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## Review

Evaluating the impact of heavy metals on antimicrobial resistance in the primary food production environment: A scoping review<sup>☆</sup>Elena Anedda<sup>a,b</sup>, Maeve Louise Farrell<sup>a,c</sup>, Dearbháile Morris<sup>a,c</sup>, Catherine M. Burgess<sup>b,\*</sup><sup>a</sup> Antimicrobial Resistance and Microbial Ecology Group, School of Medicine, University of Galway, Ireland<sup>b</sup> Food Safety Department, Teagasc Food Research Centre Ashtown, Dublin, Ireland<sup>c</sup> Centre for One Health, Ryan Institute, University of Galway, Ireland

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## ABSTRACT

Heavy metals are naturally occurring environmental compounds, which can influence antimicrobial resistance (AMR) dissemination. However, there is limited information on how heavy metals may act as a selective pressure on AMR in the primary food production environment. This review aims to examine the literature on this topic in order to identify knowledge gaps. A total of 73 studies, which met pre-established criteria, were included. These investigations were undertaken between 2008 and 2021, with a significant increase in the last three years. The majority of studies included were undertaken in China. Soil, water and manure were the most common samples analysed, and the sampling locations varied from areas with a natural presence of heavy metals, areas intentionally amended with heavy metals or manure, to areas close to industrial activity or mines. Fifty-four per cent of the investigations focused on the analysis of four or more heavy metals, and copper and zinc were the metals most frequently analysed (n = 59, n = 49, respectively). The findings of this review highlight a link between heavy metals and AMR in the primary food production environment. Heavy metals impacted the abundance and dissemination of mobile genetic elements (MGEs) and antimicrobial resistance genes (ARGs), with MGEs also observed as playing a key role in the spread of ARGs and metal resistance genes (MRGs). Harmonization of methodologies used in future studies would increase the opportunity for comparison between studies. Further research is also required to broaden the availability of data at a global level.

## 1. Introduction

Antimicrobial resistance (AMR) is recognized as one of the greatest global threats to human and animal health. It has been estimated that the number of deaths attributed to AMR will reach 10 million per annum by 2050 if no action is taken (O'Neill, 2016). AMR is an ancient natural phenomenon, predating the use of antibiotics (D'Costa et al., 2011). In recent decades, the decreasing pace in the development of new antibiotics, and the overuse and misuse of antibiotics, have caused a rapid increase in the emergence of AMR (Acharya and Wilson, 2019; Roca et al., 2015; Squadrone, 2020). The inappropriate use of antibiotics, in both human and veterinary medicine, and in food production systems places selective pressure on microorganisms (Acharya and Wilson, 2019; Roca et al., 2015) which has resulted in microorganisms encoding several mechanisms to survive in the presence of antibiotics

(Koutsoumanis et al., 2021). These mechanisms may be innate or acquired (Brooks and Brooks, 2014). Bacteria can acquire resistance through the acquisition of mobile genetic elements (MGEs) that carry antimicrobial resistance genes (ARGs) that can be easily transferred among microorganisms via horizontal gene transfer (HGT) (Di Cesare et al., 2016; Pal et al., 2017; Squadrone, 2020).

AMR dissemination is not only influenced by the presence of antibiotics, but it has also been shown that agents in the environment, such as biocides and heavy metals, can facilitate the spread of ARGs and antimicrobial resistant bacteria (ARB) (Koutsoumanis et al., 2021; Seiler and Berendonk, 2012; Yazdankhah et al., 2018). Most heavy metals are naturally occurring elements, and are commonly classified into essential and non-essential metals (Yazdankhah et al., 2018). The first group (essential) includes metals that are necessary for organisms' cellular functions and components (Seiler and Berendonk, 2012), but can be

<sup>☆</sup> This paper has been recommended for acceptance by Da Chen.

\* Corresponding author.

E-mail addresses: [Elena.anedda@teagasc.ie](mailto:Elena.anedda@teagasc.ie) (E. Anedda), [m.farrell34@nuigalway.ie](mailto:m.farrell34@nuigalway.ie) (M.L. Farrell), [dearbhaille.morris@universityofgalway.ie](mailto:dearbhaille.morris@universityofgalway.ie) (D. Morris), [kaye.burgess@teagasc.ie](mailto:kaye.burgess@teagasc.ie) (C.M. Burgess).

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toxic if they are present in excess (Yazdankhah et al., 2018). These metals include copper (Cu), chromium (Cr), cobalt (Co), manganese (Mn), iron (Fe), and zinc (Zn). The second group (non-essential) comprises metals such as barium (Ba), aluminium (Al), and lithium (Li). Depending on the degree of toxicity, heavy metals can be also classified into less toxic, such as tin (Sn), and highly toxic metals, such as mercury (Hg), arsenic (As), cadmium (Cd), lead (Pb), and vanadium (V), which have no biological functions (Squadrone, 2020; Yazdankhah et al., 2018).

There are different ways in which heavy metals can be toxic to bacteria; they can inactivate proteins and enzymes through inappropriate binding of metal-binding sites in enzymes, they can generate reactive oxygen species (ROS), and/or they can interfere with nutrient absorption and cell structure (Yazdankhah et al., 2018; Yu et al., 2017). As a result, bacteria have evolved resistance mechanisms against these metals, such as metal complex formation or sequestration of toxic metals, detoxification through reduction of intracellular ions, and extrusion of toxic ions by efflux systems (Yazdankhah et al., 2018). Genes that confer resistance to heavy metals, metal resistance genes (MRGs), have been found in both core bacterial genomes and on MGEs of bacteria (Squadrone, 2020). Resistance to heavy metals can co-occur with AMR due to two principal phenomena: co-resistance and cross-resistance (Fig. 1). Co-resistance occurs when genes responsible for different resistances are located close to each other on the same MGE (Chapman, 2003). This means that MRGs and ARGs are encoded on the same genetic element, such as plasmids, transposons and integrons, thus increasing the potential for co-transfer to other bacteria (Baker-Austin et al., 2006; Yu et al., 2017). Cross-resistance occurs when a single mechanism confers resistance to more than one substance, such as antibiotics and heavy metals (Chapman, 2003). For example, multi-drug efflux pumps can eject both metals and antibiotics from the bacterial cell (Baker-Austin et al., 2006). Additionally, two other phenomena can contribute to the co-selection of AMR and heavy metal resistance (HMR): biofilm formation and co-regulation processes. Biofilm formation allows bacteria to survive in the presence of stress factors, such as heavy metals, because biofilm components can bind or react with metal ions, preventing their diffusion within the biofilm structure. Consequently, mutations in bacterial genomes may be induced and AMR co-selected (Yu et al., 2017). The co-regulation phenomenon occurs when the expression of ARGs changes as a result of a bacterium's exposure to a heavy metal (Yu et al., 2017). Therefore, the presence of heavy metals in the environment can play a crucial role in the dissemination and persistence of AMR.

Heavy metals naturally occur in the environment. They are elements of the earth's crust that derive from pedogenetic processes of erosion of source materials (Wuana and Okieimen, 2011), and cannot be easily degraded because of their stable characteristics (Baker-Austin et al., 2006; Srivastava et al., 2017). Heavy metals can also be released into the environment as a consequence of anthropogenic activities. Agriculture and aquaculture practices, as well as practices in livestock and horticulture ecosystems, facilitate heavy metal dissemination. Although their application is increasingly restricted due to environmental concerns this

varies between countries and regions. For example, copper compounds are used to protect plants due to their fungicidal functions (Haynes et al., 2020). In order to prevent accumulation in soil, European legislation has established a ceiling of 28 kg copper per hectare over a period of 7 years (EU Regulation, 2018/1981). Additionally, the use of copper compounds in organic agriculture is restricted in the EU, which sets a ceiling of 8 Kg copper per hectare because of its possible long-term consequences if accumulated in the soil (EU regulation 473/2002).

Moreover, to prevent negative effects on human and animal health, as well as in the environment, the use of copper compounds in animal feed is regulated in Europe. EU Regulation 2018/1039, restricts the levels of copper in animal feed. In some parts of the world, copper is used in footbaths on dairy farms to prevent and treat dermatitis, although this practice has been banned in the EU since 2006 (Thomsen et al., 2008). Copper-based antifouling paints are applied in aquaculture to protect cages and nets from the growth of marine microorganisms (Yu et al., 2017). Moreover, heavy metals are added in animal and fish-feed to promote animal health and growth (Seiler and Berendonk, 2012); for instance, zinc oxide, as well as copper sulphate, can be added in pig feed to improve post-weaning performance, although the administration of high levels of zinc oxide was prohibited in the EU from June 2022 (EMA, 2017) (Regulation (EU) 2019/6). Metals added in feed are not fully absorbed, resulting in their presence in animal faeces, which can then be disseminated to the environment through land spreading of agricultural waste. Additionally, the environment can be polluted by heavy metals because of the presence of industrial facilities and factories, or the use of synthetic fertilizers that have heavy metal impurities (Yu et al., 2017). Thus, these factors and practices, including land application of animal manures and biosolids from sewage sludge as fertilizers, direct excretion of faeces on lands, heavy metals use as fungicide, fertilizer, and additives in animal feed, all contribute to heavy metal pollution worldwide (Yazdankhah et al., 2018). The presence of heavy metals may influence ARB and ARG dissemination via co- and cross-resistance mechanisms in the primary food production environment, and consequently among plants, animals and humans through transmission routes such as food, water and soil (Koutsoumanis et al., 2021).

Considering that the food production environment is interconnected with human and animal environments, a "One Health" approach, which is a strategy encompassing human, animal and environment concerns (World Health, 2014a), is required to tackle AMR. It is therefore important to understand how agronomic practices and/or the natural presence of heavy metals may influence AMR entering the food chain. This scoping review aims to summarise the evidence regarding the impact of heavy metals as a selective pressure for the dissemination of AMR in the primary food production environment. Whilst a link between HMR and AMR has been demonstrated (Baker-Austin et al., 2006; Pal et al., 2017), there is currently limited information about how this link impacts on AMR in the primary food production environment.

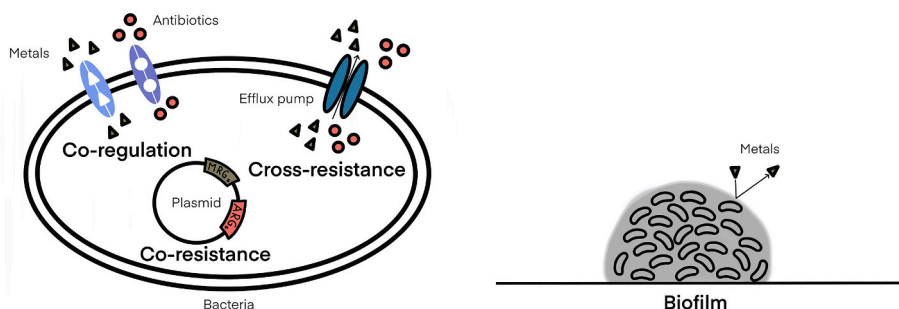


Fig. 1. Mechanisms associated with co-resistance to heavy metals and antimicrobials.

## 2. Methods

### 2.1. Literature identification: research question and data sources

The review protocol employed for the identification of scientific articles was prepared according to pre-established guidelines (Sargeant and O'Connor, 2020) and adapted from previous studies (Chique et al., 2020; Farrell et al., 2021; Hooban et al., 2020). Through discussion with a review panel, which included three members, the following research question was formulated:

'Do heavy metals have an impact on AMR in the primary food production environment?'

Based on this question, combined search terms related to 'heavy metals', 'AMR', 'dissemination' and 'primary food production environment' were selected to define search strings. Initially, Scopus was used to assess the terms' efficacy and relevance to the research question. As a result of discussion with the review panel, further terms were added to the search strings, while others were considered not suitable. A list of included and excluded terms is available in [Supplementary Table 1](#). Once a list of the most appropriate terms was compiled, search strings ([Supplementary Table 1](#)), with relevant subject headings and Boolean operators such as 'AND' and 'OR', were developed for Scopus, Embase, Medline, PubMed and Biosis (Web of Science) databases. In each database, the field 'TITLE-ABS-KEY' was applied to the search string aiming to explore the terms in these three sections; except for the Biosis database where the terms were searched in the topic field using the field tag 'TS'. Additionally, in the Embase and Medline databases, in order to capture narrower terms, the command 'explode' was used to extend a term which holds more specific terms; whereas in PubMed, 'MeSH' indexing facilitated finding terms that describe the content of an article. Following this, all articles were exported to the reference management software (Endnote 20), and duplicates removed.

Supplementary searches were conducted via grey literature sources using the search term 'antimicrobial resistance and heavy metals' on the following websites: EFSA ([www.efsa.europa.eu](http://www.efsa.europa.eu)), FSAI ([www.fsai.ie](http://www.fsai.ie)), INAP ([www.gov.ie](http://www.gov.ie)), ECDC ([www.ecdc.europa.eu](http://www.ecdc.europa.eu)), WHO (<https://www.who.int/>), CDC ([www.cdc.gov](http://www.cdc.gov)), FDA ([www.fda.gov](http://www.fda.gov)), FAO (<https://www.fao.org/>), OIE (<https://www.woah.org/>), EPA ([www.epa.ie](http://www.epa.ie)), FSA ([www.food.gov.uk](http://www.food.gov.uk)) and EMA ([www.ema.europa.eu](http://www.ema.europa.eu)).

### 2.2. Screening and inclusion/exclusion criteria

The article screening consisted of two phases: the first was the evaluation of article titles and abstracts, considering pre-defined inclusion criteria; the second was the assessment of full-texts, after implementing the defined specific exclusion criteria. As shown in [Table 1](#), the

**Table 1**  
List of inclusion and exclusion criteria applied for article screening.

Inclusion criteria	Exclusion criteria
Investigation of antimicrobial resistance	Not relevant to the primary food environment
Determination of heavy metal concentration or detection of heavy metal resistance genes	Heavy metal not investigated
Focus on agricultural/food production environment	Antimicrobial resistance not investigated
Published between 2006 to 2021	Antimicrobial resistance and heavy metal link not investigated
English language	Investigation of AMR and heavy metal for a limited and restricted process not related with the scope of this research (e.g. anaerobic thermophilic digestion process)
	Not primary research (review)
	Inappropriate method used

three main inclusion criteria applied were: (i) focus on antimicrobial resistance; (ii) measurement of heavy metal concentration; (iii) studies undertaken in agricultural/food production environment. Moreover, two limitations were added as inclusion criteria: (iv) articles published since 2006 and (v) texts written in English. Articles published since 2006 was applied as a limitation to ensure the most recent and advanced methodologies, while the English language was chosen for practicality. The articles excluded during the second phase included: studies that analysed samples not related to the primary food production environment; studies that did not investigate AMR or heavy metal concentrations or their association; studies that were focused on a specific process that may have an impact on AMR dissemination and heavy metals but that were not within the scope of this research (e.g. anaerobic thermophilic digestion process); studies that used inappropriate methods; studies that were not primary research ([Table 1](#)).

### 2.3. Data extraction

All relevant data included in the screened research articles were extracted and organised into categories in MS Excel spreadsheets. The main fields included (i) year of publication, (ii) country where analysis was conducted, (iii) type of sample analysed (e.g. soil, water, manure, and other primary food production environmental samples), (iv) sampling location details (e.g. industrial area, mining area, agricultural fields, and other sites), (v) characterization of AMR, (vi) detection of HMR, (vii) measurement of heavy metals, (viii) heavy metals examined and (ix) mobile genetic element detection (e.g. integrons, plasmids, transposons, and other MGEs) ([Supplementary Table 2](#)).

## 3. Results

### 3.1. Literature screening

We identified 2868 articles ([Fig. 2](#)) by applying the search strings ([Supplementary Table 1](#)) in the following databases: Scopus, Embase, Medline, Pubmed and Biosis (Web of Science). Following removal of duplicates that occurred across multiple databases and screening of titles and abstracts, the articles were subjected to full text review according to pre-established inclusion/exclusion criteria ([Table 1](#)). The reasons why articles were excluded after full text screening are listed in [Fig. 2](#).

Several investigations, for example, were excluded because they examined the impact of heavy metal on AMR as part of a specific process outside the scope of this research (n = 19). [Lu and Lu \(2019\)](#) for instance, analysed the effect of different parameters, including heavy metal concentration, on AMR during anaerobic composting of swine manure, while [Zhang et al. \(2018a\)](#) investigated the impact of copper on the fate of ARGs, MRGs and MGEs during the aerobic co-composting of tylosin fermentation dregs. Another two articles were excluded due to the use of mathematical modelling to provide a general mechanistic framework ([Arya et al., 2021](#); [Gothwal and Thatikonda, 2021](#)), which were therefore incomparable with the other studies. Three articles were considered as a result of the grey literature search, but did not include original data and were therefore not considered in subsequent analysis. The final number of articles considered relevant for inclusion was 73.

### 3.2. Data analysis

The articles included were published over a period of 13 years (from 2008 to 2021) and showed a rapid increase since 2017 ([Fig. 3](#)).

Geographically, most of the investigations were conducted in Asia, with China being the country where the most studies were performed (n = 44), followed by Brazil, India and Iran (n = 4 in each country). Three studies were undertaken in Poland and two each in Australia, Portugal and UK, respectively, while one relevant study was conducted in each of the following countries: USA, Korea, Russia, Italy, Spain, Finland, Denmark and Congo ([Fig. 4](#)).

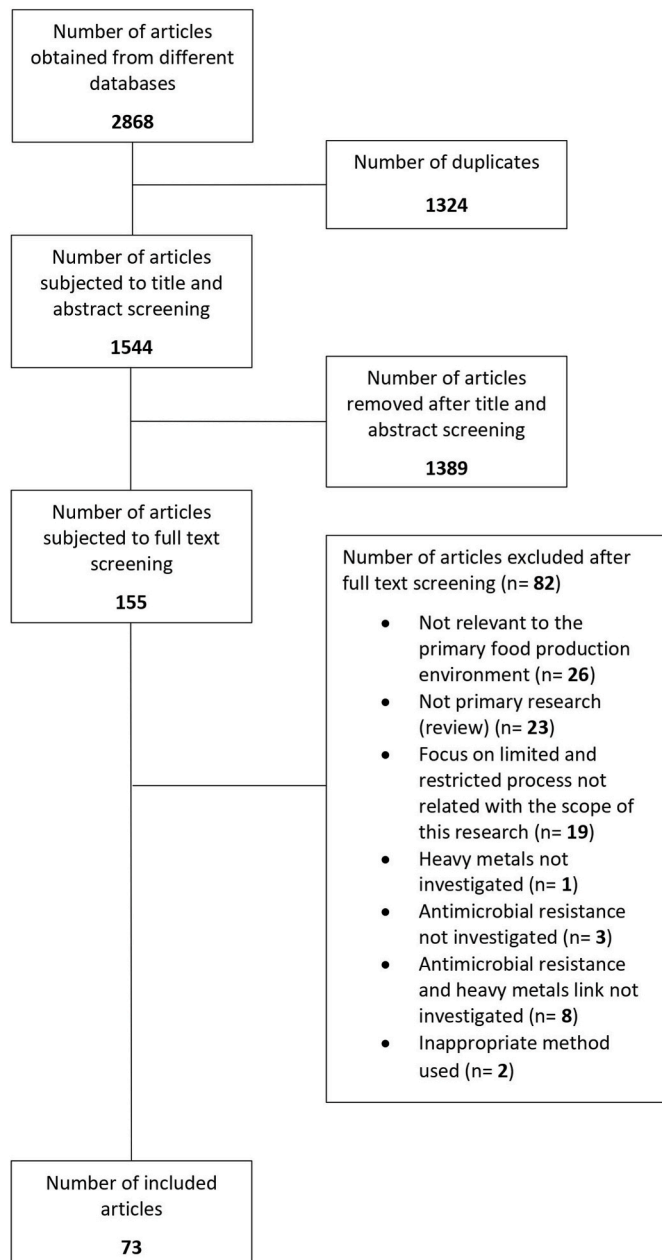


Fig. 2. Flowchart delineating the review protocol applied for article screening, including exclusion criteria, leading to the final studies included.

In terms of the objective of the studies, they were mainly focused either on investigating the impact of heavy metals on AMR ( $n = 50$ ; 68.5%) or on the detection of MRGs and ARGs ( $n = 23$ ; 31.5%). In the first case, different approaches were adopted. For example, [Hu et al. \(2017\)](#) employed a temporal approach to investigate the impact of nickel exposure on the diversity and abundance of ARGs over a period of 4–5 years. [Younessi et al. \(2020\)](#) used a spatial approach to compare the prevalence of ARB in poultry manure between antibiotic-using and antibiotic-free farms; while a quantitative approach was applied in many of the studies, such as the one undertaken by [Li et al. \(2019\)](#) who investigated the effect of sub-lethal levels of heavy metals on antimicrobial resistance.

Overall, there were diverse sample types analysed in the included studies. Soil was the sample analysed most, followed by water (including sea, river and lake water) and manure. Sampling locations also differed between the included articles. In some cases ( $n = 22$ ) samples were

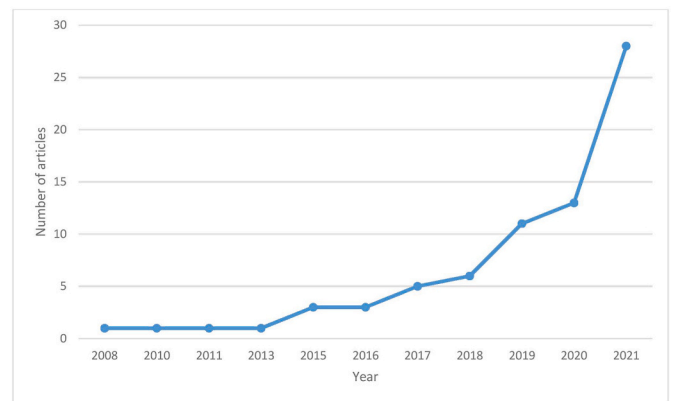


Fig. 3. Line chart showing the number of included articles per year of publication.

collected from areas subjected to treatments, for example soil intentionally amended with heavy metals ([Zhao et al., 2020](#)), or with manure for nutrient addition purposes or study purposes. Other studies ( $n = 18$ ) focused on the analysis of samples collected from areas close to industries or mines. For example, [Zhang et al. \(2018b\)](#) collected soil samples from seven agricultural areas with different distances from a lead-zinc smelting plant which electrolyzes lead, silver, gold, and other metals, while [Safari Sinegani and Younessi \(2017\)](#) analysed soil samples from three different mining sites, two iron ore mines and one lead-zinc mine. Twenty-six investigations were performed on samples collected from areas with no association with either industries or mines, nor were they subjected to treatments with heavy metals or manure, and therefore focused on the natural presence of heavy metals. An example of this is represented by the study conducted by [Gallo et al. \(2019\)](#) on soil samples obtained from sites for which there were no reports of contamination by heavy metals. In seven cases no data regarding sample locations were provided ([Fig. 5](#)).

Although AMR was evaluated in all the articles included, HMR was only investigated in a subset of the studies. Some studies examined the heavy metal concentration only. However, in 22 articles both HMR and HM concentration were analysed.

Considering the techniques employed, different approaches for the detection of AMR and HMR were applied; some studies used phenotypic methods only ( $n = 14$ ), others used a combination of phenotypic and genotypic methods ( $n = 13$ ), while the majority used genotypic methods only ( $n = 46$ ), seven of which used a metagenomics approach. Culture-based analysis, such as minimum inhibitory concentration (MIC) and antibiotic sensitivity testing through disk diffusion, were the phenotypic methods used to detect AMR and HMR. The genotypic methods used to detect ARGs and MRGs were mainly polymerase chain reaction (PCR) and whole genome sequencing (WGS). In a few cases, AMR and HMR were assessed using other methods, for example a novel cultivation-independent assay (BrdU-PICT) or a GeoChip hybridization assay ([Berg et al., 2010](#); [Zhang et al., 2021](#)).

In one case ARGs were not studied, instead the presence of antibiotics and their correlation with MRGs and MGEs was investigated using high performance liquid chromatography/tandem mass spectrometry system and statistics analysis instead ([Lu and Liu, 2021](#)). In two studies the methods used to detect AMR were not specified; both studies conducted ARG conjugative transfer experiments in heavy metal contaminated environments in which the donors harboured plasmids that carried specific ARGs ([Pu et al., 2021a](#); [Zhang et al., 2018c](#)). Specifically, [Pu et al. \(2021a\)](#) evaluated conjugative transfer using the donor *Pseudomonas putida* KT2442, which harboured the IncP plasmid RP4 that carried kanamycin, ampicillin, and tetracycline resistance genes; while, [Zhang et al. \(2018c\)](#) employed the donor *E. coli* S17-1 which harboured the plasmid pCM184-Cm that carried ARGs to ampicillin, tetracycline,



Fig. 4. Worldwide distribution of included studies.

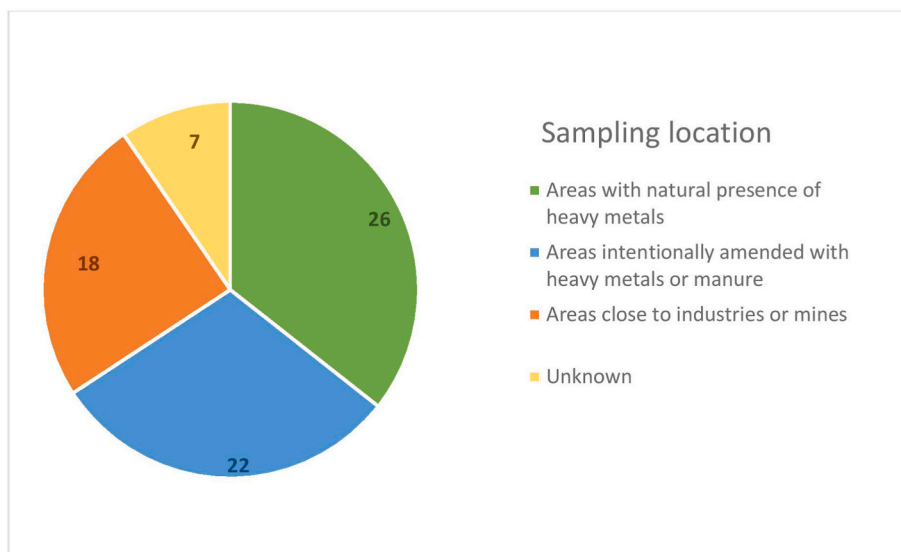


Fig. 5. Breakdown of sampling location type.

and chloramphenicol.

Regarding the methods applied to measure heavy metal concentrations, this analysis was mainly evaluated using a single technique; however, seven studies used two or even three different methods, depending on the type of heavy metal being assessed. Inductively Coupled Plasma (ICP), including ICP-optical emission spectrometry and ICP-optical mass spectrometry, was the method mostly used to assess heavy metals (n = 32). Another method applied was Atomic Absorption Spectrometry (AAS), including flame-AAS, Hydra-C-AAS, Hydride

generation-AAS and cold vapour-AAS (n = 20). While atomic fluorescence spectrometry (liquid chromatography-AFS and cold vapour-AFS) was used in five studies, X-Ray fluorescence was used in only two studies. HM concentration was not measured in 21 investigations, which instead were focused on HMR analysis. In ten out of these 21 studies, the HM concentration was established in advance. Moreover, data regarding the methods used to detect HM were not provided in two cases. The choice of method applied depends on the type of sample and metal to be analysed. For example, ICP is a method that allows detection of metals at

very low concentrations; particularly, it can analyse multiple metals at one time, while AAS can't (Jin et al., 2020; VELEZ, 2009). AFS, for example, can only be applied for certain metals, such as arsenic and mercury (Jin et al., 2020).

The type and number of heavy metals investigated differed between studies, 54% (n = 40) examined four or more heavy metals, 20% (n = 15) analysed two or three metals, while the remaining 24% (n = 18) focused on a specific single metal, namely copper, zinc, arsenic, cadmium, nickel or mercury. Among all the studies, copper was the most common heavy metal analysed (n = 59), followed by zinc, for which concentration and/or resistance were assessed in 49 studies (Fig. 6B). Both copper and zinc concentrations were determined primarily using ICP and AAS methods, while regarding copper and zinc resistance characterization, MIC was the method mostly used for the detection of resistance phenotypes, followed by PCR and WGS.

The classes of antibiotics tested varied among the studies (Fig. 6A). The antibiotics mostly tested belonged to the tetracycline class (n = 51) and beta-lactam class (n = 43). Sulphonamides and quinolones were also commonly tested (n = 36), as well as aminoglycosides (n = 30) and macrolides (n = 28). Most studies (n = 54, 73%) focused on resistance to several classes of antibiotics, only in seven cases was the focus on one individual class of antibiotic. For example, Buta et al. (2021) evaluated the presence of ARGs encoding for resistance to eight different antibiotic classes, while Gothwal and Thatikonda (2017) tested seven different antibiotics, but all belonging to the quinolones class.

In most studies, AMR plus HMR were analysed using a genomic approach that did not involve bacterial culture analysis. This meant that the majority of the studies were not focused on the analysis of specific bacterial species or groups. Only a few investigations targeted the analysis of specific bacteria. For instance, Mustafa et al. (2021) studied HMR and its association with AMR in *Salmonella* Typhimurium isolates. In contrast, Laffite et al. (2020) investigated the impact of anthropogenic activities on the occurrence of beta-lactamase and carbapenemase genes in river sediments, although the host bacterial species were not specified.

The majority of studies (n = 51, 70%) assessed the presence of MGE, particularly integrons (n = 38), plasmids (n = 21), transposons (n = 18)

and insertion sequences (n = 8). Among the integrons, class 1, which is frequently linked with ARGs and MRGs (Gillings et al., 2015), was the most commonly detected (n = 30), followed by class 2 (n = 14), 3 and 4 (n = 2 and n = 1, respectively). Four studies assessed the presence of plasmid mediated quinolone resistance (PMQR) (Gallo et al., 2019; Siddiqui et al., 2020; Tuo et al., 2018; Xu et al., 2017) and seven examined plasmid mediated beta lactam resistance (Figueiredo et al., 2019; Otinov et al., 2020; Pu et al., 2021a; Pu et al., 2021b; Wang et al., 2020; Wilson et al., 2019; Zhang et al., 2018c). In some cases (n = 9), an experiment was conducted to evaluate the mobility of resistance genes (conjugation transfer of ARGs) (Supplementary Table 2).

All of the included studies identified a link between AMR and heavy metals. Depending on the aim and the type of study conducted, the findings obtained varied among the studies. The majority (n = 34) established that heavy metals promote the spread of MGEs and/or ARGs, and a correlation between a specific heavy metal and ARGs or antibiotics has been established. For example, Hao et al. (2021) stated that heavy metals were the most influential environmental factors that affect the distribution of ARGs and MGEs, followed by polycyclic aromatic hydrocarbons and soil features, after statistical analysis. In particular, the authors identified significant positive correlations between the abundance of multidrug ARGs and metals, such as chromium, cobalt and arsenic. Hu et al. (2017) provided evidence that ARG abundance increased as a result of nickel exposure and correlated with its concentration. Overall, copper and/or zinc, which were the main metals analysed, have been associated with tetracycline (Duan et al., 2019; Figueiredo et al., 2019; He et al., 2017; Mazhar et al., 2021; Mustafa et al., 2021; Safari Sinegani and Younessi, 2017; Wu et al., 2020; Zhao et al., 2019a; Zou et al., 2021), sulphonamide (Duan et al., 2019; Figueiredo et al., 2019; He et al., 2017; Hubeny et al., 2021; Wu et al., 2020; Zhao et al., 2019a; Zou et al., 2021), betalactam (Figueiredo et al., 2019; Mazhar et al., 2021; Mustafa et al., 2021; Wu et al., 2020; Zhao et al., 2019b; Zou et al., 2021), aminoglycoside (Figueiredo et al., 2019; Wang et al., 2021; Zhao et al., 2019b; Zou et al., 2021) and macrolide resistance genes (Wang et al., 2021; Zou et al., 2021). The same antibiotics have been associated with nickel and arsenic, but nickel has also been associated with multidrug and vancomycin resistance (Hu et al., 2017;

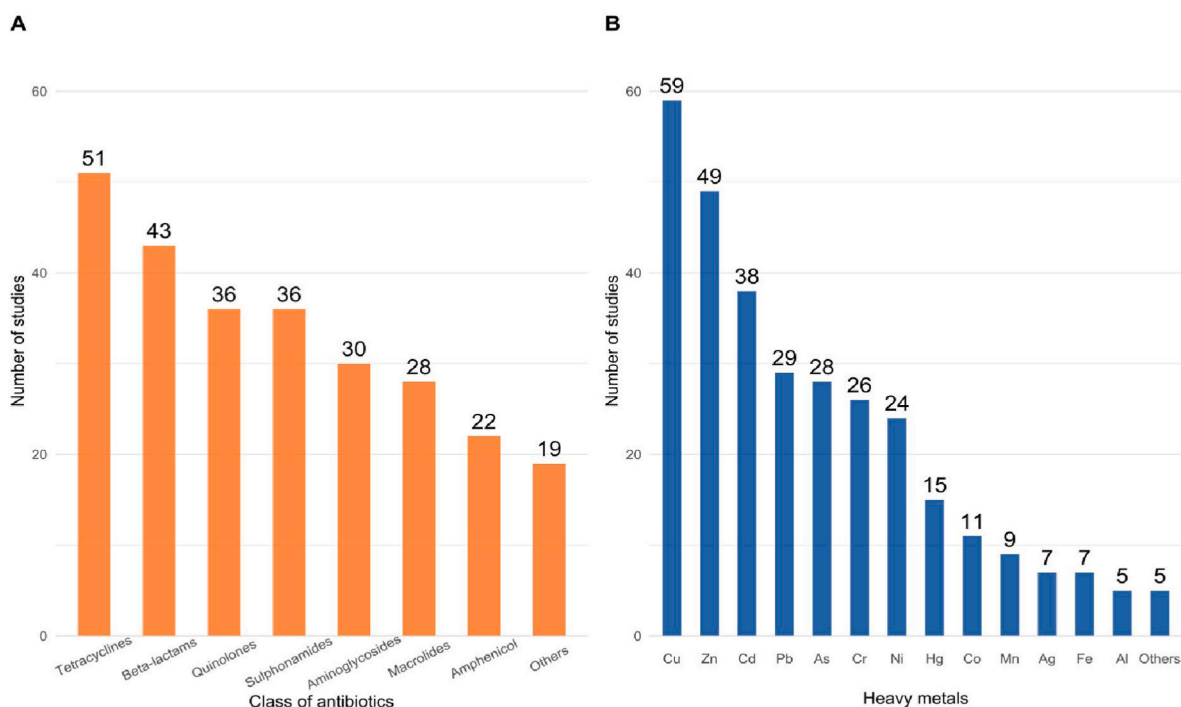


Fig. 6. (A) Class of antibiotics examined in the included studies. (B) Heavy metals investigated in the studies included in this review.

Safari Sinangani and Younessi, 2017; Zhao et al., 2019b; Zou et al., 2021), while arsenic was also linked with streptomycin, chloramphenicol and multidrug resistance (Figueiredo et al., 2019; Hao et al., 2021; Mazhar et al., 2021; Wang et al., 2021; Zhao et al., 2019b).

The role of MGEs on the dissemination of ARGs and MRGs was demonstrated in nine studies. Anjum et al. (2011) demonstrated that bacteria with an IncP plasmid had the capacity to transfer MRGs and ARGs through a conjugative mechanism, therefore they proposed that these plasmids are key for the dissemination of multi-drug resistant bacteria in contaminated alluvial soil. Sixteen investigations showed a positive correlation between HMR and AMR, while 14 studies stated that heavy metals, such as copper and arsenic, apply selective pressure on AMR. For instance, one study revealed a high association between copper tolerance and resistance to antibiotics like ampicillin, erythromycin and vancomycin through statistical correspondence analysis (Glibota et al., 2019), showing a positive correlation between the occurrence of heavy metals and antimicrobial resistance. The role that heavy metals may play as a selective pressure on AMR was also demonstrated, for example, in a study which indicated that arsenic exposure exerted a selective pressure on the bacterial resistome in soil (Cao et al., 2020).

#### 4. Discussion

This scoping review aimed to interrogate existing literature with regard to the impact of heavy metals on AMR in the primary food production environment. Traditionally much more attention has been given to AMR development and transmission between humans and animals than in natural and agricultural settings; however, this has changed in the last decade (Koutsoumanis et al., 2021). This increased interest in the role of the environment is reflected in the rapid increase in the number of relevant studies since 2016 included in this scoping review. The importance of AMR in environmental settings was highlighted in the 2015 World Health Organization AMR Action Plan (World Health, 2015), that recognized AMR as a worldwide threat to human health. The studies included in this scoping review considered different types of environmental samples: soil, water and manure principally; but also examined the characteristics of the environment where they were collected from, for example the vicinity to mines or industries, or exposure to a specific treatment. Through the analysis of the included studies, an association between AMR and the presence of heavy metals was generally observed. In some cases, a direct correlation was established, such as promotion of ARGs dissemination induced by heavy metals; while in other cases, the role of MGEs on the spread of ARGs and MRGs was demonstrated.

##### 4.1. Limitations in geographical distribution of studies included

Given the inclusion criteria in the review, there was a limitation in the geographical representativeness. The majority of studies identified were undertaken in China. There may be a number of reasons for this. China is the highest producer and consumer of antibiotics in the world (Duan et al., 2019). In 2013 the annual consumption of antibiotics in China was reported to be 160,000 ton, of which 84,000 ton was used in livestock (Yue et al., 2021), with a significant increase in the use of antibiotics in veterinary settings from 46% in 2007 to 52% in 2013 observed (Tiimub et al., 2021). Furthermore, despite the implementation of the Animal Medicine Management Regulations in 2004, it has been reported that antibiotics may be overused as treatments and animal growth promoters in China (Zhou et al., 2016). For thousands of years China, like many countries, has applied varied agricultural practices, including raw manure soil amendment and irrigation with wastewater (Zhou et al., 2017). Since most antibiotics are poorly absorbed in the animal and human intestine, they can be released in the environment through faeces and urine (He et al., 2017). Hence, long-term manure application may enhance the levels of ARGs (Tuo et al., 2018).

Moreover, heavy metal pollution of soil, especially in the south of China, is a growing concern, notably for cadmium and arsenic which are the most common heavy metals in this area due to the presence of mines and industries (Zhao et al., 2020). This pollution may be caused by industrialization, that lead to the development of mining factories and smelting plants; and anthropogenic activities, such as use of heavy-metal-contained pesticides, application of manure on farmland, and abandonment of mine tailings (Cao et al., 2020; Qiao et al., 2021). It has been reported that accumulation of heavy metals in agricultural soil in China can be caused by the use of sewage irrigation, which represents an important source of water due to the deficiency of water sources in this country (Wu et al., 2022). Additionally, the use of fertilizers and insecticides have increased heavy metal concentration in soil, especially cadmium, nickel, copper and mercury, which are used in crops, such as rice (Shifaw, 2018; Su et al., 2022).

A number of studies were undertaken in mining-affected areas or close to mineral mines and smelting plants where metal concentrations are more likely to be elevated (Xu et al., 2017; Zou et al., 2021). Heavy metal pollution of water is also of concern; it has been found that heavy metals are responsible for 20% of water pollution incidents in China (Wu et al., 2017). China has acknowledged the growing concern about AMR and heavy metals in the environment, and through its National Action Plan to Contain Antimicrobial Resistance (2016–2020), it has increased research in order to address knowledge gaps (World Health, 2014b).

In accordance with EU legislation (Directive 2003/99/EC, Commission implementing Decision (EU) 2013/652 and 2020/1729), AMR in *Salmonella*, *Campylobacter jejuni* and indicator commensal *E. coli* from major food-production animals is monitored annually by EU member states (Koutsoumanis et al., 2021). Moreover, the use of antibiotics in animal feed as growth promoters has been banned since 2006 in the European Union, as well as in the United States since 2014 (Yue et al., 2021) with more recent restriction on the use of heavy metals in livestock production (EMA, 2017) (EU Regulation, 2019/6) (EU Regulation, 2018/1039). Despite the presence of these regulations and surveillance systems, there have been limited investigations on AMR and heavy metal linkages in Europe. Anthropogenic activities have caused an increase in heavy metal pollution in Europe; for example, almost 85% of metals discharge into water resources stemmed from human activities (Silva et al., 2021). Additionally, heavy metals are used as animal growth and health promoters, as disinfectants, antiseptics and preservatives in agriculture and aquaculture sectors (Figueiredo et al., 2019), even though their use is increasingly restricted and varies worldwide. Similarly, in low and low middle income countries (LMIC) there is limited monitoring of heavy metal concentrations and AMR; for example, in India the waste generated from industrial, urban and agricultural activities is often discharged into water systems without any treatment (Laffite et al., 2020; Siddiqui et al., 2020), but there is limited understanding of how this may impact on the dissemination of AMR or ARGs.

##### 4.2. Diversity of methods applied to detect antimicrobial and heavy metal resistance reflects studies' heterogeneity

The use of phenotypic and/or genotypic methods to detect AMR or HMR has various advantages and disadvantages. Although the use of culture-based analysis is time-consuming, requires trained personnel, and may be subject to human error (Shanmugakani et al., 2020), it is generally lower cost than genotypic methods, and provides data to guide treatment decisions (Boolchandani et al., 2019; Shanmugakani et al., 2020). However, culture-based analysis can only be applied for cultivable bacteria and not for studying microbial communities that are characterized by uncultivable microorganisms (Boolchandani et al., 2019). In contrast, genotypic methods are more rapid, and provide accurate results on the detection of AMR and HMR genetic mechanisms (Anjum et al., 2018). Molecular techniques are supported by the building of resistance gene databases; however, there is still limitation

on the harmonization of data feeding into databases, and they may not detect all resistance mechanisms because of the considerable genetic diversity and the continuous discovery of new genes (Boolchandani et al., 2019).

The methods applied in the studies included in this review included phenotypic, genotypic and a combination of both. This results in a heterogeneity in the studies, which consequently means it is difficult to compare them with each other.

In investigations that demonstrated heavy metals as a selective pressure on AMR dissemination ( $n = 14$ ), several methods were applied. Most of these articles detected AMR and HMR using only genotypic methods, of which PCR was the most predominant. For example, Hu et al. (2016), observed that AMR in agricultural soil changed considerably after 4–5 years of copper contamination, through the use of high throughput quantitative PCR (HT-qPCR). It would be of interest to determine if this observation also resulted in phenotypic alterations. Three articles employed phenotypic methods to investigate AMR and HMR. For example, MIC analysis was applied to investigate the effects of metal such as arsenate, copper and zinc on AMR in bacterium LSJC7 (Chen et al., 2015). LSJC7, a sequenced strain of Enterobacterales, was already known to harbour arsenate and tetracycline resistance genes, demonstrating the value of linking phenotypic and genotypic analysis. Four studies applied both phenotypic and genotypic methods. For instance, Li et al. (2019) stated that metals, such as copper, zinc and silver, at sub-lethal concentrations led to an increase in AMR via genetic mutations. This study was conducted by analysing the MIC of antibiotics and metals in a 96-well microplate, and the genetic profile of the resistant mutants through whole genome sequencing.

The majority of the articles that suggested that heavy metals promote the dissemination of MGEs and/or ARGs applied only genotypic methods to detect AMR and HMR. The use of genotypic methods allows the detection of new resistance variants, with an accurate and rapid approach. Through HT-qPCR and metagenomic analysis, Wang et al. (2021) demonstrated the role of heavy metals, such as arsenic, copper, zinc and lead, on the co-selection of ARGs, especially at high metal concentrations, and asserted that ARGs abundance and profile could be altered by the presence of heavy metals. Exclusively phenotypic methods, such as antibiotic susceptibility test by disc diffusion and minimum inhibitory concentration of heavy metals, were performed in the study conducted by Safari Sinegani and Younessi (2017) which identified heavy metals as one of factors responsible for the high abundance of AMR in agricultural soil. The use of phenotypic methods is constructive to establish the prevalence of AMR in bacterial populations, to evaluate how resistance profiles have changed over time, and to identify new resistance phenotypes.

Further investigations established a positive association between heavy metals and AMR using phenotypic methods or a combination of phenotypic and genotypic approaches. A strong correlation between cadmium and gentamycin resistance was detected in one study, which evaluated AMR by agar dilution test, and the HMR by MIC in animal faeces samples, and suggested a future genomic investigation (Marazzato et al., 2020). Back et al. (2020) identified the important role of co-selection of tetracycline resistance and zinc resistance in CC398 livestock-associated (LA)-methicillin-resistant *Staphylococcus aureus* (MRSA) in Korea's swine population using both phenotypic and genotypic methods to analyse AMR and HMR. It would be of interest to investigate the relationship between AMR phenotypes and genotypes in order to determine if there is a match between them, and to identify the most accurate and fastest approach to conduct such comparative analysis.

It has been demonstrated that within the same study phenotypic and genotypic results on AMR and HMR analysis can be mismatched, indicating there is not always concordance between the phenotypic results and resistance genes profile (Do Nascimento et al., 2017; Neuert et al., 2018; Schwan et al., 2021); therefore, a combination of culture-based analysis and genotypic methods may produce results with a more

complete overview. The extensive diversity of methods used makes the comparison of studies difficult; it would be preferable to establish a harmonised procedure for the detection of AMR and HMR in the environment, and measuring heavy metal concentrations, to enable more informative comparative analysis.

#### 4.3. Heavy metals influence the abundance and spread of mobile genetic elements

The presence of MGEs, such as integrons, plasmids, transposons and insertion sequences, were examined in almost 70% ( $n = 51$ ) of the studies included in this review. A significant number of these investigations ( $n = 32$ ) established the role of heavy metals, such as copper, zinc, arsenic, cadmium and lead, in affecting the distribution of ARGs, and the abundance and spread of MGEs in the environment ( $n = 22$ ); so that heavy metal content positively related with ARG abundance, in samples which included agricultural soil, urban soil, soil from livestock, water and animal faeces.

Several studies demonstrated that heavy metals can influence ARG dissemination by facilitating the spread of MGEs. It was shown that cadmium at sub-inhibitory concentrations enhanced conjugative transfer of the RP4 plasmid from *P. putida* KT2442 to the microbial community in fresh water (Pu et al., 2021a). Cadmium treatment has been shown to lead to an increase in cell membrane permeability, and affected the mRNA expression levels of genes involved in conjugation, repressing some genes that downregulate the expression of the genes required for conjugation (Pu et al., 2021a). Wang et al. (2020) observed that heavy metals, including copper and zinc, promoted conjugative transfer of the multi-resistance plasmid RP4 through bacterial cell membrane damage. A further study provided evidence of the ability of heavy metals, including copper and zinc, to promote ARG transfer through conjugation in the water environment. In this case, heavy metals were involved in generation of reactive oxygen species, alteration of expression of conjugation related genes, and an increase of in cell membrane permeability (Zhang et al., 2018c). Otinov et al. (2020) highlighted that some metal oxide nanoparticles (MONPs), such as zinc-oxide (ZnO) and boehmite (AlOOH), can enhance the efficacy of transformation and conjugation, respectively, while others, such as titanium dioxide (TiO<sub>2</sub>) can inhibit transfer mechanisms.

Furthermore, other studies established that exposure to heavy metals can result in an increase in the abundance of MGEs such as integrons, insertion sequences and transposons, which are related to the transfer mechanisms of ARGs among bacteria. For example, cadmium was shown to increase the abundance of ARGs and *intI1* in oxytetracycline polluted soil and lettuce tissue (Guo et al., 2021). Tongyi et al. (2020) observed that the abundance of *intI1*, insertion sequences, and ARGs increased, along with the gradient of zinc concentration in the soil, indicating that zinc can affect ARG mobility. In urban soils heavy metals, such as arsenic, cadmium, cobalt, chromium, copper, mercury, nickel and zinc, can alter MGE abundances (Zhao et al., 2019b).

In contrast to these studies that consider MGEs as a main factor influencing ARG dissemination, Qiao et al. (2021) proposed that MGEs have only an indirect effect on AMR dissemination by influencing the microbial community composition and that, instead, was considered the main driver of ARG spread.

Overall, the role of heavy metals on the dissemination of MGEs, and consequently ARGs, is evident. Through the regulation of gene expression, the production of reactive oxygen species and the alteration of cell permeability, heavy metals can affect MGE spread, and thus even AMR dissemination.

## 5. Conclusion

The results obtained in this review demonstrate a link between heavy metals and AMR in the primary food production environment. Although the studies included differed in their objectives, sample types, sampling



locations and methods applied, all asserted the important role of heavy metals on AMR dissemination and persistence. Some studies demonstrated that heavy metals can apply selective pressure on AMR; others that heavy metals can promote the dissemination of MGEs and/or ARGs in the environment; and others that MGEs play an important role in ARGs and MRGs dissemination. However, a better understanding of how heavy metals can influence AMR and ARGs in the primary food production environment is needed to inform the development of effective mitigation measures. Limited investigations regarding heavy metals and AMR dissemination in the environment have been conducted to date in many regions of the world. Therefore more research should be undertaken to better understand the mechanisms through which heavy metals impact on AMR, particularly in the context of feed and food production. Harmonising the approaches to AMR and HMR analysis and measurement of heavy metal concentration is critical. The increased availability of metagenomic analysis provide the opportunity of obtaining more in-depth information on AMR profiles in microbial communities, linked with physicochemical properties such as heavy metal concentrations, in different ecological niches.

### Credit author statement

**Elena Anedda:** Writing - Original Draft, Investigation, Resources, Data Curation, Visualization, **Maeve Louise Farrell:** Resources, Writing - Review & Editing, Visualization, **Dearbháile Morris:** Conceptualization, Validation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition, **Catherine M. Burgess:** Conceptualization, Validation, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

### 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

No data was used for the research described in the article.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2023.121035>.

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