

目次

疫情期间人为源减排对城市大气氧化性的影响 .....朱剑蓝, 秦墨梅, 朱嫣红, 胡建林 (617)

不同天气形势对南京地区双高污染的输送及潜在源区分析 .....秦阳, 胡建林, 孔海江 (626)

不同方法判定南京臭氧生成敏感区的差异 .....陈柑羽, 李勋, 李琳, 秦墨梅, 谢鸣捷, 王鸣, 李婧楠, 胡建林 (635)

2006~2021年夏半年上海臭氧浓度特征及其大环流背景分析 .....郑庆锋, 梁萍, 段玉森, 林燕芬, 张宋嘉, 徐卫忠 (645)

基于大气成分观测网的山西省近地面O<sub>3</sub>体积分数分布特征 .....李莹, 王淑敏, 裴坤宁, 闫世明, 孙鸿博, 张逢生, 高兴艾 (655)

伊宁市夏季大气臭氧生成机制及减排策略 .....王文婷, 谷超, 李丽明, 李新琪, 郑镇森, 耿春梅, 王晓丽, 杨文 (668)

运城市四季VOCs特征、来源及臭氧形成敏感物种 .....阴世杰, 刘新罡, 刘亚非, 李晨露, 张晨, 张欢, 王正, 程强 (678)

郑州市冬夏季污染过程中大气VOCs污染特征、来源解析及活性分析 .....赖梦洁, 张栋, 于世杰, 宋鑫帅, 李晓, 张瑞芹 (689)

郑州市PM<sub>2.5</sub>中有机酸的污染特征、来源解析及二次生成 .....李子涵, 董喆, 尚璐琪, 孔梓涵, 李晓, 张瑞芹 (700)

中国三大城市群PM<sub>2.5</sub>浓度非线性变化分析 .....吴舒祺, 顾杨阳, 张天岳, 赵文吉 (709)

基于LEAP模型的临港新片区中长期碳排放预测及减排潜力分析 .....吴琼, 马昊, 任洪波, 郭明星, 陈鹏, 李琦芬 (721)

碳交易背景下中国华北地区碳代谢格局变化 .....郑宏媚, 沈方, 许光耀, 关欣 (732)

考虑区域特点和车型差异的氢燃料电池汽车全生命周期减碳预测分析 .....马菁, 蔡旭, 张春梅, 兰利波, 陈轶嵩, 付佩 (744)

我国主要河流水系硝态氮污染特征及定量源解析 .....韦英怀, 胡敏鹏, 陈丁江 (755)

不同时空尺度下土地利用结构与空间格局对苏州水质的影响 .....谭娟, 熊丽君, 王卿, 任志文, 朱丹丹, 王敏 (768)

深圳市2015~2021年雨源型河流水质时空变化及其对降雨的响应 .....韦必颖, 成建梅, 苏晓煜, 程天舜 (780)

河南黄河改道区浅层地下水化学特征与主控污染源解析 .....王帅, 任宇, 郭红, 曹文庚, 李祥志, 肖舜禹 (792)

北京西山岩溶地下水化学特征及成因分析 .....郭高轩, 代垠东, 许亮, 朱琳, 欧志亮, 戚琦, 辛宝东 (802)

店埠河流域地表水-地下水化学特征及其成因分析 .....郑涛, 秦先燕, 吴剑雄 (813)

张家口地区枯水期地下水化学特征及其成因机制分析 .....金爱芳, 殷秀兰, 李长青, 李文娟, 庞菊梅, 金晓媚 (826)

黄河中下游典型抗性细菌及抗性基因污染分布 .....闵威, 高明昌, 孙绍芳, 宋茜茜, 邱立平 (837)

制药废水中抗生素抗性的污染特征、检测手段和控制方法 .....彭安萍, 高虎, 张新波 (844)

水体组分对聚苯乙烯纳米颗粒聚沉行为的影响 .....汤端阳, 郑文丽, 陈关潼一, 陈思莉, 陈尧, 赵晓丽, 汪浩 (854)

富磷废弃钙基生物炭对水体中铅的去除 .....刘天, 吕思璐, 杜兴国, 程敏, 谢燕华 (862)

壳聚糖改性生物炭的制备及其对水溶液中Cd<sup>2+</sup>的吸附机制 .....姜凌, 安靖玥, 岳小琼, 李亚雄, 夏秋乐, 祝婷文佳, 柴丽红 (873)

硼掺杂介孔炭吸附四环素的效能与机制 .....邹震, 许路, 乔伟, 唐茂森, 金鹏康 (885)

磁性含磷油茶壳生物炭对水中磺胺甲噁唑的吸附特性 .....韩帅鹏, 唐李文, 刘勤, 林家亮, 李晓慢, 程建华, 胡勇有 (898)

广东省高分辨率温室气体排放清单及特征 .....卢清, 唐明双, 廖彤, 黄志烟, 钟庄敏, 宋佩珊, 沈劲, 张智胜, 梁小明, 孙家仁, 陈来国 (909)

辽河口“退塘还湿”修复区生态系统CO<sub>2</sub>交换及其环境调控 .....刘思琪, 陈虹, 邢庆会, 程浩, 韩建波, 徐雪梅 (920)

生物炭施用两年后对热带地区稻菜轮作土壤N<sub>2</sub>O和CH<sub>4</sub>排放的影响 .....胡煜杰, 唐瑞杰, 胡天怡, 陈琦琦, 汤水荣, 阮延正, 孟磊 (929)

生物炭改良盐碱地研究与应用进展 .....魏盈, 焦乐, 张鹏, 刘福德, 肖辉, 董辰辰, 孙红文 (940)

免耕对农田土壤团聚体的影响研究: Meta分析 .....徐芝萍, 饶越悦, 孟艳, 温媛, 孟维伟, 王旭清, 李宗新, 刘开昌, 代红翠 (952)

黔中喀斯特地区典型县域碳储量时空演变及多情景模拟预测: 以普定县为例 .....李月, 罗红芬 (961)

不同改良剂对酸性紫色土团聚体和有机碳的影响 .....李越, 徐曼, 谢永红, 王颖, 黄容, 谢军, 王子芳, 高明 (974)

Ca改性生物炭对土壤磷赋存形态影响及稳定化机制 .....张超, 翟付杰, 单保庆 (983)

秦岭中段不同恢复阶段弃耕农田植物多样性变化及其驱动因素 .....闫成龙, 薛悦, 王艺菲, 康海斌, 王得祥 (992)

我国典型制药厂污染场地中抗生素的污染特征及生态风险 .....杨炳彬, 黄争, 赵建亮, 何良英, 刘有胜, 胡立新, 石义静, 应光国 (1004)

广州市土壤多环芳烃污染特征及风险评估 .....邹子航, 陈莲, 张培珍, 王雨菡, 王振江, 林森, 唐翠明, 罗国庆, 钟建武, 李智毅, 王圆 (1015)

基于源导向的土壤重金属风险评价及管控因子分析 .....潘泳兴, 陈盟, 王楠楠 (1026)

基于Monte-Carlo模拟的湖南省典型工厂周边农田土壤重金属区域潜在生态风险特征及来源解析 .....罗豪杰, 潘俊, 陈小霞, 张敏, 沈良辰, 李歆, 丁平, 蔡丹, 蔡立梅, 胡国成 (1038)

基于参数优化和蒙特卡罗模拟的砷污染地块健康风险评估 .....袁贝, 刘虎鹏, 杜平, 陈娟, 张云慧, 张昊 (1049)

基于APCS-MLR和PMF模型的赤泥堆场周边耕地土壤重金属污染源解析 .....沈智杰, 李杰芹, 李彩霞, 廖泽源, 梅楠, 罗程钟, 王定勇, 张成 (1058)

PE-Cd复合污染土壤中Cd释放迁移特征及机制 .....王迪, 徐绍辉, 邵明艳, 林青 (1069)

氯代乙烯的厌氧微生物还原脱氯特性 .....李伟, 刘贵平, 刘峻, 吕良华, 乔文静, 余欣, 张晓琦, 蒋建东 (1080)

昭通市农田土壤和蔬菜重金属污染评价及相关性分析 .....张好, 董春雨, 杨海婵, 孙思静, 韩宇, 黄祖志, 张乃明, 包立 (1090)

钝化剂对轻中度镉污染农田的安全利用效果 .....王晓晶, 张东明, 曹阳, 吕家琰, 代允超 (1098)

氧化石墨烯负载铁锰复合材料对镉污染土壤的钝化修复 .....袁婧, 吴骥子, 连斌, 袁峰, 孙淇, 田欣, 赵科理 (1107)

关键生育期施加外源灌溉水对水稻镉吸收转运的影响 .....周霞, 胡雨丹, 周航, 陈琼, 谭文韬, 曾鹏, 辜娇峰, 廖柏寒 (1118)

外源锌对镉胁迫下玉米幼苗生长及根系构型分级的影响 .....张辉红, 魏畅, 柳海涛, 张静静, 刘芳, 赵颖, 张雪海, 李鸽子, 姜瑛 (1128)

稀土元素铈对镉胁迫下小麦幼苗生长的缓解效应 .....张静静, 徐正阳, 焦秋娟, 范丽娜, 刘芳, 赵颖, 宋佳, 化党领, 李鸽子, 柳海涛 (1141)

根施伯克氏菌对小麦镉吸收转运的两段式阻控作用 .....郭佳佳, 王常荣, 刘仲齐, 黄青青, 张长波, 黄永春, 薛卫杰, 孙约兵 (1150)

高密度聚乙烯微塑料与氯嘧磺隆对大豆生长和根际细菌群落的复合胁迫效应 .....胡晓玥, 滑紫微, 姚伦广, 杜丽, 牛秋红, 李玉英, 闫路, 陈兆进, 张浩 (1161)

微塑料的人体富集及毒性机制研究进展 .....包亚博, 王成尘, 彭吾光, 依代倩, 向萍 (1173)

机器学习在微塑料识别与环境风险评估中的应用研究进展 .....白润昊, 范瑞琪, 刘琪, 刘勤, 严昌荣, 崔吉晓, 何文清 (1185)

微塑料与农田土壤中典型污染物的复合污染研究进展 .....侯宇晴, 李冰, 王金花, 宋文慧, 王兰君, 王军, 朱鲁生 (1196)

水中微/纳塑料电化学检测及去除的研究进展 .....郑伟康, 刘振中, 项晓方 (1210)

基于分布式认知理论的农户面源污染治理支付意愿影响因素 .....郭晨浩, 李林霏, 夏显力 (1222)

《环境科学》征订启事(825) 《环境科学》征稿简则(836) 信息(897, 1106, 1149)

# 黄河中下游典型抗性细菌及抗性基因污染分布

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**摘要:** 以黄河中下游某城市段的黄河水体作为研究对象, 对其中可培养总细菌、典型抗性细菌[阿莫西林(AMX)抗性细菌、磺胺甲噁唑(SMZ)抗性细菌]及其对应的典型抗性基因[ $\beta$ -内酰胺类抗性基因( $bla_{CTX-M}$ )、磺胺类抗性基因( $sulI$ 、 $sulII$ )]、1种整合酶基因  $intI1$  的季节及沿程分布进行调研分析. 结果表明, 该市黄河流域可培养总细菌、AMX 抗性细菌和 SMZ 抗性细菌受温度与人类活动影响显著, 其菌属组成与数量具有明显的时空分布特征, 主要以芽孢杆菌属(*Bacillus*)和假单胞菌属(*Pseudomonas*)为主. 抗性基因丰度随温度降低呈下降趋势,  $\beta$ -内酰胺类抗性基因在总基因中占比高于磺胺类基因, 磺胺类基因中以  $sulI$  为优势基因. 相关性分析表明, I 型整合子是加速抗性基因传播的重要因素. 研究有助于了解该地黄河水体抗性污染现状, 可为黄河中下游流域抗性基因风险评估提供理论支撑.

**关键词:** 黄河流域; 抗性基因(ARGs); 抗性细菌(ARB); 时空分布; 荧光定量 PCR(qPCR)

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## Distribution of Typical Resistant Bacteria and Resistance Genes in Source Water of the Middle and Lower Reaches of the Yellow River

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**Abstract:** The Yellow River water of an urban area located in the middle and lower reaches of the Yellow River was taken as the research object, in which the seasonal and along-range distribution of total culturable bacteria, typical antibiotic resistant bacteria (amoxicillin resistant bacteria and sulfamethoxazole-resistant bacteria), and their corresponding typical resistance genes [ $\beta$ -lactam resistance gene ( $bla_{CTX-M}$ ) and sulfonamide resistance genes ( $sulI$  and  $sulII$ )], as well as  $intI1$  were investigated. The results showed that the total culturable bacteria,  $\beta$ -lactam-resistant bacteria and sulfonamide-resistant bacteria in the Yellow River Basin were significantly affected by temperature and human activities. The composition and quantity of their genera had obvious spatiotemporal distribution characteristics, in which *Bacillus* and *Pseudomonas* were dominant in the composition and number of bacteria. The abundance of resistance genes decreased with the decrease in temperature. The proportion of  $\beta$ -lactam resistance genes in the total genes was higher than that of sulfonamide genes, and  $sulI$  was the dominant gene in sulfonamide genes. Correlation analysis showed that class I integron played an important role in accelerating the spread of resistance genes. This study offers insight into the status quo of water resistance pollution in the Yellow River and provides theoretical support for the risk assessment of resistance genes in the middle and lower reaches of the Yellow River Basin.

**Key words:** Yellow River Basin; antibiotic resistance genes (ARGs); antibiotic resistant bacteria (ARB); spatial and temporal distribution; quantitative-polymerase chain reaction (qPCR)

水环境中抗生素的长期赋存会诱导抗生素抗性细菌(antibiotic resistance genes, ARGs)的增殖, 进而导致环境中抗性基因(antibiotic resistant bacteria, ARB)大量传播<sup>[1,2]</sup>. 抗性细菌和抗性基因作为新型环境污染物质, 近年来在国际上引起广泛关注<sup>[3]</sup>. 抗性基因可通过垂直基因转移<sup>[4]</sup>不断累积, 并有可能通过水平基因转移的方式<sup>[5,6]</sup>从抗性细菌传递到致病菌中, 引起抗药性的散播和流行<sup>[7]</sup>, 给生态系统安全和人类健康带来严重威胁<sup>[8,9]</sup>.

近年来, 我国各大流域都发现存在不同程度的抗性基因与抗性细菌污染问题<sup>[10-15]</sup>. 黄河作为中国的母亲河, 在中国经济社会发展和生态安全方面具有十分重要的地位<sup>[16]</sup>, 黄河中下游流域畜禽养殖业发达, 而导致抗生素污染问题尤其突出<sup>[17]</sup>. 但是目前

有关黄河中下游流域水体抗性细菌与抗性基因的污染问题缺乏系统的研究.

因此, 本文以黄河中下游某城市段的黄河水体为研究对象, 对其中可培养总细菌、典型抗性细菌(AMX 抗性细菌、SMZ 抗性细菌)、典型抗性基因( $sulI$ 、 $sulII$ 、 $bla_{CTX-M}$ )及 16S rDNA 含量的季节及沿程分布进行调研分析, 以期对黄河中下游流域水体抗性细菌与抗性基因污染风险评价提供参考, 并为全面控制黄河中下游水环境中典型抗性细菌和抗性基

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因污染提供可靠数据支撑和依据.

## 1 材料与方法

### 1.1 实验试剂及仪器

主要试剂如下: E. Z. N. A Soil DNA Kit (Omega, 美国), TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver. 4.0、SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) 和 TaKaRa Ex Taq (TaKaRa 日本), TIAN prep Mini Plasmid Kit II、pGM-T 克隆试剂盒、D2000 DNA Maker 和  $6 \times$  loading buffer (天根生化科技有限公司, 中国), PCR 引物 (Invitrogen, 美国), 磺胺甲噁唑和阿莫西林 (源叶生物, 中国).

主要仪器如下: 梯度 PCR 仪 (Multigene™ optimax, Labnet 美国), 荧光定量 PCR 仪 (LightCycle Nano, Roche 瑞士), 凝胶成像仪 (MiniBIS Pro, DNR, 以色列), 核酸微量定量仪 (Nanodrop2000, Thermo Scientific, 美国).

### 1.2 水样采集

黄河自西向东流经该市, 根据该市地形特点, 沿黄河流向设立 6 个取样点 (A ~ F 点, 如图 1 所示), 采样时间分别为夏季 (6 月)、秋季 (10 月) 和冬季 (12 月), 环境温度分别在  $28 \sim 35$ 、 $16 \sim 25$  及  $-5 \sim 8$  °C, 采样时尽量避免降水与温差的影响; 使用提前消毒和反复冲洗后的 10 L 聚乙烯塑料桶于采样点河流中央地带采样, 避免河岸沉积物对水样的污染. 每个采样点在距离表层水体 1 m 的位置取 3 个平行样品, 取后避光用冰袋保存运送到实验室尽快完成分析<sup>[18]</sup>.

### 1.3 细菌检测

利用异养菌平板计数法来测定不同水样中可培养总细菌 (不添加抗生素 R2A 琼脂培养基<sup>[19]</sup>)、可培养 SMZ 抗性细菌 (添加  $64 \text{ mg} \cdot \text{L}^{-1}$  磺胺甲噁唑 R2A 琼



图 1 黄河中下游某城市段取样点分布示意

Fig. 1 Distribution of sampling sites in a city in the middle and lower reaches of the Yellow River

脂培养基) 和可培养 AMX 抗性细菌 (添加  $16 \text{ mg} \cdot \text{L}^{-1}$  阿莫西林 R2A 琼脂培养基)<sup>[20,21]</sup> 的数量. 筛选出单菌落后, 保存在 15% 的甘油中, 委托上海博尚生物公司测序.

### 1.4 抗性基因检测

#### 1.4.1 水样 DNA 的提取

本实验中所涉及实验材料事先置于灭菌器中高压灭菌, 将不同采样点采集的水样用真空泵抽滤, 利用  $0.22 \mu\text{m}$  孔径的醋酸纤维素滤膜收集水中细菌, 使用 E. Z. N. A Soil DNA Kit (Omega 美国) 试剂盒提取样品 DNA.

#### 1.4.2 PCR 反应

PCR 反应体系 ( $50 \mu\text{L}$ ) 包括 Ex Taq 酶  $0.125 \mu\text{L}$ ,  $10 \times$  Ex Taq Buffer ( $20 \text{ mmol} \cdot \text{L}^{-1} \text{ Mg}^{2+}$  Plus)  $2.5 \mu\text{L}$ , dNTP Mixture ( $2.5 \text{ mmol} \cdot \text{L}^{-1}$ )  $2.0 \mu\text{L}$ , 模板 DNA  $2.0 \mu\text{L}$ , 上下游引物各  $1.0 \mu\text{L}$ , ddH<sub>2</sub>O  $16.375 \mu\text{L}$ . 反应程序为: 预变性 ( $94^\circ\text{C}$ , 5 min)、变性 ( $94^\circ\text{C}$ , 30 s)、退火 (30 s)、延伸 ( $72^\circ\text{C}$ , 30 s)、循环 (36 次) 和延伸 ( $72^\circ\text{C}$ , 7 min). 相关引物序列及相关信息如表 1 所示.

表 1 目标引物序列  
Table 1 Target primer sequence

目标基因	引物名称	引物序列 (5'-3')	扩增片段	文献
<i>sulI</i>	<i>sulI</i> -F	CGCACCGGAAACATCGCTGCAC	163	[22]
	<i>sulI</i> -R	TGAAGTCCGCCCGCAAGGCTCG		
<i>sulIII</i>	<i>sulIII</i> -F	TCCGGTGGAGGCCGGTATCTGG	191	[22]
	<i>sulIII</i> -R	CGGGAATGCCATCTGCCTTGAG		
<i>bla<sub>CTX-M</sub></i>	<i>bla<sub>CTX-M</sub></i> -F	ATGTGCAGYACCAGTAARGTKATGGC	300	[23]
	<i>bla<sub>CTX-M</sub></i> -R	ATCACKCGGRTCCGCCXGGRAT		
<i>intI1</i>	<i>intI1</i> -F	GGCTTCGTGATGCCTGCTT	146	[24]
	<i>intI1</i> -R	CATTCCTGGCCGTGTTCT		
16S rDNA	1369F	CGGTGAATACGTTTCYCGG	143	[23]
	1492R	GGWTACCTTGTTACGACTT		

1.4.3 抗性基因定量检测及计算

本文利用荧光定量PCR技术(qPCR)对目标基因进行定量检测. 荧光定量PCR反应体系(20 μL)包括SYBR Green PCR混合染料 10 μL, 上下游引物各 0.8 μL, 模板DNA 2 μL, ddH<sub>2</sub>O 6.4 μL. 反应程序设定为: 预变性(95℃, 5 min)、变性(95℃, 15 s)、退火(20 s)、循环(45次). 利用产物的溶解曲线验证样品扩增特异性.

将携带目标基因的质粒梯度稀释 10 倍, 绘制 qPCR 标准曲线. 相关目标基因的标准曲线和扩增率如表 2 所示.

表 2 目标基因定量所需的标准曲线与扩增效率<sup>1)</sup>

Table 2 Standard curve and amplification efficiency of target genes for qPCR

目标基因	标准曲线	R <sup>2</sup>	扩增效率/%
<i>sulI</i>	$y = -3.502 2x + 40.64$	0.993 2	95
<i>sulII</i>	$y = -3.314 3x + 43.54$	0.992 0	100
<i>bla<sub>CTX-M</sub></i>	$y = -3.513 4x + 46.68$	0.995 0	92
<i>intI1</i>	$y = -3.436 7x + 48.96$	0.997 4	95
16S rDNA	$y = -3.544 3x + 36.78$	0.997 8	92

1) y 为检测 Ct 值, x 为细胞拷贝数的 lg 值

水环境样品中抗性基因的量可用绝对丰度和相对丰度按公式(1)和公式(2)计算:

$$\text{水样抗性基因的绝对丰度} (\text{copies} \cdot \text{mL}^{-1}) = \frac{\text{模板拷贝数} (\text{copies} \cdot \mu\text{L}^{-1}) \times \text{DNA模板总量} (\mu\text{L})}{\text{水样体积} (\text{mL})} \quad (1)$$

$$\text{水样抗性基因的相对丰度} = \frac{\text{水样中抗性基因的绝对丰度}}{\text{16S rDNA的绝对丰度}} \quad (2)$$

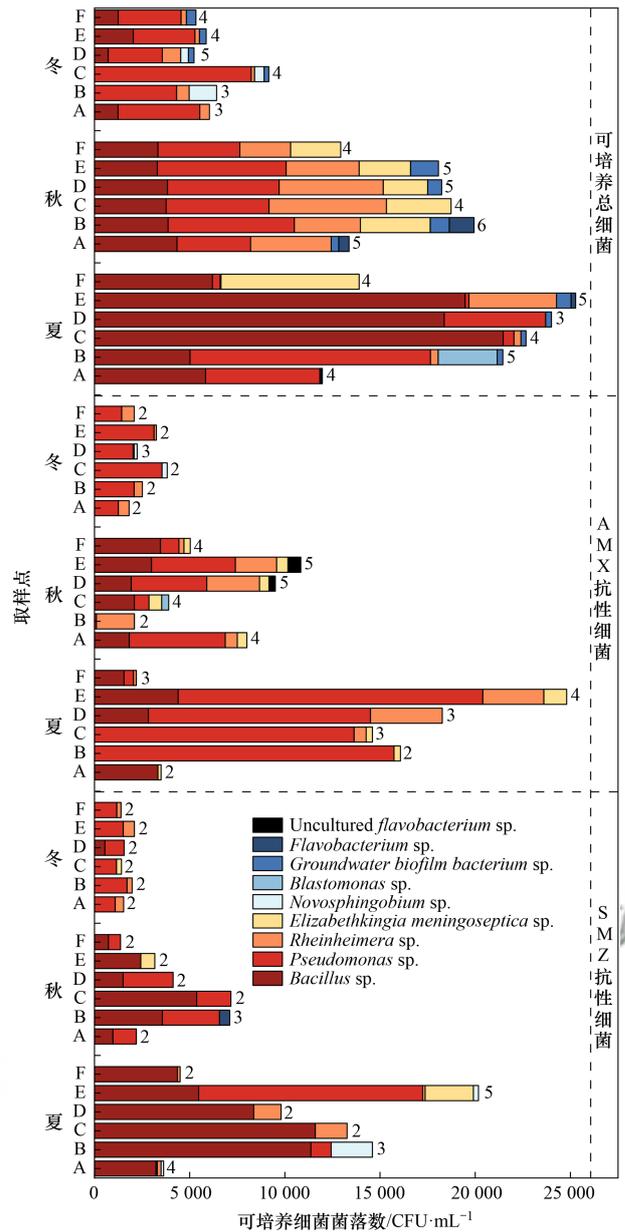
2 结果与讨论

2.1 典型抗性细菌时空分布特征

2.1.1 典型抗性细菌时间分布特征

不同季节黄河中下游某市黄河沿程取样点中可培养总细菌、AMX 抗性细菌和 SMZ 抗性细菌数量及分布状况如图 2 所示. 可培养总细菌、AMX 抗性细菌和 SMZ 抗性细菌数量在该市水体中具有明显的季节性特征, 随着温度降低(夏季到冬季), 各取样点 3 种菌的菌属数与菌落数量均呈现下降趋势, 表明温度下降对抗性细菌活性影响较大.

如表 3 所示, 随温度降低, 可培养总细菌数量逐渐减少, 由夏季的  $1.99 \times 10^4$  CFU·mL<sup>-1</sup> 降低至冬季的  $0.63 \times 10^4$  CFU·mL<sup>-1</sup>, 数量减少 68% 以上, 但菌属数始终维持在 3~6 种之间, 夏季总细菌菌属以芽孢杆菌属(*Bacillus*)和假单胞菌属(*Pseudomonas*)为主, 随着温度下降, 秋季芽孢杆菌属丰度由 64.03% 下降至



柱状图右侧数字表示各取样点检测到的不同菌属的数量

图 2 黄河水各季沿程可培养细菌分布

Fig. 2 Distribution of culturable bacteria along the Yellow River in different seasons

表 3 各季可培养细菌菌落数平均值/CFU·mL<sup>-1</sup>

Table 3 Mean number of culturable bacterial colonies in each season/CFU·mL<sup>-1</sup>

类型	夏季	秋季	冬季
总细菌	$2.08 \times 10^4$	$1.70 \times 10^4$	$6.35 \times 10^3$
AMX 抗性细菌	$1.38 \times 10^4$	$7.03 \times 10^3$	$2.63 \times 10^3$
SMZ 抗性细菌	$1.12 \times 10^4$	$4.19 \times 10^3$	$1.66 \times 10^3$

13.78%, 假单胞菌属、莱茵海默氏菌属(*Rheinheimera*)和脑膜脓毒性菌(*Elizabethkingia meningoseptica*)占比上升, 冬季时假单胞菌属成为主要菌属(68.94%). AMX 抗性细菌丰度受温度影响较大, 由夏季到冬季, 其平均丰度由  $1.32 \times 10^4$  CFU·

mL<sup>-1</sup>降低至  $0.26 \times 10^4$  CFU·mL<sup>-1</sup>, 下降 80% 以上. 同时, 平均菌属数量由 3 种下降为 2 种. 夏季菌属以芽孢杆菌属 (15.25%)、假单胞菌属 (72.43%) 和莱茵海默氏菌属 (9.76%) 为主, 冬季低温下以假单胞菌属为主要菌属, 占比超过 85%. SMZ 抗性细菌丰度变化与 AMX 抗性细菌相类似, 平均丰度随着温度降低, 由  $1.10 \times 10^4$  CFU·mL<sup>-1</sup> 降低至  $0.17 \times 10^4$  CFU·mL<sup>-1</sup>, 芽孢杆菌属逐渐减少, 假单胞菌属成为主要菌属. 以上结果表明温度对可培养总细菌、AMX 抗性细菌和 SMZ 抗性细菌具有较强选择性, 假单胞菌属抗性细菌具有良好的低温抵抗能力.

从检测结果看, AMX 抗性细菌数在总体上高于 SMZ 抗性细菌, 这一现象可能是由于华东地区  $\beta$ -内酰胺类抗生素使用量远高于磺胺类<sup>[25]</sup>. 徐艳等<sup>[26]</sup>报道石家庄汪洋沟地区地表水总细菌和磺胺类抗性细菌丰度分别在  $8.40 \times 10^3 \sim 2.71 \times 10^5$  CFU·mL<sup>-1</sup> 和  $5.33 \times 10^1 \sim 8.32 \times 10^4$  CFU·mL<sup>-1</sup> 之间; 欧丹云等<sup>[27]</sup>研究九龙江下游流域四环素与氟甲砜霉素抗性细菌丰度在  $8.41 \times 10^3 \sim 1.14 \times 10^5$  cells·mL<sup>-1</sup> 及  $1.61 \times 10^2 \sim 5.88 \times 10^2$  cells·mL<sup>-1</sup> 之间; Moore 等<sup>[28]</sup>研究北爱尔兰 Lagan River 中四环素和  $\beta$ -内酰胺类等 12 种抗生素抗性细菌丰度在  $10^{3.8} \sim 10^{4.3}$  CFU·mL<sup>-1</sup> 之间. 对比可知, 该市黄河水中总细菌与 SMZ 抗性细菌的赋存水平处于中等水平.

### 2.1.2 典型抗性细菌空间分布特征

沿程条件下黄河中下游某市黄河沿程取样点中抗性细菌数量变化及分布状况如图 2 所示. 各季节中 3 类细菌数量从 A~F 点检测的沿程菌落数具有明显的区域特征, 即沿河水流向呈现先上升后下降趋势. 从图 2 可知, A 和 F 点的 3 类细菌菌属数量最低, 而 E 点 3 类细菌菌属数量最高. 可培养总细菌年平均菌落数的低值区出现在 A 点, 可培养总细菌数为  $1.05 \times 10^4$  CFU·mL<sup>-1</sup>, 沿流向到取样点 C 可培养总细菌数量上升至  $1.68 \times 10^4$  CFU·mL<sup>-1</sup>, 随后在 F 点下降至  $1.07 \times 10^4$  CFU·mL<sup>-1</sup>. 该市黄河中 AMX 抗性细菌与 SMZ 抗性细菌年平均菌落数变化趋势与可培养总细菌相似, 在 E 点达到最高值, F 点取得最低值. 由图 1 可知, A 点和 F 点位于该市郊区, 人口较稀疏, 而 E 点位于人口密集的城市下游, 受人类活动干扰较大. 以上数据表明, 抗性细菌的空间分布特征受人类活动影响显著.

## 2.2 典型抗性基因时空分布特征

### 2.2.1 典型抗性基因绝对丰度变化特征

为避免纯培养方法的局限性, 对黄河中两种磺胺类抗性基因 (*sulI*、*sulII*), 一种  $\beta$ -内酰胺类抗性基因 (*bla<sub>CTX-M</sub>*) 和一种整合酶基因 (*intI1*) 进行了定量检测, 结果如图 3 所示.

4 种基因绝对丰度的变化在总体上仍呈由夏至冬逐渐衰减的趋势, 这与 Luo 等<sup>[24]</sup>报道的抗性基因季节变化规律是一致的. 磺胺类抗性基因受温度变化影响较大, 夏季各取样点 *sulI* 和 *sulII* 丰度平均值为  $2.07 \times 10^5$  copies·mL<sup>-1</sup> 和  $8.75 \times 10^4$  copies·mL<sup>-1</sup>, 到冬季 *sulI* 和 *sulII* 丰度平均值仅剩  $3.63 \times 10^3$  copies·mL<sup>-1</sup> 和  $4.98 \times 10^2$  copies·mL<sup>-1</sup>, 下降率达 98.25% 和 99.43%. *bla<sub>CTX-M</sub>* 与 *intI1* 基因同样遵循上述规律, 但温度对  $\beta$ -内酰胺类抗性基因和 I 型整合子 *intI1* 的影响不如磺胺类抗性基因明显, *intI1* 在第三季里总平均丰度最高, 达  $2.47 \times 10^5$  copies·mL<sup>-1</sup>, 这可能与整合子具有广泛的抗生素抗性有关<sup>[29]</sup>. 徐秋鸿等<sup>[14]</sup>对夏季长江口近岸地区水样进行了研究, 结果表明水样中抗性基因的绝对丰度平均值为  $2.32 \times 10^4 \sim 7.66 \times 10^4$  copies·mL<sup>-1</sup>. 张丹丹等<sup>[30]</sup>研究了敖江下游各类抗生素抗性基因的分布特征, 目的基因绝对丰度介于  $7.7 \times 10^4 \sim 1.5 \times 10^6$  copies·mL<sup>-1</sup> 之间. Na 等<sup>[31]</sup>对北黄海中 *sulI*, *sulII* 进行了表征, 其丰度范围在  $3.3 \times 10^4 \sim 3.55 \times 10^4$  copies·mL<sup>-1</sup> 之间. 胡亚茹等<sup>[32]</sup>检测到华东地区某饮用水源地中 *sulI* 和 *sulII* 的平均丰度分别为  $2.10 \times 10^5$  copies·mL<sup>-1</sup> 和  $1.70 \times 10^4$  copies·mL<sup>-1</sup>. 上述长江、海河流域人口密集, 抗生素使用和排放量<sup>[25]</sup>, 致使其抗性基因污染较为严重, 而该市黄河流域抗性基因污染则处于国内一般水平.

从空间角度分析, 各抗性基因沿流向呈先降低后上升趋势. 上游段 (A 点和 B 点) *sulI*、*sulII*、*bla<sub>CTX-M</sub>* 和 *intI1* 在夏秋中的丰度平均值相对较高, 分别为  $9.87 \times 10^4$ 、 $5.04 \times 10^4$ 、 $1.46 \times 10^5$  和  $4.37 \times 10^5$  copies·mL<sup>-1</sup>, 该段是农村禽、畜、渔养殖密集区, 且夏秋季为畜禽疫病防治重点时期, 抗生素类药物使用量大且面源污染严重, 可能是加重该段河流中抗性基因富集的主要原因<sup>[33-35]</sup>; 中游段 (C 点和 D 点) 上述 4 种基因有所下降; 城市下游 E 点受城市人类活动影响其抗性基因丰度再次上升, 达到最高值  $2.48 \times 10^5$ 、 $1.18 \times 10^5$ 、 $1.65 \times 10^5$  和  $4.50 \times 10^4$  copies·mL<sup>-1</sup>; 在远离城市的 F 点, 抗性基因恢复到低丰度水平. 上述分析表明: 水环境中整合子和抗性基因的赋存水平与人类活动、城市开发及抗生素释放密切相关<sup>[28,36-38]</sup>.

### 2.2.2 典型抗性基因相对丰度变化特征

抗性基因相对丰度可更好地反映各抗性基因的分布情况, *sulI*、*sulII* 和 *bla<sub>CTX-M</sub>* 的相对丰度计算结果如图 4 所示. 随着温度降低, 3 种抗性基因相对丰度均呈先减少后增多趋势. 夏季 *bla<sub>CTX-M</sub>* 抗性基因相对丰度为  $1.53 \times 10^{-3}$ , 秋季略有下降, 但冬季相对丰度迅速上升至  $1.36 \times 10^{-2}$ , 表明 AMX 抗性细菌对低温具有较强适应性, 导致该类抗性基因大量赋存. 磺胺

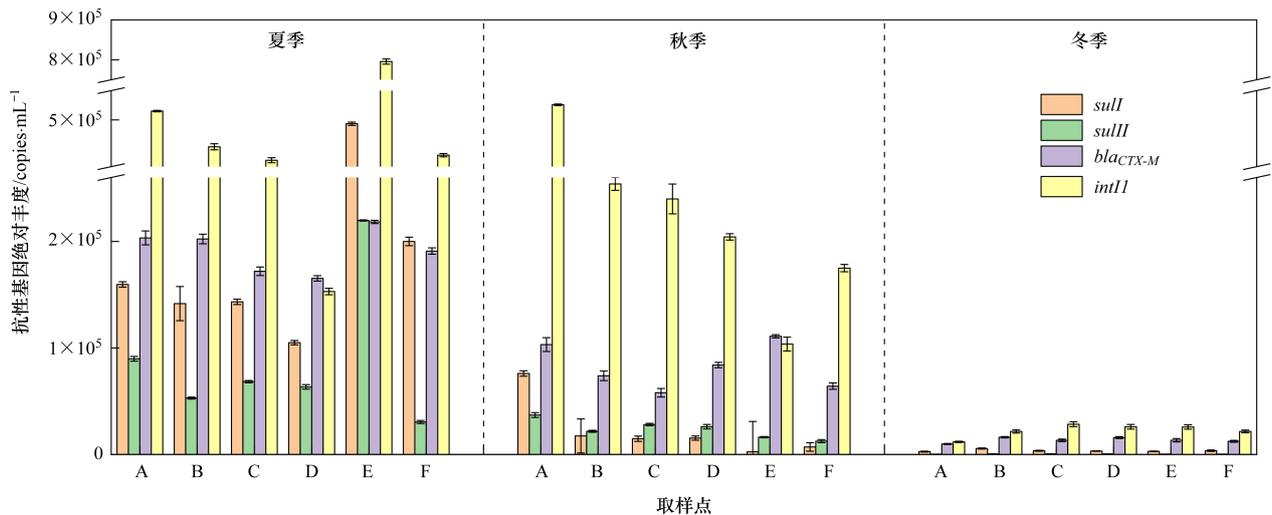


图3 黄河水沿程抗性基因绝对丰度

Fig. 3 Absolute abundance of antibiotic resistance genes along the Yellow River

类抗性基因主要以 *sulI* 为主, 夏季 *sulI* 相对丰度均值为  $1.50 \times 10^{-3}$ , 而 *sulII* 仅为  $6.09 \times 10^{-4}$ . 冬季时两者增至  $3.66 \times 10^{-3}$  和  $4.82 \times 10^{-4}$  水平, 含量差距更为明显. 冬季该市黄河流域进入枯水期, 水面结冰导致常规方法检测不到的厌氧菌与异养菌大量繁殖<sup>[39]</sup>, 可能是造成冬季 *bla<sub>CTX-M</sub>* 与 *sulI* 的平均相对丰度高于夏秋季的原因.

3类抗性基因相对丰度随沿程总体呈现先下降后上升的趋势. 其中, 上游(A点和B点)的高相对量与养殖业抗生素的集中使用有关, 而城市中心采样

点(C点和D点)3类抗性基因相对丰度略微下降, 结合总细菌变化趋势, 推测人类活动导致河流中细菌大量繁殖, 进而16S rDNA检测值升高, 而沿程下游中细菌丰度下降, 相对值有所上升. 对比已有研究, 该流域段 $\beta$ -内酰胺类抗性基因相对丰度高于太湖水体<sup>[40]</sup>与黄浦江表层水体<sup>[41]</sup>. 磺胺类基因相对丰度与海河流域<sup>[24]</sup>相近, 低于北江流域<sup>[42]</sup>, 而Pruden等<sup>[3]</sup>检测科罗拉多州北部河流受磺胺类基因污染最严重的地区中 *sulI* 和 *sulII* 相对丰度仅为  $10^{-3}$  和  $10^{-4}$ . 可见国内各流域抗性基因污染问题必须予以高度重视.

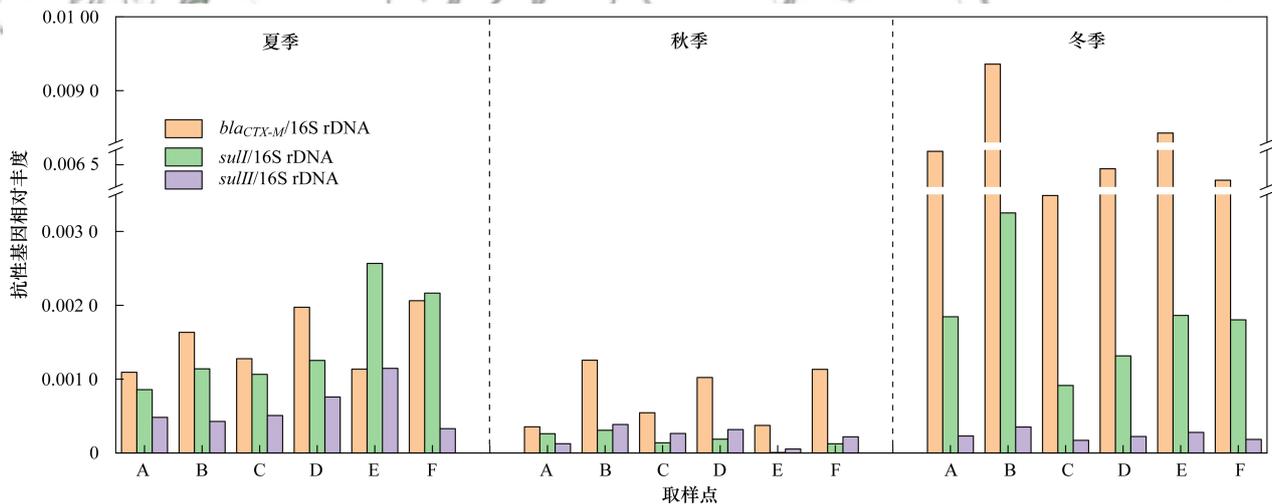


图4 黄河水沿程抗性基因相对丰度

Fig. 4 Relative abundance of antibiotic resistance genes along the Yellow River

### 2.3 I型整合子与抗性基因相关性分析

整合子是实现抗性基因水平转移的重要遗传单位<sup>[43, 44]</sup>, 本研究采用Spearman相关性检验<sup>[45]</sup>对I型整合子基因和其他抗性基因进行了相关分析, 结果如表4所示. 可知磺胺类和 $\beta$ -内酰胺类抗性基因均与 *intI1* 呈极显著相关性 ( $P < 0.01$ ), 说明整合子仍是加

速抗性基因传播的重要因素<sup>[44]</sup>. Luo等<sup>[24]</sup>对海河流域进行了磺胺类基因与I型整合子相关性分析, 结果与本研究的相同. 然而, 前人对长江口近岸地区<sup>[14]</sup>和温榆河流域<sup>[46]</sup>的分析表明 *sulII* 与 *intI1* 无显著相关性, 与本研究的结果不符. *sulI* 可能作为I型整合子3'-CS的组分, 故与 *intI1* 具有更强的相关性<sup>[47]</sup>. 除整

合子外, *sulII* 和  $\beta$ -内酰胺酶基因的水平播散还与 ISCR 元件耦合, 而 ISCR 结构使得抗性基因更容易在质粒上整合, 导致抗性基因的加速传播<sup>[48]</sup>.

表 4 I 型整合子与抗性基因相关性分析<sup>1)</sup>

Table 4 Correlation analysis between *intI1* and antibiotic resistance genes

类型	项目	<i>sulI</i>	<i>sulII</i>	<i>bla<sub>CTX-M</sub></i>
黄河水	<i>r</i>	0.851**	0.891**	0.853**
	<i>P</i>	< 0.01	< 0.01	< 0.01

1) *r* 表示相关系数; \*\* 表示极显著性差异, *P* < 0.01

### 3 结论

(1) 该市黄河流域夏秋冬三季中可培养总细菌、AMX 抗性细菌、SMZ 抗性细菌在菌属组成与数量变化上均有明显的时空分布特征, 这是由于温度变化和人类活动影响造成的。

(2) 该流域段抗性基因污染情况处于一般水平, 其中  $\beta$ -内酰胺类抗性基因 (*bla<sub>CTX-M</sub>*) 的检出丰度明显高于磺胺类抗性基因, 磺胺类抗性基因中 *sulI* 为优势基因。

(3) 相关性分析表明, 磺胺类抗性基因 (*sulI*、*sulII*) 和  $\beta$ -内酰胺类抗性基因 (*bla<sub>CTX-M</sub>*) 均与 *intI1* 具有显著相关性 (*P* < 0.01), 表明整合子基因仍是加速抗性基因传播的重要因素。

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## CONTENTS

Impacts of Anthropogenic Emission Reduction on Urban Atmospheric Oxidizing Capacity During the COVID-19 Lockdown	ZHU Jian-lan, QIN Mo-mei, ZHU Yan-hong, <i>et al.</i>	( 617 )
Transport and Potential Sources Regions of Double High Pollution in Nanjing by Different Synoptic Situations	QIN Yang, HU Jian-lin, KONG Hai-jiang	( 626 )
Differences of Three Methods in Determining Ozone Sensitivity in Nanjing	CHEN Gan-yu, LI Xun, LI Lin, <i>et al.</i>	( 635 )
Characteristics of Ozone Concentration in Shanghai and Its Associated Atmospheric Circulation Background During Summer Half-years from 2006 to 2021	ZHENG Qing-feng, LIANG Ping, DUAN Yu-sen, <i>et al.</i>	( 645 )
Distribution Characteristics of Near Surface Ozone Volume Fraction in Shanxi Province Based on Atmospheric Composition Observation Network	LI Ying, WANG Shu-min, PEI Kun-ning, <i>et al.</i>	( 655 )
Photochemical Mechanism and Control Strategy Optimization for Summertime Ozone Pollution in Yining City	WANG Wen-ting, GU Chao, LI Li-ming, <i>et al.</i>	( 668 )
Characteristics, Sources, and Ozone-sensitive Species of VOCs in Four Seasons in Yuncheng	YIN Shi-jie, LIU Xin-gang, LIU Ya-fei, <i>et al.</i>	( 678 )
Pollution Characteristics, Source Analysis, and Activity Analysis of Atmospheric VOCs During Winter and Summer Pollution in Zhengzhou	LAI Meng-jie, ZHANG Dong, YU Shi-jie, <i>et al.</i>	( 689 )
Pollution Characteristics, Sources, and Secondary Generation of Organic Acids in PM <sub>2.5</sub> in Zhengzhou	LI Zi-han, DONG Zhe, SHANG Lu-qi, <i>et al.</i>	( 700 )
Nonlinear Variations in PM <sub>2.5</sub> Concentration in the Three Major Urban Agglomerations in China	WU Shu-qi, GU Yang-yang, ZHANG Tian-yue, <i>et al.</i>	( 709 )
Medium and Long-term Carbon Emission Projections and Emission Reduction Potential Analysis of the Lingang Special Area Based on the LEAP Model	WU Qiong, MA Hao, REN Hong-bo, <i>et al.</i>	( 721 )
Dynamic Analysis on Carbon Metabolism of the Northern Region of China Under the Background of Carbon Emission Trading Policy	ZHENG Hong-mei, SHEN Fang, XU Guang-yao, <i>et al.</i>	( 732 )
Carbon Reduction Analysis of Life Cycle Prediction Assessment of Hydrogen Fuel Cell Vehicles: Considering Regional Features and Vehicle Type Differences	MA Jing, CAI Xu, ZHANG Chun-mei, <i>et al.</i>	( 744 )
Nitrate Pollution Characteristics and Its Quantitative Source Identification of Major River Systems in China	WEI Ying-huai, HU Min-peng, CHEN Ding-jiang	( 755 )
Effects of Land Use Structure and Spatial Pattern at Different Temporal and Spatial Scales on Water Quality in Suzhou Creek	TAN Juan, XIONG Li-jun, WANG Qing, <i>et al.</i>	( 768 )
Spatial-temporal Variation in Water Quality of Rain-source Rivers in Shenzhen from 2015 to 2021 and Its Response to Rainfall	WEI Bi-ying, CHENG Jian-mei, SU Xiao-yu, <i>et al.</i>	( 780 )
Chemical Characteristics of Shallow Groundwater in the Yellow River Diversion Area of Henan Province and Identification of Main Control Pollution Sources	WANG Shuai, REN Yu, GUO Hong, <i>et al.</i>	( 792 )
Chemical Characteristics and Genetic Analysis of Karst Groundwater in the Beijing Xishan Area	GUO Gao-xuan, DAI Yin-dong, XU Liang, <i>et al.</i>	( 802 )
Hydrochemical Characteristics and Its Origin of Surface Water and Groundwater in Dianbu River Basin	ZHENG Tao, QIN Xian-yan, WU Jian-xiong	( 813 )
Hydrochemical Characteristics and Genesis Mechanism of Groundwater in the Dry Period in the Zhangjiakou Area	JIN Ai-fang, YIN Xiu-lan, LI Chang-qing, <i>et al.</i>	( 826 )
Distribution of Typical Resistant Bacteria and Resistance Genes in Source Water of the Middle and Lower Reaches of the Yellow River	MIN Wei, GAO Ming-chang, SUN Shao-fang, <i>et al.</i>	( 837 )
Contamination Characteristics, Detection Methods, and Control Methods of Antibiotic Resistance in Pharmaceutical Wastewater	PENG An-ping, GAO Hu, ZHANG Xin-bo	( 844 )
Effect of Water Components on Aggregation and Sedimentation of Polystyrene Nano-plastics	TANG Duan-yang, ZHENG Wen-li, CHEN Guan-tong-yi, <i>et al.</i>	( 854 )
Lead Removal from Water by Calcium-containing Biochar with Saturated Phosphate	LIU Tian, LÜ Si-lu, DU Xing-guo, <i>et al.</i>	( 862 )
Preparation of Chitosan-modified Biochar and Its Adsorption Mechanism for Cd <sup>2+</sup> in Aqueous Solution	JIANG Ling, AN Jing-yue, YUE Xiao-qiong, <i>et al.</i>	( 873 )
Efficacy and Mechanism of Tetracycline Adsorption by Boron-doped Mesoporous Carbon	ZOU Zhen, XU Lu, QIAO Wei, <i>et al.</i>	( 885 )
Adsorption Properties of Magnetic Phosphorous Camellia Oleifera Shells Biochar to Sulfamethoxazole in Water	HAN Shuai-peng, TANG Li-wen, LIU Qin, <i>et al.</i>	( 898 )
High Resolution Emission Inventory of Greenhouse Gas and Its Characteristics in Guangdong, China	LU Qing, TANG Ming-shuang, LIAO Tong, <i>et al.</i>	( 909 )
Ecosystem CO <sub>2</sub> Exchange and Its Environmental Regulation of a Restored Wetland in the Liaohe River Estuary	LIU Si-qi, CHEN Hong, XING Qing-hui, <i>et al.</i>	( 920 )
Effects of Biochar Application Two Years Later on N <sub>2</sub> O and CH <sub>4</sub> Emissions from Rice-Vegetable Rotation in a Tropical Region of China	HU Yu-jie, TANG Rui-jie, HU Tian-yi, <i>et al.</i>	( 929 )
Research and Application Progress of Biochar in Amelioration of Saline-Alkali Soil	WEI Ying, JIAO Le, ZHANG Peng, <i>et al.</i>	( 940 )
Effect of No-tillage on Soil Aggregates in Farmland: A Meta Analysis	XU Yi-ping, RAO Yue-yue, MENG Yan, <i>et al.</i>	( 952 )
Spatio-temporal Evolution and Multi-scenario Simulation of Carbon Storage in Karst Regions of Central Guizhou Province: Taking Puding County as an Example	LI Yue, LUO Hong-fen	( 961 )
Effects of Different Modifiers on Aggregates and Organic Carbon in Acidic Purple Soil	LI Yue, XU Man, XIE Yong-hong, <i>et al.</i>	( 974 )
Effect of Ca Modified Biochar on the Chemical Speciation of Soil Phosphorus and Its Stabilization Mechanism	ZHANG Chao, ZHAI Fu-jie, SHAN Bao-qing	( 983 )
Plant Diversity Changes and Its Driving Factors of Abandoned Land at Different Restoration Stages in the Middle of the Qinling Mountains	YAN Cheng-long, XUE Yue, WANG Yi-fei, <i>et al.</i>	( 992 )
Contamination Characteristics and Ecological Risk of Antibiotics in Contaminated Sites of Typical Pharmaceutical Factories in China	YANG Jiong-bin, HUANG Zheng, ZHAO Jian-liang, <i>et al.</i>	( 1004 )
Pollution Characteristics and Risk Assessment of Polycyclic Aromatic Hydrocarbons in Soils of Guangzhou	ZOU Zi-hang, CHEN Lian, ZHANG Pei-zhen, <i>et al.</i>	( 1015 )
Quantifying the Contribution of Soil Heavy Metals to Ecological and Health Risk Sources	PAN Yong-xing, CHEN Meng, WANG Xiao-tong	( 1026 )
Potential Ecological Risk Characteristics and Source Apportionment of Heavy Metals in Farmland Soils around Typical Factories in Hunan Province Based on Monte-Carlo Simulation	LUO Hao-jie, PAN Jun, CHEN Xiao-xia, <i>et al.</i>	( 1038 )
Health Risk Assessment for an Arsenic-contaminated Site Based on Monte Carlo Simulation and Parameters Optimization	YUAN Bei, LIU Hu-peng, DU Ping, <i>et al.</i>	( 1049 )
Pollution Source Apportionment of Heavy Metals in Cultivated Soil Around a Red Mud Yard Based on APCS-MLR and PMF Models	SHEN Zhi-jie, LI Jie-qin, LI Cai-xia, <i>et al.</i>	( 1058 )
Characteristics and Mechanism of Cd Release and Transport in Soil Contaminated with PE-Cd	WANG Di, XU Shao-hui, SHAO Ming-yan, <i>et al.</i>	( 1069 )
Characterization of Reductive Dechlorination of Chlorinated Ethylenes by Anaerobic Consortium	LI Wei, LIU Gui-ping, LIU Jun, <i>et al.</i>	( 1080 )
Analysis of Heavy Metal Pollution Evaluation and Correlation of Farmland Soil and Vegetables in Zhaotong City	ZHANG Hao, DONG Chun-yu, YANG Hai-chan, <i>et al.</i>	( 1090 )
Safe Utilization Effect of Passivator on Mild to Moderate Cadmium Contaminated Farmland	WANG Xiao-jing, ZHANG Dong-ming, CAO Yang, <i>et al.</i>	( 1098 )
Simultaneous Immobilization of Cadmium and Arsenic in Paddy Soils with Novel Fe-Mn Combined Graphene Oxide	YUAN Jing, WU Ji-zi, LIAN Bin, <i>et al.</i>	( 1107 )
Effects of the Application of Irrigation Water Containing Zn at the Key Growth Period on the Uptake and Transport of Cd in Rice	ZHOU Xia, HU Yu-dan, ZHOU Hang, <i>et al.</i>	( 1118 )
Effects of Exogenous Zinc on Growth and Root Architecture Classification of Maize Seedlings Under Cadmium Stress	ZHANG Hui-hong, WEI Chang, LIU Hai-tao, <i>et al.</i>	( 1128 )
Mitigative Effect of Rare Earth Element Cerium on the Growth of Zinc-stressed Wheat ( <i>Triticum aestivum</i> L.) Seedlings	ZHANG Jing-jing, XU Zheng-yang, JIAO Qiu-juan, <i>et al.</i>	( 1141 )
Two-stage Inhibition Effects of <i>Burkholderia</i> sp. Y4 Application on Cadmium Uptake and Transport in Wheat	GUO Jia-jia, WANG Chang-rong, LIU Zhong-qi, <i>et al.</i>	( 1150 )
Effects of Combined Stress of High Density Polyethylene Microplastics and Chlorimuron-ethyl on Soybean Growth and Rhizosphere Bacterial Community	HU Xiao-yue, HUA Zi-wei, YAO Lun-guang, <i>et al.</i>	( 1161 )
Human Accumulation and Toxic Effects of Microplastics: A Critical Review	BAO Ya-bo, WANG Cheng-chen, PENG Wu-guang, <i>et al.</i>	( 1173 )
Overview of the Application of Machine Learning for Identification and Environmental Risk Assessment of Microplastics	BAI Run-hao, FAN Rui-qi, LIU Qi, <i>et al.</i>	( 1185 )
Research Process on the Combined Pollution of Microplastics and Typical Pollutants in Agricultural Soils	HOU Yu-qing, LI Bing, WANG Jin-hua, <i>et al.</i>	( 1196 )
Research Progress in Electrochemical Detection and Removal of Micro/Nano Plastics in Water	ZHENG Wei-kang, LIU Zhen-zhong, XIANG Xiao-fang	( 1210 )
Factors Influencing Willingness of Farmers to Pay for Agricultural Non-point Source Pollution Control Based on Distributed Cognitive Theory	GUO Chen-hao, LI Lin-fei, XIA Xian-li	( 1222 )