REVIEW PAPER

THRESHOLDS OF METAL AND METALLOID TOXICITY IN FIELD-COLLECTED ANTHROPOGENICALLY CONTAMINATED SOILS: A REVIEW

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Received: March 3th, 2021 / Accepted: May 25th, 2021 / Published: July 1st, 2021
https://DOI-10.24057/2071-9388-2021-023

ABSTRACT. Ecotoxicological studies of soil metal toxicity conventionally rely on the use of uncontaminated soils gradually enriched with metals in the form of soluble salts. Although this method is very useful in many ways, it is continually complicated by the difficulty of extrapolating laboratory results to actual field-collected soils exposed to decades of contamination. Although many studies emphasize the importance of using field-contaminated soils for toxicity bioassays, the number of studies actually conducted based on this premise is relatively small. This review provides an in-depth recompilation of data on metal toxicity thresholds in field-contaminated soils. We have summarized the EC_{10}, EC_{25}, and EC_{50} values for metals, i.e., values of metal concentrations that reduce the response of specific organisms by 10%, 25%, and 50% of the value in uncontaminated soils. In our summary, most studies show that total metal content can predict organismal responses as well as bioavailable fractions. These results are consistent with the intensity/capacity/quantity concept proposed for plant nutrient uptake. In addition, microorganisms are thought to be more sensitive to metals than plants and invertebrates. However, our analysis shows that there is no statistically significant difference between the sensitivity of microorganisms and other organisms (plants and invertebrates) to any metal or metal pool. We expect that this information will be useful for environmental assessment and soil quality decisions. Finally, we encourage future studies to analyze dose-effect relationships in native field-collected soils with varying degrees of metal contamination from long-term anthropogenic pollution.

KEYWORDS: artificially polluted soil; metal-enriched soil, metal-spiked soil; field-collected soil; field-contaminated soil; ecotoxicity thresholds

CITATION: Javier Santa-Cruz, Patricia Peñaloza, Maria V. Korneykova, Alexander Neaman (2021). Thresholds of Metal and Metalloid Toxicity In Field-Collected Anthropogenically Contaminated Soils: A Review. Geography, Environment, Sustainability, Vol. 14, No 2, p. 6-21
https://DOI-10.24057/2071-9388-2021-023

ACKNOWLEDGMENTS: The study was funded by the Russian Foundation for Basic Research (RFBR), project number 20-14-50002. The authors also wish to thank Evgenii L. Vorobeichik for helpful comments and Andrei Tchourakov for editing this article.

Conflict of interests: The authors reported no potential conflict of interest.

SCOPE OF THE REVIEW

Ecotoxicology analyzes the effects of chemicals on organisms in the environment. Its ultimate goal is to protect the structure and functioning of ecosystems. It is achieved by assessing any exposure to a single species of certain test organisms and then extrapolating the resulting effective concentrations to safe levels for populations and communities (van Gestel 2012). In turn, soil ecotoxicology is an interdisciplinary field of science that studies the toxicological effects of chemicals on soil ecology (Hooper and Anderson 2008) to reduce the risks that certain human activities pose to soil ecosystems. In particular, soil contamination by metals and metalloids has become a serious threat to the environment in the era of industrialization (e.g., Korkina and Vorobeichik 2018). In the discussion that follows, the term "metal" includes metalloids (such as arsenic) for the sake of simplicity.

This review provides an in-depth recompilation of data on metal toxicity thresholds in soils exposed to decades of contamination. In the discussion that follows, the latter type of soils is referred to as "field-collected" or "field-contaminated" soils. We conducted an exhaustive review of the literature reporting dose-effect relationships in field-collected soils and omitted all studies that used metal-spiked soils, i.e., uncontaminated soils gradually spiked in a laboratory setting with metals in the form of soluble salts. We summarized the EC_{10}, EC_{25}, and EC_{50} values for metals, i.e., values of metal concentrations that reduce the response of specific organisms by 10%, 25%, and 50% of the value in uncontaminated soils. In our review, we analyzed studies that clearly stated the effective values of metal concentrations in soil. We also reviewed studies in which EC_x values for metals in soil could be estimated using either reported regressions or the dose-effect relationships shown in the figures.

Most of the responses summarized in this review relate to the individual level of biological organization, as there were not enough responses reported at lower organizational levels (i.e., molecular and cellular) and higher organizational levels (i.e., population, community, and ecosystem). Similarly, this review did not include studies in which it was not possible to determine the effects of any particular metal on organismal responses. In other words, we excluded studies that reported pollution index thresholds rather than thresholds for a particular metal.

SPIKED VERSUS FIELD-CONTAMINATED SOILS

Ecotoxicological studies of soil metal toxicity conventionally rely on the use of spiked soils. Although this method is very useful in many ways, it is continually complicated by the difficulty of extrapolating laboratory results to actual field soils exposed to decades of contamination (e.g., Neaman et al. 2020). Our comprehensive review of scientific literature conducted earlier (Santa-Cruz et al. 2021) revealed that all studies without exception had greater metal toxicity in spiked soils than in field-contaminated soils. Importantly, this observation held equally true for different types of organisms (e.g., plants, invertebrates, and microorganisms). To give but one example, the average effective concentration 50% (EC_{50}) of total copper in spiked soils (354 ± 39 mg kg^{-1}) was statistically lower than in field-collected soils (987 ± 491 mg kg^{-1}, $p<0.05$) when plant responses were used as bioindicators of toxicity.

It is a well-known fact that when metals are first introduced into the soil in the form of soluble salts, they exhibit high solubility and toxicity, which gradually decrease. In the scientific literature, this effect is called "aging" (also spelled "ageing"). Even though the concept of metal aging dates back to the 1990s (e.g., Ford et al. 1997), there is still little understanding of the physical, chemical, and biological processes that govern the transformation of metal ions into less soluble or so-called "fixed" forms (McBride and Cai 2016).

In order to overcome the difficulties presented by divergent metal toxicity in spiked versus field-collected soils, some researchers employed artificial aging of metal-spiked soils under both laboratory and field conditions. But the necessary duration of aging of metal-spiked soils until ecotoxicity bioassays may be considered realistic remained unclear. The study of McBride and Cai (2016) demonstrated that soils amended with 200-400 mg kg^{-1} of soluble Cu or Zn salts retained a significant degree of phytotoxicity even after 10 years of field aging. Likewise, the study of Martinez and Martinez-Villegas (2008) revealed that copper solubility decreased in copper-alumina-organic matter mixed systems aged for over 8 years. Therefore, it is safe to say that metal aging is a very slow process that does not yield easily to artificial replication.

TOTAL VERSUS "BIOAVAILABLE" METAL POOLS

It is believed that total metal concentrations in polluted soil are not sufficient to predict its potential toxicity. Several studies have attempted to forecast the so-called "bioavailable" metal fraction in soil by correlating organism responses with different metal pools in soil (e.g., Lillo-Robles et al. 2020). Assessment of "bioavailable" metal fractions in soil is often done using distilled water or chemically non-aggressive neutral salts. Other methods utilize pore water extracted by the Rhizon soil moisture samplers or the technique of diffusive gradients in thin films (DGT). In the discussion that follows, we will refer to these bioavailable fractions as "extractable" when the researchers chose to express them in mg kg^{-1} of soil, or "soluble" when the researchers expressed them in mg L^{-1} of soil solution (or extraction solution). Soil solution-free metal activities may also be used for assessing metal availability to organisms. In the following discussion, pMe^{2+} refers to the negative logarithm of Me^{2+} ion activity, where Me^{2+} represents Cu^{2+} or Zn^{2+} or Pb^{2+} ion. In is important to emphasize that the lower value of pMe^{2+} signifies the higher activity of the free Me^{2+} ion.

Appendices A-E contain the summary of studies that reported correlations between organism responses and various metal pools in soil. However, the data are inconsistent, making interpretations difficult. Yet most of the studies demonstrate that total metal content can predict organism responses just as well as bioavailable fractions (either extractable, soluble, or pMe^{2+}). These findings are consistent with the intensity/capacity/quantity concept proposed for nutrient uptake by plants (Marschner 1993), as discussed in more detail below.

The quantity factor refers to the total element content in the soil. The intensity factor is the concentration of elements in the soil solution, taking into account that this fraction is immediately delivered to the roots at any given time. In turn, the capacity factor is the kinetics of element release, i.e., the buffering capacity of the soil to supply element ions from the solid phase into the soil solution. These are the factors that are known to govern the phytoavailability of nutrients in soils (Fig. 1).

In other words, the absorption of elements by plants depends not only on their concentrations in the soil solution (intensity), but also on the total content of the elements in soil (quantity), and their supply kinetics (capacity). The same is true for metal phytoavailability in soil, which is similarly driven by the intensity/capacity/quantity factors (e.g., Prudnikova et al. 2020). Likewise, in the study of Sauvé et al. (1996), plant tissues accumulated an average of 2,000 times the amount of total copper dissolved in the solution. This is only possible if copper in the soil solution is buffered by desorption-dissolution mechanisms (Sauvé 2002). For this reason, it is safe to assume that the same factors also control metal availability to soil organisms (such as invertebrates).

In summary, metal toxicity in soil depends on the diverse soil metal pools available to supply metal ions to the soil solution at the time when plant roots or soil organisms uptake metal ions. For this reason, total metal content can predict organism responses just as well as the so-called "bioavailable" fractions.

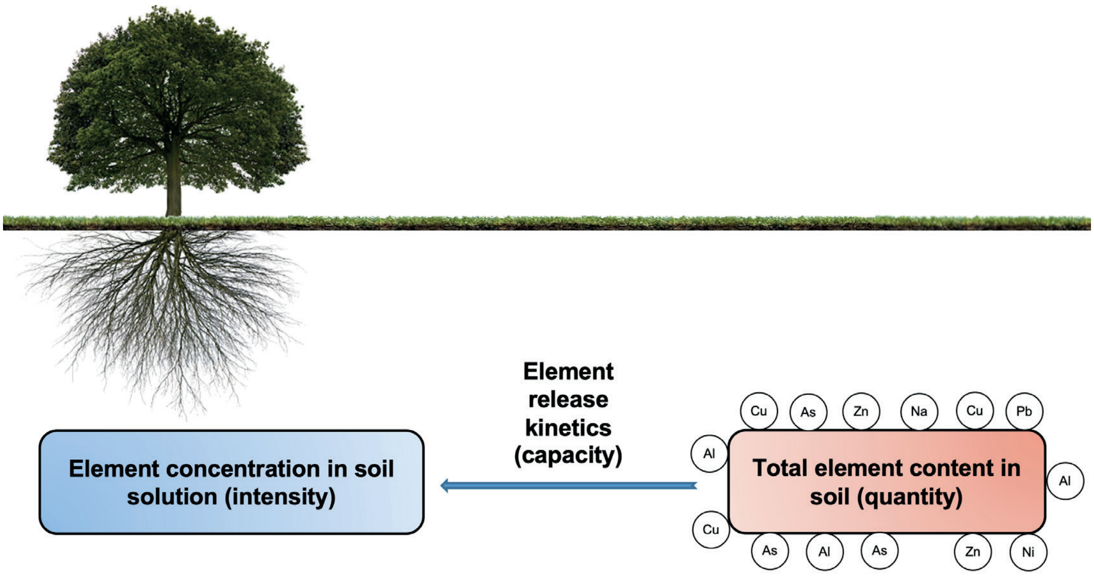

Fig. 1. Intensity, quantity, and capacity factors of element phytoavailability in soils (adapted from Neaman et al. 2021)

METAL TOXICITY THRESHOLDS

A single effective concentration value for a specific organism response is clearly insufficient to undertake any noteworthy agricultural or ecological endeavor. For this reason, Checkai et al. (2014) proposed to average the effective concentration values for different species and responses. However, this approach ignores the concept of the hierarchical cascade of biological responses to any given stress. According to this concept, the severity of chemical exposure to metals correlates with the complexity of specific levels of biological organization (e.g., Spurgeon et al. 2005). Lower organizational levels (i.e., molecular, cellular, and individual) are more sensitive to different types of stress than higher organizational levels (i.e., population, community, and ecosystem). Table 1 summarizes the studies reporting EC_{50} values for metals of at least two levels of biological organization, revealing the following order: molecular < cellular < individual.

Thus, an argument can be made that effective concentration values should not be averaged out for responses registered at different levels of biological organization. As mentioned above, most of the responses summarized in this review pertain to the individual level, whereas the number of responses registered at other levels was not sufficient to analyze them separately (Online Supplementary Material). For this reason, Table 2 sums up the responses of different species from all the levels, grouped by three types of organisms: plants, invertebrates, and microorganisms.

It is worth noting that the biggest challenge in using field-collected soils for ecotoxicity assessment has to do with the presence of several metals in the polluted soil. Regression analysis is one of the conventional methods employed to discern the impacts of various metals in field-contaminated soils. For instance, in the study of Bustos

Table 1. Plant and earthworm hierarchical biological responses to copper and zinc

Metal	Study	Species	LBO	EP	Total (mg kg^{-1})	pMe^{2+}
					EC_{50}	EC_{50}
Cu	Kolbas et al. (2014)	*Helianthus annuus*	M	CC	-	6.6
			M	Chl*a*/Chl*b*	-	6.6
			M	ChlTot	-	6.7
			I	LA		5.7
			I	SH DW	-	5.2
	Scott-Fordsmand et al. (2000b)	*Eisenia fetida*	C	NRRT	163	-
			I	CQ	517	-
Zn	Spurgeon et al. (2005)	*Lumbricus rubellus*	M	GE	616	
			C	NRRT	645	-
			I	CQ	3236	-

"Total" refers to total metal content in the soil, whereas pMe^{2+} refers to the negative logarithm of Me^{2+} ion activity, where Me^{2+} represents Cu^{2+} or Zn^{2+} ion. The lower value of pMe^{2+} signifies the higher activity of the free Me^{2+} ion. LBO: Level of biological organization; M: molecular; C: cellular; I: individual; CC: carotenoid content; Chla/Chlb: chlorophyll a/chlorophyll b ratio; ChlTot: total chlorophyll content; CQ: cocoon quantity; GE: gene expression (*mt-2*); LA: leaf asymmetry; NRRT: neutral-red retention time; SH DW: shoot dry weight.

et al. (2015), the authors correlated metal concentrations in earthworm tissues with earthworm responses. The conclusion was that the toxicity for *Eisenia fetida* in soils under study may be largely attributed to arsenic, whereas copper had only a secondary effect, contrary to what one would expect in soils affected by copper mining.

Another approach to sorting out the impacts of various metals in field-contaminated soils is to compare the obtained foliar metal concentrations with normal ranges. This approach was used to demonstrate that phytotoxicity in the Port Colborne site (Ontario, Canada) was attributable mostly to nickel, whereas the impacts of other metals (such as copper and cobalt) were minor (Dan et al., 2008, Kukier and Chaney 2004). In addition, a study by Hamels et al. (2014) evaluated the relative contribution of each individual metal to field-contaminated soil toxicity using a toxic unit approach. Specifically, for each metal, the toxic unit was calculated as the ratio of total metal concentration to the corresponding EC_{50} derived from single-metal spiked-soils for the same plant species.

However, there are several studies that have not demonstrated the effects of a single contaminant on biological responses (Appendices F-N). Thus, the data presented in these studies should be treated with caution. For this reason, we decided to exclude these studies from the summary in Table 2, considering only those studies that demonstrate the effects of a single contaminant on biological responses.

Interestingly, microorganisms are generally believed to be more sensitive to metals than plants and invertebrates (Giller et al. 1999). However, our analysis reveals that there is no statistically significant difference between the sensitivity of microorganisms and other organisms (plants and invertebrates) to any metal or metal pool. Moreover, removing both low organization level responses (i.e., molecular and cellular) and high organization level responses (i.e., population, community, and ecosystem) from the analysis has almost no effect on the result (not shown).

It is important to emphasize that invertebrates are more sensitive to copper than plants, based on the total metal pool data. This is a strong argument in favor of using invertebrates as indicators of soil quality.

It is worth noting that zinc was less toxic than copper when judged by the soluble pool data. Measurements of total zinc content support this view, although there was only one available study of total zinc content. This finding is consistent with our own results, validating the alleviating effects of zinc on copper toxicity to plants and soil microorganisms in copper-polluted soils that are attributable to copper-zinc antagonism (Stowhas et al. 2018, Stuckey et al. 2021). As for nickel, the results are contradictory. Total pool data indicate that copper is more phytotoxic than nickel. While the statistical difference was significant at $\alpha = 0.1$, it is still valid given the high data variability.

However, soluble pool data suggest that nickel is more phytotoxic than copper (*p < 0.001*). The free ion activity data indicate the same trend as the soluble pool data, however, there was only one data for free Ni^{2+} ion activity. This

Table 2. Summary of effective concentrations (EC_{50}) for plants, invertebrates and microorganisms. For this summary, we considered only studies that demonstrates the impact of a single pollutant on biological responses

Metal pool	Metal	Plants	Invertebrates	Microorganisms
Total ($mg\ kg^{-1}$)	As	-	22	313
	Cu	987 ± 491 aB*	303 ± 108 bB**	408 ± 174 ab
	Ni	1710 ± 510 A*	-	-
	Pb	-	960 ± 505 A**	-
	Zn	9820	-	-
Extractable ($mg\ kg^{-1}$)	Cu	330 ± 520	-	139
	Ni	607	-	-
	Pb	-	19 ± 24	-
	Zn	1423 ± 2059	-	-
Soluble ($\mu g\ L^{-1}$)	Cu	382 ± 213 B***	-	-
	Ni	9.3 ± 6.3 C***	-	-
	Pb	-	24,784 ± 37,159	-
	Zn	2579 ± 270 A***	-	5971 ± 4878
Free ion activity (pMe^{2+})	Cu	6.1 ± 0.3	-	-
	Ni	6.8	-	-
	Zn	-	-	-

The term "metal", for the sake of simplicity, includes metalloids (such as arsenic). pMe^{2+} refers to the negative logarithm of Me^{2+} ion activity, where Me^{2+} represents Cu^{2+} or Ni^{2+} ion. The lower value of pMe^{2+} signifies the higher activity of the free Me^{2+} ion. Lowercase letters in the same row indicate statistically significant differences between the types of organisms (p <0.05). Uppercase letters in the same column for the same metal pool indicate significant differences between the metals (* *p < 0.1*, ** *p < 0.05*, ** *p < 0.001*).

finding lends support to the study of Tarasova et al. (2020), which concluded that nickel impacted plant growth more severely than copper in Cu-Ni-smelter polluted soil.

FUTURE RESEARCH NEEDS

Although it is clear that scientific research should give preference to field-contaminated soils over spiked soils, a limited number of studies with field-contaminated soils have been conducted so far. As mentioned above, the biggest challenge in using field-collected soils for ecotoxicity assessment has to do with the presence of several metals in the polluted soil. In some cases, it might even be outright impossible to gauge the impact of any specific metal (e.g., Prudnikova et al. 2020).

We suggest that future research in this area should focus on contaminated sites with a single predominant metal contaminant. For example, historic industrial sites where wood has been treated with copper sulfate provide an excellent opportunity to encounter soils contaminated primarily with copper. One such site, located in Hygum, Denmark, has been extensively studied and copper toxicity thresholds for earthworms, and microorganisms have been established (e.g., Mirmonsef et al. 2017, Sauvé 2006). The Hygum site is believed to be polluted largely by copper (Scott-Fordsmand et al., 2000b). Although the site has been the subject of several studies, none of them have shown explicitly that there are no other metals in the investigated soils. Given that arsenic- and chrome-based products were also common in wood preservation in the past (Jakobs-Schonwandt et al. 2010), additional soil chemical analysis at the Hygum site might be warranted. Since wood treatment with copper sulfate is a common practice around the world, we assume that historical wood treatment operations can be found in many other countries. Another possibility is to study copper toxicity in vineyards, where copper may be a major metal contaminant due to the use of copper sulfate as a fungicide (Schoffer et al. 2020).

There are other sites contaminated with one dominant metal contaminant that have been described in the literature but have not been sufficiently studied. For example, Al-Hiyaly et al. (1990) described a site with contamination from electric pylons where zinc could reasonably be assumed to be the dominant metal contaminant. However, the authors did not attempt to establish thresholds for zinc toxicity in that study. Thus, future studies at this and similar sites around the world should be encouraged.

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Appendix

Appendix A. Correlation coefficients between different arsenic soil pools and biological responses

Study	Coefficient	Species	Endpoint	As_{total}	$As_{soluble}$
Wang et al. (2020)	Pearson (r)	Native microbes	ACP K_a	-0.70**	-0.49
			ACP V_{max}	-0.70*	-0.70*
			BG K_a	-0.49*	ns
			BG V_{max}	-0.70*	ns
			DHA K_a	ns	-0.70*
			DHA V_{max}	-0.70*	-0.70*

Significance level: * $p < 0.05$, ** $p < 0.01$, ns: not significant. ACP K_a: acid phosphatase catalytic efficiency; ACP V_{max}: acid phosphatase maximum reaction rate; ALP K_a: alkaline phosphatase catalytic efficiency; ALP V_{max}: alkaline phosphatase maximum reaction rate; BG K_a: β-glucosidase catalytic efficiency; BG V_{max}: β-glucosidase maximum reaction rate; DHA K_a: dehydrogenase catalytic efficiency; DHA V_{max}: dehydrogenase maximum reaction rate.

Appendix B. Correlation coefficients between different copper soil pools and biological responses

Study	Coefficient	Species	Endpoint	Cu_{total}	$Cu_{extractable}$	$Cu_{soluble}$	pCu^{2+}
Kolbas et al. (2014)	Pearson (r)	*Helianthus annuus* (Sunflower)	CC	-0.88***	-	-0.91***	0.89***
			Chl*a*/Chl*b*	ns	-	ns	0.51**
			ChlTot	-0.89***	-	-0.91***	0.89***
			LL	-0.37*	-	-0.36*	0.64**
			R DW	-0.79***	-	-0.81***	0.76***
			SH DW	-0.56**	-	-0.51**	0.62**
			TLA	-0.44*	-	-0.41*	0.55**
Konečný et al. (2014)	Spearman (ρ)	*Enchytraeus crypticus*	JQ	-0.97***	-0.89***	-	-
Kolbas et al. (2018)	Pearson (r)	*Helianthus annuus* (Sunflower)	ChlTot	ns	-	ns	-
			R DW	-0.81**	-	-0.85**	-
			SH DW	-0.86**	-	-0.94**	-
			TAC	ns	-	ns	-
			TLA	-0.84**	-	-0.92**	-

Significance level: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant. CC: carotenoid content; Chl*a*/Chl*b*: chlorophyll a/ chlorophyll b ratio; ChlTot: total chlorophyll content; EL: epicotyl length; JQ: juvenile quantity; LA: leaf asymmetry; LL: leaf length; R DW: root dry weight; SH DW: shoot dry weight; SH L: shoot length; TAC: total antioxidant capacity; TLA: total leaf area; WC: water content.

Appendix C. Determination coefficients (R^2) of regressions between different copper soil pools and biological responses

Study	Species	Endpoint	Cu_{total}	$Cu_{extractable}$	$Cu_{soluble}$	pCu^{2+}
Konečný et al. (2014)	*Enchytraeus crypticus*	JQ	0.93*	0.80*	-	-
Verdejo et al. (2015)	*Lolium perenne* (Perennial ryegrass)	R L	0.40*	-	0.40*	0.33*
		SH L	0.58*	-	0.24*	ns
Verdejo et al. (2016)	*Lactuca sativa* (Lettuce)	SH L	0.19*	-	ns	ns
Mondaca et al. (2017)	*Avena sativa* (Oat)	SH L[a]	0.36*	-	ns	ns
		R DW	0.36*	-	ns	ns
		SH DW	0.44*	-	ns	ns
		SH L[c]	0.35*	-	0.20*	0.19*
	Brassica rapa (Turnip)	SH DW[a]	0.46*	-	ns	ns
		SH La	0.47*	-	ns	ns
		R L	0.67*	-	ns	ns
		SH DW[b]	0.67*	-	0.27*	ns
		SH L[b]	0.64*	-	0.31*	ns
		SPQ	0.56*	-	0.19*	ns
Lillo-Robles et al. (2020)	Several species	PC	ns	-	0.67*	ns
		SH DW	ns	-	0.99**	0.69*
		SR	ns	-	0.78*	ns

Significance level: * $p < 0.05$, ** $p < 0.001$, ns: not significant. Test duration: [a] 21 days, [b] 42 days, [c] 62 days. JQ: juvenile quantity; PC: plant cover; R DW: root dry weight; R L: root length; SH DW: shoot dry weight; SH L: shoot length; SPQ: seeds pods quantity; SR: species richness.

Appendix D. Correlation coefficients between different lead soil pools and biological responses

Study	Coefficient	Species	Endpoint	Pb_{total}	$Pb_{extractable}$		$Pb_{soluble}$
					0.01 M $CaCl_2$	Water	
Luo et al. (2014b)	Pearson (r)	*Eisenia andrei*	SV	-0.68*	-0.83*	-0.79*	-0.78*
			JQ	ns	-0.63*	-0.47*	-0.52*
Luo et al. (2014a)	Pearson (r)	*Enchytraeus crypticus*	SV	-0.65*	-0.87*	-0.72*	-0.82*
			JQ	-0.63*	-0.81*	-0.73*	-0.85*
Luo et al. (2015)	Pearson (r)	*Platynothrus peltifer*	JQ	ns	-0.49*	-0.45*	-0.44*

Significance level: * $p < 0.01$, ns: not significant. JQ: juvenile quantity; SV: survival.

Appendix E. Correlation coefficients between different fractions of zinc in soil and biological responses

Study	Coefficient	Species	Endpoint	Zn_{total}	$Zn_{soluble}$	
					0.01 M KNO_3	ASV
Lessard et al. (2014b)	Not available	Native microbes	AA	ns	-0.87**	-0.89**
			BG A	ns	ns	-0.70*
			IA	ns	-0.80**	-0.81**
			PH A	ns	-0.84**	-0.87**
			PA	ns	-0.78*	-0.80**
			UA	ns	-0.85**	-0.87**

Significance level: * $p < 0.05$, ** $p < 0.01$. AA: arylsulfatase activity; ASV: 0.01 M KNO_3 extract measured by square wave anodic stripping voltammetry; BG A: β-glucosidase activity; IA: invertase activity; PA: protease activity; PH A: phosphatase activity; UA: urease activity.

Appendix F. Total effective concentrations (ECx) of arsenic and the properties of soils under study

Study	SO	Soil properties			M	D	Species	As_{total} (mg kg^{-1})		
		pH	CEC (cmol$_+$ kg^{-1})	OM (%)				EC_{10}	EC_{25}	EC_{50}
Invertebrates: Worms										
Bustos et al. (2015)	Chile	5.7-7.6	NA	0.7-4.9	VS	√	*Eisenia fetida*	**8**	**14**	**22**
Microorganisms										
Nordgren et al. (1986)	Sweden	3.5-5.0	NA	NA	VS	x	Native microbes	-	-	200
Wang et al. (2020)	China	4.6-8.2	8.1-22	0.5-5.3	VS	√	Native microbes	35	-	313
							Microbe mean	35	-	257

CEC: cation exchange capacity; D: the study demonstrates the impact of a single pollutant on biological responses?; "x" means "no", whereas "√" means "yes"; M: method; VS: various field-collected soils; native microbes: biological response is attributed to several soil microorganism taxa (i.e., archaea, bacteria, actinomycete, algae, fungi, and protozoa); NA: not available; OM: organic matter; SO: soil origin.

Appendix G. Total effective concentrations (EC_x) of copper and the properties of soils under study

Study	SO	Soil properties			M	D	Species	Cu_{total} (mg kg^{-1})		
		pH	CEC ($cmol_+ kg^{-1}$)	OM(%)				EC_{10}	EC_{25}	EC_{50}
Plants										
Hamels et al. (2014)	Sweden	5.0-6.1	9-16	12.1	F	√	*Hordeum vulgare* (Barley)	-	-	1260
Kolbas et al. (2014)	France	7.0-7.5	3.1-19	1.5-7.8	VS	√	*Helianthus annuus* (Sunflower)	372	-	760
Verdejo et al. (2015)	Chile	5.7-7.6	NA	0.7-5.8	VS	√	*Lolium perenne* (Perennial ryegrass)	414	750	1088
Verdejo et al. (2016)	Chile	5.7-7.6	NA	0.7-5.8	VS	√	*Lactuca sativa* (Lettuce)	445	955	1805
Mondaca et al. (2017)	Chile	5.7-7.6	NA	0.7-5.8	VS	√	Several species	421	618	840
Kolbas et al. (2018)	France	5.9-7.2	2.7-3.2	1.2-1.5	F	√	*Helianthus annuus* (Sunflower)	145	-	318
							Plant mean	**369**	**735**	**987**
Invertebrates										
a. Nematodes										
Naveed et al. (2014)	Denmark	5.9-6.6	NA	3.3-6.0	VS	√	Native nematodes	**338**	-	-
b. Springtails										
Scott-Fordsmand et al. (2000a)	Denmark	6.1-7.1	10-13	3.9-5.5	VS	√	*Folsomia fimetaria*	643	-	-
								2463[c]	-	-
Liu et al. (2018)	China	7.2	18	3.2	VS	x	Several species	187	-	723
							Springtail mean	**278**	**-**	**723**
c. Worms										
Scott-Fordsmand et al. (2000b)	Denmark	6.5-7.0	NA	NA	VS	√	*Eisenia fetida*	159	-	340
Van Zwieten et al. (2004)	Australia	6.6-6.9	NA	3.3-12	F	x	*Eisenia fetida*	-	-	131a
Maraldo et al. (2006)	Denmark	NA	NA	NA	VS	√	*Enchytraeus crypticus*	99	-	439
Konečný et al. (2014)	Zambia	5.1-6.9	3.5-15	1.7-15	VS	x	*Enchytraeus crypticus*	-	-	351
Naveed et al. (2014)	Denmark	5.9-6.6	NA	3.3-6.0	VS	√	Native earthworms	110	-	-
Delgadillo et al. (2017)	Chile	5.7-8.3	NA	0.7-10	VS	√	*Eisenia fetida*	-	-	213
Mirmonsef et al. (2017)	Denmark	NA	NA	NA	VS	√	*Aporrectodea tuberculata*	-	-	220
							Worm mean	**123**	**-**	**282**
Microorganisms										
Baath et al. (1991)	Sweden	NA	NA	NA	VS	x	Native microbes	-	-	2500[c]
Sauvé (2006)	Denmark	6.0-7.1	NA	3.7-5.1	VS	√	Native microbes	154	193[b]	285
Arthur et al. (2012)	Denmark	6.1-6.6	NA	2.7-5.1	VS	√	Native microbes	-	-	532
Naveed et al. (2014)	Denmark	5.9-6.6	NA	3.3-6.0	VS	√	Native microbes	575	-	-
							Microbe mean	**365**	**-**	**408**

a. Archaea/Bacteria										
Mertens et al. (2010)	Denmark	5.2-5.9	6.7	3.6	VS	√	AOA and AOB	-	-	2060[c]
Naveed et al. (2014)	Denmark	5.9-6.6	NA	3.3-6.0	VS	√	Native bacteria	**170**	-	-
b. Fungi										
Naveed et al. (2014)	Denmark	5.9-6.6	NA	3.3-6.0	VS	√	Native fungi	**1585**	-	-
Soil properties										
Naveed et al. (2014)	Denmark	5.9-6.6	NA	3.3-6.0	VS	√	Physical and chemical properties	**311**	-	-

AOA and AOB: ammonia-oxidizing archaea and ammonia-oxidizing bacteria community; CEC: cation exchange capacity; D: the study demonstrates the impact of a single pollutant on biological responses?; "x" means "no", whereas "√" means "yes"; F: fading with uncontaminated soil or with artificial OECD soil (sphagnum peat 10% w/w, kaolinite clay 20% w/w, quartz sand 70% w/w); M: method; VS: various field-collected soils; native microbes: biological response is attributed to several soil microorganism taxa (i.e., archaea, bacteria, actinomycete, algae, fungi, and protozoa); NA: not available; OM: organic matter; SO: soil origin. [a] Mean value for several soils. [b] EC_{20} instead of EC_{25} (not included in the mean). [c] Not included in the mean.

Appendix H. Effective concentrations (EC_x) of extractable and soluble copper pools, and free copper ion

Study	Species	Extractant	$Cu_{extractable}$ (mg kg^{-1})	$Cu_{soluble}$ (µg L^{-1})			pCu^{2+}		
			EC_{50}	EC_{10}	EC_{25}	EC_{50}	EC_{10}	EC_{25}	EC_{50}
Plants									
Hamels et al. (2014)	*Hordeum vulgare* (Barley)	0.0155 M Cohex, SSR: NA	**50**	-	-	-	-	-	-
		1 M NH_4NO_3, SSR: 1/2.5	**8.9**	-	-	-	-	-	-
		0.05 M EDTA, SSR: 1/2.5	**930**	-	-	-	-	-	-
		0.001 M $CaCl_2$, SSR: 1/10	-	-	-	**390**	-	-	-
		C_{DGT}	-	-	-	**40**	-	-	-
Kolbas et al. (2014)	*Helianthus annuus* (Sunflower)	Pore water	-	**311**	-	580	**7.1**	-	**6.3**
Kolbas et al. (2018)	*Helianthus annuus* (Sunflower)	Pore water	-	-	-	361	-	-	-
Lillo-Robles et al. (2020)	Several species	0.1 M KNO_3, SSR: 1/2.5	-	**267**	**397**	**539**	**7.5**	**6.8**	**5.9**
	Plant mean	**Pore water**	-	-	-	**471**	-	-	-
Worms									
Konečný et al. (2014)	*Enchytraeus crypticus*	0.05 M EDTA, SSR: 1/2.5 (recalculated from SSR: 1/10)	**398**	-	-	-	-	-	-
Microorganisms									
Aponte et al. (2021)	Native microbes	NA M DTPA, SSR: NA	**139**	-	-	-	-	-	-

C_{DGT}: Diffusive gradients in thin films measured concentration; NA: not available; SSR: soil/solution ratio.
Lillo-Robles et al. (2020): various Chilean field-collected soils with pH 4.9-7.1 and 0.9-8.0% organic matter. This study demonstrates the impact of a single pollutant on biological responses.
Aponte et al. (2021): various Chilean field-collected soils with pH 4.7-5.9 and 1.0-2.8% organic matter. This study demonstrates the impact of a single pollutant on biological responses.

Appendix I. Total effective concentrations (EC_x) of nickel and the properties of soils under study

Study	SO	Soil properties			M	D	Species	Ni_{total} (mg kg^{-1})	
		pH	CEC (cmol$_+$ kg^{-1})	OM(%)				EC_{25}	EC_{50}
Plants									
Dan et al. (2008)	Canada	5.7-6.9	5.0-63	6.0-28	VS	√	*Avena sativa* (Oat)	1727[a]	-
Cioccio et al. (2017)	Canada	4.6-6.1	23-54	9.6-25	VS	√	*Avena sativa* (Oat)	-	1270
		NA	NA	NA	VS	√	*Glycine max* (Soybean)	-	1590
Gopalapillai et al. (2019)	Canada	5.5-7.4	9.7-49	3.6-18	F	√	*Avena sativa* (Oat)	-	2269[a]
							Plant mean	**1727**	**1710**

CEC: cation exchange capacity; D: the study demonstrates the impact of a single pollutant on biological responses?; "x" means "no", whereas "√" means "yes"; F: fading with uncontaminated soil; M: method; VS: various field-collected soils; NA: not available; OM: organic matter; SO: soil origin. [a] Mean value for several soils.

Appendix J. Effective concentrations (EC_x) of extractable and soluble nickel pools, and free nickel ion

Study	Species	Extractant	$Ni_{extractable}$ (mg kg^{-1})		$Ni_{soluble}$ (µg L^{-1})	pNi^{2+}
			EC_{25}	EC_{50}	EC_{25}	EC_{50}
Plants						
Kukier and Chaney (2004)	Several species	0.01 M $Sr(NO_3)_2$, SSR: 1/4	-	-	**9.3**	-
Dan et al. (2008)	*Avena sativa* (Oat)	0.2 M $C_2H_2O_4$ + $(NH_4)_2C_2O_4$, SSR: 1/20	**465**[a]	-	-	-
Gopalapillai et al. (2019)	*Avena sativa* (Oat)	0.2 M $C_2H_2O_4$ + $(NH_4)_2C_2O_4$, SSR: 1/20	-	**607**[a]	-	-
		Pore water	-	-	-	**6.8**[a]

SSR: soil/solution ratio. [a] Mean value for several soils.

Kukier and Chaney (2004): Canadian field-collected soil artificially adjusted to pH 5.2-7.8 by adding $CaCO_3$ and $MgCO_3$; contains 17% of organic matter. The study demonstrates the impact of a single pollutant on biological responses.

Appendix K. Total effective concentrations (EC_x) of lead and the properties of soils under study

Study	SO	Soil properties			M	D	Species	Pb_{total} (mg kg^{-1})	
		pH	CEC (cmol$_+$ kg^{-1})	OM (%)				EC_{10}	EC_{50}
Invertebrates									
a. Mites									
Luo et al. (2015)	Netherlands	3.2-6.8	1.8-21	3.8-13	VS	√	*Platynothrus peltifer*	658	696
b. Worms									
Hui et al. (2009)	Finland	NA	NA	NA	F	x	Native enchytraeids	-	11,030[a]
Luo et al. (2014b)	Netherlands	3.2-6.8	1.8-21	3.8-13	VS	√	*Eisenia andrei*	1377	1543
Luo et al. (2014a)	Netherlands	3.2-6.8	1.8-21	3.8-13	VS	√	*Enchytraeus crypticus*	583	642
							Worm mean	**980**	**1092**
Microorganisms									
Vanhala and Ahtiainen (1994)	Finland	3.1-4.8	NA	NA	VS	x	Native microbes	-	**46,850**

CEC: cation exchange capacity; D: the study demonstrates the impact of a single pollutant on biological responses?; "x" means "no", whereas "√" means "yes"; F: fading with uncontaminated soil; M: method; VS: various field-collected soils; native microbes: biological response is attributed to several soil microorganism taxa (i.e., archaea, bacteria, actinomycete, algae, fungi, and protozoa); NA: not available; OM: organic matter; SO: soil origin. [a] Not included in the mean.

Appendix L. Effective concentrations (EC_x) of extractable and soluble lead pools

Study	Species	Extractant	$Pb_{extractable}$ (mg kg^{-1})		$Pb_{soluble}$ (µg L^{-1})	
			EC_{10}	EC_{50}	EC_{10}	EC_{50}
Invertebrates						
a. Mites						
Luo et al. (2015)	*Platynothrus peltifer*	Water, SSR: 1/5	**2.2**	**5.5**	-	-
		0.01 M $CaCl_2$, SSR: 1/5	**7.2**	**49**	-	-
		Pore water	-	-	**3040**	**6418**
b. Worms						
Luo et al. (2014b)	*Eisenia andrei*	Water, SSR: 1/5	0.4	3.0	-	-
		0.01 M $CaCl_2$, SSR: 1/5	0.4	50	-	-
		Pore water	-	-	99,000	67,550
Luo et al. (2014a)	*Enchytraeus crypticus*	Water, SSR: 1/5	0.4	1.0	-	-
		0.01 M $CaCl_2$, SSR: 1/5	1.3	5.1	-	-
		Pore water	-	-	**119**	**385**
	Worm mean	**Water, SSR: 1/5**	**0.4**	**2.0**	-	-
		0.01 M $CaCl_2$, SSR: 1/5	**0.9**	**28**	-	-
		Pore water	-	-	**49,560**	**33,967**

SSR: soil/solution ratio.

Appendix M. Total effective concentrations (EC_x) of zinc and the properties of soils under study

Study	SO	Soil properties			M	D	Species	Zn_{total} (mg kg^{-1})	
		pH	CEC ($cmol_+$ kg^{-1})	OM (%)				EC_{10}	EC_{50}
Plants									
De Knecht et al. (1998)	Netherlands	NA	NA	NA	VS	x	*Trifolium pratense* (Red clover)	-	347
Smolders et al. (2002)	Belgium	5.5-6.1	17-21	6.0-13	VS	x	*Triticum aestivum* (Wheat)	217	1215
Beyer et al. (2011)	United States	3.8-4.8	NA	NA	VS	x	Several species	-	2359
Beyer et al. (2013)	United States	3.6-4.2	14-16	8.0-13	F	x	Several species	-	311
Hamels et al. (2014)	Belgium / France	4.8-7.6	1.0-69	1.7-40	F	√	*Hordeum vulgare* (Barley)	-	9820[a]
							Plant mean	**217**	**1561**
Invertebrates									
Spurgeon et al. (2005)	United Kingdom	3.7-7.1	NA	NA	VS	x	Decomposer community	-	**979**
a. Springtails									
Mertens and Smolders (2013)	Belgium / United Kingdom	NA	NA	NA	VS	x	*Folsomia candida*	**507**	-
b. Worms									
Spurgeon and Hopkin (1995)	United Kingdom	5.5-7.4	NA	9.4-27	VS	x	*Eisenia fetida*	-	3605
Posthuma and Notenboom (1996)	Netherlands	5.5	NA	1.9-6.4	VS	x	Several species	-	1379

Spurgeon and Hopkin (1996)	United Kingdom	5.5-7.4	NA	9.4-27	VS	x	*Eisenia fetida*	-	1872
Nahmani and Lavelle (2002)	France	NA	NA	NA	VS	x	*Aporrectodea caliginosa*	-	2000[b]
Spurgeon et al. (2005)	United Kingdom	NA	NA	NA	VS	x	*Lumbricus rubellus*	-	1499
		5.4-7.4	NA	NA	VS	x	Native earthworms	-	1737
Mertens and Smolders (2013)	Belgium / United Kingdom	NA	NA	NA	VS	x	*Eisenia fetida*	924[a]	-
							Worm mean	**924**	**1912**
				Microorganisms					
Vanhala and Ahtiainen (1994)	Finland	4.3-7.2	NA	NA	VS	x	Native microbes	-	**2775**
				a. Bacteria					
Broos et al. (2005), Broos et al. (2004)	United Kingdom	5.2-5.7	2.5-4.7	NA	VS	x	*Rhizobium leguminosarum*	-	**403**

CEC: cation exchange capacity; decomposer community: biological response is attributed to several soil organism taxa (i.e., earthworms, isopods, microbes, mites, mollusks, myriapods and springtails); D: the study demonstrates the impact of a single pollutant on biological responses?; "x" means "no", whereas "√" means "yes"; F: fading with uncontaminated soil; M: method; VS: various field-collected soils; native microbes: biological response is attributed to several soil microorganism taxa (i.e., archaea, bacteria, actinomycete, algae, fungi, and protozoa); NA: not available; OM: organic matter; SO: soil origin. [a] Mean value for several soils. [b] EC_{100} instead of EC_{50} (not included in the mean).

Appendix N. Extractable, soluble, and free zinc ion effective concentrations (EC_x)

Study	Species	Extractant	$Zn_{extractable}$ (mg kg^{-1})	$Zn_{soluble}$ (µg L^{-1})		pZn^{2+}	
			EC_{50}	EC_{10}	EC_{50}	EC_{10}	EC_{50}
			Plants				
De Knecht et al. (1998)	*Trifolium pratense* (Red clover)	0.01 $CaCl_2$, SSR: NA	**121**	-	-	-	-
Smolders et al. (2002)	*Triticum aestivum* (Wheat)	Pore water	-	**400**	**6900**	-	-
		C_{DGT}	-	150	4410	-	-
Nolan et al. (2005)	*Triticum aestivum* (Wheat)	Pore water	-	-	-	**3.9**	**3.4**
Beyer et al. (2011)	Several species	0.01 M $Sr(NO_3)_2$, SSR: 1/4	120	-	-	-	-
Beyer et al. (2013)	Several species	0.01 M $Sr(NO_3)_2$, SSR: 1/4	52	-	-	-	-
		Mehlich 3, SSR: NA	**95**	-	-	-	-
Hamels et al. (2014)	*Hordeum vulgare* (Barley)	0.0155 M Cohex, SSR: NA	**327**[a]	-	-	-	-
		1 M NH_4NO_3, SSR: 1/2.5	**145a**	-	-	-	-
		0.05 M EDTA, SSR: 1/2.5	**3798**[a]	-	-	-	-
		0.001 M $CaCl_2$, SSR: 1/10	-	-	**2388**[a]	-	-
		C_{DGT}	-	-	2770[a]	-	-
	Plant mean	0.01 M $Sr(NO_3)_2$, SSR: 1/4	62	-	-	-	-
		C_{DGT}	-	-	3590	-	-
			Invertebrates: Worms				
Spurgeon and Hopkin (1995)	*Eisenia fetida*	Water, SSR: 1/13 to 1/17	**21**	-	-	-	-
Posthuma and Notenboom (1996)	Several species	0.01 M $CaCl_2$, SSR: 1/10	**95**	-	-	-	-
		Pore water	-	-	**21,135**	-	-

Microorganisms							
Lessard et al. (2014b)	Native microbes	0.01 M KNO_3, SSR: 1/2	-	-	**8031**	-	-
		ASV: 0.01 M KNO_3, SSR: 1/2	-	-	9481	-	-
Lessard et al. (2014a)	Native microbes	ASV: 0.01 M KNO_3, SSR: 1/2	-	-	400	-	-
	Microbe mean	**ASV: 0.01 M KNO_3, SSR: 1/2**	**-**	**-**	**4940**	**-**	**-**

ASV: measured by square wave anodic stripping voltammetry; C_{DGT}: diffusive gradients in thin films measured concentration; EP: endpoint; native microbes: biological response is attributed to several soil microorganism taxa (i.e., archaea, bacteria, actinomycete, algae, fungi, and protozoa); NA: not available; TD: test duration (days); SSR: soil/solution ratio. [a] Mean value for several soils.
Lessard et al. (2014a): various Canadian field-collected soils. The study demonstrates the impact of a single pollutant on biological responses.
Lessard et al. (2014b): various Canadian various field-collected soils with pH 3.3-7.1, CEC 15-247 $cmol_+ kg^{-1}$, and 1.6-70.3% organic matter. The study demonstrates the impact of a single pollutant on biological responses.
Nolan et al. (2005): various Australian and United States field-collected soils, with pH 3.6-8.1 and 0.2-20% of organic matter. The study, however, does not demonstrate the impact of a single pollutant on biological responses.