TCs) included tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC). Four quinolones (QNs) included norfloxacin (NFX), ciprofloxacin (CIP), enrofloxacin (ENR) and lomefloxacin (LOM). Two macrolides (MLs) included erythromycin (ETM) and roxithromycin (RTM). Standards for SDM, SMX, TC, CTC, LOM and RTM with purities of >98% were obtained from Sigma-Aldrich (St. Louis, MO, USA), while the other antibiotic standards, with purities of >98% were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Simeton (J & K Chemical Ltd., USA) was used as the internal standard to enhance the analytical precision. A stock solution (1.0 mg/mL) of Simeton was prepared in methanol solution, stored in at −20 °C prior to use.

Acetonitrile and methanol (HPLC grade) were purchased from Fisher Scientific (New Jersey, USA). Formic acid (98%) was purchased from Fluka (Bucks, Switzerland). Ultrapure water was prepared with a Milli-Q water purification system (Millipore, Billerica, MA, USA). Disodium ethylenediamine tetraacetate, citric acid and sodium citrate of analytical grade were obtained from Yaohua Chemical Reagent Factory (Tianjin, China). Citric acid buffer (pH 4) was prepared according to the procedure described by Zhou et al. (2011). Strong anion exchange (SAX) cartridges (6 mL, 500 mg) were provided by Agilent Technologies (Wilmington, DE, USA) and Oasis HLB cartridges (6 mL, 500 mg) were purchased from Waters (Milford, MA. USA). All other reagents were of analytical reagent grade.

2.3. Sample extraction and clean-up

One half-gram of each freeze-dried manure sample and 2 g of each soil sample were extracted with 10 mL acetonitrile and 10 mL citric acid buffer ($pH = 4$) in a 50 mL polypropylene centrifuge tube. The sample was vortexed for 1 min and treated ultrasonically for 15 min, followed by centrifugation. This extraction process was repeated twice. The extract was combined into a round-bottom flask, concentrated with a rotary evaporator at 50 °C to remove the organic solvent, and diluted to 100 mL with ultrapure water to make sure the organic solvent in solution had a concentration of less than 5%.

SAX and HLB cartridges were set up in tandem for cleanup of extracts. Prior to the SPE cleanup, 0.2 g of disodium ethylenediamine tetraacetate was added into each aqueous extract to chelate with metal cations. The cartridges were pre-treated with 10 mL methanol and 10 mL ultrapure water; the diluted extract was passed through the cartridges at a loading rate of about 5 mL/min. After loading the entire amount of extract, the SAX cartridge was removed and the HLB