Science of the Total Environment 920 (2024) 170991

Contents lists available at ScienceDirect

Science of the Total Environment

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

Airborne antibiotic and metal resistance genes - A neglected potential risk at e-waste recycling facilities

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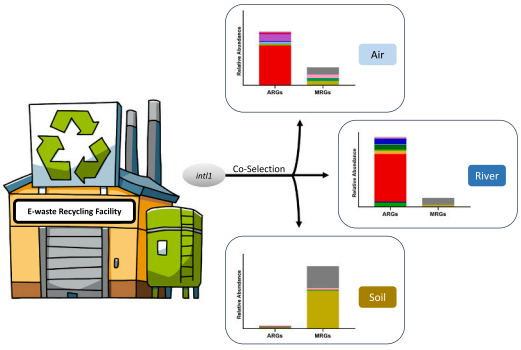
HIGHLIGHTS

- *Intl1* plays a major role in resistance genes acquisition at e-waste facilities.
- Co-selection is the driving mechanism of gene acquisition at e-waste facilities.
- Different ARGs were found in air, soil and, river samples at e-waste facilities.
- Gram-negative bacteria carry more ARGs while gram-positive bacteria carry more MRGs.
- Mercury-rich environments select for a more diverse ARG profile.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Ewa Korzeniewska

Keywords:
Antimicrobial resistance
Mobile genetic elements
Endotoxins
Heavy metals
Aerosols

ABSTRACT

Heavy metal-rich environments can promote the selection of metal-resistance genes (MRGs) in bacteria, often leading to the simultaneous selection of antibiotic-resistance genes (ARGs) through a process known as co-selection.

To comprehensively evaluate the biological pollutants at electronic-waste (e-waste) recycling facilities, air, soil, and river samples were collected at four distinct Swiss e-waste recycling facilities and analyzed for ARGs, MRGs, mobile genetic elements (MGEs), endotoxins, and bacterial species, with correlations drawn to heavy metal occurrence. To our knowledge, the present work marks the first attempt to quantify these bio-pollutants in the air of e-waste recycling facilities, that might pose a significant health risk to workers.

Although ARG and MRG's profiles varied among the different sample types, *intl1* consistently exhibited high relative abundance rates, identifying it as the predominant MGE across all sample types and facilities. These findings underscore its pivol role in driving diverse bacterial adaptations to extreme heavy metal exposure by selection and dissemination of ARGs and MRGs.

All air samples exhibited consistent profiles of ARGs and MRGs, with *bla*TEM emerging as the predominant ARG, alongside *pbrT* and *nccA* as the most prevalent MRGs. However, one facility, engaged in batteries recycling and characterized by exceptionally high concentrations of heavy metals, showcased a more diverse resistance gene profile, suggesting that bacteria in this environment required more complex resistance mechanisms to cope with extreme metal exposure.

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https://doi.org/10.1016/j.scitotenv.2024.170991
Received 27 November 2023; Received in revised form 24 January 2024; Accepted 13 February 2024
Available online 15 February 2024

Furthermore, this study unveiled a strong association between gram-negative bacteria and ARGs and less with MRGs.

Overall, this research emphasizes the critical importance of studying biological pollutants in the air of e-waste recycling facilities to inform robust safety measures and mitigate the risk of resistance gene dissemination among workers. These findings establish a solid foundation for further investigations into the complex interplay among heavy metal exposure, bacterial adaptation, and resistance patterns in such distinctive ecosystems.

1. Introduction

Electronic-waste (e-waste) recycling facilities are specialized sites that handle the disposal and recycling of electronic waste, including discarded electronic devices such as computers, mobile phones, and televisions. Together with facilities that recycle end-of-life vehicles and other scrap metal waste, these facilities pose the most rapidly increasing waste sector, because they play a vital role in managing the ever-increasing volume of electronic and metal waste generated worldwide (Owusu-Sekyere et al., 2022). The global volume of e-waste generated is increasing by 3–5 % annually, and this trend is expected to continue due to growing consumer demands for electronics, the shorter lifespans of devices, and rapid technological advancements (Shittu et al., 2021; Forti et al., 2020). It is projected to reach around 75 million metric tons per year by 2030. A significant part of generated e-waste (12 Mt. out of the 53.6 Mt. worldwide in 2019), and the greatest e-waste generation per capita, with 16.2 kg per capita, is documented in Europe and therefore Europe is a place where studies on e-waste are of significant importance (Forti et al., 2020).

Despite the important functions of e-waste recycling facilities, they also pose significant risks for workers. One of the primary risks faced by workers in these facilities is the exposure to toxic substances, including heavy metals like lead (Pb), mercury (Hg), and cadmium (Cd), as well as chemicals like brominated flame retardants (BFRs) and hydrochlorofluorocarbons (HCFCs) present in the e-waste (Forti et al., 2020). The recycling processes, such as shredding, dismantling, and smelting, can generate aerosols and fine particles that may carry these harmful substances in the air. Inhalation of toxic dust and particulate matter can lead to severe respiratory problems, lung damage, and other adverse health effects (López et al., 2022; Jain et al., 2023).

A less acknowledged concern at e-waste recycling facilities is the release of antibiotic resistance genes (ARGs) and other resistance genes into the air. Antibiotic resistance (AMR) poses a global threat, rapidly spreading around the world and jeopardizing public health. The emergence and dissemination of antibiotic resistance genes (ARGs) have led to the diminishing effectiveness of antibiotics, rendering once-treatable infections more challenging to manage (O'Neill, 2016). Currently, over 20,000 known ARGs exist, representing a diverse range of mechanisms and resistance phenotypes (Inda-Díaz et al., 2023). These genes can be found in various environments, including not only hospitals, farms, and natural ecosystems, but also heavy metal-rich environments such as Municipal Solid Wastes (MSW) landfills that contain high amounts of metals due to e-waste (Anand et al., 2021; Gwenzi et al., 2021; Li et al., 2019a; Gilbert et al., 2010; Xin et al., 2022; Wu et al., 2022; Zhou et al., 2021; Song et al., 2021; Bai et al., 2022; Aminov, 2009; Martínez, 2008; Sarkar et al., 2023; Chen et al., 2017). Studies have shown elevated levels of ARGs in the soil and water of metal-polluted areas and suggested that heavy metals were the key drivers that shaped ARG profiles in these environments (Wang et al., 2021; Seiler and Berendonk, 2012; Nguyen et al., 2019; Zhang et al., 2018; Vats et al., 2022). ARGs can also be aerosolized during the recycling processes, similar to other harmful substances and the aerosolized ARGs pose an additional risk to the workers, including potential colonization or infections by antibiotic-resistant bacteria. Furthermore, aerosolization presents the possibility for the spread and dissemination of antibiotic resistance within and beyond the facility.

ARGs can develop *de novo* by mutagenesis and studies have shown that sub-lethal concentrations of heavy metals can facilitate this process, however horizontal gene transfer (HGT) is more likely to drive the spread of ARGs in metal-rich environments (Zhang et al., 2018; Li et al., 2019b; Mazhar et al., 2021). HGT is a process where genetic material is exchanged between bacteria cells. This transfer is particularly efficient through mobile genetic elements (MGEs), DNA fragments capable of moving between bacterial genomes which include integrons, virus and plasmids (Wang et al., 2021; Wright et al., 2008; Dickinson et al., 2019).

Bacteria are developing resistance not only to antibiotics but also to heavy metals (Wang et al., 2021; Dickinson et al., 2019; Li et al., 2017; Pal et al., 2015). These metal- and antibiotic-resistances (MRGs & ARGs) often occur simultaneously, and this association is not coincidental. Their co-occurrence is driven by two mechanisms: co-resistance and cross-resistance (Dickinson et al., 2019). Co-resistance describes the phenomenon where ARGs and MRGs co-occur. Many ARGs and MRGs are, for example, located on the same plasmids, leading to co-selection during exposure to either antibiotics or heavy metals (Zhang et al., 2018; Siddiqui et al., 2020). The prominent presence of heavy metals in e-waste recycling facilities contributes to the co-selection phenomenon. MRGs confer a selective advantage to bacteria in the metal-rich environments, facilitating the maintenance and spread of ARGs. Cross-resistance, on the other hand, refers to genes that encode for mechanisms providing resistance to multiple compounds, which could be antibiotics and heavy metals. Most often, cross-resistance involves efflux pumps that can pump out specific antibiotics and heavy metals (Martinez et al., 2009; Roosa et al., 2014).

To gain a comprehensive understanding of the potential risks associated with aerosolized resistance genes, it is crucial to identify their host species. Resistant bacteria that are human pathogens would pose significantly greater risk to workers compared to environmental bacteria. This is because colonization and infections with such bacteria can lead to severe and challenging-to-treat health issues (O'Neill, 2016). Identifying and characterizing bacterial species with ARGs and MRGs can be achieved through various ways, such as sequencing and endotoxin testing. Endotoxins are components of the outer membrane of gram-negative bacteria and can be released into the air during the recycling processes (Degobbi et al., 2011; Rolph et al., 2018). In addition to being a valuable method for characterizing the hosts of resistance genes, aerosolized endotoxins themselves present a health risk as well. Inhalation of endotoxins can result in adverse respiratory effects and exacerbate respiratory conditions in individuals working in or residing near these facilities. Therefore, incorporating the measurement of endotoxin levels in the air is crucial not only for a comprehensive characterization of the microbial environment and resistance gene hosts but also to elucidate the complex biological hazards present in the air of e-waste facilities, providing a more complete picture of the overall biopollutants in these facilities. This holistic approach is essential for fully grasping the intricate environmental health risks associated with e-waste recycling.

The combination of airborne ARGs, MRGs, MGEs, and endotoxins in e-waste recycling facilities may present complex biological hazards and to our knowledge has not been studied before. We hypothesized that the co-selection with MRGs might create an increased risk of antibiotic resistance among bacteria within the facility, which could potentially result in the dissemination of antibiotic resistance to surrounding environments, and subsequently posing a risk of respiratory health issues among workers and nearby residents.

Our study is pioneering in its endeavor to comprehensively investigate these airborne biological pollutants at e-waste recycling facilities. Our objectives include quantifying the amounts of the hazardous biopollutants, evaluating the correlations between the different gene types, endotoxins, metals, and bacterial species, and assessing their potential for emission via the air. This research aims to fill a critical gap in our understanding of the environmental risks associated with e-waste recycling from a biological point of view, providing insights that can inform strategies to mitigate potential health impact on workers and neighboring communities.

2. Material and methods

2.1. Sample collection sites and methods

Four different types of e-waste and scrap metal recycling facilities were chosen for this study:

an e-waste collection facility (A)
an e-waste recycling facility (B)
a car demolishing facility (recycling of end-of-life vehicles, as well as small and big electronic devices, such as refrigerators) (C)
a battery recycling facility (D).

At each facility, several suitable locations were chosen for sample collection as can be seen in S1. At each location, a minimum of 2 samples were taken.

From February to April 2021, total suspended particles (TSP) were sampled on quartz filters with a 0.3 μm pore size (VWR International, USA) and a diameter of 80 mm using a medium-volume air sampler that has been previously used in bioaerosol studies (100 L/min, LY2050, Qingdao, China (Agarwal et al., 2023; Yue et al., 2019)) for 24 h at each location. Filters were stored at −20 °C until further analysis.

Furthermore, approximately 25 g of soil samples were taken from the surface of the ground at different locations around each facility and stored in falcon tubes at −20 °C until further analysis. At two facilities (A & C), there were nearby rivers from which samples were also taken by placing a 50 mL falcon tube approximately 10 cm deep into the running water which were then stored at −20 °C. All locations of soil and river sampling can be seen in S1.

2.2. Metal determination by ICP-OES, ICP-MS and FIMS

The quantitative determination of metals was carried out after extraction of ½ filter in a mixture of 6 mL HNO_3 67 % normatom, 1 mL H_2O_2 30 % suprapur and 1 mL high-purity water (18 MΩ cm) in a microwave oven (MLS Start 1500) at 210 °C for 30 min. The digestion solution was diluted to 50 mL with high-purity water. For the mercury determination, an aliquot of 10.0 mL was taken and stabilized with 5 % $KMnO_4$ (pink colour).

The determination of the elements Cd, Cu, Pb, Co and Zn was carried out, depending on the content, by inductively coupled plasma optical emissions spectrometry (ICP-OES, Agilent 5110) or by triple quadrupole plasma mass spectrometry (QQQ-ICP-MS, Agilent 8800) using different gas reaction modes to eliminate interferences.

For the determination of mercury, the sample solutions were prereduced with hydroxylammonium hydrochloride solution and subsequently reduced to Hg^0 using the cold vapour technique on a flow injection mercury system (FIMS-400, Perkin Elmer) by means of $SnCl_2$ and quantified.

2.3. DNA extraction

DNA extraction of the samples followed the original manufacturer's protocol of the DNeasy PowerSoil Kit (Qiagen, Germany), with the addition of AMPure magnetic beads (Beckman Coulter, USA), and the previously published method by Agarwal et al. (2023) with slight modifications as follows (Agarwal et al., 2023).

For the air samples, 1/6 of each filter was cut into small pieces and added to the PowerBead tubes. For the river samples, a volume of 45 mL was passed through a 0.2 μm membrane filter (Sartorius, Germany), which was subsequently cut into small pieces and added to the PowerBead tubes. 250 mg of each soil sample was weighed and directly added to the PowerBead tubes.

The extracted DNA was eluted in a final volume of 60 μL of double distilled water (ddH_2O) (Thermo scientific, USA). The DNA concentration was measured using the microplate absorbance reader Infinite® M Nano (TECAN, Switzerland). All DNA samples were stored at −20 °C until further analysis.

2.4. qPCR for detection of ARGs, MGEs and MRGs

qPCR was utilized to detect 17 different ARG subtypes conferring resistance to 7 types of commonly used antibiotics, two different MGEs, two bacterial reference strains, and 5 MRGs (details in S2 & S3). All primer sets were selected from previously published studies to cover a wide range of common ARG, MGE, and MRG types (Agarwal et al., 2023; Roberto et al., 2019; Yang et al., 2020a).

All qPCR reactions were performed on a BioRad real-time PCR system. The amplification of the ARGs, MGEs, and bacterial reference strains followed the established protocol described by Agarwal et al. (2023), while the amplification of the MRGs followed the established protocols described by Roberto et al. (2019) and Yang et al. (2020a). Each qPCR reaction was conducted in a volume of 20 μL using SYBR Green. For all ARGs and MGEs an initial heating step of 95 °C for 3 min was followed by 45 cycles of the following conditions: denaturation for 20 s of 95 °C, annealing for 20 s at 55–60 °C (depending on the gene, S2) and extension for 30 s at 72 °C. At the end of each run, a melting curve was done by 30 s at 95 °C, followed by a stepped temperature ramping from 65 °C to 95 °C (0.5 °C steps in 10 min). For all MRGs the following conditions were used: an initial heating step of 95 °C for 3 min followed by 40 cycles of denaturation for 30 s at 95 °C, annealing for 1 min at 56–62 °C (depending on the gene, S3) and extension for 40 s at 72 °C. For validation purposes a melting curve was done as well.

The threshold cycle (CT) values obtained from the qPCR runs were used to calculate the relative abundance of each gene by using the 2^(-ΔCT) method. This approach utilizes the CT values of each individual gene and normalizes them to the total microbial DNA abundance (CT values of the reference gene 16S), as previously described by Agarwal et al. (2023). This normalization allows for straightforward comparison of abundance rates between samples, sample types, and across different studies. To obtain the absolute abundance values for the 16S gene, plasmids containing known concentrations of the 16S were utilized. These plasmids were constructed from *E. coli* JM109 strain as described by Tao et al. (2021).

2.5. Bacterial 16S rRNA sequencing

To assess the microbial diversity in the air and soil in and around the different e-waste recycling facilities, 16S rRNA bacterial amplicon sequencing using the Illumina MiSeq 2x300bp sequencing platform (Novogene, UK) was performed. The sequencing approach targeted the V3-V4 region of the 16S rRNA gene, and a sequencing depth of 20,000 reads per sample was achieved. The exact count of qualified reads per sample, with all chimeras removed, along with the accession numbers of the sequences at GenBank (NCBI, US) can be found in S4. Prior to sequencing, all replicates from each location of the four recycling facilities were combined into pooled samples. Library preparation, sequencing and subsequent bioinformatical analysis were conducted by Novogene, UK.

2.6. Endotoxin test

The overall burden of gram-negative bacteria in the air of the e-waste recycling facilities and the potential risks associated with airborne endotoxin exposure were estimated by conducting an endotoxin test on all air samples. To process the air samples collected on filters, 1/8 of each filter was cut into small pieces and added to 1 mL of ddH_2O. The samples were then pretreated with 0.05 % Tween 20 by vortexing for 30 min and centrifuging at 10,000*g* for 1 min. The resulting supernatant was used for the endotoxin assay, with a 100-fold dilution in phosphate-buffered saline (PBS), following the Chrome-LAL assay protocol from Cape Cod, as previously described by Agarwal et al. (2023).

The measurements of endotoxin concentrations were performed using the microplate absorbance reader Infinite® M Nano (TECAN, Switzerland). The associated Magellan software (TECAN, Switzerland) was employed for data analysis and calculation of the endotoxin concentrations.

2.7. Statistical analyses

Statistical analyses were performed and graphical representations were prepared using GraphPad Prism software (v10.0.2, available at https://www.graphpad.com/) and RStudio (v4.3.0, available at http://www.r-project.org/).

To assess the statistical significance of observed differences, multiple unpaired *t*-tests were conducted, with a significance level set at $p \leq 0.05$. The results were considered statistically significant when the *p*-value fell below this threshold.

Correlation analyses were carried out using RStudio, by using the following packages for calculations and visualizations: igraph and psych. A correlation was deemed significant if the Spearman's correlation coefficient (r) exceeded 0.6, and the *p*-value was less than 0.05. Correlation analyses focusing on endotoxins at individual facilities were not limited by the Spearman's correlation coefficient or p-value and were only intended to show trends rather than statistical significance.

For calculations and visualizations of a redundancy analysis (RDA), the following packages in RStudio were employed: vegan, ggplot2, ggrepel, ade4, ggord and ggalt.

3. Results & discussion

3.1. Detection of heavy metals

In this study, air samples at four distinct e-waste recycling facilities in Switzerland were taken and the concentrations of six heavy metals were determined.

Among the four sites, the e-waste collection facility exhibited notably lower metal concentrations, with a cumulative concentration of all six metals of 62–72 ng/m^3 than the other facilities. This can be attributed to effective ventilation within the facility and the absence of recycling operations, resulting in minimal aerosol generation and subsequently lower airborne metal levels. In contrast, all other facilities involved recycling processes such as shredding, dismantling, and smelting, leading to substantially more intensive aerosol generation, with airborne metal concentrations reaching up to 383,490 ng/m^3 (highest inside the battery recycling facility).

When comparing the individual measurements in this study with standard heavy metal concentrations in the ambient air (continuously monitored at up to 67 stations across Europe and regularly published by EMEP (EMEP-CCC-3-2022.pdf, n.d.)), it becomes evident that the concentrations detected at the four e-waste facilities are significantly higher than typical ambient air concentrations (S6). While the e-waste collection facility displayed an elevation of around 1–2 log levels compared to normal ambient air concentrations, the other three facilities showed elevations of around 2–3 log levels, and in some cases even 4–5 log level elevations, such as cobalt (Co) concentrations at the battery recycling facility and e-waste recycling facility. The only exceptions are mercury (Hg), which was detected within the range of normal ambient air concentrations at the e-waste collection site and at some locations within the car demolishing site, and zinc (Zn), which was not detected at all in most sampled locations.

Copper (Cu) and lead (Pb) emerged as the most prevalent metals across all facilities (S5) due to their widespread use in copper wires, printed circuit boards, glass panels, and gaskets found in computer monitors (Dave et al., 2016; Widmer et al., 2005). Additionally, Co and Zn were found in elevated concentrations at the e-waste recycling site and battery recycling site. Notably, the latter facility exhibited exceptionally high levels of Zn, accompanied by significant amounts of cadmium (Cd) and Hg, reflecting the presence of these metals as core components in various batteries (Recknagel et al., 2014; Kuchhal and Sharma, 2019).

While metal concentrations at the e-waste collection site remained relatively consistent between indoor and outdoor sampling locations, other facilities showed substantial differences (S7). For instance, the e-waste recycling site saw a concentration increase of approximately one logarithm (log) in all metal levels during the recycling stage compared to the arrival and storage stage, with Zn solely detected at that stage. A similar trend occurred at the car demolishing facility, where all metal concentrations increased by around 1 log at locations involving recycling processes, such as near the shredder, in contrast to locations without recycling operations, e.g. the storage hall. Zn was not detected anywhere within this facility. The most significant differences between sampling locations were however observed at the battery recycling facility, with disparities of around 2–3 log levels between indoor and outdoor samples, except for Zn, which was exclusively found indoors at exceedingly high levels (more than 350,000 ng/m^3). These results clearly demonstrate that the recycling processes generate a substantial quantity of aerosolized metal particles, presenting a considerable occupational risk to facility workers.

This assumption is strengthened when comparing the measured concentrations to the guidelines established by the World Health Organization (WHO) for limit values of Cd, Pb and Hg in Europe expressed as annual time weighted averages (TWAs) (Table 1). While the limit value for Hg was only exceeded in the indoor area of the battery recycling facility, Cd and Pb limit values were exceeded at all stages of the e-waste recycling facility, as well as the indoor location of the battery recycling facility and some locations within the car demolishing facility. Some of these values exceeded the limits by more than 2 log levels, indicating a significant health risk for employees at these facilities. Conversely, locations without aerosol-generating processes carried metal concentrations within the WHO guideline values, suggesting rapid deposition of aerosolized metal particles and consequently a significantly reduced risk to nearby residents compared to exposed workers. No WHO guidelines have been established for Co, Cu and Zn. The WHO guidelines are however intended for the general population and do not specifically apply to occupational environments. In the United States on the other hand, the California Division of Occupational Safety and

Table 1
Comparison of WHO and Cal/OSHA guideline limit values for heavy metal exposure and the measured concentrations in this study.

Metal	WHO annual TWA [ng/m^3] (World Health Organization, 2000)	Cal/OSHA PEL 8-hour TWA [ng/m^3] (*Occupational Safety and Health Administration*, n. d.)	Concentration ranges in this study [ng/m^3]
Co	–	20,000	1–3428
Cu	–	100,000	14–10,528
Cd	5	5000	1–2246
Pb	500	50,000	10–13,466
Hg	1000	100,000	1–5375
Zn	–	–	0–367,399

Health Administration (Cal/OSHA) establishes permissible exposure limits (PEL) for occupational settings (Table 1) which are the legally enforceable limits in the USA. The measured concentrations of metals in the current study do not surpass these limits. The large gaps between the WHO limits and PELs reflect different assessments of the hazardous potentials of these metals. Based on the precautionary principle, it is recommendable to reduce the airborne metal concentrations and personal exposure in general.

3.2. Absolute abundance of the reference gene 16S

The absolute abundance of the reference gene 16S provides an estimate of the total bacterial load in each sample type at each facility.

In the air samples, the absolute abundance rates of 16S ranged around 10^5 copies/m^3, with the lowest rate of 1.42×10^4 copies/m^3 detected in a sample from the e-waste collection site, and the highest rate of 1.72×10^5 copies/m^3 detected in a sample from the car demolishing facility (S8). For the soil samples, the rates were consistently in the range of 10^{10} copies/g across all soil samples from all facilities. In the river samples, there was a bit more variability, with rates ranging from 3.84×10^6 copies/L in the river near the car demolishing facility to 1.37×10^7 copies/L in the river near the e-waste collection site.

These values align with findings from previous studies on 16S abundances (Tao et al., 2021; Lymperopoulou et al., 2016; Zhang et al., 2017; Tkacz et al., 2018), suggesting that the observed bacterial loads in the current study are within the normal range for the sampled environments. Only in the case of 16S abundance in river samples, some studies in the literature reported abundance rates up to 2–3 log levels higher compared to the current study, suggesting wider ranges of 16S abundance rates in this sample type (Guan et al., 2018; Yang et al., 2020b; Chen et al., 2019a).

3.3. Detection of ARGs, MGEs and MRGs in different environments

All air, soil and river samples collected in this study were analyzed to determine the relative abundance of ARGs, MGEs and MRGs. This study prioritized the relative abundance of the genes over absolute abundance to enhance comparability across samples and sample types, aiming to provide a comprehensive overview of the diverse gene patterns present in the investigated environments.

3.3.1. Air

Regarding the air samples, the battery-recycling facility exhibited the highest relative abundance of ARGs at 0.0825 ± 0.0216, while the e-waste collection site displayed the lowest one at 0.0031 ± 0.0006 (Fig. 1a).

11 ARGs were detected, among which *bla*TEM was the most prevalent across all four facilities, with *sul2* and *tetW* also commonly identified. Other ARGs were less frequently observed. These findings align with previous studies, which have shown that ARGs found in metal-rich environments often belong to beta-lactam, tetracycline, and sulfonamide resistance gene categories (Li et al., 2017; Li et al., 2015; Knapp et al., 2011; Zhao et al., 2019). However, it is important to note that the present study represents the first investigation into ARG occurrence in the air of metal-rich environments, limiting the comparisons with published literature to the results on soil and aquatic samples. A study by Li et al. (2018) investigated ambient levels of ARG in Zürich, Switzerland, among other city, and included several genes that were also part of the current study. These findings serve as valuable background levels of ARGs, enabling a comparison with our results. While many genes exhibited similar relative abundances, two genes, *sul2* and *tetW*, were notably elevated in the samples from the e-waste recycling facilities compared to urban air samples. *sul2* was elevated by around 1 log. *tetW* on the other hand was not detected at all in the urban samples in the study conducted by Li et al. (2018), while it was detected in every sample of the current study at an abundance rate of around 0.0005. These two genes emerged as among the most dominant genes in the air of e-waste facilities, suggesting their specific selection in metal-rich environments such as e-waste recycling facilities.

Interestingly, the battery recycling facility exhibited a more diverse array of ARGs, including *vanA*, *qnrS*, *mhpA* II, *floR*, and *tetG* at substantial abundances, while *merA*, a gene associated with mercury resistance, was not detected at all (Fig. 1a). A study by Zhao et al. (2021) similarly reported greater ARG diversity in mercury-polluted soil, although the specific types of ARGs differed, likely due to the variation in sample types. This result suggested that mercury-rich environments fostered a broader ARG profile most likely beyond the ARGs included in this study, which possibly reflects a more complex survival strategy adopted by bacteria in such a setting. Furthermore, the need to contend with various harmful metals and other hazards simultaneously, as observed in the present study, where elevated levels of not only Hg but also Zn, Cd, Cu, and Pb were recorded, might add to the complexity of the survival strategies.

Three of the ARGs studied here encode for efflux pumps: *acrA*, *floR*, *tetG*. Among them, *acrA* was not detected in any air samples, while *floR* and *tetG* were observed with low relative abundance rates. Consequently, it can be inferred that cross-resistance driven by efflux-pump associated ARGs may be a less significant mechanism for bacteria to develop antibiotic resistance in the air of heavy metal rich environments.

Conversely, co-selection appears to be a more likely mechanism for ARG acquisition at e-waste facilities, given the high relative abundance of MGEs detected across all facilities (Fig. 2). Particularly, *intI1* was found at high relative abundance rates in all facilities, consistent with

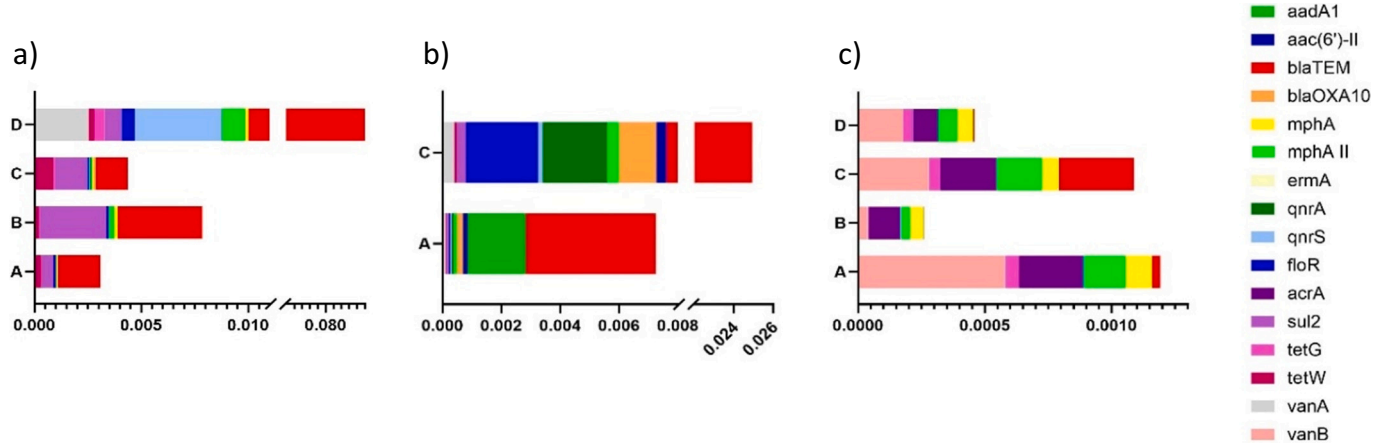

Fig. 1. Relative abundance of ARGs detected at each facility using the 2^(−ΔCT) method in the a) air; b) river; c) soil.

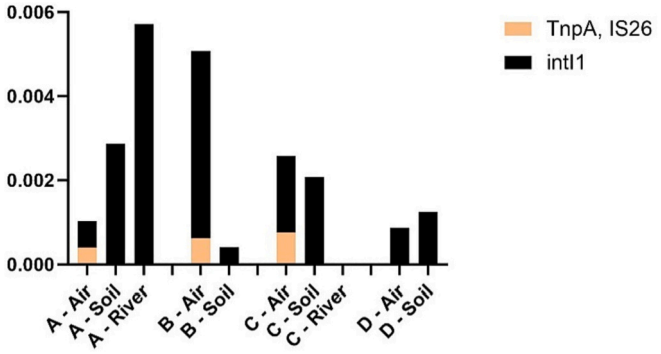

Fig. 2. Relative abundance of MGEs found at each facility in the air, soil and river using the 2^(−ΔCT) method.

prior studies highlighting its potential pivotal role in gene acquisition within metal-polluted environments (Wright et al., 2008; Zhao et al., 2019; Di Cesare et al., 2016).

Regarding the MRGs found in the air, *pbrT* and *nccA* clearly dominated across all facilities (Fig. 3). *copA* and *merA* were detected in the air at similar abundance rates as *pbrT* and *nccA* in the e-waste recycling site and car demolishing facility but were scarcely detected in the air samples from the other two facilities. These observations are consistent with previous research, that identified *pbrT*, *nccA*, and *copA* as commonly occurring MRGs in metal-rich environments (Roosa et al., 2014; Liao et al., 2023; Chen et al., 2019b).

3.3.2. Soil

The relative abundance rates of ARGs in the soil were notably lower, with approximately a 1-log difference compared to the air samples (Fig. 1c). Moreover, the diversity of ARGs in the soil significantly diverged from those observed in the air. For instance, *bla*TEM, the most abundant ARG in air samples, was only found in the soil sample of the car demolishing facility, while *vanB*, *acrA* and *mphA* II were the prevailing ARGs in the soil samples overall. Although previous studies did not identify these specific genes as the most abundant ARGs in metal-polluted soils, vancomycin, multidrug, and MLSB resistance genes have frequently emerged in prior research as well (Li et al., 2017; Li et al., 2015; Zhao et al., 2019). The uniform ARG diversity profile across all soil samples suggests that these ARGs are common in soils near e-waste recycling facilities.

The substantial divergence in ARG diversity between soil and air samples might occur due to certain bacteria being easier aerosolized than others and subsequently carrying different genes with them to the aerosol phase (Perrott et al., 2017). In addition, factors beyond heavy metal exposure might influence the ARG and MRG profiles in the different sample types (Bengtsson-Palme et al., 2018; Larsson and Flach, 2022).

Conversely, while the same two MRGs dominated in both air and soil samples (*pbrT* & *nccA*), the relative abundance rates of MRGs in the soil were considerably higher with up to 1 log differences (Fig. 3).

The present study represents the first investigation into the disparities of ARG and MRG patterns in different sample types at e-waste facilities and further research is warranted to uncover the precise mechanisms and factors influencing the differences in expression of either of these resistance genes. An intriguing similarity between the air and soil samples lies however in *intI1* being the predominant MGE in both sample types, reinforcing the notion that *intI1* plays a central role in bacterial adaptation strategies within metal-rich environments and therefore co-selection is the major mechanism for gene acquirement in the soil as well, even though the high abundance of *acrA* suggests the possibility that cross-resistance occurs more frequently in the soil than in the air (Fig. 2).

3.3.3. River

ARG abundance rates in the river samples were found to be higher

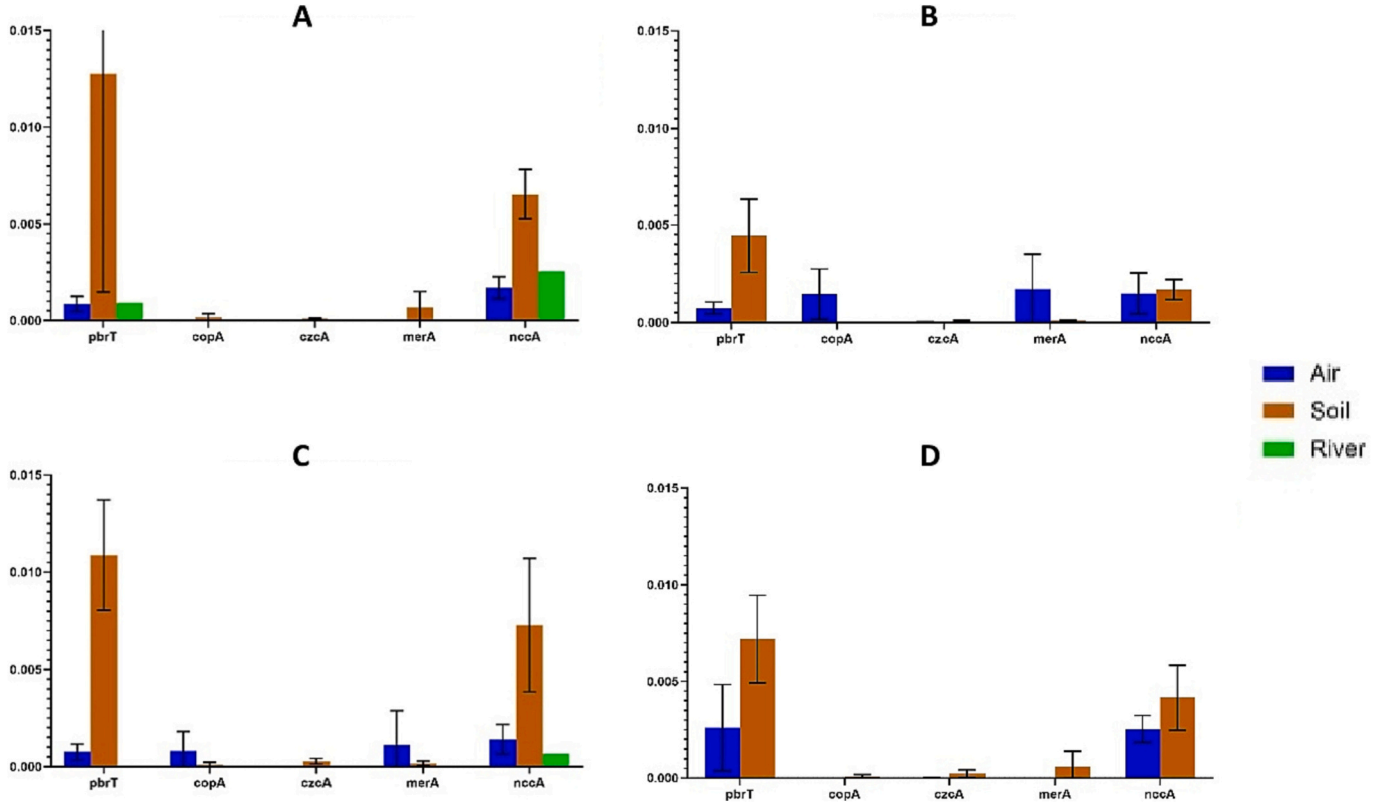

Fig. 3. Relative abundance of MRGs found at each facility in the air, soil and river using the 2^(−ΔCT) method; error bars indicate the standard deviation.

compared to those in air and soil samples (Fig. 1b). It is noteworthy that although *bla*TEM was also the most abundant ARG in the river samples, other ARGs that were less prevalent in the air samples displayed higher abundance levels in the river samples, including *aadA1*, *qnrA* and *floR*. This observation indicates that the ARGs present in the river samples may have been influenced by factors and conditions unrelated to the e-waste facilities, possibly originating upstream from the facilities. This interpretation gains credibility from the fact that the two river samples from the e-waste collection and car demolishing sites exhibited substantial differences from each other, as well as from findings in other studies examining river samples (Knapp et al., 2011; Graham et al., 2011). While the river samples near the e-waste collection site had high amount of *aadA1* and *intl1*, the river sample near the car demolishing site had higher amounts of *qnrA* and *floR* and no detectable MGE.

3.4. Detection of endotoxins

Endotoxins are components of the outer wall of gram-negative bacteria and serve as effective indicators for such bacteria. Furthermore, they present a significant health risk to exposed individuals, making them a crucial parameter for assessing biological hazards in the air (Liebers et al., 2020; Sykes et al., 2011).

In the present study however, airborne endotoxin concentrations were consistently low, ranging from 0.18 to 1.07 EU/m^3 (Fig. 4). Even though, no guidelines for ambient endotoxins exists, a Dutch committee in 1998 recommended that exposure should not exceed 50 EU/m^3 (Sykes et al., 2011). The significantly lower endotoxin concentrations detected in this study indicate that endotoxins are of lesser concern at e-waste recycling facilities in this study and pose a low risk to the health of workers.

3.5. Variations in sampling locations at each individual facility

Upon closer examination of each facility, notable variations became apparent, indicating that factors beyond heavy metal exposure may influence the presence of ARGs, MGEs, and MRGs.

While only marginal differences in abundance rates of ARGs, MRGs, MGEs, endotoxin levels, and heavy metal concentrations were observed between the two sampling locations at the e-waste collection site, more substantial differences within a facility were noted at the e-waste recycling site and car demolishing site (S9–12). This divergence could be attributes to effective ventilation within the e-waste collection facility and the fact that this site exclusively handled the collection of e-waste without any processing, whereas the other facilities engaged in significant amounts of recycling processes.

In the e-waste recycling and car demolishing facilities, certain locations, such as the recycling stage at the e-waste recycling facility or an outside location near a railway at the car demolishing site, exhibited the highest MRG and MGE abundance rates within the respective facility. Conversely, these locations showed ARG abundance rates and endotoxin concentrations that were among the lowest at each facility. In contrast, other locations, such as the storage stage at the e-waste recycling facility or the location near the shredder at the car demolishing site, displayed higher ARG abundance rates and endotoxin concentrations, while MRGs and MGEs were comparatively lower.

Despite the seemingly similar pattern in these two facilities, this trend was not associated with elevated or lower metal concentrations. This implies that factors beyond metal concentrations played a significant role in shaping bacterial coping strategies in metal-rich environments and that further research is needed to comprehensively understand the multifaceted dynamics of gene expression and acquisition in these environments.

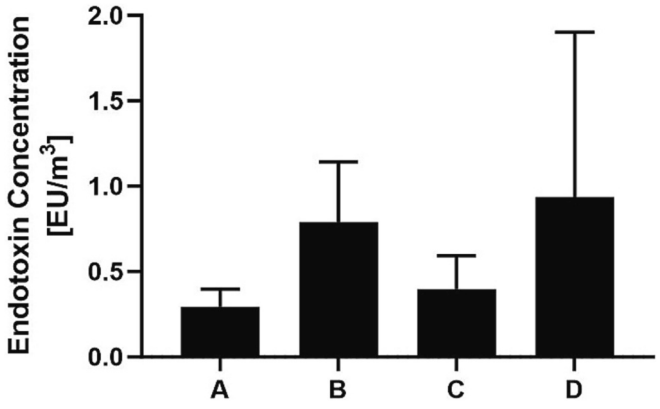

Fig. 4. Endotoxin concentration detected at each E-waste facility; error bars indicate the standard deviation.

3.6. Bacterial community composition

Bacterial community composition in both air and soil samples from all four facilities was characterized through sequencing.

At the phylum level, Actinobacteriota and Proteobacteria emerged as the two most common bacterial phyla in both air and soil (Fig. 5). However, beyond these predominant phyla, there were significant differences in bacterial composition between air and soil samples. In the air samples, the four most common phyla (Actinobacteriota, Proteobacteria, Cyanobacteria and Firmicutes) accounted for over 85 % of the bacterial composition in nearly all samples. In contrast, soil samples exhibited greater diversity, with the top 10 phyla not even comprising 80 % of the bacterial composition in most cases.

Most of the bacterial genera detected in the air and soil samples were typical environmental bacteria commonly found in terrestrial or aqueous environments, such as *Sphingomonas* and *Agrococcus* (S13). In the air samples, several genera were also associated with human colonization, particularly skin bacteria such as *Corynebacterium*, suggesting continuous shedding of these bacteria by the workers. Notably, pathogenic bacteria, including *Pseudomonas*, *Staphylococcus*, and *Acinetobacter*, which are often linked to antibiotic resistance, were also identified in the air samples, indicating a potential risk for workers at e-waste facilities to encounter antibiotic-resistant pathogens, posing health concerns (Coello Pelegrin et al., 2021; Molineri et al., 2021).

Interestingly, several bacterial genera detected in the air of the e-waste facilities, are typically predominant in extreme environments like Antarctica or deserts, such as *Psychrobacter*, *Carnobacterium*, and *Blastococcus* (Bozal et al., 2003; Leisner et al., 2007; Castro et al., 2018). The presence of these bacteria in the air of e-waste facilities suggests that they may have been transported through the air from distant locations and have adapted to the environment at the e-waste facilities.

3.7. Correlation analysis between genes, species, endotoxin, and heavy metals

In the correlation analysis of the air samples, all ARGs, MGEs, MRGs, endotoxin, heavy metals, and the top five bacterial phyla were included. A strong correlation cluster was observed around *intl1*, which showed significant correlation with several ARGs, MRGs, metals and the gram-negative bacterial phylum Firmicutes (Fig. 6). Firmicutes have previously been associated with strong correlations to ARGs, such as with *sul1* and *sul2* (Shi et al., 2020; Huerta et al., 2013). In this study the correlation cluster contained: *sul2*, Co, *copA*, *mphA*, Pb, Cd, *mphA* II, and *merA*, with the correlation strength in decreasing order. These findings suggest that especially Firmicutes may utilize *intl1* for HGT of the mentioned ARGs and MRGs, particularly in the presence of Co, Pb and Cd. Importantly, the phylum Firmicutes encompasses many human pathogens, such as *Staphylococcus* and *Clostridium*, which were also detected in relatively high abundance in the air of all e-waste facilities. Infections caused by these bacteria, especially when being antibiotic

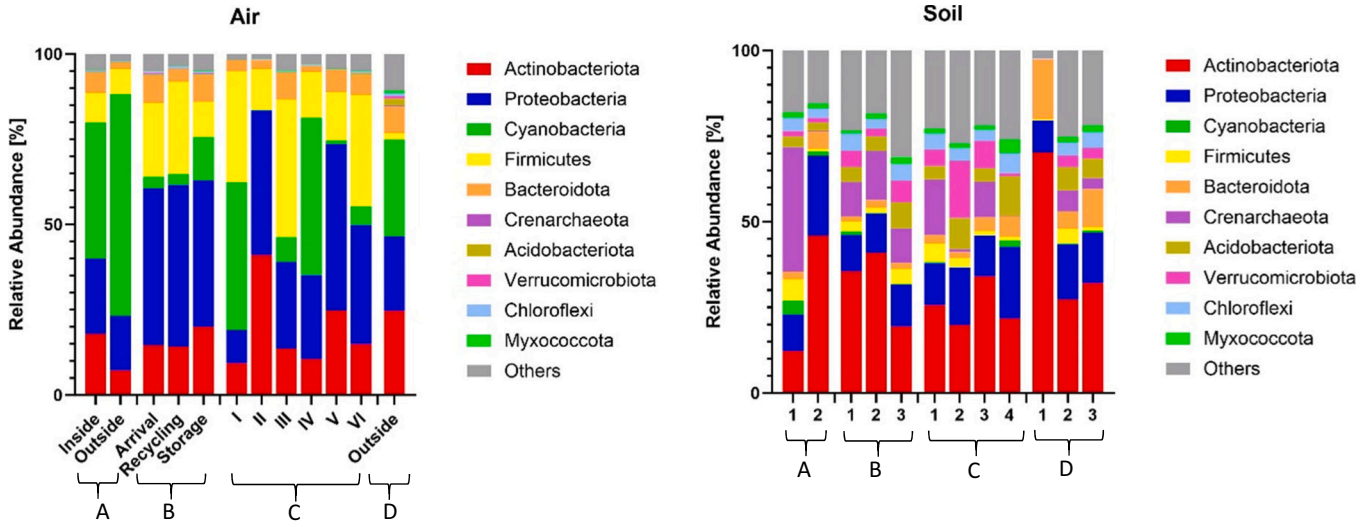

Fig. 5. Top 10 bacterial phyla found in the air and soil of each location of each e-waste facility.

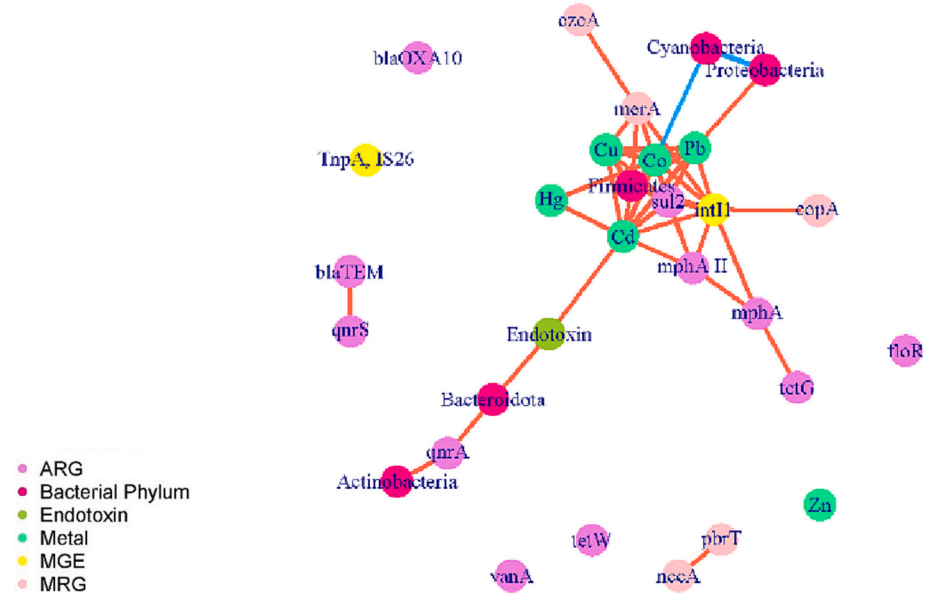

Fig. 6. Correlation analysis of ARGs, MGEs, MRGs, heavy metals and the top 5 bacterial phyla found in the air samples; red edge = positive correlation, blue edge = negative correlation.

resistant, can therefore pose a significant risk to workers at the e-waste facilities and nearby residents.

Cyanobacteria are the only bacteria that showed a negative correlation with certain factors in this analysis (to Co and Proteobacteria), indicating a lesser association with metal-rich environments.

At two facilities (e-waste recycling site and car demolishing site) it was observed that endotoxin concentrations tended to increase in locations where there was a higher relative abundance of ARGs and a lower relative abundance of MRGs. To delve deeper into this association, individual correlation analyses specifically focusing on the relationships with endotoxins were carried out. These analyses indeed revealed negative correlations between endotoxins and most MRGs, as well as positive correlations with many ARGs (S14) at both facilities. However, it is crucial to note that no statistical significance has been determined due to a low number of samples, thus interpretations are speculative. Despite this, the observation of endotoxin concentrations aligning with the prevalence of ARGs suggest that gram-negative bacteria are more likely to carry ARGs and less likely to carry MRGs. There are parallels of this interpretation with findings from Biswas et al. (2021), where gram-negative bacteria were more prone to develop resistance to antibiotics and less to metals in contrast to gram-positives that developed more metal resistance and less antibiotic resistance (Zhao et al., 2021), however this comparison should be approached with caution and further research and statistical validation are needed.

Furthermore, a correlation analysis with all ARGs, MGEs, MRGs, endotoxin, heavy metals, and the top 5 bacteria phyla detected in the soil was conducted. A big correlation cluster was again observed around *intl1* in the soil samples, showing strong correlations with *vanB*, *nccA*, *pbrT*, *bla*TEM, *bla*OXA10, *mphA* II, *acrA*, *sul2*, *tetG*, and *copA* (Fig. 7). Many genes in this cluster overlapped with those in the air sample cluster, reinforcing the notion that *intl1* is the key driver of HGT of ARGs and MRGs at e-waste facilities, regardless of their occurrence in the air or soil. Although *TnpA*/IS26 had a strong correlation with Bacteroidota and Actinobacteria in the soil, it had no correlation with any ARG or MRG, except a negative correlation with *vanA*, suggesting that *TnpA*/IS26 may have a lesser role in the spread of ARGs and MRGs at e-waste

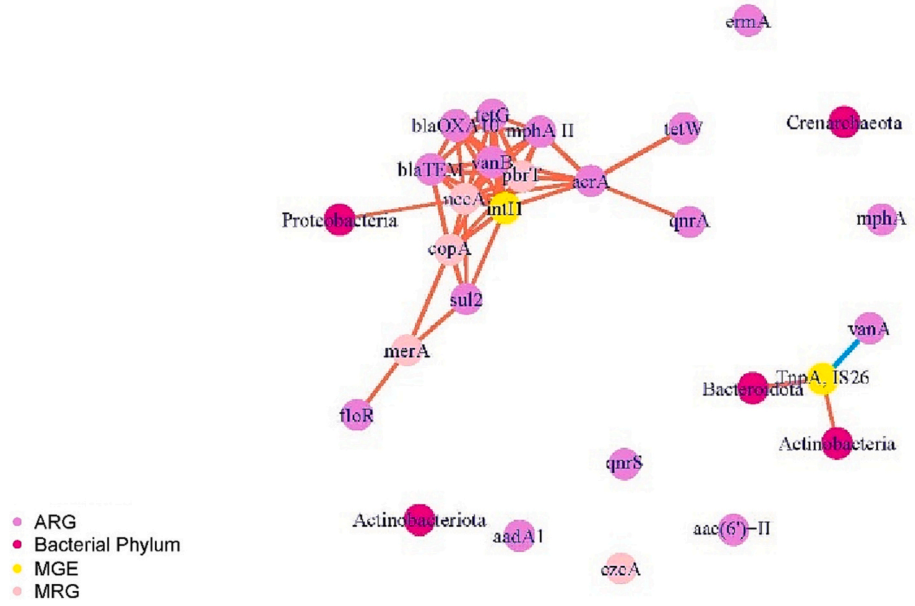

Fig. 7. Correlation analysis of ARGs, MGEs, MRGs, heavy metals and the top 5 bacterial phyla found in the soil samples; red edge = positive correlation, blue edge = negative correlation.

facilities.

3.8. RDA

In the first RDA analysis that was done with the results from the air samples only, RDA1 primarily accounted for variation between individual samples due to their location factor (Fig. 8a). The battery recycling facility exhibited significant variation and was positioned far from the other three facilities on the graph, while the other three facilities were positioned closer to each other, and their samples exhibited less variation. RDA2, on the other hand, was largely explainable by metal concentrations found at the facilities.

The plot revealed distinct clusters of genes influenced by specific facilities. The battery recycling facility contributed to a cluster on the right side of the plot, including the following genes: *bla*TEM, *qnrS*, *floR*, *vanA*, *tetG*, *nccA*, *pbrT*, and *mphA* II. These genes were detected at higher abundance rates at the battery recycling facility, with some being more than 2 log levels higher than at the other facilities (*qnrS* and *vanA*). Another cluster on the left bottom of the plot consisted of *sul2*, *merA*, *copA*, *intI1*, and *mphA*, which were most likely contributed by the e-waste recycling facility. Lastly, a cluster on the far upper left of the plot included *bla*OXA10, *tetW*, *TnpA*/IS26, and *qnrA*, which were

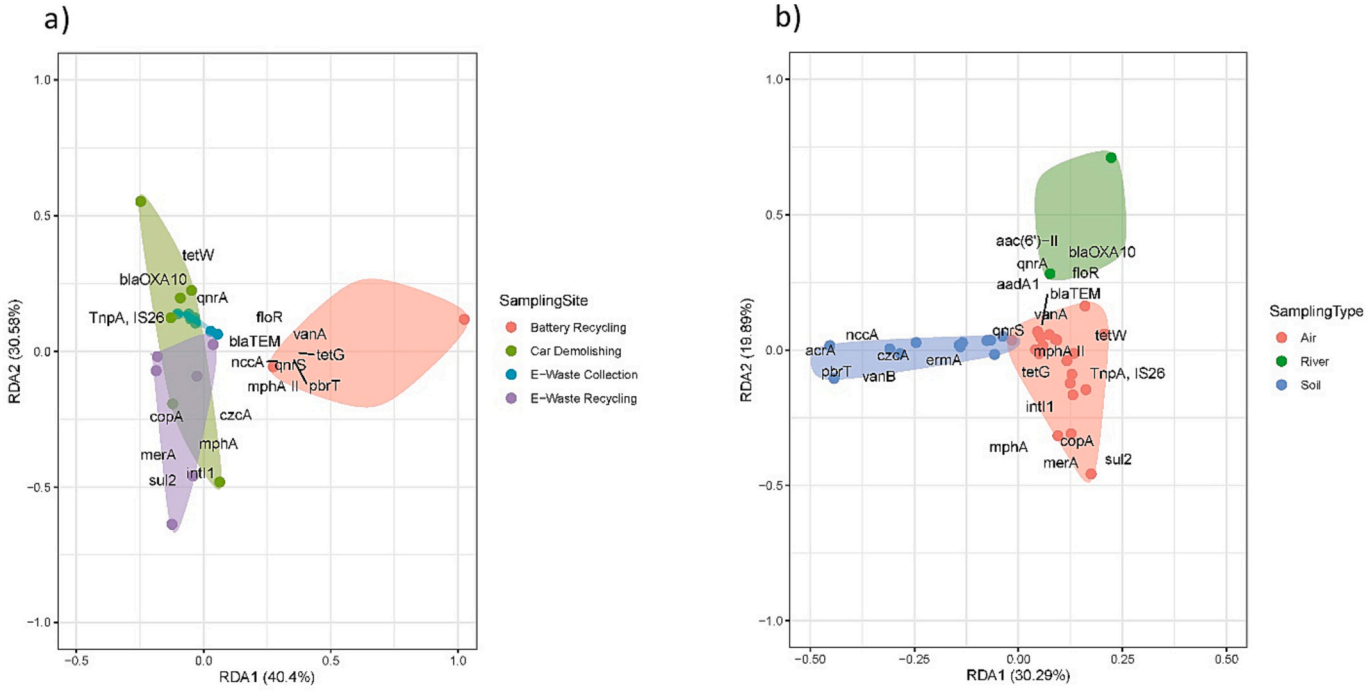

Fig. 8. RDA Analysis of ARGs, MGEs and MRGs at the four different e-waste facilities; a) heavy metal concentrations set as the environmental factor, clustered by e-waste recycling facilities; b) bacterial species on phylum level set as the environmental factor, clustered by sampling type.

predominantly contributed by the car demolishing facility. These results indicate that different e-waste facilities promote the occurrence of different genes and even though an overall similar trend can be observed, a closer examination of each facility individually is essential, especially when identifying other influencing factors.

The second RDA analysis incorporated the results from all three sample types (air, soil, river) (Fig. 8b). RDA1 primarily reflects the variation driven by the presence of distinct bacterial species within these sample types, while RDA2 is predominantly influenced by the redundancy of genes within each sample type. Notably, it is evident that the three sample types form distinct clusters with minimal overlap. In particular, the river cluster stands out as separate from the other two sample type clusters, indicating that the bacteria and genes in the river might originate upstream from the e-waste recycling facilities and are less influenced by the metal occurrence.

Overall, most MRGs were found within the cluster of the soil samples, while ARGs were more accumulated in the air sample cluster. This implies that MRGs are of greater significance for bacteria inhabiting the soil of metal-rich environments, whereas bacteria carrying ARGs might be more readily aerosolized and thus more prevalent in the air. This poses a significant risk of infections with antibiotic-resistant bacteria for workers who are continuously exposed to the facility's air.

4. Conclusion

The present study investigated potential biological hazards in the air at e-waste recycling facilities by analyzing ARGs, MGES, MRGs, endotoxins, and bacterial species and correlating them to heavy metal occurrences. Furthermore, the results were compared to the results obtained from soil and river samples collected near the e-waste recycling facilities.

In conclusion, the study found that similar types of ARGs and MRGs were present in the air of all four recycling facilities, with *bla*TEM being the most abundant ARG and *pbrT* and *nccA* being the most abundant MRGs. However, the battery recycling facility, which had the highest concentrations of heavy metals, especially Zn, Hg, and Cd, exhibited a more diverse resistance gene profile, suggesting that bacteria in this environment required a more complex and diverse mechanism to cope with the extremely high metal concentrations.

Soil and river samples had significantly different gene profiles compared to the air samples, indicating that certain bacteria might be easier aerosolized than others and subsequently carrying different genes with them to the aerosol phase.

However, regardless the sample type, *intl1* was the dominant MGE with high abundance rates, indicating that it was the key driver of ARG and MRG acquisition by co-selection at e-waste recycling facilities. This poses a risk to workers due to the increased enrichment and spread of resistant and potentially pathogenic bacteria and proper precautions are necessary, especially in aerosol-generating locations such as the vicinity of shredders and dismantling machines.

Furthermore, the study revealed that gram-negative bacteria were more likely associated with ARGs and less likely associated with MRGs. However, these associations were less correlated with metal concentrations, indicating the need for further research to identify additional influential factors.

Overall, this research provides new insights into the potential biological hazards posed by antibiotic resistance genes and metal resistance genes in e-waste recycling facilities, highlighting the importance of effective safety measures and further investigations into the complex interactions of these genes in various environmental contexts.

CRediT authorship contribution statement

V. Agarwal: Writing – original draft, Visualization, Validation, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **B. Meier:** Validation, Investigation. **C. Schreiner:** Writing – original draft, Methodology, Investigation. **R. Figi:** Writing – original draft, Methodology, Investigation. **Y. Tao:** Writing – review & editing, Software, Resources. **J. Wang:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgement

This study was supported by the Swiss National Science Foundation project titled “Emission quantification, transport modelling and risk evaluation of airborne antibiotic resistance genes from key sources in Zürich and Beijing”, grant number IZLCZ0_189880.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.170991.

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