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The unpredictable nature of microbiological responses to metals in real-world contaminated soils: A review

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Microbial responses, such as biomass or enzymatic activity, are commonly used to evaluate metal toxicity in contaminated soils. However, multiple studies have demonstrated the existence of microbial tolerance and resilience to metals. The adaptive responses of soil microorganisms to metal stress may compromise their suitability for evaluating metal toxicity in contaminated soils. Further evaluation is needed to establish the robustness of microbiological responses as metal toxicity indicators in contaminated soils. In this review, we focus on real-world contaminated soils, excluding artificially contaminated soils. We reviewed studies that reported the values of effective concentrations at 10% and 50% (EC_{10} and EC_{50}) of soil metals (either total, extractable, or soluble concentrations) for soil microbiological response in real-world contaminated soils. However, there are also studies demonstrating that the effects of soil metals on microbiological responses range from toxic (negative) in soils with metal concentrations below the mean reported EC_{10} values to stimulatory (positive) in soils with metal concentrations above the mean reported EC_{50} values. Hence, in some cases, microorganisms' responses indicate metal toxicity at low soil metal concentrations, at which toxicity is not expected. In contrast, in other cases, microorganisms are stimulated by metals at high soil metal concentrations, at which stimulatory responses are not expected. Further, soil microbiological responses can be influenced by soil physicochemical properties rather than soil metals concentrations even at metal concentrations above the mean reported EC_{50} values, at which metal toxicity for soil microorganisms is expected. In summary, the unpredictable nature of microbiological responses to metals makes them unreliable indicators of metal toxicity in real-world contaminated soils.

KEYWORDS

bacteria, ecotoxicology, environmental assessment, fungi, soil quality

Introduction

Soil contamination with metals[1] constitutes a significant global environmental challenge (González Henao and Ghneim-Herrera, 2021; Karnwal et al., 2024). The ecotoxicity of metals in soils can be assessed by a two-fold approach (ISO 17402, 2008): (1) quantifying the total metal concentration and/or an operationally defined fraction of the total metal concentration in the soil, and (2) exposing organisms to the soil and quantifying biological responses. If the obtained metal concentrations and biological response(s) are negatively correlated, threshold values for soil metal ecotoxicity may be derived.

Many ecotoxicological studies have been performed on uncontaminated soils that have been spiked artificially with soluble metal salts (e.g., Halim et al., 2021; McKee et al., 2017; Tibihenda et al., 2022; You et al., 2024). Spiked soils consistently result in increased toxicity for plants and soil organisms relative to the toxicities observed in real-world soils that have been contaminated for decades (Neaman et al., 2020; Oorts et al., 2006; Smolders et al., 2003). The prolonged residence time and associated partitioning of metals in soils, known as "aging" (Martínez and McBride, 2001), significantly impacts metal bioavailability and toxicity (Islam, 2025) in such a way that cannot be replicated in artificially contaminated soils. Rather, metal spiking tends to selectively eradicate specific microbial species over periods of months to years, leading to an artificial loss of metabolic functioning and biogeochemical processing associated with these species (Giller et al., 1999).

High metal content in soils can disrupt soil ecosystems, prompting researchers to identify the most sensitive species for detecting early signs of toxicity (Broos et al., 2005). However, there is a lack of studies on real-world contaminated soils that compare the sensitivity of different types of organisms as bioindicators of metal toxicity. The study of Naveed et al. (2014) on soil contaminated by wood treatment with copper-based fungicides demonstrated that earthworms exhibit greater sensitivity to copper than do bacteria, nematodes, and fungi. Likewise, the study of Dovletyarova et al. (2024) demonstrated that plants exhibit greater sensitivity to metals than microorganisms do.

The results of the two latter studies can be explained by the fact that the available ecotoxicological data on metal toxicity for plants and invertebrates typically correspond to the individual level of biological organization, whereas microorganism responses are usually assessed at the community level (Santa-Cruz et al., 2021). Resistance to metal-induced stress indeed varies with the level of biological organization (Spurgeon et al., 2005). Typically, populations and communities are less sensitive to stress than are individuals. Accordingly, individual-level responses of plants and invertebrates are expected to be more sensitive to metal toxicity than are the community-level responses of microorganisms.

Moreover, multiple studies have demonstrated the existence of microbial tolerance and resilience to metals, i.e., development of resistant microbial populations and adaptive mechanisms that enable survival in metal-contaminated soils (reviews of Das et al., 2022; Mallick et al., 2015; Naik et al., 2018; Narayani and Shetty, 2013). This well-established adaptive response of soil microorganisms to metal stress may compromise their suitability for evaluating metal toxicity. However, does this inference imply that microbial indicators lack sensitivity across all contamination scenarios, or are they selectively applicable to specific metals or environmental conditions?

Further investigation is warranted to establish the robustness of microbiological responses as metal toxicity indicators across a range of real-world contamination scenarios. Thus, the purpose of this review is to evaluate the validity of microbiological responses as indicators of metal ecotoxicity in real-world contaminated soils.

1 To simplify the discussion in this article, the term "metals" also includes metalloids (e.g., arsenic). The term "heavy metal" is not recommended by the International Union of Pure and Applied Chemistry and will be avoided.

TABLE 1 Mean ± standard deviation of effective concentrations at 10% and 50% (EC_{10} and EC_{50}) for soil microorganisms across various metal pools in the reviewed studies. Detailed data are available in the Supplementary material.

Metal pool	Metal	EC_{10}	EC_{50}
Total (mg kg^{-1})	Arsenic	75 ± 67	630 ± 580
	Cadmium	—	5100 ± 3444
	Copper	551 ± 335	1115 ± 1050
	Lead	—	48,650 ± 30,901
	Nickel	—	325 ± 106
	Zinc	—	1151 ± 1476
Extractable (mg kg^{-1})	Copper	—	304 ± 331
Soluble (mg kg^{-1})	Copper	0.27	0.62
	Zinc	—	7.8 ± 9.7

Materials and Methods

Our first criterion for the selection of studies was to include only real-world contaminated soils, excluding artificially contaminated soils. However, considering that sewage sludge application to agricultural fields is a widespread practice worldwide, we also included studies on soils on which sewage sludge was applied more than 7 years prior to the study. For simplicity, these sewage sludge studies will also be referred to as "real-world contaminated soils" since the period of 7 years appears to be sufficient for metal aging in soils, based on the data reported in several long-term aging studies (Ma et al., 2006; McBride and Cai, 2016; Zeng et al., 2017).

Total metal concentration in contaminated soils may not be a consistent factor in predicting potential toxicity for plants and soil biota (ISO 17402, 2008). As a result, a wide-ranging set of approaches has been developed to estimate the "bioavailable" metal fraction in soil by linking operationally defined metal pools to specific biological responses (Kim et al., 2015). The operationally defined bioavailable metal pools are targeted by extractions with water, salt solutions, chelating agents (e.g., DTPA or EDTA), or acids (e.g., HCl or CH_3COOH) (Supplementary Table 1). In the following discussion, we will refer to these bioavailable fractions as

TABLE 2 Summary of the number of studies on soil microbial indicators of metal stress—including microbial activity, microbial diversity and community structure, and plant–microbe interactions (Benedetti and Dilly, 2006), as well as soil enzyme activity as a proxy of microbial activity (Shaw and Burns, 2006). The specific authors corresponding to each study counted in the table are listed in Supplementary Tables 20–24.

Soil microbial indicator	Affected by metals		Affected by soil properties but not by metals	Inconsistent or no response, either to metals or soil properties
	Increased with increasing metal concentrations	Decreased with increasing metal concentrations		
Soil microbial number and biomass				
Total microorganism count (viable and non-viable)	0	3	1	0
Culturable microorganism count	1	5	3	2
Chloroform fumigation extraction of microbial biomass carbon	3	14	0	0
Chloroform fumigation extraction of microbial biomass nitrogen	2	3	0	0
Substrate-induced respiration	2	8	0	0
Soil microbial activity				
Basal respiration (organic matter mineralization)	4	16	2	6
Nitrogen mineralization	0	1	2	1
Nitrification	2	1	1	0
Ammonification	0	0	1	1
Soil microbial diversity and community structure				
Community level physiological profiles (Biolog or MicroResp)	3	8	4	6
Plant-microbe interaction				
Nodulating symbiotic bacteria	0	1	0	2
Free-living plant-beneficial microorganisms	0	2	0	1
Soil enzymes				
Diverse enzymes	15	47	7	5

"soluble" when the researchers used salt solutions or water, or as "extractable" when chelating agents or acids were used.

We reviewed studies that reported the values of effective concentrations at 10% and 50% (EC_{10} and EC_{50}) of soil metals (either total concentrations or bioavailable concentrations) for soil microbiological response in real-world contaminated soils (Supplementary Tables 2–9). We were able to identify studies reporting toxicity thresholds using microbiological responses in real-world contaminated soils for the following elements: arsenic, cadmium, copper, lead, nickel, and zinc, whereas data for other metals were absent. Thus, we decided to focus our review on these six elements.

A single effective concentration value for a specific biological endpoint (e.g., growth, reproduction, survival, etc.) is insufficient for decision making in agricultural or ecological contexts (ISO 17616, 2019). As an alternative, Checkai et al. (2014) recommend averaging effective concentration values across different biological endpoints. Adopting this approach, we summarized available metal toxicity thresholds for soil microorganisms (Table 1). Few studies have established toxicity thresholds of soluble or extractable soil metal fractions for microorganisms using real-world contaminated soils. We were able to identify studies only for copper (Supplementary Table 8) and zinc (Supplementary Table 9), whereas data for other metals were absent. Supplementary Tables 10–15 summarize studies reporting correlations between various metal pools in soil and microbiological responses.

Our second criterion for the selection of studies was to include studies in which metal concentrations (either total concentrations or bioavailable concentrations) were above the effective concentration at 50% (EC_{50}) for microorganisms (Table 1) for at least one metal. Supplementary Tables 16–19 present metal concentrations reported in each study, highlighting metal concentrations above the EC_{50} values for microorganisms. Therefore, in the studies quantified in Table 2, metal concentrations exceeded mean reported toxicity thresholds and metal toxicity was expected for soil microorganisms. The specific authors corresponding to each study counted in the table are listed in Supplementary Tables 20–24.

On the other hand, we noted studies reporting statistically significant negative correlations between microbiological responses and soil total copper concentrations, which were below the reported effective concentration at 10% (EC_{10}) for copper using microorganisms' responses as a bioindicator (Table 1). In other words, these studies report copper toxicity to soil microorganisms at low copper concentrations, at which toxicity is not expected. These studies are summarized in Supplementary Table 25. Conversely, we also found studies reporting statistically significant positive correlations between microbial responses with rising soil copper concentrations, even though none of the metal concentrations exceeded the mean reported EC_{10} threshold for microbial bioindicators. These findings are presented in Supplementary Table 26.

We arranged the reviewed information according to the well-established classification of microbiological responses used in soil quality assessment (Benedetti and Dilly, 2006): (1) soil microbial biomass and number, (2) soil microbial activity, (3) soil microbial diversity and community structure, and (4) plant-microbe interactions (Table 2). Additionally, Shaw and Burns (2006) posited soil enzymatic activity as a proxy of soil microbial activity. To this end, we also included studies on soil enzymatic activity in our review (Table 2). In the following discussion, the terms "microbiological responses" or "microorganism responses" refer to at least one of the five types of responses listed above.

Discussion

Metal pools governing metal toxicity for microorganisms in contaminated soils

Multiple studies show that total metal concentration of a soil can predict microorganism responses as successfully as the soluble or extractable metal pools (Supplementary Tables 10–15). Similar results have been found for predicting plant responses in metal-contaminated soils (Peñaloza et al., 2024). Plant uptake of metals depends on the metal concentration in the soil solution, the total metal concentration in the soil, and the kinetics of metal transfer between the solid and solution phases (Peñaloza et al., 2024; Prudnikova et al., 2020). It is unclear if the same conceptual understanding can be applied to metal uptake in the cells of microorganisms. For instance, the study of Khan et al. (2009) used the 1 M NH_4NO_3 soil extract following chloroform fumigation. This method is widely used to estimate soil microbial carbon (ISO 14240-2, 1997), but it was utilized in the study of Khan et al. (2009) to estimate soil copper fraction retained by the cells of microorganisms. This study suggests that microorganisms can retain more copper in their cells than the amount of copper present in the soil solution at any one moment (Supplementary Table 27).

Metal concentrations in the soil solution are regulated by precipitation and dissolution reactions, and by adsorption and desorption processes involving the solid phase (Sauvé, 2002). Analogously, metal uptake and retention by microbial cells in soils may be regulated, in part, by the capacity of the total soil metal pool (dominated by the solid phase) to supply metal ions to the soil solution at the precise time when microbial cells are assimilating metal ions. This mechanism can potentially explain why total metal concentration of a soil can predict microorganism responses as successfully as the soluble or extractable metal pools (Supplementary Tables 10–15). However, very limited data are available for metal concentrations in the cells of microorganisms (Khan et al., 2009), requiring future studies to confirm the mechanism controlling cellular uptake of metals in soil systems.

Microbiological responses to soil metals at concentrations exceeding the reported EC_{50} values

Table 2 summarizes the number of studies reporting microbiological responses to soil metals at concentrations exceeding the reported EC_{50} values. The studies are grouped by the type of microbial response (see Method section) (Benedetti and Dilly, 2006; Shaw and Burns, 2006). In many studies summarized in Tables 2 and Supplementary Tables 20–24, soil microbiological responses were sensitive indicators of metal toxicity. As expected, microbiological responses decreased with increasing soil metal concentrations, indicating metal toxicity. In some cases, it was possible to derive metal toxicity thresholds for soil microorganisms, as summarized in Table 1.

However, in many other cases, soil microbiological responses were influenced by soil properties, such as soil organic matter content, pH, and texture (Moya et al., 2025; Yáñez et al., 2022), rather than by soil metal concentrations (Table 2). Likewise, in many other cases, microbiological responses were not affected by soil metal concentrations or soil properties. In other words, in many cases, no metal toxicity for microorganisms was observed despite soil metal concentrations (either total pool or a fraction) being above the reported effective concentration at 50% (EC_{50}) for metals using microorganisms' responses as bioindicators (Table 1).

Notably, in many cases, soil microorganism responses were positively correlated (i.e., increased) with soil metal concentrations increase (Table 2). The underlying mechanism of this microbial response is unknown. The mechanism most frequently proposed in the literature is the well-established adaptive response of soil microorganisms to metal stress (reviews of Das et al., 2022; Mallick et al., 2015; Naik et al., 2018; Narayani and Shetty, 2013). Some additional possible mechanisms might include (Table 3):

1. Biogeochemical mitigation of metal toxicity: decrease of metal bioavailability in the soil under certain biogeochemical conditions, such as a decrease in metal solubility at higher pH values. Also, this mechanism includes the potential effect of

TABLE 3 Summary of possible mechanisms explaining the increasing microbial response at increasing soil metal concentrations. The mechanism most frequently proposed in the literature is the well-established adaptive response of soil microorganisms to metal stress (reviews of Das et al., 2022; Mallick et al., 2015; Naik et al., 2018; Narayani and Shetty, 2013). This table summarizes additional possible mechanisms reported in the literature. MNB stands for microbial number and biomass, SEA stands for soil enzymatic activity, MA stands for microbial activity, and MDCS stands for microbial diversity and community structure.

Study	Soils contaminated by…	Microbiological response	Proposed mechanism
Anza et al. (2021)	Multiple metals	SEA	Biogeochemical mitigation of metal toxicity
Azarbad et al. (2013)	Multiple metals	MDCS	Fungal dominance
Bai et al. (2021)	Multiple metals	MA; SEA	Biogeochemical mitigation of metal toxicity
Benidire et al. (2020)	Copper and zinc	DNA analyses	Root exudate stimulation
Bhattacharyya et al. (2008)	Cadmium and copper	MNB; MA; SEA	Biogeochemical mitigation of metal toxicity
Boteva et al. (2016)	Cadmium, chromium, cobalt, lead, and zinc	SEA	Metabolic utilization
Dai et al. (2004)	Cadmium, copper, lead and zinc	MNB	Fungal dominance
Lawlor et al. (2000)	Copper and zinc	MNB	Biogeochemical mitigation of metal toxicity
Li et al. (2009)	Cadmium and zinc	SEA	Metabolic utilization
Martínez-Toledo et al. (2023)	Arsenic, cobalt, copper, molybdenum, nickel, and zinc.	MA; SEA	Metabolic utilization
Muhlbachova et al. (2015)	Multiple metals	MNB; MA; SEA	Biogeochemical mitigation of metal toxicity
Niemeyer et al. (2012)	Cadmium, chromium, copper, iron, lead, nickel, zinc	MNB; MA	Biogeochemical mitigation of metal toxicity
Pająk et al. (2016)	Lead and zinc	MDCS	Biogeochemical mitigation of metal toxicity
Pleshakova et al. (2023)	Lead	MNB	Metabolic utilization
Stefanowicz et al. (2008)	Multiple metals	MDCS	Fungal dominance
Wang et al. (2011)	Copper and zinc	SEA	Metabolic utilization

soil organic carbon and other soil nutrients, enhancing microbial tolerance to metal contamination;
2. Metabolic utilization: essential metals, at low concentrations, can stimulate microbial metabolism as enzyme cofactors;
3. Root exudate stimulation: metals can stimulate the production of root exudates that promote microbial diversity and activity;
4. Fungal dominance: fungi outcompete bacteria in metal-contaminated soils due to their greater tolerance to metals.

With respect to the mechanism (4), we are aware of only one study (Naveed et al., 2014) that compared the sensitivity of fungi and bacteria to metal stress using DNA analysis. The following effective concentration at 10% (EC_{10}) of total soil copper content were obtained: bacteria and fungi richness of 181 and 800 mg kg^{-1}, respectively, and bacteria and fungi diversity of 171 and 2374 mg kg^{-1}, respectively. Thus, the study of Naveed et al. (2014) demonstrated that fungi are more resistant to metals than bacteria in soils contaminated with copper, calling for similar studies using DNA analysis in soils with contamination by other metals.

It might be argued that hormesis is a plausible explanation of why soil microorganism responses positively correlate with soil metal concentrations. However, hormesis is the stimulatory effect on organisms resulting from exposure to a low dose of chemicals (Calabrese and Baldwin, 2003; Kaiser, 2003; Renner, 2004), in this case, a low concentration of soil metals. Thus, the concept of hormesis cannot be applied to the case of soil metal concentrations above the effective concentration at 50% (EC_{50}), which are summarized in Table 2 and whose corresponding studies are detailed in Supplementary Tables 20–24.

Microbiological responses to soil metals at concentrations below the reported EC_{10} values

Supplementary Table 25 summarizes studies on soils with copper concentrations (total and soluble fraction) below the reported effective concentration at 10% (EC_{10}) for copper using microorganisms' responses as a bioindicator (Table 1). In these studies, copper toxicity to soil microorganisms was unexpected, given that soil copper concentrations were low. However, the studies show statistically significant negative correlations between microbiological responses and soil total copper concentrations. The underlying mechanisms explaining these negative correlations are not clear, requiring future studies.

Finally, positive correlations between total soil copper concentration and soil microbial responses were observed in the three studies summarized in Supplementary Table 26. Soil total copper concentrations were up to 496 mg kg^{-1}, which is well below the reported effective concentration at 10% (EC_{10}) for copper using microorganisms' responses as a bioindicator (Table 1). In this case, these findings can indeed be viewed as an hormetic response, i.e., a stimulatory effect on microorganisms resulting from exposure to low concentrations of soil metals (Abeed et al., 2022).

Conclusions and future research needs

This review demonstrates that the effects of soil metals on microbiological responses range from toxic (negative) in soils with metal concentrations below the mean reported EC_{10} values to stimulatory (positive) in soils with metal concentrations above the mean reported EC_{50} values. Hence, in some cases, microorganisms' responses indicate metal toxicity at low soil metal concentrations, at which toxicity is not expected. In contrast, in other cases, microorganisms are stimulated by metals at high soil metal concentrations, at which stimulatory responses are not expected. Further, soil microbiological responses can be influenced by soil physicochemical properties rather than soil metals concentrations even at metal concentrations above the mean reported EC_{50} values, at which metal toxicity for soil microorganisms is expected. In summary, the unpredictable nature of microbiological responses to metals makes them unreliable indicators of metal toxicity in real-world contaminated soils.

As discussed above, individual-level responses of plants and invertebrates are known to be more sensitive to metal toxicity than are the community-level responses of microorganisms. Indeed, the toxicity responses of plants and earthworms are largely predictable based on reported EC_{10} or EC_{50} values (Neaman et al., 2025; Schoffer et al., 2024; Tapia-Pizarro et al., 2025). Thus, we propose using plants and invertebrates, rather than microorganisms, as bioindicators of metal toxicity in real-world contaminated soils.

The following hypotheses can be tested in future research:

1. Metal uptake and retention by microbial cells in soils is regulated by the capacity of the total soil metal pool (dominated by the solid phase) to supply metal ions to the soil solution at the time when microbial cells are assimilating metal ions.

To test this hypothesis, the methodology of the study of Khan et al. (2009) can be used. Specifically, a salt solution can be used to extract the soluble metal pool from soils following chloroform fumigation to estimate the soil metal fraction retained by the cells of microorganisms. This approach will help discern the relationship between cellular metal concentrations, the concentrations of metals in the soil solution, and the total metal concentrations in soils.

2. Fungi have greater tolerance of metals than bacteria do.

To test this hypothesis, the methodology of the study of Naveed et al. (2014) can be used. Specifically, DNA analysis can be used to quantify bacteria and fungi richness and diversity in soils with an increasing gradient of metal contents. Then, effective concentrations at 10% and 50% (EC_{10} and EC_{50}) can be derived for fungal and bacterial responses, allowing the comparison of the sensitivity of fungi and bacteria to metal stress.

Author contributions

JTS: Investigation, Data curation, Methodology, Writing – original draft, Writing – review and editing, Formal Analysis. JWS: Writing – review and editing, Writing – original draft. CY: Writing – review and editing, Validation, Conceptualization, Supervision, Writing – original draft. RG: Funding acquisition, Writing – review and editing, Writing – original draft. AN: Investigation, Writing – original draft, Supervision, Conceptualization, Methodology, Writing – review and editing.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2025.1737077/full#supplementary-material

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