



## Review

# Effect of heavy metal pollution on soil microorganisms: Influence of soil physicochemical properties. A systematic review

Claudia Campillo-Cora<sup>a,b,\*</sup>, Andrés Rodríguez-Seijo<sup>a,b</sup>, Paula Pérez-Rodríguez<sup>a,b</sup>,  
David Fernández-Calviño<sup>a,b</sup>, Vanesa Santás-Miguel<sup>a,b,c</sup>

<sup>a</sup> Departamento de Biología Vegetal e Ciencia do Solo, Facultade de Ciencias, Universidade de Vigo, As Lagoas s/n, 32004, Ourense, Spain

<sup>b</sup> Instituto de Agroecoloxía e Alimentación (IAA), Universidade de Vigo – Campus Auga, 32004, Ourense, Spain

<sup>c</sup> Microbial Ecology, Department of Biology, Lund University, Ecology Building, 22362, Lund, Sweden

## ARTICLE INFO

Handling Editor: Dr X Xu

## Keywords:

Soil microorganisms  
Microbial properties  
Heavy metal  
Ecotoxicological  
Soil pollution

## ABSTRACT

This review examines the complex interaction between heavy metals and soil microorganisms, focusing on five common heavy metals (HM) (chromium -Cr-, copper -Cu-, nickel -Ni-, lead -Pb-, and zinc -Zn-) in polluted areas worldwide. The systematic review was performed following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. The literature selection procedure involved searching four databases (Web of Science, Scopus, Google Scholar, and PubMed) with a variety of search queries and inclusion and exclusion criteria. As a result of the review, 106 scientific articles that addressed Cr, Cu, Ni, Pb and/or Zn effect on soil microorganisms between 2018 and 2022 were identified. Soil microorganisms, crucial for soil functions/functioning, are impacted by heavy metal pollution, affecting essential functions such as nutrient cycling, organic matter cycling, and carbon sequestration. Various microbial properties (microbial activity -including enzymatic activity-, microbial community composition/diversity, microbial biomass/abundance), reflecting heavy metal effects, show diverse microbial responses influenced by both heavy metal pollution and soil properties (soil pH, organic matter content, texture). Although extensive research has been conducted in this field, further studies are needed to better understand the intricate relationship between heavy metal (HM) pollution, soil microbial responses, and soil properties influence. This review explores the most common methodologies and their main challenges and underscores the need for methodologies to specifically assess HM toxicity. Understanding these details is essential for developing effective strategies to mitigate the adverse effects of HM pollution on soil ecosystems.

## 1. Introduction

### 1.1. Heavy metals in soils

The term “heavy metal” is used to describe a group of metals and metalloids with a relatively high density (greater than 5 g cm<sup>-3</sup>), particularly transition metals (elements in groups 3 to 12 of the periodic table totally or partially that have filled d orbitals in one or more oxidation states), which may exhibit toxicity (ability of a substance to cause harm) and cause poisoning (negative effect that occurs from exposure) even at low concentrations [1–3]. Some authors have proposed that the term “heavy metal” is inappropriate due to its difficulty of definition and there is no consensus in the scientific community [4]. However, the utilisation of alternative terminology, such as “toxic

metals” or “trace metals”, also presents certain challenges. While “trace metals” are often associated with essential physiological functions at low concentrations, “toxic metals” refer to elements that typically have no biological role and can be harmful even at low levels [1,5]. Additionally, the term “trace metal” encompasses both heavy metal(loid)s and lower-density metals, which may not cause as many environmental and toxicological issues as heavy metals. In this review, the term “heavy metal” refers to those with high density that are known to have significant environmental and toxicological impacts. This definition includes certain higher-density metalloids, such as arsenic (As) and antimony (Sb).

At low concentrations, some heavy metals (HMs) are essential for plants (e.g., Cu, Ni, and Zn) or animals (e.g., Cr, Cu, Ni, and Zn), while others serve no biological function and may be toxic even at low doses,

\* Corresponding author. Departamento de Biología Vegetal e Ciencia do Solo, Facultade de Ciencias, Universidade de Vigo, As Lagoas s/n, 32004, Ourense, Spain  
E-mail address: [ccampillo@uvigo.es](mailto:ccampillo@uvigo.es) (C. Campillo-Cora).

such as lead (Pb) [6]. For instance, high exposure to certain heavy metal (HM), including  $\text{Cr}^{6+}$ , Cu, Ni, Pb or Zn, has been linked to the development of cancer and other serious illnesses [7]. HMs are naturally present in soils, with their concentration dependent on the parent material. However, anthropogenic activity has led to the accumulation of HMs in the soil, which have a negative impact on the ecosystem [8–10]. In general, HMs are released into the environment during industrial activities, mining, smelting, the discharge of wastewater (industrial or domestic), the combustion of fossil fuels, the application of pesticides (e. g., Bordeaux mixture) or the application of organic and inorganic fertilizers (mainly phosphatic fertilizers) [11]. When the concentration of HMs in soils reaches a level that is detrimental to food safety, crop yield, and soil organisms health, adverse effects may be observed [2].

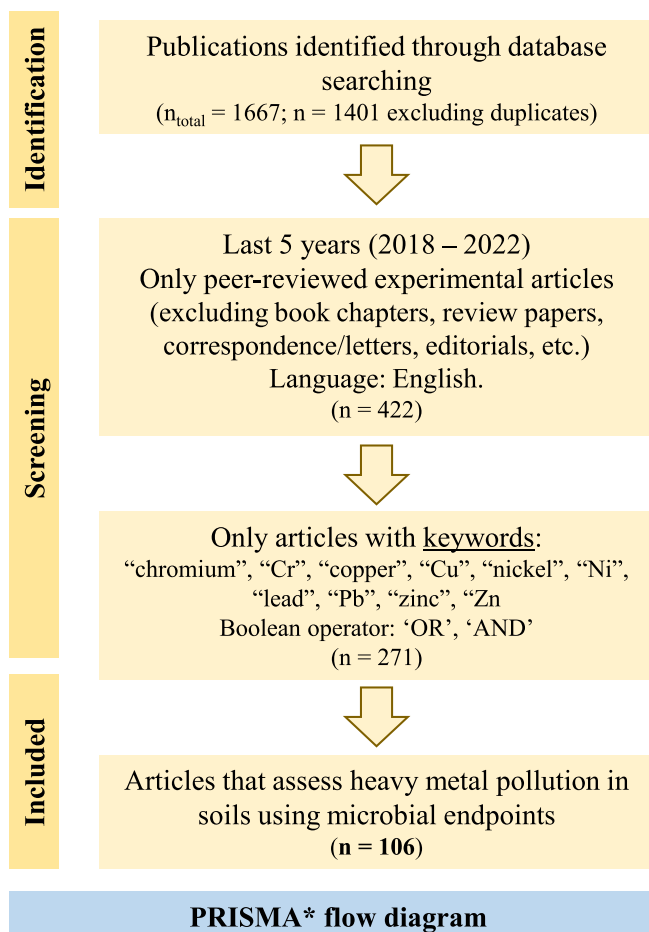
This review focuses on the study of five HMs: Cr, Cu, Ni, Pb and Zn, and their effects on soil microorganisms. These HMs were selected for study because they are very common in polluted areas and there are abundant references to them in the scientific literature. Furthermore, Cr is typically present in an anionic form, which may result in distinct behaviour and effects on microorganisms when compared to the other four HM, which are present in cationic form. Despite extensive research exists in the scientific literature concerning the effect of HM pollution on soil microorganisms, the complex relationship between HM pollution, microbial response and the influence of soil properties has not been thoroughly studied. This review aims to provide a comprehensive overview of how soil properties influence microbial response to HM pollution, advancing the understanding of these intricate interactions.

## 1.2. Soil microorganisms and heavy metals

Soil microorganisms play essential roles in several soil functions and services, including organic matter cycling, carbon (C) sequestration and maintenance of biogeochemical cycles, soil structure creation and maintenance and plant diseases control [12–14]. Soil microorganisms are often the first to come into contact with HMs in the soil matrix due to their widespread presence, mobility and high surface area/volume ratio, leading to a greater exposure to HMs [15]. When HMs accumulate in soils can compromise microbial functions and services, potentially impacting soil quality (capacity to perform essential functions and services) and ecosystem services [16–18]. Because of their sensitivity to changes and their crucial roles in soil processes, microbial properties are widely used as indicators of HM pollution and soil quality [18–20].

## 2. Review methodology

The literature search was conducted following PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) recommendations [21]. This systematic review was conducted using original research papers in peer-reviewed journals (excluding book chapters, editorials, and review papers). Searches were conducted across worldwide research databases (Scopus, Web of Science, PubMed, and Google Scholar). The research was focused on the effect of HM pollution in soils by using soil microorganisms. Multiple search inquiries were conducted using a combination of keywords including “heavy metal”, “potentially toxic element” (or “PTE”, which was also included in the search), “soil microorganisms”, “soil microbial communities” (Fig. 1). Boolean operators were used to refine the search: the truncation operator (“\*”) was used to ensure that all keyword variants were identified, and the conjunction operator “AND” and “OR” were used to combine different search items. The initial literature search was conducted between September and November 2022 and updated in November 2023. The search was limited to articles published in English between 2018 and 2022. In addition, the results were filtered based on the presence of any of the following keywords (using the Boolean operators ‘AND’ and ‘OR’): “chromium”, “Cr”, “copper”, “Cu”, “nickel”, “Ni”, “lead”, “Pb”, “zinc”, “Zn”. Finally, articles addressing the effect of HM pollution on soil microorganisms were selected and classified according to the



\*Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Fig. 1. PRISMA flow diagram [21].

microbial properties examined which, in the context of this review, are measurable characteristics that reflect the state of microorganisms in soil. These microbial properties are (1) microbial activity (Table S1, including enzymatic activity in Table S1B), (2) microbial community composition and/or diversity (Table S2), and (3) microbial biomass/abundance (Table S3). A search of the selected databases identified 1667 articles, 106 of which were retained after eliminating duplicates and meeting the above criteria (Table S4).

## 3. Literature review results

### 3.1. Microbial mechanisms in response to heavy metal pollution in soils

The presence of HMs in soils may have a negative effect on soil microorganisms. HM cations, especially those with high atomic numbers, exert their toxicity on microorganisms when they come into contact with cells, prompting the microbial mechanisms in response to HM toxicity, or when HM enter the cell [15,22,23]. Some nonessential HM cations that present similar characteristics to physiologically essential cations may substitute as cofactors of enzymes inhibiting their activity, e.g.,  $\text{Ni}^{2+}$  with  $\text{Fe}^{2+}$  or  $\text{Zn}^{2+}$  with  $\text{Mg}^{2+}$ , entering the cell [15,22]. In the case of HM oxyanions (negatively charged oxygen-containing ion), they may interfere with the metabolism of nonmetals that share similar structural configurations [22]. For example, chromate ( $\text{Cr}_2\text{O}_4^{2-}$ ) and sulphate ( $\text{SO}_4^{2-}$ ) both have a tetrahedral molecular geometry. Chromate can enter cells through uptake systems that normally transport sulphate, due to their structural similarity [22].

Some microorganisms are genetically predisposed to bioaccumulate

HMs to a certain extent (e.g., *Stenotrophomonas maltophilia* for Cr<sup>6+</sup> and Cr<sup>3+</sup> [24], *Amycolatopsis* sp. for Cu<sup>2+</sup> [25], *Stenotrophomonas* sp. for Ni<sup>2+</sup> [26], *Staphylococcus* sp. for Pb<sup>2+</sup> [26], and *Streptomyces zinciresistens* for Zn<sup>2+</sup> [27]) and may also possess the capacity to modify or detoxify HMs (e.g., *Ochrobactrum* sp. and *Paenarthrobacter* sp. for Cr<sup>6+</sup> [28]). These adaptations enable the microorganisms survive and thrive in HM-polluted soils. Microorganisms possess a variety of mechanisms to resist the toxic effects of HMs, which can act separately or in combination. These include: (i) exclusion by a permeability barrier; (ii) exclusion by efflux of active transporters; (iii) intracellular sequestration within the cytoplasm; (iv) extracellular sequestration; and (v) detoxification.

(i) Exclusion by a permeability barrier:

The permeability barrier mechanisms are not present in all microorganisms but are observed in many bacterial [29,30], archaeal [31], and fungal [32] species that have evolved adaptations to survive in HM-polluted soils [22,23].

Cells employ passive diffusion to exchange gases, including O<sub>2</sub>, N<sub>2</sub> or CH<sub>4</sub>, via non-specific pores in the membrane, facilitating the transport of essential molecules. However, certain volatile metal compounds (Me<sub>3</sub>As, Me<sub>2</sub>Hg or AsH<sub>3</sub>), synthesized by the cell, can also be excreted by passive diffusion [33].

Furthermore, modifications to the cell wall, membrane, or envelope act as additional permeability barrier mechanisms of HM exclusion [23]. For example, some microorganisms produce extracellular polymeric substances (extracellular polysaccharides, EPS), preventing interactions with vital cellular components [23,34,35]. EPS are high molecular weight compounds, mainly composed of polysaccharides, along with proteins, lipids and DNA [34]. In response to HM-stress, EPS are synthesized intracellularly and transported across the cell membrane, though they can occasionally be produced extracellularly by secreted enzymes [34]. EPS intracellular synthesis begins when a C source enters the bacterial cell (diffusion, active transport, or group translocation) and is enzymatically transformed into polysaccharides, which are then transported extracellularly through general mechanism pathway [34]. HMs can bind to negatively charged functional groups in EPS on bacterial and fungal cell walls, such as carboxyl groups (-COOH), amine groups (-NH<sub>2</sub>) and phosphates (PO<sub>4</sub><sup>3-</sup>) [34,36–38]. HM ions are adsorbed onto the cell surface by interacting with EPS on microbial cells [34, 39], through mechanisms like ion exchange, surface complexation, and inorganic microprecipitation [40,41]. The ion exchange, common for binary HM cations, involves replacing a previously bound metal cation at a free site on polysaccharides [42,43]. For example, Pb<sup>2+</sup> has been shown to adsorb to the EPS of *Klebsiella* sp. via ion exchange with the counter ions K<sup>+</sup> and Mg<sup>2+</sup> [44]. In surface complexation, active groups in cell wall EPS (e.g., carboxyl, amino, thiol, hydroxy, phosphate, hydroxy-carboxyl) bind to free HM ion [43], as seen in *Pseudomonas syringae*, where the protein CopC complexes with Cu<sup>+</sup> and Cu<sup>2+</sup> [45]. Inorganic microprecipitation involves HM precipitation on the cell wall. For example, *Pseudomonas putida* binds Pb<sup>2+</sup> and Cd<sup>2+</sup> via functional groups (hydroxyl, amide, and phosphate groups) on its cell wall, resulting in the precipitation of these HM [46]. Depending on the mechanism, it can occur in either living or dead cells.

Several bacteria and archaea possess a proteinaceous surface (S-layer) on their cell wall, comprising up to 15 % of cell proteins. This S-layer can bind large amounts of HMs, such as Pb, Cu, Cr, U, Al, or Cd [41], through nonspecific interactions with functional groups (e.g., carboxyl, phosphoryl) or specific binding sites for bivalent cations [47]. S-layer proteins may act as a nucleation center (sites that initiate the mineral formation), facilitating the formation of biominerals (naturally occurring inorganic materials produced by living organisms) [48,49].

Regarding changes in cell shape, Naik & Dubey [50] observed that *Pseudomonas aeruginosa* strain 4 EA reduced cell size under Pb nitrate exposure, likely limiting Pb uptake by decreasing surface exposure. After Cr exposure, *Pantoea agglomerans* became more oval-shaped and

aggregated closely, forming a packed structure surrounded by thick, woolly coat. This coat, serving as a protective barrier, is likely composed of EPS. By adopting this shape and forming dense aggregates, the cells reduce their surface area exposed to Cr, thereby decreasing metal uptake and minimizing potential toxic effects [51].

(ii) Exclusion by efflux of active transporters:

The efflux mechanisms are not uniformly present in all microorganisms but have been observed in many bacteria, fungi, and archaeal species [52–54].

Transmembrane transporters, or efflux pump systems, are the largest category of HM resistance systems, responsible for exporting HMs from the cytoplasm via active transporters. These mechanisms can be chromosomal or plasmid-encoded and are attributed to energy-demanding processes, such as the CBA (cation antiporter) efflux transporter, cation diffusion facilitator (CDF), P-type ATPases, and ATP-binding cassette (ABC) transporter [55,56]. In Gram-negative bacteria, CBA transporters (Resistance-Nodulation-Division -RND-protein family) form expulsion complexes with membrane fusion proteins (MFP) and outer membrane proteins (OMF), to facilitate the HM transport. CDF membrane-bound proteins in bacteria expel metal ions, and are grouped into Mn<sup>2+</sup> transporters, Fe<sup>2+</sup>/Zn<sup>2+</sup> transporters, Zn<sup>2+</sup> transporters and other metal ion transporters (excluding Fe<sup>2+</sup>/Mn<sup>2+</sup>). P-type ATPases, which are membrane proteins, directly use ATP hydrolysis for active transport of HM ions across the cell membrane [55]. ABC transporters play a crucial role exporting HMs, by activating ATP-binding domains when HM ions bind, leading to ATP hydrolysis and the expulsion of HM ions [57]. For instance, ChrA, a hydrophobic membrane protein from the CHR (chromate ion transporters, ABC transporter) superfamily, acts as a chemiosmotic pump, exporting chromate from the cytoplasm or periplasm, powered by the proton motive force [58]. Other CHR proteins also contribute to chromate resistance via chromate expulsion [59, 60].

- (iii) Intracellular sequestration within the cytoplasm: When HMs enter the cell and cannot be expelled by efflux transporters, certain microorganisms activate a cytosolic sequestration mechanism for protection [48]. This mechanism allows the uptake of high levels of HMs, such as through the synthesis of metallothionein, a low molecular weight, thiol-rich, cysteine-abundant proteins that bind HM. Some bacteria also employ methylation, transferring methyl groups to metal(loid)s [61,62]. However, only a subset of metals can be methylated, such as As, obtaining CH<sub>3</sub>AsO<sub>3</sub>H<sub>2</sub>, (CH<sub>3</sub>)<sub>2</sub>AsO<sub>2</sub>H or (CH<sub>3</sub>)<sub>3</sub>AsO, among others, decreasing its toxicity and facilitating As excretion [63]. HMs can also be accumulated in the vacuoles by fungi and certain bacteria [36,64–66]. Additionally, morphological adaptations can enhance HM tolerance. For example, *Pantoea agglomerans* exhibited changes like elongation and curvature after Cu exposure, while Pb-treated cells become dumbbell-shaped, as a possible strategy of the cells to increase metal accumulation [51].

(iv) Extracellular sequestration:

The extracellular sequestration of HMs has been documented in bacteria, fungi and archaea [67–71]. This process can involve both living cells, which actively excrete metabolites, and dead cells, where previously released compounds persist and interact with HMs, depending on the mechanism and the specific HM in question [37,41,72]. The excretion of specific metabolites reduces HM toxicity through chelation, precipitation, or crystallization, preventing HMs from entering the cell [48,64]. The EPS (nucleic acids, proteins, lipids, complex carbohydrates) secreted by soil microorganisms contain ionizable functional groups (carboxyl, phosphoric, amino, hydroxyl, acyl groups), which can adsorb HM ions through hydrogen bonds, glycolipid bonds, protein-sugar bonds, and intermolecular interactions [73]. For instance,

certain fungi and bacteria secrete melanin, which chelates cations due to the presence of carboxyl and deprotonated hydroxyl groups [48]. Microorganisms can also secrete siderophores and sulphides to bind HM ions outside the cell [36,40]. Siderophores are typically small, low-molecular-weight molecules (hydroxamates, catecholates, carboxylates) that chelate metal ions (e.g., Cu, Ni, Pb, Zn) with high affinity [55].

- (v) Detoxification: Redox reactions provide the chemical basis for biotransformation and detoxification, resulting in the formation of less mobile and less soluble metal species. These reactions can be intracellular or extracellular [74].
- In general, HM cation detoxification involves biotransformation to a less toxic and soluble form. For instance,  $Pb^{2+}$  ions can be precipitated as  $PbO$  or  $PbS$ , while  $Cu^{2+}$  ions can be reduced to  $Cu^0$  [55]. Several biotransformation mechanisms exist, such as  $H_2S$  production to form insoluble sulphides with HMs (Cu, Hg, etc.) [72].
  - In the case of Cr detoxification, the reduction of  $Cr^{6+}$  to  $Cr^{3+}$  can be mediated by bacteria. This process may occur directly through an enzymatic reaction (intracellular or extracellular) or indirectly via the metabolic products of other microbes [75, 76]. For example, the enzyme glucose oxidase from *Aspergillus niger* produces molecules (such as gluconolactone and hydrogen peroxide), which can reduce  $Cr^{6+}$  to  $Cr^{3+}$  [40,77]. Some microbes produce metabolic products that can reduce  $Cr^{6+}$  to  $Cr^{3+}$ , such as  $H_2S$  and  $Fe^{2+}$  from sulphate-reducing bacteria and iron-reducing bacteria [75,76]. The transformation process mainly relies on an appropriate electron donor that facilitates the reduction of  $Cr^{6+}$  to  $Cr^{3+}$  while minimizing the generation of reactive oxygen species (ROS), which can lead to oxidative stress [59].

Aerobic or anaerobic conditions of the environment will also determine the type of detoxification that can occur. Aerobic detoxification involves the reduction of  $Cr^{6+}$  to  $Cr^{3+}$  by various aerobic microorganism (e.g., *Bacillus subtilis* [78]). However, some metabolic processes under aerobic condition can enhance the oxidation of  $Cr^{3+}$  back to  $Cr^{6+}$ , potentially limiting detoxification. Conversely, anaerobic conditions allow for various metabolic pathways (e.g., fermentation, dissimilatory metal reduction) promoting the reduction of  $Cr^{6+}$ . In general, anaerobic conditions can be more favourable for Cr detoxification [59].

Furthermore, following exposure to a HM or due to preexisting mutations, microorganisms can adapt to HM pollution in order to prevent interaction with the HM or by modifications in cell membrane composition. For instance, the transport of chromate within the cell via the sulphate uptake pathway may be impeded in the event of mutation in the chromosome-encoded sulphate pathway [59,60]. Microorganisms can also activate DNA repair mechanisms to counteract induced damage by oxidative stress (ROS production), such as the SOS response enzymes (RecA, RecG, RuvB) [79].

### 3.2. Mode of action and effect of heavy metals on soil microorganisms

The mode of action depends on the HM, as each interacts with microorganisms differently. HMs can exert toxic effects on soil microorganisms by disrupting enzymatic functions, damaging cell membranes, and causing oxidative stress (ROS production).

In the case of Cr, once chromate enters cells, it can be reduced to less toxic species. In the reduction of  $Cr^{6+}$  to  $Cr^{3+}$ ,  $Cr^{5+}$  is generated i.e., a Cr radical with unpaired electrons in its outer shell, making it highly reactive [15,22,40,75]. This  $Cr^{5+}$  readily accepts electrons, enhances electron transfer to oxygen and induces reactions that generate free radicals (e.g., Fenton reactions). As a result,  $Cr^{5+}$  promotes the production of ROS, typically in the cytoplasm, such as superoxide ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radicals ( $-OH$ ) [59,80–83].

Following ROS production,  $Cr^{5+}$  can be oxidized back to  $Cr^{6+}$  [59, 80–83]. ROS accumulation induces oxidative due to stress lipid peroxidation, damaging cell membrane integrity and functionality, as well as DNA damage (base oxidation and strand breaks, compromising genomic stability) [59,82–84]. In proteins, ROS modify amino acids, altering their structure and activity, contributing to cellular dysfunction [82,85].  $Cr^{6+}$  induces cellular alterations (e.g., increased cell volume, rounding) by inhibiting cell division (likely due to Cr interference with nucleic acid and protein synthesis) and by depleting free thiol ( $-SH$ ) groups essential for maintaining the cytoskeleton [85].  $Cr^{6+}$  can also cause actin filament condensation, disrupting the cytoskeletal network, which leads to a loss of motility and abnormal morphology [85]. Additionally,  $Cr^{6+}$  disrupts enzyme function by displacing metal cofactors (e.g., Fe, Mn) or altering the enzyme structure (denaturing or changing three-dimensional configuration), leading to reduced metabolic processes, such as nitrification [84,86].  $Cr^{3+}$  can also affect enzyme structure and activity by reacting with carboxyl and thiol groups [87].  $Cr^{3+}$  can alter cell membrane composition, inhibits growth, causes metabolites loss, and destabilizes membrane through lipid peroxidation [88]. Furthermore,  $Cr^{3+}$  can bind to organic compounds in soil, making them inaccessible to microbial degradation [89].

Cu is an essential element for microorganisms, regulated by specific uptake proteins [90]. When Cu levels exceed binding site capacity, homeostatic mechanisms activate to expel excess Cu, but if overwhelmed, Cu binds to non-specific low-affinity sites, a phenomenon called mis-metallation [91]. This can cause severe cellular damage and death, as Cu replaces metals in metalloproteins and metalloenzymes, releasing them into the cytoplasm and disrupting protein and enzyme functions [91]. Cu can also interfere with physiological functions, denature proteins, disrupting enzymatic processes, and damaging the cell membranes. Furthermore,  $Cu^{2+}$  in soils can be temporally reduced to  $Cu^+$ , for example in presence of soil organic matter (SOM) [92] or by microbial-mediated reactions (e.g., *Pseudomonas* sp. [93]). This reduction generates ROS, leading to cellular damage and oxidative stress [94–98].

Similar to Cu, Ni can bind to metalloproteins and metalloenzymes at the binding sites intended for essential metals (e.g., Cu, Zn, Fe), disrupting their functions [99]. Ni ions can also bind to non-metallic enzymes, compromising their functionality by occupying active sites. Ni may also bind to other enzyme regions, altering their conformation and inhibiting their activity, even without interfering with metal-binding sites [99]. Ni generates oxidative stress and cellular damage through ROS production, arising from interactions with ligands (e.g., cysteine, histidine), and impairs key protective enzymes, exacerbating oxidative stress [99,100].

Lead can disrupt cell membrane integrity by binding to functional (e.g., carboxyl, hydroxyl, sulfonate, sulfhydryl, and phosphate), altering permeability and compromising essential functions [101,102]. Pb also impairs protein and nucleic acid functions by binding to sulfhydryl groups in proteins and hydroxyl groups in nucleic acids, causing conformational changes and functional disruption [101]. Pb can also inhibit enzymatic activity by interfering with essential metal cofactors, leading to enzyme inactivation [102–104]. In addition, Pb also disrupts oxidative phosphorylation, and alters osmotic balance of the cell, inhibits cell division, and can denature proteins, resulting in functional loss that severely impacts cellular structure and metabolism [101–104].

Similar to other HM cations, Zn can also damage cell membranes, induce oxidative stress through ROS production, and disrupts other cellular processes [105]. Zn alters membrane permeability by blocking protein transport pathways, disrupting ion transport and other essential cellular processes [105,106]. Zn can also bind to negatively charged groups in the microbial surface (e.g., sulphur), altering membrane fluidity, damaging specific ion pumps, and impairing the transport and exchange of cellular substances [105,106].

### 3.3. Microbial properties as indicators for heavy metal pollution in soils

The impact of HM pollution on microorganisms can be determined through several microbial properties, which serve as specific indicators of microbial response to environmental stressors [18,107]. In this review, these microbial properties were classified into three different groups: (i) microbial activity (Tables S1A and S1B); (ii) microbial community composition and/or diversity (Table S2); (iii) microbial biomass/abundance (Table S3). Tables S1–S3 summarize recent research (2018–2022) on the effects of HM (Cr, Cu, Ni, Pb, and Zn) on these microbial properties and their general trends.

Microbial activity can be determined through microbial properties like bacterial growth, fungal growth and soil basal respiration (SBR) (Table S1A). Bacterial growth is highly sensitive to HM pollution, generally decreasing as HM content in soil increases [108–111]. Fungal growth showed mixed responses: while some studies reported a negative relationship with increasing HM levels [108,112], others show a stimulatory effect [109,110]. These trends make it challenging to use fungal growth as the only indicator of soil status in the assessment of HM toxicity in soils. SBR typically decreases with higher HM content (Table S1A), but in some cases SBR does not exhibit changes in response to HM contamination [113–115]. These different responses may be influenced by soil properties (e.g., soil pH or C content) which can have a greater impact on the SBR than HM [113]. The microbial metabolic quotient ( $qCO_2$ ), calculated from SBR, reflects microbial respiration per unit of microbial biomass and generally increases with HM pollution (Table S1A).

Enzymatic activities are widely used to assess the impact of HM pollution in soils. The most common enzymatic activities (urease -URE-, dehydrogenase -DHA-, acid and alkaline phosphatase -AcP, AIP-,  $\beta$ -glucosidase (BG) and arylsulfatase activity (ARY)) show varied responses to HM pollution, including decreases, increases or no changes (Table S1B). Soil properties (like soil pH, texture or SOM content) may also influence the enzymatic response, sometimes affecting the enzymatic response more than the HM content [116–118]. However, enzymatic activities may not directly reflect viable microbial populations, since functional enzymes from dead cells can persist [119], but they remain highly valuable indicators of potential soil activity, though caution is needed in their interpretation [120,121].

The microbial community composition is commonly used to assess HM pollution effects, relying on DNA-based techniques (metagenomic sequencing, PCR of the 16S rRNA gene, PCR of the 18S rRNA gene, and ITS analysis), culture-dependent techniques and phospholipid fatty acid (PLFA) analysis (Table S2). DNA analysis shows that microbial community composition usually changes with increasing HM levels, with sensitive species declining and HM-resistant species proliferating (Table S2), such as *Aspergillus fumigatus* [122] or *Candidatus koribacter* [123]. However, some studies report no significant changes in microbial community [124]. These different responses may result from HM toxicity and the influence of soil properties on microbial community structure, (e.g., SOM content, soil pH, texture, N and P contents), which often affect microbial response more than HM level [125,126] (Table S2). On the other hand, bacterial community composition is typically more sensitive to HM pollution than fungal community composition [127,128], as fungi are generally more HM-tolerant [129]. Rajapaksha et al. [130] suggested that acidification from HM pollution might benefit fungi while suppressing more sensitive bacterial population. They also proposed that bacterial mortality releases additional C into the soil, enhancing fungal proliferation, while bacteria with increased sensitivity, cannot use this resource. Similarly, Vázquez-Blanco et al. [111] observed that fungal growth was less affected than bacterial growth in response to Cu additions. Microbial diversity indices can also reflect higher bacterial community sensitivity to HM in comparison to fungal community [131–133]. Microbial diversity indices can be determined from DNA-based analysis, such as Simpson, Shannon, and Chao1. However, the microbial community

diversity indices do not exhibit a defined trend in response to HM pollution (Table S2): while diversity generally decreases with HM pollution [127,134], some studies report increases or no significant effect [135]. This may reflect the proliferation of HM-tolerant microorganisms, masking the decline of sensitive species and leading to an apparent stability or even an increase in diversity indices. Soil properties also strongly influence microbial diversity, in some cases more than HMs [125]. Microbial metabolic activity, assessed through Community-Level Physiological Profiling (CLPP) analysis (usually determined as Average Well Colour Development, AWCD), generally declines with increasing HM content (Table S2), although it may also be enhanced by the presence of HMs due to the development of resistant populations [136].

Microbial biomass or abundance is often used in the assessment of HM pollution, through microbial biomass C (MBC) and, less commonly, microbial biomass N (MBN). Both MBC and MBN usually decrease as HM levels increase (Table S3). MBC may also be influenced by soil properties, such as soil pH or texture, alongside HM content in some cases [137]. Other properties for determining microbial biomass or abundance include PLFA analysis (Table S3). PLFA analysis shows that microbial biomass or abundance decreases with increasing HM levels [109].

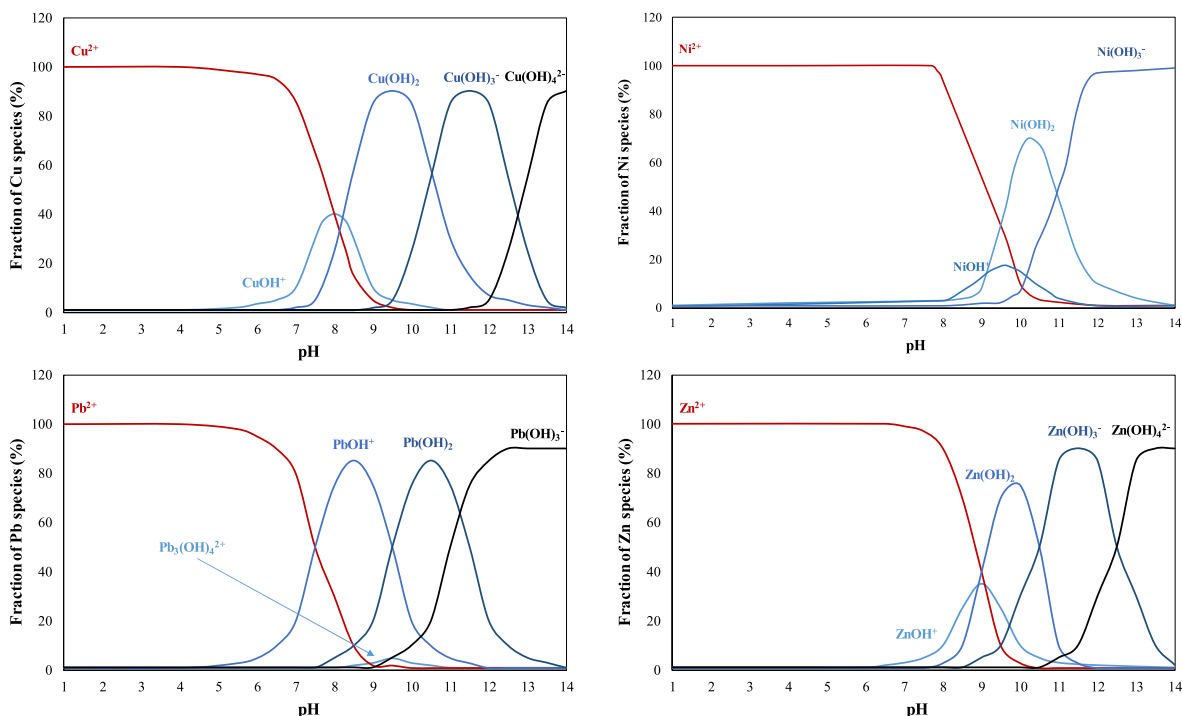
The most common microbial methods for assessing HM pollution effects (SBR, PCR -16S rRNA, 18S rRNA, ITS-, CLPP, MBC, URE, DHA, AcP and AIP activities), sometimes give inconsistent results or even show no variation (Tables S1–S3). In addition, these microbial properties are occasionally correlated with soil physicochemical characteristics, particularly soil pH, SOM content, soil texture and nutrients [113, 137–147], which can directly influence the microbial community. For example, fungal communities proliferate more in acidic soils than bacterial communities, while high SOM content is associated with higher microbial biomass [148,149]. When these methods are applied to assess HM pollution, it can be challenging to distinguish whether the observed microbial response is primarily influenced by soil properties or HM content. Additionally, soil properties may alter HM toxicity, further complicating the study the relationship between microbial response and HM pollution [150]. Another limitation is that these methods do not allow identification of specific HM or HM combination responsible for toxicity. This can be especially limiting in field studies, when multiple HMs may co-occur. In summary, these methods provide very valuable information about overall microbial responses to HM pollution, however our literature review suggests that their specificity for identifying the factors driving toxicity might be limited in certain contexts.

### 3.4. Influence of soil properties on heavy metal availability and microbial toxicity

HM toxicity depends on the available metal content in soils, and it is strongly influenced by key soil properties, particularly soil pH, SOM content and particle size distribution and type [151]. These soil properties were selected for detailed analysis in this section because there are widely recognized as the primary factors governing HM availability [151], and are frequently reported in the reviewed literature as key factors influencing the microbial response to HM pollution (Tables S1–S3). Their effects on HM availability may modulate impacts on microbial community [142,152,153], sometimes to a greater extent than HM content [154].

#### 3.4.1. Soil pH

Soil pH directly impacts all chemical processes and has a greater influence on the solubility of HM than any other property [155], as it can affect the surface charge of layer silicate clays, organic matter, or oxides of Fe and Al [5,151]. However, the effect of soil pH is complex and highly specific for each HM [5,151]. In general, the solubility of the HM that occur as free hydrated cations increases with decreasing pH, as it is shown in Fig. 2 for Cu, Ni, Pb and Zn. This may be explained by certain phenomena: (a) under acidic conditions, competition with released  $H^+$ ,



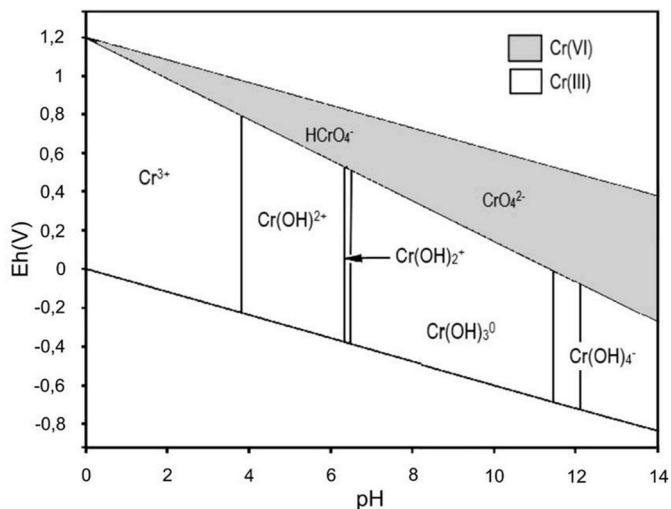
**Fig. 2.** Speciation diagram of heavy metal cations under different pH ranges: Cu (adapted from Powell et al. [157]), Ni (adapted from Dubey and Sharma [158]), Pb (adapted from Powell et al. [159]) and Zn (adapted from Powell et al. [160]). The x-axis represents the pH of the solution, and the y-axis represents % of metal species. Each curve represents a different species of the respective metal: red for the free metal cation ( $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Zn}^{2+}$ ), and blue and black lines for other metal species (e.g.,  $\text{Cu}(\text{OH})_2$ ,  $\text{Ni}(\text{OH})_2$ ,  $\text{Pb}(\text{OH})_2$ ,  $\text{Zn}(\text{OH})_2$ ). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$\text{Fe}^{3+}$  or  $\text{Al}^{3+}$  for negative sorption sites, (b) a decrease in the pH-dependent negative charge of the sorption complex, and (c) dissolution of soil components (e.g., Al, Fe and Mn hydroxides) that become unstable with decreasing pH [1,155,156].

On the other hand, HMs anions, such as Cr, are mobile in alkaline conditions, and they are increasingly sorbed with decreasing pH as soil colloids acquire an additional positive charge [1,155,156]. The solubility of Cr in water varies considerably with pH, with the lowest solubility occurring between pH 5.5 and 8.0. In unpolluted soils with an acidic to neutral pH and a redox potential (Eh) below 600 mV, the reduced form

$\text{Cr}^{3+}$  is predominant, representing the less toxic form (Fig. 3). The adsorption of  $\text{Cr}^{3+}$  is pH-dependent, so it increases as the pH rises. In strongly oxidized media, particularly at neutral to basic pH, the most stable form is  $\text{Cr}^{6+}$ , i.e., the most toxic form, with increasing solubility as pH increases. The most common species of  $\text{Cr}^{6+}$  are chromates ( $\text{CrO}_4^{2-}$ ), although it is in pH-dependent equilibrium with other forms such as dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) [161].

The effect of soil pH on metal toxicity may influence microbial community responses [163]. In the case of HM cations, lower pH leads to enhanced HM mobility (i.e., increased HM toxicity), which affects microbial community diversity [164]. Fernández-Calviño and Băăth [150] observed that Cu did not affect microbial growth in alkaline soil (pH 7.8), but in acidic soils (pH 6.9, 5.5, 4.5), bacterial growth and SBR decreased with increasing Cu levels. Other authors showed that, for the same Cu and Zn level, those forms causing higher acidification potential (e.g.,  $\text{CuSO}_4$  and  $\text{ZnSO}_4$ ), exhibited a greater toxic effect on SBR and bacterial growth compared to other tested forms, such as oxides or commercial Cu-fungicides [111,165]. Borgulat et al. [113] found that soil pH and organic C were the main factors influencing SBR, while Pb content did not significantly affect SBR. However, Pb was significantly correlated to functional diversity and metabolic activity, which were also significantly correlated to soil pH and organic C. Tang et al. [126] found that soil pH and organic C had a greater effect on microbial diversity than high Cr or vanadium (V) levels. In the case of Cr (as an anionic HM), Zhang et al. [166] determined that soil pH, together with SOM content, were the main factors for  $\text{EC}_{50}$  and  $\text{EC}_{10}$  values (Cr concentration causing 50 % or 10 % inhibition, respectively) using MBC: Cr toxicity increased as soil pH increased. These findings highlight the complex interaction between soil properties, microbial responses, and HM toxicity.



**Fig. 3.** Eh-pH diagram of chromium in aqueous solution at 25 °C and 1 atm (source: Maronezi et al. [162]). The x-axis represents the pH of the solution, and the y-axis the redox potential (Eh in V). The diagram illustrated the predominant Cr species as a function of pH and Eh.

### 3.4.2. Soil organic matter

The quantity and quality of SOM can enhance HM immobilization in soils [167,168]. SOM contains numerous functional groups, including

carboxyl (-COOH), carbonyl (C=O) and phenolic groups (Ar-OH), which exhibit a strong affinity for HM ions due to their ability to form coordinate bonds and engage in electrostatic interactions with positively charged metal ions. This affinity enables them to adsorb HM ions and form stable complexes, contributing to HM immobilization and reducing HM availability. According to the Soil Continuum Model (SCM), SOM consists of progressively decomposing organic material processed by decomposer organisms, leading to smaller molecules enriched in polar and ionizable groups [169]. Certain fractions of SOM exhibit a high affinity for HM ions due to their negative charges and diverse functional groups, which enable the formation of stable complexes [1,155,170]. The interactions between HM ions and SOM fractions reducing HM availability include ion exchange, chelation, coagulation and peptization [5,171]. Ion exchange is a nonspecific process where HM ions in soil solution are reversibly adsorbed onto charged surfaces by outer-sphere complexation (by weak covalent bonding) of SOM and can be replaced by other ions present in the solution [151,172,173]. Chelation is the process where organic molecules (chelating agents) form stable complexes with HM, involving the binding of HM to multiple donor atoms in the functional groups of SOM, resulting in stable inner-sphere complexes (highly stable structures formed by ionic, covalent bonds, or a combination of both) [1,170,172]. Coagulation is the process by which HM and SOM aggregate into larger flocs by reducing net surface charge and electrostatic repulsion, facilitating their agglomeration and stabilizing them in the soil matrix [174,175]. Peptization, the reverse process to coagulation, consists in the dispersion of the HM precipitates, e.g., metal oxides, into the soil matrix [171]. As an example, during peptization, SOM anionic fractions can replace ions or hydroxyl groups on metal oxides, altering the surface charge, reducing electrostatic attraction and improving the metal dispersion in the soil matrix [171]. Thus, SOM fractions can form organometallic complexes with HM cations, e.g., Cu, Ni, Pb or Zn, and their stability generally increases with increasing pH [170]. The dissolved fraction of organic matter (DOM) in SOM also plays a key role in several soil processes that influence the mobility, bioavailability and toxicity of HMs, and even control their speciation [1, 5,151,155,156]. DOM form complexes with HM cations reducing the HM free ions in the soil solution, reducing their toxicity and bioavailability [176–178]. For HM anions like Cr, DOM can reduce mobility, solubility, toxicity and bioavailability, by mediating the reduction of Cr<sup>6+</sup> to Cr<sup>3+</sup> in DOM or forming organometallic complexes [177,179, 180]. Hence, the SOM content influences the toxic effect of HMs on microbial communities. Stefanowicz et al. [181] found that SOM had a strong positive impact on soil microbes (SBR, MBC, URE, ARY), which was greater than that of HMs. The authors attributed this to two main reasons: i) SOM provides a variety of substrates to some microbial communities, enhancing microbial activity and ii) SOM tends to adsorb HMs, which decreases their toxicity. In a similar vein, Xian et al. [182] determined that below a certain threshold of SOM content, Pb exhibited pronounced toxicity on enzymatic activity, while beyond this threshold, SOM appeared to positively influence enzymatic activity and provided protection to the microbial community against the detrimental impacts of Pb [182]. Regarding enzymatic activities, Naylo et al. [116] found that AIP and URE were significantly correlated with total organic C and P level than HM content, while Zamulina et al. [117] observed DHA and URE were more strongly correlated with water-soluble SOM than HM levels. Guo et al. [183] suggested that increased SOM reduced HM availability, altering microbial community composition. Tang et al. [126] reported that organic C, along with soil pH, had a greater influence on microbial diversity than Cr or V contents. Borgulat et al. [113] found that organic C was significantly correlated with enzymatic activity (AcP), SBR, functional diversity and metabolic activity, sometimes more than Pb content. Zhang et al. [166] found that SOM, together with soil pH, governed Cr toxicity (measured by Substrate-Induced Respiration, SIR), with increased SOM reducing Cr toxic effects.

### 3.4.3. Soil texture

The particle size distribution exerts a great effect on HM sorption and availability, mainly due to the finer granulometric fraction [151]. Coarse-grained soils generally present lower HM sorption than fine-grained soils, with HM total content of soils generally increases with decreasing particle size [184]. The capacity to retain HMs is principally attributed to the following:

- A larger surface area of the finer fraction of soil, which can be external or internal. The external surface area is directly related to particle size and mass. The internal surface area is related to the interlaminar separation and mass, referring to the space between layers in clay minerals where metal ions can be adsorbed. This interlayer space can also expand or contract depending on, for example, moisture, allowing for additional sites for metal sorption [1,172].
- The presence of electric charges in the interlaminar space (permanent charge) or on their surface (variable charge). The permanent charge is inherent to the crystal lattices of minerals and is usually negative, irrespective of pH and ionic strength. This negative charge attracts positively charged metal ions, i.e., cations, facilitating their adsorption within the interlaminar space. Variable charge is a secondary superficial source of charge in humic substances, oxyhydroxides of iron (Fe) and aluminium (Al) and clay minerals (e.g., kaolinite). Fe and Al oxides present a positive charge at low pH (proton adsorption) and a negative charge at high pH (proton desorption) [172].

The fine-grained fraction contains a variety of soil particles, including aluminosilicates, Fe and Mn oxyhydroxides, and humic acids [1]. Aluminosilicates are characterized by a permanent negative charge [1]. The negative charge of clay minerals is very relevant for retaining HM ions. In soil solution, HMs can act as Lewis acids, i.e., electron acceptors, and therefore, an electron-pair donating surface functional group (-OH, -SH or -COOH) can form a complex with an electron-pair acceptor metal ion (M<sup>+2</sup> such as Cu<sup>+2</sup>, Ni<sup>+2</sup>, Pb<sup>+2</sup> or Zn<sup>+2</sup>) [155]. Metal oxyanions (e.g., HCrO<sub>4</sub><sup>-</sup>) may release OH<sup>-</sup> from the surface upon complexation [155]. The specific surface area depends on the amount and kinds of clay minerals, but the chemical composition and properties of clay minerals are diverse [1,172]. Other HM-reactive constituents of soils are metal hydroxides, mainly Fe and Mn, which possess a functional group (OH<sup>-</sup>) that can donate their protons to the surrounding solution and can take up HM ions in return in a pH-dependent reaction [5,151, 156]. Hence, soil particle-size distribution and composition influence HM availability for soil microorganisms, which reduces HM toxicity with decreasing particle size. Chen et al. [185] found that microbial properties (MBC, SBR, DHA) were more negatively affected by HM pollution in the coarse sand fraction than in the clay fraction. Harvey et al. [186] determined that fungal resistance to stress (*Saitozyma podzolica*) was inversely related to pore size, i.e., surface area increases, which increases Pb adsorption and decreases Pb toxicity.

### 3.5. Methodologies for assessing heavy metal toxicity in soils: advances and future Directions

When evaluating HM-polluted soils exhibiting diverse properties (particularly soil pH, organic matter, and clay content) through microbial properties, it can potentially pose challenges in discerning whether the observed differences among distinct soils are attributable to metal accumulation or variations in soil properties. One way to avoid this issue is to use methods that are specifically related to the toxicity of the HM under study by focusing on community-level responses to HM exposure.

In this context, Pollution-Induced Community Tolerance (PICT) methodology emerges as an alternative, as it is directly linked to HM toxicity (or HM combination), allowing the comparison between soils with varying physicochemical properties and the identification of the

HM (or the HM combinations) exerting toxic pressure on the microbial community [187]. Through this ecotoxicological approach, the tolerance of a microbial community to a specific HM (or combination of HM) is determined as a toxicity index. This tolerance can be quantified by comparing physiological responses (which can be determined by a wide variety of microbial endpoints) with a microbial reference community, thereby providing a robust assessment of chemical-induced structural impacts [187–189]. This PICT methodology consists of two phases: the selection phase and the detection phase. Selection phase of PICT occurs in soil (either in the field or laboratory) where microbial communities are first exposed to the HM (or HM combination). During this exposure, the selective pressure exerted by the HM(s) favours the proliferation of tolerant species, while more sensitive species are negatively affected, leading to an overall more tolerant community. The detection phase of PICT involves quantifying the microbial community through a second exposure to the HM (or HM combination) at varying concentrations, obtaining toxicity indices such as  $IC_x$ ,  $EC_x$  or  $LC_x$  (concentration that causes an inhibition, effect or lethality of  $x$  %, respectively) [187–189]. Thus, this PICT methodology allows to identify exactly the pollutant which is exerting toxicity on the microbial community. Additionally, this kind of methodologies allows for comparison between soils with different physicochemical properties; however, they may also present other limitations or biases. PICT methodology has been largely applied to study HM pollution in soils, however several aspects should be addressed in future research, such as the need of reference soils to compare the response of the microbial community [191,192], or the identification of bias or interferences in the determination of microbial community tolerance [190,193].

Regarding other ecotoxicological methodologies, the Toxicity Identification Evaluation (TIE) methodology was applied to determine the toxicity of HM in microbial communities, but only for sediments and aquatic environments [194,195]. However, there is no evidence of its application and/or adaptation to soils. The Effect-Directed Analysis (EDA), originally developed for aquatic environments analysis, was applied to soils, but only for organic pollutants [194,196]. Hence, there is a lack of evidence of TIE and EDA approaches could be successfully applied to compare HM-pollution in soils with different physicochemical properties.

#### 4. Concluding remarks

The intricate relationship between HMs and soil microorganisms is a multifaceted interplay that is influenced by various factors. Understanding the impact of HMs on soil microorganisms is vital, given the essential role of microorganisms in soil functions, including organic matter cycling, C sequestration, and maintenance of biogeochemical cycles. Microbial properties serve as valuable indicators of soil quality, reflecting changes in overall soil health. A variety of microbial parameters, including microbial activity, microbial community composition, and microbial biomass/abundance, can be used to assess the impact of HMs on soil microorganisms. The complexity arises from the diverse microbial responses observed in these parameters, which are influenced not only by HM pollution but also by soil properties.

Soil pH, organic matter content, and texture significantly influence the availability of HMs and, consequently, their impact on soil microorganisms. The intricate interplay between these factors makes it difficult to attribute observed differences solely to HM accumulation, necessitating the use of specifically related methodologies which assess HM toxicity (such as the Pollution-Induced Community Tolerance methodology). Understanding the interaction between HMs and soil microorganisms is essential to assess whether HM pollution exerts toxicity in soil, to design effective strategies to mitigate the adverse effects of HM pollution on soil ecosystems. To elucidate the details of this relationship and progress to sustainable soil practices, further research with existing (e.g., PICT, TIA, EDA) or new methodologies creation is essential.

#### Ethical approval, consent to participate and consent to publish

No applicable.

#### CRedit authorship contribution statement

**Claudia Campillo-Cora:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Andrés Rodríguez-Seijo:** Methodology, Formal analysis, Data curation. **Paula Pérez-Rodríguez:** Methodology, Investigation, Conceptualization. **David Fernández-Calviño:** Writing – review & editing, Validation, Resources, Funding acquisition, Conceptualization. **Vanesa Santás-Miguel:** Writing – original draft, Formal analysis, Data curation.

#### Availability of data and materials

The authors declare that the data supporting the findings of this study are available within the review.

#### 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.

#### Acknowledgements

The authors would like to recognize the financial support of the Consellería de Cultura, Educación e Universidade (Xunta de Galicia) through the contract ED431C 2021/46-GRC granted to the research group BV1 of the University of Vigo. Funding for open access charge: Universidade de Vigo/CISUG. Claudia Campillo-Cora holds a post-doctoral contract in Campus Auga (0623–137919) funded by the agreement Xunta de Galicia - Universidade de Vigo. Andrés Rodríguez-Seijo thanks the Ministerio de Ciencia e Innovación and the European Union NextGeneration EU<sup>+</sup>/PRTR for the postdoc grant Juan de la Cierva Incorporation 2020 (IJC2020-044197-I/MCIN/AEI/10.13039/501100011033). This work was supported by a postdoctoral fellowship (ED481B-2022-081) Vanesa Santás-Miguel financed by Xunta de Galicia. Paula Pérez-Rodríguez was funded by Juan de la Cierva contract, grant number JC2020-044426-I, from the Spanish Ministry of Science and Innovation and NextGenerationEU.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejsobi.2024.103706>.

#### References

- [1] C. Gonnelli, G. Renella, *Heavy Metals in Soils*, 3th Editio, Springer, Dordrecht, Netherlands, 2013.
- [2] P.C. Nagajyoti, K.D. Lee, T.V.M. Sreekanth, Heavy metals, occurrence and toxicity for plants: a review, *Environ. Chem. Lett.* 8 (2010) 199–216, <https://doi.org/10.1007/s10311-010-0297-8>.
- [3] M. Gerloch, E.C. Constable, An introduction to transition-metal chemistry, in: *Transit. Met. Chem.*, Wiley, Weinheim (Germany), 1994, pp. 1–19, <https://doi.org/10.1002/3527604014.ch1>.
- [4] R. Hübner, K.B. Astin, R.J.H. Herbert, 'Heavy metal'—time to move on from semantics to pragmatics? *J. Environ. Monit.* 12 (2010) 1511, <https://doi.org/10.1039/c0em00056f>.
- [5] A. Kabata-Pendias, *Trace Elements in Soils and Plants*, fourth ed., CRC Press, Taylor & Francis Group, New York, 2011. Boca Raton.
- [6] B.A. Chowdhury, R.K. Chandra, Biological and health implications of toxic heavy metal and essential trace element interactions, *Prog. Food Nutr. Sci.* 11 (1987) 55–113. <https://europepmc.org/article/med/3303135>. (Accessed 4 June 2022).
- [7] J. Briffa, E. Sinagra, R. Blundell, Heavy metal pollution in the environment and their toxicological effects on humans, *Heliyon* 6 (2020) e04691, <https://doi.org/10.1016/J.HELIYON.2020.E04691>.



- [8] Q. Zhang, C. Wang, Natural and human factors affect the distribution of soil heavy metal pollution: a review, water, air, Soil Pollut. 231 (2020) 350, <https://doi.org/10.1007/s11270-020-04728-2>.
- [9] F.X. Han, A. Baniin, Y. Su, D.L. Monts, J.M. Plodinec, W.L. Kingery, G.E. Triplett, Industrial age anthropogenic inputs of heavy metals into the pedosphere, *Naturwissenschaften* 89 (2002) 497–504, <https://doi.org/10.1007/s00114-002-0373-4>.
- [10] V. Tilwankar, S. Rai, S.P. Bajpai, A review on contamination profile of heavy metals and its role in environment, *Plant Arch.* 18 (2018) 1239–1247.
- [11] H. Ali, E. Khan, I. Ilahi, Environmental chemistry and ecotoxicology of hazardous heavy metals: environmental persistence, toxicity, and bioaccumulation, *J. Chem.* 2019 (2019) 6730305, <https://doi.org/10.1155/2019/6730305>.
- [12] J.O.G. Jönsson, B. Davidsdóttir, Classification and valuation of soil ecosystem services, *Agric. Syst.* 145 (2016) 24–38, <https://doi.org/10.1016/j.agsy.2016.02.010>.
- [13] J.H. Faber, J. Van Wensem, Elaborations on the use of the ecosystem services concept for application in ecological risk assessment for soils, *Sci. Total Environ.* 415 (2012) 3–8, <https://doi.org/10.1016/j.scitotenv.2011.05.059>.
- [14] O. Coban, G.B. de Deyn, M. van der Ploeg, Soil microbiota as game-changers in restoration of degraded lands, *Science* 375 (2022) eabe0725, <https://doi.org/10.1126/SCIENCE.ABE0725/ASSET/F5D5AAC3-DDE9-45C1-9619-20F9A9A924ED/ASSETS/IMAGES/LARGE/SCIENCE.ABE0725-F4.JPG>.
- [15] E. Puglisi, R. Hamon, S. Vasileiadis, D. Coppolecchia, M. Trevisan, Adaptation of soil microorganisms to trace element contamination: a review of mechanisms, methodologies, and consequences for risk assessment and remediation, *Crit. Rev. Environ. Sci. Technol.* 42 (2012) 2435–2470, <https://doi.org/10.1080/10643389.2011.592735>.
- [16] V. Iordache, A. Neageo, Conceptual methodological framework for the resilience of biogeochemical services to heavy metals stress, *J. Environ. Manag.* 325 (2023) 116401, <https://doi.org/10.1016/j.jenvman.2022.116401>.
- [17] N. Abdu, A.A. Abdullahi, A. Abdulkadir, Heavy metals and soil microbes, *Environ. Chem. Lett.* 15 (2017) 65–84, <https://doi.org/10.1007/s10311-016-0587-x>.
- [18] J. Tang, J. Zhang, L. Ren, Y. Zhou, J. Gao, L. Luo, Y. Yang, Q. Peng, H. Huang, A. Chen, Diagnosis of soil contamination using microbiological indices: a review on heavy metal pollution, *J. Environ. Manag.* 242 (2019) 121–130, <https://doi.org/10.1016/j.jenvman.2019.04.061>.
- [19] S. Schoenholtz, H.V. Miegroet, J. Burger, A review of chemical and physical properties as indicators of forest soil quality: challenges and opportunities, *For. Ecol. Manag.* 138 (2000) 335–356, [https://doi.org/10.1016/S0378-1127\(00\)00423-0](https://doi.org/10.1016/S0378-1127(00)00423-0).
- [20] P. Nannipieri, J. Ascher, M.T. Ceccherini, L. Landi, G. Pietramellara, G. Renella, Microbial diversity and soil functions, *Eur. J. Soil Sci.* 54 (2003) 655–670, <https://doi.org/10.1046/j.1351-0754.2003.0556.x>.
- [21] M.J. Page, J.E. McKenzie, P.M. Bossuyt, I. Boutron, T.C. Hoffmann, C.D. Mulrow, L. Shamseer, J.M. Tetzlaff, E.A. Akl, S.E. Brennan, R. Chou, J. Glanville, J. M. Grimshaw, A. Hróbjartsson, M.M. Lallu, T. Li, E.W. Loder, E. Mayo-Wilson, S. McDonald, L.A. McGuinness, L.A. Stewart, J. Thomas, A.C. Tricco, V.A. Welch, P. Whiting, D. Moher, The PRISMA 2020 statement: an updated guideline for reporting systematic reviews, *BMJ* (2021) n71, <https://doi.org/10.1136/bmj.n71>.
- [22] D.H. Nies, Microbial heavy-metal resistance, *Appl. Microbiol. Biotechnol.* 516 51 (1999) 730–750, <https://doi.org/10.1007/S002530051457>, 1999.
- [23] M.R. Bruins, S. Kapil, F.W. Oehme, Microbial resistance to metals in the environment, *Ecotoxicol. Environ. Saf.* 45 (2000) 198–207, <https://doi.org/10.1006/eesa.1999.1860>.
- [24] M.Z. Alam, S. Ahmad, Chromium removal through biosorption and bioaccumulation by bacteria from tannery effluents contaminated soil, *Clean* 39 (2011) 226–237, <https://doi.org/10.1002/clean.201000259>.
- [25] V.H. Albarracín, B. Winik, E. Kothe, M.J. Amoroso, C.M. Abate, Copper bioaccumulation by the actinobacterium *Amycolatopsis* sp. AB0, *J. Basic Microbiol.* 48 (2008) 323–330, <https://doi.org/10.1002/jobm.200700360>.
- [26] F. Aslam, A. Yasmin, S. Sohail, Bioaccumulation of lead, chromium, and nickel by bacteria from three different genera isolated from industrial effluent, *Int. Microbiol.* 23 (2020) 253–261, <https://doi.org/10.1007/s10123-019-00098-w>.
- [27] Y. Lin, X. Wang, B. Wang, O. Mohamad, G. Wei, Bioaccumulation characterization of zinc and cadmium by *Streptomyces zinciresistans*, a novel actinomycete, *Ecotoxicol. Environ. Saf.* 77 (2012) 7–17, <https://doi.org/10.1016/j.ecoenv.2011.09.016>.
- [28] M. Gan, Y. Zhou, D. Huang, P. He, B. Tang, Y. Cai, J. Zhu, The enhanced effect of key microorganisms in chromium contaminated soil in Cr(VI) reduction, *Chemosphere* 362 (2024) 142682, <https://doi.org/10.1016/j.chemosphere.2024.142682>.
- [29] W. Zeng, F. Li, C. Wu, R. Yu, X. Wu, L. Shen, Y. Liu, G. Qiu, J. Li, Role of extracellular polymeric substance (EPS) in toxicity response of soil bacteria *Bacillus* sp. S3 to multiple heavy metals, *Bioproc. Biosyst. Eng.* 43 (2020) 153–167, <https://doi.org/10.1007/s00449-019-02213-7>.
- [30] F.L. Lederer, U. Weinert, T.J. Günther, J. Raff, S. Weiß, K. Pollmann, Identification of multiple putative S-layer genes partly expressed by *Lysinibacillus sphaericus* JG-B53, *Microbiology* 159 (2013) 1097–1108, <https://doi.org/10.1099/mic.0.065763-0>.
- [31] A.R. Showalter, J.E.S. Szymanowski, J.B. Fein, B.A. Bunker, An x-ray absorption spectroscopy study of Cd binding onto a halophilic archaeon, *J. Phys. Conf. Ser.* 712 (2016) 012079, <https://doi.org/10.1088/1742-6596/712/1/012079>.
- [32] Y. Deng, L. Wang, K. Luo, D. Peng, H. Jiang, C. Jin, X. Zhou, L. Bai, Screening and identifying a cadmium-resistant fungus and characterizing its cadmium adsorption, *Pol. J. Environ. Stud.* 26 (2017) 1011–1021, <https://doi.org/10.15244/pjoes/67655>.
- [33] A. Raab, J. Feldmann, Microbial transformation of metals and metalloids, *Sci. Prog.* 86 (2003) 179–202, <https://doi.org/10.3184/003685003783238671>.
- [34] Vandana, M. Priyadarshane, S. Das, Bacterial extracellular polymeric substances: biosynthesis and interaction with environmental pollutants, *Chemosphere* 332 (2023) 138876, <https://doi.org/10.1016/j.chemosphere.2023.138876>.
- [35] C. Dang, Z. Yang, W. Liu, P. Du, F. Cui, K. He, Role of extracellular polymeric substances in biosorption of Pb<sup>2+</sup> by a high metal ion tolerant fungal strain *Aspergillus Niger* PTN31, *J. Environ. Chem. Eng.* 6 (2018) 2733–2742, <https://doi.org/10.1016/j.jece.2018.04.005>.
- [36] S. Tiwari, C. Lata, Heavy metal stress, signaling, and tolerance due to plant-associated microbes: an overview, *Front. Plant Sci.* 9 (2018), <https://doi.org/10.3389/fpls.2018.00452>.
- [37] K. Vijayaraghavan, Y.-S. Yun, Bacterial biosorbents and biosorption, *Biotechnol. Adv.* 26 (2008) 266–291, <https://doi.org/10.1016/j.biotechadv.2008.02.002>.
- [38] K.R. K. U.R. Sardar, E. Bhargavi, I. Devi, B. Bhunia, O.N. Tiwari, Advances in exopolysaccharides based bioremediation of heavy metals in soil and water: a critical review, *Carbohydr. Polym.* 199 (2018) 353–364, <https://doi.org/10.1016/j.carbpol.2018.07.037>.
- [39] P. Gupta, B. Diwan, Bacterial Exopolysaccharide mediated heavy metal removal: a Review on biosynthesis, mechanism and remediation strategies, *Biotechnol. Reports* 13 (2017) 58–71, <https://doi.org/10.1016/j.btre.2016.12.006>.
- [40] Y. Mao, H. Tan, M. Wang, T. Jiang, H. Wei, W. Xu, Q. Jiang, H. Bao, Y. Ding, F. Wang, C. Zhu, Research progress of soil microorganisms in response to heavy metals in rice, *J. Agric. Food Chem.* 70 (2022) 8513–8522, <https://doi.org/10.1021/ACS.JAF.C.2C01437>.
- [41] L. Velásquez, J. Dussan, Biosorption and bioaccumulation of heavy metals on dead and living biomass of *Bacillus sphaericus*, *J. Hazard Mater.* 167 (2009) 713–716, <https://doi.org/10.1016/j.jhazmat.2009.01.044>.
- [42] M. Priyadarshane, S. Das, Biosorption and removal of toxic heavy metals by metal tolerating bacteria for bioremediation of metal contamination: a comprehensive review, *J. Environ. Chem. Eng.* 9 (2021) 104686, <https://doi.org/10.1016/j.jece.2020.104686>.
- [43] V. Javanbakhsh, S.A. Alavi, H. Zilouei, Mechanisms of heavy metal removal using microorganisms as biosorbent, *Water Sci. Technol.* 69 (2014) 1775–1787, <https://doi.org/10.2166/wst.2013.718>.
- [44] W. Wei, Q. Wang, A. Li, J. Yang, F. Ma, S. Pi, D. Wu, Biosorption of Pb (II) from aqueous solution by extracellular polymeric substances extracted from *Klebsiella* sp. J1: adsorption behavior and mechanism assessment, *Sci. Rep.* 6 (2016) 31575, <https://doi.org/10.1038/srep31575>.
- [45] M. Koay, L. Zhang, B. Yang, M.J. Maher, Z. Xiao, A.G. Wedd, CopC protein from *Pseudomonas syringae*: intermolecular transfer of copper from both the copper (I) and copper(II) sites, *Inorg. Chem.* 44 (2005) 5203–5205, <https://doi.org/10.1021/ic0506198>.
- [46] S. Khashei, Z. Etemadifar, H.R. Rahmani, Immobilization of *Pseudomonas putida* PT in resistant matrices to environmental stresses: a strategy for continuous removal of heavy metals under extreme conditions, *Ann. Microbiol.* 68 (2018) 931–942, <https://doi.org/10.1007/s13213-018-1402-7>.
- [47] M. Vogel, S. Matys, F. Lehmann, B. Drobot, T. Günther, K. Pollmann, J. Raff, Use of specific metal binding of self-assembling S-layer proteins for metal bioremediation and recycling, *Solid State Phenom.* 262 (2017) 389–393, <https://doi.org/10.4028/www.scientific.net/SSP.262.389>.
- [48] G. Hafenburg, E. Kothe, Microbes and metals: interactions in the environment, *J. Basic Microbiol.* 47 (2007) 453–467, <https://doi.org/10.1002/jobm.200700275>.
- [49] H.C.W. Skinner, Biominerals, *Mineral. Mag.* 69 (2005) 621–641, <https://doi.org/10.1180/0026461056950275>.
- [50] M.M. Naik, S.K. Dubey, Lead-enhanced siderophore production and alteration in cell morphology in a Pb-resistant *Pseudomonas aeruginosa* strain 4EA, *Curr. Microbiol.* 62 (2011) 409–414, <https://doi.org/10.1007/s00284-010-9722-2>.
- [51] B.V. Mohite, S.H. Koli, S.V. Patil, Heavy metal stress and its consequences on exopolysaccharide (EPS)-Producing *Pantoea agglomerans*, *Appl. Biochem. Biotechnol.* 186 (2018) 199–216, <https://doi.org/10.1007/s12010-018-2727-1>.
- [52] D.H. Nies, Efflux-mediated heavy metal resistance in prokaryotes, *FEMS Microbiol. Rev.* 27 (2003) 313–339, [https://doi.org/10.1016/S0168-6445\(03\)00048-2](https://doi.org/10.1016/S0168-6445(03)00048-2).
- [53] D.H. Nies, S. Silver, Ion efflux systems involved in bacterial metal resistances, *J. Ind. Microbiol.* 14 (1995) 186–199, <https://doi.org/10.1007/BF01569902>.
- [54] G. Del Sorbo, H. Schoonbeek, M.A. De Waard, Fungal transporters involved in efflux of natural toxic compounds and fungicides, *Fungal Genet. Biol.* 30 (2000) 1–15, <https://doi.org/10.1006/fgbi.2000.1206>.
- [55] K. Mathivanan, J.U. Chandirika, A. Vinothkanna, H. Yin, X. Liu, D. Meng, Bacterial adaptive strategies to cope with metal toxicity in the contaminated environment – a review, *Ecotoxicol. Environ. Saf.* 226 (2021) 112863, <https://doi.org/10.1016/j.ecoenv.2021.112863>.
- [56] R. Choudhury, S. Srivastava, Zinc resistance mechanisms in bacteria, *Curr. Sci.* 81 (2001) 768–775.
- [57] W.-Y. Song, J. Park, C. Eisenach, M. Maeshima, Y. Lee, E. Martinoia, ABC transporters and heavy metals, [https://doi.org/10.1007/978-3-319-06511-3\\_1](https://doi.org/10.1007/978-3-319-06511-3_1), 2014.
- [58] A.H. Alvarez, R. Moreno-Sánchez, C. Cervantes, Chromate efflux by means of the ChrA chromate resistance protein from *Pseudomonas aeruginosa*, *J. Bacteriol.* 181 (1999) 7398–7400, <https://doi.org/10.1128/JB.181.23.7398-7400.1999>.
- [59] H. Thatoi, S. Das, J. Mishra, B.P. Rath, N. Das, Bacterial chromate reductase, a potential enzyme for bioremediation of hexavalent chromium: a review,

- J. Environ. Manag. 146 (2014) 383–399, <https://doi.org/10.1016/j.jenvman.2014.07.014>.
- [60] M.I. Ramírez-Díaz, C. Díaz-Pérez, E. Vargas, H. Riveros-Rosas, J. Campos-García, C. Cervantes, Mechanisms of bacterial resistance to chromium compounds, *Biometals* 21 (2008) 321–332, <https://doi.org/10.1007/s10534-007-9121-8>.
- [61] R. Bentley, T.G. Chasteen, Microbial methylation of metalloids: arsenic, antimony, and bismuth, *Microbiol. Mol. Biol. Rev.* 66 (2002) 250–271, <https://doi.org/10.1128/MMBR.66.2.250-271.2002>.
- [62] J.K. King, J.E. Kostka, M.E. Frischer, F.M. Saunders, Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments, *Appl. Environ. Microbiol.* 66 (2000) 2430–2437, <https://doi.org/10.1128/AEM.66.6.2430-2437.2000>.
- [63] M.A. Rahman, C. Hassler, Is arsenic biotransformation a detoxification mechanism for microorganisms? *Aquat. Toxicol.* 146 (2014) 212–219, <https://doi.org/10.1016/j.aquatox.2013.11.009>.
- [64] M. Zubair, M. Shakir, Q. Ali, N. Rani, N. Fatima, S. Farooq, S. Shafiq, N. Kanwal, F. Ali, I.A. Nasir, Rhizobacteria and phytoremediation of heavy metals, *Environ. Technol. Rev.* 5 (2016) 112–119, <https://doi.org/10.1080/21622515.2016.1259358>.
- [65] Y. Shi, L. Tang, Q. Shao, Y. Jiang, Z. Wang, C. Peng, T. Gu, Z. Li, The dynamic roles of intracellular vacuoles in heavy metal detoxification by *Rhodotorula mucilaginosa*, *J. Appl. Microbiol.* 135 (2024), <https://doi.org/10.1093/jambio/lxae241>.
- [66] B.P. Rosen, Transport and detoxification systems for transition metals, heavy metals and metalloids in eukaryotic and prokaryotic microbes, *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* 133 (2002) 689–693, [https://doi.org/10.1016/S1095-6433\(02\)00201-5](https://doi.org/10.1016/S1095-6433(02)00201-5).
- [67] V.I. Slaveykova, N. Parthasarathy, K. Dedieu, D. Toescher, Role of extracellular compounds in Cd-sequestration relative to Cd uptake by bacterium *Sinorhizobium meliloti*, *Environ. Pollut.* 158 (2010) 2561–2565, <https://doi.org/10.1016/j.envpol.2010.05.016>.
- [68] Z.-B. Yue, Q. Li, C. Li, T. Chen, J. Wang, Component analysis and heavy metal adsorption ability of extracellular polymeric substances (EPS) from sulfate reducing bacteria, *Bioresour. Technol.* 194 (2015) 399–402, <https://doi.org/10.1016/j.biortech.2015.07.042>.
- [69] J. Li, Z. Jiang, S. Chen, T. Wang, L. Jiang, M. Wang, S. Wang, Z. Li, Biochemical changes of polysaccharides and proteins within EPS under Pb(II) stress in *Rhodotorula mucilaginosa*, *Ecotoxicol. Environ. Saf.* 174 (2019) 484–490, <https://doi.org/10.1016/j.ecoenv.2019.03.004>.
- [70] K. Paria, S. Pyne, S.K. Chakraborty, Optimization of heavy metal (lead) remedial activities of fungi *Aspergillus penicillioides* (F12) through extra cellular polymeric substances, *Chemosphere* 286 (2022) 131874, <https://doi.org/10.1016/j.chemosphere.2021.131874>.
- [71] M. Li, X. Deng, W. Sun, L. Hu, H. Zhong, Z. He, D. Xiong, Extracellular polymeric substances of acidophilic microorganisms play a crucial role in heavy metal ions adsorption, *Int. J. Environ. Sci. Technol.* 19 (2022) 4857–4868, <https://doi.org/10.1007/s13762-021-03352-9>.
- [72] G.M. Gadd, A.J. Griffiths, Microorganisms and heavy metal toxicity, *Microb. Ecol.* 4 (1977) 303–317, <https://doi.org/10.1007/BF02013274>.
- [73] K. Yin, Q. Wang, M. Lv, L. Chen, Microorganism remediation strategies towards heavy metals, *Chem. Eng. J.* 360 (2019) 1553–1563, <https://doi.org/10.1016/j.cej.2018.10.226>.
- [74] V. Pande, S.C. Pandey, D. Sati, P. Bhatt, M. Samant, Microbial interventions in bioremediation of heavy metal contaminants in agroecosystem, *Front. Microbiol.* 13 (2022), <https://doi.org/10.3389/fmicb.2022.824084>.
- [75] A. Arishi, I. Mashhour, Microbial mechanisms for remediation of hexavalent chromium and their large-scale applications; current research and future directions, *J. Pure Appl. Microbiol.* 15 (2021) 53–67, <https://doi.org/10.22207/JPAM.15.1.32>.
- [76] I. Hwang, B. Batchelor, M.A. Schlautman, R. Wang, Effects of ferrous iron and molecular oxygen on chromium(VI) redox kinetics in the presence of aquifer solids, *J. Hazard Mater.* 92 (2002) 143–159, [https://doi.org/10.1016/S0304-3894\(02\)00006-7](https://doi.org/10.1016/S0304-3894(02)00006-7).
- [77] J.F. Gutiérrez-Corona, P. Romo-Rodríguez, F. Santos-Escobar, A.E. Espino-Saldana, H. Hernández-Escoto, Microbial interactions with chromium: basic biological processes and applications in environmental biotechnology, *World J. Microbiol. Biotechnol.* 32 (2016) 191, <https://doi.org/10.1007/s11274-016-2150-0>.
- [78] M. Shafique, A. Jawaid, Y. Rehman, As(V) reduction, as(III) oxidation, and Cr(VI) reduction by multi-metal-resistant *Bacillus subtilis*, *Bacillus safensis*, and *Bacillus cereus* species isolated from wastewater treatment plant, *Geomicrobiol. J.* 34 (2017) 687–694, <https://doi.org/10.1080/01490451.2016.1240265>.
- [79] P. Hu, E.L. Brodie, Y. Suzuki, H.H. McAdams, G.L. Andersen, Whole-genome transcriptional analysis of heavy metal stresses in *Caulobacter crescentus*, *J. Bacteriol.* 187 (2005) 8437–8449, <https://doi.org/10.1128/JB.187.24.8437-8449.2005>.
- [80] A. Lamb, G. Evans, J.R. King, Mathematical modelling of toxicity associated with intracellular chromium reduction, *Bull. Math. Biol.* 75 (2013) 1472–1500, <https://doi.org/10.1007/s11538-013-9856-z>.
- [81] J.G. Sandana Mala, D. Sujatha, C. Rose, Inducible chromate reductase exhibiting extracellular activity in *Bacillus methylotrophicus* for chromium bioremediation, *Microbiol. Res.* 170 (2015) 235–241, <https://doi.org/10.1016/j.MICRES.2014.06.001>.
- [82] X. Tang, Y. Huang, Y. Li, L. Wang, X. Pei, D. Zhou, P. He, S.S. Hughes, Study on detoxification and removal mechanisms of hexavalent chromium by microorganisms, *Ecotoxicol. Environ. Saf.* 208 (2021) 111699, <https://doi.org/10.1016/J.ECOENV.2020.111699>.
- [83] G. Hu, P. Zheng, H. Feng, G. Jia, Imbalance of oxidative and reductive species involved in chromium(VI)-induced toxic effects, *React. Oxygen Species* 3 (2017) 1–11.
- [84] F.-L. Sun, L.-L. Fan, Y.-S. Wang, L.-Y. Huang, Metagenomic analysis of the inhibitory effect of chromium on microbial communities and removal efficiency in A2O sludge, *J. Hazard Mater.* 368 (2019) 523–529, <https://doi.org/10.1016/j.jhazmat.2019.01.076>.
- [85] M. Bassi, A. Donini, Phalloidin-visualization of F-actin in normal and chromium-poisoned cells, *Cell Biol. Int. Rep.* 8 (1984) 867–871, [https://doi.org/10.1016/0309-1651\(84\)90070-5](https://doi.org/10.1016/0309-1651(84)90070-5).
- [86] Y.M. Kim, H. Park, K. Chandran, Nitrification inhibition by hexavalent chromium Cr(VI) – microbial ecology, gene expression and off-gas emissions, *Water Res.* 92 (2016) 254–261, <https://doi.org/10.1016/j.watres.2016.01.042>.
- [87] C. Viti, E. Marchi, F. Decorosi, L. Giovannetti, Molecular mechanisms of Cr(VI) resistance in bacteria and fungi, *FEMS Microbiol. Rev.* 38 (2014) 633–659, <https://doi.org/10.1111/1574-6976.12051>.
- [88] M. Pesti, Z. Gazdag, J. Belágyi, In vivo interaction of trivalent chromium with yeast plasma membrane, as revealed by EPR spectroscopy, *FEMS Microbiol. Lett.* 182 (2000) 375–380, <https://doi.org/10.1111/j.1574-6968.2000.tb08924.x>.
- [89] D.S. Ross, R.E. Sjogren, R.J. Bartlett, Behavior of chromium in soils: IV. Toxicity to microorganisms, *J. Environ. Qual.* 10 (1981) 145–148, <https://doi.org/10.2134/jeq1981.00472425001000020004x>.
- [90] J. Wyszowska, J. Kucharski, M. Kucharski, A. Borowik, Effect of cadmium, copper and zinc on plants, soil microorganisms and soil enzymes, *J. Elem.* (2014), <https://doi.org/10.5601/jelem.2013.18.4.455>.
- [91] C.I.Z. O'Hern, K.Y. Djoko, Copper cytotoxicity: cellular casualties of noncognate coordination chemistry, *mBio* 13 (2022), <https://doi.org/10.1128/mbio.00434-22>.
- [92] A.N. Pham, A.L. Rose, T.D. Waite, Kinetics of Cu(II) reduction by natural organic matter, *J. Phys. Chem. A* 116 (2012) 6590–6599, <https://doi.org/10.1021/jp300995h>.
- [93] R. Andrezza, B.C. Okeke, S. Pieniz, A. Brandelli, M.R. Lambais, F.A.O. Camargo, Bioreduction of Cu(II) by cell-free copper reductase from a copper resistant *Pseudomonas* sp, *NA, Biol. Trace Elem. Res.* 143 (2011) 1182–1192, <https://doi.org/10.1007/s12011-010-8899-3>.
- [94] C.A. Juan, J.M. Pérez de la Lastra, F.J. Plou, E. Pérez-Lebeña, The chemistry of reactive oxygen species (ROS) revisited: outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies, *Int. J. Mol. Sci.* 22 (2021) 4642, <https://doi.org/10.3390/ijms22094642>.
- [95] E.E. Farmer, M.J. Mueller, ROS-mediated lipid peroxidation and RES-activated signaling, *Annu. Rev. Plant Biol.* 64 (2013) 429–450, <https://doi.org/10.1146/annurev-arplant-050312-120132>.
- [96] E. Cabisco, J. Tamarit, J. Ros, Oxidative stress in bacteria and protein damage by reactive oxygen species, *Int. Microbiol.* 3 (2000) 3–8.
- [97] B. Halliwell, J.M. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford university press, USA, 2015.
- [98] L. Gaetke, Copper toxicity, oxidative stress, and antioxidant nutrients, *Toxicology* 189 (2003) 147–163, [https://doi.org/10.1016/S0300-483X\(03\)00159-8](https://doi.org/10.1016/S0300-483X(03)00159-8).
- [99] L. Macomber, R.P. Hausinger, Mechanisms of nickel toxicity in microorganisms, *Metallomics* 3 (2011) 1153, <https://doi.org/10.1039/c1mt00063b>.
- [100] Z. Cheng, Y.-Y.C. Wei, W.W. Sung, B.R. Glick, B.J. McConkey, Proteomic analysis of the response of the plant growth-promoting bacterium *Pseudomonas putida* UW4 to nickel stress, *Proteome Sci.* 7 (2009) 18, <https://doi.org/10.1186/1477-5956-7-18>.
- [101] A. Kushwaha, N. Hans, S. Kumar, R. Rani, A critical review on speciation, mobilization and toxicity of lead in soil-microbe-plant system and bioremediation strategies, *Ecotoxicol. Environ. Saf.* 147 (2018) 1035–1045, <https://doi.org/10.1016/J.ECOENV.2017.09.049>.
- [102] A. Mitra, S. Chatterjee, S. Katak, R.P. Rastogi, D.K. Gupta, Bacterial tolerance strategies against lead toxicity and their relevance in bioremediation application, *Environ. Sci. Pollut. Res.* 28 (2021) 14271–14284, <https://doi.org/10.1007/S11356-021-12583-9>.
- [103] A. Jaroslavecicka, Z. Piotrowska-Seget, Lead resistance in micro-organisms, *Microbiology* 160 (2014) 12–25, <https://doi.org/10.1099/mic.0.070284-0>.
- [104] P.L. Goering, Lead-protein interactions as a basis for lead toxicity, *Neurotoxicology* 14 (1993) 45–60.
- [105] J. Hou, Y. Wu, X. Li, B. Wei, S. Li, X. Wang, Toxic effects of different types of zinc oxide nanoparticles on algae, plants, invertebrates, vertebrates and microorganisms, *Chemosphere* 193 (2018) 852–860, <https://doi.org/10.1016/j.chemosphere.2017.11.077>.
- [106] A. Kumar, A.K. Pandey, S.S. Singh, R. Shanker, A. Dhawan, Engineered ZnO and TiO<sub>2</sub> nanoparticles induce oxidative stress and DNA damage leading to reduced viability of *Escherichia coli*, *Free Radic. Biol. Med.* 51 (2011) 1872–1881, <https://doi.org/10.1016/j.freeradbiomed.2011.08.025>.
- [107] M. Huang, L. Jiang, Y. Zou, S. Xu, G. Deng, Changes in soil microbial properties with no-tillage in Chinese cropping systems, *Biol. Fertil. Soils* 49 (2013) 373–377, <https://doi.org/10.1007/s00374-013-0778-6>.
- [108] D. Mónok, L. Kardos, S.A. Pabar, Z. Kotroczó, E. Tóth, G. Végvári, Comparison of soil properties in urban and non-urban grasslands in Budapest area, *Soil Use Manag.* 37 (2021) 790–801, <https://doi.org/10.1111/SUM.12632>.
- [109] Q. Zhong, C. Cruz-Paredes, S. Zhang, J. Rousk, Can heavy metal pollution induce bacterial resistance to heavy metals and antibiotics in soils from an ancient land-mine? *J. Hazard Mater.* 411 (2021) 124962, <https://doi.org/10.1016/j.jhazmat.2020.124962>.

- [110] R. Vázquez-Blanco, M. Arias-Estévez, E. Bååth, D. Fernández-Calviño, Comparing the effect of Cu-based fungicides and pure Cu salts on microbial biomass, microbial community structure and bacterial community tolerance to Cu, *J. Hazard Mater.* 409 (2021) 124960, <https://doi.org/10.1016/j.jhazmat.2020.124960>.
- [111] R. Vázquez-Blanco, M. Arias-Estévez, E. Bååth, D. Fernández-Calviño, Comparison of Cu salts and commercial Cu based fungicides on toxicity towards microorganisms in soil, *Environ. Pollut.* 257 (2020) 113585, <https://doi.org/10.1016/j.envpol.2019.113585>.
- [112] L. Xiao, Z. Yu, H. Liu, T. Tan, J. Yao, Y. Zhang, J. Wu, Effects of Cd and Pb on diversity of microbial community and enzyme activity in soil, *Ecotoxicology* 29 (2020) 551–558, <https://doi.org/10.1007/S10646-020-02205-4>.
- [113] J. Borgulat, W. Lukasiak, A. Borgulat, A. Nadgórska-Socha, M. Kandziara-Ciupa, Influence of lead on the activity of soil microorganisms in two Beskid landscape parks, *Environ. Monit. Assess.* 193 (2021) 839, <https://doi.org/10.1007/S10661-021-09503-2>.
- [114] O. Stajković-Srbinić, A. Buntić, N. Rasulić, D. Kuzmanović, Z. Dinić, D. Delić, V. Mrvić, Microorganisms in soils with elevated heavy metal concentrations in southern Serbia, *Arch. Biol. Sci.* 70 (2018) 707–716, <https://doi.org/10.2298/ABS180504034S>.
- [115] N.S. Elsayed, H. Obaid, D. Shi, P. Lei, D. Xie, J. Ni, O.K. Shalaby, C. Ni, Effect of zinc application on maize productivity and eukaryotic microorganism's diversity in a newly cultivated field, *J. Soil Sci. Plant Nutr.* 22 (2022) 3697–3707, <https://doi.org/10.1007/s42729-022-00920-x>.
- [116] A. Naylo, S.I. Almeida Pereira, L. Benidire, H. El Khalil, P.M.L. Castro, S. Ouvrard, C. Schwartz, A. Boularbah, Trace and major element contents, microbial communities, and enzymatic activities of urban soils of Marrakech city along an anthropization gradient, *J. Soils Sediments* 19 (2019) 2153–2165, <https://doi.org/10.1007/S11368-018-2221-Y>.
- [117] I.V. Zamulina, A.V. Gorovtsov, T.M. Minkina, S.S. Mandzhieva, T.V. Bauer, M. V. Burachevskaya, The influence of long-term Zn and Cu contamination in Spolic Technosols on water-soluble organic matter and soil biological activity, *Ecotoxicol. Environ. Saf.* 208 (2021) 111471, <https://doi.org/10.1016/J.ECOENV.2020.111471>.
- [118] T. Wang, Z. Yuan, J. Yao, A combined approach to evaluate activity and structure of soil microbial community in long-term heavy metals contaminated soils, *Environ. Eng. Res.* 23 (2018) 62–69, <https://doi.org/10.4491/EER.2017.063>.
- [119] M.D. Wallenstein, M.N. Weintraub, Emerging tools for measuring and modeling the in situ activity of soil extracellular enzymes, *Soil Biol. Biochem.* 40 (2008) 2098–2106, <https://doi.org/10.1016/j.soilbio.2008.01.024>.
- [120] H. Aponte, P. Meli, B. Butler, J. Paolini, F. Matus, C. Merino, P. Cornejo, Y. Kuzuyakov, Meta-analysis of heavy metal effects on soil enzyme activities, *Sci. Total Environ.* 737 (2020), <https://doi.org/10.1016/j.scitotenv.2020.139744>.
- [121] P. Nannipieri, C. Trasar-Cepeda, R.P. Dick, Soil enzyme activity: a brief history and biochemistry as a basis for appropriate interpretations and meta-analysis, *Biol. Fertil. Soils* 54 (2018) 11–19, <https://doi.org/10.1007/s00374-017-1245-6>.
- [122] M. Navas, J. Pérez-Esteban, M.A. Torres, C. Hontoria, A. Moliner, Taxonomic and functional analysis of soil microbial communities in a mining site across a metal (loid) contamination gradient, *Eur. J. Soil Sci.* 72 (2021) 1190–1205, <https://doi.org/10.1111/EJSS.12979>.
- [123] J.L.A. Shaw, J.G. Ernakovich, J.D. Judy, M. Farrell, M. Whatmuff, J. Kirby, Long-term effects of copper exposure to agricultural soil function and microbial community structure at a controlled and experimental field site, *Environ. Pollut.* 263 (2020) 114411, <https://doi.org/10.1016/J.ENVPOL.2020.114411>.
- [124] M.H. Hemmat-Jou, A.A. Safari-Sinegani, A. Mirzaei-Asl, A. Tahmourespour, Analysis of microbial communities in heavy metals-contaminated soils using the metagenomic approach, *Ecotoxicology* 27 (2018) 1281–1291, <https://doi.org/10.1007/s10646-018-1981-x>.
- [125] Z. Liu, Y. Yang, S. Ji, D. Dong, Y. Li, M. Wang, L. Han, X. Chen, Effects of elevation and distance from highway on the abundance and community structure of bacteria in soil along qinghai-tibet highway, *Int. J. Environ. Res. Publ. Health* 18 (2021) 13137, <https://doi.org/10.3390/IJERPH182413137>.
- [126] X. Tang, Y. Huang, Y. Li, Y. Yang, X. Cheng, G. Jiao, H. Dai, The response of bacterial communities to V and Cr and novel reducing bacteria near a vanadium-titanium magnetite refinery, *Sci. Total Environ.* 806 (2022) 151214, <https://doi.org/10.1016/J.SCITOTENV.2021.151214>.
- [127] Y. Cao, C. Ma, H. Chen, G. Chen, J.C. White, B. Xing, Copper stress in flooded soil: impact on enzyme activities, microbial community composition and diversity in the rhizosphere of *Salix integra*, *Sci. Total Environ.* 704 (2020) 135350, <https://doi.org/10.1016/J.SCITOTENV.2019.135350>.
- [128] H. Wang, Y. Zeng, C. Guo, Y. Bao, G. Lu, J.R. Reinfelder, Z. Dang, Bacterial, archaeal, and fungal community responses to acid mine drainage-laden pollution in a rice paddy soil ecosystem, *Sci. Total Environ.* 616–617 (2018) 107–116, <https://doi.org/10.1016/j.scitotenv.2017.10.224>.
- [129] E. Bååth, Effects of heavy metals in soil on microbial processes and populations (a review), *Water Air Soil Pollut.* 47 (1989) 335–379, <https://doi.org/10.1007/BF00279331>.
- [130] R.M.C.P. Rajapaksha, M.A. Tobor-Kapion, E. Bååth, Metal toxicity affects fungal and bacterial activities in soil differently, *Appl. Environ. Microbiol.* 70 (2004) 2966–2973, <https://doi.org/10.1128/AEM.70.5.2966-2973.2004>.
- [131] X. Pan, S. Zhang, Q. Zhong, G. Gong, G. Wang, X. Guo, X. Xu, Effects of soil chemical properties and fractions of Pb, Cd, and Zn on bacterial and fungal communities, *Sci. Total Environ.* 715 (2020) 136904, <https://doi.org/10.1016/J.SCITOTENV.2020.136904>.
- [132] X.Y. Zeng, S.W. Li, Y. Leng, X.H. Kang, Structural and functional responses of bacterial and fungal communities to multiple heavy metal exposure in arid loess, *Sci. Total Environ.* 723 (2020) 138081, <https://doi.org/10.1016/J.SCITOTENV.2020.138081>.
- [133] X. Zhang, X. Gai, Z. Zhong, F. Bian, C. Yang, Y. Li, X. Wen, Understanding variations in soil properties and microbial communities in bamboo plantation soils along a chromium pollution gradient, *Ecotoxicol. Environ. Saf.* 222 (2021) 112507, <https://doi.org/10.1016/J.ECOENV.2021.112507>.
- [134] Y. Geng, C. Peng, Z. Wang, S. Huang, P. Zhou, D. Li, Insights into the spatiotemporal differences in tailings seepage pollution by assessing the diversity and metabolic functions of the soil microbial community, *Environ. Pollut.* 306 (2022) 119408, <https://doi.org/10.1016/J.ENVPOL.2022.119408>.
- [135] T.P. Gao, Z.D. Wan, X.X. Liu, J.W. Fu, G.H. Chang, H.L. Sun, H.J. Li, Y.Y. Shen, Y. B. Liu, X.W. Fang, Effects of heavy metals on bacterial community structure in the rhizosphere of *Salsola collina* and bulk soil in the Jinchuan mining area, *Geomicrobiol. J.* 38 (2021) 620–630, <https://doi.org/10.1080/01490451.2021.1914784>.
- [136] S. Yuebing, Z. Shunan, W. Lin, L. Xuefeng, X. Yingming, Changes of enzymatic activities, substrate utilization pattern, and microbial community diversity in heavy metal-contaminated soils, *Water Air Soil Pollut.* 231 (2020) 422, <https://doi.org/10.1007/S11270-020-04798-2>.
- [137] É.R. Batista, J.J. Carneiro, F. Araújo Pinto, J.V. dos Santos, M.A.C. Carneiro, Environmental drivers of shifts on microbial traits in sites disturbed by a large-scale tailing dam collapse, *Sci. Total Environ.* 738 (2020) 139453, <https://doi.org/10.1016/J.SCITOTENV.2020.139453>.
- [138] Z. Luo, J. Ma, F. Chen, X. Li, S. Zhang, Effects of Pb smelting on the soil bacterial community near a secondary lead plant, *Int. J. Environ. Res. Publ. Health* 15 (2018) 1030, <https://doi.org/10.3390/IJERPH15051030>.
- [139] J.P. Singh, B.P. Vaidya, N.M. Goodey, J.A. Krumin, Soil microbial response to metal contamination in a vegetated and urban brownfield, *J. Environ. Manag.* 244 (2019) 313–319, <https://doi.org/10.1016/J.JENVMAN.2019.04.111>.
- [140] J. Yang, S. Wang, Z. Guo, Y. Deng, M. Xu, S. Zhang, H. Yin, Y. Liang, H. Liu, B. Miao, D. Meng, X. Liu, L. Jiang, Spatial distribution of toxic metal(Loids) and microbial community analysis in soil vertical profile at an abandoned nonferrous metal smelting site, *Int. J. Environ. Res. Publ. Health* 17 (2020) 1–17, <https://doi.org/10.3390/IJERPH17197101>.
- [141] C. Yuan, F. Li, Z. Yuan, G. Li, X. Liang, Response of bacterial communities to mining activity in the alpine area of the Tianshan Mountain region, China, *Environ. Sci. Pollut. Res.* 28 (2021) 15806–15818, <https://doi.org/10.1007/S11356-020-11744-6>.
- [142] T. Gao, H. Li, Y. He, Y. Shen, G. Li, X. Li, Y. Chen, Y. Liu, C. Li, J. Ji, J. Xu, G. Chang, The variations of bacterial community structures in tailing soils suffering from heavy metal contaminations, *Water Air Soil Pollut.* 232 (2021) 392, <https://doi.org/10.1007/S11270-021-05338-2>.
- [143] Y. He, Y. Xu, Y. Lv, L. Nie, H. Wang, Soil bacterial community structure in turfey swamp and its response to highway disturbance, *Int. J. Environ. Res. Publ. Health* 17 (2020) 1–21, <https://doi.org/10.3390/IJERPH17217822>.
- [144] M. Hur, S.J. Park, Identification of microbial profiles in heavy-metal-contaminated soil from full-length 16S rRNA reads sequenced by a pacbio system, *Microorganisms* 7 (2019) 357, <https://doi.org/10.3390/MICROORGANISMS7090357>.
- [145] B. Jiang, A. Adebayo, J. Jia, Y. Xing, S. Deng, L. Guo, Y. Liang, D. Zhang, Impacts of heavy metals and soil properties at a Nigerian e-waste site on soil microbial community, *J. Hazard Mater.* 362 (2019) 187–195, <https://doi.org/10.1016/J.JHAZMAT.2018.08.060>.
- [146] S. Li, J. Wu, Y. Huo, X. Zhao, L. Xue, Profiling multiple heavy metal contamination and bacterial communities surrounding an iron tailing pond in Northwest China, *Sci. Total Environ.* 752 (2021) 141827, <https://doi.org/10.1016/J.SCITOTENV.2020.141827>.
- [147] B. Liu, G. Su, Y. Yang, Y. Yao, Y. Huang, L. Hu, H. Zhong, Z. He, Vertical distribution of microbial communities in chromium-contaminated soil and isolation of Cr(VI)-Reducing strains, *Ecotoxicol. Environ. Saf.* 180 (2019) 242–251, <https://doi.org/10.1016/j.ecoenv.2019.05.023>.
- [148] J. Rousk, P.C. Brookes, E. Bååth, Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization, *Appl. Environ. Microbiol.* 75 (2009) 1589–1596, <https://doi.org/10.1128/AEM.02775-08>.
- [149] J. Schnürer, M. Clarholm, T. Rosswall, Microbial biomass and activity in an agricultural soil with different organic matter contents, *Soil Biol. Biochem.* 17 (1985) 611–618, [https://doi.org/10.1016/0038-0717\(85\)90036-7](https://doi.org/10.1016/0038-0717(85)90036-7).
- [150] D. Fernández-Calviño, E. Bååth, Interaction between pH and Cu toxicity on fungal and bacterial performance in soil, *Soil Biol. Biochem.* 96 (2016) 20–29, <https://doi.org/10.1016/j.soilbio.2016.01.010>.
- [151] H.B. Bradl, Adsorption of heavy metal ions on soils and soils constituents, *J. Colloid Interface Sci.* 277 (2004) 1–18, <https://doi.org/10.1016/j.jcis.2004.04.005>.
- [152] X.T. Bai, J. Wang, H. Dong, J.M. Chen, Y. Ge, Relative importance of soil properties and heavy metals/metalloids to modulate microbial community and activity at a smelting site, *J. Soils Sediments* 21 (2021) 1–12, <https://doi.org/10.1007/S11368-020-02743-8>.
- [153] R. Nikolova, M. Petkova, N. Dinev, A. Kenarova, S. Boteva, D. Berov, G. Radeva, Correlation between bacterial abundance, soil properties and heavy metal contamination in the area of non-ferrous metal processing plant, Southern Bulgaria, *BioRisk* 2022, <https://doi.org/10.3897/BIORISK.17.77458>, 2022.
- [154] C. Yáñez, J. Verdejo, H. Moya, P. Donoso, C. Rojas, E.A. Dovletyarova, O. A. Shapoval, Y.A. Krutyakov, A. Neaman, Microbial responses are unreliable indicators of copper ecotoxicity in soils contaminated by mining activities,

- Chemosphere 300 (2022) 134517, <https://doi.org/10.1016/J.CHEMOSPHERE.2022.134517>.
- [155] D.C. Adriano, *Trace Elements in Terrestrial Environments*, second ed., Springer, New York, 2001.
- [156] F.M.G. Tack, *Trace elements: general soil chemistry, principles and processes*, in: P.S. Hooda (Ed.), *Trace Elem. Soils*, Wiley, Chippenhham, Wiltshire, 2010, pp. 9–38.
- [157] K.J. Powell, P.L. Brown, R.H. Byrne, T. Gajda, G. Hefter, S. Sjöberg, H. Wanner, Chemical speciation of environmentally significant metals with inorganic ligands Part 2: the Cu<sup>2+</sup>-OH-, Cl-, CO<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, and PO<sub>4</sub><sup>3-</sup> systems (IUPAC Technical Report), *Pure Appl. Chem.* 79 (2007) 895–950, <https://doi.org/10.1351/pac200779050895>.
- [158] S. Dubey, Y.C. Sharma, Optimization of reclamation of Ni(II)-rich solutions by  $\gamma$ -alumina nanoparticles, *J. Hazardous, Toxic, Radioact. Waste* 23 (2019) 04019005, [https://doi.org/10.1061/\(ASCE\)HZ.2153](https://doi.org/10.1061/(ASCE)HZ.2153).
- [159] K.J. Powell, P.L. Brown, R.H. Byrne, T. Gajda, G. Hefter, A.-K. Leuz, S. Sjöberg, H. Wanner, Chemical speciation of environmentally significant metals with inorganic ligands. Part 3: the Pb<sup>2+</sup> + OH-, Cl-, CO<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, and PO<sub>4</sub><sup>3-</sup> systems (IUPAC Technical Report), *Pure Appl. Chem.* 81 (2009) 2425–2476, <https://doi.org/10.1351/PAC-REP-09-03-05>.
- [160] K.J. Powell, P.L. Brown, R.H. Byrne, T. Gajda, G. Hefter, A.-K. Leuz, S. Sjöberg, H. Wanner, Chemical speciation of environmentally significant metals with inorganic ligands, Part 5: The Zn<sup>2+</sup> + OH-, Cl-, CO<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, and PO<sub>4</sub><sup>3-</sup> systems (IUPAC Technical Report), *Pure Appl. Chem.* 85 (2013) 2249–2311, <https://doi.org/10.1351/pac-rep-13-06-03>.
- [161] F. Macías-Vázquez, R. Calvo de Anta, *Niveles Genéricos de Referencia de metales pesados y otros elementos traza en suelos de Galicia. Consellería de Medio Ambiente e Desenvolvemento Sostible*, Santiago de Compostela, Spain, 2009.
- [162] V. Maronezi, M.M.A. dos Santos, D.B. Faria, M.I.G. Rosa, M.C. Shinzato, Mecanismos de remoção de Cromo(VI) do solo pela interação entre matéria orgânica e Ferro(III), *Rev. Do Inst. Geológico* 40 (2019) 17–33, <https://doi.org/10.33958/revig.v40i2.645>.
- [163] X. Hu, J. Wang, Y. Lv, X. Liu, J. Zhong, X. Cui, M. Zhang, D. Ma, X. Yan, X. Zhu, Effects of heavy metals/metalloids and soil properties on microbial communities in farmland in the vicinity of a metals smelter, *Front. Microbiol.* 12 (2021) 707786, <https://doi.org/10.3389/fmicb.2021.707786>.
- [164] S.-C. Tseng, C.M. Liang, T. Chia, S.S. Ton, Changes in the composition of the soil bacterial community in heavy metal-contaminated farmland, *Int. J. Environ. Res. Publ. Health* 18 (2021) 8661, <https://doi.org/10.3390/IJERPH18168661>.
- [165] J. Rousk, K. Ackermann, S.F. Curling, D.L. Jones, Comparative toxicity of nanoparticulate CuO and ZnO to soil bacterial communities, *PLoS One* 7 (2012) e34197, <https://doi.org/10.1371/JOURNAL.PONE.0034197>.
- [166] X. Zhang, X. Zhang, L. Li, G. Fu, X. Liu, S. Xing, H. Feng, B. Chen, The toxicity of hexavalent chromium to soil microbial processes concerning soil properties and aging time, *Environ. Res.* 204 (2022), <https://doi.org/10.1016/j.envres.2021.111941>.
- [167] V. Antoniadis, E. Levizou, S.M. Shaheen, Y.S. Ok, A. Sebastian, C. Baum, M.N. V. Prasad, W.W. Wenzel, J. Rinklebe, Trace elements in the soil-plant interface: phytoavailability, translocation, and phytoremediation—A review, *Earth Sci. Rev.* 171 (2017) 621–645, <https://doi.org/10.1016/j.earscirev.2017.06.005>.
- [168] M.A. Khan, S. Khan, A. Khan, M. Alam, Soil contamination with cadmium, consequences and remediation using organic amendments, *Sci. Total Environ.* 601–602 (2017) 1591–1605, <https://doi.org/10.1016/J.SCITOTENV.2017.06.030>.
- [169] J. Lehmann, M. Kleber, The contentious nature of soil organic matter, *Nature* 528 (2015) 60–68, <https://doi.org/10.1038/nature16069>.
- [170] L. Guo, J. Nkoh Nkoh, R. Xu, A critical review of the interactions of organic carbon components with soil minerals: insight from bibliometric analysis of the environmental behaviors of heavy metal(loids), *J. Soils Sediments* 23 (2023) 2396–2416, <https://doi.org/10.1007/s11368-023-03502-1>.
- [171] J.L. Mortensen, Complexing of metals by soil organic matter, *Soil Sci. Soc. Am. J.* 27 (1963) 179–186, <https://doi.org/10.2136/sssaj1963.03615995002700020027x>.
- [172] J. Porta, M. López-Acevedo, C. Roquero, *Edafología para la agricultura y el medio ambiente*, 2003, 3rd Edito, Ediciones Mundi-Prensa, Madrid (Spain).
- [173] H.M. Selim, Transport and retention of heavy metal in soils, in: D.L. Sparks (Ed.), *Adv. Agron.*, Elsevier, 2013, pp. 275–308, <https://doi.org/10.1016/B978-0-12-407247-3.00005-6>.
- [174] J. Duan, J. Gregory, Coagulation by hydrolysing metal salts, *Adv. Colloid Interface Sci.* 100–102 (2003) 475–502, [https://doi.org/10.1016/S0001-8686\(02\)00067-2](https://doi.org/10.1016/S0001-8686(02)00067-2).
- [175] D.K. Adekeye, O.K. Popoola, S.S. Asaolu, A.A. Adebawore, O.I. Aremu, K. O. Olabode, Adsorption and conventional technologies for environmental remediation and decontamination of heavy metals: an overview, *J. Res. Rev.* 6 (2019) 505–516.
- [176] L. Weng, E.J.M. Temminghoff, S. Lofts, E. Tipping, W.H. Van Riemsdijk, Complexation with dissolved organic matter and solubility control of heavy metals in a sandy soil, *Environ. Sci. Technol.* 36 (2002) 4804–4810, <https://doi.org/10.1021/es0200084>.
- [177] O.K. Borggaard, P.E. Holm, B.W. Strobel, Potential of dissolved organic matter (DOM) to extract As, Cd, Co, Cr, Cu, Ni, Pb and Zn from polluted soils: a review, *Geoderma* 343 (2019) 235–246, <https://doi.org/10.1016/j.geoderma.2019.02.041>.
- [178] R.-Y. Kim, J.-K. Yoon, T.-S. Kim, J.E. Yang, G. Owens, K.-R. Kim, Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review, *Environ. Geochem. Health* 37 (2015) 1041–1061, <https://doi.org/10.1007/s10653-015-9695-y>.
- [179] C.-C. Chiu, C.-J. Cheng, T.-H. Lin, K.-W. Juang, D.-Y. Lee, The effectiveness of four organic matter amendments for decreasing resin-extractable Cr(VI) in Cr(VI)-contaminated soils, *J. Hazard Mater.* 161 (2009) 1239–1244, <https://doi.org/10.1016/j.jhazmat.2008.04.081>.
- [180] A. Kunhikrishnan, G. Choppala, B. Seshadri, H. Wijesekara, N.S. Bolan, K. Mbene, W.-I. Kim, Impact of wastewater derived dissolved organic carbon on reduction, mobility, and bioavailability of As(V) and Cr(VI) in contaminated soils, *J. Environ. Manag.* 186 (2017) 183–191, <https://doi.org/10.1016/j.jenvman.2016.08.020>.
- [181] A.M. Stefanowicz, P. Kapusta, S. Zubeck, M. Stanek, M.W. Woch, Soil organic matter prevails over heavy metal pollution and vegetation as a factor shaping soil microbial communities at historical Zn–Pb mining sites, *Chemosphere* 240 (2020) 124922, <https://doi.org/10.1016/j.chemosphere.2019.124922>.
- [182] Y. Xian, M. Wang, W. Chen, Quantitative assessment on soil enzyme activities of heavy metal contaminated soils with various soil properties, *Chemosphere* 139 (2015) 604–608, <https://doi.org/10.1016/J.CHEMOSPHERE.2014.12.060>.
- [183] H. Guo, M. Nasir, J. Lv, Y. Dai, J. Gao, Understanding the variation of microbial community in heavy metals contaminated soil using high throughput sequencing, *Ecotoxicol. Environ. Saf.* 144 (2017) 300–306, <https://doi.org/10.1016/J.ECOENV.2017.06.048>.
- [184] B. Huang, Z. Yuan, D. Li, M. Zheng, X. Nie, Y. Liao, Effects of soil particle size on the adsorption, distribution, and migration behaviors of heavy metal(loids) in soil: a review, *Environ. Sci. Process. Impacts* 22 (2020) 1596–1615, <https://doi.org/10.1039/D0EM00189A>.
- [185] J. Chen, F. He, X. Zhang, X. Sun, J. Zheng, J. Zheng, Heavy metal pollution decreases microbial abundance, diversity and activity within particle-size fractions of a paddy soil, *FEMS Microbiol. Ecol.* 87 (2014) 164–181, <https://doi.org/10.1111/1574-6941.12212>.
- [186] H.J. Harvey, A.M.T. Mitzakoff, R.D. Wildman, S.J. Mooney, S.V. Avery, Microbial metal resistance within structured environments is inversely related to environmental pore size, *Appl. Environ. Microbiol.* 87 (2021) 1–11, <https://doi.org/10.1128/AEM.01005-21>.
- [187] H. Blanck, A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities, *Hum. Ecol. Risk Assess.* 8 (2002) 1003–1034, <https://doi.org/10.1080/1080-700291905792>.
- [188] G. Imfeld, F. Bringel, S. Vuilleumier, Bacterial tolerance in contaminated soils: potential of the PICT approach in microbial ecology, in: *Tolet. To Environ. Contam.*, 2011, pp. 335–364. <https://www.scopus.com/inward/record.uri?eid=2-s2.0-84858156505&partnerID=40&md5=cfec5958230bbe7f48bbd599f95939f9>.
- [189] A. Tili, A. Berard, H. Blanck, A. Bouchez, F. Cássio, K.M. Eriksson, S. Morin, B. Montuelle, E. Navarro, C. Pascoal, S. Pesce, M. Schmitt-Jansen, R. Behra, Pollution-induced community tolerance (PICT): towards an ecologically relevant risk assessment of chemicals in aquatic systems, *Freshw. Biol.* 61 (2016) 2141–2151, <https://doi.org/10.1111/fwb.12558>.
- [190] J.D.S. Lefkeldt, J. Magid, P.E. Holm, O. Nybroe, K.K. Brandt, Evaluation of the leucine incorporation technique for detection of pollution-induced community tolerance to copper in a long-term agricultural field trial with urban waste fertilizers, *Environ. Pollut.* 194 (2014) 78–85, <https://doi.org/10.1016/j.envpol.2014.07.013>.
- [191] C. Campillo-Cora, D. Soto-Gómez, M. Arias-Estévez, E. Bååth, D. Fernández-Calviño, Estimation of baseline levels of bacterial community tolerance to Cr, Ni, Pb, and Zn in unpolluted soils, a background for PICT (pollution-induced community tolerance) determination, *Biol. Fertil. Soils* 58 (2022) 49–61, <https://doi.org/10.1007/s00374-021-01604-x>.
- [192] C. Campillo-Cora, D. Soto-Gómez, M. Arias-Estévez, E. Bååth, D. Fernández-Calviño, Bacterial community tolerance to Cu in soils with geochemical baseline concentrations (GBCs) of heavy metals: importance for pollution induced community tolerance (PICT) determinations using the leucine incorporation method, *Soil Biol. Biochem.* 155 (2021) 108157, <https://doi.org/10.1016/j.soilbio.2021.108157>.
- [193] C. Campillo-Cora, R. González-Feijóo, M. Arias-Estévez, D. Fernández-Calviño, Dissolved organic matter as a confounding factor in the determination of pollution-induced community tolerance (PICT) of bacterial communities to heavy metals using the leucine incorporation method, *Geoderma* 430 (2023) 116335, <https://doi.org/10.1016/J.GEODERMA.2023.116335>.
- [194] R.M. Burgess, K.T. Ho, W. Brack, M. Lamoree, Effects-directed analysis (EDA) and toxicity identification evaluation (TIE): complementary but different approaches for diagnosing causes of environmental toxicity, *Environ. Toxicol. Chem.* 32 (2013) 1935–1945, <https://doi.org/10.1002/etc.2299>.
- [195] S. Dailianis, N. Charalampous, S. Giokas, D. Vlastos, I. Efthimiou, M. Dormousoglou, C. Cocilovo, C. Faggio, A. Shehu, J. Shehu, G. Lyberatos, I. Ntaikou, Chemical and biological tracking in decentralized sanitation systems: the case of artificial constructed wetlands, *J. Environ. Manag.* 300 (2021) 113799, <https://doi.org/10.1016/j.jenvman.2021.113799>.
- [196] Q. Feng, L. Yang, J. Chen, F. Li, F. Fang, J. Guo, Z. Chen, Y. Shao, Identification of the estrogen-active compounds via integrating effect-directed analysis and non-target screening in soils of the northeastern China, *Environ. Sci. Eur.* 36 (2024) 58, <https://doi.org/10.1186/s12302-024-00885-x>.