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Zinc's Role in Mitigating Copper Toxicity for Plants and Microorganisms in Industrially Contaminated Soils: A Review

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Abstract— This review focuses on the issue of metal antagonism in soils contaminated by multiple metals as a result of industrial emissions. Building upon previous findings in aquatic ecosystems, the potential of zinc to mitigate copper toxicity in more complex soil systems is explored. A range of studies investigating the role of zinc in reducing copper toxicity to plants and microorganisms in soils contaminated by copper mining in central Chile are examined. The mechanisms underlying metal interactions in soils, including the terrestrial biotic ligand model and the intensity/capacity/quantity concept, are thoroughly discussed. Furthermore, the review underscores the pressing need for future studies to enhance our understanding and develop effective strategies for mitigating copper toxicity in industrially contaminated soils.

Keywords: heavy metals, antagonism, copper, zinc, industrially polluted soils, artificially polluted soils

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Soil metal contamination is a significant environmental threat that requires thorough investigation and implementation of advanced management strategies to mitigate metal toxicity in soils, protect ecosystem services, and ensure the production of safe agricultural commodities. The global scale of soil metal contamination is deeply troubling, as evidenced by assessments that have identified more than 5 million contaminated sites covering approximately 20 million hectares worldwide [1].

Emissions from industrial operations often result in the simultaneous pollution of the environment with multiple metals. This is primarily attributed to the fact that the ore used typically contains not only the intended metal but also associated elements. For instance, in copper mining, the use of sulfide ores results in the discharge of other chalcophile elements (i.e., elements associated with sulfur compounds) such as Zn, As, Cd, Hg, and Pb [2]. Consequently, cases of monometallic contamination can be considered the exception rather than the prevailing norm.

In terms of pollutant behavior in the environment, polymetallic pollution is distinct from monometallic pollution due to the increased complexity resulting from interactions among the elements introduced by emissions. These elements interact not only with the native elements and compounds already present in the environment but also with each other. Such interactions can take three forms: summation, enhancement of toxic effects (synergism), and reduction (antagonism) [3]. Due to limited information, the summation model is often accepted as the default approach, while synergism is less frequently considered [4–6] and antagonism is even more rarely explored [6, 7]. However, the latter scenario is crucial as it holds potential practical applications through the use of antagonistic elements to effectively reduce the toxicity of the target contaminant. This review is dedicated precisely to this aspect of the interaction of metals in polymetallic contamination, i.e., their antagonism.

The review focuses on soil contamination by copper. Copper is widely recognized as an essential element for plants, animals, and microorganisms, but its presence in high concentrations in the environment can lead to toxicity. The review draws from limited experimental data on soils contaminated by copper mining in central Chile.

A comprehensive understanding of metal antagonism requires a broader examination of the issue. Therefore, we delve into the disparities in toxic effects observed in artificially and industrially contaminated soils. We also examine which metal pool (free ions, exchangeable fraction, or total content) better predicts copper toxicity in soil for plants and microorganisms. In addition, we outline potential areas for further research, including practical applications.

We should clarify that various terms are utilized to refer to the group of pollutants under consideration, such as "heavy metals", "trace elements", "potentially toxic elements" and others. While "heavy metals" is a widely used term in scientific literature and environmental regulations, the International Union of Pure and Applied Chemistry (IUPAC) discourages its use due to its lack of precision [8]. Other terms have not gained widespread acceptance and have also faced criticism [9]. Hence, for the sake of brevity, this work employs the relatively neutral term "metal" to refer to hazardous metals.

BIOTIC LIGAND MODEL

The Biotic Ligand Model (BLM) [10] serves as the theoretical foundation for analyzing metal interactions. Initially developed for aqueous systems, its focus was on ions like Ca^{2+}, Na^{+}, Cl^{-}, HCO_3^{-}, among others [11]. Subsequently, the applicability of BLM expanded to include the behavior of metals in aquatic environments. In particular, BLM has been used to quantitatively assess how the chemical composition of the aqueous medium influences the bioavailability of metals and their transformation between different forms. Biotic ligands refer to potential uptake sites for pollutants, such as areas on the surface of gills (the primary target organ for metal uptake in aquatic organisms [12]). In our specific case, cations of other metals may compete with Cu^{2+} for biotic ligands, potentially reducing its toxicity.

The empirical basis for the theoretical framework discussed is provided by numerous data indicating that zinc reduces copper toxicity to aquatic organisms. Protective effects of zinc have been demonstrated in phytoplankton [13], algae [14, 15], mollusks [16], and fish [17]. Furthermore, zinc has been shown to mitigate copper toxicity in terrestrial plants, particularly lettuce [18–20], under hydroponic conditions.

Analogous to the Biotic Ligand Model (BLM) for aquatic systems, a Terrestrial Biotic Ligand Model (TBLM) [21] has been proposed for soils. However, soils are more complex than aquatic environments due to the presence of multiple phases, namely solid, liquid, and gaseous. As a result, metal interactions in soils are more complicated and will be further explored when discussing the concepts of intensity/capacity/quantity.

It is important to emphasize that the TBLM has exclusively been tested on artificially contaminated soils, i.e., soils intentionally contaminated during controlled experiments, starting from initially clean conditions [22]. However, as discussed below, extrapolating results from studies conducted on artificially contaminated soils to contamination scenarios arising from real-world industrial facilities presents significant challenges [23].

ARTIFICIALLY AND INDUSTRIALLY CONTAMINATED SOILS

Despite an extensive body of research on the toxicity of metals in soils, the majority of these studies are based on laboratory experiments involving artificial contamination of substrates. The standard experimental design entails introducing soluble metal salts into originally uncontaminated soil, typically creating a series of variants with progressively increasing concentrations. However, researchers have repeatedly observed significant discrepancies—sometimes by orders of magnitude—between toxicity assessments obtained from experiments with artificially contaminated soils and those with industrially contaminated soils. Our reviews [24, 25] have summarized the results of ecotoxicological studies evaluating effective concentrations at 50% (EC_{50}) of metals for plants and microorganisms derived from both artificially and industrially contaminated soils. Figure 1 shows an example of plant responses as an indicator of toxicity: on average, EC_{50} values for copper (total content) in artificially contaminated soils were markedly and statistically significantly lower than in industrially contaminated soils.

Ecotoxicologists attribute this discrepancy to the concept of metal "aging" in soils, wherein toxicity is affected by the duration of metal presence in the soil [26]. Indeed, in artificially contaminated soils, metals typically persist for a few months, rarely for a few years, whereas in areas affected by industrial emissions, metal presence can last for several decades and sometimes even centuries. Although the concept of metal aging in soils was first proposed in the 1990s [27], a comprehensive understanding of the physical, chemical, and biological processes governing the transformation of metal ions into less mobile or so-called "fixed" forms is still lacking [26]. Potential mechanisms of metal aging may include metal sorption to inorganic and organic components of the soil solid phase and precipitation of metals into less soluble forms. These processes require long time scales, measured in years and decades.

One possible explanation for the variations in toxicity assessments observed in experiments with artificially and industrially contaminated soils is the initial disparity in the chemical form of artificially introduced metals compared to those originating from industrial emissions. For instance, in one of our studies [28], results from electron probe microanalysis revealed that the primary phases of arsenic in the investigated industrially contaminated soils were less soluble iron oxides and copper sulfides. In contrast, artificially contaminated soils usually involve the addition of soluble arsenic compounds such as potassium or sodium arsenate [29].

A similar situation arises concerning the distinct responses of artificially and industrially contaminated soils to the addition of various amendments. For instance, in experiments with artificially contaminated substrates, the incorporation of calcium sulfate into the soil reduced the phytotoxicity of cadmium [30] and lead [31], possibly due to antagonistic interactions between calcium and metal ions. However, experiments with industrially contaminated soils showed that the introduction of calcium sulfate increased the phytotoxicity of metals [32]. This outcome could be attributed to calcium displacing metal cations from the soil exchangeable complex. Since calcium sulfate, unlike calcium carbonate [33], does not elevate soil pH, metal immobilization does not occur. Consequently, the addition of calcium sulfate to industrially contaminated soils enhanced metal phytoavailability.

Both examples—the differences in assessing effective concentrations at 50% and the reduction of toxicity by amendments—emphasize the importance of prioritizing the use of native soils exposed to long-term industrial emissions in ecological research, over artificially contaminated substrates. This is crucial for comprehending the behavior of metals in soils, identifying patterns of metal translocation in plants and soil-dwelling organisms, and determining factors that influence metal bioavailability and toxicity [34, 35]. Despite many researchers advocating the significance of employing industrially contaminated soils in ecotoxicological studies [36], regrettably, such an approach often remains merely a declaration and is seldom put into practice.

One of the reasons for this situation is the complexity of disentangling the contributions of individual metals to overall soil toxicity. To some extent, this complexity can be addressed by a detailed analysis of the chemical composition of organism tissues. For example, in the case of polymetallic contamination with a predominance of copper, it was shown [37] that plant responses exhibited the strongest correlation with copper tissue concentrations, while correlations with other metals were weak. Another approach to identifying the leading toxicant is to compare the regression relationships of organism responses to the levels of different elements in the soil. For example, in experiments with the earthworm *Eisenia fetida,* linear regression analysis indicated a relationship between the number of cocoons produced and total arsenic in the soil, while no significant relationship was observed with other elements [38]. This result was unexpected since it was initially assumed that copper, which is predominant in soils contaminated by copper industry, would exhibit the highest level of toxicity.

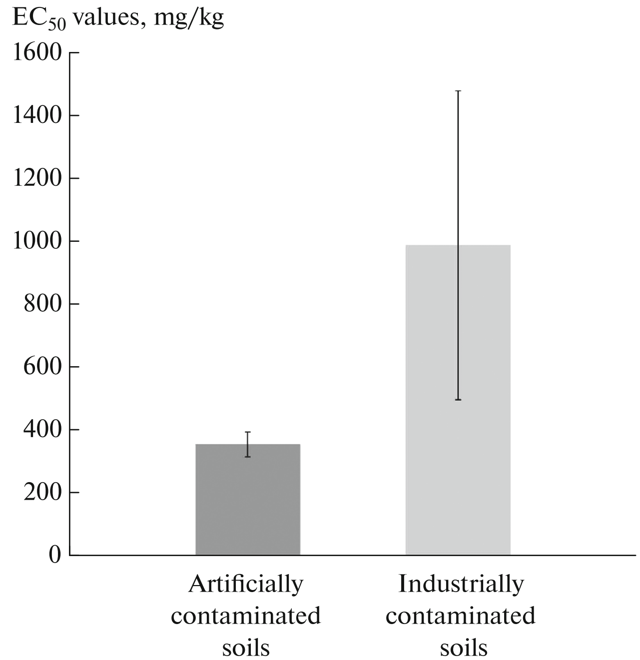

Fig. 1. Comparison of mean effective concentrations at 50% (EC_{50}) of copper (total content) for plant responses in artificially (n = 4) and industrially (n = 7) contaminated soils (ANOVA, $p < 0.05$), based on review data [24, 25]. Error bars represent standard deviation.

Regrettably, these approaches alone do not always provide a conclusive identification of the leading toxicant in instances of polymetallic contamination. Frequently, the responses of biota in ecotoxicological experiments show equally strong correlations with the content of several metals in the soil [39, 40].

SOIL METAL POOLS

It is widely recognized that the total content of nutrients in soils is insufficient to accurately predict their potential phytoavailability [41]. Similarly, this principle applies to metals, as it is widely accepted that their total content alone cannot reliably indicate their potential toxicity [42]. There are numerous instances where soils have exhibited remarkably high total metal contents, yet no signs of toxicity have been observed. A notable example of this is seen in soils impacted by the activities of the Kargaly copper mines in the Orenburg region in Russia, where copper mining and smelting occurred from the 18th to the 20th century [43]. Even today, remnants of malachite-bearing copper ore ($CuCO_3 \cdot Cu(OH)_2$) can still be found on the soil surface. Despite the extremely high total copper content in the investigated soils (~10 g/kg), the concentration of its exchangeable form was surprisingly low (<0.5 mg/kg) [44]. Remarkably, the high total copper content in these soils did not adversely affect plant growth, and the low phytotoxicity of the contaminated soils can be attributed to the limited solubility of malachite [44].

In recent decades, numerous attempts have been made to predict the bioavailable fraction of metals in soils by analyzing correlations between organismal responses and different soil metal pools [45]. A consensus has now been reached that the exchangeable forms of metals, i.e., the fraction extracted using chemically non-aggressive neutral salts (such as a 0.05 M solution of $CaCl_2$ or a 0.01 N solution of KNO_3), are the most informative for assessing their toxicity in soils [46]. For example, in the study of industrially contaminated soils and the analysis of correlations between plant responses and copper concentrations, the exchangeable form of copper in the soil was found to be the most reliable indicator of phytotoxicity compared to total and other pools of this element in the soil [47]. An alternative approach to assessing potential toxicity is to evaluate the activity of free metal ions in the soil solution [48].

On the other hand, it is important not to ignore other metal pools. The review [25] presents research results that analyze correlations between responses of different biota and major metal pools in soil. Although the data obtained are contradictory, the majority of the studies reviewed [25] show that the responses of plants and microorganisms can also be predicted by the total metal content, almost as successfully as by the bioavailable fractions (free ions, water-soluble and exchangeable forms). The following discussion will explore how these results align with the intensity/capacity/quantity concept.

INTENCITY/CAPACITY/QUANTITY CONCEPT

The concept of intensity/capacity/quantity was initially proposed to describe the processes of nutrient uptake by plants [49]. In this concept, intensity refers to the concentration of an element in the soil solution, quantity represents the total content of the element in the soil, and capacity refers to the buffering capacity of the soil, which determines the kinetics of ion release from the solid soil phase into the soil solution. It is assumed that metals present in the soil solution are available to plant roots at all times.

Therefore, the uptake of elements by plants depends not only on their concentrations in the soil solution (intensity), but also on the total content of elements in the soil (quantity) and the kinetics of their release from the solid soil phase into the soil solution (capacity). The same principle applies to the phytotoxicity of metals in soil, which is also influenced by the intensity/capacity/quantity factors [39]. It is reasonable to assume that these same factors influence the toxicity of metals not only to plants but also to soil-dwelling organisms.

Therefore, the toxicity of metals in soil can be influenced by various metal pools. Ultimately, the uptake of metals by plant roots and their phytotoxicity are contingent on the concentration of metal ions in the soil solution. However, the release of ions into the soil solution is determined by the total metal content and factors that control the kinetics of their transition from the solid phase to the liquid phase. It is precisely for this reason that the total metal content can predict plant and microorganism responses as effectively as bioavailable fractions.

COMPARISON OF COPPER AND ZINC ECOTOXICITY

A single value of the effective concentration for a specific organism and response indicator is clearly insufficient for comparing the ecotoxicity of different metals. It is more reliable to average the values of effective concentrations across different species and response indicators [50]. Table 1 presents averaged data on the ecotoxicity of copper and zinc in industrially contaminated soils, based on materials from the review by [25]. The term "extractable forms" refers to data expressed in mg/kg of soil, and "soluble forms" refers to data expressed in μg/L of soil solution or extractant. In turn, pMe^{2+} represents the negative decimal logarithm of the activity of Cu^{2+} or Zn^{2+} ions (similar to pH, lower pMe^{2+} values indicate higher activity of free metal ions).

The data from different studies (see Table 1) were highly variable, and aggregation of these data revealed numerous gaps that prevented the identification of statistically significant differences for all comparisons. However, it can be asserted that zinc is less toxic to plants and invertebrates compared to copper. Data on the activity of free metal ions also support this trend (although only one study provided estimates of EC_{50} for free zinc ions). For microorganisms, such an analysis is not possible due to a lack of data.

On the other hand, the "total" averaging of effective concentrations ignores the concept of a hierarchical cascade of biological responses to stressors, where resistance to metal-induced stress correlates with the level of biological organization [51]. Typically, lower levels (molecular, cellular, and individual) are more sensitive to stress than higher levels (population and community) [51]. Figure 2 shows the averaged EC_{50} values for different metal pools considering various levels of biological organization. The series obtained confirm the pattern observed for other stressors, i.e., an increase in metal toxicity thresholds with increasing

Table 1. Effective concentrations (EC_{10} and EC_{50}) of different copper and zinc soil pools for plants and invertebrates in industrially polluted soils, considering all levels of biological organization and only the individual level, based on review data [25]

Metal	All levels of organization				Individual level only			
	plants		invertebrates		plants		invertebrates	
	EC_{10}	EC_{50}	EC_{10}	EC_{50}	EC_{10}	EC_{50}	EC_{10}	EC_{50}
Total content, mg/kg								
Cu	369 ± 151	987 ± 491	233 ± 218 A**	458 ± 461 A***	391 ± 139	991 ± 489	253 ± 187 A*	544 ± 494 A***
Zn	217	1561 ± 2987	716 ± 295 B**	1779 ± 1091 B***	217	1472 ± 3154	716 ± 295 B*	2294 ± 1343 B***
Extractable form, mg/kg								
Cu	–	330 ± 520	–	398	–	330 ± 520	–	398
Zn	–	317 ± 901	–	70 ± 98	–	330 ± 929	–	70 ± 98
Dissolved form, μg/L								
Cu	289 ± 31	382 ± 213 A***	–	–	391	348 ± 232 A**	–	–
Zn	275 ± 177	4117 ± 2052 B***	–	21 135 ± 28 093	275 ± 177	4117 ± 2052 B**	–	21135 ± 28093
Free ion activity, pMe^{2+}								
Cu	7.3 ± 0.3	6.1 ± 0.3	–	–	6.9	6.1	–	–
Zn	–	3.4	–	–	–	3.4	–	–

Mean ± standard deviation is presented. Capital letters in the column for a specific metal pool indicate statistically significant differences between metals (* $p < 0.1$, ** $p < 0.05$, *** $p < 0.01$).

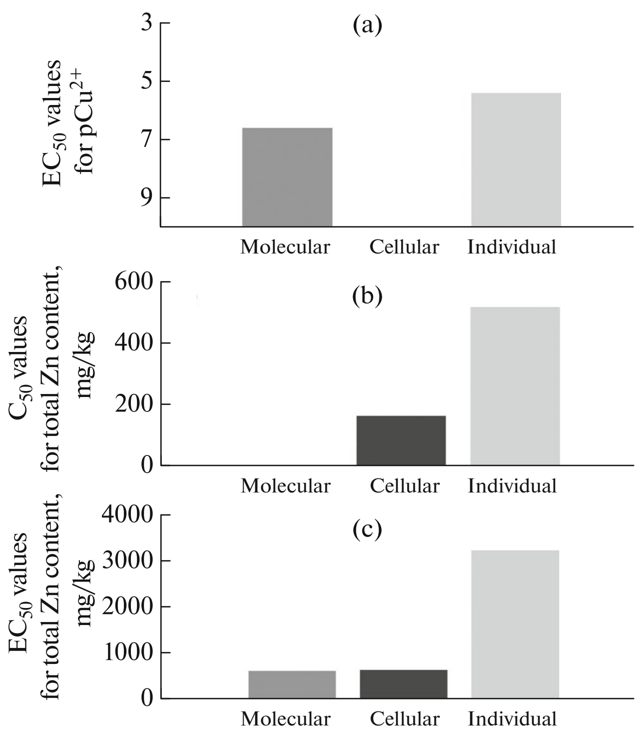

Fig. 2. Comparison of effective concentrations at 50% (EC_{50}) for responses at different levels of biological organization in industrially contaminated soils: (a) EC_{50} of copper activity for *Helianthus annuus* responses [71], (b) EC_{50} of zinc total content for *Eisenia fetida* responses [72], (c) for *Lumbricus rubellus* responses [51]. Values for pCu^{2+} are presented in reverse order to emphasize that a higher bar indicates higher activity of free Cu^{2+} ions.

levels of biological organization: molecular < cellular < individual.

Thus, averaging the effective concentration values for response parameters at different levels of biological organization can distort the assessment of metal toxicity. However, the majority of toxicity studies on copper and zinc focus solely on the individual level responses, with insufficient data available for separate analysis at other levels [25]. Nevertheless, analyzing the data solely at the individual level did not alter the interpretation of the results presented in Table 1, confirming that zinc is less toxic to plants and invertebrates than copper.

ANTAGONISM BETWEEN COPPER AND ZINC

The interaction of zinc with other metals has been relatively well studied at the cellular and individual levels. Zinc is known to counteract oxidative stress induced by cadmium [52–55]. Zinc can protect lipids from oxidative degradation caused by cadmium and copper, and it can also enhance the biosynthesis of antioxidant enzymes [15, 56, 57]. It has been hypothesized that Zn^{2+} may compete with Cu^{2+} for the same functional groups in cell membranes or proteins, leading to inhibition of copper uptake by plants [58, 59]. The close physicochemical properties of Cu^{2+} and Zn^{2+} further explain their antagonistic interactions [60].

In a study by [61], the use of zinc-containing fertilizers suppressed the uptake of copper by rice plants, demonstrating antagonistic interactions between zinc and copper. However, there is limited research on the interactions of zinc with metals in contaminated soils. We are aware of only three studies that used industrially contaminated soils and directly investigated the protective effect of zinc against copper toxicity. Let us review the results of these studies.

In the study by [62], the efficiency of symbiotic nitrogen fixation by *Rhizobium* bacteria was used as a response variable. The principle of the method was simple: beans were grown in perlite-containing (i.e., soil-free) pots and irrigated with sterile nitrogen-free nutrient solution; subsequently, the tested soil samples were introduced into the pots as inoculants. Given that symbiotic nitrogen fixation is the only nitrogen source in this system, the dry shoot mass can be used as a measure of its effectiveness. Agricultural soils contaminated by copper mining activities in the central Chile were analyzed.

It is known that high concentrations of copper and zinc inhibit nitrogen fixation in soils, with copper being more toxic than zinc. For example, the effective concentration (EC_{50}) for total metal content in sewage sludge-enriched agricultural soils with respect to symbiotic nitrogen fixation by clover was 334 mg/kg for zinc and 99 mg/kg for copper [63]. In another study [64], the minimum effective concentration of zinc for clover nitrogen fixation was even higher, ranging from 614 to 876 mg/kg. In the soils tested in the discussed study [62], the total copper content in most cases exceeded the mentioned toxicity threshold for symbiotic nitrogen fixation, while the total zinc content was significantly lower. This led to the conclusion that the zinc content in the studied soils did not reach a toxic level for symbiotic nitrogen fixation.

The results obtained in the study by [62] are presented in Fig. 3, showing a clear inverse relationship between the dry shoot mass of beans and the molar ratio of Cu/Zn in soil, while the correlation with the total copper content was not statistically significant. In other words, toxicity begins to manifest when the copper content in the soil is no longer "balanced" by zinc. The Cu/Zn ratio used in [62] is conceptually similar to the (Ni + Cu)/(Ca + Mg) ratio proposed in [65, 66] for soils contaminated by a copper-nickel smelter on the Kola Peninsula. However, in [62], the soil Cu/Zn ratio explained the variation in response parameters better than the Cu/Ca and Cu/Mg ratios.

Studies [67] and [68] demonstrated a protective effect of zinc against copper phytotoxicity. Using agricultural soils contaminated by copper mining activities in Chile [67], a negative (toxic) effect of total soil cop-

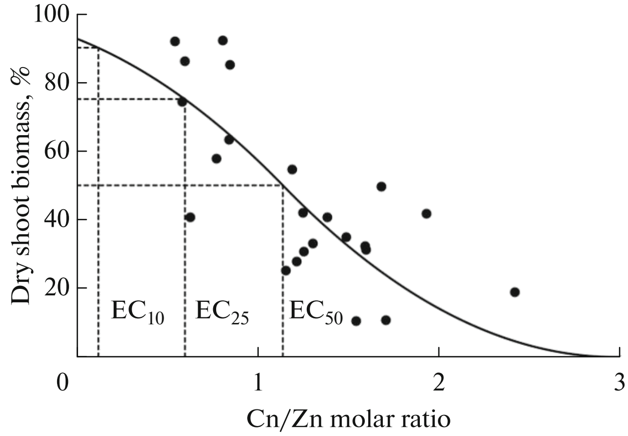

Fig. 3. Dry shoot biomass of beans as a function of the molar ratio of Cu/Zn in soil, based on the data from [62].

per content and a positive (protective) effect of total soil zinc content were observed in relation to the growth of lettuce and oat shoots (Fig. 4, Table 2). These results also led to the recommendation to use the molar Cu/Zn ratio in soil as a predictor of toxicity [67]. Importantly, the soil Cu/Zn ratio explained plant growth better than total copper content (see Table 2). In the work of [68], the Cu/Zn concentration ratio in plant tissues was a good indicator of ryegrass growth.

To assess the tolerance of biota to metals, the effective Cu/Zn ratio can be employed, which was proposed following the same principles as the effective concentration. For symbiotic nitrogen fixation, the EC_{50} for the Cu/Zn ratio was determined to be 1.2 (Fig. 3), whereas for the growth rate of oats and lettuce, the values were 5.9 and 7.0, respectively (Fig. 5). These findings are consistent with other studies indicating a greater sensitivity of soil microorganisms to metals when compared to plants [48, 69].

CONCLUSION: PROSPECTS FOR FUTURE RESEARCH

Our review has identified notable gaps in the current understanding of copper-zinc antagonism in industrially contaminated soils, despite its potential practical applications. This situation contrasts with the relatively well-studied phenomenon of copper-

Table 2. Regression relationships of plant growth responses to soil copper and zinc content, based on the data from the study by [67]

Predictor	Regression Equation	R^2	p
	Lettuce, shoot length		
Total Cu	10.80 – 0.004 Cu	0.20	0.019
Total Zn	Statistically insignificant	–	–
Total Cu and Zn	8.00 – 0.005 Cu (p = 0.001) + 0.021 Zn (p = 0.003)	0.45	0.001
Cu/Zn	11.44 – 0.810 Cu/Zn	0.38	0.001
	Oats, shoot length		
Total Cu	10.94 – 0.005 Cu	0.28	0.015
Total Zn	Statistically insignificant	–	–
Total Cu and Zn	8.27 – 0.006 Cu (p=0.002) + 0.020 Zn (p=0.013)	0.49	0.002
Cu/Zn	11.37 – 0.822 Cu/Zn	0.39	0.003
	Oats, shoot dry weight		
Total Cu	Statistically insignificant	–	–
Total Zn	Statistically insignificant	–	–
Total Cu and Zn	0.13 – 0.0001 Cu (p = 0.032) + 0.0006 Zn (p = 0.060)	0.30	0.043
Cu/Zn	0.22 – 0.022 Cu/Zn	0.23	0.028

The coefficient of determination (R^2) and significance level (p) are indicated.

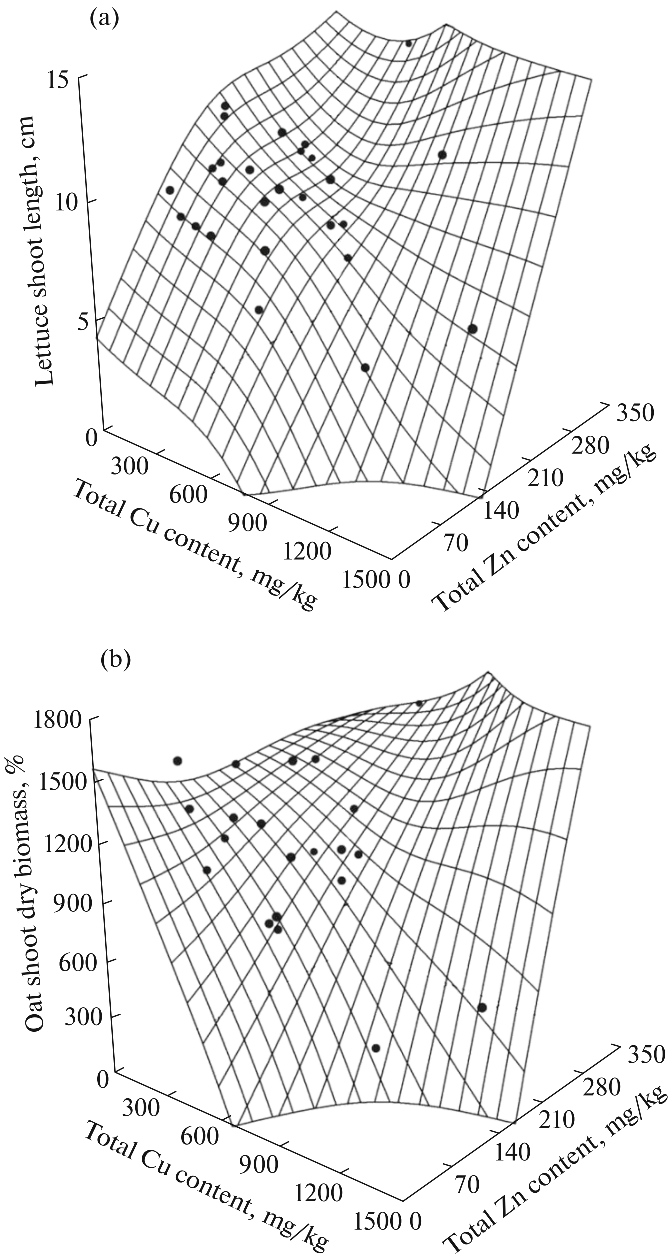

Fig. 4. Effects of total soil contents of copper and zinc on (a) lettuce shoot length and (b) oat shoot dry biomass, based on the data from [67].

zinc antagonism in aquatic ecosystems. Further research in this area can proceed in several directions.

Firstly, the results examined regarding the reduction of copper toxicity by zinc apply only to microorganisms and plants, and for a limited set of response indicators. It is essential to expand the range of response indicators tested for microorganisms and plants, and to include other species. For example, conducting experiments with earthworms is promising: one study [70] demonstrated the protective effect of zinc on cadmium uptake by earthworms in sewage sludge-enriched soils, suggesting that similar effects may apply to copper.

Secondly, the studies discussed in the review cover a very limited range of soils and industrial pollution scenarios; in effect, they examine soils of a single type in a single region. It is imperative to diversify the situations studied, including different soil properties (texture, organic matter content, pH), natural conditions, and different types of polluting industries.

Finally, the study of the effects of the artificial introduction of zinc additives, such as zinc fertilizers

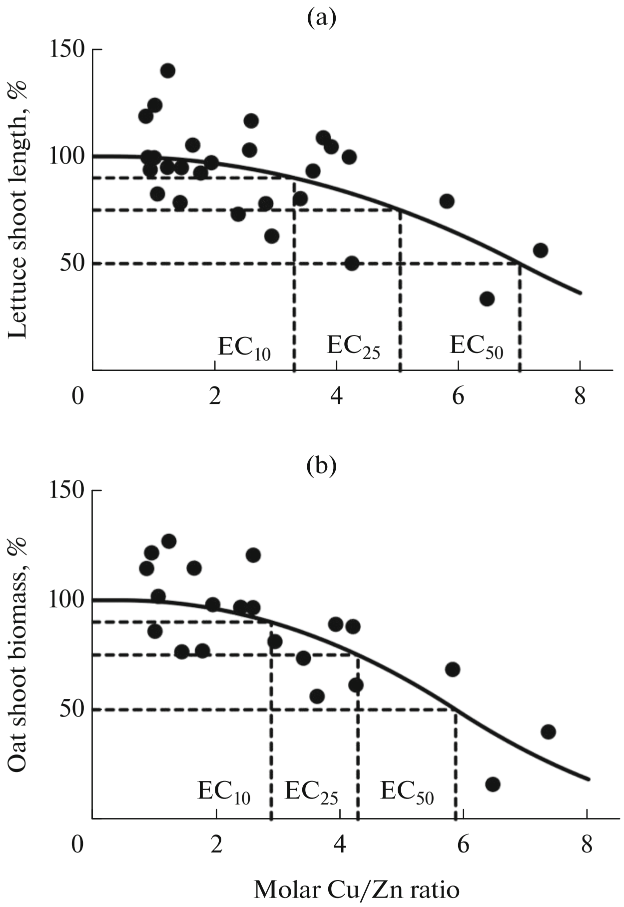

Fig. 5. Lettuce shoot length (a) and oat shoot dry biomass (b) as a function of Cu/Zn molar ratio in soil, based on the data from [67].

($ZnSO_4$), to reduce copper toxicity is promising. Surprisingly, this approach has never been tested in industrially contaminated soils. It is essential to evaluate the method's effectiveness in remediation, taking into account economic considerations, and to identify any limitations, especially the threshold levels of soil zinc contamination at which zinc fertilizer application remains viable.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The article does not contain any studies involving humans or animals in experiments performed by any of the authors.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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