ISSN 1067-4136, Russian Journal of Ecology, 2021, Vol. 52, No. 6, pp. 479–485. © Pleiades Publishing, Ltd., 2021.

Metal Ecotoxicity Studies with Artificially Contaminated versus Anthropogenically Contaminated Soils: Literature Review, Methodological Pitfalls and Research Priorities

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Received March 19, 2021; revised May 3, 2021; accepted June 7, 2021

Abstract—Most ecotoxicological studies on the toxicity of metals in soil are conducted using artificially contaminated soils, i.e., originally uncontaminated soils to which increasing amounts of metals are added in the form of soluble salts in a laboratory setting. This approach has been rightly criticized because of the difficulty of extrapolating the results to real field situations. In our literature review, all studies without exception demonstrated a higher toxicity of metals in artificially contaminated soils than in anthropogenically contaminated soils exposed to pollution a few decades ago. Therefore, the traditional approach to the analysis of metal toxicity in soils, which is based on metal enrichment, has become outdated; new studies with such soils cannot provide any original insights at this time. We encourage researchers of metal pollution from anthropogenic emissions to analyze dose-effect relationships using native field-collected soils, rather than adopting the standard approach, which is based on artificially contaminated soils.

Keywords: artificially polluted soil, metal spiking, metal-spiked soil, field-contaminated soil, ecotoxicity thresholds

DOI: 10.1134/S1067413621060126

Currently, two approaches are used for ecotoxicological studies on soil metal toxicity. The most common approach is based on the use of artificially contaminated soils, i.e., originally uncontaminated soils to which increasing amounts of metals in the form of soluble salts are added in a laboratory setting. These soils are also referred to as "metal-spiked soils" or simply "spiked soils". An alternative approach requires the use of actual soils contaminated by anthropogenic activities, such as mining and agriculture, among others. These soils are referred to as "field-contaminated soils" or "field-collected soils". The main difference between these approaches is related to the time the metal has spent in the soil, as anthropogenic pollution, in most cases, has occurred some decades ago. For the sake of simplicity, in the following discussion, the term "metal" includes metalloids (such as arsenic).

It should be noted that the approach of using artificially contaminated soils has been repeatedly and rightly criticized because of the difficulty of extrapolating results to real field situations [e.g., 1]. Researchers first noted metal toxicity discrepancies in artificially contaminated versus anthropogenically contaminated soils back in the 1980s and 1990s [e.g., 2, 3]. And yet studies based on artificially contaminated soils continue to appear in scientific journals to this day. As of September 2020, there were approximately 1.500 articles in the Web of Science database containing the words "spiked", "metal*", and "soil*" in the title, abstract or among the keywords. Approximately 500 of these articles were published in the last five years alone.

The purpose of this paper is to raise issue with scientific journals that continuously publish studies on metal toxicity analysis performed with artificially contaminated soils. We cannot acquiesce to such obsolete methodology and suggest that priority be given to metal toxicity research based on anthropogenically contaminated soils.

EXPOSITION AND CRITIQUE

In our comprehensive literature review (Table 1), all studies without exception showed a higher metal toxicity in artificially contaminated soils than in anthropogenically contaminated soils. The pattern held steady for all types of organisms, such as plants, invertebrates, microorganisms, etc. The disparity between artificially contaminated and anthropogenically contaminated soils is attributed to the fact that metal toxicity depends, among other factors, on the residence time of metals in the soil. The process of transformation of metals in the soil over time is called "aging" (or "ageing").

The concept of metal aging first appeared in the literature in the 1990s [e.g., 4]. Since then, some studies have attempted to elucidate the mechanics of this process. For instance, a model has been proposed [5] that integrates short- and long-term aging of copper added to soils. This model can predict the aging process based on four factors: soil pH, incubation time, soil organic matter content and temperature. However, this model needs to be improved by considering other factors that affect the aging process, such as moisture, plant uptake, and microbial activities [5]. Thus, one of the important research priorities, in our opinion, should be to expose the mechanism of metal aging in soils.

In addition, metal enrichment in most studies was performed using soluble metal salts, such as sulfates, nitrates, chlorides, and acetates. But this approach has been highly criticized due to the confounding effects of salinity [6]. Other studies propose soil leaching to decrease excess salinity [7]. However, this procedure is not usually carried out due its complexity and labor-intensive nature.

Some authors argue that they choose artificially contaminated soils to better understand the mechanisms of interaction of the added metal with the soil and the effect of the added metal on soil properties. But the impact of soil physicochemical properties—such as pH, organic matter content, cation exchange capacity, etc. – on metal toxicity thresholds in artificially contaminated soils has already been adequately described [e.g., 8, 9]. Therefore, a different research objective should be adopted, namely, the effect of soil physicochemical properties on metal toxicity in anthropogenically contaminated soils.

Some studies conducted long-term field experiments with soils artificially contaminated with metals. In one of these studies [10], soils spiked with 200 mg/kg of copper or zinc salts retained significant phytotoxicity after 10 years of field aging, despite conversion of the metals to less labile forms. It is noteworthy that the concentration of 200 mg/kg of copper or zinc is considerably lower than the threshold value of these metals observed in ecotoxicity experiments with anthropogenically contaminated soils [11]. This implies that the threshold values of total metals added will not represent actual field conditions unless experiments are conducted over long periods of time (i.e., decades) to allow for the aging process of the metals.

Given the above shortcomings, artificially contaminated soils can only play a very minor role with respect to environmental assessment and decision making on soil quality. Difficulties in interpreting anthropogenically contaminated soils are due to the presence of multiple metal contaminants in soils, which obscures the impact of specific metals on the responses of plants and soil organisms. However, our studies demonstrated that once metal concentrations in plant tissues were determined, it was possible to discern the toxicity effects of specific metals [e.g., 12] and even to gauge metal toxicity thresholds in some cases [13, 14]. In a similar vein, analysis of metals in the tissues of earthworms exposed to soils polluted by copper mining revealed that toxicity to *Eisenia fetida* was not caused by copper, as might be expected, but by arsenic, whereas copper had only a minor toxic effect [15].

RESEARCH PRIORITIES

Although it is clear that preference should be given to anthropogenically contaminated soils over artificially contaminated soils in scientific research, few studies have so far been conducted with anthropogenically contaminated soils [11]. As mentioned above, the major challenge in using anthropogenically contaminated soils for ecotoxicity assessment concerns the presence of various metals in the polluted soil. In some cases, it might even be impossible to measure the impact of a specific metal [e.g., 16]. Therefore, we suggest that future research in this area should focus on sites polluted with one predominant metal contaminant.

For example, historical industrial sites where wood was treated with copper sulphate offer an excellent opportunity to find soils largely contaminated with copper. One such site is in Hygum (Denmark). It has been extensively studied, with copper toxicity thresholds established for plants, earthworms, and microorganisms [e.g., 17, 18]. The Hygum site is believed to be largely polluted by copper [19]. Although the site has been the subject of several studies, none of them have explicitly demonstrated that no other metals are present in the investigated soils. Since arsenic- and chrome-based products were also common in wood preservation in the past [20], further chemical analysis of the soil at the Hygum site may be warranted. Considering that wood treatment with copper sulphate is a common practice worldwide, we assume that historical wood treatment operations can be found in many other countries.

Table 1. EC$_x$ values of metal ecotoxicity in artificially contaminated soils (stated as “A” in the table) and anthropogenically contaminated soils (stated as “B” in the table)

Reference	Target organism	EC$_x$	Endpoint	Metal	Total metal concentration, mg kg^{-1}	
					A	B
Sheppard et al. [2]	*Pinus sylvestris*	90	Survival	As	200	>500
Spurgeon and Hopkin [3]	*Eisenia fetida*	50	Survival	Cu	836	>2609
		50	Growth	Cu	601	1763
		50	Cocoon quantity	Pb	1629	2131
		50	Growth	Pb	2249	10830
		50	Survival	Zn	1078	>32 871
		50	Cocoon quantity	Zn	357	3605
Smit and Van Gestel [31]	*Folsomia candida*	50	Juvenile quantity	Zn	185	>1537
		50	Growth	Zn	462	>1537
Kjær et al. [32]	*Fallopia convolvulus*	50	Germination	Cu	312	>928
Pedersen et al. [33]	*Folsomia candida*	10	Juvenile quantity	Cu	50	>2500
		50	Juvenile quantity	Cu	519	>2500
	Folsomia fimetaria	10	Juvenile quantity	Cu	141	>2500
		50	Juvenile quantity	Cu	657	>2500
Scott-Fordsmand et al. [34]	*Folsomia fimetaria*	10	Adult size	Cu	1075	>2912
		10	Survival	Cu	813	>2912
		50	Survival	Cu	2141	>2912
		10	Juvenile quantity	Cu	337	>2912
		50	Juvenile quantity	Cu	994	>2912
		10	Juvenile size	Cu	957	>2912
		50	Juvenile size	Cu	1886	>2912
Scott-Fordsmand et al. [19]	*Eisenia fetida*	10	Growth	Cu	428	>1369
		10	Cocoon quantity	Cu	34	248
		50	Cocoon quantity	Cu	210	517
		10	NRR	Cu	8	69
		50	NRR	Cu	39	163
Pedersen and Van Gestel [35]	*Folsomia fimetaria*	10	Juvenile quantity	Cu	707	>2500
		50	Juvenile quantity	Cu	1414	>2500
Smolders, et al. [36]	Microbes	50	PNR	Zn	274^{b}	>34 100
Smolders et al. [37]	*Triticum aestivum*	10	Shoot growth	Zn	185	>2101
		10	Shoot growth	Zn	411	>2520
		50	Shoot growth	Zn	714	>2101
		50	Shoot growth	Zn	1224	>2520
		50	Shoot growth	Zn	814	1215
	Microbes	10	SIR	Zn	863	>2101
		10	SIR	Zn	1393	>2520
		10	SIR	Zn	303	>3741
		10	PNR	Zn	600	>2101
		10	PNR	Zn	591	>2520
		10	PNR	Zn	282	>3741
		10	MRR	Zn	972	>2520
		10	MRR	Zn	1144	>3741
Smolders et al. [38]	Microbes	10	SIR	Zn	37	>480
		50	SIR	Zn	341	>480
		10	PNR	Zn	84	>480
		10	PNR	Zn	222	>390

Table 1. (Contd.)

Reference	Target organism	EC$_x$	Endpoint	Metal	Total metal concentration, mg kg^{-1}	
					A	B
Smolders et al. [39]	Microbes	10	PNR	Zn	66	>205
		50	PNR	Zn	227	>480
		50	PNR	Zn	115	>205
		10	NMR	Zn	481	>480
		10	Basal respiration	Zn	286	>390
		10	Basal respiration	Zn	159	>205
		50	SIR	Zn	3470^{a}	>3741
		50	PNR	Zn	935	>2101
		50	PNR	Zn	1201	>2520
		50	PNR	Zn	485	>3741
		20	MRR	Zn	760^{a}	>2101
		20	MRR	Zn	1660^{a}	>2520
		20	MRR	Zn	2125^{a}	>3741
Lock et al. [40]	*Folsomia candida*	50	Juvenile quantity	Pb	2570	>5690
		50	Juvenile quantity	Pb	3210	>14400
		50	Juvenile quantity	Pb	2160	>5460
Maraldo et al. [41]	*Enchytraeus crypticus*	10	Survival	Cu	522	>1601
		50	Survival	Cu	775	>1601
		10	Juvenile quantity	Cu	35	99
		50	Juvenile quantity	Cu	341	439
Mertens et al. [42]	Microbes	50	PNR	Zn	392	>1641
Oorts et al. [43]	Microbes	50	SIR	Cu	534	>825
		50	PNR	Cu	363	>825
		50	PNR	Cu	95	>104
		50	PNR	Cu	138	239
		50	PNR	Cu	164	>196
De Brouwere et al. [44]	Microbes	10	N_2O reduction	Zn	91	>1863
		50	N_2O reduction	Zn	231	>1863
Mertens and Smolders [45]	*Eisenia fetida*	10	Juvenile quantity	Zn	569	747
		10	Juvenile quantity	Zn	902	>2520
	Folsomia candida	10	Juvenile quantity	Zn	171	>2101
		10	Juvenile quantity	Zn	458	>2520
		10	Juvenile quantity	Zn	165	507
Ruyters et al. [46]	*Enchytraeus albidus*	50	Juvenile quantity	Cu	571	>689
	Hordeum vulgare	50	Root elongation	Cu	538	>689
		50	Root elongation	Cu	240	>448
		50	Root elongation	Cu	432	>435
	Solanum lycopersicum	50	Shoot growth	Cu	469	>513
		50	Shoot growth	Cu	426	>435
		50	Shoot growth	Cu	190	>455
Hamels et al. [47]	*Hordeum vulgare*	50	Shoot growth	Zn	1273^{b}	9820^{b}
		50	Shoot growth	Zn	2040	>6100
		50	Shoot growth	Cu	340^{b}	1375^{b}

MRR—maize residue respiration; NMR—nitrogen mineralization rate; NRR—neutral red retention time; PNR—potential nitrification rate; SIR—substrate induced respiration. ^{a} Estimate based on illustrations; ^{b} Mean value for several soils.

Another possibility is to study copper toxicity in vineyards, where copper could be the predominant metal contaminant due to the use of copper sulphate as fungicide [21]. Also, it might be warranted to study metal toxicity in places naturally enriched by metals due to the presence of outcrops of metallic mineralization, such as copper ores in northern Greece [22]. Given the current paucity of studies on metal toxicity thresholds based on anthropogenically contaminated soils [11], future studies with soils with a predominant metal contaminant would broaden the general understanding of metal toxicity patterns in plants, earthworms, and microorganisms under various edaphoclimatic conditions.

Some early studies in the 1980s [e.g., 23, 24] reported soil enrichment with copper from pig slurry. In fact, copper was commonly added to pig fattening rations to act as a growth promoter [23]. Thus, it was claimed that the application of pig slurry to soils caused copper to be the predominant metal contaminant. However, recent studies [e.g., 25, 26] report that livestock feed is supplemented with both copper and zinc. Thus, application of animal manure to soils results in soil enrichment with both copper and zinc. But in our recent studies [27, 28] we found that the responses of plants and soil microorganisms depend on the ratio of soil copper content to zinc content (Cu/Zn ratio), and that zinc alleviates copper toxicity to these organisms. Thus, if the application of animal manure to soils produces soils with different Cu/Zn ratios, this could be an interesting new topic to explore in further studies on the protective effects of zinc on copper toxicity to plants and soil organisms.

There are other sites polluted with a predominant metal contaminant that have been described in the literature but have not been sufficiently studied. For example, Al-Hiyaly, et al. [29] presented a site with contamination from electricity pylons, where zinc could be reasonably expected to appear as the main metallic contaminant. However, the authors did not attempt to establish zinc toxicity thresholds in that study. Therefore, future research should be encouraged at this and similar sites around the world.

As for the available studies on metal toxicity thresholds in anthropogenically contaminated soils, most of them report threshold values for total metal concentrations, rather than exchangeable/soluble metal concentrations. Several researchers also concluded that metal toxicity is determined by the pool of exchangeable/soluble metals in the soil, rather than by the total pool of metals in the soil [e.g., 30]. For this reason, future studies should be encouraged to establish metal toxicity thresholds based on exchangeable/soluble fractions, especially in soils with contrasting properties in terms of pH and organic matter content.

CONCLUSION

In our view, the traditional approach to the analysis of metal toxicity in soils, which is based on metal enrichment, has become obsolete. Indeed, we do not believe that further studies with artificially contaminated soils can provide any original insights at this time. Therefore, we are of the opinion that studies using soils artificially enriched with metals should be strongly discouraged for ecotoxicological research.

Some scientific concepts have a shorter shelf life than others. It is normal for some notions to wither and die in the process of evolution of scientific discoveries. For example, the idea of humus fractionation was very popular in Russia in the past. However, the current guidelines of the journal Eurasian Soil Science, which is published in Russia, explicitly prevent the publication of articles on humus fractionation because the concept is now considered obsolete. Similarly, guidelines for other journals could clearly specify that research based on soil metal enrichment would not be considered for publication because the concept is largely obsolete.

In conclusion, we encourage authors, reviewers, and editors to exercise much more scientific rigor with respect to ecotoxicological studies on soil metal toxicity conducted with artificially contaminated soils.

ACKNOWLEDGMENTS

The research team acknowledges Andrei Tchourakov for editing this article.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest.

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