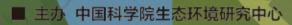


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**ENVIRONMENTAL SCIENCE** 

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不同国家农用地土壤环境标准比较与启示 李勖之,姜瑢,王国庆,陈玉东,龙涛,林玉锁



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# 德兴铜矿区抗生素抗性基因污染特征及其驱动因子

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摘要:环境抗生素抗性基因(ARGs)污染作为一类新型污染物受到广泛关注.本研究采用高通量荧光定量 PCR(HT-qPCR)技术,研究了江西省德兴铜矿矿区周边 7 个采样点 ARGs 和可移动遗传元件(MGEs)的种类、丰度和影响因素.结果表明,德兴铜矿区具有多样的 ARGs,个别点位 ARGs 最大检出数达 70 种.在相对丰度水平上,个别点位 ARGs 相对丰度达到 0.085. ARGs 与 MGEs 相对丰度呈显著正相关(P<0.05), TnpAO4 和 Intil 是德兴铜矿最主要的 MEGs,推测水平基因转移是采样区环境 ARGs 传播的重要机制. Pearson 相关性分析和 RDA 分析结果表明,重金属 Cu 含量与 ARGs 检出种类和丰度均呈显著正相关关系(P<0.05),提示德兴铜矿区 Cu 的高含量存在可能是该区域 ARGs 形成的重要驱动因子.

关键词:德兴铜矿;重金属;抗生素抗性基因(ARGs);驱动因子;高通量荧光定量 PCR(HT-qPCR)

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# Pollution Characteristics and Driving Factors of Antibiotic Resistance Genes in Dexing Copper Mine

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Abstract: Environmental antibiotic resistance genes (ARGs) are a type of emerging pollutant that has been widely concerning. However, investigations into the contamination of ARGs in mining areas have been scarce. Here, the types, abundances, and influencing factors of ARGs and mobile genetic elements (MGEs) were investigated in soil/sediment of the Dexing copper mine area in June 2019 by using high-throughput quantitative polymerase chain reaction (HT-qPCR). Furthermore, the influence of heavy metals and MGEs factors on ARGs was studied using the multivariate statistical analysis method. The results showed that there were a variety of ARGs in the Dexing copper mining area, and the maximum detected number of ARGs was 70. At the relative abundance level, the relative abundance of individual sites reached 0.085. In the Dexing copper mine, multidrug, MLSB,  $\beta$ -lactamases, tetracycline, and aminoglycoside resistance genes were the dominant ARG classes based on their numbers. The efflux pump was the most dominant resistance mechanism, followed by antibiotic deactivation and cellular protection. There was a significant positive correlation between the abundance of ARGs and MGEs (P < 0.05), and TnpA04 and Inti1 were the most important MEGs in Dexing copper mine samples, indicating that horizontal gene transfer might be an important mechanism for the spread of environmental ARGs. The results of Pearson correlation analysis and RDA analysis showed that the content of Cu was significantly positively correlated with the detected numbers and abundance of ARGs (P < 0.05), suggesting that the high content of Cu in the Dexing copper mining area might be an important driving factor for the formation of ARGs.

Key words; Dexing copper mine; heavy metals; antibiotic resistance genes (ARGs); driving factors; high-throughput quantitative polymerase chain reaction (HT-qPCR)

早在 1943 年,青霉素就被引入临床治疗. 到目前为止,已有数千种抗生素被应用于医疗、水产养殖和动植物生产等领域. 然而,由于抗生素的不合理应用导致的细菌耐药性增强和抗生素抗性基因(antibiotic resistance genes, ARGs)加速传播日益严重<sup>[1]</sup>. 全球因微生物耐药性引起的健康问题,导致每年需要花费大约 1 万亿欧元支出<sup>[2]</sup>. 预测到 2050年,每年因耐药性微生物感染致死的人数将达上千万人<sup>[3]</sup>. 2006年 Pruden等<sup>[4]</sup>的研究第一次将 ARGs作为新型环境污染物提出之后,许多学者在医疗废水<sup>[5]</sup>、养殖废水<sup>[6,7]</sup>、污水处理厂<sup>[8~10]</sup>、地下水<sup>[11]</sup>、消流海洋<sup>[12]</sup>、地表水<sup>[13]</sup>、沉积物<sup>[14~16]</sup>、土壤<sup>[17~20]</sup>和空气中<sup>[21,22]</sup>都检测到 ARGs 的存在,而一旦含有 ARGs 的菌群成为优势菌种,将对当地生态环境安全和人类健康造成极大的危害.

土壤中含有种类丰富的微生物,也是 ARGs 巨大的储存库,研究者们从远离人类活动干扰的北极、阿拉斯加冻土中提取到的 DNA 中发现存在对 β-内酰胺类、四环素类、糖肽类和氯霉素类等抗性的基因<sup>[23-25]</sup>,说明 ARGs 存在于原始的自然环境中. 林岚等<sup>[15]</sup>的研究通过分析西太平洋深海和黄海沉积物中抗性基因含量的差别,发现人类活动导致的环境变化会使微生物在面对环境选择压力时能够产生耐受性,加速 ARGs 的传播.

抗生素是环境抗生素抗性的选择压力已经成为

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普遍共识[26],而重金属近年来也作为环境微生物抗 生素抗性选择压力而被大量研究.有研究表明,土壤 中的重金属 Cu 会提高大环内酯类抗性基因[27]、四 环素类抗性基因和万古霉素抗性基因[28]的丰度,土 壤中 Zn 和 Cu 的累积可能导致磺胺类与四环素类 抗性基因的丰度增加<sup>[29]</sup>. 基因水平转移是 ARGs 在 环境中传播扩散的重要机制[30],可移动的基因元件 可通过接合、转化和转导等方式将 ARGs 从一株菌 转移到另一株菌中,从而使后者获得抗性.矿区环境 受到采矿活动影响,重金属污染较严重,目前针对矿 区土壤环境 ARGs 的相关研究还比较少,本研究通 过高通量荧光定量 PCR(HT-qPCR)技术,对亚洲最 大露天铜矿——江西省德兴铜矿周边土壤和沉积物 环境中 ARGs 和可移动元件 (mobile genetic elements, MGEs)的种类和丰度进行分析,并探究环 境重金属和 MGEs 对检出抗性基因种类和丰度的 影响.

#### 1 材料与方法

#### 1.1 研究区域概况

德兴铜矿位于江西省上饶市德兴市信江流域乐安河附近,是亚洲最大的铜矿之一<sup>[31]</sup>,也是第一大伴生金矿和伴生银矿,目前生产规模为处理矿石 10万t·d<sup>-1</sup>.德兴市素有"铜都"、"银城"和"金山"之称.自唐、宋以来,德兴铜矿区域就开始了铜矿开采,粗放式开采导致整个区域内零星遍布了数以百万方计的历史遗留酸性废渣和逾千个废弃矿洞<sup>[32]</sup>.

#### 1.2 样品采集

2019年6月,在江西省德兴市德兴铜矿区设置7个采样点,编号1~7,7个采样点布设如表1和图1所示.每个采样点采集表层(0~10 cm)土壤1 kg,装入灭菌塑料袋,放置低温箱带回实验室进行下一步处理.

#### 表 1 德兴铜矿区土壤/沉积物采样点情况

Table 1 Soil/sediment sampling sites of Dexing copper mine

10	abic i Sonz scument sampini	ig sites of Dexing copper finite
采样点	名称	经纬度
1	富家坞采矿场附近菜地土	$117^{\circ}42'42.\;17''\mathrm{E},\;29^{\circ}00'57.\;21''\mathrm{N}$
2	富家坞采矿场山脚土	$117^{\circ}42'41.03''\mathrm{E},29^{\circ}00'58.38''\mathrm{N}$
3	富家坞采矿场大坞河沉积物	117°42′41.63″E, 29°00′58.25″N
4	德兴铜矿中学附近土	$117^{\circ}42'37.3''$ E, $29^{\circ}02'16.96''$ N
5	德兴铜矿住宅小区土	$117^{\circ}41'12.74''\mathrm{E},29^{\circ}03'31.77''\mathrm{N}$
6	大坞河人乐安河口沉积物	$117^{\circ}40'29.~72''\mathrm{E},~29^{\circ}04'24.~62''\mathrm{N}$
7	德兴铜矿尾矿库尾沙	$117^{\circ}44'09.55''\mathrm{E},29^{\circ}03'41.41''\mathrm{N}$

#### 1.3 土壤重金属含量测定

称取土壤或沉积物样品 0.5 g,浓硝酸-浓盐酸-氢氟酸-高氯酸消解后, Pb 和 Cd 用石墨炉进行测 定, Cu 和 Zn 用火焰原子吸收分光光度法进行测定.

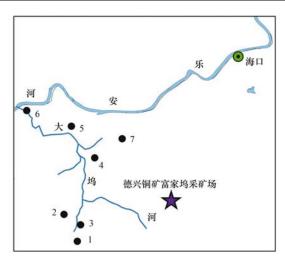


图 1 德兴铜矿区采样点分布示意

Fig. 1 Map of sampling sites of Dexing copper mine

#### 1.4 DNA 提取

将采回土样充分混匀并除去土壤中的植物根系和砾石,每个编号取 2 个平行样, -80°C 下保存至DNA 提取. 使用 FastDNA® SPIN kit for Soil 试剂盒 (MP Biomedical,美国) 提取总 DNA,实际操作参照试剂盒内说明书,提取的 DNA 采用 Nano Drop 2000 (Thermo) 进行检测. DNA 样品于 -20°C 下保存备用.

#### 1.5 ARGs 测定

利用 Wafergen SmartChip 实时 PCR 系统 (Wafergen, Fremont, CA),采用 HT-qPCR 方法,检测各样品中的 ARGs,使用了 296 对引物,包括 283 个 ARGs、12 个 MGEs 和 1 个 16S rRNA 基因. HT-qPCR 实验操作和相对基因拷贝数计算方法等见文献[10]. 将每个 ARGs 的相对基因拷贝数分别除以 16S rRNA 基因的相对基因拷贝数,得到每个 ARGs 的相对丰度.

#### 1.6 数据分析

处理 HT-qPCR 数据时,利用 OriginPro 2017 进行 ANOVA 分析、Pearson 相关性分析和热图分析 (Heatmap),利用 Rstudio 软件进行冗余分析 (RDA).

#### 2 结果与分析

#### 2.1 重金属检出含量

样品中 4 种被测重金属含量见表 2 和图 2. 7 个采样点中, Zn、Cu、Cd 和 Pb 均有超背景值的点位. 其中, 6 号点位  $\omega(Zn)$ 、 $\omega(Cu)$  和  $\omega(Cd)$  均为各点位最大值, 分别为 83. 73、630. 50 和 1. 54  $mg\cdot kg^{-1}$ ,  $\omega(Pb)$  也很高, 仅次于 5 号采样点的样品,为 36. 3  $mg\cdot kg^{-1}$ ; 6、3 和 7 号采样点  $\omega(Cu)$  分别达到 630. 50、506. 58 和 440. 99  $mg\cdot kg^{-1}$ , 分别超

背景值的 31、25 和 22 倍. 6 号采样点为大坞河人 乐安河口沉积物,3 号采样点为大坞河沉积物,7 号采样点为德兴铜矿尾矿库尾沙,德兴铜矿区这些 采样点位的 Cu 含量远超背景值,说明人为采矿活 动加剧了土壤中重金属 Cu 的污染程度,德兴铜矿 区的重金属 Cu 污染不容忽视.

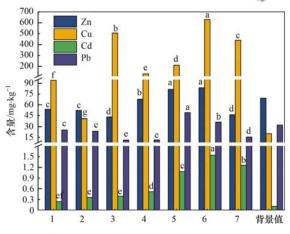
表 2 德兴铜矿区土壤/沉积物指标检测结果 $^{1)}/mg\cdot kg^{-1}$ 

Table 2 Heavy metal contents of Dexing copper

mine	sampling	sites/mg·	kg - 1
•			

	1 0	0 0		
采样点	$\omega(Zn)$	ω(Cu)	<b>ω</b> ( Cd)	<b>ω</b> ( Pb)
1	53.87c	94. 04f	0. 24ef	25. 52 c
2	52. 23 c	40. 98g	0.36e	23.67c
3	43. 27d	506. 58b	0.39e	11.41e
4	67. 85b	130.76e	0.52d	11.64e
5	81. 58a	211. 44d	1.08c	49. 11a
6	83. 73a	630. 50a	1. 54a	36. 30b
7	46. 12d	$440.99\mathrm{c}$	1. 26b	15. 68d
江西省土壤元素背景值	69. 4	20. 3	0. 108	32. 3

#### 1)不同字母小写字母表示采样点间差异显著(P<0.05)



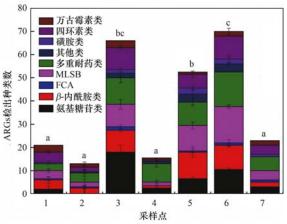
横坐标数字表示采样点;不同小写字母表示 采样点间差异显著(P<0.05)

图 2 德兴铜矿区土壤重金属指标检测结果

Fig. 2 Heavy metal contents of Dexing copper mine sampling sites

#### 2.2 ARGs 检出种类

德兴铜矿 7 个采样点中,共检测到 9 类主要类型的 ARGs,包括氨基糖苷类(aminoglycosides)、β-内酰 胺类(β-lactamases)、喹诺酮类氯霉素类(FCA)、大环内酯类林肯酰胺类链阳性菌素 B 抗性基因(MLSB)、多重耐药类(multidrug)、磺胺类(sulfonamide)、四环素类(tetracycline)、万古霉素类(vancomycin)和其他类(others).各采样点 ARGs检出种类数分别为 21、13、66、15.5、52.5、70 和23,均值为 37,检出数量显著高于其他点位的是 6、3 和 5(P < 0.05,图 3).6号采样点为大坞河入乐安河口沉积物,3号采样点为大坞河沉积物,5号采样点为德兴铜矿住宅小区土壤.6 和 3号采样点检出数量相对较高,可能与样品中 Cu 含量较高有关.



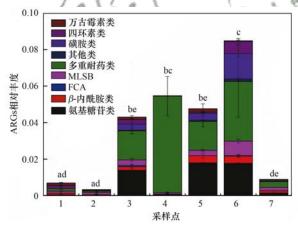
不同小写字母表示采样点间差异显著(P<0.05)

#### 图 3 德兴铜矿样品中检测到的 ARGs 种类数

Fig. 3 Number of ARGs detected in Dexing copper mine sampling sites

#### 2.3 ARGs 检出相对丰度

对德兴铜矿中 ARGs 的相对丰度水平进行分析 (图 4),结果表明,各样点中 ARGs 相对丰度范围为  $0.3\times10^{-2}\sim8.5\times10^{-2}$ ,其中 6 号采样点的 ARGs 相对丰度显著高于其他点位(P<0.05),其相对丰度达到  $8.5\times10^{-2}$ ,其次是 4.5 和 3 号采样点,其相 对丰度分别为  $5.5\times10^{-2}$ 、 $4.8\times10^{-2}$ 和  $4.3\times10^{-2}$ .



不同小写字母表示采样点间差异显著(P<0.05)

#### 图 4 德兴铜矿样品中 ARGs 相对丰度

Fig. 4 Relative abundance of ARGs in Dexing copper mine sampling sites

从 ARGs 对应的抗生素来看,德兴铜矿区检出的 ARGs 主要类别为多重耐药类、MLSB、β-内酰胺类、四环素类和氨基糖苷类抗性基因(图 5). 从 ARGs 所对应的抗性机制来看,外排泵为主要的抗性机制,其次是抗生素失活机制(图 6).

#### 2.4 ARGs 组成比较分析

基于德兴铜矿土壤中 ARGs 的组成和丰度,经 热图分析可以很好地展示 ARGs 组成差异与分布 (图7).由图7可以看出,ARGs 丰度最高的3个采 样点分别是6号(大坞河人乐安河口沉积物)、5号

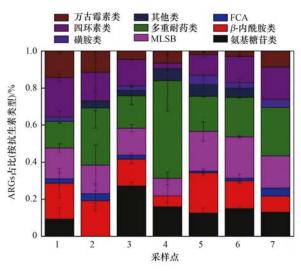


图 5 德兴铜矿样品中检测到的各类 ARGs 所占比例

Fig. 5 Proportion of various types of ARGs detected in Dexing copper mine sampling sites

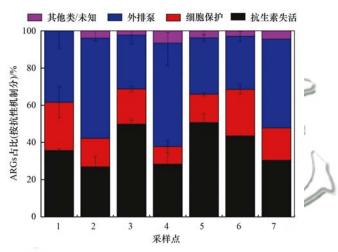


图 6 德兴铜矿样品中检测到的各类 ARGs 所占比例

Fig. 6 Proportion of various types of ARGs detected in Dexing copper mine sampling sites

(德兴铜矿住宅小区土壤)和3号(大坞河沉积物). 6号采样点中氨基糖苷类、β-内酰胺酶类、MLSB、磺胺类、四环素类抗性基因的相对丰度都较高,5号采样点中氨基糖苷类、β-内酰胺类和万古霉素类的抗性基因相对丰度较高,3号采样点中多重耐药类抗性基因相对丰度较高.

#### 2.5 MGEs 相对丰度及其与 ARGs 的相关关系

德兴铜矿各采样点 MGEs 检出相对丰度如图 8 所示. 从中可知,各采样点中 6 号采样点 MGEs 相对丰度显著高于其他点位,其次是 5 和 3 采样点. 各采样点 MGEs 相对丰度与 ARGs 相对丰度检出规律基本一致. MGEs 与 ARGs 检出丰度的 Pearson 相关性分析见表 3. 从中可知, MGEs 总丰度与 ARGs 总丰度显著正相关(P<0.05),其 Pearson 相关系数 r 为 0.79. 其中,磺胺类、MLSB 和四环素类这 3 类抗性基因与 MGEs 丰度呈极显著相关关系(P<0.01),

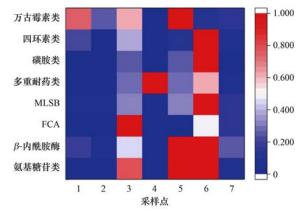
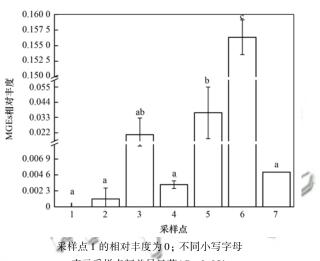


图 7 德兴铜矿采样点 ARGs 分布热图

Fig. 7 Heatmap of ARGs of Dexing copper mine sampling sites



表示采样点间差异显著(P<0.05) 图 8 德兴铜矿采样点 MGEs 相对检出丰度

Fig. 8 Relative abundance of MGEs in Dexing copper mine sampling sites

提示这 3 类 ARGs 的丰度可能与 MGEs 介导的水平 基因转移密切相关. 同时,部分抗性基因类别之间也 存在极显著的相关关系 (P < 0.01),包括  $\beta$ -内酰胺 类和氨基糖苷类 (r = 0.94),磺胺类和 MLSB (r = 0.99),四环素类和 MLSB (r = 0.95),磺胺类和四环素类(r = 0.95).

本研究中共检出 12 种 MGEs, 其组成比例如图 9 所示. 从中可知, 占比最高的 MGEs 为 *TnpA04*, 占比 47%, 其次为 *Intil* 占比 26%.

#### 2.6 ARGs 检出丰度与重金属含量的相关性

对德兴铜矿采样点各重金属浓度与 ARGs 检出丰度和种类进行 Pearson 相关性分析,结果见表 4. 从中可知,重金属 Cu 含量与 ARGs 的检出种类数呈显著正相关(*P* < 0.05), Pearson 相关系数达 0.79.

进一步对德兴铜矿采样点 ARGs 相对丰度与 4种重金属含量进行 RDA 分析. 结果表明, RDA1 与 RDA2 两个排序轴的解释度分别为 44.05% 和 38.37%, 累积解释度达到 82.42%. 4 种重金属中,只

有 Cu 对 ARGs 的检出丰度表现出显著影响 (P < 0.05,图 10). 德兴铜矿采样点主要检出的 ARGs 类型

中,氨基糖苷类和磺胺类与 Cu 含量呈正相关性. 采样点3、5 和6号与重金属 Cu 和 Cd 含量呈正相关性.

#### 表 3 MGEs 与 ARGs 检出丰度的 Pearson 相关性分析<sup>1)</sup>

Table 3	Pearson correlation	analysis between	concentrations of	of ARGs and MGEs

					,					
	MGEs	ARGs	氨基糖苷类	β-内酰胺类	FCA	MLSB	多重耐药类	磺胺类	四环素类	万古霉素类
MGEs	1	0. 79 *	0.71	0. 76 *	0. 34	0. 98 **	0. 35	0. 99 **	0. 93 **	-0.17
ARGs		1	0.73	0.65	0.37	0.76 *	0. 79 *	0.80 *	0.68	-0.15
氨基糖苷类			1	0. 94 **	0.53	0.78 *	0. 21	0.76 *	0.63	0.41
β-内酰胺类				1	0. 27	0.80 *	0.10	0. 79 *	0.64	0.41
FCA					1	0.49	0.06	0.44	0.55	0.06
MLSB						1	0. 25	0. 99 **	0. 95 **	-0.09
多重耐药类							1	0.33	0. 22	-0.43
磺胺类								1	0. 95 **	-0.12
四环素类									1	-0.13
万古霉素类										1

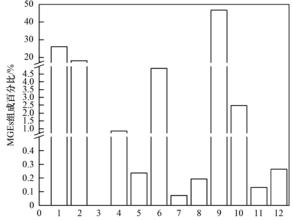
1)\*表示在0.05 水平(双侧)显著相关, \*\*表示在0.01 水平(双侧)极显著相关

#### 表 4 重金属含量与 ARGs 检出种类和丰度的 Pearson 相关性分析1)

Table 4 Pearson correlation analysis between ARGs and heavy metals

		Table 4 Tearson correta	tion analysis betw	cen micos and neavy me	tais	7/
	Zn	Cu 🅢	Cd	Pb	ARGs 种类	ARGs 丰度
Zn	1	0.11	0. 57	0.76*	0.35	0.74
Cu	_	/1∤ (5	0. 67	0.00	0. 79 *	0. 57
Cd		11.15		0.48	0.46	0. 52
Pb	10.8	11/1/2		11/2 (4)	0.36	0. 29
ARGs 种类	87 B	11/12		/ []	γ <u>1</u>	0.70
ARGs 丰度	1//	STIC		1015	- L	10 1 3 X
1 1 //6		1 11 / 11	2.0	/ 1864 1	797	- 17

1)\*表示在0.05水平(双侧)显著相关



1. Intil (clinic), 2. IntilLC, 3. Inti2(占比为 0), 4. Inti3, 5. IS613, 6. TnpA01, 7. TnpA02, 8. TnpA03, 9. TnpA04, 10. TnpA05, 11. TnpA07, 12. Tp614

#### 图 9 德兴铜矿 MGEs 组成

Fig. 9 MGEs composition of Dexing copper mine sampling sites

#### 3 讨论

本研究采用 HT-qPCR 对德兴铜矿周边土壤和沉积物中 ARGs 进行了检测与定量分析. 从 ARGs 的检出种类来看,共检出 70 种 ARGs,这与在绵阳市的紫土丘陵区畜禽养殖土壤检出 79 种 ARGs 种类结果相似<sup>[19]</sup>,高于沈阳蔬菜地土壤中检测出的 21

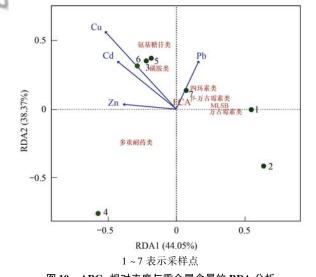


图 10 ARGs 相对丰度与重金属含量的 RDA 分析 Fig. 10 Redundancy analysis (RDA) of the relative abundance of ARGs and heavy metals

种抗性基因<sup>[17]</sup>,但低于福建省岩市境内山谷型尾矿 库水体中检测的 106 种 ARGs<sup>[33]</sup>.

在抗性基因相对丰度方面,本研究中德兴铜矿 ARGs 相对丰度介于  $0.3 \times 10^{-2} \sim 8.5 \times 10^{-2}$ ,全球 土壤 ARGs 的相对丰度水平约在  $10^{-7} \sim 10^{-2[34]}$ ,由此可见德兴铜矿的 ARGs 相对丰度属于较高水平,

与沈阳[17]和北京[18]蔬菜地土壤因过度施肥以及抗 生素残留土壤中的 ARGs 相对丰度结果类似,说明 土壤中 Cu 含量升高也可能导致 ARGs 相对丰度升 高. 由图 3 和图 4 可以看出, 6 号采样点的 ARGs 种 类和相对丰度最高,其次是3号采样点,这两个采样 点与其他采样点不同,他们汇集了大坞河旁德兴铜 矿采矿场的排水,可能形成了一个重金属元素高并 含有复杂成分的采矿工艺废液沉积的土壤环境,进 而影响了土壤中微生物的分布与结构,从而导致了 ARGs 种类和丰度的增加, 4 和 5 号采样点为人口 活动密集点,分别位于中学附近和住宅小区,其土壤 ARGs 的丰度仅次于 6 号采样点,这可能是由于人 类活动和上游铜矿排水的共同影响,导致抗生素抗 性基因的丰度提高. Ji 等[35] 对上海养殖场畜禽粪便 和周围土壤抗生素抗性基因研究表明,重金属 Cu 与人类活动都有可能通过改变土壤微生物的多样 性,从而影响土壤中抗生素抗性基因的种类和丰度.

本研究中, ARGs 与 MGEs 丰度呈显著正相关 (表3).这与 Ma 等[36]的研究通过高通量测序技术 和宏基因组学分析不同生态系统中 ARGs 与 MEGs 丰度之间相关性得出的结论相似. 由图 9 可知,转座 酶基因 TnpAO4 和一类整合子基因 Intil 是德兴铜矿 最主要的2种MGEs,而TnpAO4基因和Intil基因是 常见的可能参与基因水平转移的一类 MGEs, 这与 乌鲁木齐公园土壤 MGEs 的研究结果类似[37],说明 TnpA04 基因和 Intil 基因有可能参与了 ARGs 的传 播扩散. 有研究表明,一类整合子的 Intil 基因与磺 胺类[38,39] 和四环素类[40] 等多种抗性基因存在相关 性,本研究中磺胺类、四环素类和 MLSB 这 3 类抗 性基因与 MEGs 丰度呈极显著相关关系 (P < 0.01),由此可以推测,水平基因转移可能是采样区 磺胺类、四环素类和 MLSB 等 3 类抗性基因传播扩 散的重要机制.

土壤成分复杂而多样,作为重金属和 ARGs 的储存库,土壤重金属可以综合多种土壤环境因子(如营养盐含量和 pH 值等)共同影响土壤中微生物群落结构,从而影响 ARGs 种类和丰度.有研究表明,在污水处理厂周边土壤<sup>[8,9]</sup>和养殖厂沉积物<sup>[7]</sup>等土壤环境中,重金属含量与 ARGs 丰度存在极显著的相关性,说明重金属污染可能是影响 ARGs 丰度的一个主要原因. He 等<sup>[39]</sup>通过研究养鸡场粪便、土壤、沉积物和水样中 ARGs 的含量与重金属环境参数的关系,间接揭示了重金属对 ARGs 的存在和分布起到联合筛选作用. Zhao 等<sup>[7]</sup>的研究通过分析黄河三角洲沉积物中 ARGs 与沉积物中重金属和养分的关系,直接证实了营养物和重金属的协同筛选

对海水养殖沉积物中 ARGs 动力学变化起到重要作用. 黄福义等<sup>[33]</sup>对尾矿水体环境中 ARGs 污染研究发现, Cu、TOC 和 MGEs 对尾矿库水体环境抗生素抗性基因的赋存和演变可能具有重要作用. Cu 是德兴采矿区土壤和沉积物中高富集重金属元素, 重金属 Cu 含量与 ARGs 检出种类和丰度均呈显著正相关关系, 提示德兴铜矿区 Cu 的高含量存在可能是该区域抗生素抗性基因形成的重要驱动因子.

重金属对 ARGs 的影响机制主要分为 4 大类,即协同抗性、交叉抗性、共调控和生物膜反应<sup>[41]</sup>.有研究表明,Cu、Cd、Pb 和 Zn 这 4 种重金属,会促进淡水细菌中的 RP4 质粒的结合转移<sup>[42]</sup>,而该质粒包含多种耐药性基因,包括氨苄青霉素耐药基因、卡那霉素耐药基因和四环素耐药性基因.重金属 Cu可以破坏细菌细胞膜从而促进质粒 DNA 通过菌膜,促使 ARGs 在细胞间传播.不同的重金属离子还会导致细胞内活性氧(reactive oxygen species,ROS)的生成和细胞膜通透性的增强,ROS 响应以及改变共轭转移和调节基因 mRNA 的表达,从而促进抗性基因的共轭转移<sup>[43~46]</sup>.

#### 4 结论

- (1)德兴铜矿区土壤/沉积物环境中存在着种类较丰富和丰度水平较高的 ARGs,大坞河人乐安河口和富家坞采矿场大坞河两个采样点检测的 ARGs 种类和丰度都最高,德兴铜矿区检出的 ARGs 主要类别为多重耐药类、MLSB、β-内酰胺类、四环素类和氨基糖苷类抗性基因,外排泵为主要的抗性机制,其次是抗生素失活机制.
- (2) Pearson 相关性分析和 RDA 分析表明,重金属 Cu 含量与 ARGs 检出种类和丰度均呈显著正相关关系(*P* < 0.05),提示德兴铜矿区 Cu 的高含量存在可能是该区域抗生素抗性基因形成的重要驱动因子.
- (3)德兴铜矿各采样点共检测了 12 种 MEGs, 其中 *TnpA04* 和 *Intil* 是德兴铜矿最主要的 MEGs, ARGs 与 MGEs 丰度呈显著正相关(*P* < 0.05),推测 水平基因转移是采样区环境 ARGs 产生的重要 机制.

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