The challenge of reducing metal phytotoxicity in soils affected by historical nickel-copper smelting operations in the Kola Peninsula, Russia

El desafío de reducir la fitotoxicidad de metales en suelos afectados por operaciones históricas de fundición de níquel y cobre en la Península de Kola, Rusia

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ABSTRACT

The topic of vegetative cover rehabilitation of industrial barrens sites has a worldwide significance, especially given the potential of the polluted soils themselves to be "secondary pollution sources." The goal of this study was to determine the effect of peat addition to the lime + vermiculite-lizardite combined amendment on plant growth in the soils from the vicinity of the Ni/Cu smelter in the Kola Peninsula, Russia. Given the complexity and time-consuming nature of field studies, we decided to test the selected amendments under laboratory conditions first. Surface horizons from two Spodosols (0-20 cm) were collected in the vicinity of the Ni/Cu smelter. *Lolium perenne* was cultivated in pots for 21 days under controlled conditions. The difference in plant growth with and without peat was not substantial. In other words, the addition of peat did not improve the efficacy of the lime + vermiculite-lizardite combined amendment in promoting plant growth. Therefore, the challenge of reducing ryegrass exposure to soil metals remained unsolved.

RESUMEN

El tema de la restauración de la cubierta vegetal en áreas industriales degradadas tiene una importancia mundial, debido a su potencial como fuente secundaria de contaminación. El objetivo de este estudio fue determinar el efecto de la adición de turba a la enmienda combinada de cal + vermiculita-lizardita sobre el crecimiento de las plantas en los alrededores de una fundición de níquel y cobre, en la Península de Kola, Rusia. Dada que los estudios de campo son complejos y precisan tiempo, se decidió probar primero las enmiendas en condiciones de laboratorio. Se muestrearon los horizontes superficiales de dos Spodosoles (0-20 cm) en las cercanías de dicha fundición. La especie *Lolium perenne* se cultivó en tiestos durante 21 días, en condiciones controladas. La diferencia en el crecimiento de las plantas entre los tratamientos con y sin turba no fue sustancial. En otras palabras, la adición de turba no mejoró la eficacia de la enmienda combinada de cal + vermiculita-lizardita para promover el crecimiento de las plantas en los suelos de las cercanías de la fundición. Por lo tanto, el desafío de reducir la exposición del *L. perenne* a metales en los suelos estudiados quedó sin resolver.

Palabras clave: metales pesados, fitorremediación, fitoestabilización, *Lolium perenne,* fitotoxicidad.

INTRODUCTION

Airborne pollutants emitted by non-ferrous smelters may turn surrounding land into industrial barrens (also called industrial deserts or brownfields), which are extremely harsh habitats for plant communities (Kozlov and Zvereva, 2007; Kadulin and Koptsik, 2019). Thus, the topic of vegetative cover rehabilitation on industrial barrens has a worldwide significance, especially given the potential of the polluted soils themselves to be "secondary pollution sources" (Luo *et al.*, 2014). One such barren is found in the vicinity of the nickel-copper (Ni/Cu) smelter of the Kola Mining and Metallurgical Company (67°56′ N, 32°49′ E), located on the Kola Peninsula, Murmansk region, Russian Federation (Slukovskaya *et al.*, 2020). Currently, this Ni/Cu smelter is known as the Monchegorsk Industrial Site; formerly, it was known as the Severonikel Smelter (hereafter, the smelter will be referred to as "the Ni/Cu smelter" for convenience.)

Copper and nickel are essential micronutrient for plants (López and Magnitski, 2011) but becomes toxic above a certain threshold (McBride, 1994; Adriano, 2001). One useful agent in reducing metal bioavailability in acidic contaminated soils is lime (Pardo *et al.*, 2018), which acts either by forming new solid phases (through metal precipitation or co-precipitation) or by promoting metal adsorption (McBride, 1994; Ma *et al.*, 2006). However, our preliminary experiment demonstrated that the application of lime does not sufficiently reduce metal phytotoxicity in soils collected in the proximity of the Ni/Cu smelter.

Several studies have been performed on the use of various clays, including vermiculite, as metal sorption agents in polluted soils (Malandrino *et al.*, 2011; Ma *et al.*, 2012; Xu *et al.*, 2017; Ou *et al.*, 2018). In this study, we proposed to use vermiculite-lizardite overburden waste material for reducing metal bioavailability. It is a waste product of a phlogopite open-pit mining operation located in the town of Kovdor, Murmansk region, Russian Federation (Kremenetskaya *et al.*, 2020). The enormous overburden stockpile contains several hundred thousand tons of vermiculite and lizardite mixture, posing complications for stockpile management. Thus, finding a local use for this waste material could potentially solve the problem of stockpile storage, and improve the feasibility and reduce the cost of soil remediation. The vermiculite-lizardite waste material is highly effective in reducing metal bioavailability because of its excellent metal sorption properties (Kremenetskaya *et al.*, 2012; Kremenetskaya *et al.*, 2020).

Our previous laboratory experiment demonstrated that lime and vermiculite-lizardite mixture is indeed an effective amendment to promote plant growth (shoot length and biomass) in soils collected from the surroundings of the Ni/Cu smelter (Tarasova *et al.*, 2020). However, we found that even plants grown in the amended soils displayed signs of metal phytotoxicity, suggesting that experiments with additional amendment combinations were needed. Organic amendments are effective metal sorbents suitable for remediation of metal-polluted soils (e.g., Venegas *et al.*, 2015). In this study, we chose peat as an organic amendment because of the widespread occurrence of unpolluted peat soils on the Kola Peninsula. We hypothesized that peat could be the additional amendment needed to increase the efficacy of the lime and vermiculite-lizardite mixture to promote plant growth in the surroundings of the Ni/Cu smelter.

Thus, the goal of this study was to determine the effect of peat addition to the lime + vermiculite-lizardite combined amendment on plant growth in the soils from the vicinity of the Ni/Cu smelter. We decided to test the selected amendments under laboratory conditions first, similar to what we did in a recent study (Tarasova *et al.*, 2020), before considering a field-scale investigation.

MATERIALS AND METHODS

Soils and Treatments

We used commercial dolomitic lime (BHZ brand, Russia) and commercial unpolluted peat (Veltorf brand, Russia) for our study. All-purpose fertilizer (Fertika brand, Russia) was used for one of the treatments according to manufacturer recommendations for grass species (0.1 g of fertilizer per 1 kg of substrate). The fertilizer had the following composition of macro- and micronutrients: NH_4-N 6.6%, NO_3-N 4.4%, P_2O_5 12%, K_2O 26%, MgO 0.4%, S 0.7%, Ca 0.55%, Mn 0.16%, Cu 0.08%, B 0.09%, Fe 0.16%, Zn 0.09%, Mo 0.008%.

Two topsoil samples (0-20 cm) classified as Spodosols (Soil Survey Staff, 2003) or Podzols (IUSS Working Group WRB, 2015) were collected in the proximity of the Ni/Cu smelter. They displayed signs of severe erosion: the A horizon was completely missing, whereas the E horizon was very thin. Importantly, the sampling point had little vegetation cover suggesting harsh conditions for plant growth. About 50 kg of each sample were collected and transferred to the laboratory. The samples were air-dried, sieved through a 2-mm mesh and then homogenized.

The four experimental treatments performed in the present study were as follows:

1) Unamended contaminated soil;
2) Dolomite (5% w/w) + vermiculite-lizardite (10% w/w);
3) Dolomite (5% w/w) + vermiculite-lizardite (10% w/w) + peat (5%);
4) Dolomite (5% w/w) + vermiculite-lizardite (10% w/w) + fertilizer (0.1 g kg^{-1}).

The purpose of the fourth treatment was to differentiate between the effects of peat as metal sorbent and nutrient availability modulator.

Eight replicates were set up for each treatment. Untreated and amended soils were placed into 0.5 L volume pots. The soils were moistened weekly and allowed to dry under room temperature (20-25 °C). The weekly wetting-drying cycles continued for one month to allow the amendments enough time to react in the soil. Three replicates were then subjected to chemical analyses and five were used for plant bioassay, as described in detail below.

Chemical characterization of the soils

Total elemental concentrations in soil and peat were established using the ICP-MS technique on a PerkinElmer ELAN 9000 DRC-e mass-spectrometer following microwave digestion with a mixture of concentrated $HF+HCl+HNO_3$ (Table 1). Standard reference materials, taken through the entire analysis process, provided experimental values of the trace metals of interest within 100 ± 20% of the certified values. Duplicates were performed on every 10th sample, in order to assure quality. Blanks were measured and they were always under limit of detection.

Exchangeable concentrations of Cu and Ni were established by atomic absorption spectroscopy (Agilent, model 240FS AA). The solution of KNO_3 0.01 mol L^{-1} was used as an extractant (soil/solution ratio of 1/2.5). The suspension thus obtained was shaken for 60 minutes and then filtered through ashless filter paper. Soil pH was established in the extract of KCl 1 mol L^{-1} (soil/solution ratio of 1/2.5). Peat organic matter content was estimated by loss-on-ignition at 525 °C.

Plant bioassay

Plant bioassay was carried out following a standard protocol (ISO 11269-2, 2012), as described in detail in our previous study (Tarasova *et al.*, 2020). Foliar elemental concentrations were established after 21 days of plant growth using the ICP-MS technique on a PerkinElmer ELAN 9000 DRC-e mass-spectrometer following microwave digestion with a mixture of concentrated $HF+HCl+HNO_3$. Standard reference materials, taken through the entire analysis process, provided experimental values of the trace metals of interest within 100 ± 20% of the certified values. Duplicates were performed on every 10th sample, in order to assure quality. Blanks were measured and they were always under the detection limit.

Three replicates were used for foliar analyses. However, due to the lack of plant biomass in the untreated soils, all the replicates had to be combined. Thus, only one value for the foliar concentration of each element was established in the untreated soils (Table 2).

ANOVA tests were used to compare the effects of treatments on soil and plant responses; the Tukey test was used as an *a posteriori* test ($p \leq 0.05$). To comply with the objective of the study, we analyzed only amended soils and excluded any untreated soils. The analyses were performed using the Minitab 17 program.

Table 1. Total metal concentrations and organic matter content in soils under study and in commercial peat. Background total metal concentrations in soils in the study area are also shown for comparison.

Cuadro 1. Concentraciones totales de metales y el contenido de materia orgánica en suelos estudiados y en la turba comercial. También se muestra la línea base de las concentraciones totales de metales en los suelos del área de estudio.

Variable	Unit	Soil 2*	Soil 3	Commercial peat	Background concentrations **	Background concentrations ***
Total Cu	mg kg^{-1}	209	345	4.9	12 ± 7.2	24 ± 6.9
Total Ni	mg kg^{-1}	341	357	10	18 ± 17	47 ± 16
Total Zn	mg kg^{-1}	41	86	18	48 ± 0.07	-
Total Cd	mg kg^{-1}	0.04	0.04	0.007	0.22 ± 0.16	0.51 ± 0.18
Total Pb	mg kg^{-1}	3.7	5.6	2.5	18 ± 11	26 ± 10
Total Co	mg kg^{-1}	15	26	1.07	7.4 ± 8.9	7.4 ± 4.6
Organic matter	%	6.4	4.6	91	-	-

* Soil 1 was used in our previous study (Tarasova *et al.*, 2020)
** Kashulina (2017)
*** Barcan and Kovnatsky (1998)

Table 2. Soil and plant responses (n = 3) to the amendment application. Average values and standard deviations are shown. Different letters in the same column indicate statistically significant differences between the treatments ($p < 0.05$). Due to the lack of plant biomass in the untreated soils, all replicates were united.

Cuadro 2. Respuestas del suelo y de la planta (n = 3) a la aplicación de las enmiendas. Se muestran los valores promedios y las desviaciones estándar. Letras distintas en la misma columna indican diferencias estadísticamente significativas entre los tratamientos ($p < 0{,}05$). Debido a la falta de biomasa vegetal en los suelos no tratados, todas las réplicas fueron unidas.

Treatment	Soil pH	Cu_{exch} mg kg^{-1}	Ni_{exch} mg kg^{-1}	Foliar Cu mg kg^{-1}	Foliar Ni mg kg^{-1}	Foliar Co mg kg^{-1}
Soil 2						
1	4.2 ± 0.11	104 ± 6.5	35 ± 2.5	698	1328	63
2	7.5 ± 0.10 a	4.3 ± 2.3 a	bdl	61 ± 3.1 a	22 ± 8.6 a	1.3 ± 0.60 a
3	7.4 ± 0.12 a	1.4 ± 0.94 a	bdl	61 ± 6.6 a	13 ± 0.44 a	0.6 ± 0.05 a
4	7.6 ± 0.10 a	bdl	bdl	68 ± 3.5 a	15 ± 1.2 a	0.7 ± 0.07 a
Soil 3						
1	4.3 ± 0.12	160 ± 61	63 ± 20	75	467	37
2	7.2 ± 0.16 a	0.37 ± 0.54 a	bdl	56 ± 9.1 a	9.2 ± 3.2 b	0.90 ± 0.28 a
3	7.4 ± 0.04 a	0.34 ± 0.57 a	bdl	53 ± 5.7 a	16 ± 1.4 a	1.2 ± 0.04 a
4	7.5 ± 0.09 a	bdl	bdl	56 ± 3.7 a	10 ± 0.83 ab	2.9 ± 2.7 a

bdl: below detection limit

RESULTS AND DISCUSSION

Total Ni, Cu, and Co concentrations in soils under study were several times higher than the corresponding background concentrations (i.e., metal concentrations in soils without anthropogenic impact) (Table 1). On the other hand, total Zn, Cd, and Pb concentrations were similar to background values (Barcan and Kovnatsky, 1998; Kashulina, 2017). The pH values of untreated soils were acidic (pH = 4.2-4.3, Table 2). Since acidic conditions make metals more soluble, potentially increasing their availability (Lillo-Robles *et al.*, 2020), we expected to observe very high exchangeable metal concentrations in the untreated soils and high metal concentrations in the shoots of *L. perenne* (Table 2, treatment 1). As a result, the shoot length and biomass of *L. perenne* was strongly inhibited in the unamended soil (Figures 1 and 2).

All dolomite + vermiculite-lizardite treatments had circumneutral pH values (Table 2). On account of this, these treatments displayed lower exchangeable metal concentrations both in the soil and in the shoots of *L. perenne* (Table 2) boosting plant growth in the lime and vermiculite-lizardite treated soils (Figure 1). However, the difference between Treatment 2 (without peat) and Treatment 3 (with peat) was not statistically significant ($p > 0.05$) for exchangeable Cu concentrations in soil, whereas exchangeable Ni concentrations were below the limit of detection. Likewise, the difference in plant responses between Treatment 2 (without peat) and Treatment 3 (with peat) was either not statistically significant ($p > 0.05$), or growth improvement was not substantial (Figure 2). Furthermore, the difference in plant responses between Treatment 3 (peat) and Treatment 4 (fertilizer) was either not statistically significant ($p > 0.05$), or growth improvement was not substantial (Figure 2).

We noted that early shoot growth in grasses—and *Lolium* species in particular—had a linear relationship with growth time (Thomas *et al.*, 1999). Consistent with this finding, our recent experiment under laboratory conditions were similar to those in the present study (Tarasova *et al.*, 2020), demonstrating a linear relationship between *L. perenne* shoot length and growth time in uncontaminated commercial peat. In contrast, Tarasova *et al.* (2020) revealed a non-linear relationship of shoot growth with growth time in polluted Histosol extracted from the vicinity of the Ni/Cu smelter, both before and after treatment. Given that the study of Tarasova *et al.* (2020) used substrates of peats diffe-

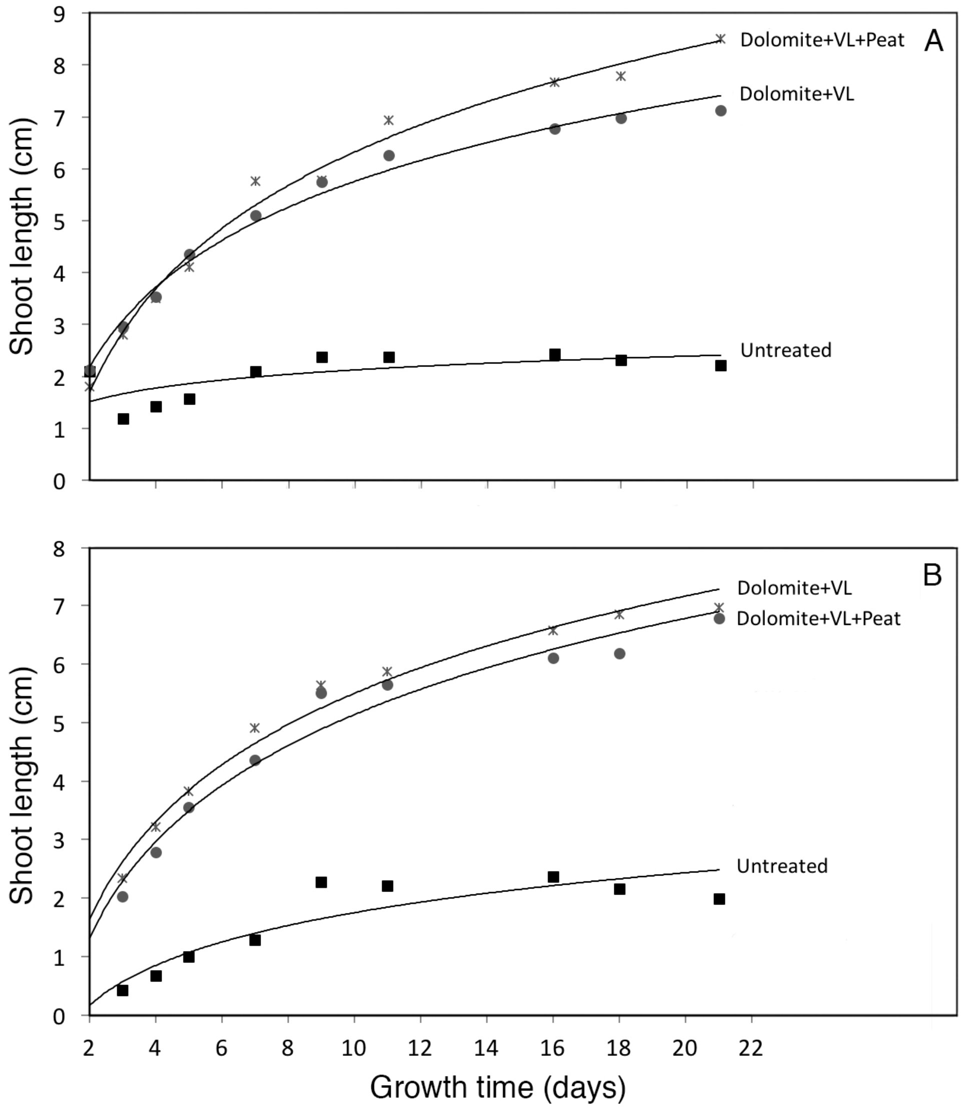

Figure 1. (A) Shoot length of *Lolium perenne* depending on growth time in Soil 2; (B) shoot length of *Lolium perenne* depending on growth time in Soil 3. Results are shown for the following treatments: (1) dolomite and vermiculite-lizardite (VL); (2) dolomite, vermiculite-lizardite (VL) and commercial peat; (3) untreated. The fourth treatment is not shown to simplify the figure and allow for better visibility and understanding.

Figura 1. (A) Longitud del brote de *Lolium perenne* en función del tiempo de crecimiento en el suelo 2; (B) Longitud del brote de *Lolium perenne* en función del tiempo de crecimiento en el suelo 3. Se muestran los resultados para los siguientes tratamientos: (1) dolomita y vermiculita-lizardita (VL); (2) dolomita, vermiculita-lizardita (VL) y turba comercial; (3) sin tratar. El cuarto tratamiento no se muestra para simplificar la figura y permitir mejor visibilidad y comprensión.

ring only in the degree of metal pollution, we infer that plant growth was negatively impacted by metal toxicity in polluted soils even after treatment.

The present study demonstrated a non-linear relationship of *L. perenne* shoot growth with growth time, in both untreated and treated soils (Figure 1). Thus, as in the case of Tarasova *et al.* (2020), we infer that slow plant growth in polluted soils was likely caused by metal toxicity, even in soils after treatment. Consistent with this argument, we found foliar Ni concentrations in *L. perenne* plants grown in all of the amended soils to be below the toxicity level of 80 mg kg^{-1} for this species (Reuter and Robinson, 1997). Likewise, foliar Co concentrations in *L. perenne* plants grown in all of the amended soils were below the toxicity level for barley of 6 mg kg^{-1} (Davis *et al.*, 1978). Thus, Ni and Co had no toxic effect on plant development in any of the treatments.

A concentration of 20-30 mg Cu kg^{-1} is considered the critical upper limit of Cu concentration in leaves, excluding highly sensitive or highly tolerant species (Kabata-

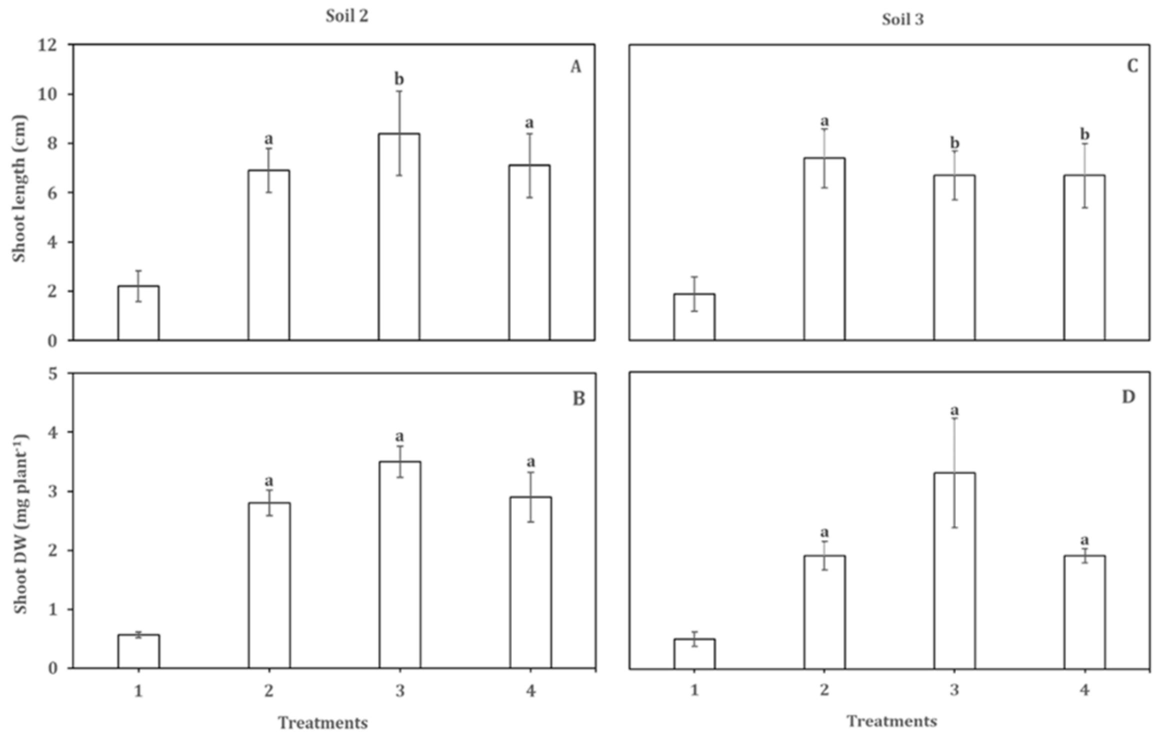

Figure 2. Effect of treatments on (A) shoot length in Soil 2, (B) shoot dry weight (DW) in Soil 2, (C) shoot length in Soil 3 and (D) shoot dry weight (DW) in Soil 3. Different lowercase letters indicate statistically significant differences between the treatments. n = 90 for shoot length; n = 5 for shoot dry weight.

Figura 2. Efecto del tratamiento sobre (A) longitud del brote en el suelo 2, (B) biomasa seca en el suelo 2, (C) longitud del brote en el suelo 3 y (D) biomasa seca en el suelo 3. Letras minúsculas distintas indican diferencias estadísticamente significativas entre los tratamientos. n = 90 para la longitud del brote; n = 5 para la biomasa seca.

Pendias and Pendias, 1992). Consistent with this finding, our previous study reported the EC_{50} (effective concentration 50%) value of 39 mg kg^{-1} for foliar Cu concentration in *L. perenne* (Verdejo *et al.*, 2015). In the present study, foliar Cu concentrations (Table 2) were higher than the upper 95% confidence interval of 47 mg Cu kg^{-1} of the reported EC_{50} value (Verdejo *et al.*, 2015). Therefore, it is likely that Cu toxicity played a part in inhibiting plant growth even in treated soils. However, given the presence of several metallic pollutants, it is hard to pinpoint the exact cause of phytotoxicity in the soils under study.

CONCLUSION

The addition of peat did not improve the efficacy of lime + vermiculite-lizardite combined amendment for promoting plant growth in the soils from the vicinity of the Ni/Cu smelter. Thus, the challenge of achieving decreases of ryegrass metal exposure in soils under study remains. In future studies, we plan to examine other inorganic amendments (e.g., phosphates, iron and manganese oxides, etc.) and test native plant populations in the study area.

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