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Research paper

Nickel phytotoxicity thresholds for ryegrass: Insights from historical copper smelting sites in Bashkortostan, Russia

Umbrales de fitotoxicidad del níquel para ballica: Hallazgos desde los históricos sitios de fundición de cobre en Bashkortostán, Rusia

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Abstract

Environmental degradation due to the operation of non-ferrous metal smelters is a significant issue in many countries worldwide. In particular, copper smelting in the Ural Mountains has led to metal contamination of soils. This study conducted an ecotoxicity evaluation using ryegrass (*Lollium perenne* L.) as a bioindicator in 25 soils collected in Kananikolskoe and Zilair villages, Southern Urals, Republic of Bashkortostan, Russia. These study sites were former copper smelters, active during the 18th-19th centuries and, thus, copper phytotoxicity was anticipated in the soils under investigation. However, the effect of shoot copper concentrations on plant growth was statistically insignificant. Among all metals, nickel was the primary determinant of phytotoxicity, echoing the findings of other studies on copper-nickel contaminated soils. The obtained 25% and 50% effective concentrations (EC_{25} and EC_{50}) for shoot nickel concentrations were 44 (28-59) and 52 (39-64) mg kg^{-1}, respectively, with values in parentheses representing the 95% confidence intervals. It is important to note that there are limited reports on nickel phytotoxicity thresholds for ryegrass, making this study a valuable contribution to ecotoxicological research. Furthermore, the reported critical nickel concentrations in ryegrass shoots vary widely, from 14 to 90 mg kg^{-1}. The EC_{25} and EC_{50} values derived in this study fall within the reported range, underscoring the reliability of the results obtained.

Keywords: Heavy metals, *Lolium perenne*, Ecotoxicity.

Resumen

*La degradación ambiental debido al funcionamiento de fundiciones de metales no ferrosos es un problema importante en muchos países del mundo. En particular, las fundiciones de cobre en los Montes Urales han provocado una contaminación del suelo por metales. Este estudio realizó una evaluación de ecotoxicidad utilizando ballica (*Lollium perenne *L.) como bioindicador en 25 suelos recolectados en las localidades Kananikolskoe y Zilair, Urales del Sur, República de Bashkortostán,*

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Rusia. Estos sitios de estudio fueron antiguas fundiciones de cobre, activas durante los siglos XVIII y XIX y, por lo tanto, se anticipó la fitotoxicidad del cobre en los suelos bajo investigación. Sin embargo, el efecto de las concentraciones de cobre en los brotes sobre el crecimiento de las plantas no fue estadísticamente significativo. Entre todos los metales, el níquel fue el principal determinante de la fitotoxicidad, similar a los resultados de otros estudios sobre suelos contaminados con cobre y níquel. Las concentraciones efectivas del 25% y 50% (CE_{25} y CE_{50}) obtenidas para las concentraciones de níquel en brotes fueron 44 (28-59) y 52 (39-64) mg kg^{-1}, respectivamente, con valores entre paréntesis representando los intervalos de confianza del 95%. Es importante señalar que existe limitada información sobre los umbrales de fitotoxicidad del níquel para ballica, lo que hace que este estudio sea una contribución valiosa a la investigación ecotoxicológica. Además, las concentraciones críticas de níquel informadas en los brotes de ballica varían ampliamente, de 14 a 90 mg kg^{-1}. Los valores de EC_{25} y EC_{50} obtenidos en este estudio se encuentran dentro del rango informado, lo que subraya la confiabilidad de los resultados obtenidos.

Palabras clave: *Metal pesado,* Lolium perenne*, Ecotoxicidad.*

Introduction

Environmental degradation due to the operation of non-ferrous metal smelters is a significant issue in many countries worldwide (Ettler, 2016). In particular, copper smelting in the Ural Mountains has led to metal contamination of soils (Semenova *et al.*, 2018; Polyakov *et al.*, 2024). The term “metals,” as used in this study, was previously referred as “heavy metals.” However, the International Union of Pure and Applied Chemistry no longer endorses the term “heavy metals” (Duffus, 2002).

It should be noted that ecotoxicological studies on the toxicity of metals in soils are usually based on uncontaminated soils that have been gradually enriched with metals in the form of soluble salts under laboratory conditions. However, the results from artificially contaminated soils can be difficult to extrapolate to real-world soils (Neaman *et al.*, 2020; Santa-Cruz *et al.*, 2021). The difficulty in interpreting real-world contaminated soils arises from the presence of multiple metal contaminants in the soil and the unclear impact of specific metals on the response of plants and soil organisms (Zhikharev *et al.*, 2022). Nonetheless, our previous studies show that once metal concentrations in plant tissues are determined, it is possible to discern the toxic effects of specific metals (Tarasova *et al.*, 2020) and, in some cases, even calibrate metal toxicity thresholds (Verdejo *et al.*, 2015).

In contrast, the study by Verdejo *et al.* (2023) demonstrated that potential ecotoxicological risks at metal contaminated sites are highly variable and must be evaluated on a case-by-case basis. Therefore, we decided to carry out an ecotoxicological assessment of soils contaminated by historical copper smelting operations in the Ural Mountains. Our attention was drawn to sites contaminated by the operation of the Kananikolsky and Preobrazhensky former copper smelters located in Southern Urals, Republic of Bashkortostan, Russia, which were active during the 18th-19th centuries (Alaeva *et al.*, 2021). For this study, we chose perennial ryegrass (*Lolium perenne* L.), an excellent bioindicator of copper toxicity (Tarasova *et al.*, 2020).

Materials and methods

Soil characterization

The soils studied were collected in Kananikolskoe and Zilair villages, where soils were contaminated by the operation of the Kananikolsky and Preobrazhensky former copper smelters, respectively. In this study, we used 25 soil samples, collected at different distances from the respective smelter (**Table 1**). Soil samples were dried at 40°C for 48 h and sieved through a 2-mm mesh. Total metal concentrations in the soils (**Table 2**) were measured by an atomic absorption spectrometer after microwave digestion with a mixture of concentrated HNO_3 and H_2O_2. Standard reference samples were used in the analysis, and experimental values of the target metals were within 100 ± 20% of the certified values.

Soil pH was measured in a 0.01 M KNO_3 extract solution (soil/solution ratio 1/2.5). An attempt was made to quantify soluble metals in the same extract solution, but their concentrations were below the detection limit of the instrument. Organic matter content was determined by the wet combustion method using sodium dichromate and sulfuric acid (Sadzawka *et al.*, 2006). The particle size distribution of sand, silt, and clay was estimated by a simplified hydrometer method (Sheldrick and Wang, 1993). Finally, the carbonate content of the soil was estimated by a titrimetric method (FAO, 2020) (**Table 1**).

Phytotoxicity assessment

The perennial ryegrass plants were grown for 21 days under controlled conditions (ISO 11269-2, 2012). The growth chamber was set with a photoperiod of 16 hours and a photosynthetically active radiation of

Table 1. Geographical coordinates, distance from each respective smelter, and soil characteristics of the sampling locations in the Ural Mountains near the Kananikolsky and Preobrazhensky former copper smelters. SOM denotes soil organic matter. Soil characteristics include pH, SOM, calcium carbonate content, and particle size distribution.

Sample	Distance, m	Coordinates (north/east)	pH	$CaCO_3$, %	SOM, %	Sand, %	Clay, %	Silt, %
Kananikolsky smelter								
1	0	52°48’46”/57°43’30”	7.2	0.26	11	61	0.82	38
2	100	52°46’51”/57°28’56”	7.3	0.32	5.9	71	5.6	23
3	100	52°47’00”/57°29’17”	7.1	0	11	61	3.3	36
4	100	52°47’00”/57°29’20”	7.2	0.68	13	51	3.9	45
6	200	52°46’58”/57°29’24”	7.2	0	5.6	61	4.9	34
7	500	52°46’51”/57°29’31”	7.2	0.21	6.6	61	2.5	37
9	3000	52°46’13”/57°29’53”	7.2	0.37	5.0	71	4.2	25
10	5000	52°46’32”/57°31’15”	7.2	0.26	5.1	61	3.3	36
11	100	52°47’70”/57°29’17”	7.2	2.1	12	56	5.4	39
12	500	52°47’21”/57°29’31”	7.2	0	21	60	15	25
13	12000	52°37’34”/57°33’14”	7.1	0.26	11	62	4.8	33
14	20000	52°37’37”/57°29’26”	6.9	6.2	14	31	3.3	66
Preobrazhensky smelter								
16	100	52°13’45”/57°25’29”	7.0	3.2	5.9	61	1.6	37
17	100	52°13’50”/57°25’32”	7.1	0.74	7.6	61	4.9	34
18	3000	52°14’38”/57°25’14”	7.2	0	14	51	2.0	47
19	2500	52°14’46”/57°28’2”	7.2	0.15	8.4	53	3.8	44
20	2000	52°14’13”/57°28’6”	7.0	0	11	52	5.8	42
21	1500	52°14’36”/57°25’50”	7.2	0.21	10	42	4.7	53
22	1000	52°14’27”/57°25’50”	7.1	0.10	11	51	3.9	45
23	500	52°13’57”/57°25’46”	7.2	1.2	13	41	2.4	56
24	1000	52°13’37”/57°26’26”	7.1	0.15	14	31	0	70
25	1500	52°13’41”/57°26’29”	7.2	1.7	15	52	1.9	46
26	2000	52°13’37”/57°26’26”	7.2	0.61	7.5	71	2.8	26
27	2500	52°13’50”/57°26’7”	7.1	0.21	6.7	51	3.9	45
28	3000	52°14’57”/57°27’25”	7.2	0.21	9.6	62	4.9	33

206 ± 38 μM m^{-2} s^{-1}. In each pot, 50 seeds were planted. Soils were watered by spraying the surface with distilled water every two days. All soils were fertilized with a commercially available universal fertilizer containing macro- and micronutrients. The low nutrient requirements of ryegrass at early growth stages (Verdejo et al., 2015) ensured that plant growth was not limited by deficiencies.

At the end of the experiment (day 21), plants were harvested by cutting them off with scissors. The number of plants was counted. The plants were thoroughly washed in the following sequence: tap water, distilled water, and distilled water again. Subsequently, the plants were dried in an oven at 70°C for 48 h to determine biomass, expressed in mg $plant^{-1}$. Shoot concentrations of elements (**Table 2**) were determined by an atomic absorption spectrometer after

Table 2. Total metal concentrations in soil, shoot metal concentrations, and shoot dry weight (DW). Note: shoot concentrations of Cr were below the detection limit (<DL).

Sample	Soil total concentrations, mg kg^{-1}						Shoot concentrations, mg kg^{-1}					Shoot DW, mg plant^{-1}
	Cd	Cr	Cu	Ni	Pb	Zn	Cd	Cu	Ni	Pb	Zn	
Kananikolsky smelter												
1	0.23	150	155	128	30	177	3.5	24	48		328	6.7
2	0.21	128	113	125	17	97	3.3	9.5	61	44	113	1.7
3	0.24	114	157	101	23	144	0.38	15	13	13	101	8.9
4	0.39	139	444	117	24	136	4.6	27	12	48	175	10
6	0.32	114	74	83	16	82	1.4	14	13	19	125	6.5
7	0.3	106	91	87	20	104	2.6	11	9.2	31	91	11
9	0.21	78	33	78	12	78	3.8	14	19	59	127	9.5
10	0.26	59	34	55	11	100	1.5	16	16	10	161	8.3
11	0.39	86	180	77	28	155	0.40	16	2.6	2.6	152	10
12	0.51	45	91	40	34	227	2.7	15	9.1	16	170	12
13 (1)	0.43	155	171	95	25	114	1.1	8.8	11	19	212	11
14	0.35	45	47	34	15	69	3.2	10	13	27	134	15
Preobrazhensky smelter												
16	1.9	166	1033	109	198	352	4.9	12	8.9	17	102	3.9
17	0.67	154	227	109	37	149	5.8	7.5	10	21	67	13
18	0.32	211	59	115	14	95	8.7	10	13	16	98	10
19	0.23	92	27	58	10	59	4.9	9.4	12	21	112	10
20	0.36	111	39	69	13	77	8.4	18	14	19	109	12
21	0.24	93	47	57	11	59	1.6	15	30	12	142	9.2
22	0.39	97	46	64	12	65	2.2	14	25	<DL	122	12
23	1.1	148	841	80	106	625	1.1	38	15	18	353	12
24	0.53	134	344	86	31	248	2.5	34	12	4.3	216	14
25	0.52	175	309	111	26	132	7.0	35	24	5.4	198	12
26	0.24	55	22	34	12	54	5.9	37	26	6.8	237	12
27	0.25	39	15	22	11	54	7.8	33	24	8.1	220	11
28	0.25	197	45	131	12	71	8.3	35	32	13	214	8.9

microwave digestion with a mixture of concentrated HNO_3 and H_2O_2.

The 25% and 50% effective concentrations (EC_{25} and EC_{50}) and their 95% confidence intervals were estimated using the Toxicity Relationship Analysis Program (TRAP) (US EPA, 2016). Shoot element concentrations were used as a dose variable, and shoot dry weight as a response variable. The reference value of 100% was based on the shoot dry weight of plants with the lowest shoot element concentrations.

Results and discussion

Most of the soils were calcareous, with a circumneutral pH and a medium-high organic matter content. Sand and silt were the predominant particle-size fractions in the soils studied (**Table 1**). The concentrations of metals in these soils varied widely. Similarly, shoot dry weight and shoot metal concentrations showed significant variation (**Table 2**).

In soils contaminated by historical copper smelting activities, copper phytotoxicity was expected. However, the effect of shoot copper concentrations on plant growth was statistically insignificant (**Figure 1**). Among all metals, nickel was the primary determinant of phytotoxicity (**Figure 2**). Similar results were reported by Tarasova et al. (2020) for copper-nickel contaminated soils: the effect of shoot copper concentrations on plant responses was not significant, whereas nickel was the element most strongly influencing plant growth.

The values of EC_{25} and EC_{50} for shoot nickel concentrations were derived using shoot dry weight as a response variable (**Figure 2**). The obtained EC_{25} and EC_{50} values were 44 (28-59) and 52 (39-64) mg kg^{-1}, respectively, where values in parentheses are the 95% confidence intervals. It is important to note that there are limited reports on nickel phytotoxicity thresholds for ryegrass, making this study a significant novel contribution to ecotoxicological research.

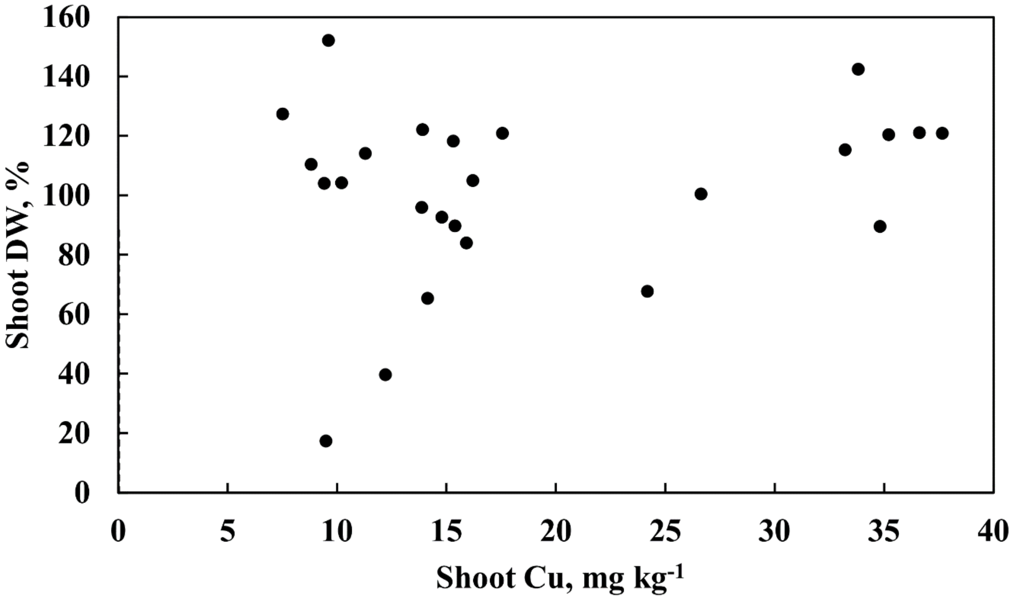

Figure 1. Ryegrass shoot dry weight as a function of its shoot copper concentration.

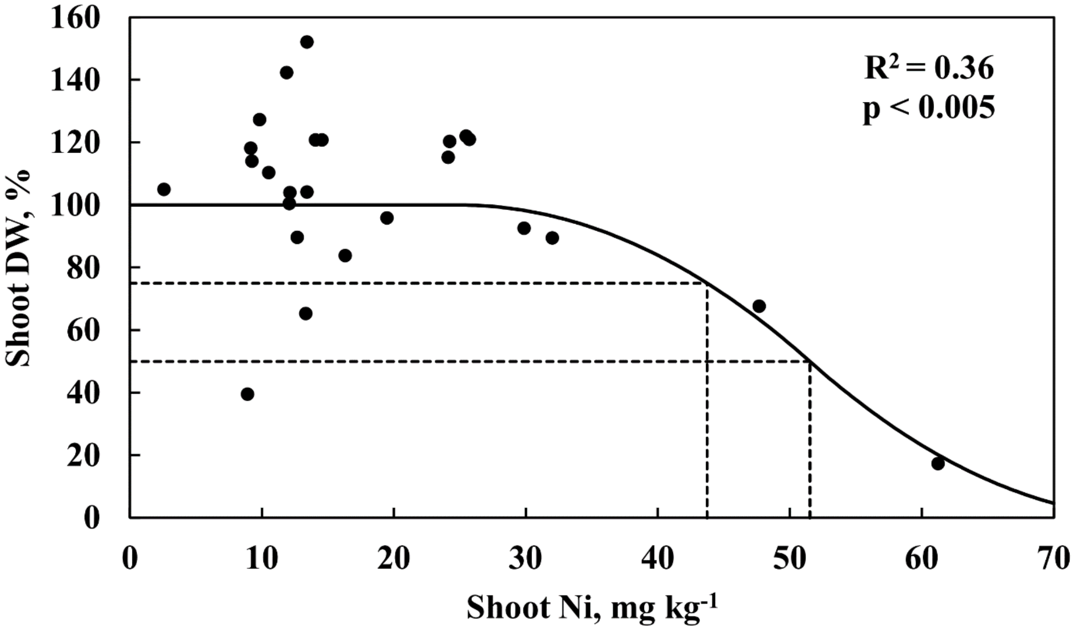

Figure 2. Impact of shoot nickel concentration in ryegrass on its shoot dry weight (DW). Effective concentrations 25% and 50% (EC_{25} and EC_{50}) are shown with dotted lines.

Existing reports on critical nickel concentrations in ryegrass shoots are inconsistent, ranging from 14 mg kg^{-1} (Davis and Beckett, 1978) to 90 mg kg^{-1} (Davis and Carlton-Smith, 1984). The EC_{25} and EC_{50} values derived in this study fall within the reported range, thereby supporting the credibility of our findings.

Practical implications and future study needs

Contaminated areas near non-ferrous metal smelters can act as sources of secondary pollution (Luo et al., 2014). Due to prolonged accumulation of pollutants and deterioration of vegetation cover, these areas lose their ability to bind metals, becoming uncontrolled sources of air and water pollution. Consequently, this study has significant practical implications for environmental assessment and decision-making regarding the contaminated soils.

Future research should focus on controlling the mobility of metals in the soil by applying various additives to reduce the concentration of metals in the soil solution. Although this method cannot eliminate metals from the soil, it can convert them into less soluble forms. A critical criterion for the success of metal immobilization in soil is the reduction of toxic effects of metals on plants and soil organisms (Dovletyarova et al., 2024).

Conclusion

This study underscores the significant environmental impact of historical copper smelting in the Ural Mountains, revealing extensive soil contamination and highlighting nickel as the primary phytotoxic metal. The derived EC_{25} and EC_{50} values for nickel (44 mg kg^{-1} and 52 mg kg^{-1}) align with existing literature, reinforcing the study's credibility. These findings emphasize the need for site-specific ecotoxicological assessments. Future research should focus on methods to immobilize metals in contaminated soils, reducing their bioavailability and toxic effects. Overall, the study provides essential insights for environmental remediation and sustainable land management.

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