

绿色和有机蔬菜基地土壤中喹诺酮类抗生素的污染特征

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摘要:利用高效液相色谱-串联质谱(HPLC-MS/MS)分析方法,探讨了广州市某绿色蔬菜基地和有机蔬菜基地土壤中4种喹诺酮类抗生素的含量与分布特征。结果表明,土壤中各化合物的检出率除洛美沙星为85%以外,其余均为100%,平均含量为0.80~24.95 $\mu\text{g}\cdot\text{kg}^{-1}$,以诺氟沙星(24.95 $\mu\text{g}\cdot\text{kg}^{-1}$)为主,其次为环丙沙星(15.40 $\mu\text{g}\cdot\text{kg}^{-1}$)和恩诺沙星(7.68 $\mu\text{g}\cdot\text{kg}^{-1}$)。4种喹诺酮类化合物总含量在7.15~122.25 $\mu\text{g}\cdot\text{kg}^{-1}$ 之间,主要分布在50~100 $\mu\text{g}\cdot\text{kg}^{-1}$ 之间,平均为48.85 $\mu\text{g}\cdot\text{kg}^{-1}$ 。有机蔬菜基地土壤中4种喹诺酮类化合物的含量均高于绿色蔬菜基地土壤。同一基地不同蔬菜土壤中喹诺酮类抗生素的含量有一定差异,但化合物组成特征差异不大。研究区土壤中喹诺酮类抗生素的含量低于兽药国际协调委员会(VICH)筹划指导委员会的抗生素生态毒性效应触发值(100 $\mu\text{g}\cdot\text{kg}^{-1}$),表明生态风险较小。

关键词:绿色蔬菜基地;有机蔬菜基地;土壤;喹诺酮类;抗生素;污染

中图分类号:X53 文献标志码:A 文章编号:1672-2043(2012)01-0125-06

Occurrence of Quinolone Antibiotics in the Soils from a Green and an Organic Vegetable Fields

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Abstract: Concentration and distribution of four quinolone antibiotics in the soil samples from a green and an organic vegetable fields in Guangzhou were investigated using high performance liquid chromatography- electrospray ionization tandem mass spectrometry (HPLC-MS/MS). Detection frequencies were 85 percent for lomefloxacin, and 100 percent for other compounds including norfloxacin,ciprofloxacin, and enrofloxacin.The average concentrations of individual compound ranged from 0.80 $\mu\text{g}\cdot\text{kg}^{-1}$ to 24.95 $\mu\text{g}\cdot\text{kg}^{-1}$. Norfloxacin was a dominant compound with an average concentration of 24.95 $\mu\text{g}\cdot\text{kg}^{-1}$, followed by ciprofloxacin and enrofloxacin with average concentrations of 15.40 $\mu\text{g}\cdot\text{kg}^{-1}$ and 7.68 $\mu\text{g}\cdot\text{kg}^{-1}$, respectively. The sum concentrations of the four compounds ranged from 7.15 $\mu\text{g}\cdot\text{kg}^{-1}$ to 122.25 $\mu\text{g}\cdot\text{kg}^{-1}$, mainly distributing within 50~100 $\mu\text{g}\cdot\text{kg}^{-1}$ and averaging at 48.85 $\mu\text{g}\cdot\text{kg}^{-1}$. The concentrations of four quinolone antibiotics in the soil samples from organic vegetable field were higher than those of that in the soil samples from green vegetable field. The dominant compounds of quinolone antibiotics in the soils grown various vegetables within the same vegetable field were generally comparative, while their concentrations varied to a certain degree. Ecological risk of the four quinolone antibiotics in the soils of studied fields was low considering that their concentrations were under the ecotoxic effect trigger value(100 $\mu\text{g}\cdot\text{kg}^{-1}$) set by the Steering Committee of Veterinary International Committee on Harmonization.

Keywords: green vegetable field; organic vegetable field; soil; quinolones; antibiotics; pollution

收稿日期:2011-04-11

基金项目: 国家自然科学基金项目(40773062, 41071211); 广东省自然科学基金重点项目(07117909, 2011020003196); 广东省科技计划项目(2005B20801002, 2006B20601003, 2010B020311006); 广东省高校高层次人才项目; 东莞市科技研究计划项目(2008108101110); 惠州市科技研究计划项目(2009B010001009); 广州市科技计划项目(2010A82070466)

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规模化动物养殖中大量使用喹诺酮类等各种抗生素以防病治病、提高饲料利用率和促进动物生长^[1-2]。抗生素使用后一般以药物原形或其代谢物随粪尿排出^[3],造成动物粪便中抗生素含量普遍较高^[4-10],成为环境中抗生素的重要来源。动物粪便常作为有机肥料用于农业生产,引起土壤抗生素污染^[5-7,11-13],其输入量甚至不亚于农药施用量^[4],在一定程度上干扰土壤微生物的群落结构与功能^[14-15]进而影响土壤肥力,甚至可被作物吸收累积^[16-17]而危及农产品质量安全。此外,土壤抗生素污染还可通过地表径流和淋滤作用进一步造成水体抗生素污染^[18-20],并引发细菌耐药性^[21],威胁动物与人类健康。因此,抗生素作为一类新兴的重要环境有机污染物,已成为近年来环境科学研究的热点,但国内外对于土壤环境污染及其生态效应的研究较少^[5-7,14-15],在我国更鲜见报道^[8,11-12,14]。特别是绿色蔬菜、有机蔬菜等经有关机构认证的蔬菜基地,虽然强调有机肥的施用,但对于动物粪肥中抗生素含量没有限制要求,因而土壤中抗生素污染问题更令人关注。为此本文针对畜禽养殖中广泛使用的喹诺酮类抗生素,探讨广州市某绿色蔬菜基地和有机蔬菜基地土壤中4种喹诺酮类抗生素的含量与分布特征,以期为土壤抗生素污染控制提供科学依据。

1 材料与方法

1.1 仪器与试剂

样品预处理和分析中所使用的仪器设备主要有固相萃取装置(VisiprepTM-DL, Supelco)、Oasis HLB 固相萃取柱(3 mL/60 mg, Supelco)、数控恒温水浴、恒温振荡器、超声波清洗器、低速离心机,以及高效液相色谱仪(Agilent 1100)、电喷雾(ESI)离子源串联质谱仪(AB4000QTRAP)。

4种喹诺酮类抗生素分别为诺氟沙星(NOR)、环丙沙星(CIP)、洛美沙星(LOM)、恩诺沙星(ENR),其标准品均产自德国 Ehrenstorfer GmbH 公司,纯度大于98%。甲醇、乙腈均为色谱纯(Sigma 公司),其他化学试剂均为分析纯。实验用水为高纯水。

喹诺酮类化合物标准品母液:准确称取0.010 0 g标准品溶于少量乙酸溶液,用乙腈/水(20/80, V/V)稀释定容至100 mL,配制成浓度为100 $\mu\text{g}\cdot\text{mL}^{-1}$ 的工作母液,于4℃冰箱中避光保存,使用期6个月。实验所用各种浓度的标准液用乙腈/水(20/80, V/V)按一定比例稀释,校正曲线工作液浓度范围为0.001~0.5 $\mu\text{g}\cdot\text{mL}^{-1}$ 。**50%硝酸镁溶液:**50 g Mg(NO₃)₂·6H₂O

溶于100 mL水,可提前配制,室温保存。10%氨水溶液:按10 mL 25%氨水加入100 mL水的比例配制,现配现用。

1.2 样品采集与预处理

土壤样品采自广州市某绿色蔬菜生产基地和有机蔬菜生产基地,前者约267 hm²,后者约33 hm²,有机肥(猪粪、鸡粪等)施用量为7.5~15 t·hm⁻²。根据种植蔬菜品种和环境条件等因素,选择13个采样地块,分别以多个点位采集表层土壤(20 cm)组成混合样。土壤样品采集后按四分法缩减,其中绿色蔬菜基地土壤样品5个,有机蔬菜基地土壤样品8个。

土壤样品在室内风干后粉碎过60目筛,按参考文献[22]方法进行预处理。准确称取1.00 g土壤样品置于10 mL离心管中,加入50%硝酸镁-10%氨水(96/4, V/V)4 mL,振荡5 min,超声提取15 min,离心(4 500 r·min⁻¹)8 min,收集上清液。残渣用上述方法反复提取2次。合并上清液,过HLB固相萃取小柱(先后过6 mL甲醇和6 mL水)萃取富集。用6 mL高纯水清洗小柱,真空干燥10 min,再用3 mL 1%乙酸-乙腈洗脱小柱。洗脱液在40℃水浴下用氮气吹至近干,用乙腈-水(20/80, V/V)定容至1 mL,溶液过0.22 μm 滤膜收集于样品瓶中待测。

1.3 HPLC-MS/MS 分析和质量控制与质量保证

色谱条件:色谱柱Ailent Eclipse Plus C18(5 μm , 2.1×150 mm)、柱温20℃;柱平衡时间30 min;流动相水:乙腈(80/20, V/V;含1%甲酸)等度洗脱,流速0.2 $\text{mL}\cdot\text{min}^{-1}$;进样量5 μL 。

质谱条件:离子化模式ESI(+);雾化气60 psi;干燥气50 psi;气帘气20 psi;离子源电压5 500 V;去溶剂温度600℃;碰撞气压水平高压。

喹诺酮类抗生素加标回收率在55%~77%之间(表1),各化合物不同加标浓度的回收率总体上差别不大,相对标准偏差(RSD,n=3)均低于10%。以混合标准品工作液系列浓度(1~500 $\mu\text{g}\cdot\text{L}^{-1}$),建立各化合物标准曲线的线性回归方程,按3倍信噪比计算得到检测限为0.04~0.20 $\mu\text{g}\cdot\text{L}^{-1}$,按10倍信噪比计算得到样品定量限为0.12~0.67 $\mu\text{g}\cdot\text{kg}^{-1}$ (表1)。

为控制实验过程中人为污染,保证操作过程准确,每10个样品间隔设置空白样、样品平行样、样品加标样,并且在进样时以固定浓度标样进行质量控制。空白样中未检出喹诺酮类化合物,平行样检测结果相对标准偏差均小于1%。整个分析流程喹诺酮类化合物回收率为72.5%~86.2%。

布特征有明显不同(图3)。有机蔬菜基地土壤中喹诺酮类抗生素平均总含量($69.9 \mu\text{g}\cdot\text{kg}^{-1}$)是绿色蔬菜基地土壤($16.9 \mu\text{g}\cdot\text{kg}^{-1}$)的4倍,单个化合物含量均高于绿色蔬菜基地土壤,这可能与有机蔬菜基地有机肥施用量较大有关。在化合物组成上,有机蔬菜基地土壤中喹诺酮类抗生素以诺氟沙星为主,其次为环丙沙星;而绿色蔬菜基地土壤中以环丙沙星为主,其次是诺氟沙星。

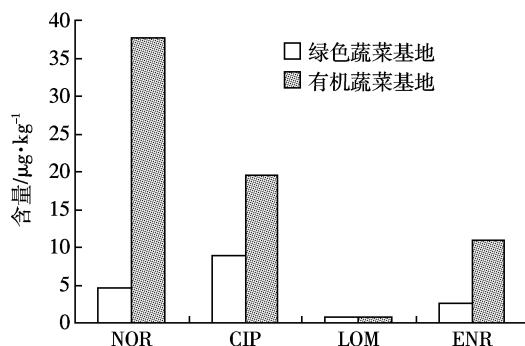


图3 两种品质蔬菜基地土壤中喹诺酮类抗生素的含量和组成特征

Figure 3 Contents and constituents of quinolones in soils from various vegetable fields

2.3 不同蔬菜土壤中喹诺酮类抗生素的含量与组成特征

同一蔬菜基地不同蔬菜品种土壤中喹诺酮类抗生素的含量有一定差异,但化合物组成特征基本相似(图4)。绿色蔬菜基地不同蔬菜土壤中喹诺酮类抗生素总含量为 $7.06\sim51.43 \mu\text{g}\cdot\text{kg}^{-1}$,菜心土壤中的总含量明显高于其他蔬菜土壤,化合物组成均以环丙沙星为主,其次是诺氟沙星。有机蔬菜基地不同蔬菜土壤中喹诺酮类化合物总含量为 $34.02\sim122.25 \mu\text{g}\cdot\text{kg}^{-1}$,

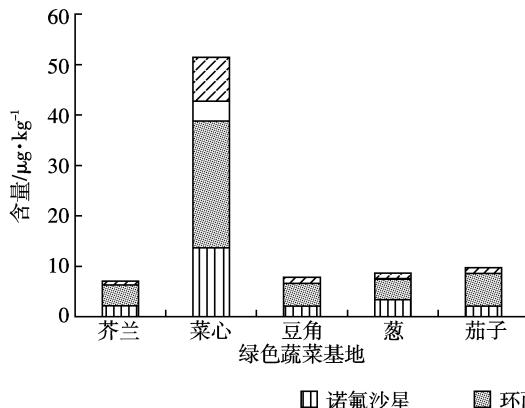


图4 不同蔬菜品种土壤中喹诺酮类抗生素的含量与分布特征

Figure 4 Occurrence of quinolone compounds in soils grown different vegetables within the same field

番薯土壤中最高($122.25 \mu\text{g}\cdot\text{kg}^{-1}$),其次为菜心土壤和芥菜土壤,芥兰土壤中最低。化合物组成有两种类型,一是以诺氟沙星为主,包括番薯土壤、绍菜土壤和芥菜土壤,二是以环丙沙星、诺氟沙星和恩诺沙星为主,包括奶白菜土壤、菜心土壤、芥兰土壤和上海青土壤。不同植物根际对土壤中有机污染物的降解情况存在明显差异^[31],如不同品种(基因型)通菜对土壤中邻苯二甲酸酯的吸收和降解存在显著差异^[33],与植物根系分泌物等生理生化特征以及根际微生物种群结构与功能的差异等因素有关。另外,同一蔬菜基地内各农户之间不同施肥、灌溉、轮作等生产方式也会造成土壤中喹诺酮类化合物的降解性、迁移性等环境行为的不同^[34],如前面已提及的关于粪肥施用条件不同会造成土壤中抗生素降解特征差异^[24-28],从而导致同一蔬菜基地种植不同蔬菜土壤中喹诺酮类化合物含量与组成的差异。

3 结论

广州市某绿色蔬菜基地和有机蔬菜基地土壤中4种喹诺酮类化合物普遍同时被检出,平均含量在 $0.80\sim24.95 \mu\text{g}\cdot\text{kg}^{-1}$ 之间,以诺氟沙星为主,其次为环丙沙星和恩诺沙星。尽管各化合物的最高含量低于抗生素生态毒性效应触发值($100 \mu\text{g}\cdot\text{kg}^{-1}$),但不能忽视其总含量超过了该值,同时需要关注各种化合物之间的联合毒性效应问题。以动物粪便作为有机肥施用的抗生素污染问题需要进一步研究。

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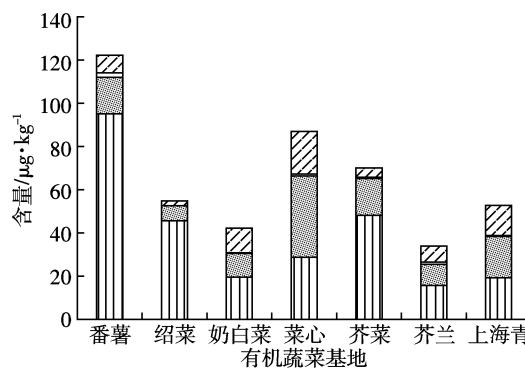


图4 不同蔬菜品种土壤中喹诺酮类抗生素的含量与分布特征

Figure 4 Occurrence of quinolone compounds in soils grown different vegetables within the same field

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