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青海湖周边地区表层土壤重金属含量和抗性基因丰度 及相关性

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摘要:土壤重金属污染以及抗性基因的流行一直是全球关注的问题,已有许多研究报道了重金属和抗性基因在土壤中的含 量,但是高原地区重金属和抗生素抗性基因(ARGs)在土壤中的含量并不清楚. 因此,调查分析了青海地区土壤中重金属和抗 性基因的环境残留量和分布情况,并且探究了土壤中重金属含量和抗生素抗性基因之间的关系. 在土壤样品中,重金属 ω (Zn)最高[平均值:(50.27±19.88) mg·kg⁻¹],其次是重金属 ω(Cd)[平均值:(30.27±9.45) mg·kg⁻¹],重金属 ω(Hg) 最 低[平均值:(0.027 ±0.019) mg·kg⁻¹]. 土壤中重金属抗性基因的亚型主要是 czcA、merA 和 merP,它们主要功能是负责对 Hg 产生耐性. 土壤中 β-内酰胺酶抗性基因相对丰度(0. 150 5) 最高,占 ARGs 总丰度的 47. 54%,四环素(tetracycline) 耐药基因占 ARGs 总丰度的 16.93%, FCA 约占 14.56%, MLSB 约占 8.77%. 可移动的遗传元件(MGEs)多样性和相对丰度均较低,仅检测出 tnpA01 基因, intl1 和 intl2 未检出. 相关性研究表明,土壤中 Cu(r = -0.533, P = 0.006) 和 Hg(r = 0.692, P = 0.006) 含量与海拔 高度呈显著负相关,其他重金属含量与海拔高度无显著相关性.此外,重金属含量与土壤类型显著相关 (P < 0.05). 土壤中重金 属 Hg 含量与 czcA(r = 0. 692, P = 0. 006)、merA(r = 0. 816, P = 0. 007)和 merP(r = 0. 594, P = 0. 02)之间存在显著正相关. 研究结 果阐明了重金属和 ARGs 在青藏高原地区的发生和分布,并发现土壤中重金属含量与抗性基因存在显著相关性.

关键词:重金属; 土壤污染; 抗生素; 抗生素抗性基因(ARGs); 海拔高度; 可移动遗传元件(MGEs) 中图分类号: X53 文献标识码: A 文章编号: 0250-3301(2023)01-0336-11 DOI: 10.13227/j. hjkx. 202202175

Heavy Metal Content and Resistance Gene Abundance and Related Properties in the Surface Soil around Oinghai Lake

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Abstract: Soil heavy metal pollution and the prevalence of resistance genes have been a global concern, and thus many studies have reported the content of heavy metals and resistance genes in soils; however, the contents of heavy metals and antibiotic resistance genes (ARGs) in the soil in highland areas is still unclear. In this study, the environmental residues and distribution of heavy metals and resistance genes in the soil in Qinghai were analyzed, and the relationship between the concentration of heavy metals and antibiotic resistance genes in the soil was explored. Among the soil samples, the content of heavy metal zinc was the highest [mean: (50.27 ± 19.88) mg·kg⁻¹], followed by the content of heavy metal cadmium [mean: (30.27 ±9.45) mg·kg⁻¹], and the content of heavy metal mercury was the lowest [mean: (0.027 ± 0.019) mg·kg⁻¹]. The subtypes of heavy metal resistance genes in soils were mainly czcA, merA, and merP, whose main function was to be responsible for developing mercury resistance. The relative abundance of β-lactamase resistance genes (0.1505) was the highest in soil, accounting for 47.54% of the total abundance of ARGs; tetracycline resistance genes accounted for 16.93% of the total abundance of ARGs, FCA accounted for approximately 14.56%, and MLSB accounted for approximately 8.77%. The diversity and relative abundance of movable genetic elements (MGEs) were low, and only the tnpA01 gene was detected; intl1 and intl2 were not detected. Correlation studies showed that Cu content (r = -0.533, P = 0.006) and Hg (r = 0.692, P = 0.006) in soil were significantly negatively correlated with altitude, whereas other heavy metals were not significantly correlated with altitude. In addition, heavy metal content was significantly correlated with soil type (P < 0.05). There was a significant positive correlation between heavy metal mercury content in soils and czc4 (r=0.692, P=0.006), mer4 (r=0.816, P=0.007), and merP (r=0.594, P=0.006) 0.02). The results of this study elucidated the occurrence and distribution of heavy metals and ARGs in the Tibetan Plateau region and found that the content of heavy metals in the soil was significantly related to resistance genes.

Key words: heavy metals; soil pollution; antibiotics; antibiotic resistance genes (ARGs); altitude; movable genetic elements (MGEs)

土壤重金属污染一直是环境治理的热点问 题[1,2]. 重金属在各种环境介质中的含量较低,因此 也被认为是微量元素[3]. 重金属污染已遍及世界许 多地区,尤其是中国等发展中国家[4]. 与世界其他 地区重金属污染相比,我国土壤重金属污染并不严 重,然而,相较于某些重金属,我国似乎正在以更快 的速度增加[5]. 此外,重金属污染是隐蔽的、持续的 和不可逆的[6]. 中国是最大的砷矿开采国、生产国 和使用国,土壤砷污染较为严重[7].

抗生素存在于多种环境介质中[8,9]. 抗生素抗 性基因(antibiotic resistance genes, ARGs)污染在近 二十年逐渐走入大众视野. 因此, ARGs 被定义为一 种新型的环境污染物[10]. 如今, ARGs 已成为全球环

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境健康关注的焦点[11].有研究表明,环境中耐抗生素细菌可能会对人类健康产生风险[12,13].自 1940年青霉素问世以来,抗生素在人类疾病的治疗和预防中发挥了至关重要的作用[14].我国每年使用抗生素总量约16.2万t,其中兽用抗生素占总量的52%,主要用于治疗动物疾病和促进动物生长[15,16].医用和兽用抗生素的滥用导致环境中ARGs的种类和数量不断增加,而ARGs也普遍存在于现代环境介质和人体微生物中[17,18].如今,抗生素耐药性已成为大多数人面临的重大公共卫生问题[19]. Knapp等[20]的研究表明,自 1940年以来,所测的各种ARGs明显增加,尤其是四环素.

随着抗生素污染问题的进一步加剧,有研究表明,土壤重金属含量与 ARGs 存在一定的相关性,并对 ARGs 丰度产生一定影响^[21,22]. 早于抗生素时代的微生物群落对抗生素极为敏感,而它们的移动遗传元件(movable genetic elements, MGEs)含有很少的抗性基因^[23]. 此外,有研究表明,环境中的耐药菌具有对抗生素产生耐药性的特征,这可能通过水平基因转移赋予病原体耐药性^[24,25]. 因此,分析环境中 ARGs 和可移动遗传元件的综合分布,对了解现代抗生素耐药性的演化和传播具有重要意义.

目前国内外对抗性基因的研究主要集中在人为

活动密切、海拔低的工业化地区,对于高海拔地区 土壤重金属和抗性基因之间的关系研究较少.因此, 本研究目的是了解青海湖周边地区土壤中重金属和 ARGs 的污染水平和分布,分析环境因子和重金属 与 ARGs 的关系.

1 材料与方法

1.1 研究区域概况

青海湖位于青海省西北部的青海湖盆地内,既 是中国最大的内陆湖泊,也是中国最大的咸水湖.青 海湖具有高原大陆性气候,光照充足,日照强烈:冬 寒夏凉,暖季短暂,冷季漫长,春季多大风和沙暴: 雨量偏少,雨热同季,干湿季分明. 湖区东部和南部 气温稍高,年均温在0.3~1.1℃之间; 西部和北部 稍低,年均温在 -0.8~0.6℃之间,平均最高气温在 6.7~8.7℃之间,平均最低气温在 - 6.7~4.9℃之 间,极端最高气温为 25℃和 24.4℃,极端最低气温 为-31~-33.4℃. 湖区全年降水量偏少. 但东部和 南部稍高于北部和西部,东部全年降水量是412.8 mm,南部是359.4 mm,西北部370.3 mm,西部是 360.4 mm 和 324.5 mm. 全年蒸发量达1 502 mm,蒸 发量远远超过降水量. 湖区降水量季节变化大,降水 多集中在5~9月,雨热同季.采样点环境因素如表 1 所示.

表 1 采样点位信息概述

| 编号 | 点位名称 | 海拔/m | 年均降雨量/mm | 年均温度/℃ | 土壤 pH 值 |
|-----|----------|-------|----------|--------------|---------|
| S1 | 德令哈 | 2 586 | 169 | 5. 3 | 8. 2 |
| S2 | 胡杨林保护区 | 2 787 | 25. 2 | 5. 1 | 8. 7 |
| S3 | 格尔木 | 2 815 | 41.5 | 4 | 8. 7 |
| S4 | 共和2 | 2 732 | 400 | 3. 2 | 8. 39 |
| S5 | 西海1 | 3 310 | 366. 4 | 4 | 7. 8 |
| S6 | 西海 2 | 3 210 | 350 | 4 | 7. 5 |
| S7 | 刚察 | 3 280 | 370. 5 | 0.91 | 8. 42 |
| S8 | 共和(青海湖边) | 3 215 | 450 | 3. 2 | 8. 2 |
| S9 | 沱沱河 | 4 528 | 283. 1 | -4.4 | 7. 5 |
| S10 | 藏羚羊观景台 | 4 596 | 365 | -6 | 8. 9 |
| S11 | 可可西里无人区 | 4 609 | 367. 8 | -5.8 | 9. 1 |
| S12 | 五道梁 | 4 613 | 301.4 | -5.9 | 8. 1 |
| S13 | 沱沱河无人区 | 4 635 | 283. 1 | -4.5 | 7. 8 |
| S14 | 玛多 | 4 218 | 585. 5 | -4. 1 | 7. 6 |
| S15 | 鄂陵湖 | 4 577 | 516. 9 | -5 | 7. 8 |

1.2 重金属的测定

本研究分别测定了 Pb、Cu、Zn、Cr、Cd、Hg 和 As 这 7 种重金属在土壤中的含量与分布,方法 如下.

样品的制备:除去样品中的枝棒、叶片和石子等异物,将釆集的样品进行风干、粗磨和细磨至过孔径 0.15 mm(100 目)筛.

微波消解:用干净的药匙取适量的土壤样品于比色管(50 mL),加入新配王水 10 mL(3:1, HCl: HNO₃,体积比),摇匀使其充分接触,放置 12 h 后加盖,在 100℃的水浴锅中进行消煮反应,反应时间为 2 h. 待其冷却后,用超纯水定容至 50 mL,混匀后过滤(0.45 mm).

电感耦合等离子体质谱法检测:过滤液用电感

耦合等离子体质谱仪(ICP-MS, Agilent 7500a, Agilent, Santa Clara, California)测量样品中重金属的 浓度. 校准曲线的范围(R = 0.9997)选自 $0.05 \sim 10$ mg·L-1. 所有化学分析均设置平行样品,以确保实 验的准确性.

1.3 抗性基因的测定

本研究测定了氨基糖苷类(aminoglycoside)、β-内酰胺酶类(β -lactamase)、喹诺酮类氯霉素类 (FCA)、重金属类(heavy metal)、遗传元件类 (MGE)、大环内酯类林肯酰胺类链阳性菌素 B 抗 性基因(MLSB)、其他类(other/efflux)、磺胺类 (sulfonamide)、四环素类(tetracycline)、万古霉素 类(vancomycin)共10类80种ARGs.其中,共检测 了3种 MGE, intl1和 intl2未检出, tnpA01基因仅 在部分样品中检出. 土壤抗性基因的测定由启因生 物科技有限公司完成,方法如下.

DNA 提取:根据制造商的使用说明,使用土壤 基因组 DNA 快速提取试剂盒(TIANNAMP Soil DNA Kit)来进行土壤样品 DNA 的提取工作. 由于样品中 的 DNA 含量较低,为了避免单一 DNA 样品提取过 程产生偏差,所以进行多次 DNA 制备.

实时荧光定量 PCR:使用 Step OnePlus™实时荧 光定量 PCR(Thermo)对 16S rRNA 基因和 ARGs 进 行定量分析,每个土壤样品均设3组平行样.反应 体系由 5 μL 的 TB GreenTM Premix Ex TaqTM II (Tli RNaseH Plus) (Takara, Code No. RR820A), 0.4 µL 正向引物(优化的最终浓度为 0.4 mmol·L⁻¹), 0.4 μL 反向引物(优化的最终浓度为 0.4 mmol·L⁻¹). 1 μL 模板 DNA、3 μL ddH,O 组成. Real-time PCR 的升温程序为:在95℃下初始变性30 s,在95℃下 变性 5 s,在退火温度下退火 30 s,一共进行 40 个循 环,最后在72℃延伸30 s. 通过溶解曲线验证扩增

特异性,溶解曲线采集温度为60~98℃,每次0.5℃ 读数一次. 本研究制备了定量 PCR 的标准样品以进 行阳性对照,通过标准曲线来计算每个样品目的基 因拷贝数.

ARGs 丰度计算方法: ARGs 丰度的表现形式分 为绝对定量和相对定量两种. 绝对定量是指单位样 品中ARGs的基因拷贝数(对于土壤为每g),计算 方法见式(1)^[26]. 相对定量是指单位菌体中 ARGs 所占的比例(无量纲),计算方法见式(2)[26].

土壤中 ARGs 的绝对丰度 = $\frac{模板数 \times DNA 总量}{}$

(1)

土壤中 ARGs 的相对丰度 = 土壤中 ARGs 的绝对丰度 (2)16S rRNA 拷贝数

1.4 统计分析

从各地区的气象局网站获取降雨、气温等数 据,用于相关环境因素对微生物群落分布和 ARGs 相对丰度时空分布差异影响和探究. 采用地理信息 系统绘图软件(ArcGIS 10.6, ESRI, Redlands, California) 绘制青海采样点空间分布. 采用 Excel 2019 和 SPSS 25.0 对土壤重金属含量、理化性质、 微生物相对丰度和抗性基因相对丰度数据进行处 理,采用相关性分析和冗余分析分析土壤微生物与 环境因子间的关系及抗性基因与微生物种群丰度间 的关系.

2 结果与分析

2.1 青海湖周边土壤样品中重金属含量分布

15 个土壤样品中 Pb、Cu、Cr、Zn、Cd 和 Hg 等 重金属的含量及分布见表 2. 土壤样品中 ω(Zn) 最 高,范围为20~79 mg·kg⁻¹,平均值为(50.27 ±

表 2 采样点土壤样品中重金属含量¹⁾/mg·kg⁻¹

Table 2 Heavy metal content in soil samples at sampling sites/mg·kg⁻¹ 采样点编号 Pb Cu Cr Zn Cd Hg As S118 20.4 36 66 0.12 0.02 14.4 0.077 S2 10 10.6 22 22 0.09 10.1 S318 15.7 27 47 0.12 0.037 33.2 S4 16 14.9 38 72 0.12 0.03312.5 S5 47 15 14.2 35 0.12 0.023 11.4 S6 16 16.7 40 59 0.14 0.024 13.4 S7 15 67 11.3 11.7 32 0.15 0.028 S8 20 19.4 46 74 0.14 0.06214 S9 2.7 7.2 23 79 0.16 0.011 31.6 S10 5.9 20 0.027 10.4 8 18 S11 24 19.3 39 53 0.02 15.1 S12 7 17 28 0.005 7.11 5.1 13 24 0.09 S13 3. 2 15 0.01 20.8 S14 16 17.0 35 56 0.021 0.17 19.6 S15 14 13.7 31 40 0.014 20.2

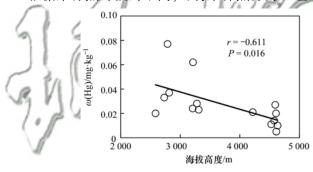
^{1)&}quot;一"表示污染物含量低于检出限

19.88) $mg \cdot kg^{-1}$. ω (Hg) 最低,范围为 0.005 ~ 0.077 $mg \cdot kg^{-1}$, 平均值为 (0.027 ± 0.019) $mg \cdot kg^{-1}$. 土壤中 Pb 和 Cr 含量相对较高,这可能是由于 Pb 和 Cr 高积累因子导致的^[27]. 在 Ogiyama 等^[28]的研究中,粪肥改良的耕地中 Zn 和 Cu 含量分别为 72 ~ 170 $mg \cdot kg^{-1}$ 和 18 ~ 109 $mg \cdot kg^{-1}$,显著高于青海地区土壤重金属含量,表明未受人为污染的土壤中重金属污染较低.

青海湖周边土壤样品中 ω (As)较高,范围为7.11~33.2 mg·kg⁻¹,平均值为(16.34±7.59)mg·kg⁻¹,部分地区高于国家标准[国家一级标准为 ω (As) \leq 15 mg·kg⁻¹].杜昊霖等^[29]在西藏的研究也发现,土壤中的As含量为中国土壤背景值的4.73倍,世界土壤背景值的8.82倍.根据表2可知,As在不同采样点含量(mg·kg⁻¹):S3为33.2、S9为31.6、S11为15.1、S13为20.8、S14为19.6和S15为20.2.以上采样点土壤中的As含量均高于国家一级标准,由此推测青海地区As污染较为严重.

2.2 重金属含量与海拔的关系

根据采样点海拔的不同,可将采样点分为3组



(L: < 3000 m、M: 3000 ~ 4000 m 和 H: > 4000 m). 分别对应采样点为 L: S1~S4、M: S5~S8 和 H: S9~S15. 计算各区域不同重金属的含量平均值(见表3), 土壤重金属 Hg 在不同组间差异明显,海拔较低的采样点重金属 Hg 含量较高,海拔较高的采样点重金属 Hg 含量较低. 本研究采用 SPSS 软件分析土壤重金属含量与海拔的相关性,结果表明土壤中重金属 Cu 和 Hg 的含量与海拔高度呈显著负相关(P<0.05,如图 1),而其他重金属含量与海拔高度无显著相关性.

表 3 不同海拔区域重金属含量和标准差1)

Table 3 Heavy metal content and standard deviation

| | at dif | ferent altitudes | |
|---------------------|-------------------|------------------|-------------------|
| 重金属 | 低海拔组 | 中海拔组 | 高海拔组 |
| Pb | 15.500(3.786) | 16.500(2.380) | 15. 571 (7. 547) |
| Cu | 15.400(4.016) | 15.500(3.306) | 10. 200 (6. 374) |
| \mathbf{Cr} | 30.750(7.544) | 38. 250(6. 131) | 25. 429 (9. 554) |
| Zn | 51.750(22.515) | 61.750(11.587) | 42. 857 (21. 169) |
| Cd | 0.113(0.015) | 0.138(0.126) | 0.140(0.044) |
| Hg | 0.042(0.025) | 0.034(0.019) | 0.015(0.008) |
| As | 17. 550 (10. 581) | 12.525(1.379) | 17. 830 (8. 008) |
| | | | |

1)括号外数值表示含量,单位为mg·kg-1,内数值表示标准差

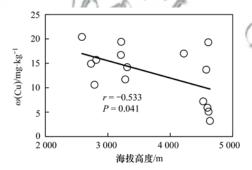


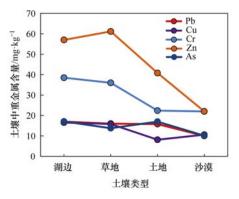
图 1 海拔高度与重金属含量的相关性

Fig. 1 Correlation between altitude and heavy metal content

2.3 影响青海湖周边土壤重金属含量差异的因素

2.3.1 土壤类型对重金属含量的影响

根据采样点土壤类型的不同,将土壤由含水率从 高到低分为湖边(S15、S8)、草地(S1、S4、S5、S6、 S7 和 S14)、土地(S9、S10、S11、S12 和 S13)和沙漠(S2)这4 种土壤类型. 除重金属 Hg 以外,其余重金属含量均随土壤类型变化出现下降的趋势(如图 2). 沙漠土壤中 Hg 含量明显高于其他土壤,达到 0.077



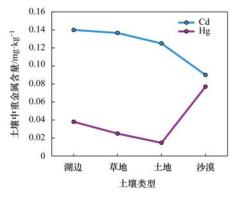


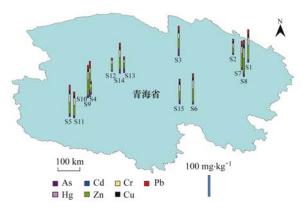
图 2 不同土壤类型重金属含量分布

Fig. 2 Distribution of heavy metal content by soil type

mg·kg⁻¹,其余重金属含量显著低于其他土壤类型.

2.3.2 大气的长距离迁移对重金属含量的影响

青海省只有少数地方有重金属污染源,由此推 测青海地区重金属污染可能是由远距离迁移造成 的. 从采样点的地理位置来看(如图3),甘肃省和四 川省附近采样点重金属含量较高,如 S1 (154.94 $\text{mg} \cdot \text{kg}^{-1}$) S6 (145. 264 $\text{mg} \cdot \text{kg}^{-1}$) S4 (153. 553) $mg \cdot kg^{-1}$), S8 (173.602 $mg \cdot kg^{-1}$), S3 (141.057 mg·kg⁻¹)和 S14(143.791 mg·kg⁻¹). 重金属含量低 的采样点远离四川省和甘肃省,如 S2 (74.867 $mg \cdot kg^{-1}$) \ S13 (76.1 $mg \cdot kg^{-1}$) \ S12 (64.215 mg·kg⁻¹)和 S10(62.327 mg·kg⁻¹). 李志涛等^[30]的 研究表明,四川省硫铁矿区周边土壤重金属 $\omega(Cd)$ 、 ω(Hg), ω(As), ω(Pb), ω(Cr), ω(Cu) π ω(Zn) 的平均值分别为:1.55、0.261、12.2、46.2、115、 74 和 113 mg·kg⁻¹. 陈文轩等^[31]的研究同样测定了 四川省农田土壤中不同种重金属的含量, $\omega(Cd)$ 、 ω(Hg), ω(As), ω(Pb), ω(Cr), ω(Cu) π ω(Zn) 的平均值分别为 0.268、0.108、9.65、32.28、 60.87、30.89 和 107.58 mg·kg⁻¹,甘肃省农田土重 金属 ω (Cd)、 ω (Hg)、 ω (As)、 ω (Pb)、 ω (Cr)、 ω(Cu)和ω(Zn)的平均值分别为 0.213、0.084、 10.46、27.52、63.01、26.83 和 86.43 mg·kg⁻¹.本 研究重金属 As 的含量与杜昊霖等[29] 在西藏土壤中 的研究结果接近,表明青藏高原地区土壤中 As 的含 量背景值较高. 青海地区土壤重金属含量分布符合 远距离迁移趋势,即近距离高且远距离低.由此推 断,除土壤本身含有的重金属外,青海地区土壤重金 属来源还包括周边地区(如四川省和甘肃省等)的 大气远距离迁移.



柱状图表示采样点重金属含量,不同颜色表示不同重金属种类 图 3 采样点分布示意

Fig. 3 Sample site distribution plot

2.4 青海湖周边土壤样品中 ARGs 的含量分布 土壤样品中发现的 ARGs 按抗性类型分类(如 图 4),丰度最高的是 β-内酰胺酶(β-lactamase)抗性

基因(相对丰度为0.1505),占总 ARGs 的 47.54%. 四环素 (tetracycline) 耐药基因占 ARGs 总丰度的 16.93%,FCA 约占 14.56%,MLSB 约占 8.77%,其他种类耐药基因占比较低. 四环素耐药基因显著高于磺胺类耐药基因,该结果与 Hu 等[32]的一致. 对于磺胺类 ARGs,相对丰度的变化趋势为sull;对于四环素耐药基因的变化趋势为tetW > tetG > tetL > tetR > tetX > tetQ,该结果与 Ji 等[33]的相似. 有研究表明,磺胺类和四环素类抗性基因是牛粪中最常见的抗性基因. 在美国,牛粪中磺胺和四环素抗性基因的相对丰度分别约为 $10^{-6} \sim 10^{-5}$ 和 $10^{-3} \sim 10^{-2[34]}$.



Fig. 4 Relative abundance of different types of ARGs

各采样点 ARGs 种类数分别为 37、28、20、26、20、20、28、39、23、28、25、35、23、35 和 25,81、88、S12 和 S14 号采样点显著高于其他点位,S1、88和 S14 号采样点重金属含量相对较高,可能是导致这些点位 ARGs 种类较多的原因^[35].维恩图显示了不同海拔[图 5(a)]和不同土壤类型[图 5(b)]样品中独特和共有的 ARGs. 3组海拔区域共有的ARGs有38种,低海拔、中海拔和高海拔地区分别观察到5、4和9种独特的ARGs.沙漠土壤中ARGs的多样性显著低于其他土壤类型,土壤类型对ARGs 多样性的影响显著大于海拔高度.

2.5 影响青海湖周边土壤 ARGs 相对丰度差异的 因素

2.5.1 海拔对土壤 ARGs 相对丰度的影响

ARGs 相对丰度与海拔高度呈显著负相关, ARGs 丰度差异随海拔升高逐渐减小. 氨基糖苷类 (aminoglycoside) 抗性基因的相对丰度与海拔高度呈显著负相关[r = -0.762, P < 0.05, 图 6(a)]. 由组间分析可知, H组土壤样品中检测到的 ARGs 相对丰度数量最少(在 80 个目标 ARGs 中, 平均值为 0.0052 ± 0.034 , n = 15). 从图 6(b) 可以看出,抗性基因相对丰度与海拔显著相关. H组 $(0.0052\pm0.034$, n = 7)和 M组 $(0.0111\pm0.0080$, n = 4)中检测到的 ARGs 平均数量显著低于 L组 (0.0588 ± 0.0080)

0.1059, n = 4). 这些结果表明海拔的变化会改变 ARGs 的相对丰度.

2.5.2 重金属含量对土壤 ARGs 相对丰度的影响 青海省土壤重金属 Cd 和 Hg 含量与 ARGs 存在 显著相关性. 重金属 Cd 与四类(氨基糖苷类、β-内酰胺酶类、MLSB 和四环素类) ARGs 存在显著负相关(如表 4),相关性系数 (r)分别为 -0.973、-0.685、-0.651 和 -0.658.

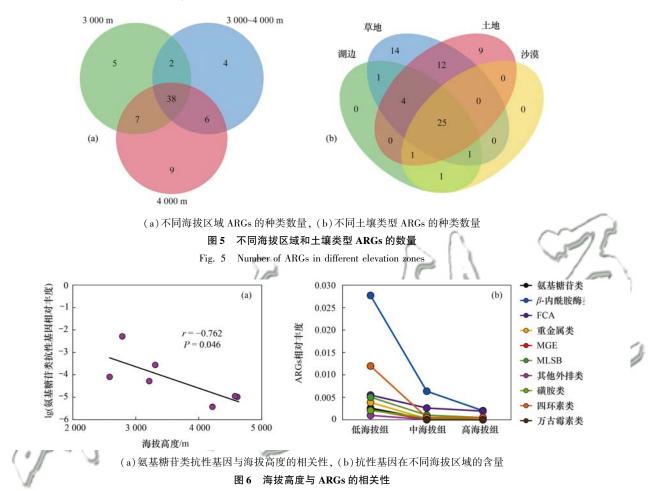


表 4 重金属 Cd 含量与抗性基因相对丰度对数值相关性分析1)

Fig. 6 Correlation of altitude with ARGs

Table 4 Cadmium content of heavy metals and relative abundance of resistance genes are analyzed as a numerical correlation

| | Cd | 氨基糖苷类 | β-内酰胺酶类 | MLSB | 四环素类 |
|----------------|---------------------|-----------|-----------|-----------|------|
| Cd | 1 | | | | |
| 氨基糖苷类 | -0. 973 ** | 1 | | | |
| β -内酰胺酶类 | -0.685 * | 0. 915 ** | 1 | | |
| MLSB | -0.651 * | 0. 865 * | 0. 810 ** | 1 | |
| 四环素类 | -0. 658 * | 0. 833 * | 0. 745 ** | 0. 838 ** | 1 |

1)* 为P < 0.05, ** 为P < 0.01,下同

土壤中重金属 Cd 含量与抗性基因亚型 blaSHV-02、blaTEM、cphA、ermK、tetW、acrA、cmlA1-01 和 mexA 存在显著相关性(如表5),与金属抗性基因相关性不显著.除 cphA 抗性基因外,重金属 Cd 含量与多种 ARGs 相对丰度对数值均为负相关性.由此推测,土壤中 Cd 含量升高可能会抑制 ARGs 的产生,具体影响效应需后续实验证明.

青海省土壤重金属 H_g 含量与抗生素抗性基因存在显著相关性(P < 0.05). 3 类抗生素抗性基因

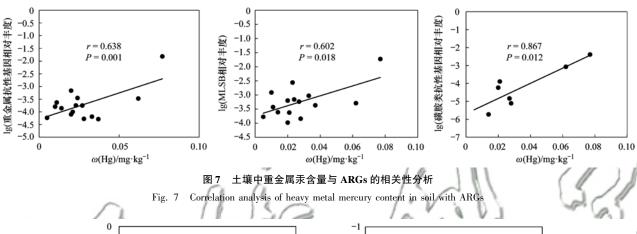
(重金属类、MLSB 和磺胺类)与土壤 Hg 含量呈正相关(如图 7),相关系数(r)分别为 0.638、0.602和 0.867.由此表明,土壤中 Hg 含量的变化会对ARGs 丰度产生一定影响.

土壤中重金属 Hg 的含量与重金属抗性基因亚型存在显著相关性(如图 8),尤其与抗汞基因相关(P < 0.05). 土壤重金属 Hg 的含量与 cadB、czcA、merA 和 merP 这 4 种重金属抗性基因呈显著正相关,相关系数 r = 0.881 (P = 0.004)、r = 0.692 (P = 0.004)

表 5 重金属 Cd 含量与抗性基因亚型相对丰度对数值相关性分析

| Table 5 | Numerical correlation anal | lysis of the relative abundance of | cadmium content and i | resistance gene subtypes of heavy i | metals |
|---------|----------------------------|------------------------------------|-----------------------|-------------------------------------|--------|
| | | | | | |

| | blaSHV-02 | blaTEM | cphA | ermK | tetW | acrA | cmlA1-01 | mexA |
|--------------|------------|------------|----------|-----------|-----------|------------|------------|-----------|
| Cd | - 0. 904 * | - 0. 710 * | 0. 867 * | -0.656 * | -0.667* | - 0. 754 * | - 0. 755 * | -0.736 ** |
| bla SHV-02 | 1 | 0. 930 ** | -0.083 | 0. 645 | 0. 851 ** | 0. 832 * | 0. 645 | 0. 823 * |
| blaTEM | | 1 | 0. 102 | 0. 845 ** | 0. 957 ** | 0. 867 ** | 0. 772 * | 0. 839 ** |
| cphA | | | 1 | -0.119 | 0. 37 | -0.368 | -0.46 | - 0. 469 |
| ermK | | | | 1 | 0. 915 ** | 0. 688 * | 0. 803 ** | 0. 781 ** |
| tetW | | | | | 1 | 0. 795 ** | 0. 829 ** | 0. 762 ** |
| acrA | | | | | | 1 | 0. 78 | 0. 781 ** |
| cmlAI - OI | | | | | | | 1 | 0.814* |



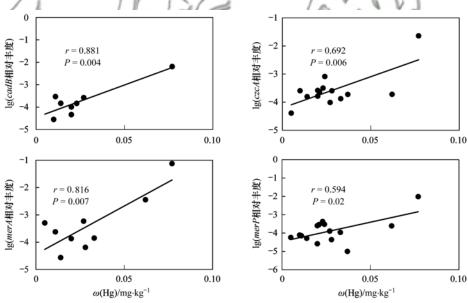


图 8 重金属汞含量与抗汞基因相对丰度相关性分析

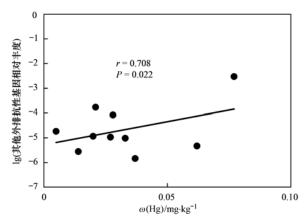
Fig. 8 Correlation analysis of mercury content in heavy metals and relative abundance of anti-mercury resistance genes

(0.006)、(r = 0.816) (P = 0.007) 和 (P = 0.02) (

土壤中汞含量的增加促进了 Hg 离子外排的机制. 土壤中重金属 Hg 的含量与其他外排基因呈显著正相关(如图 9),相关系数 r=0.708 (P=0.022). 这些基因的主要功能是产生一种排除对生物体造成损害的物质的流出机制. 根据土壤中重金属抗性基因的含量分布,S2 号样品中 Hg 含量最高,所有重金属抗性基因的丰度均增加,但 czcA (重金属

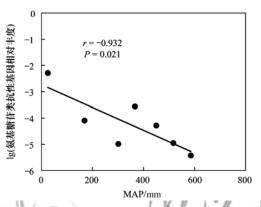
排泄基因)、merA[汞(II)还原酶]和 merP(抗汞结合蛋白)增加最为明显,说明土壤中 Hg 含量的增加促进了 Hg 离子外排的机制.

土壤中除重金属 Cd 和 Hg 外,其余重金属含量与 ARGs 也存在显著相关性. 例如,土壤中重金属 Pb 含量与 cphA 和 floR 存在显著相关性,相关系数分别为 r=0.708 (P=0.033) 和 r=-0.960 (P=0.013); 重金属 Cu 含量与 cphA 和 floR 存在显著相关性,相关系数为 r=0.784 (P=0.012) 和 r=-0.987 (P<0.001); 重金属 Cr 含量与 cphA 和



重金属汞含量与其他 外排基因的相关性分析

Correlation analysis of heavy metal mercury with the other/efflux genes



10



细菌间传播,但不同的 MGEs 携带不同种类的 ARGs^[36,37]. 青海环境中极少数 ARGs 由 MGEs 携 带,表明这些 ARGs 在细菌间转移的可能性较 低^[38]. 为了研究 ARGs 和 MGEs 之间的关系,本研究 分析了 ARGs 与 MGEs 相对丰度之间的关系. 韩柳 等[35]的研究发现 ARGs 与 MGEs 相对丰度呈显著正 相关(P<0.05). Pearson 相关分析表明 ARGs 的相 对丰度与 MGEs 的相对丰度呈显著正相关,结果表 明 MGEs 的相对丰度升高有利于 ARGs 的总丰度升

转移潜力较弱. 在受人类影响的环境中, ARGs 的种类和丰度 远高于环境背景值. 此外, MGEs 的种类和丰度也高 于没有人为影响的原始环境. 由此推测人为活动不 仅导致了环境中 ARGs 种类和相对丰度升高,更加 快了 ARGs 在多种生物体间的快速传播,对此需严

格控制抗生素类药品的滥用.

高(P<0.05, 见图 11), 该结果与 Zhang 等[39]的一

致. 青海土壤样品中仅检测到 tnpA01 基因,未检测

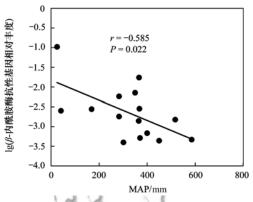
到 intl1 和 intl2.由于 intl1 和 intl2 基因负责复制、 剪裁、运输和整合抗性基因,推测青海 ARGs 水平 floR 存在显著相关性,相关系数为 r = 0.713 (P =(0.031)和 r = -0.853 (P = 0.031); 重金属 Zn 含量 与 blaSFO 存在显著相关性,相关系数为 r = -0.997(P=0.048); 重金属 As 含量与 arsM 存在显著相关 性,相关系数为 r = -0.897 (P = 0.015).

2.5.3 降雨量对土壤 ARGs 相对丰度的影响

年平均降雨量(MAP)与土壤中的 ARGs 存在 显著相关性. 采样点位 MAP 与氨基糖苷类抗性基因 和 β -内酰胺酶类抗性基因存在显著负相关,相关系 数为 -0.932 和 -0.585 (如图 10).

2.6 青海湖地区土壤 ARGs 水平转移潜力

基因水平转移是 ARGs 在细菌间传播的主要方 式. MGEs是可移动的遗传元件,可以携带ARGs在



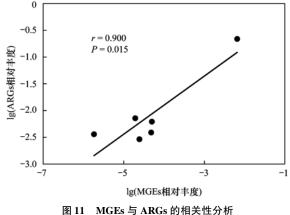
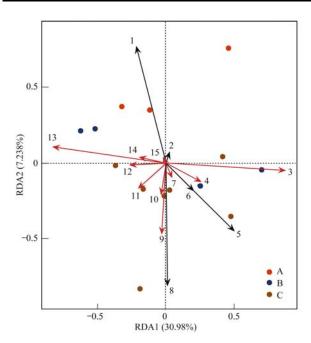


Fig. 11 Correlation analysis of MGEs with ARGS

2.7 冗余分析

本研究通过多变量冗余分析方法来研究主要环 境因子对土壤中 ARGs 的影响. 如图 12 所示,第一 轴主要由年平均降雨量(MAP)来解释,占 ARGs 总 方差的30.98%,第二轴由海拔高度和年平均温度 (MAT)来解释,占 ARGs 总方差的 7.238%. 本研究 表明, ARGs 在第一轴上载荷率较高, 表明该地区土 壤样品中 ARGs 主要受到年平均降雨量的影响.



1. 年平均温度, 2. 重金属, 3. mexF 抗性基因, 4. mexE 抗性基因, 5. 年平均降雨量, 6. pH 值, 7. merA 抗性基因, 8. 海拔高度, 9. aioA 抗性基因, 10. merC 抗性基因, 11. ermK 抗性基因, 12. tetW 抗性基因, 13. blateM 抗性基因, 14. mexA 抗性基因, 15. czcA 抗性基因

图 12 RDA 分析 Fig. 12 RDA analysis

3 讨论

本研究对青海湖周边土壤中重金属和 ARGs 进行检测和痕量分析. 共检测了 7 种重金属在土壤中的含量,其中 Zn 含量最高,汞含量最低. 彭驰等^[40]研究表明,我国城市土壤重金属ω(Pb)、ω(Cd)、ω(Cu)和ω(Zn)的平均值为 58.5、0.49、42.1 和156.3 mg·kg⁻¹,青海湖周边土壤样品中这 4 种重金属分别为 15.8、0.1291、13.0 和 50.2667 mg·kg⁻¹. 青海地区土壤重金属含量与其他城市差异较大,表明青海地区人为活动影响较小. 环青海湖土壤中重金属含量与海拔高度存在显著相关性,该结果与张利瑞^[41]等的相似. 张利瑞等^[41]对兰州市耕地土壤重金属进行研究,分析不同环境因子对土壤重金属及其风险的影响. 其结果表明,采样季平均降雨量、GDP 和海拔高度对耕地土壤重金属风险空间差异的影响最显著.

本研究共检测了 80 种 ARGs,与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^[42].通过皮尔森相关性分析发现,土壤中重金属含量与 ARGs 的相对丰度呈显著相关性,该结果与Ji 等^[33]的研究结果相符,其对上海养殖场畜禽粪便和周围土壤 ARGs 研究表明,重金属 Cu 与土壤微生物的多样性存在显著相关性,从而影响土壤中

ARGs 的种类和丰度. 除重金属外,其余环境因子与ARGs 相对丰度间也存在显著相关性,例如海拔高度和年降雨量等.

4 结论

- (1) 青海湖土壤样品中重金属 Cu 和 Hg 的含量与海拔高度存在显著相关性,其余重金属与海拔高度无著相关性.重金属含量在土壤类型分布上差异较为明显,青海地区土壤重金属除了本身固有外,可能主要来源是大气的长距离迁移.环青海湖土壤样品中重金属含量相对较低,所形成的生态风险相对较低.
- (2) 土壤样品中共检测出 80 种 ARGs,其中 β-内酰胺酶类抗性基因丰度最高. 土壤中抗性基因的相对丰度受多种环境因素的影响,年均降雨量是土壤中 ARGs 相对丰度的主要影响因素.
- (3) ARGs 与 MGEs 存在显著正相关. 由此表明,抗生素的滥用不仅会加大环境中耐药基因的储存量,还会增加耐药基因在生物链中的传播风险.
- (4) 本文分析了环青海湖周边土壤样品重金属含量与 ARGs 的相对丰度,并与其他地区进行比对,表明青海地区土壤重金属及 ARGs 的污染相对较低,所造成的健康风险相对较低.探究了重金属及环境因子与 ARGs 相对丰度的相关性,探寻了土壤 ARGs 可能的影响因素,对青海地区土壤 ARGs 污染做了系统的评估.

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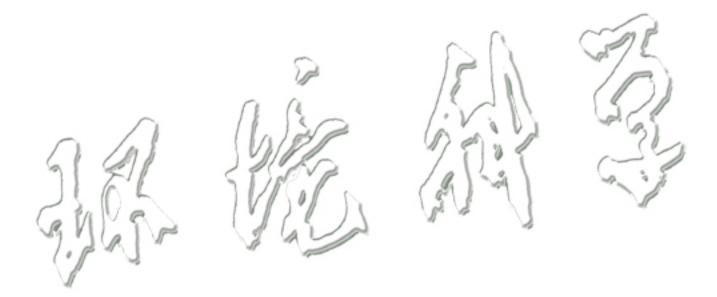
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